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NLGN1 and NLGN2 in the prefrontal cortex: their role in memory consolidation and strengthening

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Abstract

The prefrontal cortex (PFC) is critical for memory formation, but the underlying molecular mechanisms are poorly understood. Clinical and animal model studies have shown that changes in PFC excitation and inhibition are important for cognitive functions as well as related disorders. Here, we discuss recent findings revealing the roles of the excitatory and inhibitory synaptic proteins neuroligin 1 (NLGN1) and NLGN2 in the PFC in memory formation and modulation of memory strength. We propose that shifts in NLGN1 and NLGN2 expression in specific excitatory and inhibitory neuronal subpopulations in response to experience regulate the dynamic processes of memory consolidation and strengthening. Because excitatory/inhibitory imbalances accompany neuropsychiatric disorders in which strength and flexibility of representations play important roles, understanding these mechanisms may suggest novel therapies.

Introduction

Strong and long-lasting memories are created by transforming fragile, newly learned information into stable and persistent biological representations, a process known as *memory consolidation.* In addition to post-translational modifications, consolidation requires a temporally limited phase of gene expression, which is accompanied by reorganization and strengthening of synaptic connections in specific neural circuits [1,2]. Consolidation is a highly dynamic process that allows for regulation of memory strength that can occur either through repetition of learning events or, in the case of single emotionally relevant experiences, via modulation [2-4]. Consolidated memories are not permanently stable; they can destabilize again and undergo *re-consolidation* if they are retrieved in certain conditions [5]. Reconsolidation is important because it provides flexibility and opportunities to strengthen or weaken the memory. Understanding the mechanisms and circuitry that underlie the strength and flexibility of memory through regulation of consolidation and

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reconsolidation is of clinical importance: several cognitive impairments are associated with either too little (e.g., aging and Alzheimer's disease) or too much memory strength (e.g. posttraumatic stress disorder [PTSD], addiction, obsessive compulsive disorder [OCD], autism spectrum disorder [ASD], and schizophrenia).

The consolidation process involves different neural circuits depending on the type of memory. For episodic memories, which process information about contexts, spaces, things, time, and conspecifics, consolidation involves interplay between the hippocampus and regions of the prefrontal cortex (PFC) [6,7]. With time (weeks in rodents, and up to years in humans), this interplay shifts the network supporting the memory representation, disengaging the hippocampus and redistributing the memory representation over cortical areas, a process known as *system consolidation* [2,8,9]. The biological, cellular and neural plasticity mechanisms recruited in the hippocampus for memory consolidation have been extensively investigated, but much less is known about the cortical mechanisms.

Typical experimental paradigms used to model episodic memories in rats and mice are based on emotionally arousing experiences, which elicit long-term memory after a single experience, e.g., contextual fear conditioning and inhibitory avoidance (IA). Using these paradigms, molecular, electrophysiological, optogenetic and pharmacogenetic investigations have revealed that biological changes induced by learning and required for consolidation progress differently in the hippocampus and cortical areas. Furthermore, these changes are more persistent in cortical regions [10-15]. The nature of these persistent molecular and cellular changes, and where in the PFC they occur is unclear.

The rodent PFC, which contains divisions that are anatomically and functionally similar to those of humans/primates, comprises the medial PFC (mPFC, further divided into prelimbic [PL] and infralimbic [IL] subregions), orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC) [16]. As most biological characterizations on mPFC functions have been carried out in mice and rats, it is important to note that although evolutionarily more complex functional specializations are likely to exit in rats compared to mice, the cytoarchitectonic definitions of mouse and rat prefrontal cortical areas appear to be similar [17,18]. In this review, we will report studies done in both rats and mice and specify the species used.

Like all other areas of the cerebral cortex, the PFC circuitry is organized in layers and shaped by multiple subpopulations of excitatory and inhibitory GABAergic neurons, the latter representing 15-20% of the total neuronal population. Little is known about how these various cell types in the PFC respond to experience. One hypothesis proposes that experience changes the overall ratio of excitation to inhibition (E/I; e.g., [19]), and that E/I dysregulation makes a major contribution to many neuropsychiatric disorders, including PTSD, depression, addiction, anxiety, schizophrenia, and ASD [20-24]. Notably, in this regard, all these disorders are characterized by impaired behavioral flexibility.

