Published in final edited form as:

J Affect Disord. 2017 October 15; 221: 47–55. doi:10.1016/j.jad.2017.06.004.

Multi-Modal Neuroimaging of Adolescents with Non-Suicidal Self-Injury: Amygdala Functional Connectivity

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Abstract

Background—Non-suicidal self-injury (NSSI) is a significant mental health problem among adolescents. Research is needed to clarify the neurobiology of NSSI and identify candidate neurobiological targets for interventions. Based on prior research implicating heightened negative affect and amygdala hyperactivity in NSSI, we pursued a systems approach to characterize amygdala functional connectivity networks during rest (resting-state functional connectivity [RSFC]) and a task (task functional connectivity [TFC]) in adolescents with NSSI.

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The authors have no conflicts of interest.

Disclosures/Conflicts of Interest:

None

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Method—We examined amygdala networks in female adolescents with NSSI and healthy controls (n=45) using resting-state fMRI and a negative emotion face-matching fMRI task designed to activate the amygdala. Connectivity analyses included amygdala RSFC, amygdala TFC, and psychophysiological interactions (PPI) between amygdala connectivity and task conditions.

Results—Compared to healthy controls, adolescents with NSSI showed atypical amygdala-frontal connectivity during rest and task; greater amygdala RSFC in supplementary motor area (SMA) and dorsal anterior cingulate; and differential amygdala-occipital connectivity between rest and task. After correcting for depression symptoms, amygdala-SMA RSFC abnormalities, among others, remained significant.

Limitations—This study's limitations include its cross-sectional design and its absence of a psychiatric control group.

Conclusions—Using a multi-modal approach, we identified widespread amygdala circuitry anomalies in adolescents with NSSI. While deficits in amygdala-frontal connectivity (driven by depression symptoms) replicates prior work in depression, hyperconnectivity between amygdala and SMA (independent of depression symptoms) has not been previously reported. This circuit may represent an important mechanism underlying the link between negative affect and habitual behaviors. These abnormalities may represent intervention targets for adolescents with NSSI.

Keywords: Non-suicidal self-injury, neuroimaging, functional connectivity, amygdala, adolescents

Introduction

Non-suicidal self-injury (NSSI) is the act of deliberately harming one's own body tissue without suicidal intent (American Psychiatric Association, 2013; Nock et al., 2006). Onset of this behavior typically occurs in early-mid adolescence and persists into young-adulthood (Andover, 2014; Andrews et al., 2014). Worldwide prevalence has been estimated to be approximately 18% (Muehlenkamp et al., 2012). NSSI may predict negative outcomes such as persistent psychopathology and suicide (Horwitz et al., 2014; Tang et al., 2011; Victor and Klonsky, 2014). In order to promote the development of novel, biologically-informed treatments, new research is needed to advance understanding of the neurobiological mechanisms associated with this behavior.

Research studies have often examined NSSI in the context of a particular diagnosis, such as major depressive disorder (MDD; Hintikka et al., 2009) or borderline personality disorder (BPD; Cerutti, Manca, Presaghi, & Gratz, 2011). However, NSSI occurs across diagnoses and even in the absence of psychiatric diagnoses (Stanford and Jones, 2009). Some have supported NSSI as a disorder in its own right, as demonstrated by the inclusion of this behavior in the "Disorders for Further Study" section of the DSM-5 (American Psychiatric Association, 2013). Moving forward, transdiagnostic research examining core mechanisms of NSSI is needed.

Although individuals with NSSI endorse a variety of reasons for engaging in this behavior, the most common purpose of NSSI is to regulate negative affect (Klonsky, 2007). The neurobiology of negative affect is relatively well-understood and is comprised of cortico-limbic neurocircuitry (LeDoux, 2000; Phillips et al., 2003). Specifically, the amygdala is a

key limbic region that initiates the threat response, while frontal regions monitor and regulate emotional responses (Allman et al., 2001; Ghashghaei and Barbas, 2002). To date, neuroimaging research has shown that patients with NSSI have heightened amygdala responses to emotional stimuli (both negative and neutral) (Niedtfeld et al., 2010; Plener et al., 2012) and that experimental injuries (a painfully cold stimulus or small incision), lead to both attenuation of amygdala responses (Reitz et al., 2015) and to subjective relief of negative affect in adults with NSSI (Osuch et al., 2014; Reitz et al., 2015). While these studies have contributed important clues about the underlying biology of NSSI, little is known about broader amygdala network functioning in NSSI, particularly in adolescents. Given the central role of the amygdala in negative affect, the current work focuses on amygdala-centered networks.

