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# Molecular diversity underlying cortical excitatory and inhibitory synapse development

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The complexity and precision of cortical circuitries is achieved during development due to the exquisite diversity of synapse types that is generated in a highly regulated manner. Here, we review the recent increase in our understanding of how synapse type-specific molecules differentially regulate the development of excitatory and inhibitory synapses. Moreover, several synapse subtype-specific molecules have been shown to control the targeting, formation or maturation of particular subtypes of excitatory synapses. Because inhibitory neurons are extremely diverse, a similar molecular diversity is likely to underlie the development of different inhibitory synapses making it a promising topic for future investigation in the field of the synapse development.

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## Introduction

From both an evolutionary and a developmental perspective, brain wiring reaches an exceptional level of complexity in the cerebral cortex. The precision that characterizes this process is truly astonishing and even more so if one considers its outcome, us. Cortical circuitries, honed over hundreds of million years of evolution, are composed of an interconnected multitude of neuronal cell types which fall in two broad categories: excitatory pyramidal neurons and inhibitory GABAergic interneurons.

The elaborate but partially stereotyped connectivity patterns of different neuronal types are perfectly suited to fulfill specific functional roles and underlie the cortex's unique computational prowess [1]. Such specificity implies not only cellular but also synaptic diversity that is built upon molecular diversity and emerges through a tightly regulated sequence of developmental processes. Each of these steps gradually restricts the number of potential synaptic partners and further sculpts specific synaptic properties. Regardless of its multiplicity, it is generally agreed that synapse development involves two broad sequential phases. First, mostly genetically determined processes lead to a transient and relatively nonspecific contact that is stabilized by molecular interactions. Afterward, during synapse maturation, a series of progressively more activity-dependent processes kick in. In this review, we will focus on the developmental molecular mechanisms that generate synapse diversity in the cerebral cortex, with a particular emphasis on the differences and similarities existing between excitatory and inhibitory synapses.

## 4. Title freely adapted from Oscar Wilde's 1895 play 'The Importance of Being Earnest'. The importance of being Axon[4]: first contact between synaptic partners

Axons terminals are endowed with the ability to discriminate their correct synaptic targets among a dense array of potential partners. A key role in mediating the first contact between synaptic partners is played by transmembrane cell adhesion molecules (CAMs) that serve both as permissive adhesion substrates and as recognition tags. For example, distinct cadherins and leucine-rich repeat (LRR) proteins are expressed in different cell types and can regulate input-specific target selection [2,3]. Classical guidance cues like Semaphorins and their receptor neuropilins have been associated with proximal and distal pyramidal cell dendritic targeting [4]. In addition, recent work showed that the cell-adhesion G protein-coupled receptor of alpha-latrotoxin latrophilin-1 and the transmembrane protein teneurin-3 are required for the specific targeting of entorhinal cortex afferents to CA1 pyramidal dendrites and CA1 hippocampal axons to distal subiculum, respectively [5•,6].

[4] Title freely adapted from Oscar Wilde's 1895 play 'The Importance of Being Earnest'.

Cortical inhibitory neurons also exhibit exquisite target specificity. A classic example is represented by SST-positive Martinotti cells, which are particularly abundant in neocortical layer V and possess ascending axons that arborize in layer I where they establish synapses onto the dendritic tufts of pyramidal neurons [7]. In the cerebellum, chemoaffinity-based recognition strategies ensure the correct targeting of inhibitory axons [8]. Although it is possible that similar mechanisms function across different cortices, the molecules regulating inhibitory target specificity in the cerebral cortex still await discovery.

## 5. Title freely adapted from Oscar Wilde's 1895 play 'An Ideal Husband'.An Ideal Husband, Act I[5]: forming a synapse

Once matching synaptic partners are in contact, a coordinated assembly of molecules on both sides of the synapse takes place. This is mediated by synaptic organizers which, in addition to having a cohesive role, initiate bidirectional trans-synaptic signaling events that trigger a near-complete program for pre-synaptic and post-synaptic differentiation [9].

