change in α-catenin, the Mena/VASP-independent role of vinculin in actin filament alignment at AJs [1], and the effects of actin stabilization on the supramolecular organization of cadherin–catenin complexes. Pushing outward, it will be interesting to determine whether different cell types use different mechanisms to achieve the same end, while exploring levels of baseline tension on AJs and differing actin architectures in cells in different tissues and in different cultured cell lines (Figure 1B). The role of tricellular junctions is also a topic for further exploration. Furthermore, cells in tissues also need to contend with force generated at basal focal adhesions, and the balance between this force and the AJ forces will be important to consider. Finally, it will be exciting to take these new insights *in vivo*, exploring the roles of vinculin in morphogenesis and examining events where Ena/VASP proteins are already known to influence morphogenesis, such as dorsal closure in *Drosophila* [17], and investigating how cells accommodate differences in tension across tissues [18] (Figure 1C) or, in a planar-polarized way, within individual cells (for example, [19]).

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Neural Circuits: Interacting Interneurons Regulate Fear Learning

A recent study has found that, during associative fear learning, different sensory stimuli activate subsets of inhibitory interneurons in distinct ways to dynamically regulate glutamatergic neural activity and behavioral memory formation.

Takaaki Ozawa and Joshua P. Johansen

Aversive experiences are powerful triggers for memory formation and adaptively change our behavior. For some individuals, however, aversive learning becomes excessive leading to anxiety disorders such as post-traumatic stress disorder, which has a lifetime risk of 7–8% in US citizens and even higher (14–16%) in soldiers with combat experience [1]. Auditory fear conditioning is a powerful model for investigating the neural circuits of aversive learning and possibly for understanding pathological anxiety disorders: during auditory fear conditioning, animals learn that an auditory tone (conditioned stimulus, CS) predicts the occurrence of an aversive outcome such as a mild electrical shock (unconditioned stimulus, US) [2–6] (Figure 1A). Excitatory glutamatergic neurons in a brain region called the amygdala are known to store fear memories and contact other regions to produce fear responses. There are, however, other cell types within the amygdala, the γ-aminobutyric acid (GABA)ergic interneurons, which can inhibit neural communication locally and modulate the function of glutamatergic neurons.

It was not clear from previous research how the coordinated activity of these different intermixed cell populations in the amygdala participated in fear memory formation. Wolff *et al.* [7] addressed this question by taking advantage of a combination of techniques including optogenetics, *in vivo* recordings of single cell electrical activity, and behavioral

analyses. Their results show that the interplay of two different types of interneurons, those expressing either parvalbumin (PV) or somatostatin (SOM) proteins, regulates glutamatergic neurons to fine tune the fear system and control the strength of fear memory formation.

Memory storage mediating fear conditioning occurs in a subnucleus of the amygdala called the lateral nucleus, and possibly in the basal nucleus (we shall refer to these together below as the basolateral amygdala). During fear conditioning synaptic connections between the auditory system and basolateral amygdala neurons are strengthened when the glutamatergic neurons are strongly activated by the aversive US [2–6,8,9]. The basolateral amygdala also contains PV+ and SOM+ GABAergic inhibitory interneurons, which can control the activity of glutamatergic neurons [10–13]: PV+ cells provide perisomatic inhibition of pyramidal neurons; and SOM+ cells inhibit distal dendrites of pyramidal neurons. In addition to their inhibitory connections with glutamatergic neurons, GABAergic cells also contact other interneurons in the basolateral amygdala [10–13], providing a possible mechanism to disinhibit principal neurons [14].

Related to this, recent work [15,16] on other neural systems has found that inhibition of interneurons by salient experiences disinhibits glutamatergic output neurons and facilitates learning or adaptive behavior. Interestingly, distinct subtypes of basolateral amygdala interneurons can be differentially activated by the same stimulus, with some showing inhibitory and others excitatory responses to an aversive outcome [17]. This suggests that the activity of specific types of interneurons in the basolateral amygdala can be regulated in different ways by the same sensory experience. But how do these different neuronal populations interact in the local basolateral amygdala circuit during fear conditioning and what effect does this have on memory formation?

