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Contextual fear conditioning depresses infralimbic excitability

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ABSTRACT

Patients with posttraumatic stress disorder (PTSD) show hypo-active ventromedial prefrontal cortices (vmPFC) that correlate with their impaired ability to discriminate between safe and dangerous contexts and cues. Previously, we found that auditory fear conditioning depresses the excitability of neurons populating the homologous structure in rodents, the infralimbic cortex (IL). However, it is undetermined if IL depression was mediated by the cued or contextual information. The objective of this study was to examine whether contextual information was sufficient to depress IL neuronal excitability. After exposing rats to context-alone, pseudoconditioning, or contextual fear conditioning, we used whole-cell current-clamp recordings to examine the excitability of IL neurons in prefrontal brain slices. We found that contextual fear conditioning reduced IL neuronal firing in response to depolarizing current steps. In addition, neurons from contextual fear conditioned animals showed increased slow afterhyperpolarization potentials (sAHPs). Moreover, the observed changes in IL excitability correlated with contextual fear expression, suggesting that IL depression may contribute to the encoding of contextual fear.

1. Introduction

The increased fear responses in patients with posttraumatic stress disorder (PTSD) are associated with reduced ventromedial prefrontal cortex (vmPFC) activity (Milad et al., 2009; Rougemont-Bücking et al., 2011). However, it is unclear if this vmPFC hypo-activity is caused by the traumatic experience or is present prior to the traumatic experience. Either mechanism could lead to the development of PTSD, since low vmPFC activity is associated with decreased inhibition of the amygdala resulting in hyperactivation of the amygdala and subsequent increased fearful behavior (Milad et al., 2009; Rougemont-Bücking et al., 2011).

Studies done in the rodent homologue to the human vmPFC, the infralimbic cortex (IL) (Koenigs & Grafman, 2009; Milad & Quirk, 2012; Milad, Rauch, Pitman, & Quirk, 2006), found that auditory fear conditioning depresses the excitability of IL neurons (Cruz, López, & Porter, 2014; Santini, Quirk, & Porter, 2008). This mechanism mimics the depressed vmPFC observed in patients with PTSD and demonstrates that aversive learning can depress vmPFC neurons. Interestingly, fear conditioning does not induce synaptic depression in IL (Pattwell et al., 2012; Sepulveda-Orengo, Lopez, Soler-Cedeño, & Porter, 2013) indicating that intrinsic rather than synaptic plasticity is the key determinate of IL excitability after aversive learning. Furthermore, pharmacological manipulation of IL intrinsic excitability is sufficient to reduce conditioned-fear expression (Santini & Porter, 2010; Santini, Sepulveda-Orengo, & Porter, 2012) indicating that the depression is functionally important.

Since our previous studies used auditory fear conditioning (Cruz et al., 2014; Santini et al., 2008), we could not determine whether contextual or cued information was depressing IL excitability. Although IL is more known for its role in the extinction of fear memory (Burgos-Robles, Vidal-Gonzalez, Santini, & Quirk, 2007; Milad & Quirk, 2002; Vidal-Gonzalez, Vidal-Gonzalez, Rauch, & Quirk, 2006), a recent study suggests that IL contributes to the contextual discrimination of fear conditioning memory (Zelikowsky et al., 2013). The depression of IL excitability after fear conditioning could convey contextual information which is key to determining which cues signal danger (Bouton, 2004; Bouton & Bolles, 1979). To examine this possibility, we investigated whether contextual information alone could depress IL excitability by combining a contextual fear conditioning paradigm with whole-cell patch-clamp recordings of IL neurons.

2. Methods

2.1. Contextual fear conditioning

Male Sprague Dawley rats (postnatal day 30 to P45) were group housed on a 12 h light/dark schedule with free access to food and

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water. All procedures were approved by the Institutional Animal Care and Use Committee of the Ponce Health Sciences University. On day 1, the contextual fear conditioned group (COND) was exposed to contextual fear conditioning consisting of a three minute exploration phase followed by three 0.7 mA scrambled footshocks (0.5 s in duration) with two minutes between shocks. A control group of rats (EXPOSURE) received the same contextual exposure time as the COND group but without shocks. An additional control group, the pseudoconditioned group (PSEUDO), received three consecutive shocks and was immediately removed from the conditioning context. On day 2, all groups of rats were placed in the conditioning context for two minutes and tested for contextual fear memory.

2.2. Patch-clamp recordings in prefrontal slices

Animals were sacrificed immediately after the test on day 2 and whole-cell recordings of IL neurons in prefrontal slices were done as previously described (Santini et al., 2008). Prefrontal slices were maintained at room temperature (21–23 °C) in artificial cerebrospinal fluid (ACSF) at least 1 h before experiments. The composition of the incubating and recording ACSF was the following: 126 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_4, 1 mM $MgSO_4$, 26 mM $NaHCO_3$, 20 mM glucose, and 2 mM $CaCl_2$ and bubbled with 95% O_2 and 5% CO_2. Whole-cell recordings of layer V pyramidal neurons were done blind with respect to group assignment using $KMeSO_4$-based internal solution: 150 mM $KMeSO_4$, 10 mM KCl, 0.1 mM EGTA, 10 mM HEPES, 0.3 mM GTP, and 0.2 mM ATP (pH 7.3, 291 mOsm). Neuronal responses to depolarizing current pulses were measured from a holding potential of −70 mV and were not corrected for the junction potential of 9 mV. Responses were filtered at 4 kHz, digitized at 10 kHz, and saved using pCLAMP9 (MultiClamp 700A, Axon Instruments, Union City, CA). As shown in Table 1, all groups had similar series resistance (R_a) and input resistance (R_{in}), which was measured from a 5 mV, 50 ms depolarizing pulse in voltage-clamp mode at a holding of −60 mV. The excitability of IL neurons was determined from responses to 800 ms depolarizing current pulses ranging from −40 to 350 pA at 10 pA increments with an intertrial interval of 5 s. The number of action potentials evoked by each current step was counted from individual responses. Fast afterhyperpolarizing potentials (fAHPs), medium afterhyperpolarizing potentials (mAHPs), and slow afterhyperpolarizing potentials (sAHPs) were measured as previously described (Santini et al., 2008). The amplitude of the fAHPs was measured in the second and third current evoked spikes within the 800 ms pulse and was assessed by subtracting the voltage at the peak of the fAHP from the threshold potential for spike initiation. The mAHPs and sAHPs were measured after the end of the 800 ms current pulse. The mAHP was measured as the peak of the AHP, and the sAHP was measured as the average potential during a 50 ms period beginning 280 ms after the end of the 800 ms depolarizing pulse (Sah and Louise Faber, 2002) in traces with the same number of spikes (2 spikes) (Santini et al., 2008). The first interspike interval (ISI), threshold, and fAHP were measured from the traces that showed the maximum number of evoked spikes. All recorded neurons were filled with biocytin and post hoc confirmed to be IL pyramidal neurons.

