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Human serotonin transporter availability predicts fear conditioning

Fredrik Åhs [a,b,*], Andreas Frick [a], Tomas Furmark [a], Mats Fredrikson [a,b]

[a] Department of Psychology, Uppsala University, Uppsala, Sweden
[b] Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden

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ABSTRACT

Serotonin facilitates fear learning in animals. We therefore predicted that individual differences in the capacity to regulate serotonergic transmission in the human neural fear circuit would be inversely related to fear conditioning. The capacity to regulate serotonergic transmission was indexed by serotonin transporter availability measured with [^{11}C]-DASB positron emission tomography. Results indicate that lower serotonin transporter availability in the amygdala, insula and dorsal anterior cingulate cortex predicts enhanced conditioned autonomic fear responses. Our finding supports serotonergic modulation of fear conditioning in humans and may aid in understanding susceptibility for developing anxiety conditions such as post-traumatic stress disorder.

1. Introduction

While central serotonin modulates negative affect (Ressler and Nemeroff, 2000), its etiological role in anxiety disorders is not fully understood. To understand the neural mechanisms of anxiety, animal studies often use fear conditioning paradigms, whereby a neutral cue subsequently elicits fear after associative aversive learning. In both animals (Maren and Quirk, 2004) and humans (Sehlmeyer et al., 2009; Mechias et al., 2010), the neural circuit underlying fear conditioning includes the amygdala, the anterior cingulate cortex (ACC) and the insula. The fear circuit of the brain, particularly the amygdala, is rich in serotonergic neurons (Barnes and Sharp, 1999) and several lines of evidence link serotonergic functions to fear conditioning. For example, blocking the serotonin transporter by administering acute doses of selective serotonin reuptake inhibitors (SSRIs) enhances fear conditioning by increasing extracellular serotonin in rodents (Burghardt et al., 2007), and reducing serotonin availability by acute tryptophan depletion compromises fear conditioning in humans (Attar et al., 2012). Molecular genetic studies demonstrate that markers of increased synaptic serotonin like, the short allele variant of the serotonin transporter gene-linked polymorphic region (5-HTTLPR), predict superior fear conditioning when compared to the long allele (Garpenstrand et al., 2001; Lonsdorf et al., 2009). The short allele of 5-HTTLPR has been coupled to conditioned fear responses in the insula (Hermann et al., 2012) and the amygdala (Klucken et al., 2013) paralleling findings in serotonin transporter knockout mice (Pang et al., 2011). In addition, high serotonin concentrations in the amygdala are associated with increased neural reactivity to emotional pictures in multimodal neuroimaging studies of serotonin transporter availability (Rhodes et al., 2007) and serotonin-1A receptor density (Fisher et al., 2006). Thus, serotonin may facilitate fear conditioning by modulating neural processing in the fear circuitry.

While previous studies support serotonergic modulation of conditioning, direct evidence relating serotonin in the human brain to fear conditioning is lacking. We therefore evaluated if fear conditioning is related to serotonin transporter availability, measured with [^{11}C]3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile ([^{11}C]-DASB) positron emission tomography (PET). Increased [^{11}C]-DASB uptake is associated with reduced synaptic serotonin (Lundquist et al., 2005), and low systemic serotonin levels attenuate conditioning (Attar et al., 2012). These findings formed the basis for our prediction of a negative correlation between conditioned skin conductance responses and [^{11}C]-DASB binding potential (BP) in the fear circuitry including the amygdala, insula and the anterior cingulate cortex (Sehlmeyer et al., 2009; Mechias et al., 2010).

To test this prediction, we combined serotonin transporter PET imaging with human fear conditioning data. During fear conditioning, a fear cue, CS+, consistently predicted the delivery of an aversive electric shock, while a control cue, CS−, was never paired with shock. Our primary measure of fear learning was the difference in autonomic responses between the CS+ and CS−.

* Corresponding author at: Department of Psychology, Uppsala University, Box 1225, 751 42 Uppsala, Sweden. Tel.: +46 18 471 5758; fax: +46 18 471 2400.
E-mail address: fredrik.ahs@psyk.uu.se (F. Åhs).

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2. Methods

2.1. Participants

Eight men and 8 women with a mean (±SD) age of 35 (±9.4) years were recruited to participate in the study. Exclusion criteria included psychiatric disorder, organic brain disorder, somatic disease, left-handedness, substance abuse, and pregnancy. The Structured Clinical Interview for DSM-IV (SCID; First et al., 1996) was administered to assess psychiatric symptoms. All participants refrained from tobacco, alcohol, and caffeine for 12 h, and from food for 3 h, before the PET investigations. None had participated in a PET study previously. The local ethical and radiation safety committees approved the study, and written informed consent was obtained from the participants.

2.2. Fear conditioning

All sixteen subjects underwent fear conditioning where two pictorial stimuli, a circle and a triangle counterbalanced for reinforcement, were displayed for 10 s. Prior to conditioning each stimulus was displayed 3 times to reduce orienting responses. During fear conditioning, the conditioned stimulus (CS+) always co-terminated with a 0.5 s electric shock delivered to the right forearm through two Ag–AgCl cup electrodes. The control stimulus (CS−) was never paired with shock. Each CS-type was displayed 6 times in a pseudo-randomized order with no more than two consecutive trials of each category. Because conditioned responses are not acquired during the first stimulus pairings, only the last 4 trials were used when computing skin conductance responses. No explicit information regarding contingencies was given. The intertrial interval varied between 15 and 35 s with a mean of 26 s. The intensity of the shock was determined by a work up procedure and terminated when subjects reported that the shock was uncomfortable but not painful. If subjects reported pain the shock level was adjusted accordingly. Fear conditioning was completed within 16–20 weeks after PET.

