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Filling the (SR)GAP in Excitatory/Inhibitory Balance

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In this issue of *Neuron*, Fossati et al. (2016) report that through its domain structure, SRGAP2A, a Rho-GTPase-activating protein, can co-regulate excitatory and inhibitory synapse development, offering a putative evolutionary genetic mechanism for preserving excitatory/inhibitory balance during speciation.

Maintaining proper balance of excitation and inhibition (E/I balance) is critical for information processing and plasticity in the nervous system. This requires a neuronal infrastructure with specific proportions of excitatory and inhibitory synapses. Despite variations in synapse densities, the ratio of excitatory and inhibitory synapses in cortical layers appears to be fairly conserved between species (DeFelipe et al., 2002), suggesting that evolutionary genetic mechanisms maintain this conservation. Yet we know little regarding such mechanisms and how they might operate. They could potentially act to alter the density of one synapse type, resulting in compensatory changes in the other. Alternatively, common mechanisms could co-regulate both synapse types, resulting in balanced changes to both during speciation.

Many genes have been shown to regulate either excitatory or inhibitory synapse formation (Paradis et al., 2007). In some instances, different isoforms of the same gene promote different synapse types (Craig and Kang, 2007). One gene with a human-specific paralog that has been shown to play a role in excitatory synapse development is Slit-Robo GTPase-activating protein 2 (*SRGAP2*), a mammalian Rho-GAP. Its human paralog, *SRGAP2C*, is a partial duplication of *SRGAP2*, and its protein product antagonizes the function of the ancestral SRGAP2 (SRGAP2A) (Charrier et al., 2012). Interfering with SRGAP2A function by heterologous expression of human SRGAP2C in mouse neocortical neurons leads to synaptic attributes associated with human brain development, namely, higher dendritic spine density and delayed maturation of excitatory synapses. In a study published in this issue of *Neuron*, Fossati et al. (2016) follow up on the finding that SRGAP2A interacts with Gephyrin, a postsynaptic scaffolding molecule at inhibitory synapses (Okada et al., 2011), and demonstrate that similar to the case for excitatory synapses, inhibition of SRGAP2A also increases the density of inhibitory synapses and delays their maturation. They further characterize different domains of SRGAP2 that confer coordinated regulation of excitatory and inhibitory synapses. Thus, the same human-specific partial duplication of SRGAP2 influences both excitatory and inhibitory synapse development, providing an evolutionary mechanism for coordinated regulation of these two synapse classes and offering an elegant solution for maintaining E/I balance during the expansion of synapse numbers in the human lineage.

Excitatory synapses are formed on dendritic spines, which typically have an enlarged head connected to the dendrite through a neck. The shape and size of spine head and neck are a reflection of the maturation status of excitatory synapses. In contrast, there is no morphological surrogate that can serve as an anatomical proxy for inhibitory synapses. As a consequence, very little is known about their density, distribution, and regulation. Inhibitory synapses can be visualized as symmetric synapses by electron microscopy; however, the low-throughput nature of this technique limits its use in mechanistic investigations. Recently, fluorescently labeled Gephyrin was shown to be a reliable marker for inhibitory synapses (Chen et al., 2012), allowing visualization of their distribution across the dendritic arbor of labeled neurons. Fossati et al. (2016) take advantage of this labeling strategy to examine the effect of SRGAP2A inactivation, which should effectively mimic expression of the SRGAP2C human paralog, on inhibitory synapses. Using in utero electroporation to fluorescently label a sparse subset of layer II/III neurons in mouse somatosensory cortex, they expressed a cell fill (TdTomato) to visualize spine morphology and EGFP-tagged Gephyrin to identify inhibitory synapses. Small hairpin RNA (shRNA) against *Srgap2a* was expressed from the same plasmid as the cell fill so that all the labeled neurons were knocked down for SRGAP2A throughout development. In control neurons, by 3 weeks after birth inhibitory synapse density and distribution resembled that of adults with ~two to three inhibitory synapses per 10 μm of dendrite, and 25% of them localized to dendritic spines. At the same age, SRGAP2A knockdown neurons had ~four inhibitory synapses per 10 μm, with ~40% of them on spines, and the average size of the Gephyrin puncta was smaller. Inhibitory synapse development has not been well characterized, so one can only presume that the smaller synapse size and higher frequency of spine synapses reflect the persistence of an immature state. By the time the mice reach adulthood, the density of inhibitory synapses remains elevated, but their average size is no longer different from wild-type controls. The authors conclude that in the absence of SRGAP2A, inhibitory synapse maturation is delayed, and this is independent of SRGAP2A's role in limiting synapse number.

