further characterize the co-localization of Ucn3$^+$ and NPY1R$^+$ INs in greater detail. It is also important to identify neurotransmitters or neuromodulators that can trigger mechanical itch in the circuits. Is the neuropeptide Urocortin-3 an itch mediator?

Notably, the TLR5$^+$-Ucn3$^+$ circuit may also converge with other itch pathways, including spontaneous itch and chronic itch. Pan et al. (2019) demonstrated that mechanical itch in histamine-induced alloknesis and several models of chronic itch are largely abolished in Ucn3$^+$ IN-ablated mice. Spontaneous itch in chronic models was also greatly attenuated after Ucn3$^+$ IN ablation. Furthermore, inhibition of TLR5$^+$-LTMR with intradermal flagellin/QX-314 greatly attenuated both histamine and calcipotriol induced alloknesis. Given a critical role of GRPR$^+$ INs in chemical itch, chronic itch, and spontaneous itch (Sun et al., 2009), it is important to know whether Ucn3$^+$ INs synapse on GRPR$^+$ neurons to mediate spontaneous itch. However, GRPR$^+$ neurons are not required for Y1R$^+$ IN-mediated mechanical itch (Acton et al., 2019).

Taken together, the mechanical itch pathway identified by Pan et al. (2019) provides a critical step forward in our understanding of the microcircuits responsible for distinct forms of itch. TLR5$^+$ LTMRs appear to mediate both mechanical allodynia (type I) and mechanical itch (type II) (Figure 1). It remains to be investigated whether NPY$^+$ and Y1$^+$ INs also receive inputs from TLR5$^+$ LTMRs. Merkel cells are touch receptors and regulate mechanical itch, and notably, alloknesis in aging and dry skin is associated with a loss of Merkel cells (Feng et al., 2018). It will be interesting to investigate the possibility of a functional link between Merkel cells and TLR5$^+$ Aβ-LTMRs in mechanical itch. Recently, Sakai and Akiyama (2019) reported that silencing TLR5$^+$ Aβ fibers with co-injection of flagellin and QX-314 could provoke mechanical itch. It is likely that Aβ-LTMRs could have both positive and negative regulations of mechanical itch, depending on whether the spinal cord inhibitory gate is open under the different physiological or pathological conditions.

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Are You There, Cortex? It's Me, Acetylcholine

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It is not well understood how associations between two temporally removed stimuli form. In this issue of *Neuron*, Guo et al. (2019) implicate basal forebrain cholinergic neurons as providing a link between auditory cues and the aversive outcomes they predict.

We decide between courses of action based on their expected outcomes. Yet, how we come to expect future events given predictive cues is not well understood; especially challenging to neuroscientists is how the brain connects two stimuli when they are temporally removed from each other. What areas are involved in learning these contingencies? Who is learning from whom? What are the resultant changes? In this issue of *Neuron*, Guo et al. (2019) uncover a means by

which the primary auditory cortex may learn from the cholinergic basal forebrain (BF) that two stimuli are associated with each other across time.

Primary sensory cortical areas are often considered low-level feature detectors merely representing components of the external world. However, through associative learning (i.e., instances in which an agent learns that one stimulus predicts another), responses within these areas can potently change. For instance, neurons within the primary visual cortex (V1) can represent the time between a visual stimulus and a water reward after learning (Hussain Shuler, 2016). Additionally, the tuning within primary auditory cortex (A1) has been shown to change when auditory cues are paired with salient outcomes (Weinberger, 2004). These results indicate that primary sensory areas can and do play important roles within associative learning and are integral to an animal's ability to learn about its environment. How might these cortical networks learn to update their representations? In particular, how might learning happen when the sensory events occur well before the predicted outcomes? While the BF cholinergic system has been implicated in providing such answers, few studies provide a mechanistic understanding of how changes are imparted to a cortical network (Hussain Shuler, 2016). In their comprehensive work, Guo et al. (2019) seek to define a role for BF cholinergic cells in an associative learning task.

While the majority of cells within its constituent subregions are not cholinergic, the collection of nuclei composing the BF is the major source of acetylcholine (ACh) for cortex (Mesulam et al., 1983). Seminal recordings within the nucleus basalis of Meynert (NBM, a BF nucleus with the most cholinergic corticopetal neurons; Mesulam et al., 1983) demonstrate that neurons in this structure strongly respond to salient outcomes, reinforce movements preceding these outcomes, and are able to influence neuronal excitability for prolonged periods of time (Richardson and DeLong, 1991). Such results gave rise to several hypotheses related to the role of ACh in learning. However, it has not been until recent technical advances that scientists have been able to target or identify cholinergic cells within an awake, behaving animal. With these techniques, researchers have discovered that cholinergic axons within cortex can engender timing activity (Liu et al., 2015), provide contextual information (Kuchibhotla et al., 2017), and affect ongoing cortical activity (Eggermann et al., 2014). Additionally, such advances have allowed for the disambiguation of BF responses from *cholinergic* BF responses in neural records. For example, using selective expression of the light-activated cation channel channelrhodopsin-2 (ChR2) within cholinergic cells, Hangya and colleagues were able to record from BF cholinergic cells and found that these cells were largely responsive to both rewarding (water) and aversive (air puff) outcomes (Hangya et al., 2015).

