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# Developing Connections for Affective Regulation: Age-Related Changes in Emotional Brain Connectivity

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#### **Abstract**

The regulation of affective arousal is a critical aspect of children's social and cognitive development. However few studies have examined the brain mechanisms involved in the development of this aspect of "hot" executive functioning. This process has been conceptualized as involving prefrontal control of the amygdala. Here, using functional magnetic resonance imaging (fMRI), we investigated the brain mechanisms involved in the development of affective regulation in typically-developing, 5- to 11-year-old children and an adult comparison sample. Children and adults displayed differing patterns of increased anterior cingulate cortex and decreased amygdala activation during episodes in which emotion regulation was required. Specifically, amygdala activation increased in adults, but decreased in children during recovery from a frustrating episode. In addition, we used effective connectivity analyses to investigate differential correlations between key emotional brain areas in response to the regulatory task demands. We found reliable increases in effective connectivity between the anterior cingulate cortex and the amygdala during periods of increased demand for emotion regulation. This effective connectivity increased with age.

# Keywords

Emotion Regulation; fMRI; Emotion Induction; Amygdala; Anterior Cingulate; Effective Connectivity

Recent research into the development of executive functioning suggests that this broad construct can be divided into two distinct, but interacting, subcategories. First, "cool" executive function is thought to be accessed by purely cognitive problems, such as number processing, sorting, and rule use, while "hot" executive function is considered to be related to problems involving the regulation of affect and motivation (see Hongwanishkul, Happaney, Lee, & Zelazo, 2005; Zelazo & Müller, 2002). Although the interaction of emotion and cognition is quite complex and often absent of clear boundaries (Pessoa, 2008), both of these aspects of executive functioning have been related to distinct regions of the prefrontal cortex (PFC). Cool executive functioning is generally linked to the dorsolateral prefrontal cortex (DLPFC) while hot executive functioning is conceptualized as originating in the ventral and medial prefrontal cortex (VMPFC). The current study investigated the

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brain development of one aspect of hot executive functioning, the regulation of negative affect, from the perspective of affective arousal and effective PFC connectivity.

Proficiency in the management of affective arousal is a critical facet of early social and cognitive development. During childhood, infants move from a necessary dependence upon the emotional support of their caregivers (Rothbart, Ziaie, & O'Boyle, 1992) to the development of self-sufficient mechanisms for emotion regulation at school age (Denham, 1998). Behavioral studies have examined both children's control of overt emotional behavior (Carlson & Wang, 2007; Cole, 1986; Saarni, 1984) and modulation of internal emotional state (Harris, Olthof, Meerum, & Terwogt, 1981). However, only recently have researchers begun to examine neural mechanisms for the development of affect regulation (for reviews see Lewis & Stieben, 2004; Lewis & Todd, 2007; Thompson, Lewis, & Calkins, 2008). Characterizing age related changes in the typical and atypical development of affective brain regulation is an important and active area of investigation (e.g., see Cole, Martin, & Dennis, 2004; Lewis & Stieben, 2004).

Numerous investigations have characterized both emotional reactivity and regulation in the adult brain and some research has indicated changes in the structure and function of key brain regions across development. Brain lesion (e.g. Adolphs, Tranel, Damasio, & Damasio, 1994) and functional magnetic resonance imaging (fMRI) studies (e.g. Morris et al., 1996) point to the role of the bilateral amygdala as critical for the perception of positive and negative emotion, while more current research has conceptualized this area as more of a "salience detector" of relevant emotional stimuli in the immediate environment (see Anderson and Phelps, 2000, 2002; Adolphs, 2008). From a developmental perspective, recent research suggests that amygdala activation to emotional faces increases between the ages of 3.5-8.5 years (Todd, Evans, Morris, Lewis, & Taylor, in press), consistent with the hypothesis that amygdala reactivity underlies an increasing sensitivity to emotionally relevant information. In turn, regions of the VMPFC, especially the anterior cingulate cortex, have been implicated in the modulation of this emotional arousal (e.g. Beauregard, Lévesque, & Bourgouin, 2001; Lange et al., 2003; Ochsner, Bunge, Gross, & Gabrieli, 2002). From a developmental perspective, the PFC, as a whole, is among the last brain areas to fully develop, with continued synaptogenesis (Rakic, Bourgeois, & Goldman-Rackic, 1994) and increasing white matter volume (Pfefferbaum, Mathalon, Sullivan, Rawles, Zipursky, & Kim, 1994; Shaw, Greenstein, Lerch, Clasen, Lenroot, Gogtay, et al., 2006) until late adolescence or early adulthood. This protracted developmental course has been linked to improvements in various complex cognitive skills (Caviness, Kennedy, Richelme, Rademacher, & Filipek, 1996; Diamond, 1988; Shaw et al., 2006), which are known to be important for the modulation of affective arousal (Gross, 1998). FMRI studies investigating the development of self-regulation have demonstrated that differential recruitment of regions of the PFC may underlie decreased executive function of emotion in children relative to adults (Bunge, Dudukovic, Thomason, Vaidya, & Gabrieli, 2002; Casey, Geidd, & Thomas, 1997; Luna, Thulborn, Munoz, Merriam, Graver, Minshew, et al., 2001).

