Endogenous opioid system modulates proximal and distal threat signals in the human brain

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Conflicts of interest
None

Abstract

BACKGROUND Fear promotes rapid detection of threats and appropriate fight-or-flight responses. Endogenous opioid system is an inhibitory neurotransmitter circuit modulating responses to pain and psychological stressors. Opioid agonists have also anxiolytic effects. Fear and anxiety constitute major psychological stressors for humans, yet the contribution of the opioid system to acute human fear remains poorly characterized.

METHODS: We induced intense unconditioned fear in the subjects by gradually exposing them to a living constrictor snake (threat trials) versus an indoor plant (safety trials). Haemodynamic responses were recorded from 33 subjects during functional magnetic resonance imaging (fMRI). 15 subjects underwent positron emission tomography (PET) with agonist radioligand [^{11}C]carfentanil with high affinity for mu-opioid receptors (MORs). Separate sessions were performed with repeated threat and safety exposure. Pupillary arousal responses to snake and plant exposure were recorded in 36 subjects. Subjective fear ratings were measured throughout the experiments.

RESULTS: Self-reports and pupillometric responses confirmed significant experience of fear and autonomic activation during the threat trials. fMRI data revealed that proximity with the snake robustly engaged brainstem defense circuits as well as thalamus, dorsal attention network and motor and premotor cortices. These effects were diminished during the repeated exposures. PET data revealed that [11C]carfentanil binding to MORs was significantly higher during the fear versus safety conditions, and the the acute haemodynamic responses to threat were dependent on baseline MOR binding in the cingulate gyrus and thalamus. Finally, baseline MOR tone predicted dampening of the haemodynamic threat responses during the experiment.

CONCLUSIONS: Preparatory response during acute fear episodes involves a strong motor component in addition to the brainstem responses. These haemodynamic changes are coupled with deactivation of the inhibitory opioidercic circuit, highlighting the role of MORs in modulating the human fear response.

Keywords: Emotion, fear, opioids, positron emission tomography

Introduction

Fear acts as a survival intelligence promoting rapid detection of threats and appropriate fight-or-flight responses by managing information processing priorities and proximity with potentially harmful targets such as predators (Mobbs et al., 2015; Vuilleumier, 2005). The mammalian fear circuit consists of a complex set of midbrain and medial temporal lobe structures, particularly periaqueductal grey, hypothalamus and amygdala, interacting with prefrontal systems accessing conscious feelings thus allowing coping with acute and distal threats (Fullana et al., 2016; Tao et al., 2021; Tovote et al., 2015; Zhou et al., 2021). These systems operate flexibly depending on the proximity of the threat. Functional magnetic resonance imaging (fMRI) studies have shown that the imminence of threat switches activation from ventromedial prefrontal cortex (vmPFC) towards periaqueductal grey matter (PAG), reflecting a shift from complex planning of avoidance strategies to automated fight-or-flight response when the predator enters the circa-strike zone (Mobbs et al., 2007; Mobbs et al., 2010). In contrast, activity in the subgenual anterior cingulate cortex is associated with episodes of courage – attempting to regulate the fear and move closer to a its target (Nili et al., 2010).

Majority of the imaging studies on brain basis of human fear have however been conducted with biologically unspecific fMRI, and the neuromolecular mechanisms behind acute human fear and its regulation remain elusive. A bulk of studies however suggests that the endogenous mu-opioid receptors system could play a key role in regulating the human fear responses (see review in Meier et al., 2021). Among the three types of opioid receptors (μ, δ, and κ receptors), the μ receptors (MORs) mediate the effects of endogenous β-endorphins, endomorphins, enkephalins, and exogenous opioid agonists. The predominant action MORs in the nervous system is inhibitory, but they also have excitatory effects. Multiple OR subtypes are abundantly expressed in the amygdala (Colasanti et al., 2011) MOR expression is also high throughout the human emotion circuit (Nummenmaa & Tuominen, 2018). MOR system is engaged during various positive and negative emotions (Nummenmaa et al., 2018a; Prossin et al., 2016; Saanijoki et al., 2017; Tuulari et al., 2017) and baseline MOR tone modulates haemodynamic responses to negative affect (Karjalainen et al., 2017; Karjalainen et al., 2018; Sun et al., 2022).

Endogenous MOR tone is negatively associated with subclinical anxiety, suggesting that perturbations in the MOR system might make individuals vulnerable to psychological stressors and subsequent development of anxious symptomatology (Nummenmaa et al., 2020). This is further corroborated by clinical data showing MOR down-regulation in the amygdala and anterior cingulate cortex in PTSD patients exposed to combat versus healthy controls (Liberzon et al., 2007). Relatedly, uncontrolled cohort studies have shown that acute opioid agonist administration following a traumatic event inhibits development of post-traumatic stress disorder (PTSD), likely due to inhibition of fear conditioning following the traumatic event (Bryant et al., 2009; Holbrook et al., 2010; Saxe et al., 2001). Finally, pharmacological experiments have established that when exposed to natural threat (spider), subjects receiving opioid antagonist naloxone kept more distance to the spider, supporting the role of MOR activation in fear suppression (Arntz et al., 1993; Eippert et al., 2008; Kozak et al., 2007; Merluzzi et al., 1991). However, there is currently no direct in vivo evidence on the role of the MOR system activation on unconditioned fear in humans, and the contribution of endogenous MOR system tone on phasic neural responses during acute threat episodes remain unresolved.

The current study

Here we tested whether MOR system is engaged during naturalistic unconditioned threat – exposure to a live snake. We measured endogenous MOR availability with high-affinity agonist radioligand [11C]carfentanil after a neutral baseline state (exposure to a household plant) and after a 10-step modified exposure treatment mimicking clinical exposure therapy for snake phobia (Merluzzi et al., 1991). During a separate fMRI experiment we repeatedly exposed the subject to the live snake versus a neutral control object (household plant) with varying proximities and modelled the haemodynamic responses as a function of subjective fear levels and proximity to the threat. We show that i) acute unconditioned fear engages the endogenous MOR system, ii) that this response is paralleled by haemodynamic activity in the midbrain, thalamic and cortical nodes of the human fear circuit, iii) that the magnitude of the haemodynamic responses to fear is dependent on the baseline MOR tone, and iv) that baseline endogenous MOR tone predicts the suppression of the fear responses during late versus early phases of the experiment.