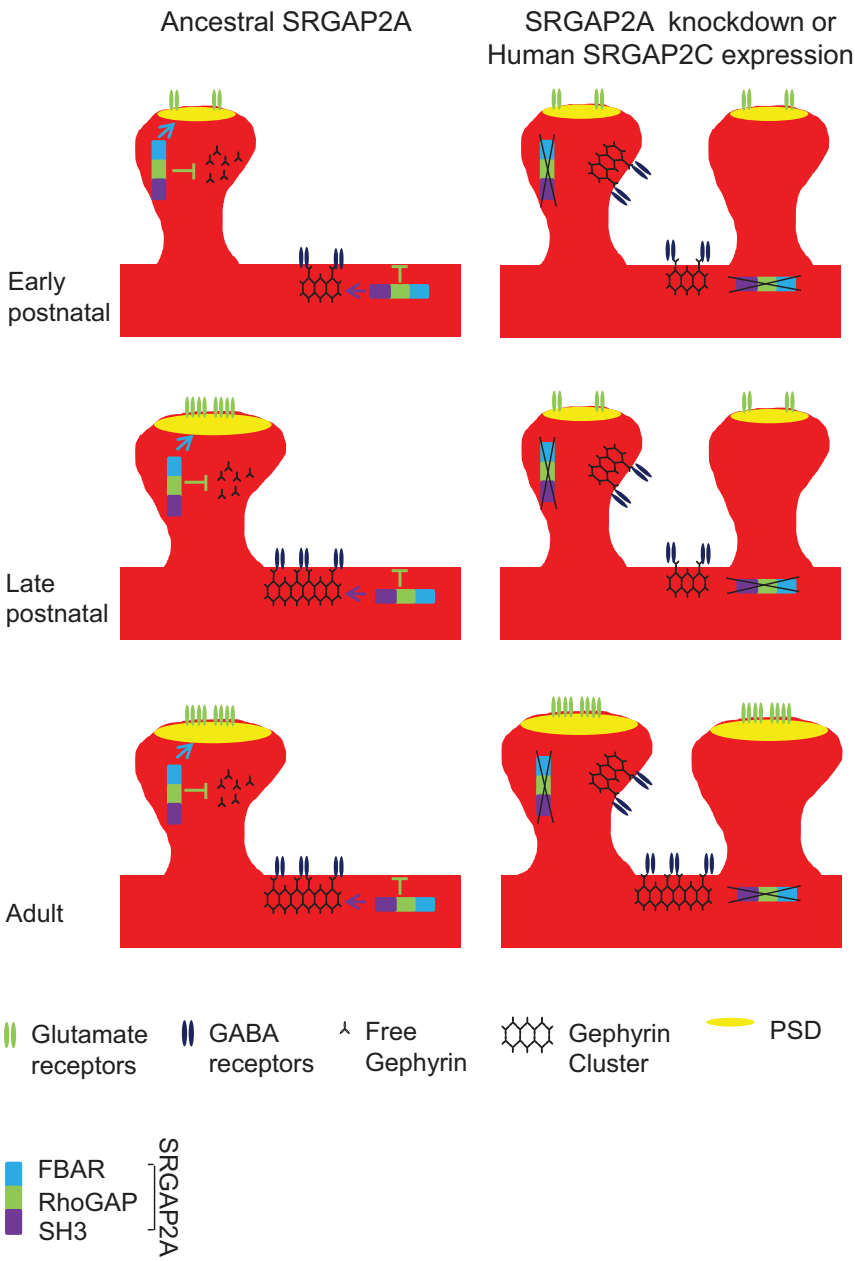

Figure 1. SRGAP2A-Mediated Regulation of Excitatory and Inhibitory Synapse Density and Maturation

In normal mice, excitatory and inhibitory synapses increase in size during development and reach adult levels by the late postnatal period (left panel). The FBAR and SH3 domains of SRGAP2A promote maturation of dendritic spines and inhibitory synapses, respectively. The Rho-GAP domain suppresses clustering of Gephyrin and initiation of new spines. When SRGAP2A is knocked down or is antagonized by SRGAP2C expression (right panel), Rho-GAP-mediated suppression is relieved and the density of spines and Gephyrin clusters increases. Loss of FBAR and SH3 domain function delays all synaptic maturation. Synapse maturation is protracted as compared to wild-type, but synapses reach wild-type size by adulthood. Consequently, adult synaptic densities are elevated, but excitatory/inhibitory ratios are maintained.

