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Enhanced Olfactory Sensory Perception of Threat in Anxiety: An Event-Related fMRI Study

Elizabeth A. Krusemark · Wen Li

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Abstract The current conceptualization of threat processing in anxiety emphasizes emotional hyper-reactivity, which mediates various debilitating symptoms and derangements in anxiety disorders. Here, we investigated olfactory sensory perception of threat as an alternative causal mechanism of anxiety. Combining an event-related functional magnetic resonance imaging paradigm with an olfactory discrimination task, we examined how anxiety modulates basic perception of olfactory threats at behavioral and neural levels. In spite of subthreshold presentation of negative and neutral odors, a positive systematic association emerged between negative odor discrimination accuracy and anxiety levels. In parallel, the right olfactory primary (piriform) cortex indicated augmented response to subthreshold negative (vs. neutral) odors as a function of individual differences in anxiety. Using a psychophysiological interaction analysis, we further demonstrated amplified functional connectivity between the piriform cortex and emotion-related regions (amygdala and hippocampus) in response to negative odor, particularly in anxiety. Finally, anxiety also intensified skin conductance response to negative (vs. neutral) odor, indicative of potentiated emotional arousal to subliminal olfactory threat in anxiety. Together, these findings elucidate exaggerated processing of olfactory threat in anxiety across behavioral, autonomic physiological, and neural domains. Critically, our data emphasized anxiety-related hyper-sensitivity of the primary olfactory cortex and basic olfactory perception in response to threat, highlighting neurosensory mechanisms that may underlie the deleterious symptoms of anxiety.

Keywords Olfaction · Threat · fMRI · Piriform · Perception · Anxiety

Adept threat analysis affords critical ecological advantage to virtually all organisms, but excessive threat reactivity could lead to devastating disabilities in humans, often observed in anxiety disorders. A wealth of anxiety research has identified a constellation of cognitive biases as a result of exaggerated threat responsivity (Mathews and MacLeod 1994, 2005). These aberrations are largely categorized into cognitive systems of attention, memory, and interpretation (Mathews and MacLeod 1994). Nevertheless, recent investigations using neuroscientific methods have implicated threat processing anomalies in early sensory perception stages, highlighting the intriguing possibility that threat processing in anxiety deviates from the norm as soon as sensory input registers in the brain.

Several neuroimaging studies identified heightened response in the visual cortex to phobic objects in anxiety (Lipka et al. 2011; Ahs et al. 2009; 2004, Straube et al. 2005; Etkin et al. 2004; Dilger et al. 2003; Paquette et al. 2003). These visual aberrations typically paralleled excessive amygdala response also observed in those studies and were thus interpreted as a byproduct of elevated emotional responses, which exaggerated visual response via amygdala reentrant projections to the visual cortex. Temporally precise electrophysiological data from our laboratory and others' have demonstrated selective threat processing in the visual cortex as early as 100 ms, isolating this visual hyper-responsivity to threat to basic visual

E. A. Krusemark (✉) · W. Li (✉)
Department of Psychology, Brogden Hall,
University of Wisconsin-Madison,
Madison, WI 53706, USA
e-mail: ekrusemark@wisc.edu
e-mail: wenli@psych.wisc.edu

W. Li
Waisman Center, University of Wisconsin-Madison,
Madison, WI, USA

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analysis (Krusemark and Li 2011; Mueller et al. 2009; Holmes et al. 2008; Li et al. 2008a, b). This early rise of perceptual bias in anxiety appears to precede the known amygdala response latency to threat (>100 ms; Krolak-Salmon et al. 2004; Oya et al. 2002), therefore accentuating a sensory hypothesis of threat bias in anxiety—sensory encoding of threat is distorted in anxiety, which directly and indirectly influences downstream operations, resulting in various anxiety symptoms.

