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Previews

Tracing a Path for Memory in the Hippocampus

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The hippocampal activity supporting trace fear conditioning has long been mysterious, but a leading hypothesis posits "time-cell"-like sequential patterns. In this issue of *Neuron*, Ahmed et al. (2020) present new data suggesting that, at least during the first session of learning, a subset of neurons coalesce to selectively encode the task but without expressing reliable sequences.

Anyone who has had a mosquito in their tent knows the scenario. The silence of nighttime in nature is broken by the high-pitched whine of wings. It gets louder and louder, then it stops, and a moment later a sharp poke is felt on an exposed part of your face. How is it that we learn to associate the mosquito's bite, which is painful, with the sound of its wings? For nearly four decades, it has been known that making the connection between a "conditioned stimulus" (CS, the sound of the mosquito in our example) and an "unconditioned stimulus" (US, a subsequent positive or negative outcome) requires a functioning hippocampus if there is a delay or "trace" period between when the CS ends and the US starts. How the neurons in the hippocampus support this learning still remains mysterious. Using a task compatible with two-photon imaging, Ahmed and colleagues (Ahmed et al., 2020) in this issue of *Neuron* illuminate the structure and dynamics of the hippocampal network during trace fear conditioning.

In the broadest terms, learning, memory acquisition, and recall have historically been split into two categories. Declarative/explicit learning concerns the acquisition, processing, and recapitulation of discrete facts and experiences—knowledge that a mosquito's buzz predicts a bite that subsequently leads to an itchy spot ("semantic knowledge") or remembering the specific details of the experience of a particularly dense mosquito swarm (an "episodic memory"). Nondeclarative/implicit learning involves event-driven skill/habit acquisition (e.g., classical conditioning). The latter form of learning in the strictest sense does not involve the hippocampus; however, with even the slightest modifications (e.g., introducing any form of temporal relationship), the hippocampus becomes involved (Clark, 2011).

Much of what we know about the temporal dynamics of neural activity in area CA1 (the primary output of the hippocampus) comes from ensemble recordings during navigation. These experiments have revealed that as an animal behaves in an environment, individual neurons are active in allocentrically defined regions known as their place field. The result is that over the timescale of seconds, if one observes many neurons, one sees sequences of activity as an animal locomotes through their place fields (Joo and Frank, 2018). These sequences are quite sparse—in a given environment, typically a subpopulation of 20%–30% of excitatory projection neurons in CA1 will be active, and given the limited size of place fields, substantially fewer in any small time window. Different environments or spatial contexts evoke activity from distinct, randomly chosen subpopulations. One of the important discoveries of the last decade is that these same sequences can be observed in animals that are not locomoting but rather running in place in order to pass the time during a delay period ("time cells") (Eichenbaum, 2017). Place cell activity is one of the two important states that are observed in awake animals. The second state is observed during "quiet wakefulness," periods around reward consumption, grooming, etc. During this state, the sparse CA1 ensemble exhibits sharp-wave ripples (SWRs)—occasional ~50–150 ms bursts of dense activity during which many neurons are simultaneously active (Joo and Frank, 2018). During many SWRs, the neurons activate in a sequential order that recapitulates or "replays" the order in which they were activated during movement. Rapid, selective silencing of neural activity using closed-loop signal processing during these periods has demonstrated SWRs to be crucial to on-going learning (Joo and Frank, 2018). The field has concluded that structured, sequential activity in the hippocampus underlies spatial navigation, decision making, and more complex learning in a "cognitive map" (Pezzulo et al., 2017; Schiller et al., 2015).

In addition to trace fear conditioning, another simple learning paradigm has historically been used to study the cellular and molecular bases of memory. In contextual fear conditioning experiments, an aversive stimulus is delivered in a small chamber that is decorated with memorable sensory cues. When a rodent is subsequently exposed to this chamber, they exhibit their memory of the previous negative experience by freezing. Using remarkable technological developments in molecular biology, it has recently been shown that the activity of specific neurons in area CA1 and other hippocampal subfields is both necessary and sufficient to create this behavioral response. Specifically, activating "engram" neurons tagged during the initial aversive experience causes mice to freeze, and engram neural ensembles can be observed to be selectively active when a mouse is freezing (Cai et al., 2016). This suggests that memories might be expressed by simple ensemble activity, without sequential structure. However, Wu and

