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Reliable non-invasive measurement of human neurochemistry using proton spectroscopy with an anatomically defined amygdala-specific voxel

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ABSTRACT

Given the central role of the amygdala in fear perception and expression and its likely abnormality in affective disorders and autism, there is great demand for a technique to measure differences in neurochemistry of the human amygdala. Unfortunately, it is also a technically complex target for magnetic resonance spectroscopy (MRS) due to a small volume, high field inhomogeneity and a shared boundary with hippocampus, which can undergo opposite changes in response to stress. We attempted to achieve reliable PRESS-localized single-voxel MRS at 3T of the isolated human amygdala by using anatomy to guide voxel size and location. We present data from 106 amygdala-MRS sessions from 58 volunteers aged 10 to 52 years, including two tests of one-week stability and a feasibility study in an adolescent sample. Our main outcomes were indices of spectral quality, repeated measurement variability (within- and between-subject standard deviations), and sensitivity to stable individual differences measured by intra-class correlation (ICC). We present metrics of amygdala-MRS reliability for n-acetyl-aspartate, creatine, choline, myo-Inositol, and glutamate + glutamine (Glx). We found that scan quality suffers an age-related difference in field homogeneity and modified our protocol to compensate. We further identified an effect of anatomical inclusion near the endorhinal sulcus, a region of high synaptic density, that contributes up to 29% of within-subject variability across 4 sessions (n = 14). Remaining variability in line width but not signal-to-noise also detracts from reliability. Statistical correction for partial inclusion of these strong neurochemical gradients decreases n-acetyl-aspartate reliability from an intraclass correlation of 0.84 to 0.56 for 7-minute acquisitions. This suggests that systematic differences in anatomical inclusion can contribute greatly to apparent neurochemical concentrations and could produce false group differences in experimental studies. Precise, anatomically-based prescriptions that avoid age-related sources of inhomogeneity and use longer scan times may permit study of individual differences in neurochemistry throughout development in this late-maturing structure.

Introduction

The amygdala is a core component of the social brain, indicating salience of social and emotional stimuli (e.g. Adolphs et al., 2005; Dalton et al., 2005). It is critical to emotional expression, not only of fear and anxiety (Davis and Whalen, 2001), where dendritic spine density parallels development of anxiety-like behavior from stress (Mitra et al., 2005), but also is essential to appetitive conditioning previously attributed to the dopamine system alone (Ambroggi et al., 2008). In humans, it continues to develop through late adolescence and into the middle twenties (Giedd et al., 1996, 1997; Nacewicz et al., 2006; Schumann et al., 2004) and its abnormal development has been quantitatively linked to the socio-emotional impairments of autism (Dalton et al., 2005; Munson et al., 2006; Nacewicz et al., 2006) and to the time course of depression and affective disorders (Frodl et al., 2003; McEwen, 2003; van Eijndhoven et al., 2009; Velakoulis et al., 2006).

Advances in the field of magnetic resonance spectroscopy (MRS) raise the possibility of longitudinal study of multiple, simultaneously measured neurochemicals without the radiation exposure of positron emission tomography. Non-invasive, localized proton MRS also permits prospective correlation with emotional and social behavior, a feat unachievable with post-mortem samples. A reliable MRS measurement of the amygdala could provide insight into the neuronal and glial differences that compose the gross volumetric and functional changes in normal and abnormal adolescent development, greatly advancing our understanding of this major target of psychiatric and behavioral therapy.

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Although current MRS techniques allow precise localization to specific brain regions, much MRS research spans swaths of cortex so large as to be almost neuroscientifically uninteresting. At an average of volume of 1.8–2.0 mL (Brierley et al., 2002), the amygdala is considerably smaller than the standard volume for spectroscopy acquisitions (typically 8 mL). A provocative finding by Oz et al. (2006) in a study of substantia nigra, an area of comparable technical difficulty, was that decreasing partial volume of adjacent tissue improved detection of a known neurochemical signature of the region. This is even more important in the medial temporal lobe, since the amygdala and hippocampus undergo opposite cytoarchitectural changes, expansion and constriction of the dendritic tree, respectively, in animal models of stress/depression (reviewed by McEwen, 2007). Recent findings in a rhesus monkey model indicate vastly different patterns of heritability for functional activation in the amygdala and hippocampus under stressful challenge conditions (Oler et al., 2010). In human work, gross volumetry reveals differential adolescent growth trajectories for amygdala and hippocampus that are also sexually dimorphic (Giedd et al., 1996, 1997). From a neuroscience perspective, it is therefore imperative to measure amygdala neurochemistry exclusive of hippocampal neurochemistry.