An important next step is to take a systems approach to characterize amygdala networks underlying NSSI. Functional connectivity within neural networks is measured by the correlation between brain regions in the pattern of spontaneous blood oxygen level dependent (BOLD) signal over time. “Positive” functional connectivity (positive correlations) within a network is believed to signify that the brain regions are serving similar goals, while “negative” functional connectivity (negative correlations) signifies that the brain regions are serving opposing goals (Fox et al., 2005). Functional connectivity can be measured at rest (resting state functional connectivity; RSFC) and during the duration of a task (task functional connectivity; TFC). Further, connectivity may increase or decrease during specific task conditions; this can be measured using psychophysiological interactions (PPI). While overall TFC provides longer (duration of the entire task) time scale information, PPI provides shorter (during specific task blocks) time scale information. Both approaches are important for understanding the dynamics of amygdala functional connectivity in the context of negative emotion in adolescents with NSSI.

Aside from one study that reported heightened amygdala activation in response to negative pictures in adolescents with NSSI (Plener et al., 2012), the majority of research on neural networks underlying NSSI has been conducted in adults. Although no adult NSSI studies have yet examined RSFC in amygdala networks, two studies have examined amygdala TFC in adults with BPD and NSSI compared to healthy controls (HC). First, Reitz et al. found that the NSSI group showed impaired amygdala-frontal connectivity that normalized (or increased) several minutes following a small incision on the forearm (Reitz et al., 2015). Second, Niedtfeld et al. found enhanced amygdala-frontal TFC following a painfully cold stimulus and presentation of negative scenes (Niedtfeld et al., 2012). In recent years, it has become increasingly clear that functional connectivity within neural networks is not static, but changes across time and across contexts (Chang and Glover, 2010; Cole et al., 2013). Therefore, a combined approach using RSFC, TFC, and PPI to understand adolescent NSSI holds potential to reveal a deeper understanding of neural networks underlying this behavior by characterizing network aberrations both at rest and in emotionally salient conditions.

Given the evidence for impaired fronto-limbic connectivity in adults with NSSI and the ongoing maturation of these networks during adolescence (Cunningham et al., 2002; Giedd et al., 1999), examination of the neural connectivity in adolescents with NSSI is warranted. As reviewed by Somerville, Jones, and Casey, adolescence is a period of dynamic change in

behavior (e.g. increased risk-taking and heightened emotionality), and in neural circuitry, including dynamic interactions involving amygdala, prefrontal cortex, and striatum (Somerville et al., 2010). In adolescence, the development of subcortical structures responsible for risk-taking and heightened sensitivity to negative emotion outpaces the development of prefrontal regions, leading to an imbalance in the regulation of emotional or reward-driven responses. The fact that these significant changes during adolescence coincide with the typical onset of NSSI highlights the importance of examining neural networks in adolescents with NSSI. It is possible that NSSI arises in the context of aberrant development of neural systems. Critical next steps include incorporation of longitudinal work examining the neurodevelopmental trajectory associated with this behavior. The hope is that this line of work will eventually serve to guide targeted intervention and prevention strategies to restore normative neurodevelopment before NSSI becomes further entrenched.

The purpose of this study was to examine amygdala networks in adolescents with NSSI using resting-state and task fMRI. To avoid confounds related to categorical diagnoses, our sample was selected based on history of NSSI behavior as opposed to psychiatric diagnosis. Our analyses focused on data from female subjects because although recruitment was open to both males and females, only one male with NSSI enrolled. For the task, we selected a negative emotion face-matching task, which has been shown to activate the amygdala (Hariri et al., 2002). We compared adolescents with NSSI versus HC on amygdala RSFC, amygdala TFC, and changes in amygdala TFC during specific task conditions (PPI). We hypothesized that adolescents with NSSI would show lower amygdala-cortical functional connectivity compared to controls both during rest and during a negative emotion task, particularly involving frontal regulatory regions.

Because we hypothesized aberrations in connectivity including frontal regulatory regions, we were interested in whether connectivity was associated with available clinical measures implicating behavioral and emotional regulation. Thus, we conducted exploratory analyses to examine whether connectivity measures within regions that showed significant group differences were related to clinical measures of impulsivity and emotion regulation within the NSSI group. We predicted that deficits in emotion regulation and impulse control would be associated with connectivity findings.

Method

Participants

Females aged 13–21 years with NSSI and age-matched HC were recruited for this cross-sectional study. The rationale for this large age range was to capture the timeframe in which NSSI is typically at its peak. Recruitment strategies included community postings and referrals from local mental health services. Inclusion criteria for the NSSI group included a history of engaging in NSSI at least 4 times, with at least 1 episode occurring in the last month. Exclusion criteria for both groups included a history of bipolar, pervasive developmental, or psychotic disorders, current pregnancy or breastfeeding, unstable medical illnesses, active suicidal intent, presence of MRI-incompatible features, a positive urine drug screen, and intelligence quotient (IQ) of less than 80 as measured by the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999). Additional exclusion criteria for

HC included any history of self-injurious behavior (suicidal or non-suicidal) and any current or past DSM-IV psychiatric diagnoses.