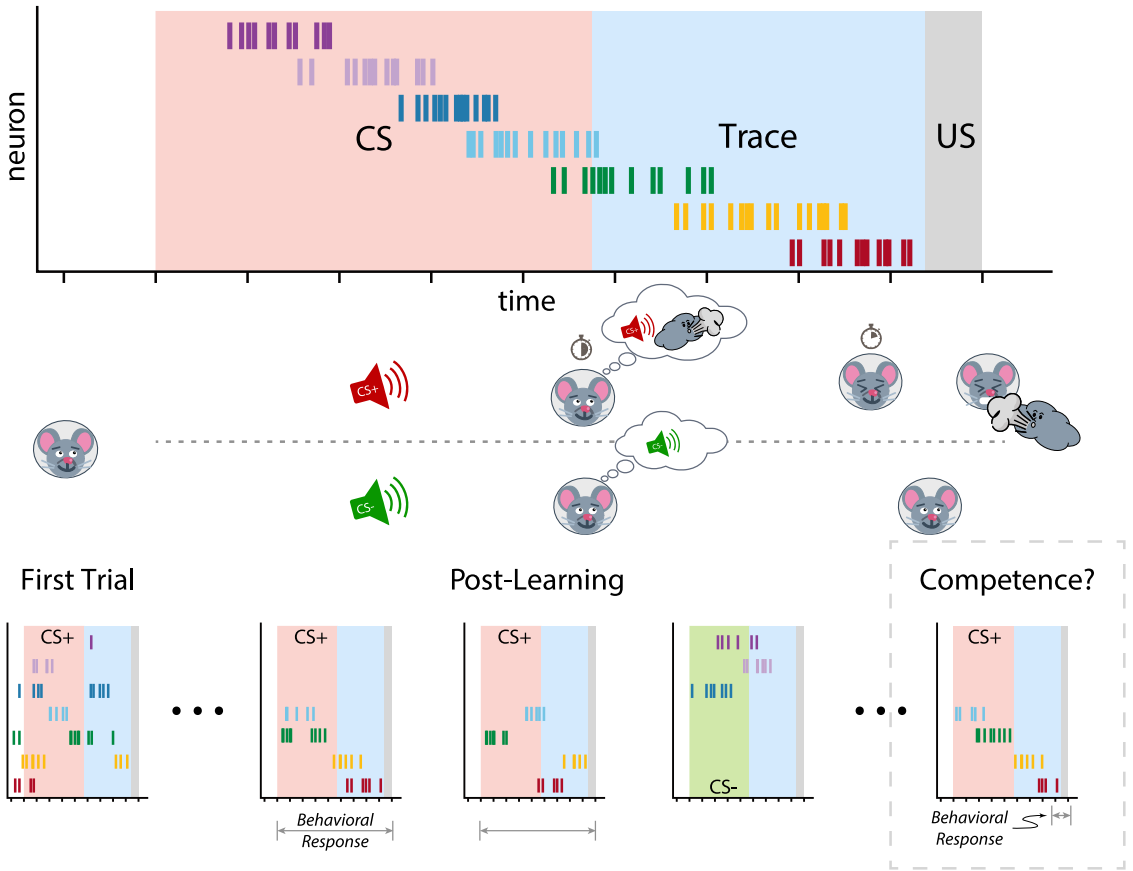

Figure 1. Trace-Fear Conditioning in the Short Term *Is Not* Encoded by Sequential Hippocampal Activity

In trace conditioning paradigms, a sensory cue (the "conditioned stimulus," CS+) is associated with an aversive stimulus ("unconditioned stimulus," US) that is administered after some delay from the end of the cue (the trace period). On some trials, a different, safe cue (CS−) is given. Longer delays during the trace period make learning this association hippocampally dependent, but the pattern of ensemble neural activity that supports the learning has been unknown. (Top) Most theories had postulated that sequences of activity expressed by "time cells" might allow animals to bridge from CS to US. (Bottom) Ahmed et al. (2020) show that, following learning, ensemble activity encodes the stages of the task but that hippocampal neurons do not express expected sequential activity. This suggests that during the initial stages of learning, coarse behavioral responses (withholding licking in this case) do not require sequences of hippocampal activity. Perhaps later, after behavioral responses eventually become more precise, these sequences might emerge.

colleagues in 2017 reported that during avoidance—a slightly different expression of the memory of a contextual fear memory—the hippocampus replays sequences of place cells correlated with the location of the aversive stimulus (Wu et al., 2017).

