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Research paper

Altered time course of amygdala activation during speech anticipation in social anxiety disorder

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ABSTRACT

Background: Exaggerated anticipatory anxiety is common in social anxiety disorder (SAD). Neuroimaging studies have revealed altered neural activity in response to social stimuli in SAD, but fewer studies have examined neural activity during anticipation of feared social stimuli in SAD. The current study examined the time course and magnitude of activity in threat processing brain regions during speech anticipation in socially anxious individuals and healthy controls (HC).

Method: Participants (SAD n=58; HC n=16) underwent functional magnetic resonance imaging (fMRI) during which they completed a 90 s control anticipation task and 90 s speech anticipation task. Repeated measures multi-level modeling analyses were used to examine group differences in time course activity during speech vs. control anticipation for regions of interest, including bilateral amygdala, insula, ventral striatum, and dorsal anterior cingulate cortex.

Results: The time course of amygdala activity was more prolonged and less variable throughout speech anticipation in SAD participants compared to HCs, whereas the overall magnitude of amygdala response did not differ between groups. Magnitude and time course of activity was largely similar between groups across other regions of interest.

Limitations: Analyses were restricted to regions of interest and task order was the same across participants due to the nature of deception instructions.

Conclusions: Sustained amygdala time course during anticipation may uniquely reflect heightened detection of threat or deficits in emotion regulation in socially anxious individuals. Findings highlight the importance of examining temporal dynamics of amygdala responding.

1. Introduction

Excessive anxiety in both the presence and anticipation of social situations is a central feature of social anxiety disorder (SAD). Exaggerated anticipatory anxiety can lead socially anxious individuals to avoid social situations or engage in safety behaviors, thus maintaining SAD symptoms by preventing new learning and reinforcing the maladaptive belief that social apprehension is warranted (Hofmann, 2007; Wells et al., 1995). Cognitive models of SAD posit that socially anxious individuals engage in negatively biased anticipatory processing prior to entering social situations (e.g., expecting a negative outcome from an interaction), which enhances anxiety and increases avoidance behaviors (Clark and Wells, 1995; Hinrichsen and Clark, 2003). Given the role of anticipatory anxiety as a maintenance factor for SAD, it is important to better understand the neural bases of anticipatory processing in social anxiety.

Studies of the functional neuroanatomy of anxiety and emotional reactivity in SAD have revealed altered neural activity in response to social stimuli, including heightened amygdala responses to harsh (e.g., angry, disgusted) faces compared to happy faces (Phan et al., 2006; Stein et al., 2002), exaggerated amygdala reactivity to harsh faces compared to healthy controls (e.g., Klumpp et al., 2010), and greater amygdala and insula activity in response to faces with angry expressions compared to neutral ones (Straube et al., 2004). Indeed, amygdala and insula regions frequently show hyperactivation across provocation and affective processing study designs in individuals with anxiety disorders, including SAD (Etkin and Wager, 2007; Miskovic and Schmidt, 2012). In addition to amygdala and insula, anterior

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cingulate cortex and medial prefrontal cortex regions are also implicated in fear and anxiety neural circuitry (Etkin, 2012), and SAD individuals exhibit altered functioning in these areas in response to threatening or negative stimuli (Brühl et al., 2014). Specifically, studies largely show increased activity in anterior cingulate regions (e.g., Amir et al., 2005; Labuschagne et al., 2012; Phan et al., 2006; but see Pujol et al., 2013) and medial prefrontal cortex areas (e.g., Stein et al., 2002; Straube et al., 2004; Labuschagne et al., 2012) compared to controls, consistent with evidence for these regions in identifying and expressing negative emotion (Etkin et al., 2011).