Evidently, this body of work has predominantly examined visual analysis, yet it is unclear how other sensory processing of threat is altered by anxiety. Of particular relevance here are potential olfactory biases to threat in anxiety, given that smells evoke powerful emotional responses in humans (Yeshurun and Sobel 2010; Bensafi et al. 2002; Schiffman 1974), and the olfactory system is intimately related to the limbic emotion system (Zald and Pardo 2000; Carmichael et al. 1994). In nonhuman animals, the olfactory system is principally involved in effectively detecting, locating, and potentially identifying predators in the in the environmental surround (Kats and Dill 1998). In fact, the olfactory-mediated defense system is so prominent that the mere presence of predator odors could evoke potent fear and anxiety responses in the animal (Blanchard and Blanchard 1988), thereby accentuating a unique association between olfactory threat processing and anxiety that could serve as a viable model of anxiety in humans. We therefore posit that, in humans, detection of a particular malodor may signal danger of a noxious airborne substance or a decaying object that carries disease, and excessive anxious response to such threats could underlie phobias and obsessive compulsive disorders. Therefore, research in human olfactory perception of danger signals would provide significant insights to potential causal mechanisms of anxiety. Even more importantly, those previous studies rarely targeted sensory perception specifically, leaving it still obscure how anxiety distorts sensory perception of threat and consequently, behavioral detection, and discrimination of threat.

To this end, combining assessment of state-level anxiety, psychophysical testing, and event-related functional magnetic resonance imaging (fMRI) techniques, we examined behavioral and neural correlates of sensory perception of threat in anxiety. Importantly, we utilized highly diluted, subthreshold negative odor cues to emphasize anxious hyper-acuity to olfactory threat signals. To evaluate our sensory perceptual model of anxiety, we tested the hypothesis that primary olfactory (piriform) cortex in anxious subjects would exhibit heightened response and strengthened functional connectivity to emotion regions in the presence of negative odors, paralleled by sharpened discrimination of negative from neutral odors. Functional connectivity analysis affords inference of functional coupling between two regions in a given condition. Using this methodology, we examined whether olfactory sensory processing in piriform cortex shows greater coactivation with emotional responses in limbic regions as a function of negative odor stimulation and anxiety.

Method

Participants

A total of 16 healthy subjects (mean age, 20.93; range, 18–28 years, eight males) with normal sense of smell provided informed consent to take part in the study, which was approved by the University of Madison Institutional Review Board. Two subjects who showed significant above-chance odor discrimination were excluded from further analysis, leaving 14 subjects (eight males) in the final sample.

Stimuli

We selected four neutral odorants (acetophenone, guaiacol, anisole, eugenol) and two negative odorants (trimethylamine, TM; valeric acid, VA) to form three odor conditions—neutral pure odor, neutral odor mixture, and negative odor mixture. The mixtures contained a primary neutral odor (at 5% dilution in mineral oil) and a highly diluted neutral (for neutral mixture) or negative odor (for negative mixture). These odors (TM and VA) at suprathreshold concentrations have been repeatedly demonstrated in our lab as strongly aversive odors (Li et al. 2007, 2010). The concentrations for the secondary odorants ranged from 0.00015% to 0.001% (see Fig. 1a), which were intended to render these odorants as subthreshold components of the mixtures. As illustrated in Fig. 1a, these six odorants constituted two sets of combinations that were counterbalanced across subjects. As such, the negative and neutral mixtures contained the same neutral base odorants, differing only in the weak component of negative versus neutral odorants.

Procedure and Analysis

Pretesting: Odor Discrimination Task

Individuals first underwent a triangular odor discrimination task outside the scanner. In each trial, participants were presented with three bottles (two contained the same pure base odorant and the other bottle, a neutral or negative mixture). Participants were instructed to choose the bottle containing the odor mixture. There were 20 trials (ten each for negative and neutral mixtures, randomly presented). Triangular discrimination accuracy was submitted to *t* tests and correlational (nonparametric Spearman) analyses.

