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Oscillations in the Basolateral Amygdala: Aversive Stimulation Is State Dependent and Resets the Oscillatory Phase

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Crane JW, Windels F, Sah P. Oscillations in the basolateral amygdala: aversive stimulation is state dependent and resets the oscillatory phase. *J Neurophysiol* 102: 1379–1387, 2009. First published July 1, 2009; doi:10.1152/jn.00438.2009. Slow oscillations (<1 Hz) in neural activity occur during sleep and quiet wakefulness in both animals and humans. Here we show that in urethan-anesthetized animals, neurons in the basolateral amygdala *in vivo* display a slow oscillation between resting membrane potential (down-state) and depolarized potentials (up-states) occurring at a frequency of ~0.3 Hz. This oscillation is insensitive to the holding potential and continues unabated under voltage clamp, indicating that up-states are synaptically driven. Somatosensory stimulation (footshock) delivered during the down-state evoked an all-or-none transition into an up-state. When delivered during down-states, footshocks triggered up-states and reset the phase of the neural oscillation, effectively synchronizing activity in the basolateral amygdala. This phase reset was reproduced by posterior thalamus stimulation, confirming that it was mediated by aversive sensory input. In contrast, a footshock delivered during the up-state was ineffective in stimulating BLA neurons. We conclude that oscillatory activity in the basolateral amygdala is driven by ensembles of cortical neurons. These ensembles gate the response of amygdala neurons to aversive stimulation in a state-dependent manner. Aversive stimulation is effective when the network is in the down-state but ineffective when the network is in an up-state.

INTRODUCTION

Spontaneous oscillations in neural activity over a wide range of frequencies are a common feature of the mammalian brain and have been found in both cortical and subcortical regions (Buzsaki 2006; Steriade 1997). These oscillations have been linked to memory formation and consolidation, spatial navigation, and binding of disparate sensory information (Singer and Gray 1995). Of these, the low-frequency (<1 Hz), slow oscillation is most prominently seen during slow-wave-sleep and certain forms of anesthesia (Steriade et al. 1993b) but also during quiet wakefulness (Petersen et al. 2003) as well as in acute brain slices maintained *in vitro* (MacLean et al. 2005; Sanchez-Vives and McCormick 2000; Watson et al. 2008). At the single-cell level, slow oscillations are seen as spontaneous fluctuations in membrane potential characterized by two phases: down-states, where the membrane potential is near the resting membrane potential, and up-states, where neurons depolarize to near spike threshold (Cowan and Wilson 1994; Steriade et al. 1993c; Wilson and Kawaguchi 1996). While the exact functional roles of these slow oscillations is not clear, entry of neurons into the up-state has been reported to alter cellular

processing of sensory inputs (Anderson et al. 2000; Petersen et al. 2003; Watson et al. 2008).

The amygdala is a temporal lobe structure that plays a key role in assigning emotional valence to sensory stimuli (Davis and Whalen 2001; LeDoux 1995). It is a complex structure with many interconnected nuclei that have extensive cortical (McDonald 1998) and subcortical (Sah et al. 2003) connections. Unimodal sensory information as well as highly processed information from cortical association areas enters the amygdala via the basolateral complex (BLA) (McDonald 1998; Sah et al. 2003) where it is locally processed to generate appropriate behavioral and visceral responses to environmental stimuli (Davis and Whalen 2001; LeDoux 2003). The amygdala plays a central role in fear conditioning, where a neutral sensory stimulus, such as a tone, is contingently paired with an aversive stimulus, typically a footshock. This association is rapidly learned and as a result, the animal subsequently responds to the previously neutral stimulus with fear (LeDoux 2003; Maren 2001; Sah and Westbrook 2008).

Unit recordings *in vivo* have shown that sensory input can drive neurons in the BLA to threshold (Romanski et al. 1993). However, how different sensory stimuli are processed within the BLA is not well understood. In the present study, we examine the processing of aversive stimuli (footshocks) within the BLA. By making whole cell recordings *in vivo*, we show that principal neurons in the BLA show spontaneous oscillatory activity that is largely subthreshold. We show that brief footshocks given when neurons are in the down-state evoke an all-or-none transition to up-states and reset the oscillatory phase of ongoing oscillations. In contrast, footshocks delivered during up-states are completely ineffective. These results demonstrate a bidirectional relationship between ongoing network activity and the processing of sensory inputs within the BLA.

METHODS

Data were obtained from Wistar rats (P16–P23; 45 ± 10 g). Animals were housed under standard laboratory conditions with a 12-h light/dark cycle (lights on at 6 AM) and food and water available *ad libitum*. In all procedures the care and experimental use of animals was in accordance with protocols approved by the University of Queensland Animal Ethics Committee.

Surgery

Animals were anesthetized with urethan (2 g/kg ip). Once a sufficient level of anesthesia was obtained, animals were mounted in a stereotaxic frame and their body temperature maintained at 37°C. Following the exposure of the skull surface, a hole was drilled unilaterally at empirically determined coordinates corresponding to the location of the left BLA (2.5–2.6 mm posterior and 4.3 mm lateral

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to midline) and the dura carefully retracted. Another hole was drilled on the contralateral side of the skull to allow a ground wire to be placed on the cortical surface. Footshocks (5–8 mA, 1 ms) were delivered via two 25-gauge needles connected to an isolated current generator (Digitimer) and inserted into the footpad contralateral to the side of the BLA recording (i.e., the right footpad). Identical procedures were used for barrel cortex recordings except that stereotaxic co-ordinates were altered (1.0 mm posterior to bregma and 4.5 mm lateral to midline).

For experiments involving prefrontal cortex or posterior thalamus stimulation, animals were prepared as for BLA recordings. Another hole was then drilled corresponding with either the prefrontal cortex (2.5 mm anterior to bregma; 0.5 mm lateral to midline) or the posterior thalamus (4 mm posterior to bregma; 20° lat). A concentric bi-polar electrode was then lowered into the prefrontal cortex (3 mm ventral to brain surface) or the posterior thalamus (5.8 mm).

