Parvalbumin Interneuron Plasticity for Consolidation of Reinforced Learning

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Parvalbumin (PV) basket cells are widespread local interneurons that inhibit principal neurons and each other through perisomatic boutons. They enhance network function and regulate local ensemble activities, particularly in the γ range. Organized network activity is critically important for long-term memory consolidation during a late time window 11–15 h after acquisition. Here, we discuss the role of learning-related plasticity in PV neurons for long-term memory consolidation. The plasticity can lead to enhanced (high-PV) or reduced (low-PV) expression of PV/GAD67. High-PV plasticity is induced upon definite reinforced learning in early-born PV basket cells, whereas low-PV plasticity is induced upon provisional reinforced learning in late-born PV basket cells. The plasticity is first detectable 6 h after acquisition, at the end of a time window for memory specification through experience, and is critically important 11–15 h after acquisition for enhanced network activity and long-term memory consolidation. High- and low-PV plasticity appear to regulate activity in distinct local networks of principal neurons and PV basket cells. These findings suggest how flexibility and stability in learning and memory might be implemented through parallel circuits and networks.

Parvalbumin (PV) basket cells are widely distributed and abundant GABAergic inhibitory interneurons that provide powerful local feedforward and feedback inhibition onto principal excitatory neurons (Bartos et al. 2007; Hu et al. 2014). In addition, PV basket cells inhibit each other reciprocally through perisomatic innervation and are dynamically coupled electrically through gap junctions (Bartos et al. 2007; Hu et al. 2014). PV basket cells filter activation of principal neurons, and networks of PV basket cells have major roles in regulating local ensemble activities, including θ and γ oscillations (Fuchs et al. 2007; Cardin et al. 2009; Sohal et al. 2009; Kuhlman et al. 2010; Isaacson and Scanziani 2011; Buzsáki and Wang 2012; Lee et al. 2012; Hu et al. 2014). Synaptic regulation of PV basket cells has been implicated in adult learning, and the maturation state of PV basket cells has been implicated in critical period-type plasticity (Hensch et al. 1998; Hensch 2005; Di Cristo et al. 2007; Southwell et al. 2010; Kuhlman et al. 2013). We recently discovered that PV basket cells show robust plasticity upon reinforced forms of learning in the adult (Donato et al. 2013), and that two subpopulations of PV basket cells generated during the first/second half of neurogenesis in the median ganglionic eminence show plasticity upon definite (early-born)/provisional (late-born) learning (Donato et al. 2015). PV neuron plasticity only becomes detectable with a delay of ~ 6 h after the initial learning event, and persists for 2–3 d (Donato et al. 2015; Karunakaran et al. 2016). In this review, we focus on the function of this

unusual form of cellular plasticity and on what it reveals about circuit and systems mechanisms of long-term memory consolidation.

Analysis of learning-related plasticity markers such as pERK, cFos, and Arc expression in the hippocampus after fear conditioning revealed a second peak of expression from +9 to +15 h after acquisition (Trifilieff et al. 2006, 2007; Bekinschtein et al. 2007; Katzev et al. 2010, 2013; Caroni et al. 2014; Karunakaran et al. 2016). Interfering with dopamine D1 receptor (D1R) signaling or organized network activity at +12 h after fear conditioning suppressed long-term consolidation of fear memory, providing evidence for the existence of a late time window at +12 h critically important for long-term memory consolidation (Girardeau et al. 2009, 2014; Rossato et al. 2009; Carr et al. 2011; Jadhav et al. 2012; McNamara et al. 2014). As discussed below, learning-related PV neuron plasticity appears to be specifically important to support offline network activity essential for long-term memory consolidation upon reinforced learning during the late time window at +12–15 h.