Neuroimaging studies examining anticipatory anxiety in SAD are more limited, but fear and emotion processing regions have been similarly implicated. In a positron emission tomography study comparing SAD individuals speaking privately prior to giving a public speech (the anticipation group) to those speaking privately after giving a speech (the comparison group), anticipatory anxiety was associated with enhanced regional cerebral blood flow in the left amygdaloid-hippocampal region and right dorsolateral prefrontal and left inferior temporal cortices, suggesting altered fear network activity (Tillfors et al., 2002). However, this study was limited by its small sample size (n=9), lack of control group, and presence of speaking during the anticipation phase. A second small (n=8) fMRI study examining neural activity during speech anticipation compared to rest (a counting-breathing relaxation task) showed increased activation in temporal lobe and limbic regions, including amygdala, during anticipation in SAD individuals compared to healthy individuals, and decreased activation in left dorsal anterior cingulate and medial prefrontal cortex areas (Lorberbaum et al., 2004). However, this paradigm was limited by its use of a rest phase as a control condition. Boehme et al. (2013) improved upon this fMRI paradigm by including a novel control anticipation task in which participants anticipated reading a word aloud so that experimenters could ostensibly "test" their equipment, compared to a 40s speech anticipation task. Compared to controls, SAD participants exhibited increased right insula and decreased left ventral striatum activity during speech versus control anticipation, as well as heightened right amygdala activity during the first half of speech anticipation (but not in the second half), suggesting a variation in the association of amygdala activity with anticipation over time in SAD participants.

The current study aimed to examine neural activity during speech anticipation in SAD individuals, expanding upon previous findings in two main ways. First, we aimed to better distinguish between neural processing during anticipation of a non-threatening versus threatening task. In previous studies, participants were informed prior to entering the scanner that they would be delivering a speech or series of speeches (e.g., Boehme et al., 2013). While this design allows for multiple trials of control and speech anticipation, it may have the unintended consequence of eliciting speech anticipatory anxiety and related neural patterns well before neural scanning of speech anticipation begins and during control anticipation, in effect "contaminating" the control condition. In the current paradigm, we informed participants of an upcoming speech only after they completed the control anticipation task and immediately prior to the speech anticipation task in the scanner.

Second, we examined the time course, or change in brain activity over time, during anticipation. Previous studies have largely focused on the magnitude or amplitude of neural reactivity rather than variability or time course of neural responses, despite the fact that timing is an important element of anticipatory anxiety (Grillon et al., 1993; Straube et al., 2009). We chose to focus on the temporal neural dynamics in a small set of *a priori* regions of interest (ROIs) that have been implicated in previous studies of anticipatory anxiety and threat processing in social anxiety (Boehme et al., 2013; Miskovic and Schmidt, 2012), namely, bilateral amygdala, insula, ventral striatum, and dorsal anterior cingulate cortex (dACC).

The time course of amygdala activity may be of particular importance for anxious populations. A previous study found that SAD individuals exhibit altered amygdala temporal response patterns to negative and positive emotional faces, such that amygdala responses occurred later in SAD versus control participants (Campbell et al., 2007). In another fMRI study of 120 participants, heightened trait neuroticism was associated with more prolonged amygdala activation following the presentation of negative images, but was not associated with initial amygdala reactivity (Schuyler et al., 2012). In other words, slower amygdala "recovery" rather than elevated amygdala reactivity to negative images correlated with trait neuroticism. Based on these findings, we expected that SAD individuals would have not only more elevated but also more sustained or prolonged amygdala activation to threat during speech anticipation compared to healthy controls. Additionally, we expected that heightened and more sustained amygdala activity would be associated with more severe social anxiety symptoms. For other ROIs, we hypothesized that SAD individuals would show heightened insula activity and reduced ventral striatum activity compared to controls. We also examined whether dACC activation in SAD was reduced (replicating Lorberbaum et al. [2004] results) or elevated (e.g., Phan et al., 2006) compared to controls. Beyond main effects of group, hypotheses regarding the time course of activity in non-amygdala regions were largely exploratory.

2. Methods

2.1. Participants

Participants were recruited as part of a study comparing two behavioral treatments for SAD (see Craske et al., 2014). SAD participants met DSM-IV criteria for principal or co-principal SAD with a clinical severity rating (CSR) of 4 or higher according to the Anxiety Disorders Interview Schedule (Brown et al., 1994). HC participants could not meet DSM-IV criteria for any Axis I disorder. All participants were between 18 and 45 years of age, either medication free or stabilized on medication, not undergoing behavioral therapy, English-speaking, and right-handed. Exclusion criteria included active suicidal ideation or severe depression (CSR > 6), psychiatric hospitalization within the past five years, serious medical conditions or pregnancy, history of psychosis or bipolar disorder, substance abuse or dependence within the past 6 months, claustrophobia, and non-removable metal in body.

