Fetsch et al. (2014) found in this control experiment: an offset in the motion coherence of the real stimulus biases the monkeys' choices and confidence ratings just like microstimulation did.

In an elegant control experiment, Fetsch et al. (2014) sought to break the system apart. Instead of using low currents to stimulate a small patch of neurons with similar preferred orientations, the authors now injected a large amount of current that recruited a wider population of neurons including disparate preferred motion directions. This widespread activation resulted in a large increase in the number of sure bet choices, indicating that monkeys experienced noisy motion information and less confident decisions. The result illustrates at least two important issues. First, it demonstrates that monkeys are capable of reporting a large decrease in confidence and, second, it shows that the behavioral consequences of microstimulation are exquisitely dependent on the selectivity of the stimulated neurons. Large stimulation currents, instead of injecting additional information, indiscriminately recruit neuronal populations whose contributions can mask subtle sensory representations.

The results reported by Fetsch et al. (2014) demonstrate that the mechanisms that read sensory evidence have access to the additional information added by microstimulation at the level of MT/MST. Future experiments should be aimed to identify the downstream neuronal circuits that read this evidence to decide whether to choose a safe bet or to risk for a larger reward. Importantly, these circuits must have learned, during behavioral training, the association between the amount of accumulated evidence and the likelihood that a given answer will be correct. What are the neuronal correlates of this learning? The answer will likely include the orchestrating functions of the frontal cortices, and also the modulatory effects of subcortical projection systems (de Lafuente and Romo, 2011; Schultz, 2013).

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Oscillatory Substrates of Fear and Safety

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Oscillatory activity in the basolateral amygdala (BLA) is critical for emotional behavior. In this issue of *Neuron*, Stujenske et al. (2014) describe novel dynamics of BLA theta-gamma-coupled neuronal oscillations associated with conditioned and innate fear.

Neuronal theta (4–10 Hz) and gamma band (30–80 Hz) synchrony, which can be detected in the neocortex and associated areas of mammals including humans, subserves several cognitive functions. Notably, these two oscillatory bands can interact with each other, and their interplay represents a fascinating area of investigation. During specific sensory and cognitive experiences, the power of the gamma rhythm is modulated by theta oscillations, as theta provides an ideal substrate of timing, suitable to define the onset of a stimulus. Specifically, the theta-gamma code could be relevant for the recall of a memory linked with salient stimuli, such as a reward or a noxious stimulus. Phase-amplitude cross-frequency coupling (CFC) between theta and gamma is an index of the modulation of the gamma power by the phase of the theta oscillations (Canolty et al., 2006). Stronger CFC can be detected in the hippocampus when an animal learns the association between an item and its spatial context (Tort et al., 2009).

The BLA, hippocampus (HPC), and medial prefrontal cortex (mPFC), three interconnected brain structures involved in fear and anxiety, display synchronized theta oscillations correlated with fear memory (Seidenbecher et al., 2003). Although it is known that BLA gamma

oscillations are evoked by fearful stimuli (Courtin et al., 2014), it is not known whether gamma-band synchronization is involved in the coordination of the BLA, HPC, and mPFC activities during fear memory retrieval. Recently, Joshua Gordon and colleagues performed a fear discrimination experiment that detected two types of associative fear responses in mice: one group were "generalizers" that froze equally during a cue that predicted footshock (CS+) and a cue that predicted the absence of footshock (CS−); a separate group were "discriminators" freezing significantly more during the CS+ than the CS− (Likhtik et al., 2014). This paradigm may be relevant for psychiatric disorders in humans, as fear generalization is strongly linked with generalized anxiety disorders (Cha et al., 2014). Furthermore, Likhtik et al. (2014) probed innate anxiety through an open-field test, which revealed a group of anxious and a group of nonanxious mice. They demonstrated that an mPFC-driven theta input to the BLA was crucial to reduce BLA neuronal firing in situations of supposed safety, for example, when the mice were in the peripheral (i.e., less anxiogenic) part of the open field or in response to the CS−, the cue that represents safety in the conditioned fear paradigm. However, this work did not clarify whether gamma oscillations played a role in this mPFC-BLA anxiolytic interaction. Could phase locking of the gamma rhythm to the theta oscillations participate in the processing of an associative fear memory?

