Biological Substrates of Emotional Reactivity and Regulation in Adolescence During an Emotional Go-Nogo Task

Todd A. Hare, Nim Tottenham, Adriana Galvan, Henning U. Voss, Gary H. Glover, and B.J. Casey

Background: Adolescence is a transition period from childhood to adulthood that is often characterized by emotional instability. This period is also a time of increased incidence of anxiety and depression, underscoring the importance of understanding biological substrates of behavioral and emotion regulation during adolescence. Developmental changes in the brain in concert with individual predispositions for anxiety might underlie the increased risk for poor outcomes reported during adolescence. We tested the hypothesis that difficulties in regulating behavior in emotional contexts in adolescents might be due to competition between heightened activity in subcortical emotional processing systems and immature top-down prefrontal systems. Individual differences in emotional reactivity might put some teens at greater risk during this sensitive transition in development.

Methods: We examined the association between emotion regulation and frontoamygdala circuitry in 60 children, adolescents, and adults with an emotional go-nogo paradigm. We went beyond examining the magnitude of neural activity and focused on neural adaptation within this circuitry across time with functional magnetic resonance imaging.

Results: Adolescents showed exaggerated amygdala activity relative to children and adults. This age-related difference decreased with repeated exposures to the stimuli, and individual differences in self-ratings of anxiety predicted the extent of adaptation or habituation in amygdala. Individuals with higher trait anxiety showed less habituation over repeated exposures. This failure to habituate was associated with less functional connectivity between ventral prefrontal cortex and amygdala.

Conclusions: These findings suggest that exaggerated emotional reactivity during adolescence might increase the need for top-down control and put individuals with less control at greater risk for poor outcomes.

Key Words: Adolescence, affect regulation, amygdala, development, emotion, prefrontal cortex

Adolescence is a period of heightened emotional reactivity (1) and vulnerability to poor outcomes (e.g., suicide, anxiety, and depression) (2). New insights into the biological basis of emotional reactivity have been provided by human neuroimaging studies. These studies have shown heightened activity in subcortical limbic regions like the ventral striatum and amygdala in adolescents relative to adults with exposure to both positive and negative information (3–5). In addition, immature prefrontal function has been shown in adolescents relative to adults in emotional contexts (4–6). Thus, the combination of enhanced bottom-up emotional processing in subcortical regions and less-effective top-down regulation from prefrontal regions might lead to an imbalance between emotion processing and control systems during adolescence. This imbalance might play a role in the increased risk for affective disorders during this period (7–10).

Although increased emotional reactivity is a characteristic of adolescence in general, there is a great deal of individual variability in emotional reactivity and regulation. For example, trait anxiety has been shown to influence behavioral and neural responses involved in emotion regulation. Studies of fear-potentiated startle in anxious adults have shown exaggerated emotional responses relative to less-anxious individuals (11). In addition, anxious individuals are less efficient at directing attention away from irrelevant emotional information (12,13). Adults and children with high anxiety have been shown to have elevated activity in brain regions implicated in emotion (e.g., amygdala) in response to negative or threatening information (e.g., fearful facial expressions) compared with low-anxiety individuals (14–17). The amount of functional coupling (functional connectivity) between the amygdala and prefrontal regions has been shown to correlate with levels of trait anxiety in adults, such that individuals with tighter coupling have lower trait anxiety (18), emphasizing the importance of prefrontal–amygdala interactions in emotion regulation. Extending these findings to adolescence might be particularly relevant, given that adolescence is a period when less-proficient prefrontal regulation of hyperactive limbic circuitry might create a condition of vulnerability to pathological processes leading to the onset of affective disorders. Examination of the neurobiological basis of individual differences related to emotional reactivity might help to explain why some adolescents experience more difficulties than others in emotion regulation leading to poor outcomes (e.g., onset of anxiety disorder or depression, adolescent suicide).

In the current study, we examined the biological substrates of developmental and individual differences in emotion regulation from childhood to adulthood with functional magnetic resonance imaging (fMRI). Specifically, we looked at initial reactivity and subsequent regulation/adaptation of limbic regions with repeated presentations of affective stimuli. While in the scanner, participants performed an emotional go-nogo task that required them to detect fearful, happy, or calm emotional expressions (target expression) while ignoring nontarget expressions. Anxiety levels in adults and adolescents were measured with the Spielberger state-trait anxiety inventory (19), a well-validated self
report. We hypothesized that adolescents would show exaggerated amygdala responses to emotional expressions compared with children and adults. In addition, we hypothesized that less habituation of the amygdala response to repeated presentations of affective stimuli and immature levels of connectivity between prefrontal control regions and the amygdala would be associated with higher trait anxiety during adolescence.