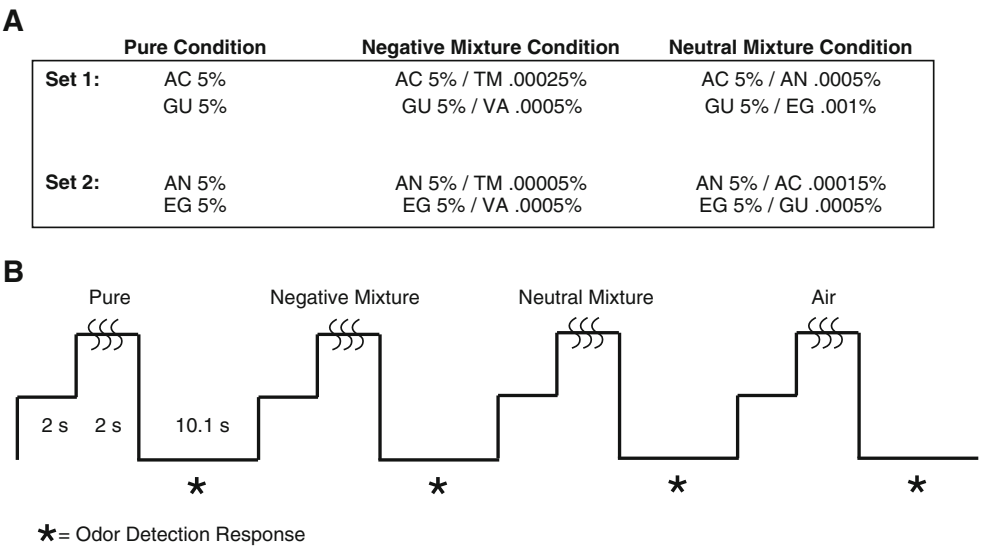

Fig. 1 Olfactory stimuli (**a**) and experimental paradigm (**b**). *AC*: acetophenone; *GU*: guaiacol; *AN*: anisole; *EG*: eugenol; *TM*: trimethylamine; *VA*: valeric acid

fMRI Experimental Paradigm

Participants then completed an odor detection task in the scanner (see Fig. 1b). At the beginning of each trial, a visual cue with "Get Ready" appeared for 2 s to prepare subjects for odor delivery, followed by another cue reading "Sniff Now", presented 200 ms before odor delivery of 2 s. Participants then responded with a button box to indicate the presence or absence of odor. Odors and room air were delivered using an MRI-compatible eight-channel computer-controlled olfactometer (airflow set at 1.5 L/min), permitting rapid odor delivery in the absence of tactile, thermal, or auditory confounds (Li et al. 2006, 2008c). Stimulus presentation and response recording were executed using Cogent software (Wellcome Dept., London, UK) as implemented in Matlab (Mathworks, Natick, MA). Each of the three odor conditions was presented for 30 times and the room air condition 15 times, in pseudo-random order such that no condition was repeated over three trials in a row. Trials recurred with a stimulus onset asynchrony of 14.1 s.

Physiological Measurement

During scanning, physiological measures including skin conductance response (SCR) and respiration were acquired in all participants using a BioPac MP150 system and accompanying AcqKnowledge software (BioPac Systems, CA). SCR was recorded through two Ag-Cl electrodes placed on the second and third toes of a subject's right foot. Offline data analysis of SCR waveforms was conducted in Matlab, after low-pass filtering (0.5 Hz) to eliminate MRI scanning artifacts. Evoked SCR responses were characterized by the maximum of the SCR deflection in the interval between 1 and 6 s after stimulus onset. Similar to prior methods (Li et al. 2008c; Flykt et al. 2007), only trials with a minimal evoked deflection of 0.015 μS were included in the SCR analysis. Skin conductance values were trimmed to exclude responses beyond two standard deviations from the individual mean response and square-root-transformed to normalize the data.

Respiration was measured using a breathing belt affixed to the subject's chest to record abdominal or thoracic contraction and expansion. Subject-specific sniff waveforms were baseline-adjusted by subtracting the mean activity in the 1,000 ms preceding sniff onset and then averaged across each condition. Sniff inspiratory volume, peak amplitude, and latency to peak were computed for each condition in Matlab. Both SCR and respiratory parameters were later entered into repeated-measures ANOVAs for statistical analysis.

Anxiety Assessment

Upon completion of the odor detection task and while still in the scanner, subjects rated their current level of anxiety using the Subjective Units of Distress Scale (Wolpe and Lazarus 1966) on an analogue scale from 0 (the most relaxed, calm you have ever been) to 100 (extremely anxious and distressed; the most anxious you have ever been). This measure is commonly used in clinical settings to assess online self-reports of anxiety and discomfort (Muhlberger et al. 2001; Edelman and Chambless 1993). Here, we used this measure to reflect state anxiety of the subjects while performing the task (we avoided self-report of anxiety earlier in order to prevent possible confounds related to assessment and reporting of anxiety).

fMRI Image Acquisition

Gradient-echo T2-weighted echoplanar images (EPI) were acquired with blood-oxygen-level-dependent (BOLD) contrast on a 3 T GE MR750 MRI scanner, using an eight-channel head coil with sagittal acquisition. Imaging parameters were TR/