Materials and methods

To validate that a live snake would be a potent fearful stimulus, we first conducted an online survey where we asked subjects to report i) how much they are afraid of snakes (1 = not at all, 10 = extremely much) and how much fear and related symptoms they would experience when exposed to a snake or a neutral control object (a household plant) art different proximities. This revealed that on average people (n = 786) were moderately strongly afraid of snakes (M = 5.43, SD = 2.80). The fear distribution was however clearly bimodal with a third of the subjects having moderately low (< 3) and the other third moderately high (> 7) fear for snakes (**Figure S1)**. Both subjective experience of fear and somatic fear-related sensations were estimated to be higher when the snake was closer to the subject (**Figure S2)**. This confirmed that the live snake exposure would be a valid and potent model for unconditioned fear in the general Finnish population.

Subjects

The study was approved by the ethics board of the hospital district of Southwest Finland, and conducted according to Good Clinical Practice and the Declaration of Helsinki. All subjects signed written informed consent and were informed that they had the right to withdraw at any time without giving a reason. The participants were compensated for their time and travel costs. We recruited only young females since age and sex influence MOR system function (Kantonen et al., 2020). Females are also on average more afraid of snakes than men (Fredrikson et al., 1996; McLean & Anderson, 2009; Tucker & Bond, 1997).

Subjects were recruited via advertisements on email lists, social media, and bulletin boards. Initially 177 subjects contacted the researchers and were pre-screened for the fear for snakes with a questionnaire based on the short version of SNAQ (Polák et al., 2020; Zsido et al., 2018). This scale contains seven Likert-scale items from the SNAQ (scaled from 0 to 100) tapping emotional response to snakes (e.g. “The way snakes move is repulsive”, “I’m more afraid of snakes than any other animal”). If their average score in the questionnaire was over 70 out of 100, they were deemed eligible for the imaging experiment and were screened for imaging exclusion and inclusion criteria. To maximize the sample size, the lower cut-off for the behavioral only study (eye tracking) was set at 45. Sample sizes for different parts of the study are shown in **Figure S3**. A total of 51 female subjects were studied: 15 in the PET-fMRI

experiment (mean age 26.0 years, range 20-42, snake fear scores mean = 79.9, SD = 9.5, Body mass index (BMI) mean 23.2, SD = 2.2) 18 for the fMRI only experiment (mean age 23.3 years, range 19-27, snake fear scores mean = 75, SD = 13.1, BMI mean 23.6, SD = 3.3) and 18 for the behavioral only experiment (mean age 25.7 years, range 20-37, snake fear scores mean = 58.4, SD = 8.1, BMI mean = 23.7, SD = 2.5, 2 left handed) for eye tracking experiment. The exclusion criteria included a history of neurological or psychiatric disorders, smoking or use of nicotine products, alcohol and substance abuse or current use of medication affecting the central nervous system; also breastfeeding or an attempt to become pregnant and the standard MRI and PET exclusion criteria for subject in imaging experiments. The study physician screened the subjects for PET imaging for eligibility, and a psychologist screened them for psychiatric disorders with the MINI questionnaire 6.0 (Sheehan et al., 1998).

Measuring Autonomic Fear Response with Eye Tracking

Eye movements were measured with Eye Link II system with 250 Hz sampling rate and spatial accuracy better than 0.5 degrees. Recordings were done in a dimly and constantly lit room. The subjects were seated and their heart rate belt around her chest, the chin on a chin rest, the hand on a marked place at the table. Next, the eye tracker was set up and calibrated and validated using standard 9-point calibration. The experimenter instructed the subject to keep staring at the fixation cross and to concentrate on the emotions that a snake or a plant evoked. They were stressed that the snake or plant would never actually touch them. Half of the trials involved a snake and half a plant. On each trial, the snake or plant was brought to the participant's field of view either far (screen), moderate (hand) or close (face) proximity with the subject. The experiment consisted of 5 x 12 snake and 5 x 12 plant trials presented in randomized order.

Each the trial begun with drift correction and recorded spoken instruction (2.2-2.7 s) indicating the type of the next trial. Both the experimenter and the subject heard the sounds, and the experimented moved the object accordingly to the indicated proximity. Subjects were instructed to keep their eyes fixated at the cross shown at the center of the screen throughout the trial. Gaze position and pupil size were measured throughout the 10 s trial, after which the subject reported on VAS scale 0-10 (0 = not at all, 10 = extremely) their current level of fear. During the VAS scale, the object was out of subject's sight. The eye tracker was recalibrated after each 12 trials and detrended before each trial. For analysis, subject wise pupil size time series were cleaned from blinks using in-house code based on PhysioData Toolbox (Kret & Sjak-Shie, 2019), baseline corrected (10 ms), and mean pupil sizes were compared between 3 time windows (3-4 s, 5-6 s, 7-8 s) across the conditions (snake vs. plant) and proximities (close, moderate, far).

PET and MRI measurements

Fear Exposure Protocol for PET

Unconditioned fear was induced using a modified version of the fear exposure therapy protocol involving 10 steps (Table S-1) with progressively increasing proximity of the snake leading to potentiation of the fear response. Subjects were first informed about the next step so that they could evaluate if they would be ready to move to the next one. If the subject evaluated the next step as too intense, the current step was repeated. Duration of each step was 60 s. Fear ratings were obtained at 10 seconds and 50 seconds during each step.