PV NEURON PLASTICITY OF OPPOSITE SIGNS UPON PROVISIONAL AND DEFINITE REINFORCED LEARNING

Reinforced trial-and-error learning tasks provide attractive experimental paradigms to investigate how plasticity is flexibly adjusted during learning. Thus, effective acqui-

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sition and flexible combination of potentially task-relevant information is essential during early phases of trial-and-error learning, whereas reliable application of validated routines dominates toward learning curve completion (Kaelbling et al. 1996). We found that at the circuit level, these contrasting requirements are correlated to shifts in the configuration of local PV-expressing basket cell networks, which show pronounced plasticity induced by certain forms of learning (Fig. 1; Donato et al. 2013). Thus, PV network configurations enriched in neurons expressing low levels of PV and GAD67 and showing relatively high inhibitory connectivity onto them are induced locally early during trial-and-error incremental learning, when

they are required for effective learning (Fig. 1A–E). Such “low-PV configurations” promote acquisition and retention of new memories and structural synaptic plasticity (Donato et al. 2013). In contrast, configurations enriched in neurons expressing high levels of PV and GAD67 and showing high excitatory connectivity onto them are induced upon Pavlovian conditioning and toward completion of trial-and-error learning protocols. Such “high-PV configurations” interfere with the acquisition of new incidental memories (Familiar Object Recognition test) and structural synaptic plasticity (Fig. 1D; Donato et al. 2013). The PV plasticity was not detected in tasks involving memory formation in the absence of reinforce-

Figure 1. Learning-induced plasticity of PV basket cells. (A) Experience-related plasticity of PV neurons. (Arrows) High- (dark blue), intermediate high- (blue), intermediate low- (gold), and low-PV (yellow) neurons. Relative contents of PV neurons in control (Ctrl), enriched (EE, 3 wk), and fear-conditioned (cFC, day 1) mice. (B) PV/GAD67 signal relationship in individual PV neurons from Ctrl, EE, and cFC mice. (C) Local densities of excitatory and inhibitory synaptic puncta as a function of PV immunoreactivity signal. (D) PV neuron plasticity induced upon pharmacogenetic activation or inhibition of dCA3 PV neurons (values 31 h after ligand delivery) and impact on hippocampal memory. (E) Morris water maze learning curve (left) and PV network configuration in CA3b during MWM learning (right). (F) Persistence of low-PV configuration in spite of spatial learning when external cues are rearranged daily, from training day 5 on. Schematic of match–mismatch protocol (left), corresponding learning curve (center), and PV network configuration on day 10 for match–mismatch protocol (right). (Adapted from Donato et al. 2013.)

ment, suggesting that it might be specifically induced upon reinforced learning. Furthermore, the plasticity was specifically detected in brain areas known to have a role in the particular form of learning (e.g., hippocampus for the water maze but not for a motor skill task such as staying on an accelerating rotating rod, and primary motor cortex but not hippocampus for the motor skill task).

Two additional observations warrant mentioning here. First, when a variation of the standard water maze protocol was introduced so that, from training day 5 on, mice had to adjust each day to a new position of the hidden platform relative to allocentric cues on the surrounding walls, mice rapidly adjusted to this more challenging protocol, but the high-PV plasticity consistently observed once mice had reliably learned the spatial map did not occur (Fig. 1F; Donato et al. 2013). This observation suggests that a shift to a high-PV network configuration might specifically be induced when mice effectively manage a task in a way that does not anymore involve unexpected features (such as the relationship between allocentric cues and the position of the hidden platform). Rather than reflecting performance, high-PV plasticity might therefore reflect the absence of deviations and unexpected value-related findings in a trial-and-error task. The second informative observation is that the learning-related plasticity does not seem to induce any distinct new phenotype within the population of local PV neurons. Instead, the plasticity appears to shift the relative prevalence of PV neurons with a given distribution of PV/GAD67 expression of excitatory versus inhibitory synaptic puncta onto them (Donato et al. 2013, 2015). This suggests the presence of consistent, predictable and reversible learning-induced shifts in the relative prevalence of local PV neurons with relatively high- or low-PV/GAD67 expression levels. Given the high degree of connectivity between individual local PV neurons and principal neurons and among the PV neurons themselves, these observations suggest that the plasticity induced upon certain types and stages of learning might influence the functioning of local principal and inhibitory neuron networks.