However, invoking a change in the overall E/I ratio in the PFC to explain neuropsychiatric disorders is rather simplistic, and not commensurate with the specific organization of brain structures and the complexity of their associated cognitive functions. While the E/I shift

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model provides an important starting point, it begs for a deeper mechanistic understanding, and especially how experience changes E/I.

Here, we will discuss recent studies investigating PFC excitation and inhibition mechanisms in memory processes. We will focus on three questions: First, do both excitatory and inhibitory neurons in the PFC critically contribute to memory consolidation, and if so, how? Second, how do PFC excitatory and inhibitory synapses change upon learning or memory consolidation? And third, are these changes affected by memory retrievals that lead to strengthening or weakening of the memory? The answers to these questions would provide valuable insight into the mechanisms of memory strength and flexibility.

Do both excitatory and inhibitory neurons in the PFC critically contribute to memory consolidation? If so, how?

The combined use of cell type–specific transgenic rodent lines and optogenetic/chemogenetic techniques allows for cell type–specific control of neuronal activation. Using these approaches, research in the field has begun to identify how different neuronal populations in the PFC are involved in long-term memory formation, modulation, and flexibility. In addition to excitatory pyramidal neurons, the microcircuits of the PFC include several types of inhibitory neuron subpopulations that have distinct regulatory functions: fast-spiking parvalbumin positive (PV+) neurons, which provide strong perisomatic inhibition onto excitatory pyramidal neurons; somatostatin positive (SST+) neurons, which inhibit the dendritic branches of both excitatory and inhibitory neurons; and a heterogeneous subpopulation that expresses 5HT3aR, including vasoactive intestinal peptide positive (VIP +) neurons that specifically target PV+ and SST+ neurons, thereby disinhibiting excitatory neurons [25] (Figure 1).

Studies selectively targeting either excitatory or inhibitory neurons in the PFC, particularly PV+ interneurons, in a variety of behavioral paradigms led to the general conclusion that both neuronal populations play a critical role in memory formation and expression. Pioneering optogenetic experiments by Yizhar et al. (2011) [19] revealed that prolonged activation of mPFC excitatory neurons, resulting in an increased E/I ratio, impairs social interaction and Pavlovian fear conditioning, and that the compensatory elevation of inhibitory cell excitability partially rescues social deficits. These observations supported the hypothesis that elevated E/I contributes to neuropsychiatric diseases. More recently, Courtin et al. (2014) [26] showed that phasic inhibition of PV+ neurons in the PL cortex during fear memory retrieval enhances fear expression and synchronization of pyramidal neuron output upon presentation of a conditioned stimulus, whereas activation of PV+ neurons partially suppresses fear expression. These data suggested that PV+ neurons coordinate the activity of prefrontal projection neurons to the basolateral amygdala (BLA) to drive fear expression, again underscoring the conclusion that inhibitory neurons shape behavioral responses. Using the activating version of Designer Receptors Exclusively Activated by Designer Drugs (DREADD-hM3Gq), a chemogenetic tool that is used to increase the excitability of the target neurons in the presence of the synthetic ligand clozapine-N-oxide (CNO), Warthen et al. (2016) [27] found that increasing the excitability of the mPFC is sufficient to enhance

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memory for food reward. However, they observed no effects on social interaction, locomotion, or anxiety behavior. Similarly, Yau et al. (2015) [28], using a conditioned suppression of lever pressing for food as a behavioral output to assess Pavlovian conditioned fear, showed that enhanced excitability of mPFC excitatory neurons does not affect fear learning, consolidation or retrieval, but is important for fear prediction error. On the other hand, using the silencing version of DREADD (DREADD-hM4Di) to decrease the excitability of PV+ neurons, Perova et al. (2015) [29] reported that elevation of E/I in the mPFC promotes learned helplessness under stress. In sum, notwithstanding some discrepancies possibly due to differences in the techniques used for stimulation or silencing and/or the choice of behavioral paradigms, all of these studies converged onto the idea that E/I balance in the PFC makes an important contribution to memory formation and retention and/or behavioral flexibility.