Assessment

After completing informed consent or assent (as appropriate), all participants completed a comprehensive diagnostic assessment, which were conducted by trained clinicians or graduate students under the supervision of a licensed psychologist. Interviews were conducted separately with adolescents and parents, and included Kiddie Schedule for Affective Disorders and Schizophrenia- Present and Lifetime Version (K-SADS-PL; Kaufman et al., 1997) for participants under 18 years and the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID; First, Spitzer, Gibbon, & Williams, 2002a, 2002b) for participants 18 or older. NSSI was measured using the self-report Inventory of Statements About Self-Injury (ISAS; Glenn & Klonsky, 2011) and the clinician-administered Deliberate Self-Harm Inventory (DSHI; Gratz, 2001). These two measures were used to provide a consensus on frequency, type, and severity of self-injury for each participant in the NSSI group. Additional self-report measures included the Difficulties in Emotion Regulation Scale (DERS; Gratz & Roemer, 2004) the Barratt Impulsiveness Scale (BIS; Patton, Stanford, & Barratt, 1995), and the Beck Depression Inventory-II (BDI; Beck, Steer, & Brown, 1996).

MRI Data Acquisition

Data were acquired at the Center for Magnetic Resonance Research at the University of Minnesota using a Siemens 3T TIM Trio scanner and a 32-channel receive-only head coil. A five-minute structural scan was acquired using a T1-weighted high-resolution magnetization prepared gradient echo (MPRAGE) sequence: Repetition time (TR)=2530ms; echo time (TE)=3.63ms; inversion time (TI)=1100ms; 1mm slices, field of view (FOV)=256, flip angle=7 degrees. Resting-state and task fMRI data were obtained using the WU-Minn Human Connectome Project consortium (<http://www.humanconnectome.org/>) multi-band EPI sequence with: 64 oblique axial slices; 2mm isotropic voxel; TR=1320 ms; TE=30 ms; flip angle=90°, FOV=212 mm; multiband factor=4. To minimize effects of task-based fMRI on resting-state fMRI, the resting state scan was acquired prior to the task.

Resting State fMRI—Participants were instructed to remain awake with their eyes closed during the resting-state scan. The scan acquired 260 volumes, which lasted 5 minutes and 43 seconds.

Emotion Face-Matching Task—The emotion face-matching task (Hariri et al., 2002) was projected onto a screen inside the bore of the scanner. This task used both affective stimuli, which were Ekman faces (Ekman and Friesen, 1975) depicting anger and fear, and control stimuli, which consisted of horizontal and vertical ellipses. Participants were instructed to look at the picture in the top row and use a response box to select one of the two pictures in the bottom row that matched. Participants were asked to match the shapes for the control stimuli and the emotions for the affective stimuli. The task consisted of thirteen, 24-second blocks (3 fixation, 5 shape, and 5 emotion), which were counterbalanced. The task acquisition included 294 volumes, which took 6 minutes and 28 seconds.

Rest and Task fMRI Processing and Analysis

A summary of rest and task fMRI preprocessing steps is shown below with a more detailed description found in the Supplementary Materials. To the extent possible, a standardized processing pipeline was used on the rest and task data.

Rest and Task fMRI Steps for Preprocessing

1. Brain extraction and anatomical parcellation of T1 data using FreeSurfer (including right and left anatomically based amygdala ROIs)
2. Quality assurance and manual corrections of anatomical parcellation of T1 data
3. Brain extraction, motion correction, and distortion correction of fMRI data using FSL Topup
4. Computation of DVARS and framewise displacement metrics
5. Exploratory independent component analysis of individual data conducted using the FSL tool MELODIC (for the purpose of de-noising the data)
6. Removal of noise components indicated by step #4 using FSL regfilt
7. Registration of FreeSurfer-generated ROIs for CSF, WM, and right and left amygdala to the fMRI using BBRegister
8. Extraction of mean BOLD time series within these ROIs using *fslmeans*

First-Level Analyses

RSFC: To examine RSFC of the left and right amygdala, we conducted a seed-based, whole-brain approach using methods previously described (Cullen et al., 2014). We used FreeSurfer to create anatomically-based right and left amygdala ROIs, which were registered to the preprocessed rsfMRI data. The average BOLD time series across voxels in these regions were extracted and used as primary regressors in separate (right and left) general linear model analyses of each voxel's time series. Additional steps included spatial smoothing (5mm Gaussian kernel), prewhitening, and registration to anatomical data and MNI standard space for later group analysis. Nuisance regressors included in each voxel's analysis using nine time series: WM, CSF, indicators of volumes of excess motion (as described above), and the six motion parameters. This resulted in whole-brain RSFC maps for each amygdala ROI.

