et al., 2014) may provide important insights into how precisely OA and R47A04^{aSP2} neurons influence the dynamic movements and sensorimotor transformations that comprise fly social interactions.

Previous studies of OA and aggression in *Drosophila* were all consistent with OA (like its norepinephrine analog) broadly regulating arousal, and thereby having an indirect effect on aggression. By combining careful behavioral analysis with sophisticated genetic and neural circuit manipulations, the study from Watanabe et al. now reveals that OA can modulate specific networks that control social behaviors, biasing the output of the network in favor of driving aggression. It is tempting to speculate that similar mechanisms might underlie the effects of norepinephrine on social behaviors in larger brains, including those of humans.

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Synapse-Specific Encoding of Fear Memory in the Amygdala

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Input specificity is a fundamental property of long-term potentiation (LTP), but it is not known if learning is mediated by synapse-specific plasticity. Kim and Cho (2017) now show that fear conditioning is mediated by synapse-specific LTP in the amygdala, allowing animals to discriminate stimuli that predict threat from those that do not.

In order to survive, animals must be able to discriminate dangerous stimuli from those that are safe. That is, when confronted with an aversive and potentially lethal event, animals must learn the specific stimuli in the environment that predict danger so that they can mobilize adaptive defensive responses to those stimuli in the future. Without a mechanism for learning specific stimulus-outcome relationships, fear and defensive behavior broadly generalize to many stimuli and settings. This is a maladaptive state of affairs that may underlie fear and anxiety disorders.

Pavlovian conditioning is a fundamental form of learning that permits animals to encode specific stimulus-outcome associations and produce adaptive behavior in anticipation of those outcomes. For example, during fear conditioning, an innocuous stimulus, such as an acoustic tone (i.e., the conditioned stimulus [CS]), that has come to predict an aversive outcome, such as an electric shock (i.e., the unconditioned stimulus [US]), produces a host of defensive responses, including freezing behavior. Importantly, animals will readily learn to discriminate a CS (e.g., a CS+) that predicts the US from one that does not (e.g., a CS−).

Decades of work have now revealed the neural circuits underlying Pavlovian fear conditioning (Herry and Johansen, 2014). Sensory information from many brain areas, particularly the thalamus, hippocampus, and cortex, converge in the basolateral complex of the amygdala (BLA; including the lateral, basolateral, and basomedial nuclei). Considerable work indicates that long-term potentiation (LTP) at synapses transmitting CS information to the BLA underlies fear conditioning. That is, the ability of the once neutral CS to generate defensive behavior is mediated by an LTP-mediated increase in synaptic transmission onto BLA principal neurons (Bocchio et al., 2017).

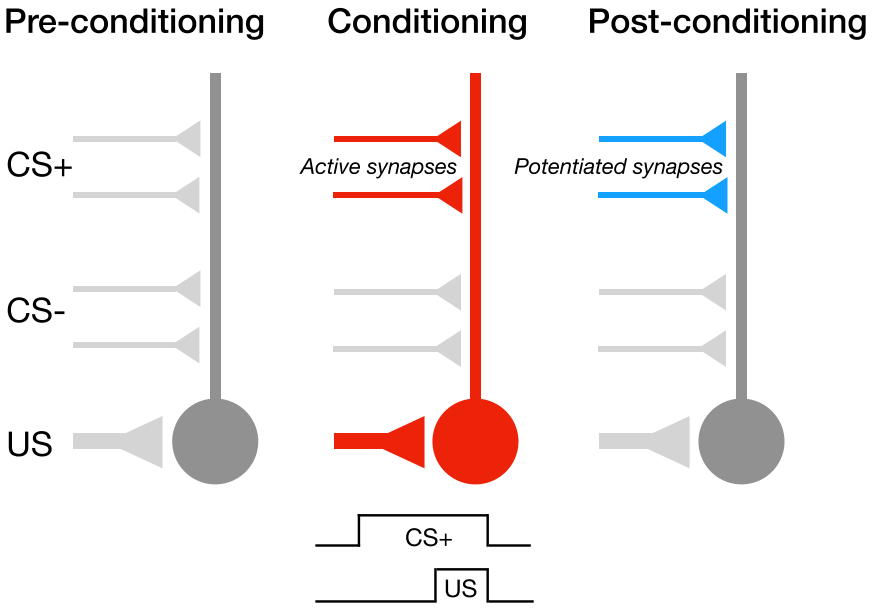

Figure 1. Input-Specific LTP in the Lateral Amygdala during Discriminative Fear Conditioning

Prior to fear conditioning, sensory afferents carrying information concerning conditional stimuli (CS+, CS−) from the auditory cortex (ACx) and medial geniculate nucleus (MGN) converge on principal neurons in the lateral amygdala (LA). During conditioning, coincident pre- and post-synaptic activity (red, active synapses and post-synaptic neurons) leads to input-specific long-term potentiation (LTP) at auditory afferents carrying CS+, but not CS−, information. Potentiated CS+ synapses (blue) allow the formerly innocuous CS to drive fear responses by depolarizing LA principal neurons.

