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Flipping the switch from electrical to chemical communication

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Abstract

Immature neurons in many brain regions are electrically coupled through gap junctions, which are lost as chemical synaptic transmission matures. This developmental uncoupling is now shown to require NMDA receptor activation.

Early in brain development, neurons communicate with one another, even before synapses have formed. At this stage, electrical coupling through gap junctions is widespread and may contribute to neuronal and circuit maturation¹. A transition from electrical to chemical synapses has been documented for multiple brain areas, but the cues and mechanisms that mediate the switch have remained elusive. In this issue, Arumugam and colleagues² provide exciting new insight into the signaling pathways involved in this developmental regulation of gap-junction coupling in hypothalamic neurons.

Gap junctions are specialized cell-cell contacts composed of membrane channels that join the cytosol of neighboring cells, allowing electrical current to flow between them³. Because they are permeable to small molecules (< 1 kDa), such as the second messengers inositol 1,4,5-triphosphate (IP₃) and cyclic AMP (cAMP), gap junctions provide a means to coordinate not only electrical activity but also metabolic and signaling processes in coupled cells⁴. Although neuronal gap junction coupling is not exclusive to the immature brain, in mammals it is most extensive and widespread before and during the time of synapse formation and occurs in many areas, including the retina, cortex, thalamus, brainstem and spinal cord³. Neurons joined by gap junctions form functional assemblies with coordinated patterns of spontaneous activity and changes in intracellular calcium levels⁵. These early gap junction-mediated activity patterns are thought to be important for neurogenesis, neuronal maturation and synaptogenesis.

Arumugam and colleagues examined developmental changes in gap junction coupling in magnocellular neurons of the rat hypothalamus. They measured expression levels of the neuronal connexin protein Cx36 and looked for functional gap junctions by assessing the ability of coupled neurons to pass small dyes or neuronal tracers between them (dye coupling). By both measures, they found that electrical coupling increases *in vivo* during the first two postnatal weeks and then decreases during the third and fourth weeks, a time of intense synapse formation (Fig. 1).

Blocking NMDA receptors attenuates the loss of gap junction coupling in developing spinal motoneurons⁶, so the authors asked whether signaling through NMDA receptors might be

important for the developmental decrease in coupling in the hypothalamus. They chronically blocked NMDA receptors *in vivo* by injecting newborn rats daily with the antagonist dizocilpine (MK-801), and they measured gap-junction coupling two and four weeks later. NMDA receptor blockade had no effect on the initial increase in coupling during the first two postnatal weeks, but it significantly reduced subsequent uncoupling: in four-week-old animals treated with MK-801, the expression of Cx36 as well as the incidence of dye coupling were higher than in untreated controls. This effect seemed to be neuron specific because the expression of the protein Cx43, a connexin expressed by glia, was not altered by MK-801.

These results extend the findings in spinal motoneurons⁶ and support the idea that NMDA receptor activation contributes to developmental uncoupling. However, systemic MK-801 treatment can disrupt other developmental processes—such as weight gain, motor development and synaptic refinement—that may indirectly affect uncoupling. To avoid these potential confounds, Arumugam and colleagues repeated the experiments in primary cultures of hypothalamic neurons, which showed a sequence of coupling and uncoupling similar to that observed in the intact brain. The NMDA receptor antagonist DL-2-amino-5-phosphonovalerate (AP5) did not affect the initial increase in coupling during the first 16 days *in vitro*, but like MK-801 treatment *in vivo*, AP5 nearly abolished uncoupling during the subsequent two weeks. For additional confirmation, the authors examined gap junction development in cultures from mice lacking the NMDA receptor subunit NR1, which is required for functional receptors. Electrical coupling failed to decrease after 16 days *in vitro* in these cultures.

Having established a crucial role for NMDA receptors, Arumugam and colleagues asked which intracellular signaling pathways might link NMDA receptor activation to Cx36 downregulation and functional uncoupling. Using a battery of drugs, they identified a requirement for calcium/calmodulin-dependent protein kinases II and IV (CaMKII/IV) and protein kinase C (PKC), both known for contributing to activity-dependent neuronal plasticity. In contrast, cAMP-dependent protein kinase (PKA) and extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK), which are also involved in activity-dependent plasticity, were not required for uncoupling. Calcium influx through NMDA receptors seems to be sufficient to trigger CaMKII/IV- and PKC-mediated uncoupling, because it was not prevented by blocking ionotropic non-NMDA glutamate receptors or by blocking voltage-gated calcium channels.

