Variation in Serotonin Transporter Expression Modulates Fear-Evoked Hemodynamic Responses and Theta-Frequency Neuronal Oscillations in the Amygdala

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Background: Gene association studies detect an influence of natural variation in the 5-hydroxytryptamine transporter (5-HTT) gene on multiple aspects of individuality in brain function, ranging from personality traits through to susceptibility to psychiatric disorders such as anxiety and depression. The neural substrates of these associations are unknown. Human neuroimaging studies suggest modulation of the amygdala by 5-HTT variation, but this hypothesis is controversial and unresolved, and difficult to investigate further in humans.

Methods: We used a mouse model in which the 5-HTT is overexpressed throughout the brain and recorded hemodynamic responses (using a novel in vivo voltammetric monitoring method, analogous to blood oxygen level–dependent functional magnetic resonance imaging) and local field potentials during Pavlovian fear conditioning.

Results: Increased 5-HTT expression impaired, but did not prevent, fear learning and significantly reduced amygdala hemodynamic responses to aversive cues. Increased 5-HTT expression was also associated with reduced theta oscillations, which were a feature of aversive cue presentation in controls. Moreover, in control mice, but not those with high 5-HTT expression, there was a strong correlation between theta power and the amplitude of the hemodynamic response.

Conclusions: Direct experimental manipulation of 5-HTT expression levels throughout the brain markedly altered fear learning, amygdala hemodynamic responses, and neuronal oscillations.

Key Words: serotonin transporter, fMRI, amygdala, fear, tissue oxygen, theta oscillations

The serotonin (5-hydroxytryptamine; 5-HT) transporter (5-HTT) is a key determinant of brain 5-HT function as it controls 5-HT availability at the synapse. There is a large natural variation in 5-HTT expression in the human population, approximately threefold between individuals (1). Current thinking is that this variation, in large part driven by the 16 or more 5-HTT gene polymorphisms discovered to date, is the source of large individual differences in personality, behavior, and brain disorder susceptibility. In this regard, gene association studies have reported that a common insertion/deletion polymorphism (producing long (l) and short (s) variants, respectively) in the 5-HTT gene upstream promoter region (5-HTTLPR) generates high (l/l) and low (s/s) expressing variants, with the s/s genotype conferring increased risk for anxiety-related traits (2) and affective disorders, especially when combined with environmental factors (3,4), and the l/l genotype conferring reduced risk. As is common with gene-association studies, these findings are confounded by failed replications (5,6) and need to be underpinned by a convincing neural substrate.

An attractive theory is that 5-HTT variation has an impact on emotionality and affective disorder susceptibility through modulation of the amygdala. This idea derives largely from human functional magnetic resonance imaging (fMRI) studies, which detect lower amygdala blood oxygen level–dependent (BOLD) responses to aversive cues in l/l versus s carriers (7,8). However, a recent meta-analysis of available published and unpublished data sets found that the association between 5-HTT variation and amygdala reactivity was of borderline statistical significance (with no significant genotypic difference in 21 of 34 samples) and resolvable only through further large-scale, and thus impractical, imaging studies to control for study design and subject heterogeneity (9).

At the heart of the 5-HTTLPR debate lie two fundamental and as yet unanswered questions: 1) Does variation in 5-HTT expression influence how aversive cues are processed, and, if so, 2) what are the underlying neuronal mechanisms? Here, as an alternative to gene-association studies, we used a mouse model of genetically altered 5-HTT expression to address these questions. 5-HTT overexpressing mice (5-HTTOE) have two- to threefold greater 5-HTT expression than wild-type (WT) mice (10), mirroring the natural variation in humans (1,11,12), with 5-HTTOE mice approximating the human l/l genotype (10). Here we investigated aversive learning and amygdala activity in 5-HTTOE and WT mice during Pavlovian fear conditioning.

We recorded amygdala activity in two ways. First, to allow direct comparison with human neuroimaging, we recorded

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amygdala hemodynamic responses in behaving 5-HTTOE and WT mice. BOLD fMRI cannot be performed in freely moving rodents but recent advances in tissue oxygen (T_{O2}) voltammetry offer equivalent hemodynamic measurements. T_{O2} signals are driven by the same neurovascular mechanisms as the BOLD signal and therefore provide a close hemodynamic surrogate, via intracerebrally implanted carbon paste microelectrodes (13–16). Recently, we have shown that T_{O2} signals in the amygdala display a brain-region-specific discrimination between aversive and neutral cues during fear conditioning in rats (17). Simultaneously in the same mice, we measured neuronal activity in the form of local field potentials (LFPs).

Here we show that increased 5-HTT expression impairs fear learning and reduces amygdala hemodynamic responses and theta oscillations evoked by aversive cues. Finally, we show that the hemodynamic response amplitude is strongly correlated with theta oscillatory power in WT mice, but not in 5-HTTOE mice, suggesting a plausible neural basis for the 5-HTTLPR-related differences in human BOLD signals.

Methods and Materials

For a full description of the methods, see Supplement 1.