DISTINCT PV NEURON SUBPOPULATIONS IMPLEMENT PLASTICITY UPON PROVISIONAL AND DEFINITE LEARNING

The observed opposite learning-induced shifts toward either more or less PV/GAD67 expression and excitatory/inhibitory synaptic puncta distributions within the ensemble of local PV neurons might reflect concerted global network shifts possibly involving most PV neurons. Alternatively, there might be PV neurons susceptible to low-PV plasticity during provisional learning, and other PV neurons susceptible to high-PV plasticity upon definite learning. Indeed, using BrdU-mediated birth-dating during medial ganglionic eminence development, we found that PV basket cells consist of two previously unrecognized subpopulations, which are specified during the first and second half of neurogenesis in mouse subpallium and differ in their connectivities and plasticity regulation (Fig. 2A–D; Donato et al. 2015). Early-born PV basket cells

generated during the first half of neurogenesis show comparatively high-PV/GAD67 levels and excitation/inhibition connectivity ratios under baseline conditions, which in most cases further increase upon learning-induced plasticity (Fig. 2A–C). Furthermore, early-born PV neurons show plasticity upon cFC and at the end of maze learning, but not upon environmental enrichment (EE) or during maze learning (Fig. 2C). Early-born PV basket cells appear to account for characteristic features of mature fast-spiking PV neuron networks such as narrow synchronization windows and learning-related θ – γ entrainment within and among brain systems (Bartos et al. 2007). Thus, high-PV and GAD67 levels enhance fast and high-frequency firing properties of PV neuron networks important for γ band network activity (Fuchs et al. 2007; Cardin et al. 2009; Kuhlman et al. 2010). In contrast, late-born PV basket cells specified during the second half of neurogenesis show low-PV/GAD67 levels and excitation/inhibition connectivity ratios under baseline conditions, which in most cases further decrease upon learning-induced plasticity. Furthermore, late-born PV neurons show plasticity upon EE, during maze learning and when critical period-type plasticity is induced (Fig. 2A–C). Notably, early-born PV neuron cell plasticity is specifically regulated by excitation, whereas late-born PV neuron cell plasticity is specifically regulated by PV neuron inhibition (Donato et al. 2015). Furthermore, cell plasticity in early-born neurons mainly involved alterations in the densities of excitatory synaptic puncta, whereas cell plasticity in late-born neurons mainly involved alterations in inhibitory synaptic puncta densities onto PV neuron dendrites. These matched specificities of regulation were particularly striking in comparisons of how cFC or EE induced PV cell plasticity in CA3/CA1 versus DG. Thus, although cFC consistently involved early-born neuron plasticity and EE consistently involved late-born neuron plasticity, the signs of PV (and *Mef2a*) changes were opposite in the hippocampal subdivisions, and this was reflected in opposite signs of excitatory (and inhibitory) connectivity regulation in the two subpopulations (Donato et al. 2015).

The consistent experience-related plasticity regulation of the two subpopulations of PV basket cells raised the issue of whether these might also show distinct output targets related to behavioral function (Kepecs and Fishell 2014; Lee et al. 2014). Indeed, we found that early-born PV neurons preferentially target deep cells in the pyramidal layer of hippocampal CA1, whereas late-born PV neurons preferentially target superficial cells (Fig. 2D). The selective connectivity between early-/late-born PV neurons and early-/late-born pyramidal cells is reminiscent of selective connectivity among principal neuron subpopulations in hippocampal dentate gyrus, CA3 and CA1, suggesting that it might reflect circuit assembly principles based on relative schedules of neurogenesis and neuronal maturation (Deguchi et al. 2011). Although the functional implications of the selective output connectivity of PV neuron subpopulations remain to be determined, our findings would be consistent with the notion that the distinct excitation/inhibition input ratios onto early- and late-born PV neurons and the specific regulation upon learning