Seventeen HC participants and 71 SAD participants entered the study and completed the fMRI scan. Of these, 1 HC and 11 SAD participants did not complete the speech anticipation task due to technical errors; thus 16 HC and 60 SAD participants were included in the present study. Participants were 50% female with a mean age of 27.8 years (SD=6.6) and were 49.3% Caucasian, 24.0% Asian/Pacific Islander, 14.7% Hispanic/Latino, 2.7% Black/African American, and 9.3% other race. HC and SAD participants did not differ by gender, age, or ethnicity ($ps > .26$). The majority (82.5%) of SAD participants were unmedicated.

2.2. Procedure

Participants completed the ADIS-IV and a battery of questionnaires, including the Liebowitz Social Anxiety Scale (Liebowitz, 1987). Eligible participants then completed a laboratory assessment, which included a computer dot probe task and public speaking task, followed by an fMRI scan approximately one week later. During the fMRI, participants completed several tasks to assess emotional reactivity and emotion regulation, including the control and speech anticipation tasks below.

2.3. Control anticipation task

Prior to entering the scanner, participants were told that they would

be completing an anticipation task in the scanner during which they would "mentally anticipate and prepare for" completing one of the tasks they had completed during their laboratory assessment as soon as they exited the scanner. All participants were then informed that they had been "randomly assigned" to complete the computer dot probe task. In fact, anticipation of this task served as the control anticipation task. While in the scanner, participants anticipated completing the computer dot probe task while viewing 90 s of a "live feed" video of the room in which they were ostensibly going to complete the task. The video portrayed a room with several computers and three people facing computers and silently working. This procedure allowed us to measure neural activity during anticipation of a non-social evaluative task prior to participants' knowledge that they would be asked to complete a speech. Immediately following the control anticipation task, participants rated their anxiety level on a scale of 0–100.

2.4. Speech anticipation task

After completing the control anticipation task in the scanner, participants were then informed through a two-way microphone that they had been mistakenly assigned to the wrong condition and that they were actually going to give a speech immediately following the scan. Participants then redid the task while anticipating giving a speech and viewing a 90 s "live feed" video of the room and audience to which they were ostensibly going to speak. The video began with one audience member present, a second and third audience member entered at 15 s, followed by a fourth audience member at 42 s. The video portrayed audience members entering the room and sitting around a conference table. The staggered entrance of audience members throughout the 90s block allowed us to better examine the temporal dynamics of neural activity during anticipation.

After the task, participants rated their anxiety level on a scale of 0–100 and were removed from the scanner. Participants then rated how much they believed they would actually be completing a speech on a scale of 0–8 (0=did not believe at all, 8=completely believed) and completed a free writing task to reflect on what they had been thinking about during the speech anticipation task (see Supplementary Material, Appendix A). We then informed participants that they would not actually be delivering a speech and fully debriefed them regarding the purpose of the deception instructions.

2.5. Measures

2.5.1. Liebowitz social anxiety scale (LSAS)

The LSAS (Liebowitz, 1987) is a 24-item scale that measures the dimensional severity of social anxiety disorder symptoms by assessing fear and avoidance across a number of social situations. We used the self-report version of the LSAS, which has shown high validity and internal consistency (Fresco et al., 2001; Rytwinski et al., 2009).

2.6. fMRI recording and pre-processing

Magnetic resonance images were acquired using a Trio 3.0 T MRI scanner. Participants' heads were secured in place using pillows. A high-resolution T2-weighted structural image (spin-echo, TR=5000 ms, TE=34 ms, matrix size=128×128, resolution 1.6 mmx1.6 mmx3 mm, FOV=200 mm, 36 slices, 3 mm thick, flip angle=90°, bandwidth=1302 Hz/Px) was acquired coplanar with the functional scans (gradient-echo, TR=3000 ms, TE=25 ms, flip angle=90°, matrix size=64×64, resolution 3.1 mmx3.1 mmx3.0 mm, FOV=200 mm, 36 slices, 3 mm thick, bandwidth=2604 Hz/Px). Functional images for each participant were realigned to the first functional volume collected to correct for head motion, co-registered to the high-resolution structural images, and normalized into stereotactic space as defined by the Montreal Neurological Institute. We performed quality assurance on each participant's series of scans; participants with more than 2.5 mm max displacement were dropped from analyses (2 SAD participants), leaving 58 SAD participants and 16 HC participants in the current analyses.