Subjects

Male 5-HTTOE and WT mice were generated on a CBA × C57BL/6J background, as described previously (10). Mice were approximately 5 months old at the time of surgery. The experiments were conducted in accordance with the United Kingdom Animals Scientific Procedures Act (1986) under project license 30/2561.

Surgery

Mice were surgically implanted with a carbon paste electrode (CPE, 200-μm diameter) into the basolateral amygdala to measure T_{O2} and a silver electrode (125-μm diameter) into the basolateral amygdala of the contralateral hemisphere to measure LFPs, as described previously (14,17). Right/left electrode positions for T_{O2}/LFP recordings were counterbalanced across mice. Coordinates were −1.35 mm anterior/posterior, ±3.10 mm medial/lateral, and −5.00 mm dorsal/ventral, relative to bregma. Auxiliary and reference electrodes (200-μm diameter silver wire) were implanted into parietal cortex. A pedestal plug (MS363, Plastics One, Roanoke, Virginia) was secured with dental cement and skull screws. Mice were allowed to recover for at least 7 days after surgery.

T_{O2} Measurements

T_{O2} signals were measured using constant potential amperometry, as described previously (14,17,18). When a constant potential (−650 mV relative to reference) is applied to an electrode implanted into the brain, O_2 is electrochemically reduced on the electrode's surface, inducing a current directly proportional to the local O_2 concentration (19). Like the fMRI-BOLD signal, the T_{O2} signal is determined primarily by changes in local cerebral blood flow (16,20).

Fear Conditioning Procedures

Two different fear conditioning paradigms were used. A separate cohort of unoperated mice (n = 11 per genotype) were tested on a standard rodent fear conditioning paradigm to see if 5-HTT overexpression affected fear learning. Mice received two training trials (30-second tone followed by .3 mA, .5-second shock) in one context followed 24 hours later by two tone-alone presentations in a novel context.

The operated mice (n = 42; 22 WT, 20 5-HTTOE) were tested on a discriminative fear-conditioning paradigm. This behavioral paradigm differs from the standard fear-conditioning paradigm described above in that mice must learn to discriminate between two distinct auditory cues (tone and white noise), with one cue paired with footshock (conditioned stimulus; CS+) and the other cue never paired with footshock (CS–). This discriminative approach is commonplace in human fMRI (21,22) and rodent electrophysiologic studies of fear (23,24). Because any stimulus could potentially evoke amygdala activity, the CS– provides the necessary nonaversive control stimulus with which to compare CS+ evoked responses, akin to a subtraction task in fMRI.

Discriminative fear conditioning was performed over 5 consecutive days. On Day 1 (pre-exposure), mice were presented with the auditory cues (five 2900-Hz tones and five white noise stimuli, both 30 seconds in duration, presented in pseudorandom order), with no shocks administered. On Days 2 through 4 (training), the mice were placed into a different context and presented with the same auditory cues, but now one cue (tone or white noise, counterbalanced across mice) was always paired with coterminating footshock (.3 mA, .5 seconds), whereas the other cue was not. On Day 5 (fear memory recall), mice were placed into a novel context and presented with the auditory cues with no shocks administered. During all days, cue-evoked freezing behavior and amygdala T_{O2} responses and LFPs were recorded simultaneously in the same mice.

Data Analysis

Behavior was recorded with a video camera and freezing was measured using Videotrack (Viewpoint, Champagne Au Mont D'Or, France) or NIH Image (25). A freezing "difference score" was calculated as follows: percent freezing during the 30-second cue presentation minus percent freezing during the 30 seconds before cue presentation (i.e., positive freezing scores indicate increased freezing to the cue and negative freezing scores indicate decreased freezing to the cue relative to the precue period).

Cue-evoked T_{O2} responses were calculated by subtracting the mean T_{O2} signal in the 5 seconds before CS onset (i.e., baseline) from the T_{O2} signal during the 30-second CS presentation. This yielded a 30-second ΔT_{O2} signal, which was then divided into fifteen 2-second timebins (i.e., 0–2, 2–4, 4–6 . . . 28–30 seconds), with each data point equal to the mean value during each 2-second timebin (Figure S2 in Supplement 1) (17).

LFPs were band-pass filtered between 1 and 45 Hz. Power spectra were calculated using a fast Fourier transform over the first 10 seconds of CS presentation and were averaged over the five CS+ versus the five CS– trials on each day for each mouse. To compare across mice, spectra were normalized by expressing the power in each frequency bin as a proportion of the total power between 1 and 45 Hz (Figure S2 in Supplement 1).

Histology

Electrode placements were determined at the end of the experiment. Mice were transcardially perfused with physiologic saline (.9% NaCl), followed by 10% formol saline (10% formalin in .9% NaCl). Coronal sections (40 μm) were cut on a freezing microtome and stained with cresyl violet. Only mice with confirmed electrode placements in the basolateral amygdala were used in the T_{O2} and LFP analyses (Figure S1 in Supplement 1).