REVIEW

The cell biology of synapse formation

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In a neural circuit, synapses transfer information rapidly between neurons and transform this information during transfer. The diverse computational properties of synapses are shaped by the interactions between pre- and postsynaptic neurons. How synapses are assembled to form a neural circuit, and how the specificity of synaptic connections is achieved, is largely unknown. Here, I posit that synaptic adhesion molecules (SAMs) organize synapse formation. Diverse SAMs collaborate to achieve the astounding specificity and plasticity of synapses, with each SAM contributing different facets. In orchestrating synapse assembly, SAMs likely act as signal transduction devices. Although many candidate SAMs are known, only a few SAMs appear to have a major impact on synapse formation. Thus, a limited set of collaborating SAMs likely suffices to account for synapse formation. Strikingly, several SAMs are genetically linked to neuropsychiatric disorders, suggesting that impairments in synapse assembly are instrumental in the pathogenesis of neuropsychiatric disorders.

Synapses: Fundamental computational units that wire circuits

Synapses are asymmetric intercellular junctions that mediate rapid point-to-point communication between neurons, and thereby connect neurons into circuits (Fig. 1 A). Synapses not only transfer information from one neuron to the next, but also process this information during transfer. The computational properties of synapses differ and cannot be predicted based on their ultrastructure or location (Monday et al., 2018; Gjorgjieva et al., 2016). As a result, it is difficult to deduce a circuit's input/output relations (and functions) from its wiring diagram without understanding its synaptic components (Fig. 1 B).

A neuron often fires bursts or trains of action potentials that vary in frequency and length (its "spike code"). The spike code of a presynaptic neuron is computed into distinct postsynaptic responses at various output synapses. The diverse properties of the input and output synapses of a neuron depend on trans-synaptic interactions between the neuron and its synaptic partners. For example, output synapses of a presynaptic neuron exhibit distinct patterns of short-term synaptic plasticity, which is generally controlled by presynaptic mechanisms, depending on the identity of the postsynaptic neuron. This observation suggests that the postsynaptic neuron "instructs" the short-term plasticity of the presynaptic neuron (Reyes et al., 1998; Rozov et al., 2001; Koester and Johnston, 2005). Moreover, synaptic computations are continuously readjusted by various forms of short- and long-term synaptic plasticity, and synapses themselves are subject to turnover. Thus, circuits are not hard-wired, but malleable computational structures.

Neurons communicate not only rapidly via synapses, but also more slowly via nonsynaptic signals. These neuromodulatory signals include neuropeptides, transmitters diffusing outside of the synaptic cleft (e.g., monoamines and acetylcholine), and diffusible molecules (e.g., endocannabinoids, retinoic acid, and nitric oxide). Neuromodulatory signals control neural circuits and amplify the complexity of synaptic information processing. For example, in *Caenorhabditis elegans*, a mechanosensory stimulus is communicated to muscle by a neuropeptide, not a synaptic neurotransmitter, illustrating how powerful nonsynaptic communication can be in driving a behavior (Tao et al., 2019).

Design of synapses

All chemical synapses exhibit the same overall structure (Fig. 1 A): a presynaptic terminal abuts a postsynaptic specialization, separated by a uniform 15–20-nm synaptic cleft. The presynaptic side is specialized for Ca^{2+}-triggered neurotransmitter release, whereas the postsynaptic side is dedicated to neurotransmitter reception. Presynaptic specializations are generally formed by axons, but can also be assembled by dendrites in dendrodendritic synapses in many brain regions, in particular the olfactory bulb and thalamus (Shepherd, 2009; Cox and Beatty, 2017). Postsynaptic specializations are most frequently formed on dendritic spines (excitatory synapses) or dendritic shafts and neuronal soma (inhibitory synapses), but can also be formed on axons. Synapses exhibit diverse properties, such as the neurotransmitter type, release probability, postsynaptic receptor composition, and presence of neuromodulatory receptors (e.g., γ-aminobutyric acid type B [$GABA_B$] or endocannabinoid receptors). As a result of different locations and properties, the brain comprises hundreds of synapse types.

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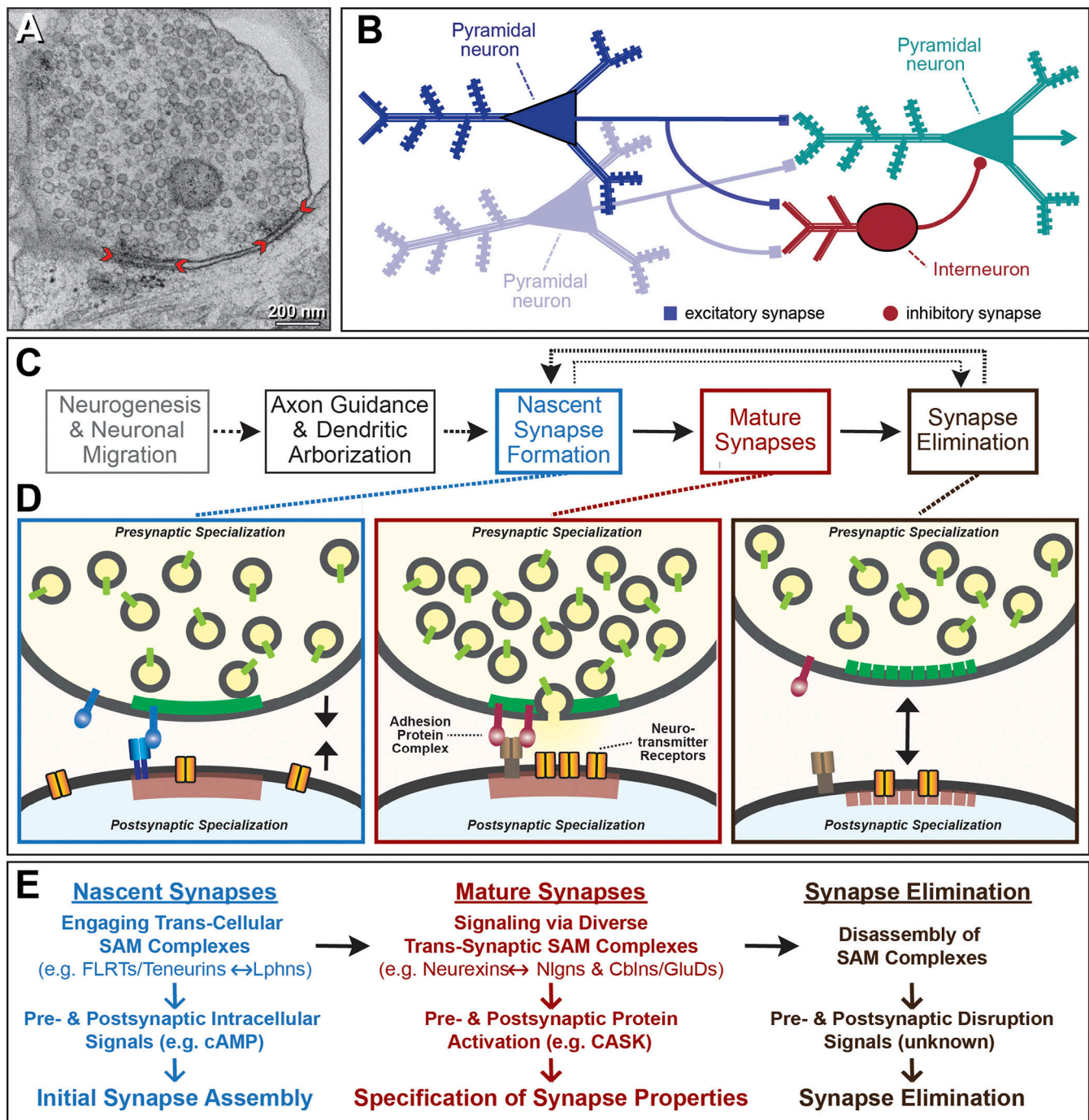Correspondence to Thomas C. Südhof: tcs1@stanford.edu.

Figure 1. **Synapses are communication nodes that connect neurons into circuits. (A)** Electron micrograph of a human synapse with two synaptic junctions to illustrate the canonical features of all synapses: An intercellular junction in which a presynaptic varicosity that is filled with synaptic vesicles contacts a postsynaptic dendrite that contains multiple trafficking organelles as well as ribosomes (image courtesy of Dr. Christopher Patzke). Red arrows indicate synaptic junctions. Most neurons form thousands of input and output synapses. **(B)** Schematic view of a cortical microcircuit in which two pyramidal neurons both directly excite a postsynaptic pyramidal neuron and indirectly inhibit it via an interneuron. If the presynaptic neurons fire in bursts and trains, as is commonly observed in brain, the postsynaptic pyramidal neuron will exhibit differential increasing or decreasing responses depending on whether the various excitatory and inhibitory synapses are facilitating or depressing. **(C)** Flowchart of the lifecycle of a synapse. After neurons are born, migrate to their appropriate positions, and extend dendrites and axons, neurons form synapses. Synapses initiate as nascent contacts that mature into functional but plastic synaptic connections and are eliminated under control of unknown signals. Synapse turnover rates vary, but many synapses are continuously renewed. **(D)** Schematic of nascent synapses (left), mature synapses (center), and synapses being eliminated (right). In nascent synapses, transneuronal interactions mediated by SAMs such as latrophilins are proposed to initiate the intracellular signaling cascades that organize synaptic specializations. Subsequent synapse maturation and shaping of synapse properties (center) is controlled by a different set of SAMs such as neurexins. During synapse elimination, SAM interactions weaken, which may induce separation of synaptic junctions and withdrawal of synaptic processes. **(E)** Schematic of how SAMs organize synapse formation and synapse elimination. CASK, calcium/calmodulin dependent serine protein kinase; Cblns, cerebellins; GluD, δ-type glutamate receptor; Lphns, latrophilins; Nlgns, neuroligins.