Neuroscientists also diverge from clinical spectroscopists in their definition of "reliability". As elegantly explained by Kreis (2004), traditional clinical studies require that the effect size of a group difference (usually a disease) be greater than the sum of within-individual variances (variance in repeatability plus between-session variance) plus between-individual variance. This approach asks the question: Are reliably resolvable peaks sufficiently stable across time, anatomy *and individuals* to see a clinically important effect? In affective neuroscience, however, sensitive detection of reliable physiological differences between healthy individuals has been used successfully to characterize behavioral traits (Tomarken et al., 1992), and these sensitive measures then used to look at pathology. This does not require the near-perfect sensitivity and specificity of single-subject diagnostics, but it requires statistical preservation of relative differences within a group (correlations). Rather than viewing it as error, this approach puts the between-individual variance in the numerator and asks the question: Are reliably resolvable peaks sufficiently stable within each individual to detect inter-individual differences that may relate to behavioral traits or adolescent development? We characterize measurement reliability first by evaluating the standard deviation of repeated measurements. This indicates overall technique capability. We then turn our attention to the ICC to relate measurement error to the size of stable between-subject differences in healthy populations.

We designed a series of studies to optimize MRS precision and sensitivity by matching the prescription to the amygdala anatomy of each participant, letting acquisition volume vary slightly but using strictly-defined criteria for spectral quality (signal to noise ratio, SNR, and spectral resolution in terms of line width of unsuppressed water, LW). We attempted to isolate amygdala from hippocampus by adapting a method from prostate chemical shift imaging, which uses a slightly overprescribed excitation volume overlapped with outer volume suppression pulses to null signal and artifact from undesired tissues (e.g. Mueller-Lisse and Scherr, 2007). We reasoned that a smaller voxel would increase anatomical precision (reduced partial volume effects) and improve shimming (see Kreis, 2004 for review), while the lower signal could be improved with additional signal averages. We validated the resultant amygdala-MRS protocol in two studies of 1-week test–retest stability. We also conducted a feasibility study to determine age-dependence of spectral quality, since our goal is a measure capable of tracking individual differences in amygdala neurochemistry during adolescent development.

Methods

Participants

Participant demographics for studies 1, 2 and 3 are listed (Table 1). All participants provided written consent or assent as part of a procedure approved by the Human Subjects Institutional Review Board of the Wisconsin School of Medicine and Public Health. Participants for study 2 were all typically developing males recruited as part of an effort to characterize amygdala neurochemistry in typical and autistic adolescent development and included 2 individuals per year of life from age 10 to 24 (two 10-year olds, two 11-year olds, etc.). Follow-up analyses were carried out on data from an additional 3 participants, aged 19.5, 19.9 and 27.2 y at the time of optimized scan, from another ongoing longitudinal study of typical and autistic adolescent amygdala development. One individual was common to studies 1 and 3, otherwise participants were unique to each study; effects in all study 3 analyses were similar with and without this individual.

Amygdala-specific MRS

Data were collected on a GE Signa 3.0 T scanner with 8-channel head coil for studies 1 and 2, and data for study 3 were collected on a GE X750 3.0 T scanner with 8-channel head coil. A system of oblique anatomical acquisitions was used to provide sagittal and axial localizers that were consistently oriented along the superior–inferior axis of the amygdala. First, a T1-weighted partial coverage scan (256 × 192 matrix, resampled to 256 × 256, over 240 mm field of view with 64 1-mm slices; 3 m 55 s) covering the right half of the brain was acquired in an oblique sagittal plane parallel to the interhemispheric sulcus (to account for head tilt in the coronal plane and rotation in

Table 1
Sample demographics for three studies of amygdala-MRS.

	Study 1 Preliminary reliability study	Study 2 Feasibility study with children	Study 3 Optimized reliability study
Sample Size	n = 11	n = 30	n = 14
Age Mean + SD	29.3 + 9.5 y	17.3 + 4.8 y	28.9 + 6.5 y
(range)	(20.6–51.6 y)	(10.0–24.0 y)	(18.5–43.4 y)
Acquisition Volume[a]	1.85 + 0.5 cc	2.6 + 0.7 cc	2.6 + 0.3 cc
(Excitation Volume)	(2.9 + 0.9 cc)	(3.6 + 0.9 cc)	(3.2 + 0.2 cc)
GM	83 + 6%	70 + 9%	76 + 5%
WM	14 + 7%	27 + 9%	16 + 5%
CSF	3 + 2%	3 + 3%	8 + 3%
Spectral Quality	80.2 + 24	139 + 67	202 + 47
LCModel (SNR[b]/LW)			
LW	0.064 + 0.015	0.051 + 0.010	0.050 + 0.009
SNR	4.9 + 1.1	8.0 + 2.3	9.7 + 1.2

[a] Effective Acquisition Volume is Excitation Volume minus overlapping outer volume suppression bands.
[b] Signal to noise ratio for NAA methyl group calculated as $(\mathrm{Max}/[2 * \mathrm{RMS}_{\mathrm{Residual}}])$ (Provencher, 1993). All values reported as mean + standard deviation. Ages are expressed in years and fractions of years.