These studies have only begun to reveal the contributions of specific mechanisms in each neuronal population. To achieve deeper insights into the mechanisms in the PFC, we must also take into consideration the fact that different PFC subregions have distinct, even opposing, functions in behavioral responses. One clear example is the mPFC in the context of fear memories: the PL cortex promotes fear expression, whereas the IL cortex promotes fear extinction, the decrease in the expression of fear-conditioned response upon repeated non-reinforced exposures [30,31].

How do changes in PFC excitatory and inhibitory synapses drive memory consolidation and storage? Are these changes affected by memory retrievals that lead to strengthening or weakening of the memory?

Given the fact that different inhibitory neuronal subpopulations can synapse onto excitatory and different types of inhibitory neurons, which can result in different E/I outcomes, it is important to understand the underlying precise network of synapses at each cell type. To better understand the mechanisms operating in the PFC in association with learning, memory consolidation, and memory strengthening, we must therefore identify the mechanisms that drive experience-dependent changes in the E/I balance of neurotransmission at glutamatergic and GABAergic synapses of specific neuronal subpopulations. Key synaptic proteins highly conserved in evolution between rodents and humans that control excitation and inhibition are the post-synaptic cell adhesion molecules neuroligin 1 (NLGN1) and NLGN2. NLGN1 is enriched at excitatory synapses while NLGN2 is enriched at inhibitory, dopaminergic and cholinergic synapses [32]. NLGNs form homodimers, and their extracellular domain binds to neurexins (NRXNs) present at presynaptic terminals, while their intracellular regions anchor to scaffolding proteins, such as the postsynaptic density protein-95 (PSD95) and gephyrin [32]. In addition to NLGN1 and NLGN2, two other NLGNs, NLGN3 and NLGN4, have been identified. NLGN3 is found at both excitatory and inhibitory synapses, and may form hetereodimers with both NLGN1 and NLGN2 [33]; NLGN4 is poorly conserved from rodents to humans, has low level of expression in the mouse and found to localize to glycinergic synapses [32]. These structural features enable NLGN1 and NLGN2 to coordinate the assembly of glutamate and GABA/glycine receptors at the postsynaptic site with the maturation and function of the

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presynaptic specialized structures (Figure 2). As their dysregulation alters the properties of synapses and disrupts neural networks without completely abolishing synaptic transmission, it appears that NLGN1 and 2 are required for synapse function, rather than synapse formation [34].

Recent studies have provided important insights about the regulatory mechanisms that NLGNs can provide. Conditional genetic deletions of all three major NLGN isoforms, individually and in combination, in cultured mouse hippocampal and cortical neurons indicated that lack of NLGNs causes small or no change in synapse numbers, but significantly impairs synapse functions [35]. Conditional knockout of NLGN1 in newborn or juvenile mice results in a significant impairment in NMDAR- and L-type Ca^{2+} channel-dependent LTPs [36]. Finally, activity and learning leads to histone modifications regulating NRXN1 alternative splicing, hence controlling NRXN1 binding to NLGN1 in the mouse dentate gyrus [37]. Together, these results suggest that NLGN1 and NLGN2 have isoform-specific functions at excitatory and inhibitory synapses. Moreover, at least for NLGN1, these functions appear to contribute to a variety of activity-dependent responses [32].

The regulation and role of NLGN1 in learning and memory are still in the process of being understood. Clinical studies have revealed that *NLGN1* genetic variants are associated with neuropsychiatric disorders such as ASD [38,39], memory loss and depression in Alzheimer's disease [40], and PTSD [41], indicating that NLGN1 plays a role in cognition. Notably, carriers of the *NLGN1* variant strongly associated with PTSD exhibited greater neural activation in limbic and prefrontal regions, as well as increased functional connectivity between the amygdala and the dorsal–lateral PFC in response to fearful face stimuli [41]. In transgenic mouse models, loss or overexpression of NLGN1 impairs spatial memory, as determined by performance in the Morris water maze test, indicating the importance of optimal NLGN1 level in hippocampal-dependent memory tasks [42,43]. Notably in this regard, NLGN1 knockout mice also exhibit increased repetitive behavior, with small deficits in social interaction and pain sensation, accompanied by deficits in hippocampal LTP [42]. These findings paralleled studies in rats, in which local viral expression was used to target the amygdala in fear conditioning [44]. Therefore, NLGN1 in multiple brain regions plays a critical role in memory formation.