The effect of SRGAP2A knockdown on inhibitory synapse development has striking parallels to its effect on excitatory synapses. In both cases, there is an increase in synapse density and a delay in maturation (Charrier et al., 2012). While it is possible that changes to one synapse type are a homeostatic adaptation to changes in the other, the domain structure of SRGAP2A suggests that it has the potential to concomitantly regulate both synapse types. SRGAP2A has an N-terminal F-BAR domain containing an EVH1 motif, a canonical binding site for the excitatory synaptic scaffolding molecule Homer, and the C-terminal SH3 domain has previously been shown to interact with Gephyrin. In addition, SRGAP2A has a central Rho-GAP domain that can inactivate small GTPases belonging to the Rho family. Fossati et al. (2016) show that immunoprecipitation of Homer or Gephyrin from brain lysates pulls down SRGAP2A, confirming its interaction with both classes of synaptic proteins. When either the EVH1 or SH3 domain is mutated, the interaction of SRGAP2A with Homer and Gephyrin, respectively, is lost.

The authors then go on to delineate the roles of the EVH1, SH3, and Rho-GAP domains in particular aspects of excitatory and inhibitory synapse formation and maturation. They use a gene replacement strategy in which they knock down endogenous *Srgap2a* with shRNA and replace it with an shRNA-resistant *Srgap2a* with or without mutations specific to each of its functional domains. Mutations in the EVH1 domain prevented rescue of the delayed excitatory synapse maturation elicited by SRGAP2A knockdown, and mutations in the SH3 domain prevented rescue of inhibitory synapse maturation. Neither domain seemed to play a major role in determining synaptic densities (Figure 1). In contrast, mutations in the Rho-GAP domain had no effect on maturation of either excitatory or inhibitory synapses but were critical for regulating the density of both synapse classes (Figure 1). These findings strongly argue that the changes to inhibitory synapse maturation and density elicited by antagonizing SRGAP2A function do not represent homeostatic adaptation to changes in excitatory synapse development, but rather are a direct consequence of SRGAP2A's unique structural and functional ability to co-regulate both synapse types. Another interesting implication of the SRGAP2A mutant analyses is that the regulation of synapse maturation and synapse density is separable. Interaction of SRGAP2A with Homer and Gephyrin does not influence synapse densities,

but contributes to the maturation of pre-existing synapses, with domain specificity for either excitatory or inhibitory synapses. The Rho-GAP domain does not influence synapse maturation, but influences synapse densities of both synapse types.

It remains to be determined whether the Rho-GAP domain influences both synapse types by inactivating the same downstream small GTPase. Rho family GTPases have previously been implicated in Gephyrin clustering as well as excitatory synapse development. Collybistin, a regulator of Gephyrin clustering, is an activator of the Rho family GTPase Cdc42. Overexpression of activated Cdc42 in cultured neurons produces numerous small Gephyrin puncta, reminiscent of the SRGAP2A inhibitory knockdown phenotype (Tyagarajan et al., 2011). Overexpression of activated Rac1, another Rho GTPase, can induce a high density of very thin spines (Tashiro et al., 2000), reminiscent of the SRGAP2A excitatory knockdown phenotype. The Rho-GAP domain of SRGAP2A interacts strongly with Rac1 and only weakly with Cdc42 (Guerrier et al., 2009), but it is still possible that this weak interaction is sufficient to sequester Cdc42 and attenuate Gephyrin clustering. Thus, there is a strong precedent for activated Rho-GTPases acting to increase excitatory and inhibitory synapse densities with qualitatively similar morphological features as the SRGAP2A knockdown. Rescue of the SRGAP2A phenotype with different constitutively active Rho-GTPases could address whether suppression of excitatory and inhibitory synapse formation by SRGAP2A relies on common downstream mechanisms.

The suppression of synapse formation by SRGAP2A and its antagonism with SRGAP2C raises other interesting questions. Does SRGAP2 determine the set point for total number of synapses in a neuron, or does it act as a "synaptogenic" molecule that promotes the initiation or progression of synapse formation? When in human development does SRGAP2C- SRGAP2A antagonism cause synaptic changes, and how is this antagonism relieved? Does it differ in evolutionarily older circuits, such as those in the hindbrain, as compared to the newer circuits of neocortex? Manipulating the temporal patterns of SRGAP2A and SRGAP2C expression and understanding their interaction, along with the elucidation of their downstream mechanisms for regulation of synapse formation, offer an exciting avenue for future research.

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Should I Stay or Should I Go?

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In this issue of *Neuron*, Lee et al. (2016) assessed the brain-wide effects of stimulating the direct and indirect pathway by optogenetic activation of D1 and D2 striatal neurons. This work demonstrates the exquisite power of combining cell-type-specific perturbation methods with focal and whole-brain measurements of brain activity.

Cristiano Ronaldo cuts from the right flank toward the center of the pitch, followed by a majestic dribble knocking out three defenders. He then swings his left foot, sending the ball curling into the right corner of the goal just out of the goalkeeper's reach. Such brilliant action on the soccer field is orchestrated not by the instructions of the player's trainer or the cheers of the fans, but by a tightly choreographed chain of neurons activated in his central nervous