A fundamental property of LTP is that it is only induced at synapses at which there is correlated pre- and post-synaptic activity (e.g., presynaptic neurotransmitter release coupled with postsynaptic depolarization). It has been argued that the "input specificity" of LTP might serve as a putative mechanism for the selectivity of conditioned fear responses to CSs that predict danger over those that do not (Shin et al., 2006). However, despite decades of work on the relationship between LTP and learning, it is not known whether learning in behaving animals is associated with input-specific LTP.

To address this question, Kim and Cho (2017) used an elegant behavioral labeling approach to identify and characterize plasticity at synaptic inputs into the lateral amygdala (LA) after discriminative auditory fear conditioning. To this end, they injected Fos-CreER^{T2} knockin mice with a Cre-dependent adeno-associated virus (AAV-DIO-eYFP) in the auditory cortex (ACx) or medial geniculate nucleus (MGN) of the thalamus, the primary auditory afferents of the LA. One week later, they exposed the animals to discrete auditory stimuli (4 or 12 kHz pure tones) after tamoxifen administration to label tone-responsive neurons in the ACx and MGN. Tone exposure recruited substantial numbers of eYFP-positive neurons in the ACx and MGN, and a substantial proportion (~12%–15%) of those neurons were found to project to LA. Moreover, different populations of ACx/MGN neurons were recruited by tones of different frequencies, revealing frequency-dependent labeled lines projecting to the LA.

After verifying the behavioral labeling approach, Kim and Cho (2017) next characterized the synaptic function of the labeled auditory inputs to the LA in acute brain slices. For these experiments, they used AAV constructs expressing channelrhodopsins (Chrimson or ChR2) injected into ACx or MGN, respectively, to functionally label auditory inputs into LA. Using whole-cell patch recordings, they found that optical stimulation in either pathway evoked monosynaptic glutamatergic excitatory post-synaptic potentials (EPSPs) in a subset of LA neurons (~36%).

With the ability to functionally label discrete auditory inputs to LA neurons, the authors could next address the penultimate question of whether auditory fear conditioning produces input-specific synaptic plasticity the LA. Prior to behavioral training, CS+-responsive neurons in the ACx/MGN were functionally labeled. After 3 weeks, mice were then trained with a conditioning procedure that yielded a reliable behavioral discrimination with animals freezing at high levels to a CS+ paired with the US, but not to a CS− that was presented alone (without the US). After conditioning, the animals were sacrificed and post- and pre-synaptic indices of synaptic plasticity in CS+ afferents to the LA in conditioned and non-shocked mice were assessed. Critically, Kim and Cho (2017) found that AMPA/NMDA ratios, a postsynaptic correlate of potentiated glutamatergic transmission, were increased in CS+ (but not CS−) afferents to LA neurons. Moreover, increases in AMPA/NMDA ratios were specific to CS+ inputs and were not expressed in other auditory afferents to the LA. None of the experiments revealed changes in presynaptic indices of synaptic efficacy. Interestingly, these changes in synaptic efficacy were most pronounced in ACx inputs to the LA. Ultimately, these experiments reveal for the first time that discriminative auditory fear conditioning induces input-specific LTP in CS+ afferents to the LA (Figure 1).