Figure 2. Developmentally specified subpopulations of PV basket cells showing plasticity upon definite or provisional learning. (A) Relationship between schedule of neurogenesis and PV/GAD67 levels in adult hippocampal CA3b. (Left) Representative example of PV neuron in adult hippocampal CA3b labeled with BrdU, and scatter plot of PV labeling values for cells (individual dots) labeled at E9.5, E11.5, E13.5, and E15.5. (Right) Labeling value distributions as a function of birth date. (B) Grafting E13.5 MGE cells into E11.5 host. Adult distribution of PV labeling intensities in donor neurons closely matched to late-born PV neurons of host. (C) Time schedule of neurogenesis versus regulation of PV levels upon cFC or EE. (D) PV signals of perisomatic boutons at deep- and superficial-layer CA1 pyramidal cells. (Left) Representative examples of bouton signal distributions at deep- and superficial-layer pyramidal neurons. Scale bars, 15 μ m (top) and 5 μ m (bottom). (Right) PV-level distributions of perisomatic boutons at deep- and superficial-layer CA1 pyramidal cells upon cFC or EE. (Adapted from Donato et al. 2015.)

might reflect information flow through functionally distinct microcircuits (Fig. 3; Mizuseki et al. 2011; Lapray et al. 2012; Knierim et al. 2014; Cembrowski et al. 2016; Lagler et al. 2016; English et al. 2017; Soltesz and Losonczy 2018).

LEARNING-INDUCED PV NEURON PLASTICITY: DELAYED ONSET AND PERSISTENCE FOR DAYS

To investigate which aspects of learning and memory might be influenced by learning-induced plasticity in local PV neurons, we monitored the time course of high-PV plasticity induced in mouse hippocampal CA3 upon cFC. In ventral (vH) and dorsal hippocampus (dH), PV neuron labeling distributions were not detectably altered up to +4.5 h (i.e., 4.5 h after acquisition) and had reached peak high-PV values at +6 h. These were maintained until +30 h, had decreased to half-maximal values around +36 h, and reached again baseline values at +48 h after acquisition (Fig. 4A). Low-PV plasticity induced upon 1 d of MWM training also only became detectable at +6 h. Notably, and like for high- or low-PV plasticity induced upon learning, pharmacogenetically induced PV plasticity only became detectable at +6 h (and not yet at +4.5 h)

upon induction (Karunakaran et al. 2016). Unlike plasticity induced upon learning, elevated contents of high- or low-PV neurons begun to decline from +10 to 11 h, and had returned to baseline values at +15 h (Fig. 4B). These observations suggested that long-lasting PV plasticity possibly required for long-term memory consolidation might depend on additional signals delivered locally. Given the role of late dopamine (DA) signaling in fear memory consolidation, we hypothesized that this might involve local DA signaling acting through D1/5 receptors. Indeed, local delivery of a specific D1 receptor agonist to vH at +12 h rescued and extended both the high- and the low-PV shifts up to at least +24 h (Fig. 4B; Karunakaran et al. 2016). Notably, local delivery of D1 agonist to vH of naive mice induced dramatic increases in the fraction of pDARPP-32+ and pERK+ PV neurons, whereas corresponding changes in non-PV neurons were much more modest (pDARPP-32) or absent (pERK), suggesting that D1R might signal in PV neurons to modulate PV neuron plasticity (Karunakaran et al. 2016).