Left and right amygdala and insula ROIs were defined anatomically (AAL library; Tzourio-Mazoyer et al., 2002); left and right ventral striatum ROIs were defined using a 6 mm radius sphere surrounding peak coordinates from Boehme et al. (2013; left: x=-4; y=10; z=6), as this study similarly compared SAD vs. control participants anticipating a speech; and the dACC ROI was defined using the automated anatomical atlas (AAL library; Tzourio-Mazoyer et al., 2002) with a rostral boundary of y=+36 and a caudal boundary of y=0 (Vogt et al., 2003). Statistical maps demonstrating task-induced activation are provided in Supplementary Material (Appendix B). We used the Marsbar toolbox (Brett et al., 2002) to extract the time course of each ROI. Raw image intensity values were converted to percent signal change (relative to the mean per voxel of the whole time course) to allow averaging of time courses across participants. Time courses were extracted for control anticipation (90 s) and speech anticipation (90 s) for each individual. To examine the association between amygdala activity and social anxiety severity, the mean and standard deviation of each participant's amygdala activity throughout the speech anticipation task was computed and used in these analyses.

2.7. Statistical analyses

The time course of each ROI was analyzed using repeated measures multi-level modeling in Stata 12.1 (StataCorp, 2011). We chose a multi-leveling modeling approach because it accounts for the hierarchical nature of repeated measures data and is robust to issues that may result from uneven sample sizes, including heteroscedasticity (Quené and Van den Bergh, 2004). For each ROI, three separate analyses were run using time course data corresponding to the time points of anxiety provocation (i.e., the entrance of audience members during the speech anticipation task): 0–15 s (Audience 1; TRs 1–5), 18–42 s (Audience 2; TRs 6–14), and 45–90 s (Audience 3; TRs 15–30). Corresponding time course data from the control anticipation task (Control 1: 0–15 s, Control 2: 18–42 s, and Control 3: 45–90 s, respectively) were included as covariates in each model. Time (TR), Group (HC or SAD), and their interaction (TimexGroup) were included as predictors, and believability score 0–8 rating of belief about giving a speech) was included as a covariate. We modeled intercepts and slopes (Time) as random effects in order to account for individual differences in the delay in the haemodynamic response and the shape of response. We examined the quadratic effect of Time and its interaction with Group first; if there was no quadratic effect, we then examined the linear effect. To control for multiple comparisons, a false discovery rate of 5% (FDR; Benjamini and Hochberg, 1995) was employed on Group and Time x Group *p*-values within each ROI, as these results were the focus of our hypotheses. As psychotropic medication can affect cerebral blood flow (e.g., Furmark et al., 2002), we ran follow-up analyses for each ROI to examine whether time course activity differed between medicated versus unmedicated SAD participants. To examine the association between amygdala activity and social anxiety symptoms, we implemented multiple regression analyses, with LSAS score as the dependent variable, and Group, mean and *SD* of right or left amygdala BOLD responses across speech anticipation, and their interaction with Group, as predictors.

3. Results

3.1. Behavioral responses

SAD participants' anxiety ratings following the control anticipation and speech anticipation tasks were significantly higher than those of HCs ($ps < .001$; see Table 1), and both groups demonstrated a significant increase in self-reported anxiety from the control to speech

Table 1
Comparison of mean LSAS, speech believability rating, and self-reported anxiety ratings following control and speech anticipation tasks between SAD and HC. SAD=social anxiety disorder group; HC=healthy control group.

	SAD (n=57)	HC (n=16)	t	p	d
Liebowitz Social Anxiety Scale (*SD*)	81.7 (19.0)[a] Range=25.3–128	17.6 (7.9) Range=0–28	13.1	< .001	4.4
Mean anxiety level after control anticipation (*SD*)	26.9 (21.8) Range = 0–95	4.0 (5.9) Range=0–20	4.1	< .001	1.4
Mean anxiety level after speech anticipation (*SD*)	51.6 (21.1) Range=0–99	15.0 (15.7) Range=0–50	6.4	< .001	2.0
Mean believability rating (*SD*)	5.3 (2.2) Range=1–8	4.4 (2.4) Range=0–8	1.3	.20	.4

[a] Questionnaire data from one SAD participant is missing, thus SAD n =56 for this analysis.

anticipation task (*ps* < .01). Believability ratings did not significantly differ between HC and SAD participants (see Table 1).