Electrophysiology

Recording pipettes (6–7 M Ω ; shank length of 7.5 mm) were fabricated from borosilicate glass using a Sutter P-87, three-stage pipette puller and were filled with an internal solution containing (in mM) 135 KMeSO₄, 7 NaCl, 10 HEPES, 2 Mg₂ATP, 0.3 Na₃GTP, 0.3 EGTA, and 0.3% neurobiotin; pH 7.3; osmolarity 290–300 mOsm. Series resistance varied between 20 and 50 M Ω during recording. No correction was made for the junction potential. Signals were amplified using either a Multiclamp 700B or Axopatch 1D (Molecular Devices), filtered at 5 kHz and digitized at 10 kHz using an Instrutech ITC-18. Data acquisition and subsequent analysis were performed using Axograph X (V1.0.8, Axograph Scientific). Stimulation of the prefrontal cortex or the posterior thalamus was achieved with an isolated voltage generator. Single stimulations (100 μ s, 20 V) were triggered manually at appropriate time points during the recordings obtained from BLA principal neurons.

Data analysis

Neuron firing properties were measured as the voltage response to a series of negative and positive current steps (20 pA increments, 600 ms). Axograph software was used to determine the width of action potentials at half of their peak amplitude, the duration and peak amplitude of both spontaneously occurring and footshock-induced up-states. The fast Fourier transform function of Axograph was used on 60-s current-clamp traces filtered at 30 Hz to obtain the power spectrums used for this study. Autocorrelograms were calculated using the Matlab signal analysis toolbox in 1-ms steps. Membrane potential variance calculations were performed on 250-ms segments in either the up- or down-state. For each cell, the average variance values were obtained from 10 to 15 up-states and down-states. To assess if footshocks led to reset of the oscillatory phase (Fig. 4A), we first calculated the mean up-state to up-state duration (effectively the wavelength of the oscillation) and mean down-state duration for that neuron before delivery of the footshock by averaging times over 1 min of recording. The time of the footshock was then calculated as a percentage of the mean down-state. The time from the footshock to the onset of the second up-state was then expressed as a percentage of the mean up-state to up-state duration. That is, the time of the first cycle of the footshock-evoked oscillation. As an internal control for these measurements (Fig. 4D), we randomly picked three locations during the down-state (30, 50, and 80% of the mean) and measured the interval to the onset of the second up-state (similar to the interval measured following delivery of a footshock). This interval was then expressed as a percentage of the mean up-state to up-state duration and plotted as the increase in oscillatory wavelength against the location within the down-state.

All average traces shown are taken from 5–10 traces and all measurements are given as means \pm SE.

Histology

After completion of the recording, animals were *trans*-cardially perfused with 2% sodium nitrite solution (in 0.1 M phosphate-buffer, pH 7.4) followed by 50 ml of 4% formaldehyde (in 0.1 M phosphate-buffer, pH 7.4). Brains were then removed and postfixed overnight in 4% formaldehyde at 4°C, followed by cryoprotection in 20% sucrose (in 0.1 M phosphate-buffered saline, pH 7.4) overnight at 4°C. Serial coronal forebrain (100 μ m) sections were cut using a freezing microtome. All sections were washed four times (10 min each) in 0.1% phosphate-buffered saline (PBS) containing 0.1% Triton X-100 before being washed for 30 min in 3% H₂O₂/PBS solution. After further washes in PBS/Triton (5 \times 5 min), sections were incubated in a 3% bovine serum albumin/PBS solution and then transferred immediately into a solution of avidin-biotin-horseradish peroxidase complex from the Vector Elite ABC kit (6 μ l A and 6 μ l B/ml of 1% BSA in PBS). Following an overnight incubation at 4°C in this solution, the sections were washed in PBS/Triton (3 \times 10 min) followed by a wash (1 \times 5 min) in sodium acetate buffer (pH = 6.0). Sections were then exposed for 15 min to a 2% NiSO₄ solution (in sodium acetate buffer, pH 6.0) containing 2 mg/ml D-glucose, 0.4 mg/ml NH₄Cl and 0.025% 3,3'-diaminobenzidine. This was followed by another incubation in the same solution but with the addition of glucose oxidase (0.2 μ l/ml). The subsequent production of H₂O₂ initiates the peroxidase reaction resulting in the deposition of NiSO₄-DAB. At a suitable time point, based on the background level of staining seen, all were washed in sodium acetate buffer to stop the reaction and then exposed to a final series of washes in PBS (3 \times 10 min). All sections were then mounted onto Superfrost Plus glass slides, exposed to a Nissl stain (to allow cytoarchitecture to be visualized), cleared in xylene and coverslipped using Depex mounting medium. Neurobiotin-filled cells were then visualized and images acquired using Zeiss Axiovision 4.5 software. In the occasions where a cell was not fully recovered the location of pipette tracts was used to determine the location of the recording. The placement of the recorded cell within the BLA was determined by reference to Paxinos and Watson rat brain atlas. Only recordings that were determined to have occurred in the BLA were included in the final analysis.

RESULTS

Whole cell recordings were made from neurons within the BLA *in vivo* (Fig. 1). Neurons had a resting membrane potential of -55 ± 1 mV and a mean input resistance of 83 ± 6 M Ω ($n = 63$). In response to depolarizing current injection neurons displayed a range of firing properties, from cells that only fired single spikes to those that fired multiple spikes with varying degrees of spike frequency adaptation (Fig. 1B). Action potentials had a half-width of 1.53 ± 0.07 ms and were followed by a shallow afterhyperpolarization (Fig. 1B). These cells were classified as principal neurons as their firing properties are similar to those previously reported for principal neurons *in vivo* (Paré et al. 1995) as well as in acute slices *in vitro* (Faber et al. 2001; Washburn and Moises 1992) and their dendrites were spiny (Fig. 1A). Our fully recovered neurons were randomly distributed throughout the basolateral complex (Fig. 1C). In three cases, recordings were obtained from neurons identified as interneurons. These cells had relatively aspiny dendrites, faster action potentials (half-width: 0.77 ± 0.08 ms) and discharged high-frequency trains of action potentials in response to depolarizing current injections (data not shown) (Mahanty and Sah 1998; Pare and Gaudreau 1996; Woodruff and Sah 2007). Due to the very small number of recordings obtained from BLA interneurons, we have confined our analysis to principal neurons.