Given the dependence on D1R signaling by the PV neuron plasticity induced upon pharmacogenetic activation or inhibition of PV neurons, we hypothesized that PV neuron plasticity induced upon learning might be sustained through endogenous DA signaling onto D1R. Indeed, local delivery of D1R antagonist any time between

Figure 3. Distinct local microcircuit connectivities for early- and late-born PV neurons in hippocampal CA1. (A) Position of deep (green) and superficial (red) pyramidal neurons in CA1. (B) Schematic representation of connectivity involving deep/superficial and early-/late-born PV neurons. (C) Proposed sequence of recruitments (1–4) for a hypothetical superficial (1) to early-born PV neuron (2) to deep pyramidal neuron (3) local circuit. Panel 4 indicates how the microcircuit might support the formation of neuronal ensembles in superficial pyramidal neurons.

+10 and +16 h after cFC or water maze learning led to a complete loss of any detectable local PV plasticity within 10–15 min. Furthermore, local delivery of D1R antagonist to vH at +12 h upon cFC suppressed any subsequent freezing to context. This finding led us to determine when local delivery of D1R antagonist to vH influences freezing to context upon cFC. These experiments revealed the existence of two sensitive time windows to influence consolidation of learning, one up to +5–6 h after acquisition, and the second one between +10 and +15 h (Karunakaran et al. 2016). The first time window was closely correlated to the delay period preceding the appearance of detectable PV neuron plasticity, whereas the second one was reminiscent of a previously described time window involving immediate early gene expression and offline network activity essential for long-term memory consolidation (Fig. 4C). The unusual features of delayed onset, and prolonged persistence of the PV plasticity raise the question of what might be its roles in learning and memory.

SIX-HOUR TIME WINDOW UPON ACQUISITION TO DEFINE WHAT WILL BE CONSOLIDATED

The 5- to 6-h time window after learning, when PV plasticity is not yet detectable, coincides with a time when memories of related experiences are merged into shared neuronal assemblies (Cai et al. 2016; Rashid et al. 2016). As recently reported, this corresponds to a time window when information acquired separately, but linked through shared elements such as context or task-relevant objects, is combined to determine whether and what is being learned (time units for learning) (Chowdhury and Caroni 2018). The duration of the time units depends on cFos protein expression and function in local neuronal assemblies, which becomes detectable 45 min after acquisition, and is maintained against proteasome-mediated degradation during about 4–5 h through processes depending on offline network activity within brain systems involved in that particular learning (Chowdhury

Figure 4. Regulation of PV neuron plasticity by D1R signaling. (A) Time course of high-PV neuron plasticity induced upon cFC in vH CA3. (B) Time course of high-PV neuron plasticity induced upon pharmacogenetic activation of PV neurons in CA3 and prolongation of the plasticity upon local delivery of D1R agonist. (C) Two time windows after acquisition of cFC when local delivery of D1/5R antagonist to vH interferes with long-term consolidation of fear memory (tested at +24 h). (Adapted in part from Karunakaran et al. 2016.)

Figure 5. Modulation of PV plasticity and long-term memory during early time window up to 5–6 h after acquisition of cFC. (A) Strengthening of weak 2US PV plasticity and fear memory (*left*) upon delivery of D1R agonist to vH at +2 h (*right*). (B) Suppression of long-term fear memory upon local delivery of D1/5R antagonist to vH at +3 h after acquisition of cFC. Control bar: test at +24 h. (C) Suppression of vH cFos induction at +15 h induced upon water maze learning by local induction of opposite-sign PV neuron plasticity (high-PV) in vH at +3 h. (Adapted from Karunakaran et al. 2016.)

and Caroni 2018). The 5–6 h time window that precedes detectable PV plasticity might therefore reflect a time when the magnitude, and possibly also the sign (toward high- vs. low-PV), of the PV neuron plasticity might be influenced by experience.