In one study, in which regulation of the amygdala by the PFC was investigated, Ochsner and colleagues (2002) presented images of negatively arousing scenes to their adult participants during fMRI scanning. Participants were instructed to view the image and to experience any feelings that the image produced during the first few seconds of presentation. They were then instructed to either "attend" to those emotions or try to reduce negative feelings by "reappraising" the content of the transient image. Ochsner and colleagues (2002) found less right amygdala and orbitofrontal cortex activation when participants were instructed to reappraise the negative scene rather than attend to their induced emotional state. Further, participants' self-report of reduced negative emotion correlated with increased anterior cingulate activation. A similar study conducted with child participants (Lévesque et al.,

2004) also found evidence for increased prefrontal and anterior cingulate cortex activation during suppression of emotion while watching sad film clips. Taken together, these findings support the critical role of the prefrontal and anterior cingulate cortices in the down-regulation of emotional arousal in both adults and children; however, the mechanisms by which these regulatory processes develop have generally remained unexamined.

One developmental study (Lewis, Lamm, Segalowitz, Stieben, & Zelazo, 2006) elegantly measured the regulatory effects of emotion induction using event related potentials (ERPs). This study took a different approach to the evaluation of brain mechanisms for affective modulation by inducing tonic changes in emotional state rather than transient responses to individual emotional stimuli. Children (ages 5-16 years) engaged in a go/no-go task in which they gained and lost points towards a desired prize. Go/no-go tasks are commonly employed in the child literature as a measure of attentional control (i.e. "cool" executive function) (Casey, et al., 1997); however, this task was designed to be an emotional version of the more common go/no-go paradigm, thereby measuring "hot" executive function. A temporary loss of all points triggered self-reported negative emotions, as well as an increase in frontal P3 response. In addition, source modeling suggested localization of this increased response to frontal midline regions across age. This effect was hypothesized to parallel increased functioning of the anterior cingulate cortex across development, possibly indicating incremental changes in the recruitment of this region to successfully regulate emotion. The design of this task allowed for measurement of more implicit brain mechanisms for emotion regulation, as well as the successful induction of negative emotions during a relatively ecologically valid emotionally arousing episode: loss of points during a computer game.

The current study employed a modified version of this task in a sample of 5–11-year-old children and a comparison group of adults. Adapting the paradigm of Lewis et al. (2006) for fMRI, participants played a game in which they won and lost points towards a desired prize. The long winning and losing blocks were designed to induce lasting emotional states during which fearful faces were presented as transient probes for the measurement of responses to salient emotional stimuli. Whereas tonic changes in mood are likely to influence the threshold of amygdala reactivity, amygdala activation is readily measured by activation to transient, salient stimuli. Thus, the fear face probe allowed optimal measurement of amygdala activation and the capacity to detect mood related differences in amygdala activation that could be driven by emotional arousal.

Our study sought to investigate the relationship between the amygdala and VMPFC in the service of affective regulation through connectivity analyses. Enhanced understanding of functional connectivity has greatly improved our knowledge of the neural correlates of human information processing and cognitive operations (Finglekurts, Finglekurts, & Kähkönen, 2005; Friston, 1994; Friston, Frith, Liddle, & Frackowiak, 1993). Modern techniques for studying functional connectivity (e.g., Just, Cherkassky, Keller, & Minshew, 2004; Meyer-Lindenberg et al., 2005) now allow neuroscientists to move toward brainbased, mechanistic theories of cognitive and emotional functioning. Methods (e.g. Roebroeck, Formisano, & Goebel, 2005; Friston, Harrison, & Penny, 2003) have now been developed to examine effective connectivity, which refers to the impact that activity in one region exerts over activity in another region. These techniques can be used to make inferences about the negative or positive influence and how this is affected by experimental manipulations. However, minimal research exists quantifying both regional brain activation and inter-region connectivity in relationship to changes in emotional mood state in adults, and no research studies have investigated this topic in the developing child brain. Our study represents one of the first attempts to measure emotional brain activation and effective connectivity in response to positive and negative emotion induction in young children.

When considering the aims of our study along with the rather small body of work conceptualizing affective regulation in the developmental literature, we constructed the following hypotheses:

- 1. We hypothesized that amygdala activation to the fearful face probes would increase during episodes of induced negative mood in both children and adults.
- 2. We hypothesized increased effective connectivity between the amygdala and PFC during lasting negative mood induction in both children and adults.
- **3.** Effective PFC connectivity to the amygdala was expected to positively correlate with the age of the child during negative mood induction.