Large-scale ensemble neural recordings have not been applied to trace fear conditioning paradigms. In one of the more widely studied variants, trace eyeblink conditioning, an auditory cue predicts an upcoming air puff aimed at the eye. Over the course of about a week of training, animals learn a reliable "conditioned response" (CR), blinking right before the air puff is delivered. As the challenge of generating the CR is to properly integrate temporally separated events, it has been argued that the "memory trace" is held within the hippocampus. Remarkably, in a human variant of this task, it was demonstrated that cognitive awareness of the CS-US relationship is required for non-amnesic subjects to develop a CR. So, what might be the pattern of hippocampal neural activity that supports learning the CS-US relationship? The prevalent theory within the field (Figure 1) is that the same sequential patterns of sequential activity observed when animals run during a delay period on a treadmill—time cells—would be an ideal mechanism to support linking the CS and US through the duration of the stimulus-free trace period (Eichenbaum, 2017; Kitamura et al., 2015). Beyond sequences, similarly to contextual fear conditioning, it is also likely that the trace conditioning experience triggers a global state change, which would link the activity of engram neurons to the process of producing a CR via overall heightened excitability (Cai et al., 2016). For this second function, order would be less important than identity.

Ahmed et al. (2020) show that, during the initial experience of an aversive CS-US pairing with a 15 s trace period, consistent *sequential* patterns of activity are not exhibited by CA1 neurons. However, by looking at the subset of neurons that were active at any point in time, they could reliably detect the temporal portion of the experiment (i.e., CS, Trace, US, Post). This suggests that the neural ensemble is encoding the task via coactivity of a

selected portion of the neurons—a manifold of population activity—but that trajectories of neural activity are not following consistent paths through this manifold. The emergence of these task-specific populations (Figure 1, "Post Learning") paralleled the emergence of a behavioral response (withholding licking during the CS, Trace, and US periods). Rapid formation of ensemble representations of a context parallels what has been observed with both hippocampal place and time cell formation (Eichenbaum, 2017). While it was quite clear from their data that consistent sequences (i.e., ones time locked to the onset or offset of the CS) were not apparent, Ahmed et al. (2020) further hypothesized that there might be sequential activity but that the phase of the sequences (i.e., which neurons began the patterns) might vary across trials. They tested this hypothesis using a hidden Markov model, an unsupervised machine-learning technique that has been demonstrated to be able to detect sequential patterns (Maboudi et al., 2018). Here, still, they failed to detect any evidence of temporal patterns between the neurons.

So, are sequences unimportant for trace conditioning tasks? A key distinction between historical time-cell experiments and the present work (Ahmed et al., 2020) is that in the time-cell experiments, rodents ran on a treadmill during the delay period. In contrast, Ahmed et al. (2020) use a tube to restrain mice during training. While in rabbits restrained immobility can correspond to an alert, exploratory brain state similar to what produces place cell activity in mice, it is unclear whether this happened in this task. It is possible that the physical restraint biases animals to quiet wakefulness. In that case, we might expect SWR/replay-like activity to support the withholding of licking behavior similar to what was observed in inhibitory avoidance (Wu et al., 2017). The slow kinetics of the fluorescent calcium indicators compared to electrophysiological data acquisition would make it difficult or impossible to detect sequential structure that might be present during SWRs. Moreover, the activity of individual neurons might be detected unreliably due to the smaller number of action potentials a single neuron produces during a SWR event. Thus, rapid SWR sequences might be present but undetectable.

At a higher level, however, perhaps there is another resolution to this apparent conflict. Semantic learning by definition begins with an initial experience (e.g., the experience of a mosquito bite). Perhaps learning and memory recall associated with new experiences are reflected in disordered activation of new contextual ensembles and bursts of ensemble hippocampal activity during SWRs. As multiple experiences add up to become knowledge—we should start slapping as soon as the buzzing stops—the hippocampus transitions to encoding the necessary sequential patterns in ordered place-cell-like activity. If this were true, we would expect that time-cell-like sequences would emerge at the same time as well-timed behaviors. Ahmed et al. (2020) studied only the neural activity that happens in the immediate aftermath of the onset of training—the consequences of the first dozen or two experiences of the air puff on the first day of training. In classical trace-eyeblink conditioning, it often takes hundreds of trials over several days for a precisely timed blink to emerge. Thus, perhaps if the study had been prolonged over a longer period of time (Figure 1, "Competence?"), and a US that was avoidable (i.e., an air puff in the eye that could be blocked with a blink rather than on the snout), the investigators might have noticed an eventual transition to more structured sequences during the CS and trace periods.

But perhaps this insight in turn points to a weakness of the classical trace conditioning paradigms! Many scenarios in life are more like the mosquito's buzz and bite, where there are temporal associations without precise, reliable timing. With new optical and electrode-based technologies on the horizon that allow for reliable detection of individual action potentials from thousands of CA1 neurons and modern machine-learning approaches for identifying sequential dynamics on manifolds of ensemble activity, we may soon be equipped to finally begin to understand how our brains can adapt to ever-changing situations that require integrating new experiences into continuous learning.

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