PET Data Acquisition

Subjects underwent two PET scans (threat and safety) in a counterbalanced order. The scans were done at the same time of the day on separate days. Prior to the threat scan the subjects underwent the 10-step fear exposure protocol (see above) where they were progressively exposed to closer contact with the snake. To maintain the desired fear level after the exposure and while waiting for the radiotracer injection, the snake was brought repeatedly close to subject's lap and their hand (approximately 10 minutes). To boost the fear responses, the experimenter had a casual conversation with another researcher during the cannulation asking for hydrocortisone in case the snake decides to bite. The experimenter ensured that the subject heard the conversation. The experimenter placed the hydrocortisone on a research table and dressed protective gloves. If a subject asked if the snake is dangerous, the experimenter answered: "don't worry about it, the snake has bitten only once, and we have the hydrocortisone ready". Hydrocortisone was not actually needed since the snake was not venomous. Subjects reported their fear levels at the beginning of the experiment and at every 10 minutes. The snake was kept inside the sPET canner room throughout as close as the subject's head as possible. A rubber snake was used to avoid scatter radiation load to the experimental snake. When the scanner room was emptied for CT image acquisition, the experimenter sneakily replaced the real snake with a rubber snake for radiation protection and returned the rubber snake in terrarium for the PET scan. Only 1/15 subject noticed the sham. The subject could see the snake via an angled mirror. During safety exposure, a routine PET scan was performed, and no external stimulus was presented.

MOR availability was measured with radioligand [^{11}C]carfentanil (Eriksson & Antoni, 2015) synthesized as described previously (Kantonen et al., 2021). Radiochemical purity of the produced [^{11}C]carfentanil batches was 98.3 ± 0.42 % (mean ± SD). The injected [^{11}C]carfentanil radioactivity was 253 ± 8.96 MBq and molar radioactivity at time of injection 358 ± 235 MBq/nmol corresponding to an injected mass of 0.47 ± 0.42 µg. Subjects were instructed to abstain from smoking and drinking alcohol or caffeine and to avoid physical exercise the day before and the day of the PET scans. The subjects were also told to fast for 3 hours prior to PET imaging. PET imaging was carried out with Discovery 690 PET/CT scanner (GE. Healthcare, US). The tracer was administered as a single bolus via a catheter placed in subject's antecubital vein, and radioactivity was monitored for 51 minutes. Subject's head was strapped to the scan table to prevent excessive head movement. T1-weighted MR scans were acquired in separate session to correct for attenuation and for anatomical reference.

PET Image Processing and Data Analysis

PET data were preprocessed with the Magia (Karjalainen et al., 2020) toolbox (https://github.com/tkkarjal/magia), an automated neuroimage analysis pipeline developed at the Human Emotion systems Laboratory, Turku PET Centre. Magia toolbox runs on MATLAB (The MathWorks, Inc., Natick, MA, USA), and utilizes the methods from SPM12 (www.fil.ion.ucl.ac.uk/spm/) and FreeSurfer (https://surfer.nmr.mgh.harvard.edu/) as well as in-house developed tools for kinetic modeling. PET images were first motion-corrected, and co-registrated to T1-weighted (T1w) MR images, after which MRI was then processed with Freesurfer for anatomical parcellation. [^{11}C]carfentanil uptake was quantified by non-displaceable binding potential (BP_{ND}) in 21 regions (amygdala, caudate, cerebellum, dorsal anterior cingulate cortex, hippocampus, inferior temporal cortex, insula, medulla, midbrain, middle temporal cortex, nucleus accumbens, orbitofrontal cortex, pars opercularis, posterior

cingulate cortex, pons, putamen, rostral anterior cingulate cortex, superior frontal gyrus, superior temporal sulcus, temporal pole, and thalamus). BP_{ND} was estimated with simplified reference tissue model in regional (Lammertsma & Hume, 1996) and voxel-level (Gunn et al., 1997) by using occipital cortex as the reference region. Due to the small sample size, we focused on region-of-interest (ROIs) analyses, whereas the voxel-level BP_{ND} images were used solely for the illustration. Prior to calculation of voxel-level BP_{ND} images the [^{11}C]carfentanil PET images were smoothed using Gaussian kernel to increase signal-to-noise ratio before model fitting (FWHM = 2 mm). BP_{ND} images were spatially normalized to MNI152-space and finally smoothed using a Gaussian kernel (FWHM = 6 mm). Subsequently, regional BP_{ND} across the fear and baseline conditions were compared using paired samples t-tests.

Fear Exposure Protocol for fMRI

During the fMRI experiment the snake (threat stimulus) and plant (safety stimulus) were brought to three different proximities (close, moderate and long distances) from the subject by the experimenter: 1) as close as possible to the subject's stomach (close distance) 2) by the subject's feet (moderate distance) 3) as far as possible on the other side of the room (long distance) by the NordicNeuroLab fMRI screen but still visible for the subject from the gantry via mirror. Each block lasted for 15 seconds and was followed by VAS rating for fear and a 5 s fixation block during which the experimenter heard instructions for the next condition via earphones. Each condition was repeated for 13 times. Presentation software controlled for timing of the experiment. For logistic reasons, the snake and plant were presented to the subjects only on one side of the room.

MRI data acquisition

MR imaging was conducted at Turku University Hospital. The MRI data were acquired using 3T MRI system with SuperG gradient technology (SIGNA, Premier, GE Healthcare, Waukesha, WI, USA) with the 48-channel head coil. High-resolution structural images were obtained with a T1w MPRAGE sequence (1 mm^3 resolution, TR 7.3 ms, TE 3.0 ms, flip angle 8◦, 256 mm FOV, 256 × 256 reconstruction matrix). The imaging sequences for the snake and plant scans were identical: 355 functional volumes (15 min 33 s) were acquired with a T2∗-weighted echo-planar imaging sequence that is sensitive to the blood-oxygen-level-dependent (BOLD) signal contrast (TR 2600 ms, TE 30 ms, 75◦ flip angle, 240 mm FOV, 80 × 80 reconstruction matrix, 250 kHz bandwidth, 3.0 mm slice thickness, 45 interleaved axial slices acquired in descending order without gaps).