TE=2350/20 ms; flip angle=60°; field of view, 240 mm; slice thickness 2 mm; gap 1 mm; in-plane resolution/voxel size, 1.72×1.72 mm; and matrix size, 128×128. A total of 574 volumes was obtained over the experimental session. A high-resolution T1-weighted anatomical scan was acquired after functional scanning at a resolution of $1\times1\times1$ mm^3. Finally, a field map was acquired with a gradient echo sequence, which was coregistered with EPI images to correct EPI distortions due to susceptibility.

fMRI Data Preprocessing

Imaging data were preprocessed using Analysis of Functional NeuroImages (Cox 1996). After the first six "dummy" volumes were discarded to permit T1 relaxation, images were slice-time-corrected and spatially realigned to the first volume of the session, followed by field map correction. The output EPIs were then spatially normalized to a standard EPI template in SPM8 (www.fil.ion.ucl.ac.uk/spm8). Unlike conventional normalization, we applied Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (Ashburner 2007), implemented in SPM8 as a preferable approach (relative to conventional normalization) to achieve more precise spatial registration. Normalized EPI images were resliced to 2×2×2 mm voxels and smoothed with a 6-mm full-width half-maximum Gaussian kernel.

Conventional fMRI Data Analysis

The preprocessed event-related fMRI data were then analyzed in SPM8 using the general linear model (GLM). Four vectors of onset times were created, corresponding to the three odor conditions and the air condition. These vectors were coded as delta functions and convolved with a canonical hemodynamic response function (HRF) to form four event-related regressors of interest. Condition-specific temporal and spatial derivatives of the HRF were also included to allow for such variations in the HRF. Six movement-related vectors (derived from spatial realignment) were included as regressors of no interest to account for motion-related variance. The data were high-pass filtered (cut-off, 128 s), and an autoregressive model (AR1) was applied.

Model estimation yielded condition-specific regression coefficients (β values) in a voxel-wise fashion for each subject. In a second step (a random effects analysis), subject-specific contrasts of these β values were entered into one-sample t tests, resulting in group-level statistical parametric maps of the T statistic (SPM). Activations were reported in a set of a priori regions of interest (ROIs) consisting of olfactory sensory and emotion regions: piriform cortex (anterior and posterior), amygdala, hippocampus, insula, and orbitofrontal cortex (OFC) (Gottfried and Zald 2005; Zelano and Sobel 2005; Carmichael et al. 1994). We note this limited set of regions would provide a layer of safeguard against "voodoo correlations" with the state anxiety measure (Vul et al. 2009). Activity in the piriform cortex was corrected for multiple comparisons across small volumes of interest (SVC), based on anatomical masks assembled in MRIcro (Rorden and Brett 2000) and drawn on the mean structural T1 image, with reference to a human brain atlas (Mai et al. 1997). Significance level was set at $p=0.05$ using a family wise error correction. For other regions, significance level was set at $p=0.001$, uncorrected. All coordinates reported correspond to Montreal Neurological Institute space.

Two primary contrasts were tested: (1) General odor response: three odor conditions pooled—air. (2) discrimination between negative and neutral subthreshold odors: negative mixture– neutral mixture. Applying a regression analysis at the group level, this contrast was then regressed on anxiety ratings to assess how this differentiation between negative and neutral mixtures correlated with individual levels of state anxiety, indicative of olfactory threat sensitivity in anxiety.

Psychophysiological Interaction (PPI) Analysis

To test our prediction that the crosstalk between olfactory and emotional regions (anatomically densely interconnected; Carmichael et al. 1994) would be enhanced in the presence of negative odors, especially in anxiety, we ran a PPI analysis (Friston et al. 1997) to estimate the functional connectivity between primary olfactory cortex (bilateral posterior piriform cortex (PPC); set as source regions) and emotion regions (amygdala, hippocampus, and orbitofrontal cortex; set as target regions). We utilized PPI as a method of functional connectivity analysis to directly assess how olfactory sensory processing of threat odors in the piriform cortex is coupled with emotional responses in the limbic network. The bilateral PPC were defined anatomically by the PPC ROI described above and then functionally constrained by the contrast of odor > air ($p<0.30$ uncorrected; average functional ROI size=86, and 75 voxels in right and left PPC). (Note that the threshold was adjusted to $p<0.50$ in two subjects with <30 suprathreshold voxels at $p<0.30$). A GLM was estimated with three regressors: a physiological regressor for the source region (the first eigenvariate of BOLD-signal time series extracted from all voxels in this region), a psychological regressor for the effect of the experimental context (negative vs. neutral odor mixture), and the PPI regressor that represented the interaction between the first two regressors. The six movement parameters were also included as regressors of no interest. Subject-specific contrasts of the PPI regressor were then entered into a group-level t test. Given our hypothesis of increased connectivity between olfactory and emotional regions in the context of negative odors, we only conducted the positive PPI contrast, thereby identifying regions showing a significant increase in functional coupling with PPC during

the negative versus neutral odor conditions. Statistical threshold for PPI analysis was set at $p<0.001$, uncorrected.