3.2. Amygdala responses

FDR-corrected significance levels were q*=.033 for right and left amygdala. There were significant Group×Time (linear) interactions for right amygdala (z=3.03, p=.002, 95% CI [.04, .20]; see Fig. 1) and left amygdala (z=3.30, p=.001, 95% CI [.07, .26]; see Fig. 2) during Audience 1, with no main effects of Group (*ps* > .21). Analyses of the simple effects of Time within each Group showed a linear decrease in BOLD response for both right (z=−2.75, p=.006, 95% CI [−.17, −.03]) and left amygdala (z=−3.96, p < .001, 95% CI [−.26, −.09]) in HC but not in SAD (*ps* > .22).

For Audience 2, there was a significant Group×Time^2 interaction for right amygdala (z=2.71, p=.007, 95% CI [.005, .03]; see Fig. 1), but not left amygdala (p=.39; see Fig. 2), with no main effects of Group (*ps* > .45). Follow up analyses in right amygdala indicated a stronger quadratic effect of Time in HC (z=−4.74, p < .001, 95% CI [−.04, −.02]) than SAD (z=−3.11, p=.002, 95% CI [−.02, −.004]). In left amygdala, there was a significant main effect of Time^2 across groups (χ^2 =13.09; p < .001).

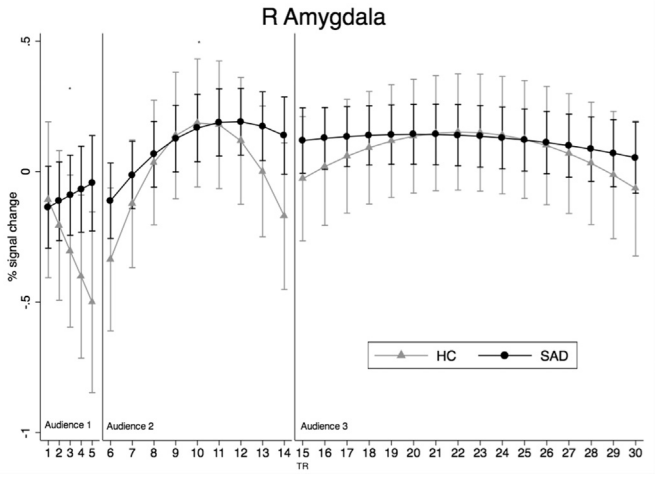

Fig. 1. Results from multi-level model analyses of right amygdala percent signal change across time during speech anticipation relative to control anticipation. HC=healthy control group, SAD=social anxiety disorder group. Audience 1=entrance of first audience member; Audience 2= entrance of 2nd/3rd audience members; Audience 3= entrance of last audience member. Asterisks mark significant FDR-corrected Group×Time interactions. Error bars indicate 95% confidence intervals.

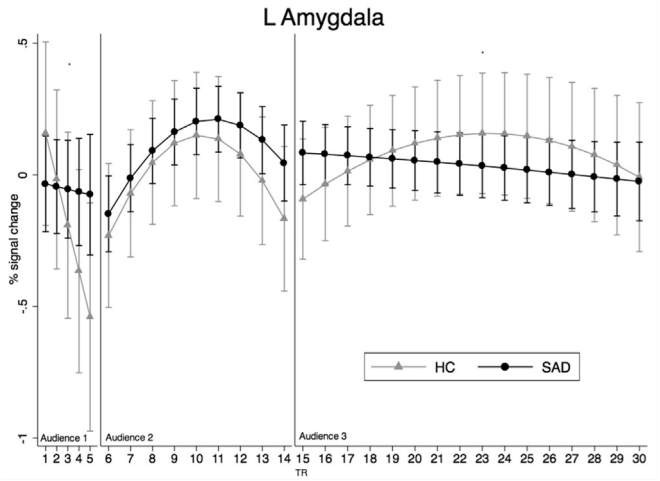

Fig. 2. Results from multi-level model analyses of left amygdala percent signal change across time during speech anticipation task relative to a control anticipation task. HC=healthy control group, SAD=social anxiety disorder group. Audience 1=entrance of first audience member; Audience 2=entrance of 2nd/3rd audience members; Audience 3=entrance of last audience member. Asterisks mark significant FDR-corrected Group×Time interactions. Error bars indicate 95% confidence intervals.

For Audience 3, there was a significant Group×Time^2 interaction for left amygdala (z=2.28, p=.02, 95% CI [.001, .007]; see Fig. 2), with no main effect of Group (*ps* > .73). The Group×Time^2 interaction for right amygdala was not significant (p=.08; see Fig. 1). Follow up analyses indicated significant quadratic effects of Time in HC in both left amygdala (z=−2.47, p=.01, 95% CI [−.01, −.001]) and right amygdala (z=−2.55, p=.01, 95% CI [−.01, −.001]) but not in SAD (*ps* > .17).