MRI data preprocessing, and analysis

The functional imaging data were preprocessed with FMRIPREP (Esteban et al., 2019) (v1.3.0.post2), a Nipype 1.1.8 (Gorgolewski et al., 2011) based tool that internally uses Nilearn 0.5.0 (Abraham et al., 2014). The following preprocessing was performed on the anatomical T1w reference image: correction for intensity non-uniformity, skull-stripping, brain surface reconstruction, spatial normalization to the ICBM 152 Nonlinear Asymmetrical template version 2009c (Fonov et al., 2009) using nonlinear registration with antsRegistration (ANTs 2.2.0) and brain tissue segmentation. The following preprocessing was performed on the functional data: co-registration to the T1w reference, slice-time correction, spatial smoothing with a 6mm Gaussian kernel, automatic removal of motion artefacts using ICA-AROMA (Pruim et al., 2015) and resampling the MNI152NLin2009cAsym standard space. To reveal brain regions encoding the threat value of the snake, the haemodynamic time series during the

snake and plant blocks were predicted with trialwise fear ratings for each subject. To reveal brain regions responding to increased proximity of the threat, we contrasted trials where intermediate proximity snake was followed by increased (close) versus decreased (far) proximity. To test for the habituation of the fear response, the haemodynamic time series for the fear response was modulated with time (1st, 2nd and 3rd third of the experiment), and fear habituation was modelled as positive and negative effects of time. For all analyses contrast images were generated for positive and negative effects and subjected to the second-level (random effects) model for population level inference. Statistical threshold was set at 0.05 (FDR corrected at cluster level).

PET-fMRI fusion analysis

To test whether baseline MOR tone is linked with haemodynamic responses during acute fear, we first extracted mean subjectwise baseline MOR availabilities across the 16 ROIs (see above). Subsequently, the ROI-wise MOR availabilities in these were correlated with the regional BOLD responses to acute fear characterizing the regional interactions between MOR and BOLD responses during fear. This enabled visualizing the regions, where the binding potential estimates predicted the BOLD responses the most accurate. A cumulative map of the MOR-dependent BOLD responses was also generated to reveal whereabouts in the brain the haemodynamic responses are most consistently associated with MOR availability.

Results

Self-reports

Subjective fear increased consistently during the exposure trials and the increase was statistically significant (mean slope 0.4, $p < .001$). Fear remained at high level until the beginning of the PET scan (mean 2.7) and was significantly higher during fear versus safety trials throughout the scan (**Figure 1**).

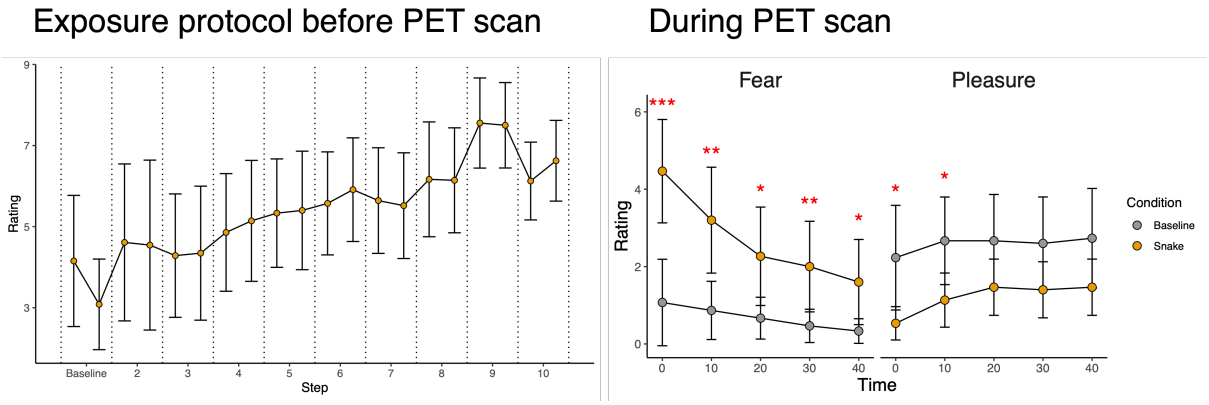

Figure 1 Mean subjective fear ratings during the exposure (left) and PET scan (right). Asterisks indicate significant differences between the baseline and snake conditions. * = $p < 0.001$, **= $p < 0.01$,* = $p < 0.05$.

Eye tracking

A Condition (Snake, Plant) X Position (Table, Hand, Face) repeated measures ANOVA was run on the mean pupil sizes separately for each time window. The ANOVA and post hoc pair-wise comparison results are shown in the **Tables S2-S7.** Eye tracking data (**Figure 2**) revealed that

threat exposure led to significant autonomic activity, as indexed by larger pupil sizes when the snake was close to the face or hand versus when it was far away, or when the subject was exposed to the plant near the hand or the face at all the analyzed time points (ps < .01) but not when it was on the table (ps > 0.05). See full statistics in the **Tables S2-7**.