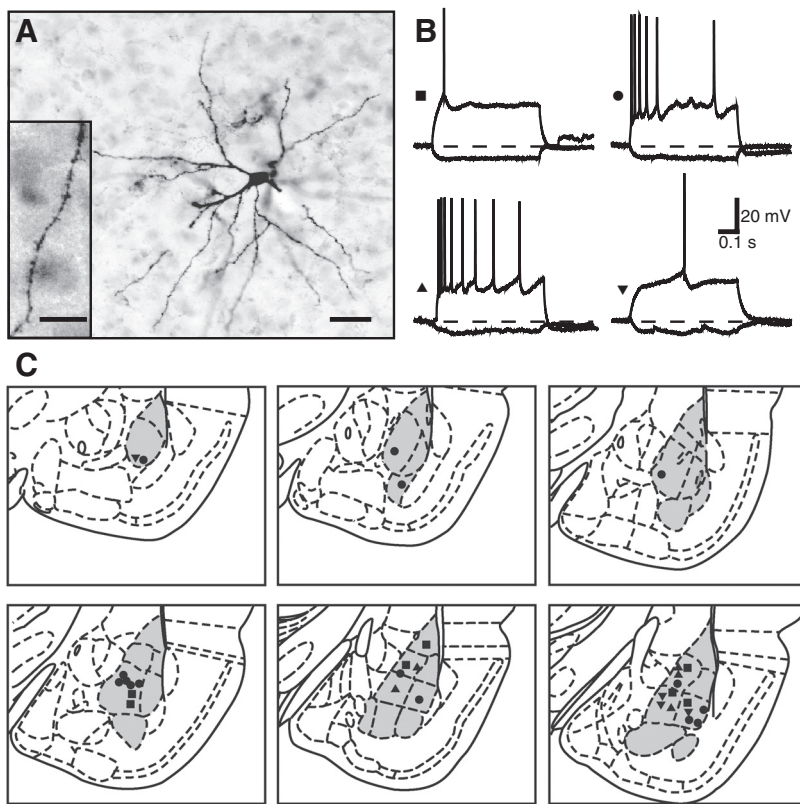


FIG. 1. Principal neurons of the basolateral amygdala display similar firing properties *in vivo* to those reported *in vitro*. *A*: photomicrograph of a neurobiotin-filled principal neuron within the basolateral amygdala, bar = 20 μm . *Inset*: high-magnification image demonstrating the presence of spines on a recovered principal neurons in the basolateral amygdala, bar = 10 μm . *B*: principal neurons can be divided into four distinct types based on their firing properties in response to a 600-ms current injection. These 4 types are: adapting (■); moderate spike-frequency adaptation (●); regular spiking (▲); and late firing (▼). *C*: location of completely recovered neurobiotin-filled cells within the basolateral amygdala, separated based on firing properties. grey, basolateral amygdala complex.

BLA principal neurons display a slow membrane potential oscillation

All neurons displayed repetitive, spontaneous fluctuations in their membrane potential (Fig. 2*A*) alternating between periods of quiescence near the resting membrane potential (down-state), and periods at depolarized-membrane potentials close to action potential threshold (up-states). The bistable nature of these changes in membrane potentials can be clearly seen in the all points histogram (Fig. 2*B*). At -60 mV, the average amplitude of the up-state was 18 ± 2 mV ($n = 7$). Action potentials were only occasionally seen during an up-state with an average of 1.1 ± 0.3 action potentials per up-state, leading to an average firing frequency of 0.5 Hz. This low spontaneous firing rate of BLA principal neurons *in vivo* is similar to that described previously (Clugnet et al. 1990; Pare and Gaudreau 1996). Autocorrelation of membrane potential fluctuations revealed a periodicity of 2.75 ± 0.19 s ($n = 12$), and consistent with this, power spectral density analysis showed a peak at 0.38 ± 0.04 Hz ($n = 8$, Fig. 2, *C* and *D*).

Sustained oscillations in membrane potential have been described in several brain regions and can arise either from the activity of ion channels intrinsic to the cell (Destexhe and Sejnowski 2003), may be driven by exogenous synaptic input (Wilson and Kawaguchi 1996), or may be a combination of the two. Moreover, depolarization of BLA principal neurons has been reported to evoke high-frequency oscillations (>1 Hz) due to activation of intrinsic voltage-dependent conductances (Pape et al. 1998; Paré et al. 1995). However, hyperpolarizing neurons by current injection did not alter the frequency of the membrane potential oscillation (data not shown), suggesting that the slow oscillation in the membrane potential is unlikely to be mediated by activation of voltage-dependent currents. In

agreement with this, switching the recording configuration to voltage-clamp (V_h ; -60 mV) revealed spontaneous bistable oscillations in the membrane current with periods of quiescence (down-states) and periods of sustained inward current (up-states) (Fig. 2, *E* and *F*). This oscillation had a periodicity similar to that displayed by the membrane potential (3.12 ± 0.10 s; Fig. 2*G*) and power spectral density analysis of membrane current revealed a peak similar to that obtained from membrane potential recordings (0.40 ± 0.06 Hz; Fig. 2*H*). Moreover, the average duration of up-states recorded under voltage-clamp (1.31 ± 0.01 s; $n = 3$) was not different from those recorded in current-clamp (1.11 ± 0.26 s; $n = 4$). Together, these results show that membrane potential oscillations in BLA neurons are driven by synaptic input. Consistent with synaptic activity driving cells into up-states, the variance of the membrane potential was much larger during up-states (6.38 ± 1.16 mV²) than during the down-state (0.28 ± 0.13 mV²; $P < 0.01$, $n = 5$). This synaptic activity also likely accounts for the larger variance of the up-state in the all points histogram (Fig. 2*B*).

Footshocks evoke up-states in BLA principal neurons

Resting oscillations in membrane potential have been described in both cortical and thalamic neurons (Steriade 2001) where the oscillatory frequency is modulated by the conscious state of the animal (Buzsáki 2006; Buzsáki and Draguhn 2004) as well as by sensory stimulation (Anderson et al. 2000; Petersen et al. 2003). The amygdala, a temporal lobe structure, plays a critical role in fear conditioning, a Pavlovian conditioning paradigm in which animals learn to associate neutral sensory stimuli with an aversive one—typically a footshock (LeDoux 2000). Within the amygdalar complex, one major