Wolff *et al.* [7] directly addressed this question by studying how different interneuron subtypes in the basolateral amygdala regulate activity of pyramidal neurons to control the acquisition of fear learning. They used an optogenetic approach and expressed light responsive proteins (opsins) in genetically distinct cell populations to control the activity of these cells with light [18,19]. Cells expressing channelrhodopsin are excited by blue light and cells expressing archaerhodopsin are inhibited by yellow light: Wolff *et al.* [7] used a virus vector to express channelrhodopsin or archaerhodopsin in either PV+ or SOM+ interneurons. They were then

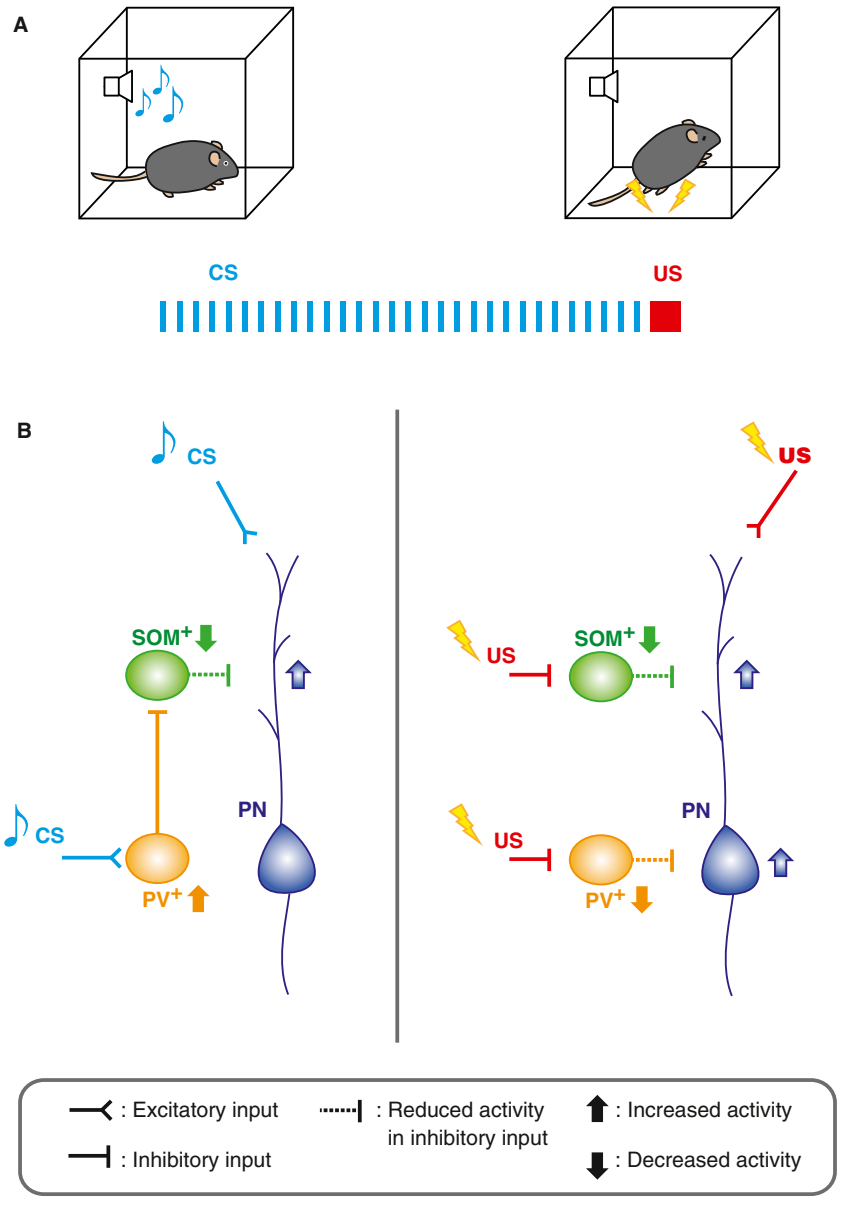

Figure 1. Working model of how amygdala interneurons interact to regulate glutamatergic cell activity and fear learning.