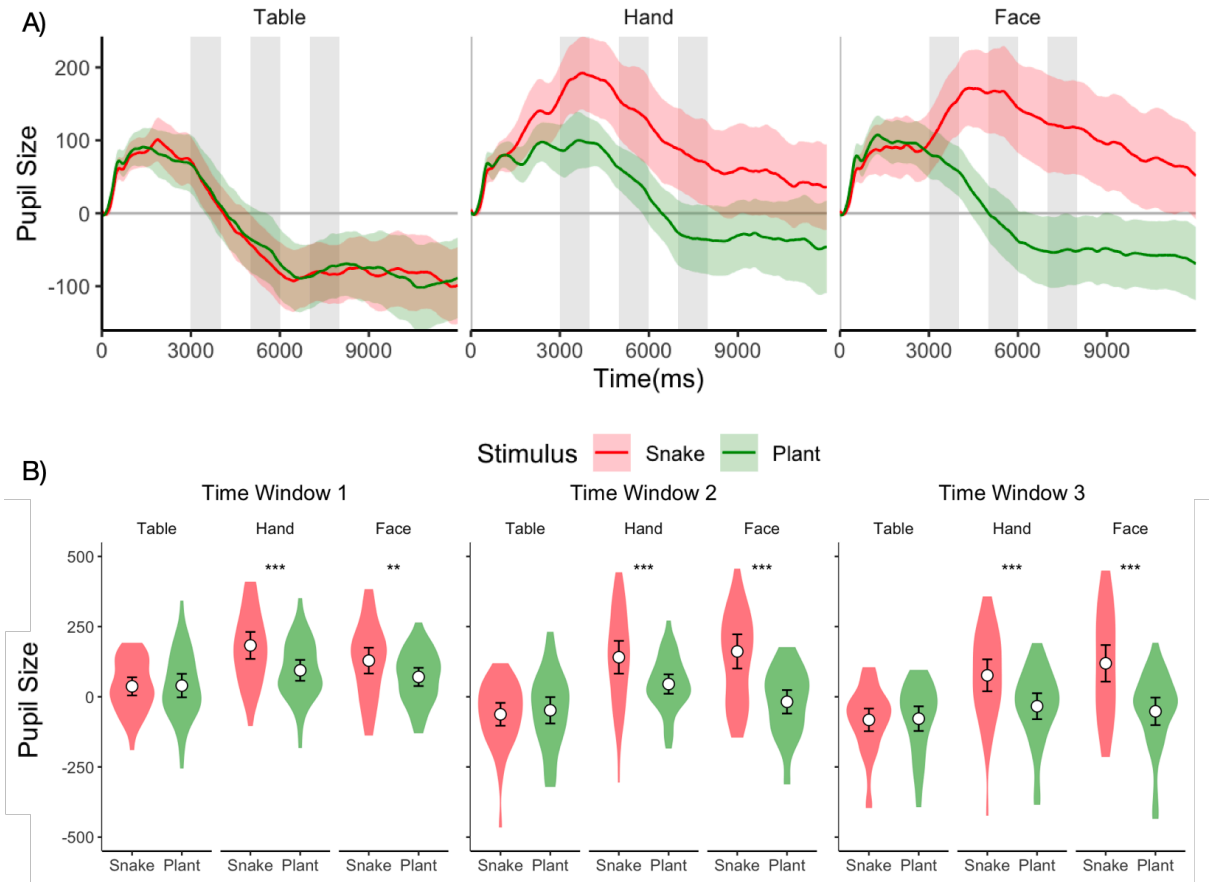

Figure 2 A) Pupillary responses during threat and safety trials at the three different distances. Solid lines show mean pupil size, shaded are represents 95% CI. Grey bars indicate the timebins (early (3-4 s), mid (5-6 s), late (7-8s)) where the pupil size was compared across conditions. B) Distributions of mean pupil sizes across the three time bins for the snake versus plant conditions. Asterisks indicate significant differences. ** p < 0.01, *** p < 0.001.

BOLD-fMRI responses associated with fear intensity

Mean fear levels were higher during snake versus plant trials, and increased as a function of the proximity of snake but not plant (*ps* < 0.001). fMRI data revealed that increasing subjective fear robustly engaged brainstem defense circuits as well as thalamus, dorsal attention network and motor and premotor cortices. Significant activations were also observed in the visual cortices. Decreasing fear levels were associated with activation in the amygdala, posterior cingulate cortex, and frontal pole (**Figure 3**).

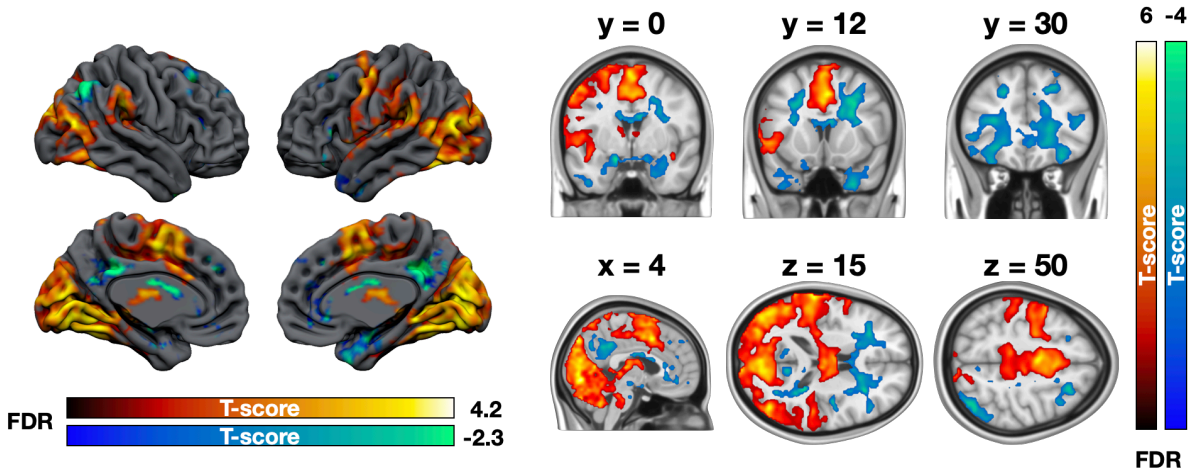

Figure 3 Brain regions responding to increasing (hot colours) and decreasing (cool colours) fear during the experiment. The data are thresholded at $p < 0.05$, FDR corrected at cluster level.

BOLD-fMRI responses associated with threat proximity

Next, we modelled the brain responses associated with increasing versus decreasing proximity of the threat. When the threat approached the subject, increased activity was observed in brainstem, cerebellum, insula, and anterior and midcingulate cortices. Additional activations were observed in primary somatosensory cortex (SI) and inferior frontal and occipital cortices (**Figure 4**). No regions were more active when the threat became more distal.

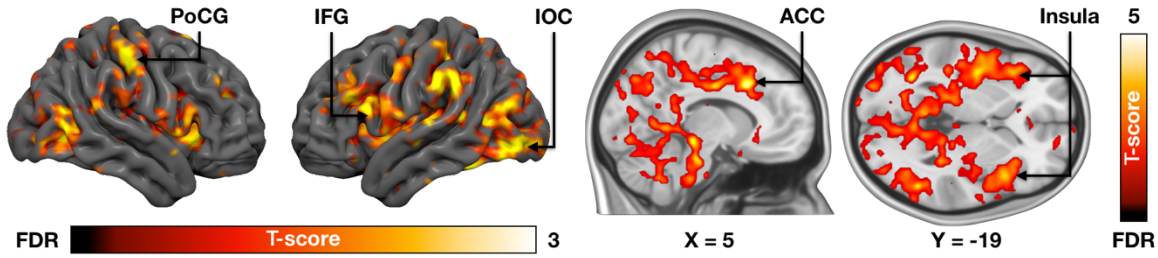

Figure 4 Brain responses to increased proximity of the threat. The data are thresholded at $p < 0.05$, FDR corrected at cluster level. ACC = anterior cingulate cortex, IFG = inferior frontal gyrus, IOC = inferior occipital cortex, PoCG = postcentral gyrus.