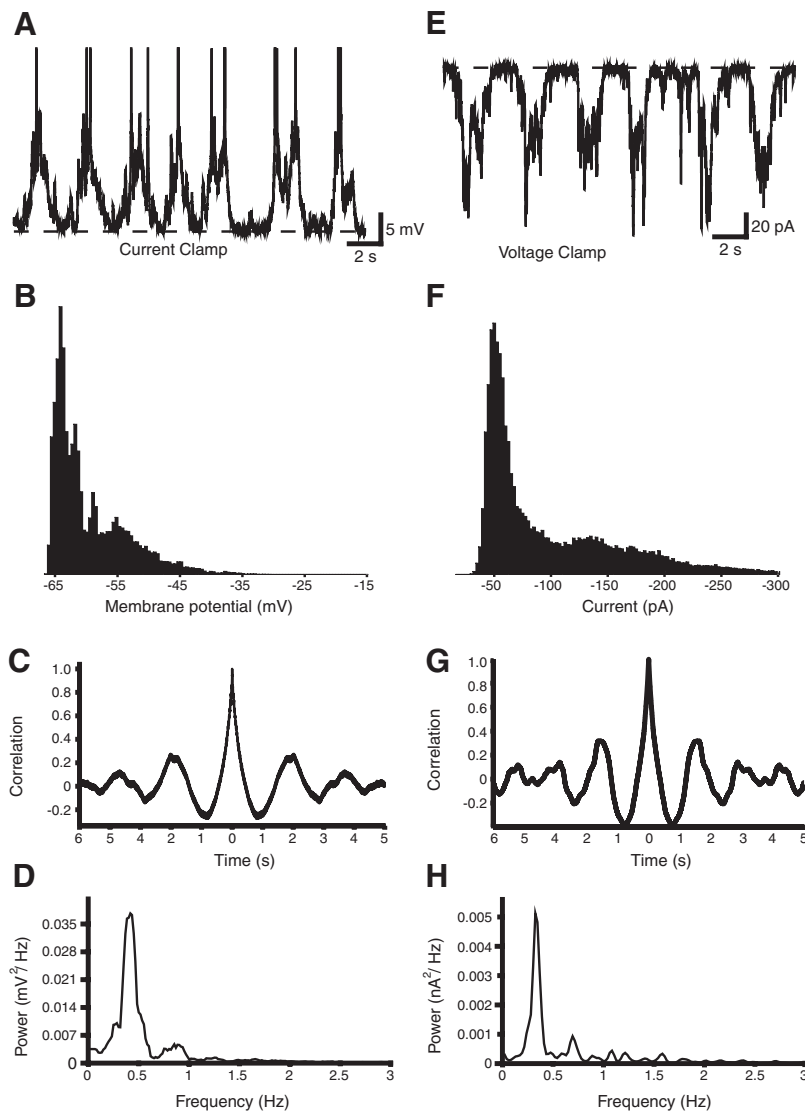


FIG. 2. Principal neurons of the basolateral amygdala display a slow oscillation between resting membrane potentials (down-state) and depolarized potentials (up-state). *A*: current-clamp recording of up- and down-states occurring within a principal neuron of the basolateral amygdala. Action potentials have been cropped for clarity. *B*: all point histogram from current-clamp recording obtained from cell in *A*. *C*: autocorrelation of current-clamp recording in *A* demonstrating clear periodicity of the membrane potential oscillation. *D*: power spectral density analysis of trace in *A* demonstrating that the oscillation frequency peaks between 0.2 and 0.5 Hz. *E*: voltage-clamp recording of neuron illustrated in *A* at a holding potential of -60 mV. The holding current shows clear rhythmic oscillations as seen in current-clamp. The all points histogram for 10 s of this current trace clearly shows 2 peaks (*F*). *G*: autocorrelation of voltage-clamp recording in *F* demonstrating a periodicity of the holding current oscillation. *H*: power spectral density analysis of trace in *E* demonstrating that, similar to the membrane potential, the oscillation frequency peaks between 0.2 and 0.5 Hz.

target of aversive sensory stimuli is the BLA (Lanuza et al. 2008; Shi and Davis 1999), and unit recordings in vivo have shown that somatosensory stimulation can drive BLA neurons to threshold (Romanski et al. 1993). We therefore tested the

effect of footshocks on BLA activity. Delivery of a single footshock (5–8 mA, 1 ms) during down-states evoked an all-or-none transition into an up-state (Fig. 3*A*). The mean latency from the footshock to the start of the up-state was $59 \pm$

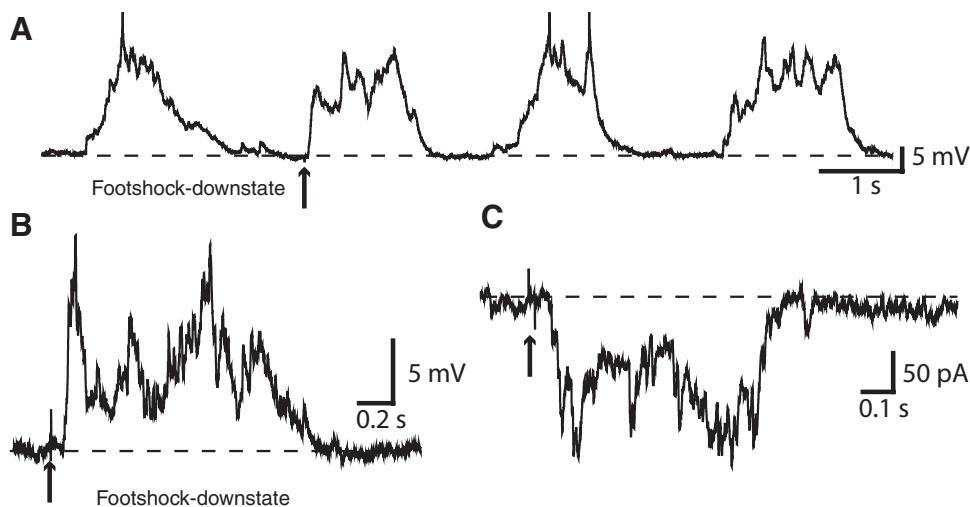


FIG. 3. Footshocks delivered during a down-state evoked an up-state. *A*: current-clamp recording demonstrating that an up-state was evoked by a footshock (\uparrow) delivered during a down-state. *B*: expanded trace of a footshock-evoked up-state. *C*: voltage-clamp recordings (holding potential of -60 mV) demonstrating that a footshock (\uparrow) delivered during a down-state induced a prolonged inward current that resembles the up-state evoked in current-clamp.

8 ms ($n = 11$). These footshock-evoked up-states were indistinguishable from spontaneously occurring up-states (Fig. 3*B*). They had a peak amplitude of 17.1 ± 1.2 mV, lasted 1.5 ± 0.1 s, and on average evoked 0.36 ± 0.14 action potentials. When neurons were held under voltage-clamp, delivery of footshock during a down-state similarly evoked a prolonged inward-current that again mimicked that occurring spontaneously (Fig. 3*C*).