What lies downstream of CaMKII/IV and PKC to mediate uncoupling? Calcium-cAMP response element binding protein (CREB), a transcriptional activator, is important for NMDA receptor-dependent, long-term neuronal plasticity^{7–9} and was thus a likely suspect in uncoupling. Both members of the CaMK family and PKC can—either directly (CaMKs) or indirectly (PKC)—phosphorylate CREB at residue S133 and enhance its transcriptional capacity¹⁰. Using a three-pronged approach that included CREB overexpression, overexpression of a dominant-negative form of CREB and administration of CREB antisense oligodeoxynucleotides, the authors showed that more CREB accelerated uncoupling, whereas less or mutant CREB attenuated it.

This inverse correlation between CREB protein levels and electrical coupling raises interesting questions about the relationship between PKC- or CaMK-dependent CREB-mediated gene expression and gap junctions. For instance, do the changes in coupling following the various types of CREB manipulation that the authors observed result from alterations in Cx36 expression or function? The authors note that the promoter region of Cx36 includes a CREB binding motif. However, CREB binding motifs are found in hundreds of other genes, only a subset of which are actually known to be regulated by CREB; therefore, CREB binding motifs do not guarantee CREB-regulated expression¹⁰. The present observation of a tight relationship between NMDA receptor activation and gap-junction uncoupling invites a more rigorous dissection of the molecular and cellular events involved.

Another interesting issue that remains to be addressed concerns the dynamics of the reciprocal relationship between gap-junctions and chemical synapses. In identified motoneuron pairs of the snail *Heliosoma*, gap junction coupling increases when cholinergic synaptic transmission is blocked and decreases when chemical synaptic transmission is increased¹¹. Is there a similar bidirectional interaction between NMDA receptors and gap junctions in hypothalamic or other mammalian neurons? For example, are they related in a self-perpetuating feedback mechanism, in which increased NMDA receptor activation is associated with decreased gap junction coupling, which in turn is associated with further increases in NMDA receptor activation? Careful examination of the time course of changes in NMDA receptor expression and activation and gap junction coupling, both across development and in response to experimental manipulations, should shed light on this pertinent issue and provide further information about underlying mechanisms.

In their cultured hypothalamic neurons, Arumugam and colleagues also found that blocking sodium-dependent action potentials with tetrodotoxin (TTX) prevented the developmental reduction in coupling, similar to the effect of NMDA receptor blockade. Although the specific mechanisms by which action potentials contribute to uncoupling still need to be investigated, one can think of at least three major possibilities. Action potentials may be required presynaptically for synchronized glutamate release and consequent NMDA receptor activation. Backpropagating action potentials that are present in many neurons¹² might also be required for sufficient depolarization in postsynaptic cells to relieve the voltage-dependent Mg^{2+} block of NMDA receptors. Action potentials may be required to trigger a retrograde synaptic signal that causes the presynaptic neuron to uncouple from its neighbors¹³. In any event, the TTX results suggest the interesting possibility that the coordination and/or the propagation of action potentials in neuronal assemblies may contribute directly or indirectly to uncoupling.

These new findings from Arumugam and colleagues constitute a big step forward in our understanding of the mechanisms that underlie early neuronal network formation. NMDA receptors are crucial for the refinement of synaptic connections throughout development. It now seems that NMDA receptors also flip the switch from electrical to chemical communication and thereby fundamentally change the mode of interneuronal communication. Many aspects concerning the nature of the link between NMDA receptor activation and gap junction loss await further investigation, and it will be interesting to know

whether similar mechanisms contribute to circuit development in other brain regions. The exciting findings from Arumugam and colleagues provide a promising starting point for addressing these important issues.

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Figure 1.

NMDA receptor–mediated uncoupling of developing hypothalamic neurons. During synaptic circuit development in the medial hypothalamus and many other brain regions, the primary mode of neuronal communication switches from one based on gap junctions (left) to one based on chemical synapses (right). Arumugam and colleagues² now show that downregulation of the neuronal gap-junction protein connexin36 (Cx36) and dye coupling (yellow) require activation of NMDA receptors, along with CaMKII/IV, PKC and CREB. In the intact brain, additional glutamatergic inputs are provided by other extrinsic sources.