The astounding diversity of synapses correlates with differences in protein composition, creating a universe of synapses sometimes referred to as the "synaptome" (Nusser, 2018; Grant and Fransén, 2020).

In brain, nearly all synapses are formed by axons *en passant*, as these axons cruise through the brain's gray matter (which incidentally makes the term "nerve terminal" as misleading as the term "circuit": presynaptic terminals are not at the end of axons, nor are circuits necessarily circular). Generally, axons form thousands of synapses that are often arranged like pearls on a string, with approximately one presynaptic specialization per 2-μm axon length (Takács et al., 2018). An axon can establish single synapses with many postsynaptic neurons or multiple synapses with a few postsynaptic neurons. Generation of multiple connections by a single presynaptic neuron onto a postsynaptic cell decreases transmission failures but limits the potential for synaptic plasticity.

All presynaptic specializations secrete neurotransmitters via principally the same release machinery, whereas postsynaptic specializations sense neurotransmitters via diverse receptor machineries (Fig. 2). The canonical presynaptic release machinery is constructed by SNARE and Sec1/Munc18-like proteins that mediate membrane fusion, synaptotagmins and their complexin cofactors that enable Ca^{2+}-triggering of fusion, and Rab3-interacting molecules (RIMs), RIM binding proteins, and Munc13s that build the active zone scaffold, tether synaptic vesicles, and recruit Ca^{2+} channels to neurotransmitter release sites (Südhof, 2012, 2013; Brunger et al., 2018; Emperador-Melero and Kaeser, 2020). This canonical presynaptic release machinery is diversified by expression of different isoforms of its various protein components, but the underlying principles are always the same, independent of neurotransmitter type. Even synapses with unusual presynaptic specializations, such as ribbon synapses or neuromuscular junctions, use the same canonical release machinery. Only one feature differentiates presynaptic terminals: the transporter proteins that fill synaptic vesicles with a neurotransmitter and associated enzymes that synthesize neurotransmitters in the first place (not needed for glutamate and glycine as general cytoplasmic components; see references above).

Postsynaptic specializations, in contrast, are specific for particular neurotransmitters and their receptors. Almost no components are shared between different types of postsynaptic specializations (Fig. 2). Four neurotransmitter receptor gene families exist: tetrameric glutamate receptors (N-methyl-D-aspartate [NMDA] receptors [NMDARs], α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate [AMPA] receptors [AMPARs], and kainate receptors), pentameric cys-loop receptors (GABA_A receptors, glycine receptors, nicotinic acetylcholine receptors, and ionotropic serotonin receptors), trimeric P2X receptors (ATP receptors), and metabotropic G protein–coupled receptors (GPCRs). Individual synapses never appear to contain more than one type of receptor (see discussion below). Most synapses (>98%) use tetrameric glutamate or pentameric cys-loop receptors (Fig. 2). Synapses using ATP neurotransmitters and P2X receptors are extremely rare. Although GPCRs often surround synaptic junctions, few GPCRs are present within postsynaptic

specializations. For example, mGluR6 glutamate receptors represent the primary glutamate sensors of some retinal photoreceptor synapses but no other synapses (Snellman et al., 2008; Martemyanov and Sampath, 2017). Few postsynaptic proteins are currently (as of 2021) known to be shared by synapses containing tetrameric glutamate and pentameric cys-loop receptors (Fig. 2). An exception is Neuroligin-3, a synaptic adhesion molecule (SAM) that binds to presynaptic neurexins and functions in both excitatory and inhibitory synapses (Budreck and Scheiffele, 2007; Zhang et al., 2015a). As a rule, therefore, synapses are composed of canonical presynaptic and diverse postsynaptic molecular machineries. As will be discussed, this gestalt has major implications for synapse formation because it suggests that postsynaptic specializations develop in response to a particular neurotransmitter signal.

Dynamics of synapse formation and elimination

During development, newborn neurons migrate to specific positions in brain and extend axons and dendrites before engaging in synapse formation (Fig. 1 C). In humans, an ∼2-yr postnatal period of exuberant synapse formation is followed by an ∼20-yr period of net synapse elimination, leading to a loss of >40% of all synapses (Huttenlocher et. al., 1982; Bourgeois and Rakic, 1993; Petanjek et al., 2011). An activity-dependent process of synapse elimination has been described for several synapses, such as the neuromuscular junction (Sanes and Lichtman, 1999), retinal inputs into the lateral geniculate nucleus (Chen and Regehr, 2000), and cerebellar climbing-fiber synapses (Kano and Hashimoto, 2009). To what extent physiological synapse elimination is generally activity dependent, however, remains unclear.

Synapses are not only made in excess and eliminated developmentally, but also turn over continuously in mature brain. Live imaging showed that ∼40% of dendritic spines on pyramidal neurons in the sensory and motor cortex are replaced every 5 d, while ∼60% of dendritic spines are stable (Attardo et al., 2015; Fig. 3). Although earlier studies observed lower rates of spine turnover in cortex (Zuo et al., 2005; Holtmaat et al., 2005), other more recent studies also detected high turnover rates (Zhou et al., 2020). Stunningly, in the hippocampus, nearly 100% of spines turn over every 2 wk (Attardo et al., 2015; Pfeiffer et al., 2018; Fig. 3). These studies monitored spines instead of synapses, but in brain, all spines are associated with synapses (usually excitatory synapses), suggesting that these astounding rates of spine replacement correspond to synapse turnover in a mature brain. Therefore, while most neurons and their long-range axonal and dendritic structures are stable in mature brain, their synaptic connections are often not. In regions such as the hippocampus, the half-life of synaptic connections may be shorter than, for example, that of late long-term potentiation (LTP; Frey and Morris, 1997). Such a high rate of synapse turnover agrees well with the continued expression of proteins implicated in synapse formation throughout life, as documented in single-cell RNA-sequencing studies (Saunders et al., 2018; Zeisel et al., 2018, Tabula Muris Consortium, 2018; Chen et al., 2020).

What molecular mechanisms sustain the rapid life cycle of synapses? Clearly, synapse formation and elimination go hand in
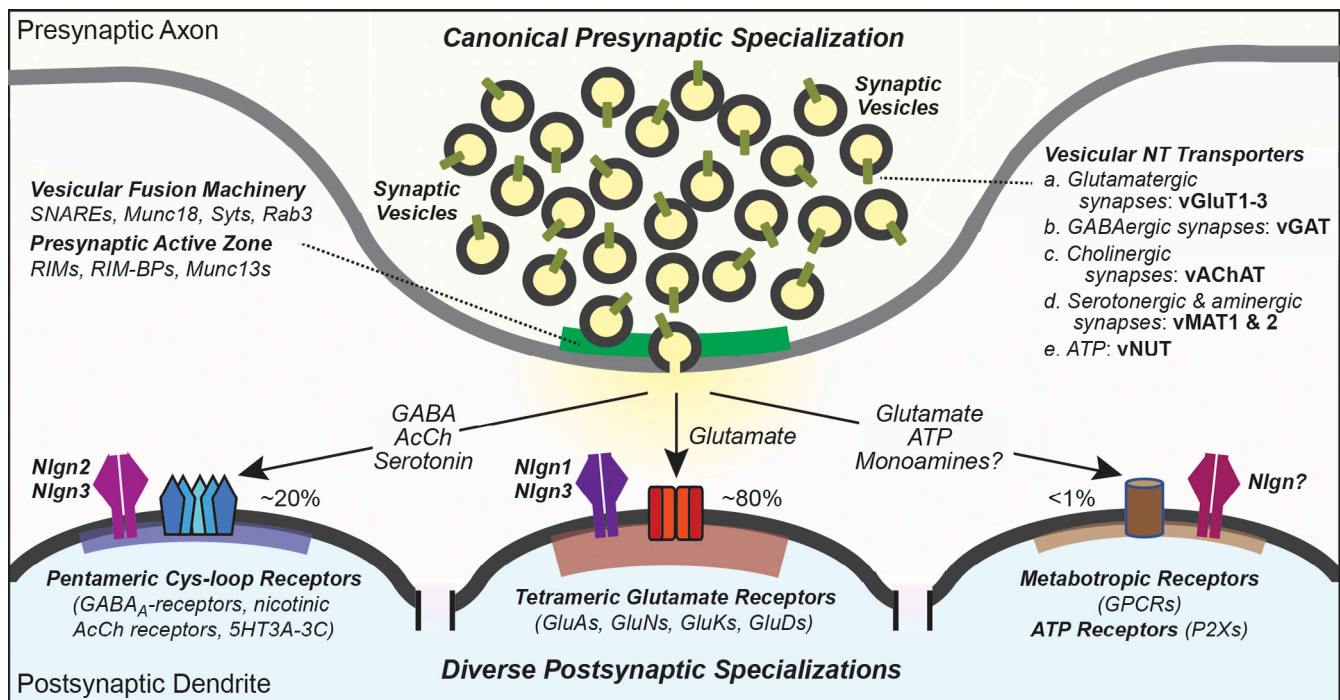
Figure 2. **Synapses are composed of presynaptic specializations containing a canonical neurotransmitter release machinery and postsynaptic specializations constructed of diverse receptors and postsynaptic densities.** The molecular composition of the presynaptic specialization is largely independent of the neurotransmitter type, with similar proteins mediating the localized and fast Ca^{2+}-dependent fusion of synaptic vesicles (Südhof, 2013). In contrast, postsynaptic specializations are diverse, with little overlap in their molecular components. Four types of receptors are associated with distinct postsynaptic molecular complexes: glutamate receptors (center) account for ~80% of synapses, pentameric cys-loop receptors (GABA_A, glycine, acetylcholine, and serotonin, left) for ~20% of synapses, and the remaining two receptor classes (metabotropic GPCRs and P2X receptors, right) for <1% of synapses (note that metabotropic GPCRs and P2X receptors are abundantly present outside of synapses). Whereas the only difference among various presynaptic specializations are the enzymes and vesicular transporters specific for particular neurotransmitters (summarized on the top right), few components of different postsynaptic specializations are currently known to be shared, including neuroligin-3, a SAM that binds to presynaptic neurexins. AcCh, acetylcholine; GluA, AMPA-type glutamate receptor; GluD, δ-type glutamate receptor; GluK, kainate-type glutamate receptor; GluN, NMDA-type glutamate receptor; Nlgn, neuroligin; Rec., receptor; STED, stimulated emission depletion; Syts, synaptotagmins.