Footshocks reset the phase of the slow oscillation

As footshocks delivered during down-states evoke a transition into an up-state, we next asked whether footshock-evoked up-states are simply inserted between two spontaneously occurring up-states (Fig. 4*Ai*) or if they might reset the phase of the membrane potential oscillation (Fig. 4*Aii*). Visual examination of our recordings suggests the latter possibility as there is no apparent change in the time from the footshock-evoked up-state to the next up-state (Figs. 3*A* and 4*B*). To determine if footshocks reset the oscillatory phase, we first measured the time of onset from a footshock-induced up-state to the next spontaneous up-state. This value was then expressed as a percentage of the average “up-state to up-state” duration for that cell and plotted against the time of the footshock relative to the down-state duration (Fig. 4*C*). If footshocks simply

insert an up-state during an ongoing oscillation, we expect the time to the next up-state (the wavelength of the evoked oscillation) to be shorter as footshocks are delivered closer to the end of the down-state.

For example, when three locations are picked during a down-state (30, 50, and 80% of the mean down-state duration) but no footshock is delivered, the wavelength of the next cycle of the oscillation (up-state to up-state duration) is effectively increased (see METHODS). Thus as one approaches the end of the down-state, the added time nears zero (Fig. 4*D*). In contrast, regardless of where in the down-state a footshock is delivered, the time from the footshock-induced up-state to the next up-state is almost identical to the mean up-state to up-state duration for that cell (Fig. 4*C*, closed circles). Indeed, across all down-state locations, the mean time to the next up-state was $98.3 \pm 3.0\%$ ($n = 5$) of the average up-state to up-state duration.

It is now well established that the major somatic pain pathways that transmit footshock information to the BLA arrives via the posterior intrathalamic nuclei (PoT) (Lanuza et al. 2008). Thus as expected, stimulation of the PoT also evoked a response that closely resembled a spontaneously occurring up-state (duration: 1.31 ± 0.04 s, peak amplitude: 12.5 ± 0.7 mV, $n = 6$) but with a shorter latency than that evoked in response to a footshock (latency: 6.35 ± 0.34 ms, $n = 6$; Fig.

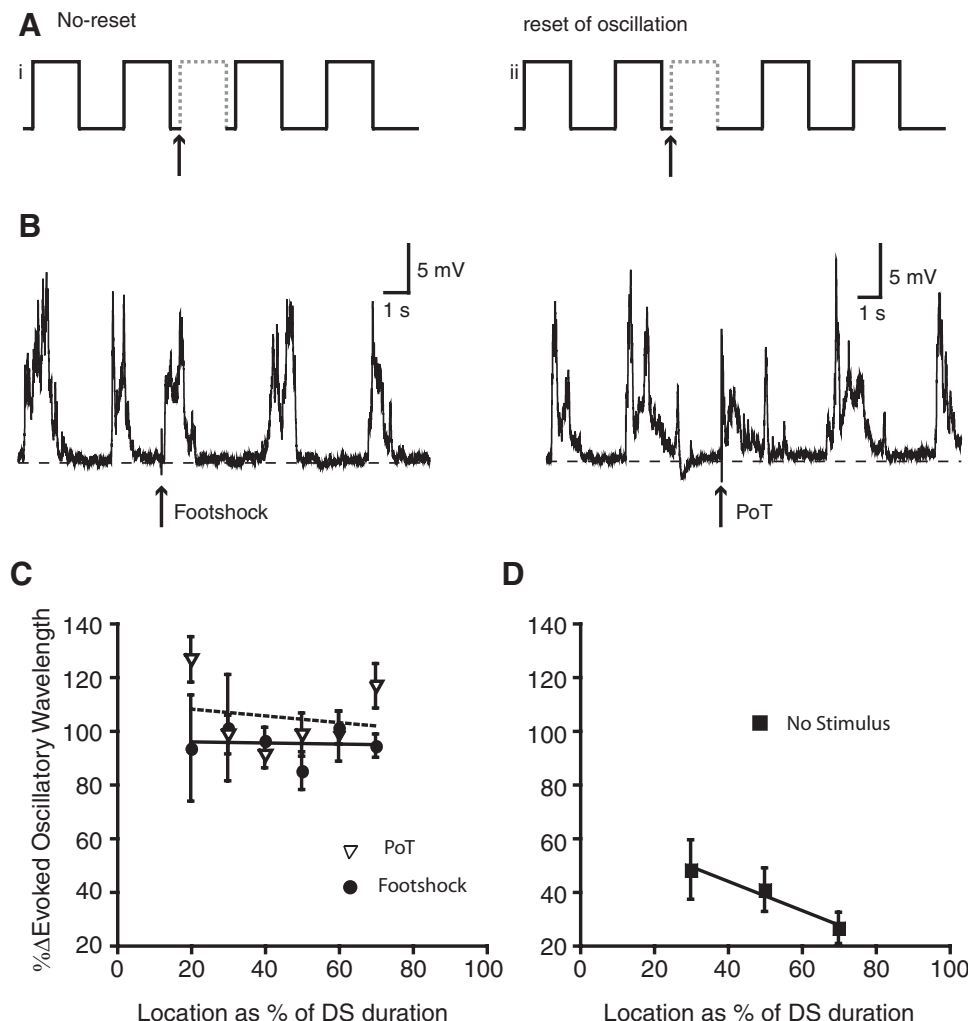


FIG. 4. Aversive stimulation reset the phase of the slow oscillation. *A*: schematic illustrating the difference between stimulus (↑) evoked insertion of an up-state (i) and stimulus evoked reset of the slow oscillation (ii). *B*: current-clamp recording demonstrating a reset of the oscillation phase in response to the delivery of a footshock (↑, trace on left) or single stimulus to the posterior thalamus (PoT, ↑, trace on right). *C*: the percentage change in the wavelength of the 1st cycle of the stimulus evoked oscillation (see METHODS) is plotted against the location of the stimulus relative to the down-state duration. For both footshock (●) and PoT stimulus (▽), the cycle duration from the stimulus-induced up-state to the next up-state was almost identical to the mean cycle duration showing a reset of oscillatory phase. In contrast (*D*), when 3 locations are picked during a down-state (30, 50, and 80% of the mean down-state duration) but no stimulus is delivered, the time to the next up-state is shorter and shorter as one approaches the end of the down-state (■).