TFC and PPI: We used FEAT to conduct TFC and PPI analyses. TFC analyses included examination of overall connectivity of the amygdala during the task, while PPI analyses are designed to assess whether specific circuits increase in synchrony specifically during task blocks (Friston et al., 1997; O'Reilly et al., 2012). For each participant, we conducted linear regression analyses in FEAT to examine TFC and PPI individually. Analyses were conducted separately for right and left amygdala. TFC analyses included mean amygdala time series as the primary regressor of interest, with the Emotion and Shape task explanatory variables included as regressors of no interest. PPI analyses included the following regressors: Emotion, Shape, mean amygdala time series, and interaction terms (the result of

multiplying the mean amygdala time series with each of the emotion and the shape regressors). For the PPI analyses, we chose to examine the emotion blocks relative to fixation and the emotion blocks relative to shape. Nuisance regressors were included: WM, CSF, volumes of excess motion, and the six motion parameters. This resulted in whole-brain RSFC maps for each amygdala ROI. Data from the resulting beta contrast maps of these analyses were then used for group comparisons.

Group Comparisons—To examine RSFC, TFC, and PPI group differences, we conducted a whole-brain, voxel-wise group comparison of the right and left amygdala FC maps separately, controlling for age, using Gaussian Random Field Theory to correct for multiple testing across voxels, specifying a cluster z-threshold of 2.3 and $p < 0.025$ (the latter parameter selected to correct for the two FC analyses [right and left amygdala]).

Follow-Up Analyses: Clinical Correlations and Depression Covariate

To test whether there was an association between amygdala connectivity and clinical measures, we conducted a series of follow-up analyses using the significant clusters from the above group analyses as individual masks to extract average z-scores from each participant's un-thresholded connectivity maps. We tested whether these measures of connectivity levels in these clusters were related to measures of impulsivity (BIS), emotion regulation difficulties (DERS), and/or frequency of NSSI episodes within the NSSI group. Using a Bonferroni correction, we set a threshold for significance at $p < 0.0042$ to correct for comparisons across the 12 different clinical subscales.

We also tested for the association between amygdala connectivity and NSSI frequency by using a consensus between the ISAS and DSHI. We decided to focus on cutting episodes for these analyses, as cutting was the primary method of NSSI among all our NSSI participants. Average weekly cutting episodes were calculated by taking the consensus of lifetime cutting episodes and dividing them by the estimated number of weeks the participant engaged in NSSI. Due to one participant tending toward extreme estimates of lifetime NSSI, we Winsorized this outlier to three standard deviations above the mean.

Given that depressive disorders are highly common among those with NSSI (Zetterqvist, 2015) and is the most common diagnosis in our sample, we repeated our group-level analyses while entering demeaned BDI scores as a covariate. Comparison of these two models' estimated group effect allows for a clearer understanding of the specificity of our findings to NSSI. However, given the overlap of depressive symptoms with NSSI, and the exclusion of major depressive disorder from the control group, BDI is highly co-linear with group; inclusion of BDI as an adjusting variable in regression models risks removing meaningful variance that had been ascribed to group differences. Thus, we present results for two sets of analyses: first, analyses that did not include the BDI covariate, and second, analyses that did include the BDI covariate.

Results

Participants

Twenty-nine NSSI and 22 HC completed all study procedures. We excluded participants due to unusable FreeSurfer parcellation ($n = 1$ HC), errors in task fMRI acquisition ($n = 1$ HC), or excessive motion during the resting-state fMRI ($n = 4$ NSSI and 1 HC) or task fMRI ($n = 4$ NSSI and 3 HC). Twenty-five NSSI and 20 HC participants were included in our final RSFC analyses and 24 NSSI and 17 HC participants were included in our final task analyses; further demographic information is provided in Table 1.

Resting-State Functional Connectivity

Amygdala RSFC—For right amygdala, NSSI had negative RSFC but HC had positive RSFC in a cluster encompassing the left angular gyrus and lateral occipital cortex. NSSI showed lower positive, while HC showed higher negative, RSFC in a cluster involving the bilateral dorsal anterior cingulate and supplementary motor area (SMA) (see Figure 1; and Table 2). For left amygdala, significant group differences were found in the following clusters: (1) right lateral occipital cortex and angular gyrus; (2) right frontal pole; (3) right inferior temporal gyrus, middle temporal gyrus, and temporal pole; (4) left middle temporal gyrus and superior temporal gyrus; and (5) left angular gyrus and lateral occipital cortex. In these regions, NSSI had lower negative RSFC but HC had higher positive RSFC with the left amygdala. Additionally, NSSI had lower positive, while HC had higher negative, RSFC between left amygdala and bilateral dorsal anterior cingulate and supplementary motor area (see Figure 1 and Table 2).

Task fMRI

Results from analyses examining group differences in whole-brain activation to the emotion task are described in the supplementary materials. Groups did not differ on performance accuracy or response time.

Amygdala TFC and PPI—When examining TFC during the entirety of the task (i.e., in the absence of a PPI with a specific task contrast), NSSI had positive, while HC had negative, connectivity between right amygdala clusters encompassing: (1) right lingual gyrus, occipital pole, and occipital and temporal fusiform; and (2) right lateral occipital cortex and superior parietal lobule. Additionally, NSSI had higher positive, and HC had lower negative, connectivity between the left amygdala clusters encompassing bilateral lateral occipital cortex and superior parietal lobule. In contrast, NSSI had lower negative, while HC had higher positive, connectivity between the left amygdala and bilateral frontal pole, medial frontal cortex, and paracingulate (see Figure 2 and Table 3).