**Methods and Materials**

**Participants**

Eighty subjects between the ages of 7 and 32 years were scanned with fMRI. Data from 6 subjects were excluded because of technical problems, leaving 60 subjects (30 female) in the initial imaging analysis (Table 1). Adolescents and adults completed the Spielberger state-trait anxiety inventory (STAI; 19) before scanning (two adults and one adolescent completed the STAI ratings immediately after the scanning session). Separate age-appropriate norms included in the STAI manual were used to standardize anxiety scores for adolescents and adults. Trait anxiety score means and SDs were 46 ± 9 and 42 ± 7 for adults and adolescents, respectively. Forty-six right-handed subjects (22 female) between the ages of 13 and 30 years were involved in the analysis of anxiety and amygdala habituation (2 adults were removed, owing to the presence of outlying values [≥ 3 SD from the mean] when the blocks were divided into early, middle, and late trials). Children were not included in this portion of the analysis, because the version of the STAI used is only appropriate for adolescents and adults. Subjects had no history of neurological or psychiatric disorders. Before participation, all subjects provided informed written consent (parental consent and subject assent for children and adolescents) approved by the Institutional Review Board of Weill Medical College of Cornell University.

**Experimental Task**

Subjects completed six runs of a go-no-go task with fearful, happy, and calm facial expressions as targets and nontargets (Figure 1). All runs included only two categories of expressions, one target and one nontarget, which were pseudorandomized across the run to control for order of presentation. All combinations of expressions were used as both targets and nontargets (four adolescents successfully completed only four runs of the task, but all four completed the fear target with calm nontarget and happy target with calm nontarget runs on which the habituation analysis was based; these four subjects were not included in the behavioral analysis, owing to failure to complete the experiment). Before each run, subjects were given instructions to respond to a particular facial expression by pressing a button but not to respond for any other expression and to respond as fast as possible without making mistakes. Stimuli were presented for 500 msec, and the intertrial interval was varied between 2 and 14.5 sec with a mean intertrial interval of 5.2 sec. Each run lasted 307.5 sec and consisted of 48 stimulus presentations in a pseudorandom order to ensure an equal number of targets in early, middle, and late trials with targets occurring on 75% of trials for all subjects.

**Stimuli and Apparatus**

Face stimuli consisted of gray-scaled fearful, happy, and calm expressions from 12 individuals (6 female) taken from the NimStim set (20) available at http://www.macbrain.org. Calm rather than neutral expressions were used on the basis of previous findings showing that pediatric populations differ from adults in their response to neutral faces (21) (Note 1 in Supplement 1). Subjects viewed images projected onto an overhead liquid crystal display (LCD) panel with the IFIS-SA system (fMRI Devices Corporation, Waukesha, Wisconsin).

**Image Acquisition**

Subjects were scanned with a General Electric Signa 3.0-T fMRI scanner (General Electric Medical Systems, Milwaukee Wisconsin) with a quadrature head coil. A high-resolution, T1 weighted anatomical scan (either a three-dimensional [3D] spoiled gradient recalled [SPGR] 256 × 256 in-plane resolution, 240-mm field of view [FOV], 124 × 1.5-mm axial slices, or a 3D magnetization prepared rapid acquisition gradient echo [MPRAGE] 256 × 256 in-plane resolution, 240-mm FOV; 124 × 1.5-mm sagittal slices) was acquired for each subject for trans-
4-mm-thick coronal slices (skip 0) with a resolution of 3.125 mm, Flip angle package (with the Analysis of Functional NeuroImages (AFNI) software Imaging Data Analysis performed on significant main effects and interactions. 2 in Supplement 1). showed no effect of anxiety on reaction time or accuracy (Note Anxiety was not included in this analysis, but a separate model measures general linear models in SPSS (SPSS, Chicago, Illinois). reaction time and accuracy were analyzed with repeated measures 2423 individual models fit for each subject. The first model encompassed all trial types and included regressors for each response type (2 responses × 3 emotional expressions = 6) by convolving the stimulus timing files with a γ-variate hemodynamic response function. Incorrect trials were included as a separate regressor for a total of 7 regressors. The second individual model included only fearful targets paired with calm nontargets and happy targets paired with calm nontargets (Note 3 in Supplement 1). Separate regressors were created for fearful targets in the early, middle, and late portions of the run (12 targets/bin) as well as calm nontargets by convolving the stimulus timing files with a γ-variate hemodynamic response function. Regressors were created in the same manner for runs containing happy target expressions paired with calm nontargets. Incorrect trials were included as a separate regressor, giving a total of nine regressors. General linear modeling was performed to fit the percent signal change time courses to each regressor. Linear and quadratic trends were modeled in each voxel timecourse to control for correlated drift.

