Connectivity Underlying Emotion Conflict Regulation in Older Adults with 5-HTTLPR Short Allele: A Preliminary Investigation

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Objective: *The serotonin transporter polymorphism short (s) allele is associated with heightened emotional reactivity and reduced emotion regulation, which increases vulnerability to depression and anxiety disorders. We investigated behavioral and neural markers of emotion regulation in community-dwelling older adults, contrasting s allele carriers and long allele homozygotes.* **Methods:** *Participants (N = 26) completed a face–word emotion conflict task during functional magnetic resonance imaging, in which facilitated regulation of emotion conflict was observed on face–word incongruent trials following another incongruent trial (i.e., emotional conflict adaptation).* **Results:** *There were no differences between genetic groups in behavioral task performance or neural activation in postincongruent versus postcongruent trials. By contrast, connectivity between dorsal anterior cingulate cortex (ACC) and pregenual ACC, regions previously implicated in emotion conflict regulation, was impaired in s carriers for emotional conflict adaptation.* **Conclusion:** *This is the first demonstration of an association between serotonin transporter polymorphism and functional connectivity in older adults. Poor dorsal ACC–pregenual ACC connectivity in s carriers may be one route by which these individuals experience greater difficulty in implementing effective emotional regulation, which may contribute to their vulnerability for affective disorders.* (Am J Geriatr Psychiatry 2014; 22:946–950)

Key Words: 5-HTTLPR, emotion regulation, neural connectivity, aging

INTRODUCTION

Executive functioning is a skill required for both successful implementation of cognitive (nonemotional) control and effective emotion regulation.[1] It is thought that the excessive, uncontrollable negative emotions experienced across anxiety and mood disorders are attributable, in part, to impaired emotion regulation.[2]

The presence of the short "s" allele of 5-HTTLPR polymorphism in the serotonin transporter gene has been associated with biased processing of emotional information, elevated limbic reactivity, and poorer prefrontal control over limbic regions.[3–5] It has also been implicated in depression, anxiety, and other stress-related disorders.[4,6] The s allele homozygotes exhibited lower dorsal anterior cingulate cortex (dACC), pregenual ACC (pgACC), posterior insula, and dorsolateral prefrontal activation during passive perception of negative emotional information compared with neutral.[7] Carriers of the s allele have also shown reduced amygdala to pgACC functional connectivity in response to emotional faces, compared with non-carriers.[3] Most of the work supporting this view, however, comes from studies of younger adults.

A significant amount of evidence shows that the 5-HTTLPR main and interactive effects attenuate with age on a broad range of affective outcomes.[8,9] Thus, results of previous observations of 5-HTTLPR effects

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on emotional regulation may not extend to older adults. Moreover, it is important to consider whether the reported effects of 5-HTTLPR on ACC connectivity in younger adults also extend to older adults.

We therefore conducted a preliminary investigation in older adults of the relationship between the 5-HTTLPR genetic polymorphism and emotion regulation, using a well-validated behavioral task together with functional magnetic resonance imaging (MRI). We used a task that required subjects to categorize face stimuli according to their emotional expression (fearful versus happy) while ignoring emotionally congruent or incongruent word labels ("fear," "happy") printed over the faces.

Emotional conflict between a word label incongruent with a facial expression creates substantially slowed reaction times.[10,11] Moreover, when an incongruent trial is preceded by an incongruent trial, reaction times are faster than if incongruent trials are preceded by a congruent trial.[10,11] It is thought that conflict from an incongruent trial triggers an upregulation of top-down control, thereby reducing conflict, indexed by faster reaction times, in the subsequent incongruent trial. Incongruent trials can thus be categorized by whether they are associated with high conflict regulation and consequently less emotional conflict (an incongruent trial [I] preceded by an incongruent trial [i]; iI) or low conflict regulation and thus more emotional conflict (an incongruent trial [I] preceded by a congruent trial [c]; cI).

In the emotional conflict task, implicit emotion regulation (i.e., of the interference caused by emotional conflict) happens through activation of the pgACC by the dACC and amygdala[10,11] in one trial (i.e., congruent, incongruent) compared with the next. The dACC is a key structure for monitoring and appraisal of nonemotional and emotional conflict and exerts control over pgACC during emotion conflict regulation.[11–13] Connectivity between these regions may be integral for successful emotion regulation, because individuals with anxiety and depression show impairments in these regions while completing this task.[14] Although prior investigations in the elderly have examined the relationship between aging and 5-HTTLPR with respect to vulnerability to stress-related cognitive and affective symptoms,[15–17] emotional reactivity,[18] and serotonin responsivity,[19] the effects of 5-HTTLPR on the neural substrates of emotional regulation in older adults are yet to be fully elucidated. In this study, we compared s carriers to long (l) homozygotes' reaction times, neural activation, and neural connectivity between dACC and pgACC to better understand the relationship between 5-HTTLPR and implicit emotion conflict regulation in older adults.

