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Persistent, generalized hypersensitivity of olfactory bulb interneurons after olfactory fear generalization

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ABSTRACT

Generalization of fear from previously threatening stimuli to novel but related stimuli can be beneficial, but if fear overgeneralizes to inappropriate situations it can produce maladaptive behaviors and contribute to pathological anxiety. Appropriate fear learning can selectively facilitate early sensory processing of threat-predictive stimuli, but it is unknown if fear generalization has similarly generalized neurosensory consequences. We performed *in vivo* optical neurophysiology to visualize odor-evoked neural activity in populations of periglomerular interneurons in the olfactory bulb 1 day before, 1 day after, and 1 month after each mouse underwent an olfactory fear conditioning paradigm designed to promote generalized fear of odors. Behavioral and neurophysiological changes were assessed in response to a panel of odors that varied in similarity to the threat-predictive odor at each time point. After conditioning, all odors evoked similar levels of freezing behavior, regardless of similarity to the threat-predictive odor. Freezing significantly correlated with large changes in odor-evoked periglomerular cell activity, including a robust, generalized facilitation of the response to all odors, broadened odor tuning, and increased neural responses to lower odor concentrations. These generalized effects occurred within 24 h of a single conditioning session, persisted for at least 1 month, and were detectable even in the first moments of the brain's response to odors. The finding that generalized fear includes altered early sensory processing of not only the threat-predictive stimulus but also novel though categorically-similar stimuli may have important implications for the etiology and treatment of anxiety disorders with sensory sequelae.

1. Introduction

Generalization of learned fear is an adaptive mechanism that promotes flexible responding to novel but potentially dangerous situations. Learned fear is studied through classical conditioning paradigms that pair a neutral sensory stimulus such as an odor (the conditioned stimulus, CS) with an aversive stimulus such as a shock (the unconditioned stimulus, US) that elicits an unconditioned defensive response. After conditioning, the defensive response will be elicited by the CS but will also generalize to non-threatening stimuli related to the CS (Dunsmoor, Mitroff, & LaBar, 2009; Dunsmoor, White, & LaBar, 2011; Lissek et al., 2008; Rajbhandari, Zhu, Adling, Fanselow, & Waschek, 2016; Resnik & Paz, 2015; Resnik, Sobel, & Paz, 2011). Generalization of conditioned fear typically falls off gradually as stimuli become more dissimilar to the CS along continuous, physical axes, such as tone frequency (Aizenberg & Geffen, 2013; Resnik & Paz, 2015; Resnik et al., 2011) or geometric size (Lissek et al., 2008, 2010, 2014), though generalization also can occur within conceptual categories (Dunsmoor & Murphy, 2015; Dunsmoor, White et al., 2011). Fear overgeneralization occurs when cues that do not actually predict dangerous outcomes evoke maladaptive fearful or defensive responses (van Meurs, Wiggert, Wicker, & Lissek, 2014). Patients with anxiety disorders exhibit broadened fear generalization compared to healthy controls (Lissek et al., 2010, 2014), suggesting that overgeneralization of learned fear may contribute to the etiology or maintenance of pathological fear (Dunsmoor & Paz, 2015; Resnik & Paz, 2015).

Most research addressing the neurobiology of conditioned fear has focused on structures such as the amygdala, hippocampus, and prefrontal cortex (Dunsmoor & Paz, 2015; Jovanovic & Ressler, 2010; LeDoux, 2000; Maren & Quirk, 2004; Phelps & LeDoux, 2005). However, fear learning also induces dramatic changes in sensory regions (Bakin & Weinberger, 1990; Chen, Barnes, & Wilson, 2011; Fletcher, 2012; Gdalyahu et al., 2012; Li, Howard, Parrish, & Gottfried, 2008; McGann, 2015; Quirk, Armony, & LeDoux, 1997; Weinberger, 2007), including CS-specific hypersensitivity in primary sensory neurons (Dias & Ressler, 2014; Jones, Choi, Davis, & Ressler, 2008; Kass, Rosenthal, Pottackal, & McGann, 2013). This plasticity can have explicitly sensory consequences, such as lowered detection thresholds (Ahs, Miller,