To determine whether the status of D1/5 receptor activation up to 5 h after acquisition might modulate the strength of PV plasticity and learning, we performed experiments in mice that underwent a comparatively weak cFC protocol. The unconditioned stimulus (US) (here foot shock) is indicated in the following as US. A 2×US (instead of 5×US) protocol resulted in a comparatively modest high-PV shift in vH at +12 and +24 h after acquisition. In parallel, when compared to a 5×US protocol, the 2×US protocol produced a reduced freezing response at +24 h, and strongly reduced freezing at +7 d. Local application of D1 receptor agonist to vH at +2 h after acquisition of 2×US fear conditioning produced a sustained enhancement of vH high-PV contents, and freezing to context at +24 h and at +7 d was now undistinguishable to fear memory upon a 5×US protocol (Fig. 5A; Karunakaran et al. 2016). These and related results provided evidence that D1/5 receptor signaling during the +0–5 h time window after acquisition modulates the strength of subsequent PV plasticity and fear memories.

In a second approach, we pharmacogenetically imposed a low-PV shift (i.e., plasticity of opposite sign to that induced by learning) at +3 h after cFC specifically in vH PV neurons. Although memory recalled at +6.5 h was not affected, the procedure led to suppression of freezing to context (i.e., of any behaviorally detectable memory) at +24 h, and up to at least 7 wk. Likewise, when high-PV plasticity was imposed pharmacogenetically at +3 h after water maze learning (a learning processes inducing low-PV plasticity), no learning-related cFos expression was detected at +15 h, and no learning was detected behaviorally on the subsequent day (Fig. 5B,C).

Therefore, although alterations in PV levels only become detectable 6 h after acquisition, PV plasticity is already modulated during the first 5 h after acquisition through endogenous D1/5 receptor signaling, providing a potential mechanism through which events occurring

subsequent to the initial acquisition process might influence long-term memory consolidation processes at +12–14 h. Notably, interventions that specifically suppressed detectable learning-induced PV plasticity from +6 h on did not affect intermediate memory tested, for example, at +6.5 h. These results suggest that requirements for consolidation of short- and intermediate-term memories differ from those for long-term memories.

TWELVE-HOUR TIME WINDOW FOR PV PLASTICITY-DEPENDENT MEMORY CONSOLIDATION

In good agreement with reports that D1R signaling 12 h after acquisition is important for long-term consolidation and strengthening of fear memories, we found that PV neuron plasticity sustained during a +11–15 h time window is critically important for enhanced ripple densities, for the cFos peak at +15 h, and for long-term memory consolidation, causally tying the PV plasticity to the occurrence of key offline network and cell assembly processes involved in memory consolidation (Fig. 6; Karunakaran et al. 2016). We further found that local D1/5 receptor signaling at +12–14 h is specifically required in PV neurons to ensure maintenance of learning-induced PV plasticity critically important for long-term memory consolidation (Fig. 6A). Incidental learning (e.g., object recognition) not involving reinforcement but involving a second peak of cFos expression at +12 h did not show PV neuron plasticity and did not depend on D1R signaling at +12–14 h for long-term memory consolidation, suggesting that a requirement for local D1/5 receptor signaling at +12–14 h is a shared feature of reinforced learning involving PV plasticity.

Mechanistically, PV basket cell recruitment enhances network activity through synchronization of principal neurons to support coordinated fast network activities such as ripples, spindles, and γ -range oscillations (Stark et al. 2014), which are critically important for long-term memory consolidation (Buzsáki 2015). The circuit mechanisms through which opposite-sign low- and high-PV

Figure 6. Modulation of PV plasticity, ripple activity, cFos expression, and long-term memory during late time window at +11–15 h after acquisition of cFC. (A) Rapid loss of high-PV neuron plasticity and loss of fear memory upon local delivery of D1/5R antagonist to vH 12 h after cFC. PV neuron analysis in vH at different time points upon cFC. (B,C) Time course of cFos expression (B) and sharp wave ripple induction (C) in vH upon cFC. (D) Enhanced ripple density at +12 h after cFC and cFos expression at +15 h after cFC are suppressed upon pharmacogenetic induction of low-PV plasticity (i.e., opposite sign to cFC) at +4 h (two left panels) or local delivery of D1/5R antagonist at +11 h (two right panels). All treatments and analyses were performed in vH. (Adapted from Karunakaran et al. 2016.)

plasticity specifically ensure long-term consolidation of provisional versus definite memories remain to be determined, but one plausible hypothesis involves distinct local networks and related neuronal assemblies in learning involving low- or high-PV plasticity (Fig. 3).