#### Methods

# **Participants**

Participants were 20 (9 male) English-speaking children ages 5–11 years (average age = 8 years, 2 months) with normal or corrected-to-normal vision. The sample included three 5year-olds, four 6-year-olds, one 7-year-old, five 8-year-olds, four 9-year-olds, one 10-yearold, and two 11-year-olds. An additional 6 children were scanned, but not included in data analyses due to excessive head movement (4), inability to complete the scan (1), or drowsiness (1). These participants did not differ in age from participants with usable data [t(24) = -.19, p = .85]. The child's primary caregiver was interviewed by phone during recruitment to ensure that all children were healthy and free of any neurological and psychiatric disorders. Parents were asked directly wether their child had any history of medical or psychiatric illness, thus ruling out the possibility of learning or language disabilities, developmental disorders, or mood disorders. Participants were recruited from local community postings and internet advertisements and were paid for their participation. In addition, they earned an age appropriate prize as part of their task participation (see below). Ethical approval for the project was obtained from the local Institutional Review Board. A parent or guardian of each participant provided written informed consent for their child's participation. Each child provided written and verbal assent.

We also tested a comparison sample of 25 (13 male) English-speaking adults ages 19–41 years with normal or corrected-to-normal vision, also free of any neurological and psychiatric disorders. All participants were recruited from local community postings and internet advertisements and were paid \$40 for their participation. Adults earned a \$10 gift certificate to a local merchant as part of their task participation (see below). All adult participants provided written informed consent.

#### **Materials and Procedure**

Before data collection and practice of the task, children were told that they would be playing to win a prize: their choice of toy from a large toy collection. After choosing their desired prize, children were given a few minutes to play with the toy and show it to their parent and/ or siblings. They were then told that they would need to earn a large amount of points during the game in order to keep their prize, and that they would earn points based on speed and accuracy. Children were not told the specific number of points which they would need to win, but if questioned, the experimenter replied, "You need a lot of points. Over a thousand, at least". They were reminded that they would not be told whether they won or lost until the end of the game. The children then practiced the emotional go/no-go task outside of the scanner to ensure understanding and proper performance during fMRI data collection. Adults were given the same task instructions as children except that they were offered their choice of gift certificate to a selected local merchant as an incentive to win.

Before scanning began, children participated in a "mock scanning" session to help ensure compliance with the requirement to remain motionless during data collection, and to help the children to feel as comfortable as possible while participating in the experiment. Children were trained to remain still while watching a children's video inside a full-size replica of our MRI scanner. During practice, custom-written software received input from a head motion sensor worn by the child and used that input to play a sound when the participant moved outside of a set threshold (3 mm). In addition to viewing approximately 10 minutes of the video, children, again, practiced the go/no-go task inside the mock scanner. With the addition of realistic scanner sounds played during children's practice session, we were able to reproduce the scanning environment in order to acclimate our young participants to the fMRI scanning procedures.

Participants took part in a novel, event-related within a block design, emotion regulation task partially adapted from Garavan, Ross, & Stein (1999) and Lewis and colleagues (2006). A go/no-go task was chosen for this study because it is often employed in the child executive function literature as a reliable measure of inhibitory control (Reed, Pien, & Rothbart, 1984). Our emotional version of the go/no-go task is illustrated in Figure 1. Throughout the paradigm, a stream of pictures of common objects (e.g. balls, shoes, umbrellas) was presented at a rapid rate. Participants were instructed to press a button when the object was presented in a green frame, but to inhibit their response when the object was presented in a red frame. Incorrect responses to both go and no-go trials were followed by a large "X" on the center of the screen and the sound of a buzzer. Periodically, fearful faces from the NimStim set of facial expressions (Tottenham et al., 2009), appeared for a onesecond duration 6–15 seconds apart (60 total trials), accompanied by a short chime. The presentation of a face served as our point of measurement for the event-related analysis. Participants were also notified of their total number of accumulated points approximately every 30 seconds throughout the task. As point totals were presented, participants heard a "slide whistle" sound indicative of point loss or point gain.