Habituation effects for fear in fMRI

We next tested whether the neural responses to fear habituated during the fMRI session. When the fear-dependent haemodynamic responses were modelled as a function the phase of the experiment (1^{st} / 2^{nd} / 3^{rd}), we observed significantly increased responses in the amygdala and brainstem (**Figure 5**). For the opposite contrast, significant effects were observed in midcingulate cortex, bilateral fusiform and parahippocampal gyri, left insula and middle temporal cortex.

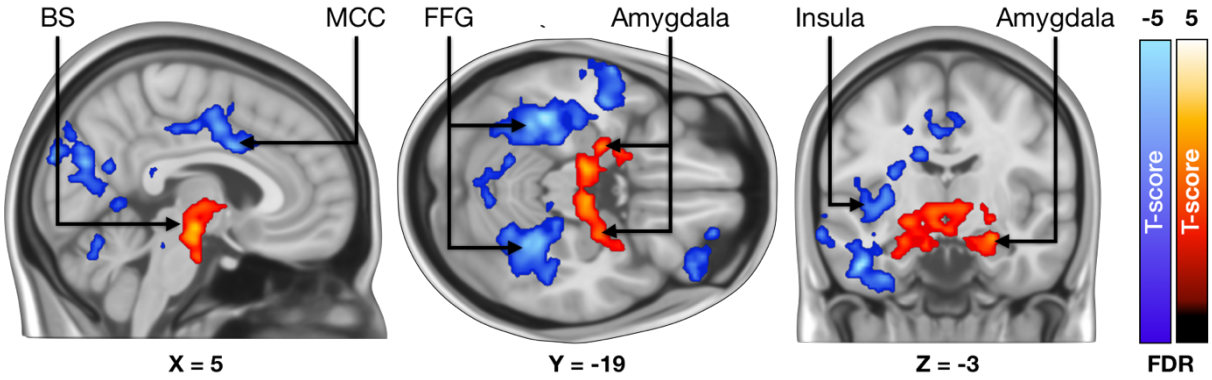

Figure 5 Brain regions where fear-dependent responses became stronger (hot colours) and weaker (blue colours) throughout the experiment. The data are thresholded at $p < 0.05$, FDR corrected at cluster level.

PET

PET data revealed that acute threat influenced the opioid system, as [^{11}C]carfentanil BPND was significantly higher during the fear versus safety conditions (**Figure 6**). This effect was observed in left thalamus, hippocampus, middle cingulate cortex, postcentral gyrus, supplementary motor area and precuneus, bilateral middle and superior frontal gyri, and fusiform and lingual gyri. No effects were observed in the opposite contrast.

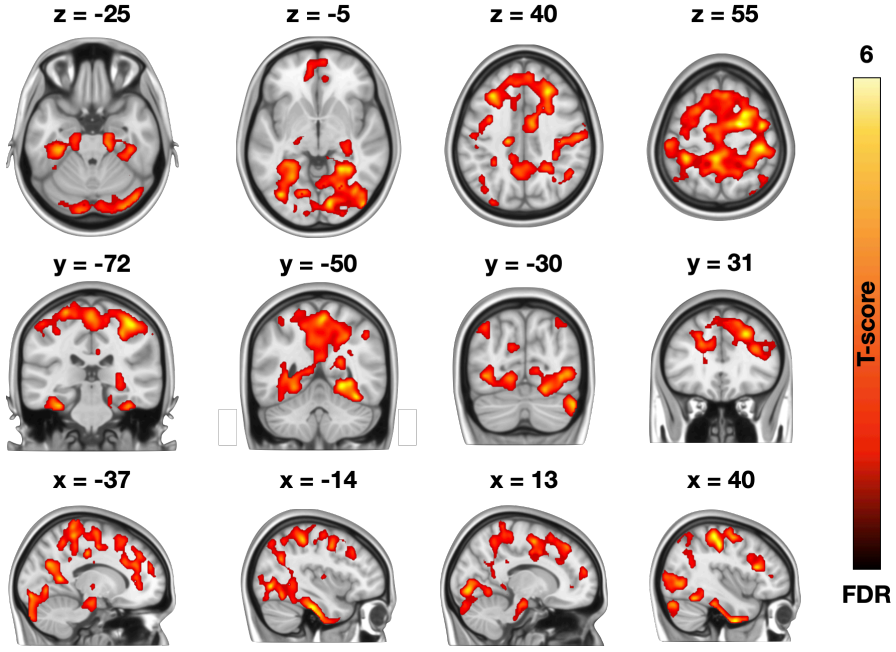

Figure 6 Changes in MOR tone during the fear versus baseline condition; [^{11}C]carfentanil BPND were significantly higher during fear versus safety conditions. The data are thresholded at $p < 0.05$, FDR corrected at cluster level.

PET-fMRI fusion analysis

Next, we analyzed the regional interactions between baseline MOR availability and BOLD responses during fear using PET-fMRI fusion analysis. This revealed a consistent and widespread positive association between MOR tone and fear-dependent haemodynamic

responses (Figure 7). These effects were observed in limbic and paralimbic regions such as amygdala and thalamus. Siginficant effects were also observed in the somatosensory and primary visual cortex and higher-level association areas, brainstem, cerebellum, insula, temporal and frontal cortices. The opposite effects were more limited and observed primarily in anterior portions of cerebellum.