Why might long-term memory consolidation depend on organized network activity and cellular plasticity induced about 12 h after acquisition, long after known biochemical and cellular processes involved in memory consolidation (Redondo and Morris 2011) have subsided? It is possible that slow biochemical processes affecting synaptic rearrangement processes involved in memory might require such a long time, but such a process would seem to put long-term memories at risk of interference through unrelated network activity. A further possibility could have been that the late time window might coincide with network processes during sleep, a brain state known to have

an important role in memory consolidation, but direct testing of this possibility suggested that the time window occurred at the same delay from the original learning process, irrespective of the time of the day or night at which it had occurred (Karunakaran et al. 2016). Instead, we propose that the late time window might serve to separate learning processes occurring on any given day from the long-lasting consequences of the learning for the animal's behavior (which includes integration with previous learning and memory processes). Such a view is consistent with our recent findings that the essential role of infralimbic cortex in subsequent alternative learning (e.g., fear extinction learning) is set up during a +11–14 h time window after fear learning through circuit and systems interactions involving specific reciprocal connectivity between prelimbic and infralimbic cortex (Mukherjee and Caroni 2018).

Figure 7. Dynamics of dH PV plasticity upon recall of cFC. All data: recall 3 d after 5US cFC; analysis in CA3b. (A–E) Analysis at +6, +12, and +24 h after recall (A), recall +1US (B), recall +5US (C), 30-min recall (extinction) (D), and 5-min recall followed 1 h later by 30-min recall (erasure) (E).

Additional findings from our laboratory focusing on the dynamics of PV neuron plasticity in area CA3 of dH upon recall of fear memory under different experimental conditions provide further support for a role of the +11–14 h long-term consolidation window in consolidating behaviorally adaptive memories. Recall of fear memory (i.e., exposure to conditioned context in the absence of foot shocks) 3 d after cFC induced low-PV plasticity at +6 h, which changed to baseline-like values at +12 h, and to high-PV plasticity at +24 h (Fig. 7A). Interfering with the PV plasticity at +12 h prevented detectable high-PV plasticity at +24 h and produced a permanent loss of the fear memory, suggesting that the late shift to high-PV plasticity was necessary for reconsolidation of the fear memory. When recall was paired to 1US instead of the 5US during fear conditioning, the low-PV plasticity persisted through the 12-h time window and had become more robust at +24 h (Fig. 7B). In contrast, if recall was paired to 5US, high-PV plasticity was induced at +6 h, and the plasticity lasted at least until +24 h (Fig. 7C). These and additional findings suggest that experience deviating from expectations (no US or only 1US instead of 5US) initially induces low-PV plasticity, which is followed by a reassessment of the new learning to either reconsolidate the original fear learning (no US), or to prepare for possible further learning (1US). In further related experiments, and extinction protocol (30 min of recall in the absence of US) induced low-PV plasticity at +6 h, which persisted through the +12-h time window (Fig. 7D). In stark contrast, an erasure protocol (5 min recall followed 1 h later by a 30-min extinction protocol) induced high-PV plasticity at +6 h, which persisted up to and beyond +24 h (Fig. 7E). Within the context of learning-related PV plasticity, extinction represents a transient adjustment to absence of US (i.e., provisional learning), whereas erasure leads to a permanent loss of the association between context and fear (i.e., definite learning).

Taken together, these findings provide evidence that PV neuron plasticity induced upon experience, and detected at +6 h, can be subsequently modified without further

behavioral experience during the +11–15-h time window of long-term memory consolidation, leading to PV neuron plasticity that reflects the long-term behavioral consequences of the experience. With other words, and consistent with our hypothesis, the late time window for memory consolidation appears to reflect a time when local plasticity is readjusted through offline network activity to match the corresponding long-term memory.