Results

Behavior: Odor Discrimination Triangular Task

As indicated in Fig. 2a–b, no subject showed supra-threshold accuracy in either the negative or the neutral triangular test (binomial tests in individual subjects—t's <1.81, p's>.10). At the group level, a one-sample t test further confirmed the subthreshold nature of the secondary odors, indicating overall chance-level performance in triangular discrimination for both negative and neutral mixtures [M (SD)=0.34 (0.19) for negative mixture and 0.34 (0.15) for neutral mixture; chance= 0.33], $t(13)$'s <0.14, p's>.89. Remarkably, however, we observed a positive correlation between differential accuracy (negative– neutral mixture) and anxiety [Spearman's rho, r= 0.57, p=0.03; Fig. 2c]. These results thus confirmed the subthreshold nature of mixture discrimination, but

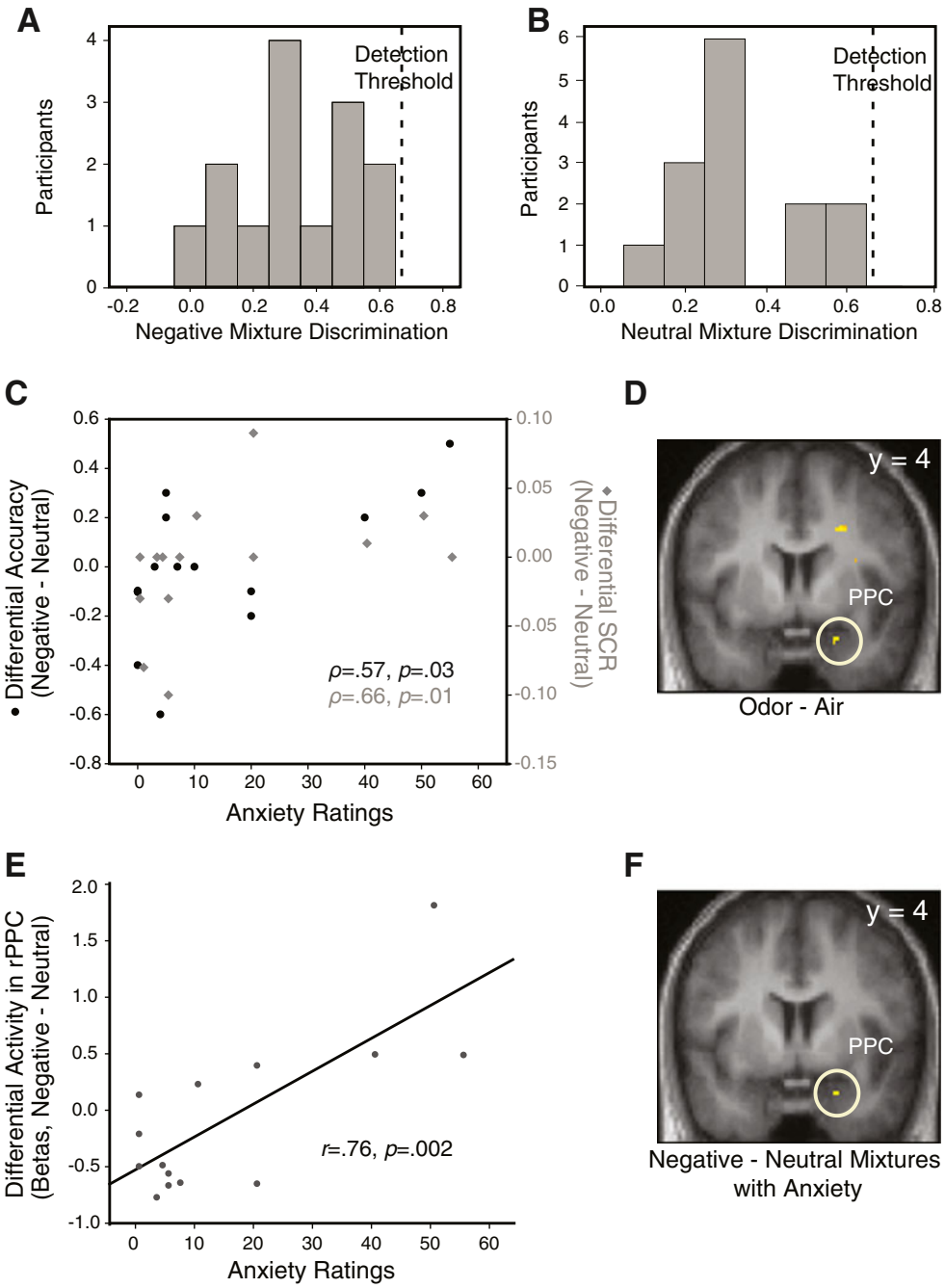

Fig. 2 Subthreshold affective odor response modulated by state anxiety. **a**, **b** Histograms for triangular discrimination accuracy: subthreshold negative and neutral odor discrimination. **c** Differential (negative–neutral mixtures) discrimination accuracy (*left axis*) and SCR (*right axis*) as a function of state anxiety. **d** General odor response in right PPC (20, 4, −22; Z=3.36). **e**–**f** Differential neural response correlated with state anxiety in right PPC (22, 4, −24; Z=3.13). Activations superimposed on the averaged T1 image (display threshold, $p<0.005$ uncorrected)

emphasized a modulatory effect of anxiety on subliminal perception of negative odors.

Physiological Results

Next, we examined the emotional effect of olfactory threat by analyzing physiological arousal during the main experiment. Similar to the triangular data, while the simple difference in SCR between negative and neutral mixtures was not significant [$t(13)=-0.74$, $p=0.47$], there was a positive correlation between differential SCR and anxiety ratings (Spearman's $r=0.66$, $p=0.01$; Fig. 2c). This index thus revealed reliable affective response to subliminal olfactory threat that increased as a function of anxiety.

Respiratory parameters were also examined, which indicated that the odor conditions did not affect sniff volume, peak amplitude, or latency, independently or interactively with anxiety, F's <1.40, p's>0.26. Thus, we confirmed that subjects made constant sniffs throughout the experiment per our instructions and accordingly excluded sniff-related confounds in the reported effects.