This task was designed to maintain the same level of difficulty for all participants. To this end, an algorithm was used to keep all children functioning at approximately the same error rate (50  $\pm$  10%). The algorithm was piloted with 25 5-year-olds (outside of the scanner) to ensure that children this young could successfully complete our task. The error rate for the task was maintained by adjusting the stimulus duration dynamically (see Table 1). Stimulus duration increased with each error made on a no-go trial and decreased with each correct response. Unbeknownst to participants, the task contained three blocks designed to induce different types of emotion. Each block lasted approximately four minutes. During Block 1 (Winning), participants saw their points steadily increase to well over 1000 with a stimulus interval set between 800 - 1150 ms. This block was designed to induce positive emotions of success in our participants, even though the task was designed to be challenging and engaging throughout. Changes in the point-adjustment algorithm and stimulus presentation speed (600 – 950 ms) caused increased task difficultly and point loss during Block 2 (Losing), which was intended to induce negative emotions of frustration. Children lost all of their previously earned points during this block. With a return to the more generous algorithm in Block 3 (Recovery), participants regained their points and ultimately won their desired prize. Although there was notable point gain, we designed this block to represent a recovery period after an emotionally negative event (Lewis et al., 2006). Each of the fourminute blocks served as points of measurement for our block analysis. The experiment was constructed with three long blocks in order to allow participants the necessary time to feel the induced positive or negative emotions due to lengthily periods of gaining or losing points, respectively.

The emotional go/no-go task was identical for adult participants, except adjusted to an adult level of difficulty. In the adult version of this task, blocks were 6 minutes long and displayed 120 fearful and neutral faces as probes (40 per block, 20 of each emotion). The stimulus presentation speed was also adjusted to a faster adult level with winning and recovery blocks presenting stimuli at 600–950ms and the losing block presenting stimuli at 400–800ms.

# **fMRI** Data Acquisition

Scanning was performed on a Siemens 3 Tesla Allegra head-only scanner (Siemens, Erlangen, Germany). High-resolution, T1-weighted anatomical images were acquired using an MPRAGE sequence (TR = 1630 ms; TE = 2.48 ms; FOV = 20.4 cm;  $\alpha$  =  $8^{\circ}$ ; image matrix =  $256^2$ ; voxel size =  $0.8\times0.8\times0.8$  mm; 224 slices). Whole-brain functional images were acquired using a single-shot, gradient-recalled echoplanar pulse sequence (TR = 2000 ms; TE = 30 ms;  $\alpha$  =  $73^{\circ}$ ; FOV = 20.4 cm; image matrix =  $64^2$ ; voxel size =  $3.2\times3.2\times3.2$  mm; 35 slices) sensitive to blood oxygenation level-dependent (BOLD) contrast. Runs consisted of the acquisition of 224 successive brain volumes beginning with 2 discarded RF excitations to allow for steady-state equilibrium.

The BrainVoyager QX 2.1 software package (Brain Innovations, Maastricht, The Netherlands) was used for all analyses. The following preprocessing procedures were performed on raw images prior to data analysis: slice scan time correction (using cubic spline interpolation), high-pass temporal filtering to remove nonlinear drifts of three or fewer cycles per time course, three-dimensional motion correction to detect and correct for small head movements by spatial alignment of all volumes to the first volume by rigid body transformation, and spatial data smoothing using a Gaussian kernel with a 4 mm full width at half-maximum. Head movements never exceeded 3 mm for all participants included in this analysis. Functional data were coregistered to the anatomical volume by alignment of corresponding points to obtain optimal fit and were then transformed into Talairach space (Talairach & Tournoux, 1988).

A multiparticipant statistical analysis was performed by multiple linear regression of the time course of the BOLD response in each voxel. The general linear model of the experiment was computed for each participant's z-normalized volume time courses. Model predictors were defined by convolving an ideal boxcar response with a gamma-function model of the hemodynamic response (Friston et al., 1995). Boxcar values were equal to 1.0 during the face presentation and were 0.0 during presentation of objects. Activation maps were visualized on a Talairach-transformed template brain, with only clusters of more than 8 contiguous voxels displayed at a resolution of 1 mm<sup>3</sup>.

# **Manipulation Check**

In order to ensure that both positive and negative emotions were successfully induced during this task, participants were interviewed regarding their emotional experiences during participation. We asked a few short, free-response questions to follow up on the participants' opinions of the task. One hundred percent of children recalled losing all their points during the game and 95% (all but one child) reported feeling negative emotions during that episode. Ninety percent reported feeling positive emotions when they discovered that they would, indeed, win their prize. We counted negative and positive reports of emotion as anything with negative (e.g. "bad", "angry", "upset", "I didn't like it", etc.) or positive (e.g. "good", "happy", etc.) valence respectively.

#### Results

# **Event-Related Analysis**

In order to test our first hypothesis regarding amygdala response to salient emotional stimuli during mood induction, we first employed a hypothesis-driven anatomical region-of-interest (ROI) approach.

**Children**—Using the standard Analysis of Functional Neuroimages ROIs (AFNI; National Institutes of Mental Health; Bethesda, MD), we localized areas within the bilateral amygdala and lateral fusiform gyri (FFG) that were significantly more responsive to the presentation of fearful faces [t = 2.07, p < .04, q < .05] than to our baseline measurement of objects during the full go/no-go task. Active regions of the right lateral FFG were localized to the expected location of the fusiform face area (Kanwisher, McDermott, & Chun, 1997; Puce, Allison, Asgari, Gore, & McCarthy, 1996). For this analysis, from which we derived our functional regions of interest for subsequent analyses, we used the false discovery rate procedure (Genovese, Lazar, & Nichols, 2002) [FDR(q) < .05] to control for multiple statistical comparisons.