CONCLUSION

PV basket cells, the most abundant local inhibitory interneurons in the brain, show dramatic cellular and structural synaptic plasticity upon reinforced learning. The plasticity comes in two flavors: one (“high-PV”) that leads to increased expression of PV neuron functional markers such as PV and GAD67 (Volman et al. 2011; Lazarus et al. 2015) and increased densities of excitatory synaptic puncta onto PV neurons, and one (“low-PV”) that leads to decreased expression of PV/GAD67 and increased densities of inhibitory synaptic puncta onto PV neurons. High-PV plasticity is implemented by early-born PV neurons, whose plasticity is induced by changes in PV neuron excitation, whereas low-PV plasticity is implemented by late-born PV neurons, whose plasticity is induced by changes in PV neuron inhibition. Low-PV plasticity is specifically induced upon provisional reinforced learning, whereas high-PV plasticity is specifically induced upon definite reinforced learning. PV neuron plasticity becomes detectable at the end of time units for learning, once experience and signaling collected during 5–6 h upon initial acquisition determine whether and what will be learned. Notably, the PV plasticity is critically important for long-term consolidation of reinforced memories during a time window +11–15 h after acquisition. The mechanisms through which PV plasticity ensure long-term memory consolidation appear to involve support of offline network activity specifically required for enhanced ripple activity

and immediate early gene expression in neuronal assemblies thought to encode the corresponding memories (Singer and Frank 2009; van de Ven et al. 2016; Ognjanovski et al. 2017; Rothschild et al. 2017; Xia et al. 2017). Long-term consolidation of nonreinforced (incidental) memories also depends on a time window around +12 h, when cFos in corresponding assemblies needs to be re-expressed, but the memories do not depend on local D1R signaling and PV neuron plasticity for long-term consolidation. These findings suggest the existence of fundamental differences between reinforced and nonreinforced memories and their consolidation, possibly reflecting the more consequential impact of reinforced memories for behavior.

Enhanced functionality of early-born PV neuron networks upon definite learning might promote consolidation of strong memories within and between brain systems. Such a mechanism might account for the enhanced γ -phase coupling between entorhinal cortex and hippocampal CA1 that was detected during late phases of hippocampal learning (Igarashi et al. 2014). Selective connectivity to and from specific functional subpopulations of principal neurons might ensure specific stabilization of neuronal assemblies encoding definite learning memories. Likewise, reduced functionality of late-born PV neuron networks might be permissive to bind assemblies of more flexible and possibly less strongly interconnected neuronal assemblies. This form of plasticity appears to be the counterpart of critical period plasticity and of how EE supports learning and brain function (Pizzorusso et al. 2002). Like for definite learning assemblies, provisional memory assemblies likely involve dedicated subpopulations of principal neurons and specific connectivity to and from early-born PV neurons.

These findings raise a number of questions for further research. One of them involves the likely prospect that provisional and definite learning might be implemented through partially separate circuits within and possibly also between brain areas. Identifying the subpopulations of neurons and the connectivity principles involved in such functional subcircuits will likely provide fundamental insights into how the brain deals with coexisting variability and stability in learning and memory (Grosmark and Buzsáki 2016; Clopath et al. 2017). A second important question involves the systems mechanisms for memory consolidation during the late time window (+11–15 h) after acquisition. Elucidating principles of how previous knowledge might be integrated with recent memories during offline network activity to support adaptive learning and behavior will not only provide important advances into mechanisms of learning and memory, but might also provide valuable entry points to begin to investigate mechanisms of long-term systems consolidation in the brain. Finally, it might be useful to consider the possibility that long-term cellular plasticity involved in learning and memory might not be exclusive to PV basket cells.

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