There were no differences in left or right amygdala time course between medicated versus unmedicated SAD participants during any segment of speech anticipation (*ps* > .26).

3.3. Dorsal anterior cingulate cortex responses

For dACC, there were no significant Group×Time effects except during Audience 3 (z=−2.23, p=.015, 95% CI [−.02, −.002]; see Fig. 3); this result remained significant after FDR correction (q*= .017). Follow-up analyses showed a linear increase in BOLD activity over

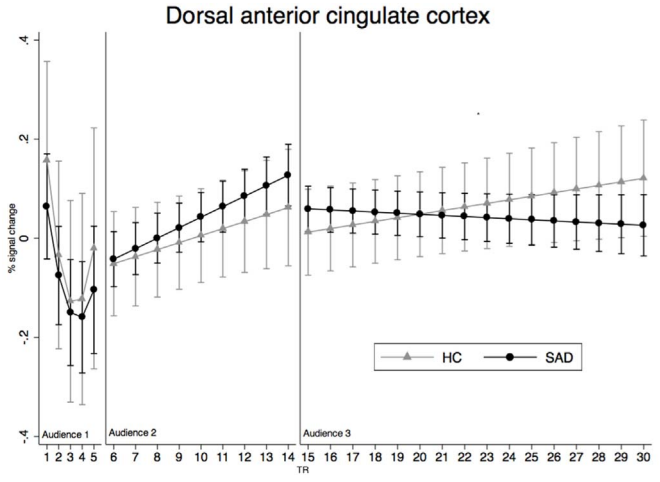

Fig. 3. Results from multi-level model analyses of dorsal anterior cingulate percent signal change across time during speech anticipation relative to control anticipation. HC=healthy control group, SAD=social anxiety disorder group. Audience 1= entrance of first audience member; Audience 2= entrance of 2nd/3rd audience members; Audience 3= entrance of last audience member. Asterisks indicate a significant FDR-corrected Group×Time interaction. Error bars indicate 95% confidence intervals.

Time during Audience 3 in HC (z=2.11, p=.04) and not in SAD (p=.22). There were no main effects of Group during any segment ($ps > .49$). There was a significant quadratic effect of Time across both groups during Audience 1 (χ^2=10.06, p=.002) but not Audience 2 (p=.06). Time course activity did not significantly differ between medicated versus unmedicated SAD patients during any segment ($ps > .06$).

3.4. Insula responses

There were no Group×Time effects for left or right insula during any segment, with the exception of left insula during Audience 3 (z=−2.37, p=.02, 95% CI [−.01, −.001]), though this result did not survive FDR correction. Follow-up analyses in left insula indicated a linear increase in BOLD activity over Time during Audience 3 in HC (z=2.64, p=.008) and not in SAD (p=.93). There were no main effects of Group in either left or right insula ($ps > .23$). There were quadratic effects of Time across groups during Audience 1 in both right insula (χ^2 =5.06, p=.02) and left insula (χ^2 =5.01, p=.03) and a linear effect of Time across groups during Audience 2 for right insula (z=3.06, p=.002) and left insula (z=3.87, $p < .001$). There was no effect of Time during Audience 3 in right insula (p=.07). Time course activity did not significantly differ between medicated versus unmedicated SAD patients during any segment ($ps > .06$).

3.5. Ventral striatum responses

For left and right ventral striatum, there were no significant effects of Group×Time ($ps > .06$). For main effects of Group, left ventral striatum showed higher activation in HC than SAD during Audience 1 (χ^2=4.44, p=.04) and left and right ventral striatum showed higher activation in SAD than HC during Audience 3 (χ=4.59, p=.03 and (χ^2=4.59, p=.03), but these results did not remain significant after FDR correction. Across groups, there were significant quadratic effects of Time in left and right ventral striatum during Audience 1 (χ=9.71, p=.002 and χ^2 =11.5, $p < .001$, respectively) and Audience 2 (χ^2=*8.72*, p=.003 and χ^2 =7.28, p=.007, respectively), and a linear effect of Time in right ventral striatum (z=2.39; p=.02) but not left ventral striatum (p=.12) during Audience 3, showing an increase in BOLD response over Time. Differences between medicated versus unmedicated SAD patients emerged only in left ventral striatum during Audience 3 (z=−2.07; p=.04), but removing medicated patients from analyses did not change results.