Next, we looked at each block individually to investigate the effects of emotion induction on the processing of fearful faces (20 faces per block). In each of the three blocks, we were able to localize a region of the right FFG that was responsive to the brief presentation of faces [Block 1: t = 2.46, p < .01, q < .05; Block 2: t = 2.39, p < .02, q < .05; Block 3: t = 2.44, p < .01, q < .05; see Figure  $q = 2^{1}$ . Within the left amygdala, we found activation to faces; however, this activation differed as a function of block. Significant activation above the object baseline was found in Blocks 1 and 2, but not in Block 3 [Block 1: t = 2.46, t = 2.

A second event-related analysis was completed to investigate the effects of prefrontal control across blocks, independent of face presentation. Here, we examined the 2-second period of time preceding the face presentation for each block. As expected, in comparison to the face presentation, we saw significantly less activation in the left amygdala and right FFG in the 2 seconds preceding the face (t = 2.34, p < .02, q < .05). In addition, we found notable change in prefrontal activation across blocks. In Block 2 (Losing), we observed an increase in VMPFC and orbitofrontal cortex activity before the face was presented in comparison to the Winning Block 1 [t = 1.96, p < .05, uncorrected]. Comparing Block 1 (Winning) and the similarly challenging Block 3 (Recovery), we saw increased activation in the pre-face period in Block 3 in the VMPFC [t = 1.96, p < .05, uncorrected)]. No significant differences across blocks were found in the anterior cingulate cortex, which was highly active throughout. This was likely related to the general difficulty of the executive function related task, leading to a ceiling effect.

**Adults—**We completed a similar analysis and localized areas within the bilateral amygdala and lateral fusiform gyrus that were responsive to the presentation of faces (regardless of emotion) across the entire task [t = 2.28, q < .05] for our adult participants. Next, we looked at each block individually to investigate the effects of emotion induction on the processing of fearful and neutral faces (40 faces per block, 20 fearful/20 neutral). Within each of the three blocks we localized a region of the FFG responsive to the brief face presentation [Block 1: t = 2.54, p < .02, q < .05; Block 2: t = 2.60, p < .01, q < .05; Block 3: t = 2.22, p < .03, q < .05; see Figure 2]. This activation was essentially uniform, with no significant differences between blocks [left FFG: F = 1.49, p = .18; right FFG: F = 1.140, p = .34]

<sup>&</sup>lt;sup>1</sup>Images of brain activation in this manuscript are masked to display results of a priori regions of interest.

insuring processing of the presented face throughout the task. Within the bilateral amygdala, we found activation to faces, however, this activation differed as a function of block [right amygdala: F = 3.11, p < .001; left amygdala: F = 2.68, p < .01]. The bilateral amygdala were significantly less active to faces in Block 2 (Losing) than to faces presented in Blocks 1 and 3 [t = 1.96, p < .05]. Finally, a comparison between Block 1 (Winning) and Block 3 (Recovery) revealed significantly greater face-related activation of the right and left amygdala in Block 3 [t = 1.96, p < .05].

In addition, within our functional ROIs, we found greater activity in a region of the right amygdala for fearful, relative to neutral, faces [t = 2.58, p < .01]. This difference in activation varied across blocks. In Block 1, the bilateral amygdala responded more to fearful than neutral faces [t = 1.96, p < .05]. However, in Block 2, the bilateral amygdala displayed equal activation to fearful and neutral faces. Finally, during Block 3, we again found that the bilateral amygdala responded more to fearful than neutral faces [t = 1.96, p < .05].

#### **Block Analysis**

In order to test our second hypothesis regarding effective VMPFC-amygdala effective connectivity during mood induction, we employed Granger Causality Mapping to map the sources of influence to and from our amygdala seed regions in both children and adults. Granger Causality theory states that a discrete time series X "Granger-causes" a discrete time series Y if the past values of X improve the prediction of the current value of Y, given that all other sources of influence have been taken into account (Roebroeck et al., 2005). Temporal information from the data is thus used to define direction of influence without establishing a model of assumed regional connectivity. Our Granger Causality analysis was conducted at the group level to generate a t statistic image of the Granger Causality Map for each block. For each Granger map, p-values were subjected to a multiple-comparison correction (FDR(q) < .05; Genovese et al., 2002). Note that there is some debate in the literature concerning the validity of the Granger Causality method for measuring temporal precedence amongst functionally connected regions (see David et al., 2008). That is, owing to interregional variation in timing of the hemodynamic response, Granger Causality Mapping may determine temporal precedence between regions in which neuronal firing is instantaneously coupled. This limitation to the Granger method, however, is not relevant when examining differences in Granger Causality maps between two or more experimental conditions. This approach was taken in the current study.