Regarding memory disorders, recent studies in rats showed that NLGN1 interacts with amyloid-β oligomers (AβO), forming a complex with GluN2B-containg N-methyl-D-aspartate (NMDA) receptors [45]. In mice, AβO interaction with NLGNs appears to mediate hippocampal synapse damage and memory loss [46], and, in rats, amyloid-induced neuroinflammation enhances epigenetic-mediated inhibition of NLGN1 expression, leading to glutamatergic dysfunction in the hippocampus and memory loss [47]. Given the fundamental roles of NLGN1 in memory and memory disorders, a next important question to be addressed is how experience regulates the expression of NLGN1 in specific cell populations, and how long the changes persist in order to promote memory consolidation and flexibility, or their dysregulation in memory disorders.

The role of NLGN2 in memory formation and storage also remains poorly characterized. As with *NLGN1*, genetic alterations in *NLGN2* are linked to severe cognitive disorders,

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implying that it is also important for cognitive functions. Genome-wide analyses in humans have identified copy number variations (CNVs) and missense single nucleotide polymorphisms (SNPs) in *NLGN2* that are associated with developmental disorders, including ASD and schizophrenia [48,49]. These human phenotypes have been in part recapitulated in transgenic mouse models. *Nlgn2* knockout mice exhibit profound developmental delays in multiple metrics such as tail length, age of eye opening, and body length, as well as increased ultrasonic vocalization in pups [50]. NLGN2-overexpressing mice, on the other hand, have reduced lifespan and offspring viability, as well as limb clasping, repetitive behaviors, and impaired social interactions that resemble aspects of Rett syndrome and ASD [51]. Both knockout and overexpression of NLGN2 in mice increase anxiety-like behaviors and alter social behaviors, suggesting that synaptic inhibition plays a critical role in anxiety regulation [50-53]. Finally, viral-mediated overexpression of NLGN2 in the mouse hippocampus increases adult neurogenesis, while decreasing performance in the water maze task [54]. In sum, NLGN2 has emerged as a key molecule in brain development, anxiety as well as cognition.

A few recent studies explored the roles of NLGN1 and NLGN2 specifically in the PFC. Liang et al. (2015) [55] reported that virus-mediated conditional knockout of *Nlgn2* in the mPFC of adult mice reduces anxiety or increases impulsivity-like behavior in the open arms of an elevated plus maze, and impairs fear conditioning. These behavioral changes are accompanied by an increased ratio of evoked E/I synaptic currents. Together, these data suggest that anxiety and fear learning may be highly sensitive to subtle changes in inhibitory synaptic function or plasticity in the mPFC [56]. Moreover, conditional *Nlgn2* knockout, despite reducing inhibition, decreases experience-dependent induction of immediate-early genes, including *c-Fos*, *Egr1* and *Npas4* in the mPFC, suggesting that chronic reduction in local inhibition may impede experience-evoked mPFC activation. Tzanoulinou et al. (2016) [57] found that peripubertal stress in rats reduces NLGN2 expression in the mPFC, whereas virus-mediated overexpression of NLGN2 in mPFC rescues the attention deficits induced by peripubertal stress. In control rats that did not experience peripubertal stress, NLGN2 overexpression in the mPFC also impaired attention, indicating the importance of optimal inhibition in attention tasks.

In sum, although ablation or overexpression of NLGN1 and NLGN2 in the PFC indicate that these proteins are necessary for memory retention and responses to stress and attention [58], their roles in specific processes that regulate memory strength and flexibility, such as memory consolidation, reconsolidation, and modulation, remain to be understood.