Group level analyses were conducted on the regression coefficients from the individual analysis after transformation into the standard coordinate space of Talairach and Tournoux (22) with parameters obtained from the transformation of each subject’s high-resolution anatomical scan. Talairached transformed images had a resampled resolution of 3 mm³.

Two separate group level linear mixed effects (LME) models were conducted with the 3dLME program within AFNI. The 3dLME program uses functions from the R software package (http://www.R-project.org). The first LME model included the factors age group, gender, emotion, and response. Trait anxiety was not included in this model, because children did not complete the STAI. The second LME model included the factors age group, trait anxiety, emotion, and trial. Gender was not included as a factor in this second analysis, owing to insufficient sample sizes, but a separate model showed no effect of gender on habituation (Note 4 in Supplement 1). Directionality of main effects and interactions was examined with post hoc t tests in SPSS on the average coefficients extracted from the regions of interest (ROIs).

Correction for multiple comparisons was applied at the cluster level following Monte Carlo simulations conducted in the Alphasim program within AFNI. Clusterwise false-positive rates of \( p < .05 \) corrected for multiple comparisons were determined for whole brain analyses as well as analyses restricted to the amygdala and ventral prefrontal cortex (vPFC) (Note 5 in Supplement 1). Corrected \( p \) values are indicated with an asterisk (*) throughout text. Coordinates presented in the text and supplemental figures represent center of mass for the ROI. Values in parentheses within the results section are means and SEMs.

A functional connectivity analysis was performed with the average time series from 16 voxels in the left amygdala where habituation was correlated with trait anxiety shown in Figure 4. Linear trend removal was first conducted on the time-series in every voxel throughout the brain. The AFNI program 3dfim+ was then used to calculate correlations between the time-series for the entire run at each voxel and the mean detrended time-series from voxels within the seed point after removing potential confounds, including motion parameters, average whole brain signal, signal from the ventricles, and signal from deep brain white matter and their derivatives.Correlation values were normalized with Fisher Z-transformation before group analysis.

Figure 2. Greater amygdala reactivity in adolescents. Mean amygdala activity for both target and nontarget expressions was greater for adolescents than adults and children. Scatter plot shows mean magnetic resonance (MR) signal in the amygdala on the y-axis. The x-axis represents age in years. Age group is coded with adults as squares, adolescents as circles, and children as triangles.
Results

Behavioral Results

The effect of emotional expression on reaction time was examined with a repeated measures general linear model (GLM), including age group (children, adolescents, adults) and gender (male, female) as between-subjects factors and emotion (fear, happy, calm) as the within-subjects factor. There were main effects of emotion \( F(2,65) = 42.00, p < .001 \) and age \( F(2,66) = 19.43, p < .001 \) on reaction time. There was also an interaction between emotion and age on reaction time \( F(4,130) = 6.13, p < .001 \). There was no effect of gender on reaction time. Post hoc t-tests showed that the main effect of emotion was due to faster reaction times for happy \((592 \pm 15)\) relative to fear \((634 \pm 20)\) and calm \((642 \pm 19)\); \( t(73) = 6.43, p < .001 \). There was no effect of gender on reaction time. Post hoc t-tests showed that adults \((606 \pm 16)\); \( U = 123, N1 = 16, N2 = 32, p < .005 \) and adolescents \((552 \pm 17)\); \( U = 67, N1 = 16, N2 = 26, p < .001 \) responded faster than children \((787 \pm 53)\). The interaction between age and emotion was due to relatively slower responses by adolescents \( Z = 1.03 \pm .01; t(56) = 2.29, p < .05 \) and children \( Z = 1.05 \pm .01; t(46) = 3.53, p < .001 \) for fearful target faces compared with adults \( Z = 1.00 \pm .01 \). The repeated measures GLM examining accuracy can be found in Supplement 1 (Note 6 in Supplement 1).