**Children**—To evaluate differences in brain functional connectivity between task blocks, an analysis was performed comparing activity among the Winning, Losing, and Recovery blocks. Within the emotional go/no-go task, each block lasted approximately 4 minutes. To ensure that sufficient emotion was induced during our time of measurement, the first 30 seconds of each block were excluded from analysis. Therefore, taking into account individual differences in participant task duration, Block 1 (Winning) was defined as minutes .5–4, Block 2 (Losing) was defined as minutes 4.5–8, and Block 3 was defined as minutes 8.5–12.

The left amygdala activation that was found to be related to the presentation of faces across blocks was selected as a reference region in order to map sources of influence. A priori hypotheses led us to focus interest on the anterior cingulate/VMPFC. During Block 1, we did not observe significant effective connectivity between the left amygdala and anterior cingulate/VMPFC [t = .56, q > .05; see Figure 3]. However, in Block 2, we observed a striking increase in connectivity, indicating that activity in the VMPFC preceded the rise and fall of the left amygdala [t = .49, q < .05]. During Block 3, significant anterior cingulate

connectivity emerged as VMPFC connectivity increased [t = .43, q < .05]. Here again, activity in the anterior cingulate cortex and VMPFC preceded left amygdala activity.

**Adults**—For adults, each block lasted approximately 6.25 minutes. To ensure that sufficient emotion was induced during our time of measurement, the first minute of each block was excluded from analysis. Therefore, taking into account individual differences in subject task duration, Block 1 (Winning) was defined as minutes 1–6.25, Block 2 (Losing) was defined as minutes 7.25–12.35, and Block 3 was defined as minutes 13.35–18.70.

Here, the right amygdala activation related to presentation of faces across blocks was selected as a reference region to map sources of effective connectivity. During Block 1, like in our child sample, we did not observe significant effective connectivity between the amygdala and anterior cingulate cortex or any other regions (see Figure 3). However, in Block 2, we observed a striking increase in connectivity, indicating dominant influence of the anterior cingulate cortex/ VMPFC on the amygdala. During Block 3, significant anterior cingulate cortex connectivity remained, but did not increase like in our child sample. Activity in the anterior cingulate cortex preceded amygdala activity.

#### **Effective Connectivity Correlations with Age**

Finally, in order to test the effects of age on prefrontal to amygdala connectivity during emotion regulation, Granger causality maps were computed at the individual subject level for all children in our study during the recovery period (Block 3). An ANCOVA was then computed on these maps with child age (in months) as a correlate to identify areas of the prefrontal cortex in which connectivity to the amygdala increased with age. We identified a large section of the anterior cingulate/inferior frontal gyrus (BA 32, 47; 657 voxels) in which preceding influence of the PFC on the amygdala increased as children in our cross-sectional sample aged r(18) = .59, p = .006 (Figure 4).

### **Discussion**

Our study provides additional support for earlier models of brain mechanisms for "hot" executive functioning by demonstrating decreased amygdala activation coupled with increased VMPFC activation in response to emotional challenges. Amygdala responses to fearful faces varied as a function of emotion induction across the three blocks of our task. Although adults and children cannot be compared directly, children demonstrated amygdala suppression during the recovery period while adults demonstrated amygdala hyperactivity. Effective connectivity analyses found heightened coupling of the amygdala and frontal cortical regions during episodes of increased regulation in response to task demands. For children, this effect greatly increased during the recovery block, but for adults, this effect was maintained from the losing to recovery periods. Further, this effective connectivity in a key region of the PFC increased with age in our child sample.

Confirming our first hypothesis regarding increased amygdala activation to fearful faces during episodes of negative emotion and replicating and extending the findings of previous studies (e.g. Ochsner et al., 2002), we found differing amygdala activation to transient, but salient, emotional stimuli in relation to longer periods of induced emotion. Although we found equivalent right FFG responses to fearful faces across all three blocks of the task, left amygdala activation varied throughout. During Block 1, while subjects were steadily gaining points and likely experiencing positive emotions, the left amygdala was responsive to fearful faces. In Block 2, task difficulty increased, and participants lost all previously earned points. The left amygdala remained active in response to the presentation of faces during this task. Finally, when task difficulty returned to the more generous Block 1 levels, the amygdala response was depressed in children, likely reflecting down-regulation of the amygdala