Skin conductance was recorded through two Ag–AgCl electrodes filled with isotonic electrolytic gel using Psylab (Contact Precision Instruments Inc., London, UK) using 100 Hz sampling rate. The signal was high pass filtered at 0.1 Hz. First interval responses show good temporal test–re-test stability (Fredrikson et al., 1993) ($r_{xy} = 0.72$) and skin conductance responses were quantified in a standard manner by subtracting the maximum value 1–4.5 s after CS onset from the mean skin conductance level in the 0.5 s time window immediately preceding CS onset (Dawson et al., 2000). SCRs less than 0 were treated as zero-responses. SCR was log-transformed (log(SCR + 1)) to reduce the influence of extreme values and deviation from normality. Because one participant had a value deviating > 5 SD she was excluded and the equipment failed for one additional participant, leaving 14 evaluated and entered into the statistical analyses.

The difference in SCR between CS+ and CS− was used as fear conditioning index because the CS− controls for reactivity differences unrelated to fear learning and the difference specifically reflects the fear memory (Agren et al., 2012).

2.3. Positron emission tomography

Participants were injected with an average of 405 (±23) MBq of [^{11}C]-3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile ([^{11}C]DASB). Scanning was performed using a 32-ring ECAT EXACT HR+ camera (Siemens/CTI, Knoxville, Tennessee), which enables the acquisition of 63 contiguous planes of data with a distance of 2.46 mm, resulting in a total axial field of view of 155 mm. Subjects were positioned in the scanner with the head fixated and a venous catheter for tracer injections was inserted. A 10-minute transmission scan was performed using three retractable germanium (^{68}Ge) rotating line sources. The tracer [^{11}C]-DASB was administrated as a rapid bolus injection and image acquisition started simultaneously. Data were acquired in three-dimensional (3D) mode during 60 min (1 × 60 s, 4 × 30 s, 3 × 60 s, 4 × 120 s, 2 × 180 s, 8 × 300 s frames).

2.4. Calculation of [^{11}C]-DASB regional binding potentials

Dynamic images were reconstructed using ordered subsets expectation maximization with six iterations, eight subsets and a 4 mm Hanning filter. Motion correction was applied to the dynamic data using Voiager (GE Healthcare, Uppsala, Sweden). The binding potential (BP) for each voxel was calculated using the reference Logan method (Logan et al., 1996). Cerebellum was used as reference region because it only has trace levels of serotonin transporters (Kish et al., 2005). The region was defined on a PET image summed over of all 22 frames using the PVElab software (Svarer et al., 2005), an observer independent approach for automatic generation of regions of interest. The analysis was performed in the time window 40–60 min post bolus injection and BP was estimated as the distribution volume ratio minus one. The BP of [^{11}C]-DASB is highly reproducible (Frankle et al., 2006; Kim et al., 2006) and could therefore be assumed to be stable between the time of PET-scanning and fear conditioning.

2.5. Regions of interest (ROIs)

Anatomically a priori defined ROIs included fear circuit areas (Sehlmeyer et al., 2009) comprising the amygdala, the anterior cingulate cortex (ACC), and the insula. The left and right amygdala ROIs were defined as a 5 mm radius sphere centered on the center co-ordinate (left amygdala: −22, −4, −15; right amygdala: 22, −4, −15) of the statistical maxima of the CS+ vs. CS− comparison in previous human neuroimaging studies on differential fear conditioning (Buchel et al., 1998; Carter et al., 2006; Cheng et al., 2006, 2007; Knight et al., 2004; LaBar et al., 1998; Milad et al., 2007; Petrovic et al., 2008; Phelps et al., 2004; Straube et al., 2007; Tabbert et al., 2005, 2006, 2011). The insula and ACC ROIs were defined in MNI space using the Automated Anatomical Labeling (AAL) library from the Wake Forest University (WFU) Pickatlas (Maldjian et al., 2003). ROIs were defined bilaterally as we did not have any hypothesis regarding laterality.

Rhodes et al. (2007) have previously used the lingual gyrus as a control region to assess the specificity of correlations between amygdala [^{11}C]-DASB BP and amygdala responses to emotional facial expressions. Therefore, we also here used the lingual gyrus to evaluate the specificity of the correlations between fear conditioning and [^{11}C]-DASB BP in the fear circuit. No correlation between [^{11}C]-DASB BP and fear conditioning was expected in the lingual gyrus. The lingual gyrus was also defined using the AAL library within WFU PickAtlas software. In total, five ROIs were investigated: right amygdala, left amygdala, bilateral ACC, bilateral insula and bilateral lingual gyrus.

2.6. Preprocessing of [^{11}C]-DASB BP images

The [^{11}C]-DASB BP images were coregistered to the summation image of all 22 [^{11}C]-DASB frames for each subject. The summation images were then normalized to the SPM PET template using affine transformation, and the transformation parameters were applied to the BP images resulting in MNI normalized BP images. The BP images were subsequently smoothed with an 8 mm isotropic Gaussian kernel.

2.7. Statistical analysis

[^{11}C]-DASB BP images were entered into a regression model in SPM8 (Wellcome Department of Cognitive Neurology, University College London, www.fil.ion.ucl.ac.uk) with the difference in SCR to the CS+ relative to the CS− used as covariate of interest. Because of our *a priori* prediction of an inverse relation between [^{11}C]-DASB binding potential and fear conditioning in the amygdala, we used a probability level of $P < 0.05$ corrected for multiple comparisons within the ROI using family