4B). Moreover, as seen with footshocks (Fig. 4B), the time interval between the PoT-induced up-state and the next spontaneous up-state was almost identical to the average up-state to up-state duration (Fig. 4C). Across all down-state locations, the mean duration was $102.7 \pm 3.0\%$ ($n = 5$) of the average up-state to up-state duration. Together these results demonstrate that both footshocks and PoT-stimulation evoke a transition into an up-state and reset the phase of the slow oscillation in BLA principal neurons.

As delivery of a footshock activates many ascending afferents, we tested the possibility that the footshock evoked up-state may be due to a global shift in activity in sensory cortex. As such, we recorded from neurons within barrel cortex ($n = 6$) as these neurons do not receive direct footshock-related inputs but do display slow oscillations in membrane potential (Petersen et al. 2003). As expected, neurons in layer 2/3 of barrel cortex displayed a slow oscillation in membrane potential (Fig. 5A). However, delivery of footshocks had no effect on membrane potential (Fig. 5A, $n = 6$). To confirm that footshocks in these animals were effective, we made recordings from BLA neurons in the same animals and found footshock evoked up-states as described in the preceding text (Fig. 5, B and C).

Response to footshock stimulation is state dependent

We have shown that in the anesthetized animal, neurons in the BLA display oscillatory changes in membrane potential that alternate between down-states near the resting membrane potentials and depolarized up-states. Delivery of a footshock when neurons are in the down-state evoked a transition into an up-state that is largely subthreshold and few or no action potentials are evoked. Thus it might be expected that footshocks given during an up-state, when the cell is nearer to

spike threshold, would evoke many more action potentials. However, when footshocks were delivered during the up-state, there was no apparent effect on membrane potential (Fig. 6, A and B). It is possible that the lack of apparent effect on membrane potential to footshocks delivered during an up-state is due to a reduction in neuronal input resistance as a result of the increased synaptic activity (Destexhe et al. 2003). However, testing the cell's input resistance by current injection (100 pA, 94 Hz) did not reveal significant changes during up-states with the input resistance being $63 \pm 3 \text{ M}\Omega$ in the down-state and $70 \pm 3 \text{ M}\Omega$ ($n = 6$) in the up-state, suggesting that ascending footshock information is not reaching BLA neurons when the network is in the up-state. This apparent lack of change in input resistance may be surprising given that up-states are driven by trains of synaptic input. However, BLA principal neurons have large dendritic trees (Faber et al. 2001; Washburn and Moises 1992), and, as our recordings are undoubtedly somatic, it is likely that we were unable to detect changes in input resistance in the dendritic tree where synaptic inputs driving the up-state are located (see following text). However, when footshocks were delivered during the up-state in voltage-clamped neurons ($V_h: -60 \text{ mV}$), no current response was detectable (not shown) in agreement with the idea that footshock evoked ascending information is not reaching BLA neurons when the network is in the up-state. We considered two scenarios to account for this observation. One possibility is that when BLA neurons are in the up-state, ascending footshock information is blocked before reaching the BLA (e.g., at the thalamus). Alternatively, BLA principal neurons may simply not be able to respond to further afferent stimulation once an up-state has been initiated. To test this possibility, we stimulated alternative afferents to BLA neurons. The BLA receives a large input from the medial prefrontal cortex

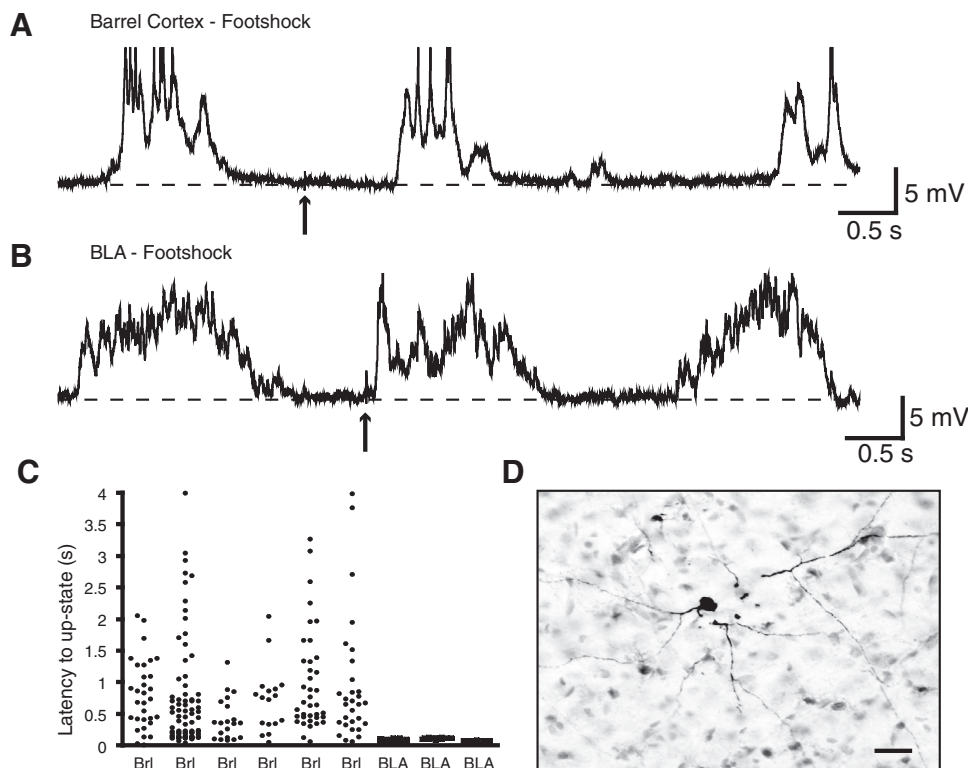


FIG. 5. Footshocks do not induce global shift into an up-state. *A*: current-clamp recording from a barrel cortex neuron. Footshock (\uparrow) delivered during a down-state had no effect on the membrane potential. *B*: current-clamp recording from a basolateral amygdala principal neuron obtained directing after the recording from the cell in *A*. Footshock (\uparrow) delivered during a down-state evoked an up-state. *C*: plot displaying the latency from footshocks to the next up-state in both barrel cortex (Brl) and principal neurons of the basolateral amygdala (BLA). In contrast to the basolateral amygdala, the onset of up-states in the barrel cortex were not time-locked to delivery of a footshock. *D*: example of neurobiotin-filled neuron from the barrel cortex. Bar = 20 μm .