For our PPI analyses, there were no significant group differences between the amygdala time series and Emotion condition.

Follow-Up Analyses: Clinical Correlations and Depressive Symptoms Covariate

No significant associations were found between significant group difference clusters and clinical measures of impulsivity and emotion regulation that survived our correction for multiple comparisons ($p < .0042$).

Within the NSSI group, average weekly cutting episodes was positively associated with left amygdala TFC with bilateral frontal pole, medial frontal cortex, and paracingulate $r(22) = .598, p = .002$.

Depressive disorders were the most common primary diagnosis among our NSSI sample (Table 1). After repeating our analyses with demeaned BDI scores as a covariate, three clusters from our RSFC analyses remained significant (see Table 2). These clusters included: (a) lower positive RSFC in NSSI and higher negative RSFC in HC between left amygdala and bilateral dorsal anterior cingulate and supplementary motor area; (b) lower negative RSFC in NSSI and higher positive RSFC in HC between left amygdala and right inferior temporal gyrus, middle temporal gyrus, and temporal pole; and (c) lower negative RSFC in NSSI and higher positive RSFC in HC between left amygdala and right angular gyrus and occipital cortex.

Discussion

The present study utilized a novel approach to understanding the neural circuitry of NSSI in adolescents by examining both RSFC and TFC with a focus on neural circuits relevant to processing negative affect. To our knowledge, this is the first study to use either of these strategies to explicate neurobiology in a sample of adolescents with NSSI. During rest and task conditions, those with NSSI showed aberrant amygdala connectivity with a variety of cortical regions. Consistent with our hypotheses, our amygdala connectivity findings included areas of the frontal lobe during both rest and task, which is key in providing executive control including the regulation of limbic structures. In addition, we also found greater amygdala connectivity with the SMA in the NSSI group during rest, which was not anticipated. However, since the SMA is involved in planning of complex movements, this circuit may be of high relevance as it may at least partially explain the link between negative affect and habitual behaviors in adolescents with NSSI.

Amygdala-Frontal RSFC and TFC

Our amygdala-frontal connectivity findings support previous research suggesting amygdala-frontal network deficits. Namely, adults with BPD and NSSI show decreased frontal activation and “normalization” (or increase) of amygdala-frontal connectivity in response to pain stimuli as well as compromised white matter microstructure in the frontal lobe (Grant et al., 2007; Niedtfeld et al., 2010; Reitz et al., 2015). These circuits have also been found to be atypical in adolescent depression (Cullen et al., 2010; Musgrove et al., 2015). Our RSFC results suggest that disrupted amygdala-frontal connectivity persists in the absence of emotional stimuli and, as evidenced by the TFC results, may become more prominent in emotionally salient contexts. Because this finding transcends both resting and task conditions, amygdala-frontal hypoconnectivity appears to be a pervasive deficit among those

with NSSI that may represent difficulty in regulation of negative affect, and the reliance upon self-injury as a self-soothing strategy. Interestingly, greater weekly episodes of cutting was associated with greater amygdala-frontal TFC within the NSSI group. This is contrary to expectations, but may be associated with NSSI being used for affect regulation. The fact that these findings did not hold after controlling for depression symptoms indicates that these neural circuit abnormalities are highly linked with the depression symptoms that are commonly experienced in adolescents with NSSI. Taken together, the findings of this study support the evidence of aberrant amygdala-frontal RSFC and TFC, which may largely reflect neural mechanisms underlying both NSSI and related depression symptoms in these adolescents.

Heightened Amygdala-SMA RSFC

Adolescents with NSSI also demonstrated greater amygdala RSFC than HC in some regions including the SMA and bilateral dorsal anterior cingulate. Further, this finding persisted despite controlling for depression severity. This indicates a potentially high level of specificity for greater amygdala-SMA RSFC among those with NSSI. The SMA is involved in the planning of complex movements, and therefore is likely invoked in the moments before and during the act of NSSI. Hyperconnectivity in this circuit could lead to (or result from) an excessive influence of negative affect upon the planning of movements, potentially increasing the likelihood of engaging in NSSI. It is possible that impaired connectivity between fronto-limbic regions coupled with hyperconnectivity between amygdala and SMA could underlie the entrenchment of NSSI behaviors and represent a potential treatment target. It should also be noted that it would be hypothesized that this finding would also occur in the context of our task. However, we did not have this finding during this task. Future research using a task that involves more self-relevant stimuli may aid in answering this question. Examples of such tasks may include social exclusion paradigms (e.g. cyber ball) or listening to negative feedback. Thus, the results of this study will await replication before firm conclusions are justified.