To dissect the PFC mechanisms involved in memory consolidation and strengthening, our laboratory used inhibitory avoidance (IA) in rats [11]. IA is a contextual fear conditioning-based paradigm in which the animals learn to avoid a context previously paired with a footshock. This episodic type of memory undergoes hippocampal–cortical system consolidation, and lends itself well for molecular and behavioral investigations. We found that IA training led to a significant increase in both NLGN1 and NLGN2 levels in the PL cortex 6 days after training (Figure 3A). Blocking NLGN2 function with an excess of NLGN2 extracellular domain injected into the PL cortex once every 2 days after training significantly disrupted IA memory consolidation, but the same manipulation targeting

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NLGN1 had no effect. These data implied that a prolonged action of NLGN2, but not NLGN1, during the first week after training is required in the PL cortex for IA memory consolidation. This lingering effect of NLGN2 raises a few questions. First, is PL NLGN1 involved at all in memory consolidation? If so, what is its critical temporal window of action? Second, why is lasting synaptic inhibition necessary for consolidation of an IA memory? One possible explanation is that the increase in NLGN2-mediated inhibition disinhibits excitatory neurons, leading to an increase in excitation that mediates IA memory consolidation. This hypothesis is plausible, as it was supported by our experiments showing that IA training is accompanied by the induction of immediate early genes (such as activity-regulated cytoskeletal-associated protein, Arc/Arg3.1) in the PL cortex.

We previously showed that reconsolidation significantly strengthens IA memory if the memory is retrieved (or reactivated) with three brief context exposures given once every two days during the first week after IA training [59]. Using this paradigm, we found that, in comparison with memory consolidation (rats trained and remaining in the home cage following training), memory strengthening caused no additional changes in NLGN1 levels in the PL cortex.

By contrast, PL NLGN2 levels decreased to control levels, suggesting that memory strengthening is accompanied by a sustained increase in excitation and a reduction of inhibition in the PL cortex (Figure 3A). These results were confirmed by a survey of Arc induction. The decrease in NLGN2 expression after retrievals suggested that changes in NLGN2 levels dynamically accompany memory strengthening. Furthermore, it suggested that the reduction in NLGN2 after retrieval occurs in cell subpopulations and synapses distinct from those in which NLGN2 was upregulated during consolidation: if NLGN2 during consolidation serves to disinhibit inhibitory neurons, a decrease in NLGN2 expression in the same synapses would reverse that effect, leading to reduced excitation, which is the opposite of what the data showed. Indeed, memory strengthening evoked by retrievals was accompanied by increased Arc induction in the PL cortex. One model that may explain these data is that training and retrieval-induced memory strengthening are paralleled by dynamic regulation of NLGN1 and NLGN2 in distinct cell subpopulations (Figure 4). Consistent with this idea, we found that blocking NLGN2 at each memory reactivation did not disrupt memory consolidation (unlike when given without the retrievals), but only blocked memory strengthening (Figure 3B). Notably, we also observed that the blocking effect of NLGN2 on memory strengthening was reversed by a reminder shock (RS), and the memory fully reinstated. Because reinstatement after RS is a typical behavioral response associated with memory extinction, we reasoned that the mechanisms mediated by NLGN2 allow for memory strengthening actually by inhibiting extinction. This explanation was confirmed experimentally using an extinction paradigm [11]. Therefore, we concluded that the experience-dependent regulations of NLGN1 and NLGN2 in distinct cell populations is key for a flexible response to fear and aversive experiences [11]. Cell population-specific changes may therefore accompany the lack of extinction flexibility, as is found in PTSD.

In sum, our results demonstrate that it is important to identify the subregions, cortical layers and cell subpopulations of the PFC in which NLGN1 and NLGN2 expression shifts in

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response to experience. These synaptic maps reflect the stability, strength, and flexibility of specific behaviors; hence, their dysregulation may underlie neuropsychiatric disorders.

Conclusions

Our understanding of the local PFC mechanisms underlying cognitive function remains limited. An important goal for future work will be the mapping of NLGN1 and NLGN2 regulation in relation to memory encoding, consolidation, and strength modulation. Furthermore, manipulations of neuron subpopulation-specific NLGN1 and NLGN2 could be used to drive memory strengthening or weakening. Because the PFC does not function in isolation, local changes will influence and be influenced by connected brain regions.