3.6. Associations between amygdala activity and social anxiety symptoms

Mean left and right amygdala activity during speech anticipation did not significantly predict LSAS score ($ps > .38$). Controlling for mean activity, *SD* of right amygdala activity was negatively associated with LSAS score (t=−2.02, p=.047), as was *SD* of left amygdala activity (t=−2.13, p=.037), indicating that less variability in amygdala activation correlated with greater symptom severity. There were no significant interactions between Group and Mean or Group and *SD* of left or right amygdala activity (all $ps > .38$), indicating that the relationship between variability in amygdala activation and LSAS score did not significantly differ between groups. Believability score was not significantly associated with mean or *SD* of BOLD activity in left or right amygdala ($ps > .49$).

4. Discussion

This study examined the time course of activity in emotion processing brain regions (specifically amygdala, insula, ventral striatum, and dorsal anterior cingulate cortex) during speech anticipation in socially anxious individuals compared to healthy controls. Our paradigm, which involved having participants anticipate giving a speech while viewing a "live feed" video of audience members entering the room where they would subsequently speak, increased self-reported anxiety in both socially anxious and healthy control individuals, relative to a control anticipation task, during which participants viewed a "live feed" video of a computer room and anticipated completing a computer task. There were significant differences in the time courses of left and right amygdala activity in socially anxious compared to healthy control individuals for speech anticipation relative to control anticipation despite no differences in the overall magnitude of amygdala activation between groups. Furthermore, less variability in left and right amygdala activity throughout speech anticipation, but not mean amygdala activation, was associated with greater social anxiety symptom severity. Time courses of activation in other regions of interest were largely similar between groups, with differences emerging only in dorsal anterior cingulate after correcting for multiple comparisons.

4.1. Amygdala responses

Amygdala results partially supported our hypotheses, with socially anxious individuals showing more sustained time courses of left amygdala activity during speech anticipation compared to controls during the first and last segments of speech anticipation (when viewing one and four audience members) but not the middle (when viewing two to three audience members), and more sustained time courses of right amygdala activity compared to controls during the first and middle segments of speech anticipation (when viewing one, two and three audience members). However, we found no differences in the overall magnitude of amygdala responses between groups. Additionally, while less variability in amygdala responding was associated with heightened social anxiety symptoms, mean amygdala activity was not. These findings reiterate the important role of the amygdala in threat processing in social anxiety disorder, and moreover, suggest that the time course or variability of amygdala response, rather than merely the magnitude of response, is an important marker of amygdala dysfunction during threat anticipation. Indeed, our findings demonstrate that differences in amygdala time course may exist even when there are no differences in the magnitude of activation.

Models of threat imminence processing (Fanselow and Lester, 1988) highlight the role of amygdalae in responding to upcoming threat ("post-encounter threat"; Mobbs et al., 2009) and to cues that signal potential or more distal threat (Herrmann et al., 2016), both relevant to speech anticipation. In healthy individuals, amygdala activation generally occurs rapidly in response to threat followed by fast deactivation (Breiter et al., 1996; Fischer et al., 2003). Though rapid amygdala activation and habituation to social stimuli have also been found in individuals with social anxiety (Sladky et al., 2012) and other anxiety disorders (Swartz et al., 2014), these studies have only focused on simple stimuli (images of faces) rather than complex social stimuli in the context of an upcoming speech as in the current study. Indeed, we found that, for healthy control participants, amygdala activity fluctuated according to the entrance of audience members during speech anticipation, with quick amygdala deactivation occurring in the first segment, and quick amygdala activation and subsequent deactivation in both the middle and last segments of speech anticipation; whereas for socially anxious individuals, amygdala deactivation did not occur in the first segment of speech anticipation, and amygdala responses were largely more sustained and less dynamic throughout the rest of the task. Moreover, less dynamic responding (less variability in amygdala activity) was associated with heightened social anxiety symptom severity, potentially reflecting less psychological and neural "flexibility" (Kashdan and Rottenberg, 2010). Conceivably, amygdala responding is more sustained in social anxiety disorder because individuals perceive imminent or distal threatening cues throughout anticipation, not just when an anxiety-producing stimulus is introduced. This explanation is consistent with the role of amygdalae in processing threat cues and maintaining vigilance toward emotional