Amygdala-Temporal and Occipital RSFC

The NSSI group showed lower negative amygdala RSFC than HC in the temporal lobe, a region involved in processing explicit emotional memories (LeDoux, 2000). Group differences were also found between the amygdala and angular gyrus and occipital cortex (NSSI showed negative, while HC showed positive, RSFC). Interestingly, both findings of amygdala-temporal and amygdala-occipital connectivity remained significant despite controlling for depressive symptoms, indicating that these circuits may have a high level of specificity for NSSI. However, it is presently unclear as to how these circuits are involved, particularly given the lack of replication regarding the functioning of healthy participants who found negative RSFC between the amygdala and angular gyrus and occipital cortex (Roy, et al., 2009) and the lack of association with self-report clinical measures. While this discrepancy could be explained by differences in connectivity analyses or due to developmental differences between the studies, longitudinal studies with uniform methods are needed to clarify this question.

Amygdala-Dorsal Cingulate RSFC

Consistent with previous work in healthy adults (Roy et al., 2009), our HC sample demonstrated negative RSFC with the dorsal anterior cingulate. The dorsal anterior cingulate is part of the salience network, which is responsible for integrating and monitoring the importance of internal and external stimuli. Increased functional connectivity between amygdala and dorsal anterior cingulate has been found during fear memory consolidation (Feng et al., 2014). Interestingly, healthy males show greater *negative* RSFC between the amygdala and dorsal anterior cingulate after being exposed to cold press versus before (Clewett et al., 2013). It is possible that the positive RSFC seen in this circuit in our sample of adolescents with NSSI could reflect spontaneous processing of negative emotional memories or thoughts about self-injury during the resting period.

Differential Amygdala-Occipital RSFC: Task versus Rest

The NSSI group showed greater overall TFC between left amygdala and regions of the occipital cortex, including the fusiform, which is a key region in the processing of facial stimuli. This contrasts with our finding of lower amygdala-occipital connectivity during rest. However, it should be noted that the locations of these occipital clusters differ across the two sets of results. Our finding of increased amygdala-occipital TFC adds to prior work showing increased amygdala-fusiform TFC while viewing fearful faces in adults with social anxiety disorder (Frick et al., 2013). Elevated amygdala-occipital TFC while processing negative facial emotion information could underlie a heightened tendency to perceive facial information as negative; speculatively, this could have relevance to adolescents with NSSI, who often have difficulties with interpersonal relationships (McMahon et al., 2010). Taken together, further research is warranted to better characterize amygdala-occipital connectivity across psychiatric disorders, and to explore whether this circuit could represent a candidate treatment target for patients with NSSI.

Strengths and Limitations

Unlike many previous studies of NSSI, which investigate the behavior in the context of a specific diagnosis, the present study examines the neural circuitry of NSSI across diagnoses. Additionally, we used a dynamic approach to understanding this behavior by examining neural networks in two different contexts. We employed a rigorous strategy to reduce spurious findings due to noise in both our RSFC and TFC data, including denoising and conservative motion correction methods, and a uniform processing stream across rest and task data sets.

One limitation of this study includes its cross-sectional design. Longitudinal designs are needed to more thoroughly understand the neural mechanisms associated with the predisposition, onset, and maintenance of this behavior. In doing so, we may understand the neural processes that unfold in individuals at high risk and thus develop prevention strategies that are tailored to these individuals. Further, larger samples are needed to provide increased power to detect associations between neurobiological and clinical measures. It is also important to note that nearly half of our NSSI group was receiving psychiatric medication at the time of the study, thus potentially obscuring our results.

Additional limitations include the lack of a psychiatric control group, which limits the ability to associate the findings to NSSI, as differences may be attributed to other forms of psychopathology. Although this study did present findings that controlled for depression, this creates an issue of co-linearity in which correcting for depression results in the removal of substantial meaningful variance between groups. Fortunately, efforts within our lab are currently underway to examine the neurobiology of NSSI using a design that incorporates psychiatric controls.

In regard to associating our connectivity findings with NSSI frequency, further development of more thorough and valid measures of NSSI are needed. We hope that the findings generated by this study will serve as a guide for selection of behavioral, self-report, and physiological measurements to further understand the role of these aberrant neural circuits in the pathophysiology of NSSI.

Conclusion

The present study represents an important step in our understanding of the neurobiology of NSSI. Our findings suggest widespread amygdala-cortical connectivity anomalies during rest and an emotionally salient task. While deficits in amygdala-frontal connectivity (driven by depression symptoms) replicates work in depression, hyperconnectivity between amygdala and SMA (independent of depression symptoms) has not been previously reported. This circuit may represent an important mechanism underlying the habitual behaviors influenced by negative affect. Additional research with larger sample sizes and more comprehensive measurements are necessary to replicate these findings and further elaborate how these circuits relate to the key psychological and neurobiological system abnormalities underlying NSSI. Such research is necessary to pave the way for developing novel, biologically based interventions for adolescents with NSSI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Source:

The study was funded by National Institute of Mental Health grant 1R21MH094558 (Dr. Cullen) and the University of Minnesota Academic Health Center Faculty Research Development Grant Program. The funding sources supported the roles of design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation of the manuscript. The funding sources were not involved in the decision to submit the manuscript for publication.