hand. Synapse elimination may be initiated by disengagement of SAMs and discontinuation of SAM signaling (Fig. 1 D). For example, deletion of cerebellins in the forebrain has no effect on excitatory synapse formation but causes a delayed loss of synapses in some brain areas (Seigneur and Südhof, 2018). This observation is consistent with a signaling role for cerebellins in shaping synapses, a role whose absence induces synapse elimination. It seems likely that microglia play a major role in synapse elimination (Paolicelli et al., 2011) and that the interaction of neural CD47 with microglial signal-regulatory protein-α drives synapse elimination (Ding et al., 2021). Moreover, synapses may be "opsonized" via the classic complement pathway during synapse elimination (Stevens et al., 2007). Polymorphisms in the complement factor C4 gene were identified as a genetic risk factor for schizophrenia (Sekar et al., 2016), suggesting that schizophrenia could involve impairments in complement-mediated synapse elimination (Druart and Le Magueresse, 2019; Presumey et al., 2017). However, the observed effect sizes are small, and no other complement factor has been linked to schizophrenia. More importantly, mice without a classic complement pathway exhibit fairly normal synapse numbers (Chu et al., 2010), and humans that lack the complement factor C3 (which is central to all complement activities) appear to suffer from severe immune disorders, but not from neurological impairments (Botto et al., 1992; Singer et al., 1994; Matsuyama et al., 2001). At present the precise roles of complement, microglia and SAMs in synapse elimination is thus unclear.

Neurotransmitter cotransmission and neurotransmitter switching

Dale's principle suggested that a given neuron releases the same neurotransmitters at all of its synapses (Dale, 1935; Strata and Harvey, 1999). Indeed, most neurons release only either glutamate or GABA. Some neurons, however, release multiple neurotransmitters or switch neurotransmitters. Among others, cotransmission has been observed for GABA and glutamate (Root et al., 2014 and 2018), GABA and acetylcholine (Takács et al., 2018), GABA and dopamine (Maher and Westbrook, 2008; Tritsch et al., 2012), glutamate and acetylcholine (Moore et al., 2015; Lamotte d'Incamps et al., 2017), and glutamate and dopamine (Hattori et al., 1991; Sulzer et al., 1998; Stuber et al., 2010; Silm et al., 2019). A fascinating cell biology emerges from these observations.

The neurotransmitter type of a synapse is likely determined by the neurotransmitter that is transported into synaptic vesicles. Mammals encode five classes of vesicular neurotransmitter
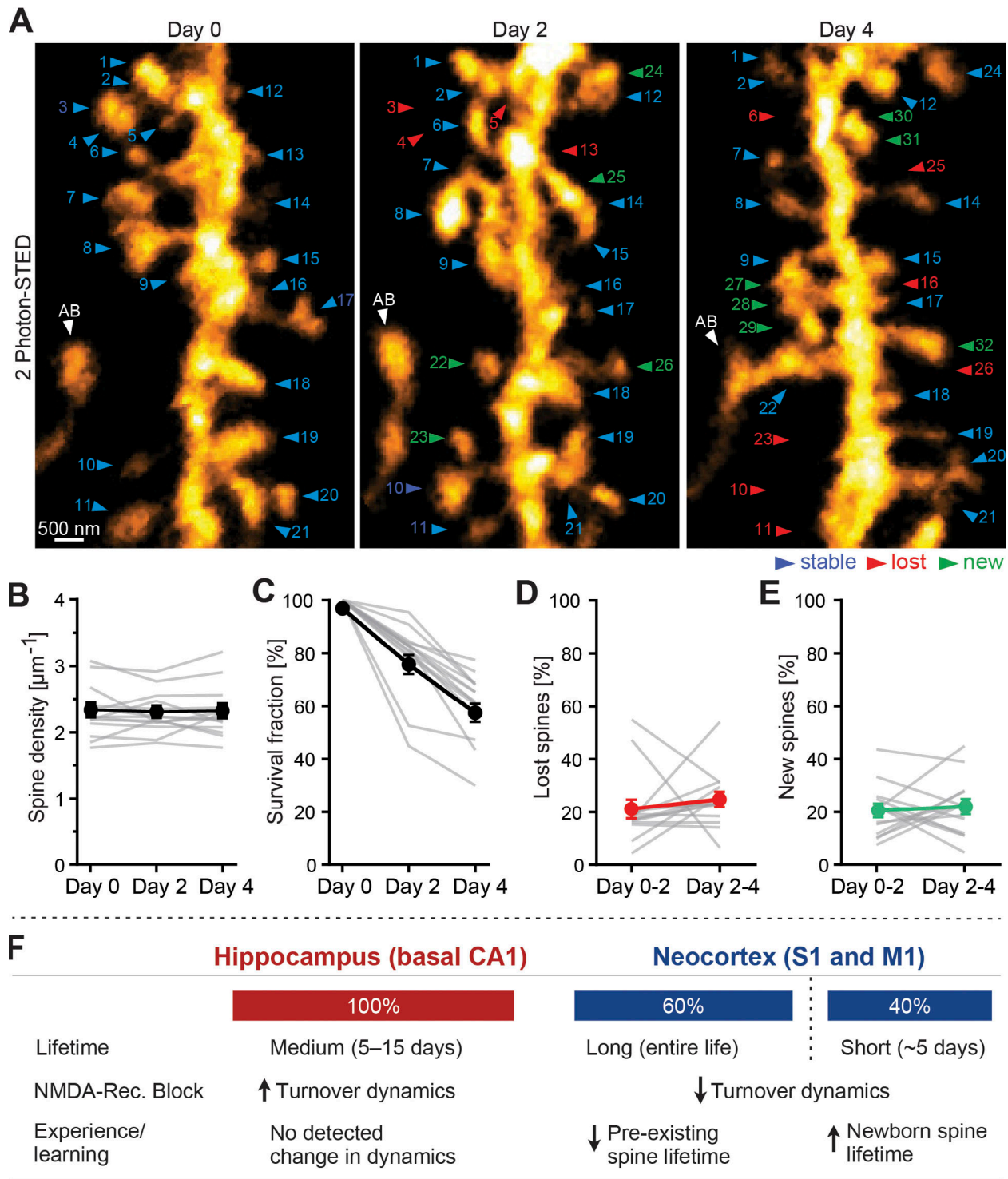
Figure 3. **Synapses, monitored via spines as proxies, are continuously replaced under physiological conditions, with different turnover rates in various brain regions. (A)** 2-Photon stimulation emission depletion (STED) images of spines on basal dendrites of CA1 pyramidal neurons in vivo at three time points separated by 2 d, illustrating rapid turnover of spines (blue arrowheads, stable spines; red arrowheads, lost spines; green arrowheads, new spines; white arrowhead, axonal bouton [AB]). Data in A–E are from Pfeiffer et al. (2018). **(B and C)** Quantification of the density (B) and survival fraction (C) of dendritic spines over 4 d (n = 14 dendrites, 3 mice). **(D and E)** Fraction of lost spines (D) and new spines (E) measured over the first or second 2-d period. Thin gray lines represent the measurements of single dendrites. **(F)** Summary of the relative turnover rates of dendritic spines in the hippocampus and cortex as determined by Attardo et al. (2015). Rec., receptor.

transporters: vesicular glutamate transporters (vGluTs), vesicular GABA transporters (vGATs; for both GABA and glycine), vesicular acetylcholine transporters (vAChTs), vesicular monoamine transporters (vMATs; for all biogenic amines, including serotonin and histamine), and vesicular nucleotide transporters (vNUTs; for ATP; Fig. 2; reviewed in Omote et al., 2016). Strikingly, if a neuron uses multiple neurotransmitters that are transported by different vesicular transporters, these transporters are invariably sorted into distinct synaptic vesicles (Takács et al., 2018; Granger et al., 2020; Silm et al., 2019). As a result, the corresponding neurotransmitters are stored in separate synaptic vesicles and released independently by vesicle exocytosis from the same neuron. Thus, the vesicular transporter type confers an identity to synaptic vesicles. By the same rule, cotransmitters that use the same vesicular transporters (e.g., GABA and glycine, or adrenaline and noradrenaline) are stored in the same vesicles. The only exception to this rule appears to be ATP, which is co-stored with acetylcholine and biogenic amines in many vesicles (Whittaker, 1984).