content (Davis and Whalen, 2001). Another possibility is that socially anxious individuals lack the emotion regulation capacity to effectively down regulate anxious responding, leading to slower amygdala "recovery" from emotional stimuli (e.g., Schuyler et al., 2012), similar to the prolonged behavioral and physiological responses to emotional stimuli seen in social anxiety (e.g., Beidel et al., 1985; Eckman and Shean, 1997). Our findings that less variability in amygdala responding correlates with heightened social anxiety symptoms parallel psychophysiological findings that socially anxious individuals exhibit reduced heart rate variability (Alvares et al., 2013; Pittig et al., 2013), a measure of autonomic flexibility that is thought to index emotion regulatory capacity (Appelhans and Luecken, 2006). Future studies should examine whether variability in amygdala activity reflects a biological index of emotion dysregulation in social anxiety.

4.2. Insula responses

In contrast to previous studies showing heightened insula activity during threat processing in social anxiety disorder (e.g., Boehme et al., 2013; Lorberbaum et al., 2004), our results showed largely similar activity between groups, in terms of both magnitude and time course. Group differences emerged only during the last segment of speech anticipation, with a greater increase in left insula activity over time in controls compared to socially anxious individuals. However, this difference did not remain significant after correcting for multiple comparisons. One possibility for our lack of group differences is that our task immediately followed the instruction that participants would be giving a speech, so insular activation may have reflected processing of bodily sensations and perceived feelings (Craig, 2009) as both groups digested this information and prepared for the upcoming speech. However, additional research examining different anticipation paradigms is required before conclusions can be drawn about the timing of anticipation task instruction on neural activation.

4.3. Dorsal anterior cingulate and ventral striatum responses

We found evidence for a steeper increase in dorsal anterior cingulate activation over time in controls compared to socially anxious individuals during the last segment of speech anticipation only. These findings do not replicate previous studies showing either increased (e.g., Phan et al., 2006) or decreased (Lorberbaum et al., 2004) magnitude of dorsal anterior cingulate activation in socially anxious individuals in response to threat. One possibility is that increasing dorsal anterior cingulate activity in this context reflects an adaptive increase in cognitive control and preparation for the upcoming speech in healthy controls as the end of the task nears (Aarts et al., 2008), though more evidence is needed. For ventral striatum, we found no evidence of group differences in the time course of activation, and no main effects of group survived correction for multiple comparisons. Task differences between our study and those finding reduced ventral striatum activity (Boehme et al., 2013) in socially anxious individuals may have contributed to our different results. For example, there may be differences in anticipatory processing during repeated trials of anticipation versus a single extended period of anticipation used in the current study. Further research is needed to examine the role of both dorsal anterior cingulate and ventral striatum regions during anticipation.

4.4. Strengths and limitations

Strengths of the current study include its relatively large sample size, use of a clinical sample, and a novel anticipation task. Limitations include the use of a 3-second TR, which prohibited us from identifying changes in functional activity that may have occurred at a higher temporal resolution. However, our statistical approach mitigated this limitation by allowing us to model the shape of activity rather than examining differences between groups at specific time points. A second limitation is that we restricted our analyses to empirically determined *a priori* regions of interest, so not all regions that are involved in emotion processing were examined. We used a region-of-interest approach because the focus of our study was temporal dynamics of activation, and thus our task design was not optimized for detecting signal magnitude for a whole-brain approach. Third, as our task instructions involved deception, participants only completed one block each of control and speech anticipation, and these tasks had to be presented in the same order across all participants. Additionally, it is possible that the novelty of additional audience members entering the room in the speech anticipation task contributed to more dynamic amygdala responding during speech versus control anticipation; however, this factor would not explain differences in amygdala responding between socially anxious individuals and healthy controls. Finally, the inclusion of both unmedicated and medicated participants in our sample is a limitation; however, follow-up analyses revealed minimal differences activation between unmedicated and medicated participants.

4.5. Summary

In summary, the present study found a more sustained time course of amygdala activation during speech anticipation in socially anxious individuals compared to healthy controls and limited evidence for altered time course activity in other regions examined. These results suggest that amygdala time course may uniquely reflect either heightened perceived threat or deficits in emotion regulation or inhibitory capacity in socially anxious individuals during anticipation. In addition, consistent with studies showing that amygdala activity is highly variable over time to anxiety-provoking stimuli (e.g., Phillips et al., 2001), these findings highlight the importance of examining amygdala time course and variability in amygdala responding.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jad.2016.11.014.

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