The authors would like to graciously thank the participants and families that participated in this study.

This work was carried out in part using computing resources at the University of Minnesota

Supercomputing Institute. This manuscript is in partial fulfillment of M. Westlund Schreiner's dissertation.

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- Those with NSSI show impaired amygdala-frontal connectivity during rest and task.
- Those with NSSI show greater amygdala-supplementary motor area (SMA) connectivity.
- Impaired amygdala-frontal connectivity in NSSI is explained by depressive symptoms.
- Increased amygdala-SMA connectivity is not explained by depressive symptoms.
- Amygdala-SMA connectivity may be important in our understanding of NSSI.

Figure 1. Left and Right Amygdala RSFC

Top Left: Clusters indicate brain regions where adolescents with NSSI had greater left amygdala RSFC than HC: bilateral cingulate and supplementary motor area. *Bottom Left:* Clusters indicate regions where adolescents with NSSI had lower amygdala RSFC than controls: (1) right lateral occipital cortex and angular gyrus; (2) right frontal pole; (3) right inferior temporal gyrus, middle temporal gyrus, and temporal pole; (4) left middle temporal gyrus and superior temporal gyrus; and (5) left angular gyrus and lateral occipital cortex. *Top Right:* Clusters indicate regions in which adolescents with NSSI had greater left amygdala RSFC than HC: bilateral dorsal cingulate and supplementary motor area. *Bottom Right:* Clusters indicate regions where adolescents with NSSI had lower amygdala RSFC than controls: left angular gyrus and lateral occipital cortex.

Figure 2. Left and Right Amygdala TFC

Top Left: Clusters indicate brain regions where adolescents with NSSI had greater left amygdala TFC than controls: bilateral lateral occipital cortex and superior parietal lobule.

Bottom Left: Clusters indicate brain regions where adolescents with NSSI had lower amygdala TFC than controls: bilateral frontal pole, medial frontal cortex, and paracingulate.

Right: Clusters indicate brain regions where adolescents with NSSI had greater right amygdala TFC than controls: (1) right lingual gyrus, occipital pole, and occipital and temporal fusiform; and (2) right lateral occipital cortex and superior parietal lobule.

Table 1

Participant Demographics

	RSFC Data		Task Data	
<i>Demographic Characteristics</i>	NSSI (n = 25)	Controls (n = 20)	NSSI (n = 24)	Controls (n = 17)
Age (mean years $\pm$ SD)	17.57 $\pm$ 2.49	18.01 $\pm$ 2.08	17.34 $\pm$ 2.44	17.98 $\pm$ 2.00
IQ (mean $\pm$ SD)	106.29 $\pm$ 11.25 (n = 24)	110.28 $\pm$ 9.65 (n = 18)	104.68 $\pm$ 11.18 (n = 22)	109.13 $\pm$ 9.82 (n = 15)
Right Handed – n (%) ^a	21 (88%; n = 24)	17 (100%; n = 17)	19 (86%; n = 22)	14 (100%; n = 14)
<i>Ethnicity – n (%)^b</i>				
Caucasian	23 (92%)	17 (85%)	23 (96%)	16 (94%)
African American	1 (4%)	1 (5%)	1 (4%)	1 (6%)
Hispanic	3 (12%)	0	2 (8%)	0
Asian	0	2 (10%)	0	0
Other	1 (4%)	0	0	0
Depressive symptoms (BDI-II)	26.72 $\pm$ 13.25 **	1.85 $\pm$ 4.22 **	28.96 $\pm$ 11.91 **	1.06 $\pm$ 1.30 **
<i>NSSI</i>				
Age of first NSSI (mean age $\pm$ SD)	11.58 $\pm$ 3.89 (n = 24)		12.30 $\pm$ 2.58 (n = 23)	
Lifetime Cutting Episodes (mean $\pm$ SD)	127.04 $\pm$ 190.88		132.13 $\pm$ 196.98	
Estimated Cutting Episodes per Week (mean $\pm$ SD) ^c	0.63 $\pm$ 1.08		0.74 $\pm$ 1.23	
<i>Current Diagnoses – n (%)^d</i>				
Major Depressive Disorder	13 (52%)		14 (58%)	
Depressive Disorder NOS	5 (20%)		3 (13%)	
Generalized Anxiety Disorder	6 (24%)		5 (21%)	
Anxiety Disorder NOS	1 (4%)		2 (8%)	
Social Phobia	1 (4%)		2 (8%)	
Specific Phobia	3 (12%)		2 (8%)	
Panic Disorder	2 (8%)		2 (8%)	
Post Traumatic Stress Disorder	3 (12%)		3 (13%)	
Obsessive Compulsive Disorder	2 (8%)		1 (4%)	
Eating Disorder NOS	1 (4%)		1 (4%)	
ADHD	1 (4%)		1 (4%)	
Alcohol Dependence	2 (8%)		1 (4%)	
No Current Disorder	5 (20%)		5 (21%)	
<i>Medications</i>				
Currently Medicated	10 (42%; n = 24)		10 (44%; n = 23)	
Antidepressants	7 (29%; n = 24)		8 (35%; n = 23)	
Stimulants	1 (4%; n = 24)		1 (4%; n = 23)	
Antipsychotics	1 (4%; n = 24)		1 (4%; n = 23)	