Table 1
Electrophysiological properties of IL neurons.

	PSEUDO	EXPOSURE	COND
E rest (mV)	−61 ± 1*	−56 ± 1	−55 ± 1
Threshold (mV)[a]	−39 ± 0.7*	−36 ± 1	−35 ± 1
R_{in} (MΩ)	196 ± 17	176 ± 10	191 ± 13
R_a (MΩ)	14 ± 1	13 ± 0.6	14 ± 0.6
Rheobase (pA)	135 ± 18	136 ± 14	164 ± 13
mAHP (mV)[b]	−3.5 ± 0.3	−4.5 ± 0.4	−4.8 ± 0.4
fAHP (mV)[a]	−12.9 ± 1.1	−12.9 ± 0.8	−11.4 ± 0.9
ISI (ms)[a]	43 ± 7	52 ± 10	107 ± 34

* One-way ANOVA showed a main effect of group in E rest ($F(2,55) = 7.15$, $p = 0.0017$) and threshold ($F(2,55) = 4.23$, $p = 0.02$). Post-hoc comparisons indicated that the PSEUDO group had a more negative E rest than the EXPOSURE ($p = 0.016$) and COND ($p = 0.0015$) groups, and a more negative threshold than the COND ($p = 0.015$) group.

[a] Measured in the trace that showed the maximum number of spikes.

[b] In all groups, the mAHP was measured in traces that showed 2 spikes.

2.3. Statistical analysis

Context conditioned fear was measured as the percent of time spent freezing during one-minute intervals after each shock during training and after placing the rat into the conditioning context on day 2 (FreezeScan, Clever Systems). Behavioral data were compared with repeated measures ANOVA (STATISTICA, Statsoft, Tulsa, OK) followed by Tukey HSD post hoc test. The electrophysiological data were analyzed using Clampfit (Axon Instruments, Union City, CA) and were compared with one-way ANOVA or Kruskal–Wallis test. Following a significant main effect with a one-way ANOVA or Kruskal–Wallis test, post hoc tests were performed with Tukey HSD test or Dunn test (sAHPs), respectively. Nonparametric Kruskal–Wallis test was selected for analyzing sAHPs since data showed skewness in its distribution. Chi-square test was utilized to compare the cumulative percentage of cells versus the maximum number of evoked spikes or the magnitude of the sAHP in each group. Values are reported as the mean ± the standard error of the mean (S.E.M.).

3. Results

Three experimental groups were designed to test whether contextual fear conditioning affects IL intrinsic excitability (Fig. 1A). On day 1 the COND group ($n = 5$) received contextual fear conditioning, the EXPOSURE group ($n = 7$) received contextual exposure with no shock presentations, and the PSEUDO group ($n = 3$) received 3 consecutive shocks and was immediately removed from the conditioning chamber. All animals were tested for contextual fear on day 2 and immediately sacrificed. As expected (Fig. 1B), a repeated measures ANOVA showed a significant main effect ($F(2,12) = 40.63$, $p < 0.001$) and post hoc analysis confirmed that rats from the COND group had significant higher levels of freezing to the conditioning context on day 2 compared to rats from the EXPOSURE and PSEUDO groups ($p < 0.05$). The difference in fear expression among groups indicates that only the COND group had acquired fear to the context.

3.1. Contextual fear conditioning depresses the intrinsic excitability of IL neurons

After the test for recall of contextual fear on day 2, we sacrificed the rats and assessed the intrinsic excitability of IL pyramidal neurons using whole-cell current-clamp recordings in prefrontal brain slices. Consistent with the representative responses to a 310 pA depolarizing pulse in single neurons from each group (Fig. 2A), neurons from the COND group ($n = 22$) fired significantly fewer spikes in response to depolarizing current steps compared to neurons from the EXPOSURE ($n = 22$) and PSEUDO ($n = 14$) groups (Fig. 2B). One-way ANOVAs revealed a main group effect at each step from 280 to 350 pA (280 pA: $F(2,55) = 4.12$, $p = 0.021$; 290 pA: $F(2,55) = 3.39$, $p = 0.041$; 300 pA: $F(2,55) = 3.68$, $p = 0.032$; 310 pA: $F(2,55) = 4.30$, $p = 0.018$; 320 pA: $F(2,55) = 4.06$, $p = 0.023$; 330 pA: $F(2,55) = 3.38$, $p = 0.041$; 340 pA: $F(2,55) = 3.91$, $p = 0.026$;