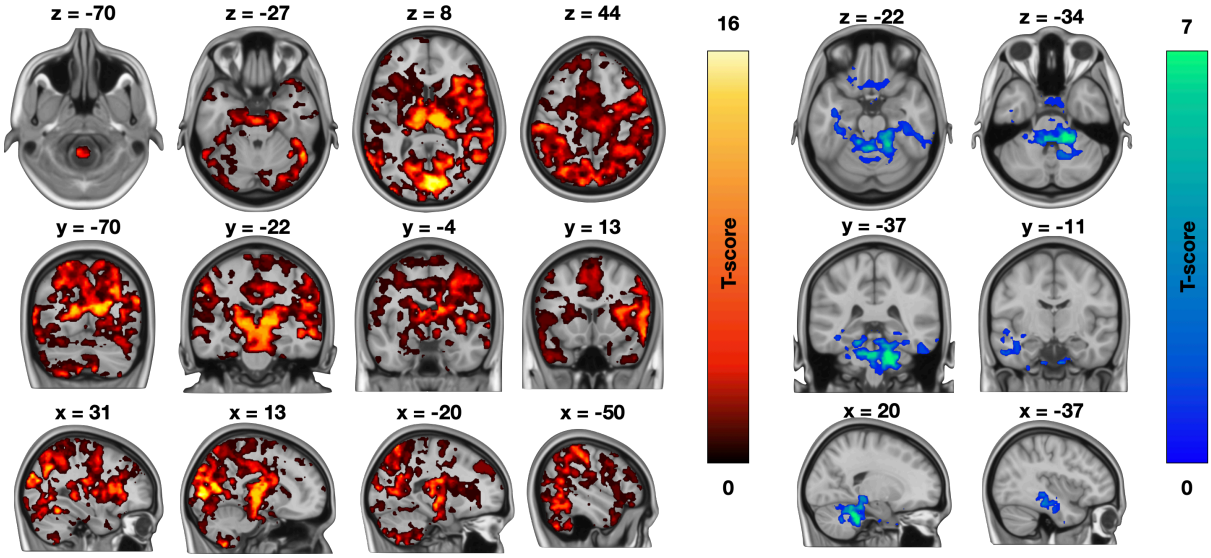

Figure 7 Regional interactions between the MOR system and acute haemodynamic reponses to unconditioned fear. Voxels in this cumulative map show the number of regions (out of 16) whose [11C]carfentanil BPND was positively (hot colours) and negatively (cool colors) associated with the subjective fear dependent BOLD-fMRI responses.

Finally, we tested whether the BOLD responses habituation to the fearful stimulus was associated with regional MOR availability. We predicted the strength of the habituation effect (i.e. changes in BOLD responses during the experiment) with mean regional MOR availabilities. This revealed that the habituation effect was stronger in subjects with higher MOR availability (Figure 8). This effect was observed bilaterally in lateral occipital cortices, linguar gyrus, thalamus, and amygdala. Right-hemispheric activations were also observed hippocampus, putamen, middle temporal cortices, insula and orbitofrontal cortex and frontal pole.

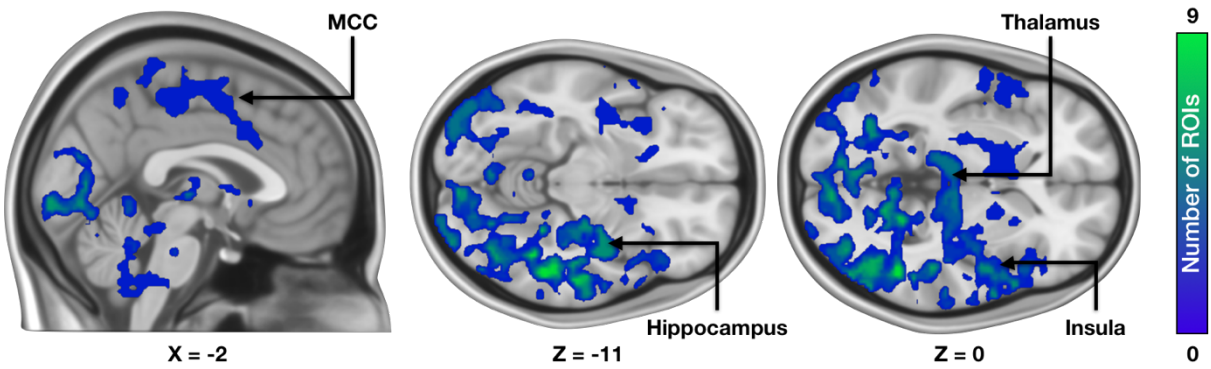

Figure 8 MOR-dependent habituation of haemodynamic fear responses. Voxels in this cumulative map show the number of regions (out of 16) whose [11C]carfentanil BPND was positivel associated with habiutation of the fear-dependent BOLD-fMRI responses throughout the experiment.

Discussion

Our main finding was that acute fear acutely activated brainstem defense circuits as well as arousal, motor, and attention systems for preparing escape and monitoring survival odds. This acute response was paralleled autonomic activation indexed by the pupillary responses as well as large-scale deactivation of the endogenous opioidergic system, as indicated by the [11C]carfentanil PET data. MOR system also modulated the acute fear response, in that high baseline MOR tone was consistently associated with stronger haemodynamic responses during acute fear. Finally, baseline high MOR tone predicted larger downregulation of cingulate, insular and hippocampal responses over the course of the fMRI experiment. Altogether these results confirm the role of the endogneous opioid system in modulating acute fear and adapting to threatening situations.

Haemodynamic responses to acute fear

Acute threat provoked by the proximity of the snake elicited strong subjective experience of fear, accompanied with autonomic activation as indexed by pupil dilation. On neural level, the acute fear response was accompanied activity in the brainstem defense circuits and thalamus. These regions managed the acute fight-or-flight responses when the threat is imminent (Mobbs et al., 2007). Significant activations were also observed in the dorsal attention networks and visual cortices. Emotions modulate attentional priorities (Vuilleumier, 2005) and engage the attention circuits consistently across individuals particularly during threatening situations (Nummenmaa et al., 2012). We also found that acute fear led to significant increase activity in the motor and premotor cortices. Emotions prepare the individual for action by adjusting the activation of the cardiovascular, skeletomuscular, neuroendocrine, and autonomic nervous system (Ekman et al., 1983; Levenson, 2003). Finally, significant effects were observed in the dorsal anterior cingulate cortex which contributes to appraisal and expression of negative emotions (Etkin et al., 2011) and integrating lower-order emotional signals into conscious representation of the emotional state (Saarimäki et al., 2016). Broadly similar effects were observed when haemodynamic responses were modelled as a function of the proximity of the threat, which was expected given that fear levels were consistently higher when the threat was nearer. Our results thus reveal that the preparatory response during acute fear episodes in fMRI involves a strong motor component in addition to the brainstem responses, indicating an automated preparation of escape behaviour.