Moreover, vesicles that contain different vesicular transporters, and are thus filled with different neurotransmitters, are sorted to different synaptic junctions with separate active zones in the same neurons (Root et al., 2014, 2018; Moore et al., 2015). As a result, even though a neuron may coexpress two neurotransmitters, these are released at different output synapses. For example, a neuron using both glutamate and GABA forms separate glutamatergic or GABAergic output synapses that contain only either postsynaptic tetrameric glutamate or pentameric GABA$_A$ receptors. Thus, stunningly, neurons coexpressing GABA and glutamate, or acetylcholine and glutamate, form separate synapses with distinct neurotransmitters.

The selective sorting of different vesicular transporters into separate vesicles that are then targeted to distinct synaptic junctions was clearly shown for acetylcholine and GABA. These neurotransmitters are co-released in the hippocampus at synapses formed by basal forebrain cholinergic neurons (Takács et al., 2018) or in the cortex at synapses formed by vasoactive intestinal peptide–positive interneurons (Granger et al., 2020). Similarly, midbrain neurons use dopamine and glutamate as cotransmitters that are packaged into distinct vesicles whose exocytosis is differentially regulated (Zhang et al., 2015b; Silm et al., 2019). Moreover, some central neurons use GABA and glutamate as cotransmitters that are packaged into separate vesicles and targeted to distinct symmetric and asymmetric synapses (Root et al., 2014, 2018).

It appears that at least in some instances, a presynaptic neuron can even selectively form synapses with distinct neurotransmitters onto different postsynaptic targets. This has been beautifully described for spinal cord motoneurons: acetylcholine is their only transmitter at the neuromuscular junction, acetylcholine and glutamate are cotransmitters at motoneuron synapses formed on Renshaw-type interneurons, and glutamate is the only transmitter for recurrent excitation between motoneurons (Moore et al., 2015; Lamotte d'Incamps et al., 2017; Bhumbra and Beato, 2018).

In general, the observation that an individual presynaptic neuron releasing two neurotransmitters forms distinct synapses

with the correct postsynaptic receptors suggests that the presynaptic neurotransmitter instructs the postsynaptic specialization. However, the example of the motoneuron indicates that the postsynaptic neuron can also determine what neurotransmitters will be used by the presynaptic neuron. As discussed in the next section, the underlying mechanisms are, however, unclear.

In addition to the use of cotransmitters that are segregated into different vesicles and secreted at distinct synaptic junctions, some neurons switch transmitters in an activity-dependent manner (Spitzer, 2017). For example, mice acquire improved motor skills after 1 wk of voluntary wheel running, which causes a switch from acetylcholine to GABA in a subset of neurons in the caudal pedunculopontine nucleus (Li and Spitzer, 2020). This reversible switch appears to change the regulation of the substantia nigra, ventral tegmental area, and thalamus by the pedunculopontine nucleus. Since different neurotransmitters use synaptic junctions with distinct types of postsynaptic specializations, the neurotransmitter switch involves formation of new synapses. Here again, the biology suggests that the presynaptic neuron instructs postsynaptic synapse formation.

Thus, we face a cell-biological challenge: How does a neurotransmitter tell a postsynaptic neuron what type of specialization to assemble? As discussed below, trans-synaptic signaling mediated by SAMs likely plays a central role, although at present our understanding of the underlying processes is limited.

Molecular logic of synapse formation: SAMs

I posit that SAMs (also called "synaptic organizing molecules") are principal agents in organizing synaptic junctions (Jang et al., 2017; Südhof, 2018; Yuzaki, 2018; Kim et al., 2021). By engaging trans-cellular interactions, SAMs are thought to nucleate nascent synapses, drive synapse maturation, control the properties of synapses, and regulate synapse elimination (Fig. 1, C and D). SAMs perform these actions by signaling in both directions (pre- to postsynaptic and post- to presynaptic). No single "master" SAM likely controls everything; instead, an orchestra of SAMs mediates assembly of diverse synaptic junctions.

Many candidate SAMs have been described (Fig. 4). Consistent with the asymmetric organization of synaptic junctions, SAMs generally form heterophilic complexes. As described above, the same basic release machinery governs presynaptic functions independently of neurotransmitter type, whereas diverse postsynaptic receptor machineries mediate postsynaptic functions in excitatory and inhibitory synapses (Fig. 2). As a result, presynaptic SAMs are mostly "hub" molecules that are present in excitatory and inhibitory synapses, like neurexins (reviewed in Südhof, 2017) and leukocyte antigen–related (LAR)-type phosphotyrosine phosphatase receptors (PTPRs; reviewed in Takahashi and Craig, 2013; Han et al., 2016; Fukai and Yoshida, 2020; Fig. 4). In contrast, postsynaptic SAMs are more diverse as ligands for these hub molecules and are often specific for excitatory or inhibitory synapses.

Broadly, SAMs perform two overlapping functions: organizing the assembly of synapses ("making synapses") and specifying synapse properties ("shaping synapses"). More SAMs shaping synapses are known than SAMs making synapses,
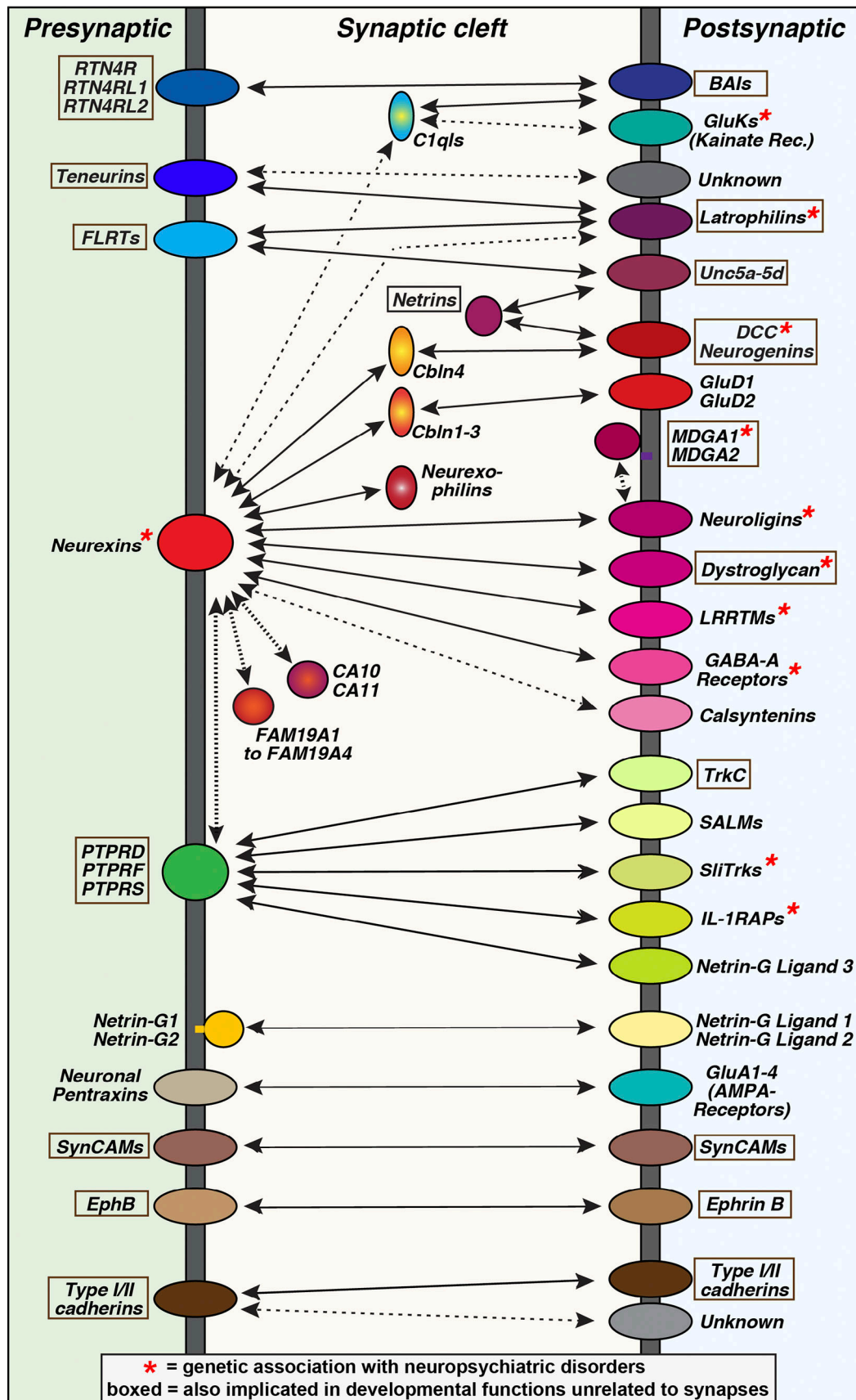
Figure 4. **Schematic diagram of candidate trans-synaptic SAM complexes governing synapse assembly.** Data were assembled from the literature and are represented graphically similar to Südhof (2018). Note that two families of presynaptic SAMs, neurexins and LAR-type receptor phosphotyrosine