Conventional fMRI Results

We first established a general olfactory response via the contrast of odors (all three odor conditions combined) versus air. Odor (vs. air) evoked significant response in the right PPC (*x, y, z*=20, 4,−22; $Z=3.36$, $p=0.03$, SVC; Fig. 2d), conforming to its known role as the primary olfactory cortex in basic olfactory registration (Li et al. 2008c; Gottfried 2006; Li et al. 2006; Zelano and Sobel 2005). The contrast between negative and neutral subthreshold odor mixtures, however, failed to indicate significant differential activity in any of the a priori emotional or olfactory regions (p's>.01, uncorrected). Nevertheless, analogous to the behavioral and physiological findings, we uncovered anxiety-dependent response differentiation in the right PPC, exhibiting a positive correlation between threat-evoked activity (negative–neutral mixtures) and anxiety levels (22, 4,−24; $Z=3.13$, $r=.76$, $p=0.058$, SVC, Fig. 2e–f). Again, the other a priori regions failed to manifest such an association (p's>.10).

PPI Results

Next, we examined whether this minute, subthreshold level of negative olfactory input could magnify the connectivity between the primary olfactory cortex and the emotional circuitry (i.e., the amygdala, hippocampus, and OFC). Using the right PPC as the seed region, a PPI analysis revealed that negative (vs. neutral) mixtures caused a significant increase in the connectivity between the right PPC and the right amygdala and right hippocampus (28, 0,−32; $Z=3.08$, $p<0.001$ uncorrected, and 24,−12, −22; $Z=3.66$, $p<0.001$, uncorrected, respectively; Fig. 3a).

Using the left PPC as the seed region, another PPI analysis yet did not yield a significant interaction effect in any of the hypothesized limbic regions, in contrast to the right-PPC PPI analysis. Nevertheless, a subject-wise regression analysis indicated a positive correlation between subjects' anxiety ratings and the heightened connectivity in negative (vs. neutral) odor context between left PPC and bilateral amygdala (L/R: −24,−2,−12/18,−4, −10; Z's=3.33/3.58, r's=.79/.82, p's<.001, uncorrected; Fig. 3b–c). Therefore, connectivity between left olfactory sensory cortex and the emotion system showed an anxiety-dependent enhancement in response to negative (vs. neutral) odor.