METHODS

Participants

Twenty-nine community-dwelling older adults ages 61–86 (mean: 70.52; standard deviation: 5.79; 14 women; education mean: 15.7 years; standard deviation: 2.2) participated, providing Stanford University–approved informed consent. Exclusion criteria included a Mini-Mental State Examination score < 27, any Axis I disorders within the past 2 years as assessed by the Structured Clinical Interview for *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision*, and use of systemic corticosteroids, psychotropic medications, short-acting anxiolytics, sedative hypnotics, or medication for treatment of dementia (inclusion/exclusion criteria as described previously).[16] Responses on the Geriatric Depression Scale[20] were also within normal limits (mean: 6.70; standard deviation: 2.52).

Participants were recruited from previous lab genetic studies to ensure adequate number of s carriers in the current sample, so we could compare s allele carriers (ss N = 9; sl N = 6) and l allele homozygotes (N = 14). (We did not enroll those with the G variant of the L allele because it could produce expression similar to s allele patterns and thus yield ambiguous results between s and l allele groups.) We considered mild cognitive impairment (MCI) status as a covariate to ensure that disease-related atrophy or connectivity changes were not unduly influencing our findings. MCI status was assessed using a cognitive battery described previously[16] and indicated that 15 participants were cognitively normal, 4 had possible MCI, and 7 had MCI.[21]

Participants had whole blood drawn at the Stanford Clinical Research Unit. DNA was extracted using the DNeasy Kit (catalogue number 69506; Qiagen GmbH D-40724, Hilden, Germany). 5-HTTLPR genotype status was determined using standard methods as previously described.[16]

Experimental Task

Inside the MRI scanner, participants completed a task where they were shown a happy or fearful face overlaid with the words "happy" or "fear." Participants were asked to identify the emotional expression of the face using a button-press while trying to ignore the distractor word; thus, the facial expression and word either matched ("congruent") or conflicted ("incongruent"; task described in more detail in Etkin et al.).[10]

Functional MRI Acquisition and Processing

Images were acquired on a 3T Signa scanner using a custom eight-channel head coil (GE Medical Systems, Milwaukee, WI). Twenty-nine axial slices (4.0-mm thickness with 0.9-mm gap) were acquired across the whole brain using a T2*-weighted gradient echo spiral pulse sequence (repetition time: 2,000 ms; echo time: 30 ms; flip angle: 80 degrees; one interleaf; field of view: 22 cm; 64 × 64 matrix). A high-resolution T1-weighted three-dimensional inversion recovery spoiled gradient-recalled acquisition in the steady-state MRI sequence was used with the following parameters: inversion time: 400 ms; repetition time: 8 ms; echo time: 3.4 ms; flip angle: 15 degrees; field of view: 22 cm; 124 slices in the coronal plane; matrix: 256 × 192; number of excitations: 1; acquired resolution: 0.9 × 0.9 × 1.2 mm. Preprocessing followed previously published procedures,[14] with the exception that normalization to the MNI152 template occurred using nonlinear methods optimally suited to atrophy found in older subjects (implemented in FSL 5.0, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/). The global signal was regressed out using signal estimates from white matter and cerebrospinal fluid. No participants had movement greater than 3 mm of translation or 3 degrees of rotation.

Analyses

Random effects group-level analyses were conducted in SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) using the contrast between the high conflict regulation trial sequence (iI) minus low conflict regulation trial sequence (cI), as previously detailed,[10–12,14] for both activation and task-related connectivity. MCI status was used as a covariate in all neuroimaging analyses.[16,21] Three participants were excluded from analyses (one was missing task data, one had MRI structural abnormalities, and one did not complete the neurocognitive testing necessary for determining MCI status). In the final sample, 12 participants were homozygous for the l allele and 14 had at least one s allele. Results were thresholded at $p < 0.001$, five voxel extent.

A psychophysiological interaction (PPI) analysis was used to examine the context-specific relationship between the activity in two regions during each trial type (e.g., congruent, incongruent) on an acquisition-by-acquisition basis.[22] PPIs allowed us to examine differences in interregional connectivity between task conditions separate from differences in activation or in general connectivity.[14] For the PPI analyses, we defined an a priori region of interest 5-mm sphere around the dACC (x,y,x = 5,33,32) by calculating a sample size–weighted average of the peak voxel coordinate for clusters in this regions from our three prior studies of healthy subjects using this task.[10–12] The pgACC cluster was identified in results of PPI connectivity contrasting iI minus cI (described in Results). In light of the frequently-implicated role of the dACC in monitoring conflict and the role of the pgACC in regulating conflict, only the connectivity results from the dACC seed region of interest to the pgACC target region of interest are discussed.