Here, we discussed the role of NLGN1 and NLGN2 in the modulation of fear memory strength, which is critical for adaptive behavior: while it is important to form long-lasting adaptive memories for the fittest behavior, overconsolidated or overstrengthened memories are maladaptive and associated with psychopathologies. Therefore, the identification of the specific circuitry and molecular mechanisms of excitation and inhibition underlying long-term memory consolidation and strengthening is important for developing specific therapeutic strategies to address the inflexibility of neuropsychiatric disorders.

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Highlights

- Learning-induced molecular changes in the PFC are critical for long-term memory.
- Neuronal excitation and inhibition in the PFC are important for memory formation.
- NLGN1 and NLGN2 cooperate to regulate memory strength.

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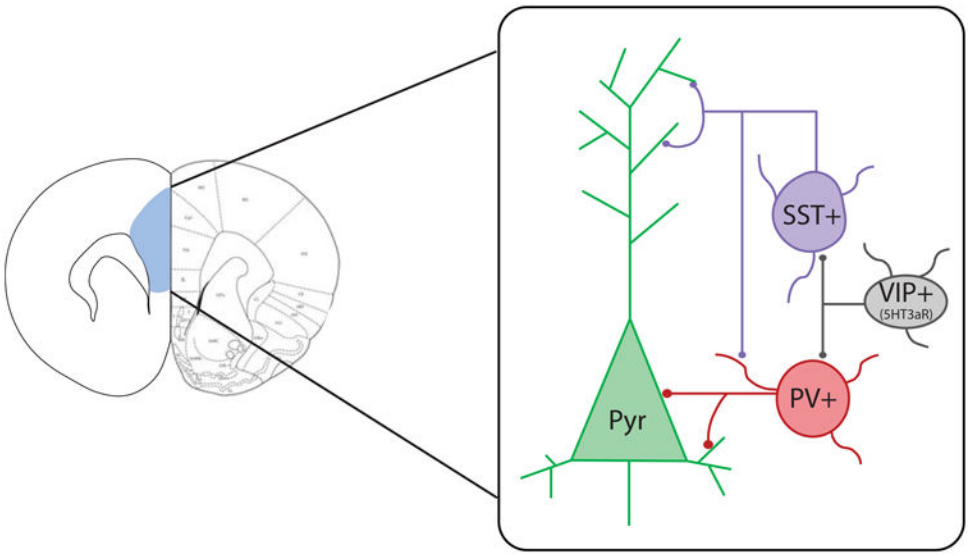

Figure 1.

Representation of excitatory and inhibitory neuron types in the prefrontal cortex (highlighted in blue, left). Excitatory pyramidal neurons (Pyr) receive perisomatic inhibition from parvalbumin-positive interneurons (PV+) and dendritic inhibition from somatostatin-positive interneurons (SST+). SST+ neurons also provide dendritic inhibition onto PV+ neurons. Vasoactive intestinal peptide–positive neurons (VIP+), a subpopulation of 5-hydroxytryptamine-3a receptor (5HT3aR)-expressing interneurons, inhibit PV+ and SST+ inhibitory neurons, resulting in disinhibition of Pyr neurons.

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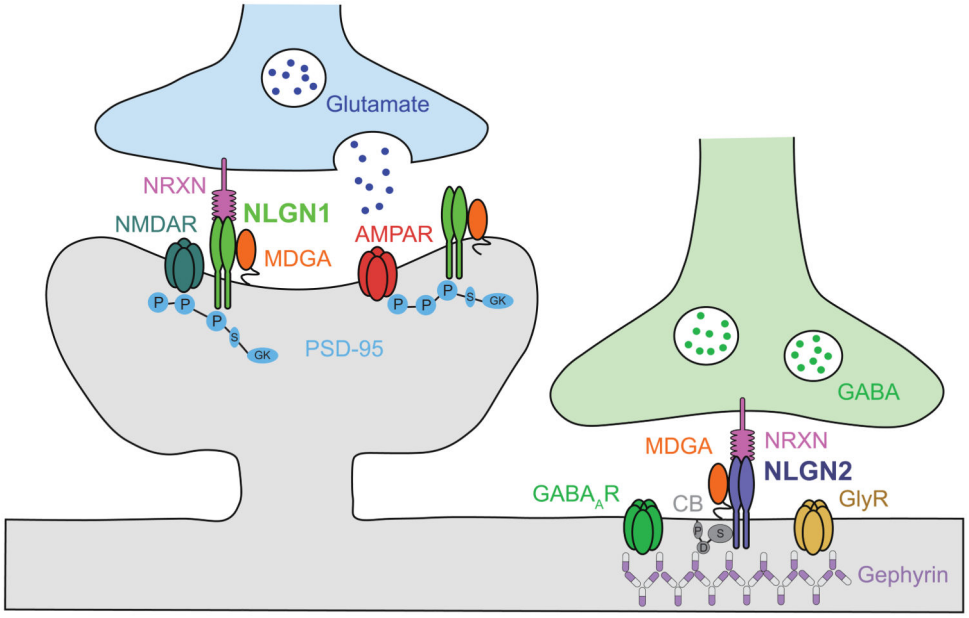