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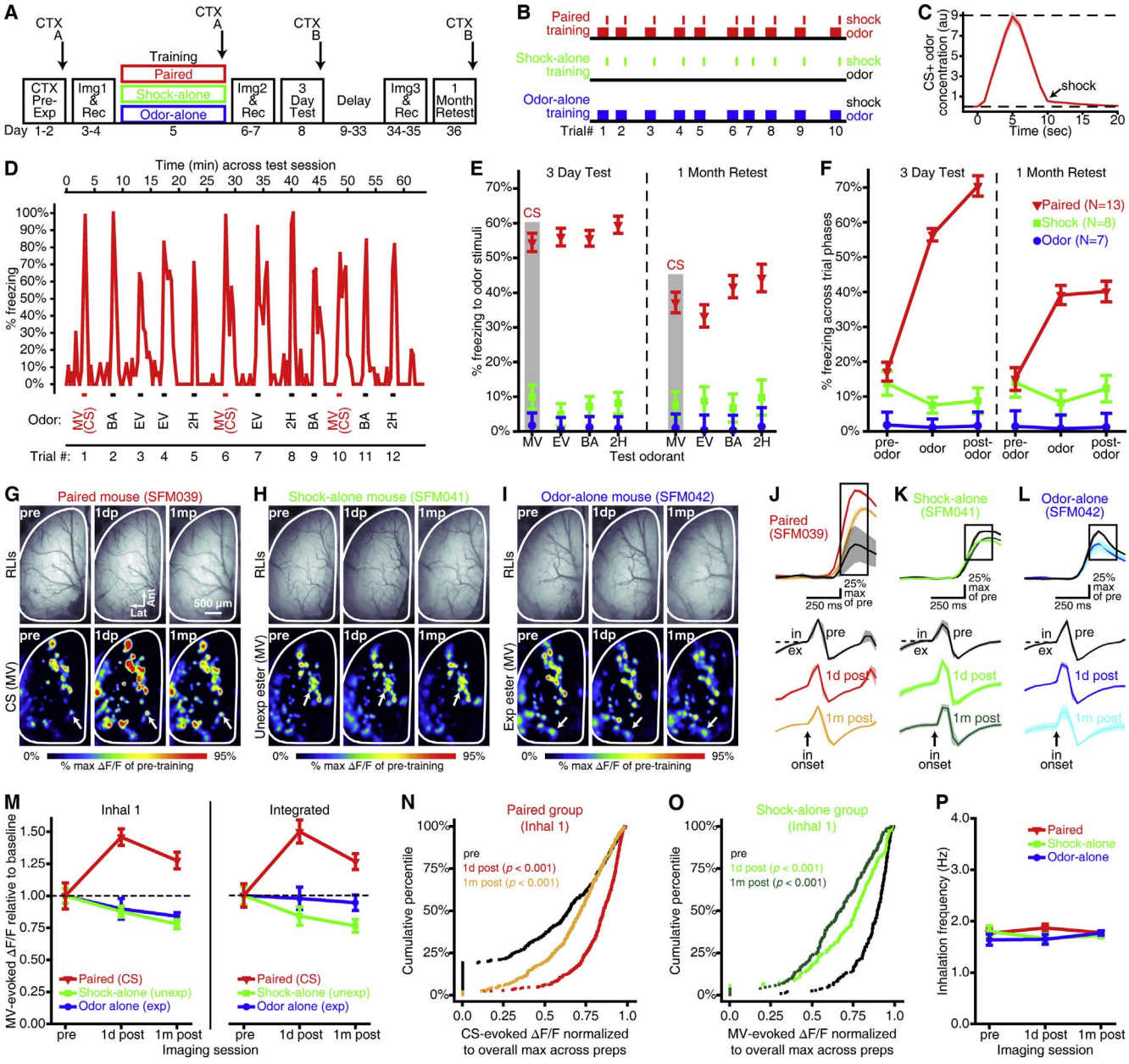

Fig. 1. Olfactory fear conditioning results in a long-lasting, generalized fear response and an enhancement of CS-evoked PG interneuron activity. (A) Experimental timeline. CTX Pre-Exp, context pre-exposure; Img, imaging; Rec, recovery. (B) Sample paired (top), shock-alone (middle), and odor-alone (bottom) training protocols. (C) Mean ± SEM CS concentration (in arbitrary units, au) across 10 paired trials. Dashed lines: 9 au, target concentration; 0 au, odor-free. (D) Representative freezing histogram that is plotted against the protocol from that paired subject's 3-day test session. Tick marks (bottom) are labeled to show odor presentations (MV/CS, EV, BA, and 2H) during all 12 trials. (E) Paired subjects exhibited odor-evoked freezing that generalized across odors, whereas comparatively little odor-evoked freezing was observed in either control group. These data are collapsed across odors in F and shown as the "odor" trial phase. (F) Freezing data are pooled across all 12 trials and separated by trial phase to show relative increases and decreases in freezing that were evoked by odor presentations in the paired and shock-alone groups, respectively. (E) and (F) show group means ± SEMs from the 3-day (left) and 1-month (right) tests. (G–I) Representative resting light images (RLIs) and pseudocolored difference maps from 1 day before (pre), 1 day after (1dp), and 1 month after (1mp) paired (G), shock-alone (H), or odor-alone (I) training. (J–L) Mean ± SEM fluorescence (top; ΔF/F) and piezosensor (bottom: in, inhalation; ex, exhalation) records correspond with the glomerular callouts in G–I. All records are aligned relative to the first inhalation after odor onset. Boxed regions indicate the frames that were used for inhalation 1-evoked activity maps (G–I) and analyses (M, left and N–O). Traces and activity maps (G–L) are averaged across 3–6 trials of MV, which was the CS for paired subjects, an unexposed ester for shock-alone subjects, and the exposed ester for odor-alone subjects. (M) Mean ± SEM CS-evoked activity during the first inhalation (left) and integrated across the entire odor presentation (right) plotted relative to baseline (dashed line) across imaging sessions. (N and O) Cumulative frequency histograms pooling glomeruli across subjects. CS-evoked PG cell activity was enhanced after paired training (N) but reduced after shock-alone training (O). *P* values are compared with pre-training baseline. (P) Mean ± SEM inhalation frequency did not differ between groups or across imaging sessions.

Gordon, & Lundstrom, 2013; Parma, Ferraro, Miller, Ahs, & Lundstrom, 2015) or altered perceptual discrimination abilities (Aizenberg & Geffen, 2013; Chen et al., 2011; Fletcher & Wilson, 2002; Li et al., 2008; Resnik & Paz, 2015; Resnik et al., 2011), but it may also be important for non-sensory functions like recruiting attention or triggering defensive behavior (McGann, 2015). Fear generalization has been presumed to reflect changes in higher-order structures responding to sensory inputs (Ciocchi et al., 2010; Dunsmoor & Paz, 2015; Dunsmoor, Prince, Murty, Kragel, & LaBar, 2011; Ghosh & Chattarji, 2015; Resnik & Paz, 2015), but sensory regions might be responsible for labeling CS-resembling stimuli as potentially threatening (Aizenberg & Geffen, 2013; Chen et al., 2011; Krusemark & Li, 2012; Miasnikov & Weinberger, 2012). Psychopathologies like post-traumatic stress disorder (PTSD) include alterations in attentional and neurosensory processing (Bryant