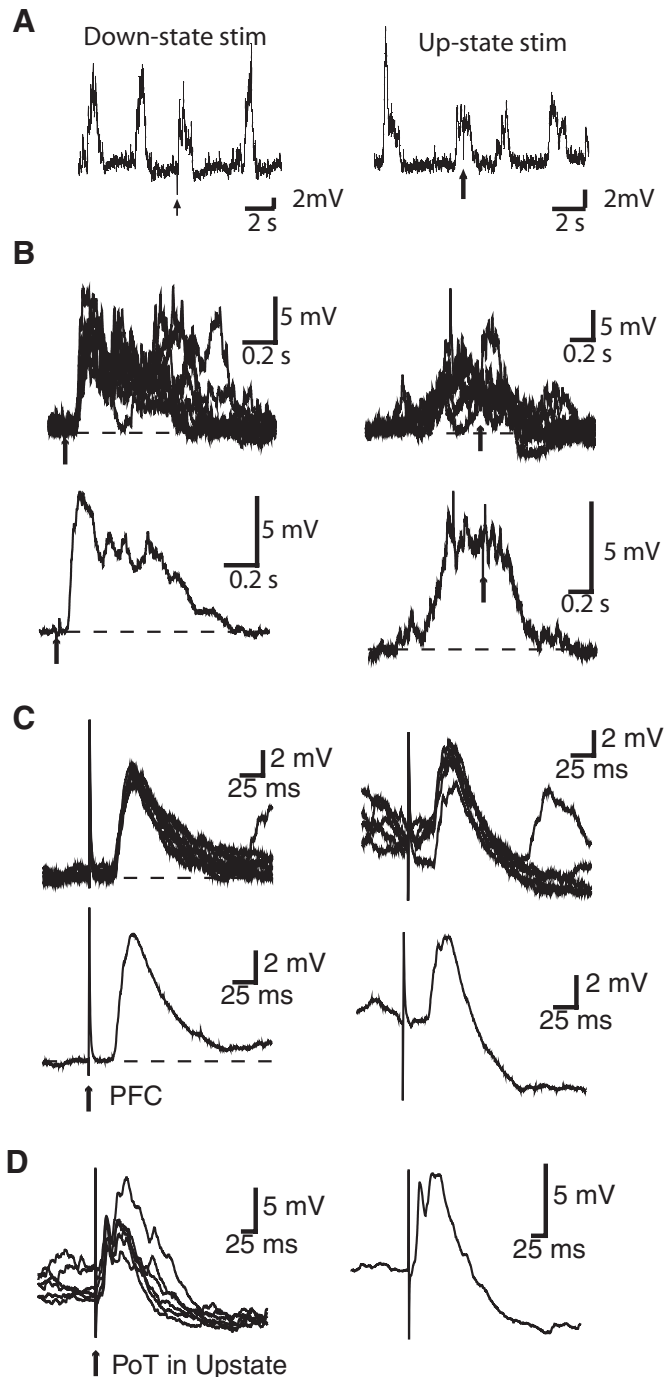


FIG. 6. Response to footshock is state dependent. *A*: delivery of footshock in the down-state evokes a transition into an up-state (trace on left) but footshock delivered in the up-state has no effect (right). *B*, left: overlaid recordings of membrane potential response to footshocks (\uparrow) delivered during down-states. The average membrane potential response to a footshock is shown in the trace below. Traces on right show overlaid membrane potential response to footshock (\uparrow) delivered during up-states. The average membrane potential response to a footshock is shown in the trace below. *C*, left: overlaid traces of membrane potential responses to medial prefrontal cortex (mPFC) stimulations presented during a down-state. The average response to stimulation in the down-state is shown below. Traces on right show overlaid membrane potential responses to mPFC stimulation during up-states. The average membrane potential response to mPFC stimulation delivered during an up-state is shown below. *D*, left: overlaid traces of membrane potential responses to stimulation in the posterior intrathalamic nuclei (PoT) during an up-state. Right: average membrane potential response to PoT stimulation delivered during an up-state.

(mPFC) (McDonald et al. 1996) and stimulation of the mPFC during the down-state produced a short latency (27.6 ± 0.8 ms, $n = 6$) excitatory postsynaptic response (mean amplitude 9.8 ± 1.2 mV, Fig. 6C) that lasted 181 ± 20 ms but did not induce up-states. In contrast to the lack of response to footshocks during the up-state, mPFC stimulation during an up-state clearly evoked a time-locked depolarization (latency: 27.4 ± 1.4 ms, $n = 4$, Fig. 6C). This response was reduced in amplitude (peak amplitude of 4.6 ± 0.8 mV, $n = 4$) from that seen in the down-state but was clearly detectable. The reduced amplitude of the depolarization is likely to be due to a combination of factors such as a lower input resistance due to active excitatory inputs, the change in driving force as well and the presence of hyperpolarizing inhibition that is enhanced due to its increase in driving force. Thus we expect that a footshock-evoked response, that is almost twice as large as that due to mPFC stimulation (17.1 ± 1.2 mV as compared with 9.8 ± 1.2 mV) would be detectable if it was present during the up-state.

Footshock-related information reaches the BLA via the PoT (Lanuza et al. 2008; Shi and Davis 1999). As we were unable to detect any response to footshocks when cells were in an up-state, we tested the effect of PoT stimulation on BLA neurons. As expected, PoT stimulation in the down-state evoked an up-state (Fig. 4). However, unlike the response to footshock, PoT stimulation in an up-state also evoked a clear response in the BLA (Fig. 6D). This response did not resemble an up-state but rather had a rapid time course similar to mPFC stimulation (Fig. 6C). Thus BLA principal neurons can clearly respond to afferent stimulation during up-states that can be detected at the soma. We suggest that the lack of a footshock response during an up-state is mostly likely due to a block of ascending footshock-related information to the BLA (see DISCUSSION).