phosphatases (PTPRD, PTPRF, and PTPRS), are hub molecules that interact with a series of postsynaptic SAM families and also bind to each other in cis (Han et al., 2020). Most candidate SAMs perform additional functions outside of synapses. Lines and arrows indicate interactions, with cis-interactions shown as dotted lines and less validated trans-interactions shown as dashed lines. DCC, deleted in colorectal cancer; EphB, Ephrin B; FLRT, fibronectin leucine-rich transmembrane; LRRTM, leucine-rich repeat transmembrane; Rec., receptor; RTN, reticulon; SALMs, synaptic adhesion-like molecules; SliTrks, Slit- and Trk-like proteins; SynCAM, synaptic cell adhesion molecule; TrkC, tropomyosin receptor kinase C.

possibly because diverse synapse properties need to be controlled by multifarious signals. The example of SPARCL1 and neuroligins illustrates the functional differentiation between SAMs that make or shape synapses (Fig. 5). SPARCL1 (a.k.a. Hevin) boosts the excitatory synapse density and the amplitude of AMPAR-mediated synaptic responses without affecting inhibitory synapses. SPARCL1 thus stimulates the making of new functional excitatory synapses (Gan and Südhof, 2020). In addition, SPARCL1 dramatically enhances NMDAR-mediated synaptic responses, suggesting that the new synapses are functionally different (i.e., contain more NMDARs). Thus, SPARCL1 acts both in the making and the shaping of synapses (Fig. 5). Neuroligins, conversely, do not influence synapse numbers but change the properties of synapses, i.e., shape synapses. Among others, neuroligin-2 deletions greatly decrease the synaptic strength at inhibitory synapses (which are untouched by SPARCL1), whereas neuroligin-1 deletions suppress NMDAR-mediated synaptic responses at excitatory synapses more than AMPAR-mediated responses. Although neuroligins and their presynaptic neurexin receptors were suggested to bind to SPARCL1 (Singh et al., 2016), SPARCL1 and neuroligins perform distinct and independent functions, suggesting that they do not physiologically interact. The phase diagrams of Fig. 4, F and G, illustrate these functional differences and interdependencies in a 2D representation, visualizing the making and shaping of synapses. Similar observations apply to many other SAMs.

Elucidating the candidacy and functions of a SAM in making and shaping synapses is not a trivial task. Three basic challenges stand out.

First, simply localizing a SAM to the synapse is not straightforward. Determining whether a protein is truly synaptic is arguably the most important need, but it requires specific antibodies and superresolution microscopy and/or immuno-EM.

Second, identifying valid protein interactions is difficult. Common approaches, such as coimmunoprecipitations and affinity measurements by surface plasmon resonance, are inconclusive. As a general rule, without the demonstration of a stable complex (for example by size exclusion chromatography coupled with multiangle light scattering or via a crystal structure) or without matching phenotypes during functional manipulations, it is difficult to distinguish sticky proteins from real ligands.

Third, identifying the synaptic functions of a SAM is challenging. Many "functional" manipulations, such as RNAi or overexpression, cause indirect nonspecific changes. Synaptic functions have to be analyzed at defined synaptic connections, requiring sophisticated electrophysiology and imaging approaches. Many SAMs, such as neurexins, perform distinct functions in different synapses. Most SAMs (except for neurexins and their multifarious ligands) have additional essential

developmental roles besides shaping synapses. It is as though a concert musician was responsible first for ushering in the audience and then for playing in the subsequent performance not just one, but multiple instruments.

Given these challenges, little is known overall at present about how SAMs orchestrate synapse formation. On top of these challenges, even the most rigorous experiments can provide ambiguous results. For example, neurexin deletions generally alter synaptic transmission without changing synapse numbers, but a discrete loss of some synapses is detected in neurexin-deficient parvalbumin-positive cortical interneurons (Chen et al., 2017) and in CA3 region neurons in mice expressing mutant neurexin-1 that lacks heparan sulfate modifications (Zhang et al., 2018). Deletion of the cerebellin neurexin ligands, conversely, causes an ~50% decrease in synapse numbers in cerebellum (Hirai et al., 2005) but only a scattered loss of synapses in other brain regions (Seigneur and Südhof, 2018). Does this mean that neurexin-neuroligin and neurexin-cerebellin interactions are "making" a small subset of synapses, or is this synapse loss secondary to the cessation of a SAM signal in the affected synapses? In support of the second hypothesis, synapses are initially formed normally by cerebellin-deficient neurons but are lost secondarily (Seigneur and Südhof, 2018). To consider these questions more deeply, next I further discuss the role of SAMs in making and shaping synapses in molecular terms.

SAMs and synaptic specificity

Synapse formation is tightly regulated. Not only are the neurons forming synapses specific, but also the subcellular locations and properties of the resulting synapses. For example, cerebellar parallel-fiber synapses always form on the distal dendrites of Purkinje cells, whereas climbing-fiber synapses always form on the proximal dendrites of Purkinje cells, with the former invariably exhibiting short-term synaptic facilitation and the latter short-term synaptic depression (Galliano and De Zeeuw, 2014). How does synapse formation produce the exquisite specificity of synaptic connections in a neural circuit? Two sequential processes are traditionally thought to establish synapse specificity: Axon guidance positions an axon adjacent to a target neuron, and partner choice then determines which neurons form synapses at at what location (e.g., distal or proximal dendrite, soma, or axon initial segment; Fig. 1, C and D). However, a third process also needs to be considered for synapse specificity: shaping of the properties of synapses that are as important for the overall performance of a neural circuit as the number and location of the synapses. These three processes collaborate to achieve the exquisite specificity of synapse formation (Südhof, 2018; Sanes and Zipursky, 2020; Chowdhury et al., 2021).

The mechanisms of axon guidance are well studied, but how axon guidance is coupled to synapse formation and which SAMs
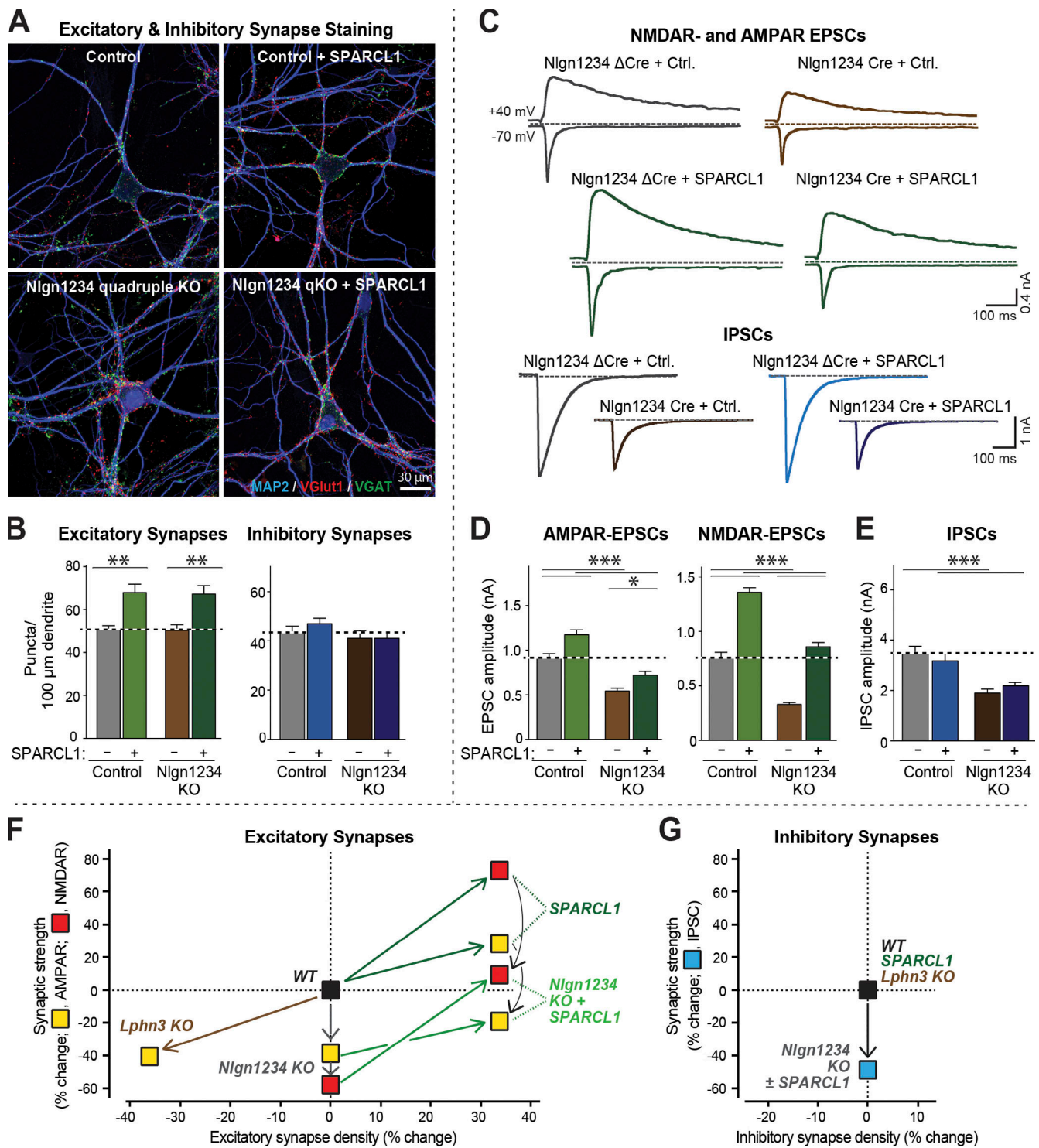
Figure 5. **Synapse numbers and properties are shaped by multiple independent molecular mechanisms: Example of the contributions of neuroligins and SPARCL1 (Hevin). (A–E)** Exemplary immunocytochemistry (A and B) and electrophysiology (C–E) experiments with cultured hippocampal neurons demonstrating that SPARCL1 and neuroligins differentially and independently control synapses. The immunocytochemistry data (A and B) show that SPARCL1 increases excitatory but not inhibitory synapse numbers, whereas deletion of all neuroligins has no effect on synapse numbers and does not impair the SPARCL1-induced increase in synapse numbers. The electrophysiology results (C–E) show that SPARCL1 increases, whereas the pan-neuroligin deletion decreases, NMDAR-mediated synaptic strength significantly more than AMPAR-mediated synaptic strength. Although these two manipulations act similarly but in opposite directions, they do not depend on each other (D). Only the neuroligin but not the SPARCL1 manipulation affects inhibitory synapse (E). Data are adapted from Gan and Südhof (2020). **(F and G)** Phase diagram of the effect of SPARCL1, neuroligins, and latrophilin-3 manipulations on excitatory (F) and inhibitory (G) synapses, as analyzed in cultured hippocampal neurons. Values were computed from Gan and Südhof (2020) and Sando et al. (2019). Numerical data in B, D, and E are means ± SEM. Statistical significance was assessed by two-way ANOVA followed by post hoc corrections. Ctrl, control; EPSC, excitation postsynaptic current; IPSC, inhibition postsynaptic current; KO, knockout. In B, D, and E, asterisks indicate statistical significance as calculated by two-way ANOVA (*, P < 0.05; **, P < 0.01; ***, P < 0.001).