To further explore the mechanisms underlying learning-induced LTP in the LA, Kim and Cho (2017) sought to determine whether increases in AMPA/NMDA ratios in CS+ afferents were restricted to post-synaptic LA neurons activated by fear conditioning as predicted by the Hebbian requirement for LTP induction. To this end, they used a dual behavioral labeling approach using a crossed FosCre^{T2}/ROSA-LSL-tdTomato mouse injected with AAV-DIO-CHr2-eYFP in ACx/MGN. Using this approach, CS+ terminals were first functionally labeled (CHr2-eYFP) during tone presentations after tamoxifen administration. Two weeks later the animals underwent fear conditioning with several conditioning trials (CS+ paired with US) to label LA neurons (tdTomato) activated by fear conditioning. After 4 days, the animals received discriminative conditioning and were sacrificed thereafter. As predicted by Hebbian models, AMPA/NMDA ratios in CS+

afferents (expressing ChR2) were only elevated in recordings from tdTomato-positive LA neurons labeled by fear conditioning, but not in non-labeled neurons. Again, increases in AMPA/NMDA ratios recorded from LA neurons active during conditioning were specific to CS+ inputs, because randomly selected ACx/MGN afferents did not exhibit changes in AMPA/NMDA ratios in either labeled or non-labeled LA neurons.

After fear conditioning, presenting CSs in the absence of the US produces an extinction of learned fear. There has been considerable debate over the underlying synaptic mechanisms responsible for fear extinction (Maren, 2015). One possibility is that extinction learning leads to a depotentiation of synapses modified by conditioning. However, the propensity of extinguished fear to return under a number of conditions (Maren et al., 2013) suggests that conditioning-induced synaptic changes must persist after extinction. To examine this possibility, Kim and Cho (2017) examined the consequences of fear extinction on AMPA/NMDA ratios in CS+ afferents to the LA. Interestingly, they found that extinction did not reduce AMPA/NMDA ratios at these synapses, suggesting that conditioning-induced potentiation of ACx/MGN synaptic transmission maintains CS-US associations even after extinction.

Of course, the ultimate question is whether input-specific potentiation of auditory synapses in the LA is necessary for CS-elicited freezing behavior after fear conditioning. To address this question, Kim and Cho (2017) explored whether dampening synaptic potentials at CS afferents to the LA would reduce conditional freezing. First, they demonstrated that low-frequency photostimulation of CS afferents led to a long-term depression of synaptic transmission in LA neurons in brain slices. Next, they implanted mice with optical cannula to deliver photostimulation to behaviorally labeled CS+ axons in the LA after fear conditioning. Importantly, low-frequency photostimulation of the LA after fear conditioning produced a highly significant decrease in CS-elicited freezing behavior. Importantly, photostimulation did not affect freezing to the CS+ when the photostimulation was delivered to CS− afferents indicating that the reduction in freezing was not a non-specific effect of LA photostimulation.

Collectively, this impressive array of experiments provides strong evidence that input-specific LTP in the LA underlies long-term memories for Pavlovian fear conditioning. Whereas previous studies have shown that fear conditioning induces LTP and concomitant changes in AMPA receptor expression in the amygdala (Rumpel et al., 2005), the study by Kim and Cho (2017) is the first to demonstrate that these changes exhibit synaptic specificity. As such, this work reveals that synaptic plasticity rules that are fundamental to LTP induction are realized in behaving animals during learning. This adds to the long list of studies suggesting that LTP underlies at least some forms of learning and memory.

Yet there are clearly limits to the number of learning phenomena that can be accounted for by activity-dependent changes in excitatory synaptic transmission. As discussed earlier, extinction learning produces dramatic decreases in freezing behavior but does not alter glutamatergic transmission at auditory afferents in the LA. Of course, extinction might be mediated by synaptic plasticity at glutamatergic synapses on inhibitory neurons that in turn inhibit LA principal cells (Trouche et al., 2013). Other conditioning phenomena, however, are not as easily understood in terms of local synaptic plasticity. For example, during ''blocking'' a CS (CS_a) that has previously been paired with a US, when subsequently paired with a different CS (CS_b) on a reinforced trial (e.g., (CS_a + CS_b) − US), impedes conditioning to CS_b. This is not predicted from Hebbian LTP induction rules, which predict strong LTP induction at CS_b synapses (Sah et al., 2008). The failure of CS_b to condition is typically understood in terms of prediction error—because CS_a fully predicts the US, little is to be learned by an incidental stimulus (CS_b) that provides no new information about the US. Clearly, phenomena such as extinction and blocking are better understood in terms of circuit processes that allow computations of prediction error based on distributed synaptic weights to arrive at adaptive behavior (McNally et al., 2011).

Although a complete mechanistic account of learning will require an understanding of molecules, synapses, and circuits, the identification of input-specific synaptic potentiation in behaviorally labeled sensory afferents of amygdala neurons after fear conditioning is an important discovery. Indeed, the current behavioral labeling approaches promise to yield critical advances in understanding how learning-induced changes in synaptic weights within distributed neural circuits contribute to higher-order learning phenomena.

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