driven by increased activity in frontal regions, but increased in adults. It is interesting to note that although Blocks 1 and 3 were equal in terms of task difficulty related to executive function, emotional context differentiated these episodes. During Block 3, participants had recently experienced frustration during point loss and were unsure that they would win their desired prize. The increase in frontal activation in Block 2, due to increased task difficulty or a tense/nervous emotional state, did not suppress left amygdala activation. However, although Block 3 provided the same level of task difficulty as Block 1, prefrontal regions increased activity and left amygdala activation returned to baseline in children. This effect may have been driven by increased demand for emotion regulation in order to win a desired prize or to better regulation due to greater expectation of success after the losing period. We also found that ACC activation was at peak during all three blocks, likely relating to the challenge of the executive function task (Carter et al., 1998). Notably, while amygdala activation to faces was suppressed in Block 3 for children, it was heighted in our adult participants. While this difference may reflect age related changes in amygdala function (Todd et al., in press), children may have also experienced differences in task motivation. Finally, this differential response could reflect differential neural mechanisms for regulation of emotion during frustrating episodes. Further studies will be needed to disentangle these possibilities.

Extending prior findings, our study investigated a second hypothesis of increased functional brain connectivity as a mechanism for "hot" executive function in children. Using a relatively new method for quantifying effective connectivity to and from the amygdala (Granger Causality; Roebroeck et al., 2005), we found that anterior cingulate activity immediately preceded left amygdala activation, but only during episodes in which participants were expected to regulate emotion, confirming our second hypothesis. During Block 1, we did not observe effective connectivity between the left amygdala and anterior cingulate cortex in adults or children. In response to the change in task difficulty and loss of desired points during Block 2, the connectivity analysis revealed section of the anterior cingulate/VMPFC in which activation immediately preceded the left amygdala response to emotional faces for subjects in both age groups. Finally, in Block 3, task demand lessened to Block 1 levels, but the anterior cingulate cortex connectivity increased greatly, coupled with a dampening in the left amygdala's response to faces in children, but not in adults. These changes in connectivity between the amygdala and anterior cingulate cortex occurred independently of general anterior cingulate activation, which was equal across blocks or VMPFC activation which was greatest in Block 2. The anterior cingulate is hypothesized to be specialized for performance monitoring and error detection (Carter et al., 1998), but also important in the processing and top-down regulation of emotion (Bush, Luu, & Posner, 2000). Therefore, increased anterior cingulate → amygdala connectivity in Block 2 was not likely due to increased task demand, given that anterior cingulate activation was equal across blocks in the 2 seconds preceding face presentation, but to changes in the need to regulate emotion. In following, Block 3 demonstrated increased anterior cingulate  $\rightarrow$ amygdala connectivity despite task difficulty and error rate being equal to that of Block 1, consistent with regulatory demand being higher during point recovery. Increased effective anterior cingulate cortex connectivity in Block 3 may represent regulation of emotional brain regions during negative mood, in comparison to the positive mood induced in Block 1. Alternatively, Whalen and colleagues (e.g. Whalen, 1998) have proposed that the amygdala is highly sensitive to the presence of ambiguity. Thus, it may be that increased connectivity in Block 3 represents prefrontal regulation of the amygdala under conditions of uncertainty (i.e. uncertainty due to possible loss of the prize).

Finally, in confirmation of our third hypothesis and the hypotheses of Bunge & Zelazo (2006) that prefrontal connectivity should increase with increasing executive function over development, we found that children differed in anterior cingulate  $\rightarrow$  amygdala effective

connectivity from adults, but also as a function of age across middle childhood. During Block 3, an episode in which participants are hypothesized to be regulating emotion while recovering from a negative episode, anterior cingulate → amygdala connectivity greatly increased for child participants, but remained constant for adults. Again, this may reflect differential regulatory brain changes in adults and children in response to this specific task or even more defined localization of PFC modulation of the amygdala. Notably, during Block 3, child anterior cingulate/inferior frontal gyrus → amygdala connectivity increased with age from a negative to a positive value around the age of 9 years. This may reflect more precise use of this region to regulate the activity of the amygdala, which may increase in precision and decrease in area during adolescence. Along these lines, a study in our own laboratory (Perlman & Pelphrey, in press), found that use of specific regions within the anterior cingulate, related to effective and ineffective modulation of emotion also changed with age. Future studies should be completed to examine this possibility.