Figure 2.

Neuroligin 1 (NLGN1) and NLGN2 cell adhesion molecules at excitatory and inhibitory synapses, respectively. NLGN1 and NLGN2 are enriched are postsynaptic membranes, and their extracellular domains adhere to distinct isoforms of neurexins (NRXN) at presynaptic membranes. Intracellularly, NLGN1 binds to the scaffolding protein postsynaptic density protein 95 (PSD95), and regulates the synaptic localization of glutamate receptors (GluR) α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and N-methyl-D-aspartate receptors (NMDARs). NLGN2 forms a complex with collybistin (CB) and gephyrin (GPHN) and regulate the synaptic localization of GABA receptors (GABAR). The cell-surface molecules MAM domain-containing glycosylphosphatidylinositol anchor proteins (MDGAs) form post-synaptic *cis*-complexes with NLGN1 and NLGN2, and negatively regulate their trans-synaptic adhesion with NRXNs [60-62]. Additional protein abbreviations: GlyR, glycine receptor; P, PDZ binding domain; S, Src homology domain (SH3 domain); GK, guanylate kinase domain; D, Dbl homology domain; P, Pleckstrin homology domain (Adapted from Bemben et al., 2015 [48]).

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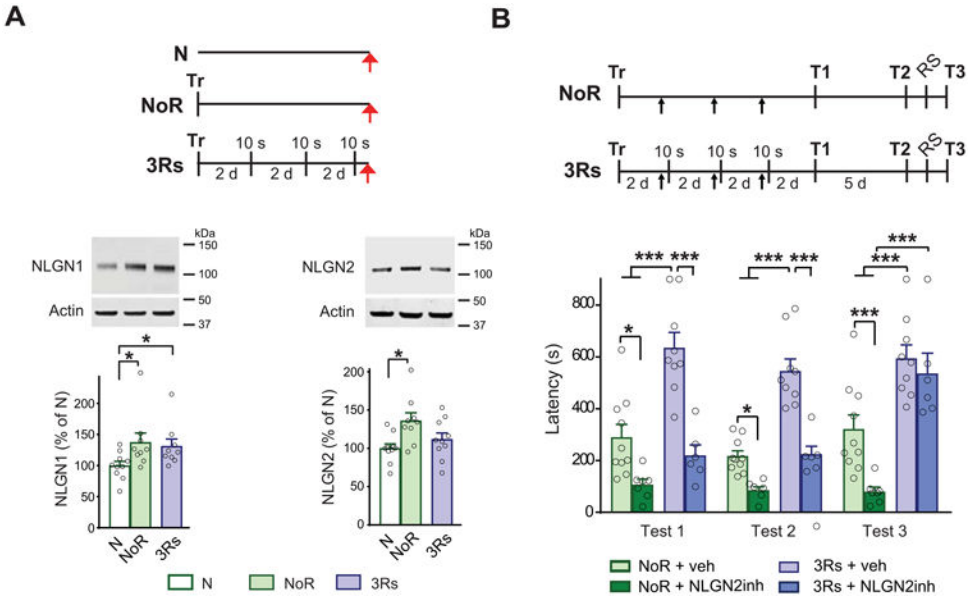

Figure 3.