Imaging Results: Age and Gender Differences

To examine developmental and gender effects, a linear mixed effects model including age group (children, adolescents, adults) and gender (male, female) as between-subjects factors and emotion (fear, happy, calm) and response (go, nogo) as the within-subjects factor was conducted on the dependent variable of blood oxygen level dependent (BOLD) signal (activity). The complete list of brain regions showing main effects and interactions is given in Table 1 in Supplement 1. Within the amygdala, there were main effects of age group \([xyz = 25 \pm 6 - 13; F(2,54) = 4.08, p < .05^*]\), emotion \([xyz = -28 2 -17\) and \(24 2 -14\); \( F(2,108) = 4.98, p < .05^*\), and response \([xyz = -26 -5 -13\) and \(25 -6 -6\)
showed that there was greater amygdala activity for fear faces in male subjects versus female subjects \( t(59) = 2.76, p < .01 \) but no significant difference between fear and happy \( t(59) = 0.99 \). There was also greater amygdala activity for nontarget than target faces \( t(58) = 6.29, p < .001 \). The interaction between gender and response was due to greater amygdala activity for nontargets in male subjects versus female subjects \( t(58) = 3.01, p < .005 \). There was no difference in amygdala activity between male subjects and female subjects for target faces.

**Neural Correlates of Response Latency**

On the basis of previous work (25) showing that increased activity in the amygdala is correlated with slower responses to fearful target faces, we conducted a linear regression analysis to determine whether amygdala activity was correlated with reaction time in the current study. We regressed the percent difference in reaction time for fearful − happy targets versus activity in the amygdala for fearful − happy targets. There was a significant association between reaction time and activity in the left amygdala, such that slower reaction times were associated with greater amygdala activity \( t(59) = 15.2 - 16, F(1,59) = 5.72, p < .05 \); Figure 3. The correlation between amygdala activity and reaction time remained significant when controlling for d’ \( r(57) = .42, p < .001 \).

To test our hypothesis that prefrontal regions were involved in modulating subcortical regions like the amygdala in the context of emotional information, we conducted another linear regression examining the percent difference in reaction time for fearful − happy faces and prefrontal activity, controlling for left amygdala activity and d’. This analysis showed that activity in the vPFC was associated with faster reaction times for fear targets \( r(58) = 6.23 - 8; F(1,56) = 8.56, p < .05 \); Figure 3.

**Individual Differences**

To examine the association between individual differences in emotional regulation and self-rated trait anxiety, a linear mixed effects model including age group (adolescent, adult) and trait anxiety (high, low) as-between-subjects factors and emotion (fear, happy) and trials (early, middle, late) as-within-subjects factors was conducted on the dependent variable of BOLD signal (activity). Within the amygdala there were main effects of emotion \( t(59) = 22 - 5 - 15 \) and \( 23 - 8 - 10; F(1,42) = 7.29, p < .05 \) and trials \( t(59) = 23 - 5 - 14 \) and \( 23 5 - 14, F(2,84) = 7.29, p < .05 \) as well as interactions among: age and emotion \( t(59) = -20 - 8 - 20, F(1,42) = 7.29, p < .05 \); anxiety and emotion \( t(59) = 19 - 4 - 12, F(1,42) = 7.29, p < .05 \); age and trial \( t(59) = -261 - 16; F(2,84) = 5.50, p < .05 \); and age, anxiety, emotion, and trial \( t(59) = -280 - 15 \) and \( 20 - 9 - 7, F(2,84) = 7.29, p < .05 \). Two regions in vPFC also showed an interaction among age, anxiety, emotion, and trial \( t(58) = -114 42 \) and \( 25 54 7, F(2,84) = 11.11; p < .05 \). Table 2 in Supplement 1 lists all brain regions showing main effects and interactions in this analysis. Post hoc tests were conducted on selected interactions of interest. The age group by trial interaction was due to greater amygdala activity in adolescents \( .44 \pm .1 \) than adults \( .07 \pm .07 \) in early trials \( t(44) = 2.47, p < .05 \) but no difference in middle \( .25 \pm .12 \) vs \( .02 \pm .05 \) or late trials \( -.02 \pm .10 \) vs \( -.06 \pm .08 \). This age group × trial interaction remained significant when controlling for reaction time (Notes 7 and 8 in Supplement 1). The interaction among age, anxiety, emotion, and trial was due to the fact that amygdala activity decreased from early to late trials (habituated) less for fear than for happy targets in more-anxious adolescents \( t(9) = 3.36, p < .01 \), but habituation did not differ as