(A) During fear conditioning the auditory CS (blue bars) is presented and this is followed closely by the occurrence of the footshock US (red square). (B) According to this interpretative model, microcircuit interactions within the lateral and basal nucleus of the amygdala (basolateral amygdala) are coordinated in specific ways depending on whether the CS or US are present. During the auditory CS period (B, left panel), parvalbumin expressing interneurons (PV+, which provide perisomatic inhibition of pyramidal neurons) are activated by the CS. This then inhibits somatostatin expressing interneurons (SOM+, which inhibit the distal dendrites of pyramidal neurons) thereby disinhibiting the processing of excitatory auditory inputs into the distal dendrites of glutamatergic principal (PN) neurons. In contrast, during the aversive US period (B, right panel), both PV+ and SOM+ interneurons are inhibited, resulting in general disinhibition of excitatory auditory inputs onto both principal cell bodies and dendrites.

able to optogenetically identify opsin expressing cells as PV+ or SOM+ interneurons based on their light responsiveness, and then examine their neural coding properties. They found that both PV+ and SOM+ cells were inhibited by shock USs (Figure 1B, right panel). The authors then optogenetically manipulated PV+ cells to examine their functional contribution to behavior and to pyramidal cell coding in basolateral amygdala neurons. They found that overriding the shock evoked inhibition in these cells by blue light/channelrhodpsin-mediated excitation during the shock period reduced both shock responses of pyramidal neurons and fear learning. In contrast, yellow light-induced inhibition of PV+ cells enhanced shock processing in pyramidal neurons and enhanced fear learning.

These results demonstrate that aversive somatosensory USs generally inhibit both basolateral amygdala PV+ and SOM+ interneurons to enhance aversive responses in glutamatergic principal neurons (Figure 1B, right panel). This mechanism appears to be important for behavioral fear conditioning, as excitation or inhibition of PV+ cell activity during the US period bidirectionally modulates fear learning. Previous work has demonstrated that strong activation of glutamatergic cells during aversive shocks is an important trigger for potentiation of auditory inputs onto lateral nucleus of the amygdala neurons and fear learning [8,9]. This uniform disinhibition of both perisomatic and dendritic regions of glutamatergic cells by aversive USs provides a possible mechanism to modulate aversive activation of these neurons to dynamically regulate basolateral amygdala neural plasticity and fear memory strength.

In contrast to the uniform inhibition produced by the aversive US, Wolff *et al.* [7] found that the PV+ neurons were activated, and SOM+ cells inhibited, by the auditory CS (Figure 1B, left panel). Surprisingly, in behavioral optogenetic experiments, they also discovered that activation of PV+ neurons during the CS period facilitated fear conditioning, while the inhibition of these cells impaired learning. In contrast, they found that optogenetic inhibition of SOM+ neurons during the CS period facilitated learning, and that optogenetic activation during the CS impaired fear conditioning. To examine the reasons for the opposing effects of auditory CS and aversive US induced PV+ cell activity on learning, the authors focused on a potential inhibitory interaction between PV+ and SOM+ interneurons. In slices of excised amygdala tissue, they showed that optogenetic activation of PV+ cells inhibited SOM+ cells, and that activation of SOM+ cells inhibited spontaneous and putative thalamic-input-evoked activation of principle neurons. Moreover, by recording from single amygdala cells in the behaving animal they demonstrated that activation of PV+ cells amplified CS-evoked responses in glutamatergic neurons while activation of SOM+ cells completely suppressed these responses.

These results suggest that, during learning, auditory CSs activate PV+ interneurons to inhibit SOM+ cells, thereby disinhibiting glutamatergic neurons and facilitating fear memory formation (Figure 1B, left panel). PV+ cells preferentially innervate the perisomatic region of glutamatergic neurons and SOM+ cells synapse primarily in glutamatergic neuron dendritic arbors [10–13]. By preferentially disinhibiting dendritic processing during the CS period, this mechanism could select which auditory inputs undergo plasticity in a branch-specific way, thereby enhancing input specificity. In addition, this process could be used to lengthen the duration of CS-induced intracellular signaling in dendrites and thereby broaden the time window over which auditory CSs can be associated with aversive USs.

An intriguing question is who is the conductor orchestrating this microcircuit symphony in the basolateral amygdala? The simple explanation is that auditory CSs and aversive USs recruit different inputs to the basolateral amygdala and/or distinct local amygdala microcircuits to regulate PV+ and SOM+ cell activity in specific ways. Neuromodulators including dopamine, noradrenaline and serotonin are known to modulate GABAergic networks and thereby gate induction of synaptic plasticity in basolateral amygdala [3,11]. Another possibility is that recruitment of specific neuromodulatory systems in basolateral amygdala control how different inhibitory neurons respond to distinct sensory stimuli. Understanding how interneuron activity is modulated in response to different sensory stimuli will be an important future research direction.