guide the construction of synapses (i.e., make a synapse) is largely unclear. Pioneering studies revealed that nonsynaptic adhesion molecules guide axons to a target cell once the axons are within the vicinity of the target region. For example, in *C. elegans*, Syg1 and Syg2, a pair of Ig-domain proteins, guide axons to their synaptic targets (Shen et al., 2004). Similarly, in the mouse retina, cadherins specify target areas for synapse formation (Duan et al., 2014). After axon guidance, synapse formation is likely initiated when SAMs instruct assembly of nascent synapses (Fig. 1 D).

A major question is whether the establishment of a synapse between particular neurons at a specific location can be mechanistically divided into a "partner choice" decision and synapse formation as such, or whether partner choice and synapse formation are mechanistically the same (Sanes and Zipursky, 2020; Südhof, 2018). As a third alternative, it is possible that synapse formation operates nonspecifically, and that nascent synapses between noncognate neurons are quickly eliminated, thereby creating specificity via a "divorce" mechanism (Fig. 1 C). Thus, three hypotheses could account for synapse specificity: A sequential partner choice → synapse establishment process, a "package deal" in which a combination of SAMs mediates both partner choice and synapse establishment (partner choice = synapse establishment), and a sequential synapse establishment → selective elimination process. For each of these hypotheses, the shaping of synapse properties could be partly inherent and partly add-ons via additional SAMs.

In considering these three hypotheses, a key observation is that synapse formation is highly promiscuous, at least under nonphysiological conditions. In heterologous synapse formation assays, expression of a SAM in a nonneuronal cell induces formation of pre- or postsynaptic specializations in cocultured neurons. Here, nearly any SAM induces heterologous synapse formation (reviewed in Südhof, 2018). Even the neuronal pentraxin receptor (a membrane-tethered pentraxin) stimulates formation of postsynaptic specializations in cocultured neurons, presumably by engaging AMPA-type glutamate receptors (Lee et al., 2017). The only specificity of heterologous synapse formation is that a given molecule induces either only pre- or postsynaptic specializations. Nearly all molecules that induce synapses in heterologous synapse formation assays are not essential for synapse formation as such when tested genetically, suggesting that in neurons, synapse formation can be induced by multitudinous signals (Jiang et al., 2021). As another demonstration of the promiscuity of synapse formation under nonphysiological conditions, neurons readily form abundant synapses with themselves ("autapses") when cultured in isolation on an island of glia (Bekkers and Stevens, 1991).

The nonphysiological promiscuity of synapse formation seems to support the notion that partner choice precedes the making of a synapse, or that synapses are formed promiscuously and noncognate synapses are then rapidly degraded. However, the package deal hypothesis positing that partner choice and synapse establishment are mediated by the activities of the same SAMs is also consistent with the nonphysiological promiscuity of synapse formation. Specifically, according to that hypothesis, neurons choose synaptic interaction partners in a hierarchical

manner based on a graded affinity among SAMs. Synapse formation only becomes promiscuous when high-affinity partners are lacking. Thus, the nonphysiological promiscuity of synapse formation does not tell us which hypothesis is correct.

What, then, do known SAM functions tell us about the partner choice and initial establishment of synapses? Many candidate SAMs were suggested to initiate synapse formation and/or encode synapse specificity, but few have endured the test of time. At present, the only SAMs that have consistently been shown to be required for establishing synapses are postsynaptic adhesion-GPCRs called latrophilins and brain angiogenesis inhibitors (BAIs; note that the name does not correspond to a known function). Like other adhesion-GPCRs, these proteins contain large extracellular domains mediating interactions with multiple trans-synaptic ligands. Deletions of latrophilin or BAI isoforms produce a severe decrease in synapse formation in specific subsets of synapses. Bai3 deletions in Purkinje cells selectively block climbing-fiber but not parallel-fiber synapse formation (Kakegawa et al., 2015; Sigoillot et al., 2015), whereas Bai3 deletions in olfactory bulb granule cells impair synapse formation of accessory bulb inputs but not of mitral cell inputs (Wang et al., 2020). Similarly, deletion of latrophilin-2 in CA1 pyramidal neurons selectively suppresses afferent synapses from the entorhinal cortex, whereas deletion of latrophilin-3 in the same neurons suppresses Schaffer-collateral input synapses (Anderson et al., 2017; Sando et al., 2019). In synapse formation, latrophilins function as GPCRs and thus as classic signaling receptors (Sando and Südhof, 2021). Latrophilin-dependent synapse formation requires interactions with presynaptic teneurins and fibronectin leucine-rich transmembranes in complexes that have been crystallographically confirmed (Lu et al., 2015; Jackson et al., 2018; Li et al., 2018, 2020; Sando et al., 2019). Puzzlingly, teneurins have also been proposed to mediate synapse formation via a homophilic trans-synaptic interaction (Mosca et al., 2012; Berns et al., 2018). However, the structure of teneurin molecules suggests that a trans-cellular interaction would be difficult to envision (Jackson et al., 2018). Moreover, no experiments in which pre- or postsynaptic teneurins were separately deleted have been reported, making it unclear whether teneurins function both pre- and postsynaptically. Overall, the exquisite specificity of different latrophilin isoforms in the formation of distinct input synapses on CA1 region neurons suggests that latrophilins contribute to synapse specificity and do not simply mediate establishment of synapses (Sando et al., 2019), favoring the package deal hypothesis outlined above.

Neurotransmitter specificity of synapses

As described above, the presynaptic neurotransmitter type determines the postsynaptic specialization in a synapse, and even in the same neuron, different types of neurotransmitters and receptors are segregated into different synapses. This observation suggests that presynaptic terminals induce postsynaptic specializations corresponding to a specific neurotransmitter type. Consistent with this notion, rapid local release of caged glutamate or GABA using photolysis induces dendritic spines and functional synapses (Kwon and Sabatini, 2011; Oh et al.,

2016). Intriguingly, local photolysis of caged-GABA stimulates generation not only of GABAergic postsynaptic specializations, but also of dendritic spines and glutamatergic specializations (Oh et al., 2016). However, at the same time, ablation of neurotransmitter release does not impede synapse formation. Specifically, abolishing evoked neurotransmitter release using genetic approaches does not block generation of spines and formation of ultrastructurally normal but nonfunctional synapses (Verhage et al., 2000; Varoqueaux et al., 2002; Sando et al., 2017; Sigler et al., 2017; Lin et al., 2018; Held et al., 2020). Moreover, uncaging of glutamate or GABA induces postsynaptic specializations only in brain slices from preadolescent mice (Kwon and Sabatini, 2011; Oh et al., 2016), whereas synapse replacement operates throughout life (Fig. 3). Viewed together, we thus have one dataset that suggests that neurotransmitter signals are instructive in synapse formation, whereas another dataset shows that neurotransmitter signals are not required for synapse formation.