---

**Figure 4.** Amygdala habituation and trait anxiety. Trait anxiety scores were negatively correlated with habituation (decrease from early to late trials) of amygdala activity \( r = -.447, p < .001 \). Amygdala habituation was calculated by subtracting activity in late trials from activity in early trials. (A) Region of the left amygdala that correlated with trait anxiety. (B) Scatter plot of the correlation between trait anxiety and amygdala habituation. The y-axis represents magnetic resonance signal in the left amygdala for early − late trials. The x-axis represents trait anxiety score.

**Table 2.** Amygdala Habitation to Target Faces

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Trait Anxiety</th>
<th>Fear</th>
<th>Happy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adolescents</td>
<td>Less Anxious</td>
<td>.45 ± .14</td>
<td>.24 ± .13</td>
</tr>
<tr>
<td></td>
<td>More Anxious</td>
<td>-.09 ± .19</td>
<td>.96 ± .25</td>
</tr>
<tr>
<td>Adults</td>
<td>Less Anxious</td>
<td>.19 ± .08</td>
<td>.55 ± .19</td>
</tr>
<tr>
<td></td>
<td>More Anxious</td>
<td>.06 ± .13</td>
<td>.11 ± .10</td>
</tr>
</tbody>
</table>

Values represent the mean difference and SEM in magnetic resonance signal between early and late trials in blocks with fear and happy targets. One outlier was removed from the mean for happy targets in more-anxious adolescents.
a function of emotion in less-anxious teens or adults (Table 2). Greater amygdala habituation to fear targets was associated with lower trait anxiety scores across both age groups \(xyz = -20 -5 -14; F(1,45) = 7.86, p < .05\); Figure 4). The pattern of activity for fear targets in the vPFC differed as a function of age and anxiety, such that less-anxious adolescents and more-anxious adults showed increased activity in early trials \((.19 \pm .08)\) versus late trials \([-11 \pm .08; t(25) = 2.70; p < .01]\), whereas activity did not differ over time for more-anxious teens and less-anxious adults.

**Functional Connectivity**

To examine the relationship between activity in the amygdala and vPFC, we conducted a functional connectivity analysis. There was a negative correlation between activity in the amygdala and vPFC for fear targets \(r(45) = 3.51, p < .05\); Table 4 in Supplement 1). A linear regression analysis showed that stronger connectivity between the vPFC (\(xyz = 2 55 -5, 21 30 3, \) and \(-17 58 4)\) and the amygdala was associated with greater habituation of amygdala activity (early – late) \(F(1,45) = 8.72, p < .05\). A conjunction analysis showed that there was overlap between the areas of vPFC showing an interaction among age, anxiety, emotion, and trial and vPFC regions where functional connectivity with the amygdala was associated with greater amygdala habituation (Figure 5).