Although it is widely accepted that amygdala contributes to emotional processing, vigilance, and relevance detection (Davis & Whalen, 2001; Sander et al., 2003), we did not observe fear or threat proximity dependent amygdala activity. This occurred despite well-powered study (n=30), potent and naturalistic fear stimulus triggering, consistent autonomic activation, modelling of the BOLD responses with trialwise subjective fear ratings. However, a large bulk of neuroimaging studies on human fear has relied on the fear conditioning paradigm. Meta-analysis of such conditioned fear responses has revealed involvement of SMA/pre-sMA, dACC, anterior insula, ventral stratum, midbrain, and thalamus. Yet, this meta-analysis did not establish significant activations in amygdala during fear conditioning (Fullana et al., 2016).

Recent well-powered fMRI studies have found similar results – lack of fear-related amygdala activity in the presence of robust peripheral physiological activation, but consistent of amygdala responses to pictures of human faces (Renée et al., 2021). Accordingly, our data add to the accumulating literature speaking against the activation of human amygdala during fear, here demonstrated under potent naturalistic fear-evoking conditions.

Opioidergic response to acute fear

Our main finding was that the MOR system responds to acute threat by downregulation as evidenced by increased [11C]carfentanil BP_{ND}, which is traditionally interpreted to occur due to lowered endogenous opioid peptide release leading to increased radioligand binding in the occupancy challenge paradigm (Nummenmaa et al., 2018b). This is, to our knowledge, the first in vivo demonstration of human MOR system responses during natural, unconditioned fear. We observed widespread increase endogenous MOR tone across limbic and paralimbic emotion circuits as well as somotomotor and frontal cortices. As with fMRI, no effects were observed in the amygdala. This downregulated endogenous opioid release is in line with prior PET studies showing similar opioid system deactivation during negative emotions (Zubieta et al., 2003) as well as large-scale PET studies focusing on individual differences that have linked downregulation of the MOR system is linked with sustained anxiety (Nummenmaa et al., 2020). Conversely, positive emotions typically lead to increased opioid release in PET sudies (Jern et al., 2023; Koepp et al., 2009; Manninen et al., 2017; Tuulari et al., 2017).

All in all, these results show that endogenous opioid system responds acutely to fear, and individual differences in MOR tone also constitute a molecular factor towards fear and possibly fear-related pathologies. Given general inhibitory role as well as calming and relaxing functions of mu receptor agonists (Nummenmaa & Tuominen, 2018), we propose that the presently observed downregulation reflects increased arousal response during the fight-or-flight situation requiring maximization of physiological and psychological resources for promoting survival. Whereas MOR system responded to sustained threat with downregulation, the PET-fMRI fusion analysis revealed that haemodynamic responses to acute fear were positively associated on baseline MOR availability. The more MORs the subjects had, the stronger their acute neural fear responses were in limbic and paralimbic regions including amygdala and thalamus, as well as cortices and higher-level association areas. This suggests that MOR system may act at different timescales during threats, with baseline tone being associated with acute reactivity, and long-term threat exposure leading to MOR downregulation.

Habituation effects

Self-reports revealed that in our healthy volunteers even the brief repeated exposure to threats (mimicking exposure therapy for phobia) was sufficient for downregulating self-reported fear towards the snake (**Figure 1**). This effect was paralleled by significant changes in the haemodynamic responses to threat in limbic and paralimbic fear circuits including brainstem, amygdala, and hippocampus over the course of the fMRI experiment. The effect was twofold: responses in the fusiform gyri, hippocampus, insula, and anterior and midcingulate cortices became weaker. Previous fMRi studies have found that activity in the ACC and insula activity is linked with the tendency to withdraw from acute threats (Nili et al., 2010), thus the present pattern may indicate dampening of the escape responses due to fear habituation. In turn, responses in the brainstem and amygdala became stronger. The amygdala effects are noteworthy as amygdala did not respond to fear imminence per se. However, as its

activity was significantly increased over the course of the experiment, these results suggest that amygdala is involved in the adaptation to acute fear in humans. Importantly, the habituation effects in the midcingulate cortex and insula were also dependent on baseline OR availability, suggesting that individual differences in baseline OR tone modulate adaptation to novel threats. This is in line with PET-fMRI studies have found that increased baseline MOR tone buffers against acute haemodynamic responses evoked by negative affect (Karjalainen et al., 2017; Karjalainen et al., 2018; Sun et al., 2022). Additionally, pharmacological studies that have found that acute MOR agonist administration effectively inhibits fear learning and development of PTSD following an acute stressor (Bryant et al., 2009; Holbrook et al., 2010; Saxe et al., 2001). All in all, our results show that the MOR system has an important role in fear regulation and it may act as an buffer against the fearful / stressing situation (Nummenmaa et al., 2020), and individual differences in MOR tone may be an important biological mechanism predisposing individuals to sustained fear and anxiety.

Limitations

Because we scanned only females, we do not know whether our results translate directly to males. The observed BP_{ND} changes may reflect receptor internalization or altered conformation, rather than occupancy by endogenous neurotransmitter. Our outcome measure (BP_{ND}) cannot directly specify which interpretation is most appropriate. As our study was conducted in healthy volunteers, we cannot tell whether the same principles of MOR-dependent fear circuit downregulation also occur in subjects with clinical phobia, and this needs to be tested in future studies.

Conclusions

We conclude that endogenous opioid system modulates acute fear responses. These effects are observed in i) endogenous MOR system tone changes during threat, as well as in the ii) capacity for the MOR tone to modulate the acute affective and somatomotor threat responses and iii) their downregulation during repeated exposure to threats in the fMRI experiment. Taken together these results highlighting the role of MORs in modulating proximate threats. Clinical studies should further elucidate whether alterations in MOR signaling contribute similarly to clinical phobias and anxiety disorders.

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