Stujenske et al. (2014) address this question in this issue of *Neuron*, investigating theta-gamma interactions throughout the BLA-mPFC-ventral HPC (vHPC) circuit in states of perceived fear and safety. To this end, they performed simultaneous electrophysiological recordings in the BLA, mPFC, and vHPC of freely moving mice undergoing a fear discrimination paradigm and an open-field to test innate anxiety. First, they disentangled two different bands of theta-nested gamma oscillatory activity in the BLA: a slow (40–70 Hz) and a fast (70–120 Hz) gamma. Next, they quantified the theta-gamma phase-amplitude CFC, reporting that both bands were strongly coupled with theta oscillations. However, "emotional" theta, classically triggered by fear memory retrieval (Seidenbecher et al., 2003), was characterized by a narrower band of slower (4–8 Hz) frequency, which only the fast gamma appeared to be coupled to, delineating a narrower band of "emotional" gamma. A strong correlation between fast gamma and a fear-related slow theta suggests that a stronger interaction between these two bands could underlie the processing of a threat by the BLA. Indeed, Stujenske et al. (2014) provide evidence that the CS+ increases BLA theta-gamma phase-amplitude coupling. Theta-gamma coupling was correlated with the freezing of the animal, with animals showing higher freezing levels also displaying stronger coupling. This finding presents the theta-gamma code as new key parameter in fear memory encoding.

Gamma power is enhanced by fear conditioning (Courtin et al., 2014). Thus, it would be tempting to propose that retrieval of a fear signal may underlie a boost in gamma power. Surprisingly, in Stujenske et al. (2014), a fear state (the CS+ for a discriminator or the center of the open-field arena for an anxious mouse) evoked a reduction of fast gamma power in the BLA. An increase in gamma power was, on the other hand, linked with a safety signal, namely the CS− for a discriminating mouse or the periphery of the open-field arena for an anxious mouse. Accurate timing for processing of threats is a crucial skill for survival. Accordingly, this study demonstrates that gamma oscillations time locked to the theta rhythm were linked to the encoding of a fear-related stimulus. Timing appears to be less crucial for a safety-related stimulus, since the fast gamma rhythm was not tightly embedded in theta oscillations but instead characterized by higher power. Were fast gamma oscillations locally generated in the BLA or rather generated elsewhere in the brain? What neuronal populations promote them? Parvalbumin-expressing (PV+) interneurons are involved in the generation of gamma oscillations (Cardin et al., 2009) and their activity shapes fear learning (Wolff et al., 2014). Could PV+ neurons account for the generation of fast gamma? Stujenske et al. (2014) examined the spiking activity of neurons recorded in the BLA; they found that one-third of the action potentials were phase locked to gamma oscillations. This was particularly emphasized in a situation of safety, where mice displayed low levels of freezing. Neurons phase locked with fast gamma often fired doublets of spikes and their action potentials tended to fall on the trough of the gamma cycle. This suggests that a discrete population of doublet-firing neurons may be implicated in the generation of emotionally salient fast gamma in the BLA. The detection of phase-locked firing also provides an indication that the extracellular rhythmic activity was likely generated locally. Although PV+ interneurons represent a strong candidate for the generation of the gamma rhythm, and PV+ basket cells often fire in doublets, further evidence will be necessary to support this hypothesis.

Could gamma synchronization provide a framework for the transmission of emotionally relevant information throughout the BLA-mPFC-vHPC circuit? Consistent with the role of gamma in cross-areas synchronization (Fries, 2009), Stujenske et al. (2014) demonstrated that slow and fast gamma oscillations were highly synchronized within the BLA-mPFC-vHPC loop. As described for the BLA, fast gamma, but not slow gamma, power was enhanced in the mPFC during safety. Does a particular structure drive phase locking and power of this gamma oscillations? Examining the directionality of the fast gamma band, Stujenske et al. (2014) described a preferential route originating from the mPFC. Previously, Likhtik et al. (2014) showed that a dominant mPFC-mediated theta input inhibits the BLA during safety states, emphasizing a role of the mPFC in controlling the BLA output. However, in principle, the mPFC could also orchestrate the onset and the power of locally generated BLA gamma oscillations. The novel results of Stujenske et al. (2014) provide compelling evidence for this. Indeed, BLA fast gamma oscillations were strongly modulated by the phase of mPFC (but not local) theta oscillations during both learned and innate safety. When a threat is present, mPFC-BLA theta synchrony triggers BLA theta-gamma coupling and reduces gamma power (Figure 1A). When safety occurs, a theta input from the mPFC to the BLA prevails, phase locking BLA fast gamma power to mPFC theta and generally enhancing BLA gamma power (Figure 1B).