**Discussion**

A neural basis for difficulties in regulating behavior in emotional contexts in adolescents was tested. The findings are consistent with a neurobiological model (26) of competition between enhanced activity in subcortical emotional processing systems and less-mature top-down prefrontal systems. The ability to engage in top-down regulation of emotional centers such as the amygdala is likely to be important during adolescence in guiding behavior in highly emotional contexts. Our findings suggest elevated amygdala activity in such situations in adolescents relative to children and adults. Differences in the strength of connectivity between top-down control and bottom-up emotion processing regions might underlie individual differences in emotion regulation especially during adolescence, when these bottom-up systems seem to be elevated in activity. Anatomical studies of brain development have shown protracted development of prefrontal regions in terms of both local decreases in gray matter density and increases in the myelination of fibers linking PFC to other brain regions (27). Both local refinements and increased connectivity are likely to improve the efficiency of emotion regulation on the basis of our findings showing that the strength of coupling between vPFC and the amygdala is correlated with greater habituation of amygdala activity during adolescence. Despite the relative immaturity of PFC during adolescence, amygdala activity decreased to near or even below baseline with repeated exposure to empty threat (fearful faces) in both adults and adolescents. These data are consistent with previous neuroimaging studies of cognitive control showing that adolescents can suppress a competing response but must recruit prefrontal regions more than adults to do so (28). The fact that adolescents respond more slowly to fear targets and show less prefrontal relative to amygdala activity for these trials than adults suggests that adolescents might be more susceptible to emotional interference relative to adults. Greater initial reactivity in subcortical limbic regions in adolescents relative to adults might explain why poor decisions might be made in the heat of the moment even though adolescents know better. Given the role of prefrontal regions in guiding appropriate actions, immature prefrontal activity might hinder decisions within an emotional context (i.e., heat of the moment).

Differences in the efficiency of prefrontal regulation might also explain the lower levels of vPFC activity in less anxious adults in the current study. Whereas less-anxious teens showed greater vPFC activity in early versus late trials mirroring the decrease in amygdala activity, less-anxious adults showed little activity in vPFC regions for fear targets. However, less-anxious adults also showed rapid habituation (decrease from early to late trials) of amygdala activity to levels below baseline. Less-anxious adults might be more efficient in regulating amygdala activity and therefore require less vPFC activity.

Theories of the neurobiological basis of affective disorders emphasize the role of circuits including the amygdala and vPFC (29–32). The current study showed differences in the amygdala and vPFC as a function of variance in trait anxiety within the normal range, and these differences might be even greater in clinically anxious populations. Functional magnetic resonance imaging studies have found greater amygdala activity in response to negatively valenced information (often fearful faces) and diminished activation of vPFC (16,33,34) in clinically anxious children and adults relative to control subjects. We and others (18) have shown that less functional connectivity between amygdala and vPFC is associated with higher anxiety. Functional coupling between the amygdala and vPFC is influenced by emotional context (35,36). This association might be especially important during adolescence when transitions from childhood
to adulthood result in increased independence (separation from care-givers) and require more self-regulation of emotion.

Increased amygdala activity has been shown during initial fear conditioning in healthy control subjects that diminishes with extinction (37,38). There is evidence for less habituation of amygdala activity (i.e., diminished activity with repeated exposures to empty threat) in clinically anxious populations than in healthy control subjects. A recent meta-analysis of studies using fear conditioning paradigms in participants with anxiety disorders revealed consistent deficits in extinction after simple fear conditioning paradigms in participants with anxiety disorders compared with healthy control subjects (40). These results are consistent with our data showing a correlation between amygdala habituation and trait anxiety in healthy adolescents and adults.

The current study required subjects to make a response that was in opposition to an affective signal (i.e., approach fearful expressions that are associated with threat) and to do so as fast as they could. Thus, optimal performance required emotion regulation and allowed us to examine individual and age-related differences in sensitivity to affective interference. Adolescents and children were relatively slower than adults when responding to fearful target faces, suggesting that adolescents and children were less efficient at overriding affective interference compared with adults. We have now shown in two separate experiments that, in the context of a go-no go task, mean reaction times for fearful facial expressions as targets are positively correlated with amygdala activity (25). Ventral PFC activity was associated with both faster reaction times to fear targets and greater amygdala habituation. Together these findings suggest that ventral prefrontal regions are engaged to regulate affective processing and facilitate appropriate responses in the presence of affective interference. Prefrontal regulation might be especially important during adolescence, owing to increased reactivity of affective processing systems like the amygdala in response to emotional information compared with children and adults. Furthermore, adolescents must deal with dramatic changes in their social environment and interactions that might serve as stressors driving activity in hypersensitive affective systems that immature prefrontal control circuitry cannot effectively regulate. Therefore, the combination of biological susceptibility and environmental context might underlie the prevalence of affective disorder onset during adolescence.

This work was supported by National Institute of Drug Abuse Grant R01 DA 18879 to BJC and National Institute of Mental Health Grant F31 MH 073265 to TAH.

Drs. Hare, Tottenham, Galvan, Voss, Glover, and Casey report no biomedical financial interests or potential conflicts of interest.

Supplementary material cited in this article is available online.