DISCUSSION

Neurons in many regions of the CNS display oscillatory activity where the underlying frequency varies with the conscious state of the animal (Buzsaki 2006; Steriade 1997). These oscillations can be seen as regular changes in membrane potential in single neurons as well as in the extracellular field potential, indicating that many neurons cycle in synchrony (Petersen et al. 2003). In particular, slow oscillations (<1 Hz) have been described in the cortex, striatum, and thalamus, where they are apparent during certain types of anesthesia as well as during slow-wave-sleep and periods of quiet wakefulness (Buzsaki and Draguhn 2004; Petersen et al. 2003; Steriade 1997, 2006). In the BLA, slow oscillatory activity has been reported using extracellular recordings during anesthesia and sleep as well as wakefulness (Collins et al. 2001; Paré and Collins 2000; Pare and Gaudreau 1996; Seidenbecher et al. 2003), whereas intracellular recordings reported higher-frequency oscillations that were evoked by membrane depolarization (Pape et al. 2005). These higher-frequency oscillations result from activation of intrinsic voltage-dependent currents (Pape et al. 2005), but how the slow oscillatory activity is evoked is not known.

In this study, we have shown that in urethan-anesthetized animals, principal neurons of the BLA have a slow oscillation in membrane potential, moving from periods near the resting

membrane potential (down-states) to periods at depolarized membrane potentials (up-states). The frequency and temporal dynamics of these oscillations are not altered by voltage-clamping the neuron indicating that intrinsic membrane conductances do not contribute significantly to either the initiation, or maintenance of up-states, but are driven by exogenous synaptic input. While our recordings have been done on young (3–4 wk) rats, slow oscillations have been described in older animals in both cortical (Steriade et al. 1993d) and amygdalar (Collins et al. 2001) regions. Extensive lesion studies have established that slow oscillations in cortical neurons are most likely generated by recurrent excitatory networks within the neocortex (Buzsaki and Draguhn 2004; Petersen et al. 2003; Steriade 2006; Steriade et al. 1993d), and projections from these neurons also drive slow oscillations in thalamic (Steriade et al. 1993a) and striatal (Wilson 1993) neurons. The BLA receives extensive excitatory inputs from many cortical regions including the perirhinal cortex (McDonald 1998). As extracellular field recordings have shown correlated oscillatory activity between the perirhinal cortex and the BLA (Collins et al. 2001), it seems likely that slow oscillations in amygdala neurons are also driven by inputs from these cortical networks.

In BLA neurons, footshocks delivered during a down-state triggered an all-or-none transition to an up-state. Following single shocks to the foot pad, we find that BLA neurons transition into an up-state with a latency of nearly 60 ms. As stimulation of the PoT evoked up-states with a latency of 6 ms, we calculate that the time for impulse propagation from the footpad to the thalamus is ~ 52 – 55 ms. This time delay is consistent with a conduction velocity of ~ 0.5 m s⁻¹, indicating that these response are likely to be due to C-fiber stimulation (Fitzgerald 1985). In a previous study in anesthetized rats, unit recordings have found that footshocks can drive BLA neurons to threshold with a first spike latency of 15–20 ms (Romanski et al. 1993) significantly faster than that seen in our study and more consistent with responses due to stimulation of A-fibers (Fitzgerald 1985). Interestingly, recordings by Romanski et al. (1993) concentrated on the ventrolateral division of the lateral amygdala with few or no recordings in the ventromedial division or the basal amygdala. In contrast, our recordings are largely in the BLA and ventromedial amygdala (Fig. 1), suggesting that there may be a sensory division of somatosensory input to the basolateral complex.

In cortical neurons, sensory stimulation evokes excitatory responses when delivered during the down-state, and these responses, while detectable, are strongly suppressed during the up-state (Petersen et al. 2003; Sachdev et al. 2004). This reduced response to stimulation can also be demonstrated in thalamocortical slices (Watson et al. 2008) and has been attributed to a combination of reduction in input resistance and increased GABAergic inhibition during the up-state (Destexhe et al. 2003; Petersen et al. 2003; Watson et al. 2008). In BLA neurons, somatosensory stimulation delivered during the down-state evoked a full up-state. This response could be reproduced by stimulation of the PoT, indicating that the footshock evoked effect results from activation nociceptive afferents (Shi and Davis 1999). By contrast, delivery of footshocks during an up-state produced no detectable response in either current- or voltage-clamp. This inability to detect a response in the up-state is unlikely to be due to shunting of synaptic inputs (Destexhe and Pare 1999) as stimulation of mPFC during

up-states evoked a robust excitatory response in BLA neurons. Moreover, stimulation of the PoT was able to evoke short-lasting responses in BLA neurons during up-states. These results indicate that oscillating cortical networks send projections to the BLA, and when the network is in the down-state, footshocks can trigger a transition into an up-state. However, it is clear that these networks are largely unresponsive to sensory stimulation when they are active (Petersen et al. 2003; Sachdev et al. 2004), precluding activation of BLA neurons when the network is in the up-state. This failure to respond is likely due to blockade of ascending sensory inputs at the thalamic level, as direct electrical stimulation of the PoT during up-states was clearly able to evoke responses.

Delivery of a single footshock (1 ms) led to the generation of an all-or-none up-state, an effect replicated by a single shock to the posterior thalamus. While this has not been previously shown in vivo, short trains of stimuli to the thalamus can generate prolonged up-states that are accompanied by action potentials in cortical slices in vitro (MacLean et al. 2005). These synaptically evoked up-states also resembled spontaneously occurring up-states and consistently activate the same assemblies of neurons within the cortex (MacLean et al. 2005). Up-states in BLA neurons are driven by synaptic inputs most likely of cortical origin. The similarity between the spontaneous and evoked up-states in BLA neurons suggests that cortical cell assemblies, linked functionally by spontaneous activity, can be synchronized by sensory stimulation. Furthermore, as single cortical afferents are unlikely to be able to drive BLA principal neurons by close to 18 mV (the average amplitude of an up-state in BLA neurons), assemblies of cortical neurons must also project to common targets within the BLA.

The initiation of the up-state by footshock resets the phase of the slow oscillation in single BLA neurons, an effect that can also be seen in extracellular field potentials (not shown), indicating that footshocks effectively synchronize firing of cells in the BLA. Thus a discrete stimulus (1 ms) can trigger a continuous rhythmic and dynamic response within the cortex and amygdala that synchronizes groups of cells for a duration that is several orders of magnitude longer than the stimuli itself. This reset is likely to be an important component in the processing of aversive stimuli by the amygdala. For example, footshock-induced resetting of network activity could function to coordinate activity of the BLA with regions involved in regulating attention. Similarly, resetting the slow oscillation could influence the formation/consolidation of associations between inputs to the BLA and the formation of emotionally salient memories.

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