This study opens new horizons to oscillatory codes underpinning emotional behavior, integrating power and theta

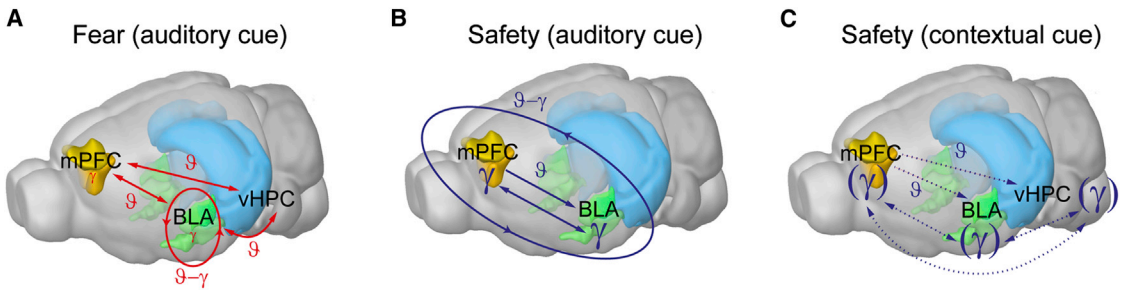

Figure 1. mPFC-BLA-vHPC Theta-Gamma Dynamics during Fear and Safety

(A) According to the data of Stujenske et al. (2014), retrieval of an auditory fear memory increases theta synchrony between mPFC, BLA, and vHPC. High mPFC-BLA theta synchrony leads to an increase in local theta-gamma coupling in the BLA. Gamma power is reduced (small size γ) in the mPFC and BLA.

(B) Stujenske et al. (2014)'s data also suggest that during safety states (presentation of the CS− to a discriminating mouse) a stronger theta input from the mPFC to the BLA promotes higher coupling of mPFC and BLA gamma power to mPFC theta phase. This, in turn, triggers higher gamma power (big size γ) and synchrony between mPFC and BLA.

(C) We speculate that gamma oscillations in the vHPC may come into play in a contextual fear conditioning paradigm. Safety states could be represented by a discriminating mouse exposed to a context predicting the absence of a footshock. Higher theta input from the mPFC to both BLA and vHPC could enhance gamma power and synchrony in all three structures. Oscillatory interactions in this panel are dashed, as they are hypothetical.

Adapted from Allen Mouse Brain Atlas—Brain Explorer 2 Website: 2014 Allen Institute for Brain Science. Allen Mouse Brain Atlas. Available from http://mouse.brain-map.org/.

phase coupling of gamma oscillations as key elements in a puzzle currently focusing on theta synchrony. Surprisingly, the vHPC, a crucial hotspot for fear and anxiety, could not be incorporated in this model, as oscillatory activity in this area did not display changes during safety. Given the importance of vHPC in the transmission of contextual information to the BLA, an intriguing hypothesis is that vHPC oscillations could encode safety in a contextual fear conditioning paradigm (Figure 1C). However, it is surprising that the vHPC did not contribute to the theta-gamma encoding of safety in a test for innate anxiety, given recent evidence (Felix-Ortiz et al., 2013).

Stujenske et al. (2014)'s data raise several novel and stimulating questions regarding the generation of gamma oscillations and their role in mPFC-BLA communication. First, do PV+ GABAergic neurons contribute to the generation of the "emotional" gamma in the BLA, and if so which subtype(s) of PV+ neurons are involved (Bienvenu et al., 2012)? In hippocampal CA1, PV+ basket cells are phase coupled to the gamma rhythm in a theta phase-dependent fashion (Lasztóczi and Klausberger, 2014), suggesting they could be the key neuron type(s) to modulate the relationship between the gamma power and theta-gamma coupling observed by Stujenske et al. (2014). Optogenetic tagging of PV+ interneurons during fear conditioning could unravel their role in the generation of emotional gamma oscillations in the BLA. Furthermore, optogenetic manipulation of mPFC-BLA circuitry could be exploited to disrupt BLA theta-gamma coupling and investigate whether this coupling is required for fear discrimination. Ultimately, mechanisms underlying a stronger mPFC theta input to the BLA will need to be explored. Findings by Likhtik et al. (2014) suggest that higher theta modulation of the BLA by the mPFC leads to inhibition of BLA principal neurons (PNs) firing, an effect consistent with classical models (Quirk et al., 2003). This inhibitory effect is probably mediated by recruitment of local GABAergic interneurons providing feedforward inhibition onto BLA PNs. The picture that emerges implicates a higher gamma power triggered by stronger mPFC theta input to PV+ interneurons. This could lead to a spatiotemporal redistribution of synaptic inhibition (Somogyi et al., 2014) impinging on BLA PNs, the main output of this nucleus.

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