Although we found evidence that effective connectivity from the anterior cingulate to the amygdala may underlie aspects of the development of "hot" executive functioning abilities in young children, our conclusions are tempered by a limitation to the current study. The construct of emotion regulation is a widely debated topic in the field of child development. There is general disagreement on the definition of this construct, the nature of its development, and how to separate regulation from aspects of general affective reactivity (Cole et al., 2004). Our methodology was aimed to put children in a real life situation in which they are typically required to regulate emotion: the loss of a desirable prize. While commendable in that it is difficult to mimic ecologically valid emotional situations within the confines of the scanning environment, this method also has some drawbacks. Specifically, it is difficult to pinpoint exactly when and how children were regulating emotion during this task. Although the brain and participant report data is suggestive of the engagement of regulatory mechanisms during our go/no-go task, without the explicit instruction to regulate emotion we cannot be sure that changes in activation and connectivity between Block 1 to Block 3 are due to increased regulation or persistent reactivity of negative emotions from Block 2. We are, therefore, somewhat limited in the interpretation of our results. However, we believe that such a complicated theoretical debate (Campos, Frankel, & Camras, 2004; Cole et al., 2004) requires a variety of approaches to better characterize the nature of the neural mechanisms involved in affective self regulation. In the future, experiments will be needed to better assess the specific regulatory strategies employed during implicit emotional tasks.

In conclusion, our study employed a novel emotion regulation paradigm to relate previous findings of variability in the amygdala and prefrontal cortex reactivity during emotion regulation to school-age children, but we also demonstrated a relationship between modulation of induced mood and anterior cingulate → amygdala connectivity. Our study implicates increased connectivity as a neural mechanism for the development of affective self regulation. Given previous research relating abnormal functional connectivity to cognitive deficits in disorders such as Autism (Just, Cherkassky, Keller, & Minshew, 2004), Schizophrenia (Meyer-Lindenberg et al., 2001), Bipolar Disorder (Almeida, Versace, Mechelli, Hassel, Quevedo, Kupfer, et al., 2009), and Alzheimer's Disease (Grady, Furey, Pietrini, Horwitz, & Rapoport, 2001) our findings have the potential to inform our understanding of regulatory brain deficits in childhood and adulthood emotional disorders by noting normative developmental changes in connectivity between these regions.

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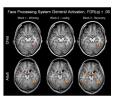
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### Figure 1.

A graphic representation of our go/no-go task. The paradigm was 12 minutes in length, for children, with 3 mood induction blocks and sporadic fearful faces presented as 1 second events



**Figure 2.**Regions of the face processing system active to the 1 second presentation of faces for each emotion induction block. Fusiform gyrus activity remained equal across blocks, but amygdala activity varied according to task demand for both adults and children.

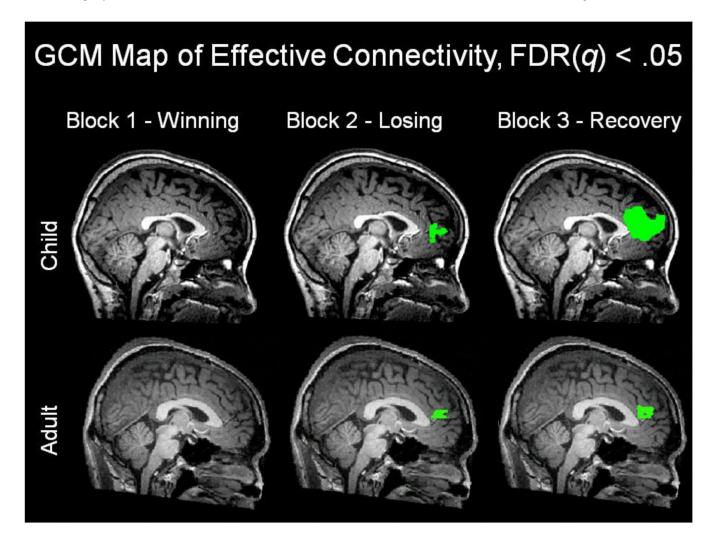


Figure 3. Granger causality mapping was employed to estimate differences in effective connectivity across blocks. Green represents active areas preceding left amygdala activity in each block. Peak anterior cingulate cortex connectivity voxel for children (Block 3: BA 32, x = -6, y = 38, z = 13).

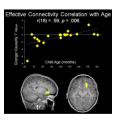


Figure 4.

Effective Connectivity from the anterior cingulated cortex/ inferior frontal gyrus to the left amygdala increased with age in children. Peak anterior cingulated cortex/inferior frontal gyrus connectivity voxel (Block 3: BA 32/47, x = 23, y = 31, z = -3).

# Table 1 Stimulus presentation and point adjustment algorithm

Stimulus presentation time and point adjustment algorithm for each of three emotion induction blocks in the child paradigm. Participants won points during Block 1, but then lost all earnings in Block 2. During Block 3, which had the same task difficulty level as Block 1, participants regained their points to win their desired prize.

	Block 1 - Winning	Block 2 - Losing	Block 3 - Recovery
Stimulus Presentation	80–1150ms	600–950ms	80–1150ms
Correct No-Go Response	-20ms	-50ms	-20ms
Incorrect No-Go Response	+70ms	+50ms	+70ms
Points for Go Trial (correct/incorrect)	+3/0	0/-15	+15/+1
Points for No-Go Trial (correct/incorrect	+7/0	0/-15	+25/+1