Neuroligin 1 (NLGN1) and NLGN2 play distinct roles in memory consolidation, strengthening, and extinction inhibition. **(A)** Schema of behavioral procedures, representative images and relative quantitative western blot analyses of PL cortex. Protein extracts were obtained from rats trained (Tr) in inhibitory avoidance (IA) and given 3 brief memory retrievals (3Rs), which consisted of 10 sec exposures to the training context without footshock, every two days. Rats were euthanized (red arrows) one hour after 3Rs or at the matched time point for the group that underwent training without retrievals and remained in the home cage after training (NoR). Naïve rats (N) served as reference controls. Data are presented as mean percentage ± s.e.m. of the mean values of the N group. One-way ANOVA followed by Newman-Keuls post hoc test; NLGN1 n = 9–10, NLGN2 n = 9–10; *$p < 0.05$ for both comparisons. **(B)** Schema of behavioral procedures is given above the graphs. Rats were trained (Tr) in IA and given 3Rs every two days or left in the home cage without retrieval (NoR) after training. Thirty minutes before each reactivation, or at matched timepoints in the NoR group, the animals received a bilateral PL cortex injection (black arrows) of NLGN2 recombinant extracellular domain (NLGN2inh) to inhibit NLGN2 function. Animals were tested for memory retention two days after the last retrieval (T1), and again five days later (T2), as shown in the schema. A reminder footshock (RS) was given in a different context with the same shock intensity one day after T2 and memory was tested one day later (T3). Data are expressed as mean latency ± s.e.m. Two-way ANOVA followed by Bonferroni post hoc test; *$p<0.05$, ***$p<0.001$ n = 7-10 (Adapted from Ye et al., 2017 [11]).

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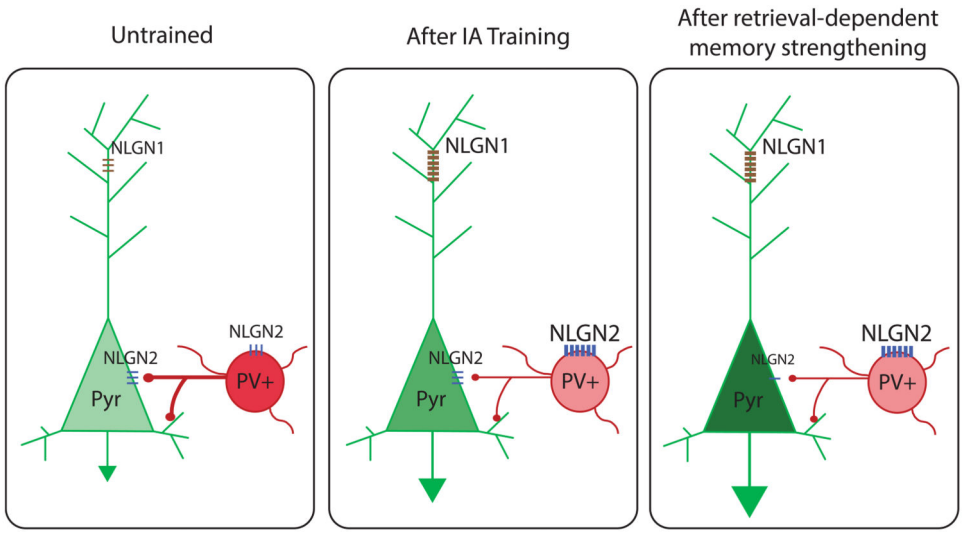

Figure 4.

Schematic representation of our working model for cell type–specific changes in NLGN1 and NLGN2 after IA training and retrieval-induced memory strengthening in the PL cortex. IA training may increase NLGN2 expression in parvalbumin-positive interneurons (PV+), which would lead to disinhibition of pyramidal (Pyr) cells, as well as NLGN1 in Pyr cells. These changes would result in increased E/I. Memory retrieval-induced strengthening would decrease NLGN2 levels in Pyr cells, while maintaining NLGN2 increased levels in PV+ neurons. These changes would further increase excitation. Together, these NLGN1 and NLGN2 changes on different neuronal populations would differentially regulate E/I balance in memory consolidation and following retrievals leading to memory strengthening.