The best example of cell adhesion molecules with both adhesive and inducing function at synapses are neuroligins and neurexins [10]. The elegant experiment that led to the discovery of the neuroligins and neurexins as potent inducers of synapse formation has become a 'classic' of neuroscience and paved the way for the discovery of several other synaptogenic adhesion complexes, such as the cell adhesion molecule SynCAM [11] or members of the leucine-rich repeat (LRR) family of cell adhesion proteins [2].

Although most synaptic organizers are ubiquitously expressed, the exceptional diversity of isoforms, ligands and interactors that they can combine in a cell-specific or circuit-specific manner critically contributes to generating synapse diversity (Figure 1). For instance, different neurexin isoforms exhibit a cell-type specific expression and pan-neurexin deletion produces dramatically diverse phenotypes at different types of synapses [12–14]. Another example is provided by how different neuroligin splice variants selectively induce glutamatergic or GABAergic presynaptic differentiation, likely through specific trans-synaptic interactions with Neurexins [15]. Selectivity may be further achieved by recruiting synapse type-specific molecules, as is the case for Neuroligin 2 which by interacting with Gephyrin recruits Gephyrin-associated proteins to inhibitory postsynapses [16]. In addition to ubiquitous synaptogenic complexes, several synapse type-specific organizers have also been identified. Although nearly all of them promote only excitatory synapse development [17–21], Sema4D-PlexinB1, Slitrk3-PTPδ and Neurexin2α-IgSF21 were identified as trans-synaptic organizing complexes selectively required for GABAergic synapse development [22,23,24•]. Interestingly, recent work showed that, despite having inducing properties, both Slitrk3 and Sema4D act in a second phase of synapse formation. In particular, Slitrk3 functions in a hierarchical and synergistic manner after Neuroligin 2 initiates the synaptic assembly [25] and Sema4D induces remodeling of the actin cytoskeleton and consequent bouton stabilization [26]. Similarly, C1q-like proteins belong to a family of extracellular synaptic organizers and have been recently shown to recruit functional postsynaptic kainate-type glutamate receptors complexes during synapse maturation (Figure 2) [27,28]. These recent findings suggest that the line traced between synapse formation and maturation is likely to be less clear-cut than what is often assumed for the sake of description.

Most synaptogenic molecules discovered so far are transmembrane adhesion molecules. However, members of the fibroblast growth factor (FGF) family have been shown to act as soluble target-derived presynaptic organizers that induce clustering of synaptic vesicles and differentiation of the presynaptic specialization [29]. Interestingly, the synaptogenic function of FGFs is synapse type-specific: FGF22 and FGF7 promote the differentiation of excitatory and inhibitory presynaptic terminals, respectively [30].

In addition to synaptic organizers, cell type-specific molecules that have adhesive but not inducing properties also contribute to synapse specificity by dictating whether a transient contact is transformed in a synapse or not. An excellent example of this selective synaptogenesis onto correct targets is how cadherin-9 regulates preferential synapse formation — rather than axon targeting — of dentate gyrus (DG) axons onto CA3 but not CA1 pyramidal neurons in the hippocampus [31].

## An Ideal Husband, Act II: synapse maturation

Excitatory and inhibitory synapses are constantly generated at a high rate in the developing cortex. Newborn synapses are functional but immature. Subsequently, a combination of genetically predetermined developmental programs [32–34] and activity-dependent processes mediates synapse maturation [35].

The maturation of a synapse involves structural and functional changes that are intimately related to both an increased efficacy of presynaptic neurotransmitter release and a mature profile of postsynaptic receptors. The basic organizing principles of synapse maturation hold true for both excitatory and inhibitory synapses (Figure 2). A critical step in this process is the recruitment of scaffolding proteins, abundant and essential

[5] Title freely adapted from Oscar Wilde's 1895 play 'An Ideal Husband'.