While Wolff *et al.* [7] examined how auditory CSs and aversive USs activate basolateral amygdala interneurons, they did not determine whether activity in these cells changes with learning. This is a critical question as it is clear that both auditory CS and aversive US responses are dynamically altered by fear conditioning. Auditory responses are gradually enhanced in ~20% of putative glutamatergic cells and this is thought to reflect synaptic plasticity within the lateral nucleus of the amygdala [2,3]. In contrast, aversive US responses gradually decrease in a subpopulation of basolateral amygdala neurons as the US becomes predicted during learning [20]. This experience-dependent reduction in aversive US processing may regulate the amount of fear learning that occurs at a given aversive US intensity. Fear learning-induced changes in basolateral amygdala interneuron coding could participate in regulating concomitant changes in auditory CS processing or aversive US processing. This could affect many processes including the specificity and strength of fear memories. It will be important in future work to examine these questions to better understand how normal fear learning occurs and possibly to gain insights into how these systems may be disrupted in disease states such as post-traumatic stress disorder.

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Limited Proteolysis: DisRUPting Proteasomal Inhibition

The 26S proteasome is a protease complex that completely degrades substrate proteins marked with a chain of ubiquitins, but is also able to perform endoproteolytic cleavage. A new study now demonstrates that regulated ubiquitin–proteasome-dependent processing ameliorates proteasomal inhibition.

Thorsten Hoppe

The proteasome is essential for ubiquitin-mediated degradation of damaged or regulatory proteins [1]. Its 20S core particle is a cylinder formed by four seven-subunit rings that contains the proteolytic sites. The unfolding and translocation of ubiquitylated substrates into the hollow 20S particle is catalyzed by two 19S cap structures, one sitting over each end of the cylinder [2]. This 'self-compartmentalized' architecture of the 26S proteasome ensures the highly selective nature of the ubiquitin–proteasome system; only ubiquitylated proteins are allowed to reach the active sites within the inner 20S core and get completely cleaved into small peptides [3].

It has been shown recently that the proteasome does not always fully degrade substrate proteins, with endoproteolytic cleavage by the proteasome sometimes initiating processing of dormant precursor proteins. These processing events might arise from internal cleavage of polypeptide loops that enter the 20S core particle of the proteasome [4]. Such an endoproteolytic cut could be a general mechanism for proteasomal degradation, since substrate turnover is often associated with the appearance of cleavage products [5]. These by-products are usually degraded with a different half-life compared with the particular full-length proteins. However, the specific topology of protein domains and/or protective binding partners can prevent proteasomal degradation of certain biologically active protein fragments.

Examples of substrates of this regulatory proteasomal cleavage mechanism — termed regulated ubiquitin–proteasome-dependent processing (RUP) — are the transcription factors NF-κB, Spt23p and Mga2p [6,7]. NF-κB governs immune and inflammatory responses linked to apoptosis and cancer. Ubiquitylation of its inactive precursor, p105, results in proteasomal degradation of the carboxy-terminal region and release of the stable p50 subunit of NF-κB [6]. Spt23p and Mga2p are distant NF-κB homologues that control fatty acid metabolism in the budding yeast *Saccharomyces cerevisiae*. Similar to NF-κB, both are produced as dormant precursors (p120) but are anchored to the endoplasmic reticulum (ER). Intriguingly, processing of the p120 forms depends on the composition of the ER membrane and liberates the active amino-terminal transcription factor domain p90 when unsaturated fatty acids are needed [7].

These data provide evidence that both conformational and functional properties of the precursor proteins influence the efficiency of RUP. Conversely, little is known about the impact of the proteasome in the coordination of this unconventional cleavage process with physiological demands. A new study by the Goldberg laboratory, published in a recent issue of *Current Biology*, now highlights a fascinating role of RUP that links processing of the transcription factor Nrf1 to proteasomal activity and vice versa [8].

In this new work, the authors addressed the previously reported, counterintuitive observation that pharmacological inhibition of the 26S proteasome often results in enhanced proteasomal activity [9]. Aside from its use as a tool to reveal cellular functions, the proteasomal inhibitor bortezomib (BTZ) is successfully used in the treatment of multiple myeloma, a cancer of antibody-generating plasma cells [10]. However, increased expression of 26S subunits in response to BTZ treatment provides