Discussion

Here we demonstrated enhanced processing of olfactory threat in anxiety across behavioral, autonomic physiological, and neural domains. Behavioral accuracy in discriminating negative odors increased systematically with state anxiety, which was paralleled by preferential response in the primary olfactory (piriform) cortex to negative odor mixtures in anxious (vs. non-anxious) subjects, indicative of remarkable olfactory perceptual acuity to threat in anxiety. Skin conductance response to negative odors was intensified by anxiety, suggesting that anxiety also heightened emotional arousal to olfactory threat cues. Relatedly, we observed intensified functional coupling between olfactory and emotional circuits in response to negative odors, especially in anxious individuals. Therefore, despite the fact that the aversive odor components, highly diluted and masked by neutral odorants, evaded reliable discrimination, these minute olfactory threats could still instigate subliminal perceptual and emotional processing, varying as a function of anxiety.

Binomial tests among individual subjects, along with a group-level t test, confirmed chance-level odor discrimination, and thus the subthreshold nature of the secondary odor components. By comparing these weak, imperceptible elements (negative vs. neutral), we were capable of assessing the extent of olfactory acuity to threat cues in anxiety. Moreover, by precluding conscious perception of these stimuli, we managed to specify mechanisms involved in subliminal and by extension, low-level odor analysis. Finally, careful monitoring of sniff parameters allowed us to rule out sniff-related confounds in the reported findings.

Therefore, while echoing previous findings of threat hyperresponsivity based on visual stimulation (1994, Mathews and MacLeod 2005), we accentuated aberrations in perceptual analysis of threat in anxiety by targeting sensory perception

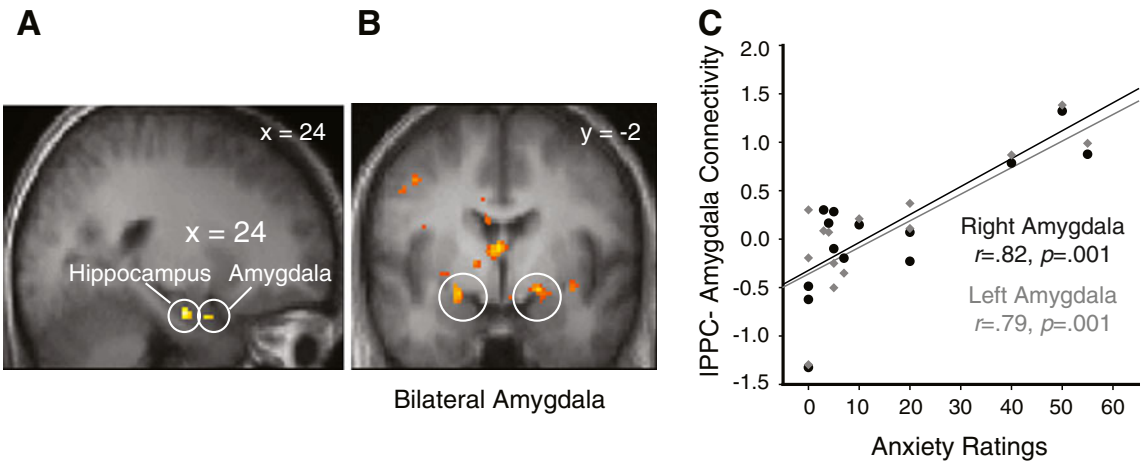

Fig. 3 PPI analyses indicated the posterior piriform functional connectivity with emotion-related regions. **a** Right amygdala and right hippocampus showed significant functional connectivity with right PPC during negative versus neutral conditions (30, −2, −32, Z=3.24; 24, −12, −22, Z=3.66). **b** State anxiety augmented connectivity between left PPC and bilateral amygdala in response to negative (vs. neutral) odor mixtures (24, −2, −12/18, −4, −10; Z's=3.33/3.58). **c** Scatterplot depicts connectivity in bilateral amygdala as a function of anxiety

of threat specifically. The heightened response in posterior piriform cortex is analogous to accounts of anxiety-modulated threat response in extrastriate/fusiform cortex in visual perception (Dunsmoor et al. 2011; Ahs et al. 2009; Straube et al. 2004), indicating a general effect across anxiety-related multiple sensory modalities. Nevertheless, we note that, unlike previous work in the visual modality, anxiety elevated threat response in the primary olfactory cortex (right PPC) without influencing the emotional circuitry (even at a highly lenient threshold of p<0.10 uncorrected). Given that amygdala responses are sensitive to stimulus intensity (Winston et al. 2005; Anderson et al. 2003; Small et al. 2003), it is possible that these extremely weak malodor elements failed to activate this area. Interestingly, these odors could nevertheless strengthen the crosstalk between the olfactory sensory cortex and the amygdala. This response pattern represents some novel evidence, suggesting that the perceptual network in anxious individuals has a keen sensitivity to threat and that this mechanism could be independent of exaggerated emotional response to threat.