To fully understand how cholinergic cells within BF affect change, Guo and colleagues first determined whether cholinergic stimulation was sufficient to change A1 cortical responses to tones (Guo et al., 2019). On a given day, in mice that express ChR2 exclusively in cholinergic cells, an auditory cue was paired with activation of cholinergic axons within A1 at a delay of 0 or 5 s. Interestingly, neurons undergo plasticity and are more responsive to the paired cue after conditioning when the two stimuli co-terminate. However, if the two stimuli were separated by 5 s, then no plasticity is seen. Guo et al. (2019) next sought to determine whether such delayed reinforcement could affect change within A1 by pairing an electric shock with an auditory tone at this delay. Indeed, such conditioning does influence cortical activity as A1 expresses plasticity to the paired tone in a similar manner to what has been previously reported (Weinberger, 2004). It was then determined that ACh is necessary for this plasticity although activation of cholinergic fibers within A1 is insufficient. To better understand the basis of these seemingly disparate results, Guo et al. (2019) identified BF cholinergic neurons that specifically project to A1 and observed their activity during behavioral conditioning using a clever array of genetic and electrophysiological techniques.

Guo et al. (2019) first determined where A1-projecting neurons existed within the BF. To do so, they injected retrograde beads into cortex and observed that the majority of cholinergic projection neurons to A1 exist within the caudal portion of the BF. While recording in this subregion of the BF, they stimulated cholinergic axons within A1 and recorded antidromic spikes within the BF. To differentiate direct activation within recorded neurons, Guo et al. (2019) used time to the first spike and jitter across laser presentations to define a population of 20 cholinergic, A1-projecting BF neurons out of a total of 1,566 neurons. With these known identities, they then replicated the finding that these neurons respond to the reinforcing outcome (Hangya et al., 2015). Furthermore, they show that within the behavioral conditioning, these neurons show strong plasticity within the conditioning trials but little plasticity afterward, suggesting that ACh is important in the induction, but not maintenance, of neural plasticity (Hussain Shuler, 2016; Richardson and DeLong, 1988).

However, in the current task design, Guo et al. (2019) are unable to determine whether the increased cholinergic activation is due to a switch between active and passive listening (within a session, auditory cues are either paired or not paired with delayed reinforcement). Alternatively, the BF cholinergic signal could carry specific information related to the CS, but to test this hypothesis, animals would need to experience two conditioned stimuli: one paired with a delayed outcome and the other not. Unfortunately, such a task requires several days of conditioning, which would make it difficult to track the same population of neurons electrophysiologically.

To mitigate this issue, Guo et al. (2019) expressed the calcium indicator GCaMP6f in cholinergic cells and performed fiber photometry within the BF. Importantly, this allows the authors to record bulk calcium transients from the same population of cholinergic cells across many days. With probes implanted, Guo et al. (2019) trained animals across several conditioning sessions where one auditory stimulus predicted a delayed shock and one auditory stimulus did not while recording neural activity. In doing so, they found that after several conditioning sessions, the cholinergic response to the paired stimulus was larger than the response to the unpaired stimulus. Furthermore, they found that there was sustained cholinergic activity that subtended the trace interval.

The results of these experiments coalesce into a fascinating constellation and reveal a possible way for cortical neurons

to express an association between two events separated in time. Specifically, Guo et al. (2019) propose that cholinergic neurons support learning by providing information about both conditioned and unconditioned stimuli as well as providing ongoing activity spanning the time until the outcome. These observations raise several questions, especially related to the sustained activity within BF after days of conditioning. For example, how does the BF, itself, learn this association between the CS and US? Are cholinergic cells a substrate for learning, or are they inheriting information learned elsewhere in the brain? Additionally, fiber photometry allows researchers to measure population activity, but it does not offer insight as to what individual cells are doing. Is the sustained cholinergic tone the result of individual neurons remaining active across an interval, or are individual cells "tiling" the time interval? Furthermore, after these many days of conditioning, are neurons in A1 also subtending the interval (as would be predicted from studies in V1; Hussain Shuler, 2016)? If so, does the incoming cholinergic signal influence circuit members to allow for such plasticity? To answer these questions, future researchers should take inspiration from this current work and use multiple, cutting-edge techniques to gain insight into brain function.

Together, this comprehensive set of experiments advances cholinergic BF neurons as a crucial player in learning and expressing associations between cues and the behaviorally relevant events that they predict.

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Why Are Sequence Representations in Primary Motor Cortex So Elusive?

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In this issue of *Neuron*, Yokoi and Diedrichsen (2019) use a finger keyboard task to show that sequences are widely represented across cortex but that only single elements are represented in primary motor cortex. These results suggest that sequence tasks primarily probe the ability to order discreet actions rather than to execute a skilled continuous sequential action.

Whether anticipating the next word in a sentence, an opponent's upcoming move, or the timing of a green traffic light, we are constantly trying to predict and respond to what will happen next. Predicting future events enables quick responses—in some cases, even circumventing inherent sensory delays in order to initiate responses simultaneously with the upcoming event. This results in the tendency to treat sets of spatial and temporal events as ordered sequences, or to learn the likelihood of associations between two or more events, in order to make inferences about the future. Many daily actions are also organized into sequences, ranging from key presses when playing the piano to the routine of