RESULTS

Behavioral

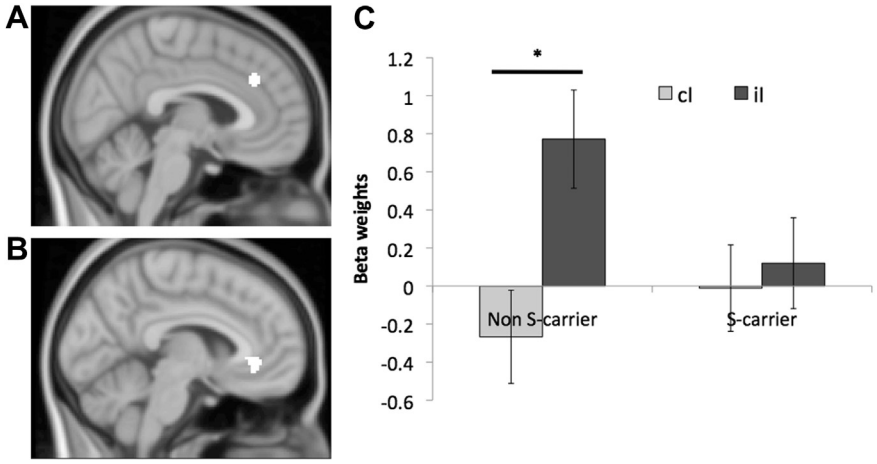
There were no significant differences between s carriers and l homozygotes in overall reaction times, emotional conflict regulation (iI-cI) reaction time differences, or for accuracy (all $F(1,24) < 1.61$, all $p > 0.21$). There were also no significant behavioral differences in the task according to MCI status (yes, possible, no; all $F(2,23) < 0.95$, all $p > 0.40$).

Neuroimaging

First, we examined neural activation between s carriers and non-carriers during emotional conflict regulation. However, no group differences were found in whole brain analyses of neural activation in response to iI compared with cI trials.

Next, we examined task context-dependent connectivity between dACC and pgACC, key functional nodes for emotion conflict regulation.[12,13] To do so, we

FIGURE 1. dACC to pgACC PPI. Connectivity between dACC and pgACC for postcongruent incongruent trials (cI) and postincongruent incongruent trials (iI) by s allele carrier status. [A] The s non-carriers increased dACC−pgACC connectivity between cI and iI trials ($t(11) = 4.24$, $p = 0.001$) but s carriers did not. [B] dACC seed, x,y,z = 5,33,32. [C] pgACC, x,y,z = −18, 36, −4. Error bars represent standard error. Coordinates are shown in MNI space. *$p = 0.001$.



carried out a PPI analysis on the contrast of iI minus cI trials in the dACC (5,33,32; Fig. 1A) between s non-carriers and s carriers. PPI results revealed greater connectivity in s non-carriers than in s carriers from dACC to the pgACC (−18, 36, −4; $z = 4.31$; 245 mm^3). Results of this contrast did not identify any target voxels located within the amygdala for the dACC seed.

To understand the relative influence of cI and iI trials to this group difference, we examined task context-dependent connectivity for the two conditions separately. Paired t tests comparing cI with iI in each genetic group illustrated (Fig. 1A) that s non-carriers increased dACC−pgACC (locations depicted in Figs. 1B, 1C) connectivity as they engaged emotion conflict regulation in iI trials relative to cI trials ($t(11) = 4.24$, $p = 0.001$) but that s carriers failed to do so ($t(13) = 0.75$, $p = 0.49$). Analogous t tests comparing iC and cC in each genetic group showed no differences between trial types (s non-carriers $t(11) = 1.17$, $p = 0.27$; s carriers $t(13) < 1$; results depicted in Supplemental Fig. 1; available online).

DISCUSSION

This is the first demonstration of an association between the 5-HTTLPR polymorphism, a gene that has been linked with depression and anxiety and functional connectivity in older adults. Our results indicate a relationship between the serotonin transporter polymorphism and neural connectivity within regions known for their prominent role in emotion regulation, independent of effects on neural activation. We found that behavioral, activation, and connectivity analyses do not always paint the same picture; some types of analyses may detect effects that are not apparent with other methods.[23] One possible explanation for the lack of correspondence between behavioral and brain measures is that we examined only one element within the circuit that contributes to behavior in this task. Additionally, measures of connectivity may be more sensitive than behavioral measures alone, thereby requiring less power for detecting effects.

By controlling for MCI status, we were able to examine the effects of genetics on affective processing independent of cognitive deficits.[16] Specifically, presence of the s "risk" allele[4] resulted in a failure to increase dACC−pgACC connectivity during emotional conflict regulation. A recent lesion study[13] demonstrated that the pgACC is causally required for emotional conflict regulation. In light of these findings, poor dACC−pgACC PPI connectivity in s allele carriers is one route by which they may have more difficulty implementing effective emotional regulation, thereby increasing vulnerability to affective disorders.[4,6] As such, our results are consistent with prior findings reporting

impaired connectivity of the pgACC in younger adult s carriers[3] and importantly extend this conclusion to community-dwelling older adults.

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