	RSFC Data		Task Data	
<i>Demographic Characteristics</i>	NSSI (n = 25)	Controls (n = 20)	NSSI (n = 24)	Controls (n = 17)
Antianxiety/Benzodiazapines	3 (13%; n = 24)		3 (13%; n = 23)	
Other Psychotropics	1 (4%; n = 24)		1 (4%; n = 23)	

^aPost-hoc analyses indicated that differing handedness did not effect study findings

^bParticipants were able to endorse more than one option for ethnicity

^cConsensus between ISAS and DSHI was calculated to determine average number of cutting episodes per week. These are pre-Winsorized scores.

^dDiagnoses include both primary and comorbid disorders

^{**}Group difference of $p < .005$ between HC and NSSI

Table 2

Location, size and peak z-values of the significant clusters in the RSFC group analyses

Seed Region	Contrast	Brain Regions	Control Mean z-stat	NSSI Mean z-stat	# of Voxels	MNI Coordinates of Peak Voxel (x, y, z)	Peak z- value	Cluster p-value	Cohen's D [Confidence Interval]
Right Amygdala	NSSI>HC	<i>Bilateral</i> /anterior cingulate cortex and supplementary motor area	-.64 ± 1.01	.39 ± .94	313	2, 10, 34	3.71	0.01	1.07 [.43–1.69]
	HC>NSSI	<i>Left</i> angular gyrus and occipital cortex	.79 ± 1.10	-.46 ± 1.11	313	-44, -56, 34	3.41	0.01	1.12 [.48–1.75]
Left Amygdala	NSSI>HC	* <i>Bilateral</i> /anterior cingulate cortex and supplementary motor area	-.95 ± 1.03	.28 ± .88	879	2, 10, 32	4.38	< 0.001	1.29 [.63–1.93]
	HC>NSSI	<i>Left</i> angular gyrus and occipital cortex	.87 ± .88	-.33 ± .79	1225	-46, -58, 34	4.19	< 0.001	1.45 [.78–2.11]
		<i>Left</i> middle temporal gyrus and superior temporal gyrus	.60 ± .58	-.49 ± .70	799	-58, -26, -8	4.33	< 0.001	1.68 [.99–2.36]
		<i>Right</i> frontal pole	.62 ± .73	-.36 ± .60	396	24, 66, 14	4.90	0.002	1.49 [.82–2.15]
		* <i>Right</i> inferior temporal gyrus, middle temporal gyrus, temporal pole	.80 ± .81	-.33 ± .72	492	50, -8, -36	4.38	< 0.001	1.47 [.80–2.13]
		* <i>Right</i> angular gyrus and occipital cortex	.98 ± 1.02	-.20 ± .78	370	40, -68, 28	4.20	0.004	1.32 [.66–1.96]

* Notates clusters that hold when entering demeaned BDI score at time of MRI as covariate

Table 3
Location, size and peak z-values of the significant clusters in the TFC group analyses

Seed Region	Contrast	Brain Regions	Control Mean z-stat	NSSI Mean z-stat	# of Voxels	MNI Coordinates of Peak Voxel (x, y, z)	Peak z-value	Cluster p-value	Cohen's D [Confidence Interval]
Right Amygdala	NSSI>HC Overall Task	<i>Right</i> lingual gyrus, occipital pole, occipital fusiform, temporal fusiform	-.55 ± .68	.52 ± .73	699	26, -66, -18	4.16	0.005	1.51 [.80–2.21]
		<i>Right</i> lateral occipital cortex and superior parietal lobule	-.39 ± .84	.65 ± .87	822	32, -66, 52	3.58	0.002	1.21 [.53–1.88]
Left Amygdala	NSSI>HC Overall Task	<i>Right</i> lateral occipital cortex and superior parietal lobule	-.36 ± .81	.74 ± .82	828	32, -60, 62	4.20	0.002	1.34 [.65–2.02]
		<i>Left</i> lateral occipital cortex and superior parietal lobule	-.41 ± .71	.64 ± .69	874	-34, -42, 38	3.38	0.001	1.50 [.79–2.20]
	HC>NSSI Overall Task	<i>Bilateral</i> /frontal pole, medial frontal cortex, and paracingulate	.73 ± .98	-.35 ± .87	663	0, 56, -4	4.04	0.008	1.18 [.50–1.85]