The subliminal nature of olfactory stimulation helps to preclude the possibility of top-down influence on sensory perception, thereby narrowing the effects down to bottom-up sensory activation. It is likely that the salience of aversive odors (even at the subthreshold level) turns on the sensory and sensory-driven attentional systems, thereby facilitating threat analysis and detection. In keeping with that, we did not observe advantaged threat processing in higher-order olfactory cortices (e.g., the olfactory OFC; Zelano and Sobel 2005; Gottfried and Zald 2005) beyond the lower-level PPC. Taken together, the current findings provide strong support for a perceptual account of threat hyper-responsivity, constituting an alternative theorization that had not been carefully considered in anxiety research.

Analogous to evidence of visual-extrastriate-amygdala connectivity enhancement in response to threat-related visual cues in specific phobia (Lipka et al. 2011; Ahs et al. 2009), we uncovered increased functional connectivity between the olfactory cortex and the emotional network in the presence of olfactory threat. This amplified communication between sensory and emotional systems could be responsible for the heightened arousal response to threat here, via increasing the output of the amygdala to brain stem structures to galvanize the sympathetic nervous system (LeDoux 1995). Given that the weak subthreshold olfactory threats here did not significantly stimulate the amygdala, this enhanced sensory–emotional coupling could serve a critical mechanism to arouse adequate physiological alertness to potential insults.

Besides the amygdala, the insula has also been implicated in anxiety disorders, especially in various specific phobias and obsessive compulsive disorders (Berle and Phillips 2006; Phillips et al. 2002). Activation in insula is also associated with multisensory disgust responses (Wicker et al. 2003; Zald and Pardo 2000; Phillips et al. 1997), which could mediate the valence-dependent neural response in highly anxious individuals here. Indeed, negative mixtures (vs. neutral mixtures) enhanced activity in left anterior insula (−26,18,−12; Z= 3.11, p=0.001), and the differential activity was positively associated with anxiety ratings (at a less stringent threshold) in bilateral insula (38,14,−20/−36,2,18; Z's=2.35/2.37, p's< 0.01). We speculate that the insula, instead of the amygdala, could have mediated threat processing in this study.

Notably, the current effects present a pattern of right-hemisphere dominance in general and threat-related olfactory

processing. The right hemisphere has been consistently linked to basic olfactory sensory processing (Li et al. 2010; Jones-Gotman and Zatorre 1993; Zatorre and Jones-Gotman 1991; 1990; Abraham and Mathai 1983) and processing in other modalities (Kanwisher et al. 1997; Mesulam 1981). The more prominent effects of negative olfactory stimulation on the right (vs. left) piriform cortex and its functional connectivity here further align with extant evidence (Davidson and Irwin 1999; Borod et al. 1998; Adolphs et al. 1996) in emotion processing, pointing to a supramodal hemispheric specialization in threat analysis. Finally, it is important to note that odors, unlike many other sensory cues, may require more nuanced assessment of valence and preference. For instance, a stinky smell associated with cheese could signal a pleasant delicacy whereas, set in a context of an untidy bathroom, this very odor could be a highly unpleasant cue of germs and contamination. Therefore, it is important in future research to assess both the affective valence and participants' subjective experience/interpretation of negative odors to tease apart these intricate facets of olfactory affective processing.

Highlighting basic sensory acuity to threat in the olfactory system in anxious people, the current study presents some novel insights into the underlying mechanisms of anxiety. Particularly, primary olfactory cortex detects minute, behaviorally imperceptible olfactory threat in anxiety, and increases its functional coupling with the emotional circuitry in this context. These sensory-driven mechanisms may constitute a neurosensory model of anxiety beyond prevalent emotion-based accounts in this field, potentially elucidating the etiology of the unfortunate and debilitating symptoms that perpetuate anxiety disorders.

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