How can we resolve this conundrum? One hypothesis is that minimal residual neurotransmitter signaling, possibly stimulated by activation of guide adhesion molecules, triggers assembly of synaptic junctions. This idea is attractive, but is not supported by evidence for residual neurotransmitter release and does not explain the specific localization of synapses, since the residual signaling is likely diffuse. A related hypothesis posits that postsynaptic receptors may selectively recruit specific types of presynaptic axons for synapse formation in conjunction with particular SAMs and activation by neurotransmitters. Indeed, this hypothesis is consistent with the photolysis experiments described above (Kwon and Sabatini, 2011; Oh et al., 2016). It would explain the observation that in spinal motoneurons that use acetylcholine and glutamate as cotransmitters, the postsynaptic cell determines whether a presynaptic terminal uses only acetylcholine (muscle cells), both acetylcholine and glutamate (Renshaw cells), or only glutamate (other motoneurons; Bhumbra and Beato, 2018). However, deletions of neurotransmitter receptors also have little effect on synapse formation. Deletion of all GABA_A receptors in cerebellar Purkinje cells did not impair GABAergic synapse formation, similar to the deletion of presynaptic GABA release (Fritschy et al., 2006). Moreover, deletion of all GABA_A receptors in cultured hippocampal neurons causes only a partial loss of GABAergic synapses (Duan et al., 2019), whereas deletion of glutamate receptors has no effect (Duan et al., 2019). On balance, the evidence thus suggests that under physiological conditions, neurotransmitter signaling does not determine the establishment or specification of synapses. How the neurotransmitter identity of a presynaptic terminal instructs the postsynaptic specialization is therefore another fundamental question that remains unsolved.

Glia in synapse formation

Extensive evidence suggests that astrocytes play a major role in synapse formation, whereas microglia contribute to synapse elimination. Astrocytic extensions often surround synaptic contacts, creating tripartite synapses in which astrocytes likely contribute to shaping synapses (for a recent review, see Noriega-Prieto and Araque, 2021). Although space constraints

prevent me from discussing these events in detail, it is noteworthy that glia also secrete powerful synaptogenic proteins (Bosworth and Allen, 2017). The specific role of these proteins, however, remains unclear, since knockout of these proteins only marginally decreases synapse numbers (Christopherson et al., 2005; Kucukdereli et al., 2011). Most of these proteins are secreted by astrocytes in trace amounts but are also present in blood, and the relation of their systemic and central nervous system functions is unexplored. For example, the synaptogenic secreted protein SPARCL1 is a blood component that is also produced at low levels by astrocytes (Fig. 5). How astrocytic proteins induce synapse formation, and what physiological significance their activities have, remains unknown. For most studies, only immunocytochemistry and few functional analyses were performed, and it is often unclear whether these candidate synaptogenic factors are indeed generating new synapses that are functional.

Shaping synapse properties

How are the diverse properties of synapses determined? Emerging evidence suggests that these properties are not autonomous functions of a synapse, but are dynamically shaped by the bidirectional signaling between pre- and postsynaptic specializations that is mediated, at least in part, by SAMs. The most extensive evidence for this view is derived from studies on neurexins, arguably the best-understood SAMs, which serve as key regulators of synapse properties.

Neurexins are presynaptic SAMs encoded by three homologous genes in vertebrates (reviewed in Südhof, 2017). Initially we simplistically proposed that neurexins are "recognition" molecules that redundantly contribute to determining neuronal identity (Ushkaryov et al., 1992; Ushkaryov and Südhof, 1993). However, two key findings quickly challenged the original view of a unitary neurexin function.

First, deletion of neurexins caused no change in brain architecture, with little synapse loss, but impaired synaptic transmission primarily by decreasing presynaptic Ca^{2+} influx (Missler et al., 2003; Luo et al., 2020). This observation indicated that neurexins are essential for organizing functional synapses, not for initiating their assembly or for conferring identity to neurons. Subsequent work using conditional deletions of neurexins in different types of neurons expanded this finding. In excitatory calyx of Held synapses (Luo et al., 2020) or inhibitory synapses formed by somatostatin-containing interneurons in cortex (Chen et al., 2017), conditional deletions of all neurexins impaired the organization of presynaptic active zones and recruitment of Ca^{2+} channels, confirming the original finding. However, deletions of all β-neurexins in the hippocampus impaired synaptic transmission by interfering with endocannabinoid signaling, suggesting a very different function (Anderson et al., 2015). Moreover, in inhibitory parvalbumin-positive interneurons in cortex, the pan-neurexin deletions suppressed synapse numbers (Chen et al., 2017). These results suggested that neurexins perform major functions at synapses that differ depending on the types of neurons involved.

Second, neurexins are expressed in thousands of isoforms that are generated by alternative promoter usage and alternative

splicing and are produced in diverse regulated patterns throughout the brain (Ullrich et al., 1995). Moreover, different neurexins and their splice variants have dramatically different functions, suggesting that it is no longer possible to talk about neurexins as a homogeneous protein family. For example, alternative splicing of presynaptic neurexins at splice site 4 (SS4) controls the postsynaptic receptor composition as analyzed in CA1 → subiculum synapses (Aoto et al., 2013; Dai et al., 2019). Presynaptic neurexin-1 containing an insert in SS4 (Nrxn1⁻SS4⁺), but not neurexin-1 lacking an insert (Nrxn1⁻SS4⁻), trans-synaptically increases postsynaptic NMDAR levels without affecting AMPARs (Dai et al., 2019). In contrast, the equivalent presynaptic neurexin-3 variant (Nrxn3⁻SS4⁺ but not Nrxn3⁻SS4⁻) decreases postsynaptic AMPAR levels without affecting NMDAR levels. Strikingly, neurexin-1 and neurexin-3 both act by binding to postsynaptic GluD1 and GluD2 using cerebellins as adaptors (Dai et al., 2021). To complicate matters, a completely different neurexin-3 function is observed in olfactory bulb synapses (Aoto et al., 2015). Here, presynaptic neurexin-3 has no effect on postsynaptic AMPAR levels in excitatory synapses but regulates the release probability of inhibitory synapses.

The overall picture that emerges is that neurexins do not perform a unitary function, but that different neurexin isoforms, generated from distinct genes via separate promoters and further diversified by alternative splicing, have distinct roles depending on the identity of the neurons in which they are expressed. These roles include a regulation of the presynaptic release machinery, postsynaptic receptor composition, and synapse numbers. Given the large number of validated trans-synaptic ligands for neurexins—more than for any other SAM (Fig. 4)—it seems likely that the diverse roles of neurexins are dependent on differential ligand interactions, but no proof for this idea is available at present.

Do other SAMs have a similarly broad role in organizing synapse properties? Initial evidence indicates that this may also apply to LAR-PTPRs. LAR-PTPRs are also expressed from three alternatively spliced genes and (again, similar to neurexins) interact with multifarious postsynaptic ligands (Fig. 4; reviewed in Takahashi and Craig, 2013; Han et al., 2016; Fukai and Yoshida, 2020). Moreover, LAR-PTPRs appear to interact with neurexins in cis, possibly via the heparan-sulfate modification of neurexins (Han et al., 2020). Deletion of all three LAR-PTPRs causes no decrease in synapse numbers, demonstrating that they alone are not essential for making a synapse, but induce an ~40% decrease in NMDAR-mediated synaptic responses without significantly altering AMPAR-mediated responses (Sclip and Südhof, 2020; Emperador-Melero et al., 2021). Although this phenotype resembles the effect of neurexin-1 SS4-alternative splicing on NMDAR-mediated synaptic responses, in the case of neurexin-1, the surface levels of NMDARs are changed (Dai et al., 2019), whereas in the case of the LAR-PTPR deletion, the surface levels of NMDARs were not impaired (Sclip and Südhof, 2020).

Signal transduction cascades organize synapses
Engagement of SAMs presumably controls synapse formation by activating cytoplasmic signals, but little is known about the processes involved. Latrophilins and BAIs, at present the best-validated SAMs in initiating synapse formation, are GPCRs. Recent data indicate that the GPCR activity of latrophilins produces cAMP, and this activity is essential for synapse formation (Sando and Südhof, 2021). This observation suggests a role for cAMP and other classic signal transduction cascades in initiating synapse formation. The use of a ubiquitous second messenger for something as specific as synapse formation may appear surprising, but cAMP signaling is highly compartmentalized and context-specific in neurons (Averaimo and Nicol, 2014; Zaccolo et al., 2021; Johnstone et al., 2018). Although enticing, little else is known about what intracellular signals induce synapses. This is a central cell-biological question that is now ready to be tackled.

Our understanding of the cytoplasmic processes regulating synapse properties is similarly limited. Much is known about the composition of presynaptic active zones and postsynaptic specializations, but how SAM-stimulated signals organize this composition is unclear. What molecular interactions align a presynaptic neurotransmitter signal, such as glutamate, with specific postsynaptic receptors, and how are these receptors coupled to a particular postsynaptic density? Again, without insight into cytoplasmic protein interactions, it will be impossible to make progress on this question. For example, it has been suggested that binding of the postsynaptic scaffolding proteins gephyrin and collybistin to the cytoplasmic tail of neuroligin-2 organizes the postsynaptic scaffold of GABAergic receptors (Poulopoulos et al., 2009). However, at a subset of GABAergic synapses, loss of GABA$_A$ receptors leads to a decrease in gephyrin clustering without a change in neuroligin-2, suggesting that neuroligin-2 alone is not sufficient to initiate the organization of GABAergic specializations via binding to gephyrin (Panzanelli et al., 2011). This agrees well with the lack of specificity of gephyrin binding to neuroligin-2. The cytoplasmic sequences of neuroligin-2 that bind to gephyrin are also present in neuroligin-1, which is present only in excitatory synapses. The signals that confer specificity of neuroligin-2 to inhibitory and neuroligin-1 to excitatory synapses, and that enable neuroligin-3 to function in both types of synapse, thus remain unknown.

Synapses in neuropsychiatric and neurodegenerative disorders
Synapses, made and shaped by multifarious trans-synaptic interactions, are arguably the most vulnerable part of the brain because of the highly polarized design of neurons. In most neurons, a complex dendritic arbor is closely connected to the cell body, whereas equally complex axons extend far away from the cell body. Dendrites are generally >10× thicker and 1,000× shorter than axons. Dendrites contain the same organelles as the cell body and are engaged in active protein synthesis and lipid metabolism, thus representing seamless extensions of the neuronal soma. Axons, in contrast, supply distant, highly compartmentalized presynaptic specializations via axonal transport over long distances. Axons contain no Golgi complex, no rough endoplasmic reticulum, and little smooth endoplasmic reticulum, limiting the presynaptic synthesis of proteins and lipids (Hanus and Ehlers, 2016; Younts et al., 2016; Hafner et al., 2019). Membrane proteins, secreted proteins, and lipids are supplied to presynaptic terminals by anterograde axonal transport from the cell body, and all material that is recycled from nerve terminals

has to be moved back to the cell body via retrograde axonal transport. As distant outstations, presynaptic terminals are therefore dependent on axonal transport. Thus the architecture of most neurons includes an inherent design fault that renders presynaptic terminals, and thereby synapses, vulnerable. This vulnerability may account for the observation that synapses are a central factor in the pathogenesis of neuropsychiatric and neurodegenerative disorders.

Advanced DNA sequencing has revolutionized the human genetics of neuropsychiatric diseases. We now know scores of genetic changes that predispose to neuropsychiatric disorders, including intellectual disability, autism, schizophrenia, and Tourette syndrome. Surprisingly, these studies implicated dysfunction of numerous genes in neuropsychiatric disorders. In many cases, the same genes predispose to different clinical entities (Taylor et al., 2020; Guang et al., 2018; Coelewij and Curtis, 2018; Keller et al., 2017; Manoli and State, 2021; Schaaf et al., 2020). Many of these genes operate in synapses. A key example is the neurexin-1 gene (*NRXN1*). One of the more common copy number variations observed in neuropsychiatric disorders localizes to chromosome 2p16.3 and inactivates only *NRXN1* expression because of the large size of the *NRXN1* gene (Südhof, 2008; Kasem et al., 2018; Hu et al., 2019). The heterozygous *NRXN1* deletion predisposes to a range of neuropsychiatric disorders. It is among the leading monogenic causes of schizophrenia, autism, and Tourette syndrome. Comparison between human and mouse neurons carrying mutations in *NRXN1* revealed that human synapses are more susceptible than mouse synapses to impairments induced by such mutations. Whereas heterozygous *NRXN1* mutations in mouse neurons produced no detectable changes, they suppressed excitatory synaptic responses in human neurons (Pak et al., 2015, 2021). These impairments were reproduced in patient-derived *NRXN1*-mutant neurons (Pak et al., 2021). These findings provide an example of the indirect relationship between genetic changes, synaptic impairments, and neuropsychiatric diseases, illustrating the challenges we face in developing new therapies for these devastating disorders.

A different picture emerges for neurodegenerative disorders, which are quintessentially related to aging. As we age, cognition declines, possibly because synapses and neurons become weaker when damage accumulates. However, recent results suggest that this is only part of what happens during aging. Pioneering studies in mice showed that age-dependent decline in cognition and synaptic plasticity could be partially reversed by exchanging the blood of old with that of young mice (reviewed in Pluvinage and Wyss-Coray, 2020). This "rejuvenation" of the brain by systemic factors cannot be explained solely by a stimulation of neurogenesis, because the synaptic plasticity changes occur in brain regions, such as the cortex, that are not subject to adult neurogenesis. It suggests that synapses age because synaptotrophic mechanisms are maintained by systemic factors that decline as we age. The mechanisms involved are unclear. At least two proteins that are present at much higher levels in the blood of young vs. old mice, SPARCL1 and thrombospondin-4, directly stimulate the formation and enhance the strength of synapses (Gan and Südhof, 2019, 2020). Whether these factors directly act on neural circuits in vivo, however, remains unknown.

When aging is associated with neurodegeneration, such as observed in Alzheimer's disease, synapses are among the first structures affected (Terry et al., 1991; DeKosky et al., 1996; Scheff and Price, 2006). At present it is unclear if the demise of synapses in neurodegenerative disorders is a nonspecific symptom, a revealing phenotype, or a diagnostic byproduct. No genes involved in neurodegeneration (except for α-synuclein) have been directly implicated in synaptic function, although presenilins and amyloid precursor protein (APP), which are causally mutated in familial Alzheimer's disease, appear to contribute to synaptic function. Mutations in amyloid precursor protein (Torroja et al., 1999; Wang et al., 2005; Priller et al., 2006) and presenilins (Zhang et al., 2009) alter synaptic functions, although the mechanisms remain unclear. Further, ApoE4 (the major genetic risk factor for sporadic Alzheimer's disease) is important for promoting synapse formation (Huang et al., 2019). It is tempting to speculate that there is a relation between the age-dependent decline in systemic factors supporting synaptic function, the aging-induced predisposition to neurodegeneration, the possible role of genes causing familial Alzheimer's disease, and the impairments in synapses observed early in neurodegeneration, but what that relation is remains unknown.

Outlook and enduring questions
Understanding the dynamics of synapses—their initial formation, the specification of their properties, their plasticity, and their turnover—is arguably one of the most important challenges in neuroscience. Efforts to meet this challenge have only started. At present, no definitive description of the basic cell-biological processes that underlie synapse formation is available.

Synapse formation is highly relevant for understanding neural circuits. How will we ever gain insight into how circuits control behavior, if we don't understand the transfer of information from one neuron to the next? Clearly, this transfer is dependent on the formation and elimination of synapses, which is a diverse and dynamic process in vivo. Among the many basic questions that need to be addressed, I would like to list a few important points.

First, what molecular logic, mediated by gene transcription and mRNA splicing, drives synapse formation? In other words, how is the specific identity of different types of synapses determined, and how is their plasticity programmed? This is of paramount importance for insight into how neural circuits are constructed.

Second, in a related question, how are synapses established? I proposed three hypotheses: that synapses are established in a canonical process following partner choice, that synapses are established nonspecifically by default and partner choice is effected post hoc by elimination of noncognate synapses, or that partner choice is part of diverse synapse establishment mechanisms mediated by distinct combinations of SAMs. Which of these hypotheses is correct is a major question to be addressed.

Third, what signal transduction pathways organize synapses? Synapse formation and elimination, independent of their mechanisms, are likely controlled by intracellular signals that are activated by SAMs, but the nature of these signals is

unknown. At present, synapse formation and elimination are black boxes: We have initial insight into some of the extracellular interactions involved, but we have no idea what actually happens in a neuron during these processes.

Fourth, what is the cell-biological basis for the design of the canonical presynaptic machinery compared with the nonoverlapping diverse composition of postsynaptic specializations? A subquestion here is how presynaptic neurotransmitters control the makeup of postsynaptic specializations, even though neurotransmitter signals don't seem to be involved.

Fifth, digging deeper into the cell biology, how does a presynaptic neuron sort different vesicular transporters into distinct vesicles that are then targeted to separate synaptic junctions? What cell-biological mechanisms allow for such exquisite specificity?

Sixth, what signals and mechanisms confer specific properties onto synapses? Clearly SAMs such as neurexins and their ligands are intimately involved, but how are they in turn regulated, and by what mechanisms do they function?

Finally, despite hundreds of papers, synaptic plasticity, especially long-term plasticity, remains an enigma. There is little insight into mechanisms besides the fact that NMDAR-dependent LTP involves recruitment of postsynaptic AMPARs and that at least in some instances, neurexins and neuroligins are necessary to render synapses competent for LTP. Moreover, there is scant evidence that long-term plasticity per se is physiologically important for a behavior, despite abundant manipulations of molecules with multifaceted roles that happen to also affect LTP. However, this lack of specific manipulations has not curtailed speculation that LTP is involved in memory, drug addiction, and scores of other human brain activities.

I hope that this review will be helpful in motivating studies in these large, and largely unexplored, areas.

Acknowledgments

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