FUNCTIONAL DEVELOPMENT OF AMYGDALAE AND ANTERIOR CINGULATE CORTEX IN EMOTION PROCESSING

By

Yuwen Hung

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Department of Psychology
University of Toronto

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Abstract

Emotion processing involves specialised brain regions allowing for effective evaluation of the social environment and for the acquisition of social skills that emerge over childhood. In humans, an important aspect of normal development is the ability to understand the facial expressions of others that signal the nature and safety of the environment. Existing functional data, however, have not characterised the developmental trajectories associated with the differing neural and cognitive-behavioural development. The current thesis investigates the functional specialisation and development of the spatial and temporal patterns in neural activities during implicit processing of facial emotions from early childhood through adulthood. The first study identified brain regions engaged in implicit processing of emotional expressions using a simple emotion-processing paradigm (target detection task) with fourteen healthy adults using magnetoencephalography (MEG) recordings.
Participants responded to a non-face target (a scrambled pattern) while ignoring the emotional face presented in a different hemifield. Results showed ACC and right-lateralised amygdala activations in early latencies in response to the unattended emotional faces related to rapid and implicit attention to the task-irrelevant facial emotions, specifically during the processing of the fearful emotion.

Based on the findings in the first study, the second study investigated the developmental patterns and age-related differences in brain activities associated with the rapid and automatic processing of the emotional expressions in MEG with twelve children 7–10 years old, twelve adolescents 12–15 years old and twelve young adults (mean age 24.4 years) using the same paradigm. The results showed that emotion processing developed early in childhood in the amygdalae, whereas the processing of fear had later maturation engaging the ACC. The results further demonstrated an age-correlated increase in development in ACC activity and an age-related laterality shift in the amygdalae related to fear processing.

The present thesis provides new evidence contributing to the understanding of the protracted but differing normal development in the emotional brain over the childhood into adulthood, and offers critical insights into understanding possible dysfunctions of these brain regions during development.
Acknowledgement

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Chapter 1: Introduction

1.1 The development of processing of emotions in faces

The ability to effectively assess one’s social environment, taking account of another’s emotional status, is crucial for successful social communication and emotional development (Cunningham and Odom, 1986). An important component of these abilities during social interaction is the processing of facial expressions due to their significant role in appropriate modulation of social behaviours (Philippot and Feldman, 1990; Vicari et al., 2000). Faces are well-validated and acknowledged representations of emotional states and signals (Ekman and Friesen, 1976; Izard, 1971). The understanding of the normal development of emotional processing skills and their underlying associated neural systems can contribute to the understanding of emerging patterns of aberrant emotional behaviour and facilitate early identification of and appropriate therapeutic intervention for them.

Children's capacity to evaluate and distinguish facial emotions is present from infancy. Studies measuring visual preference and habituation recovery have found that, as early as 3 months, infants can visually discriminate among several facial expressions of adults including happiness, anger, fear, surprise, sadness and disgust when they are shown pictures of these emotional faces (Labarbera et al., 1976; Schwartz et al., 1985; Young-Browne et al., 1977). Infants are able to discriminate happy and sad faces from surprised faces and also to discriminate between different intensities, for example, mild versus intense happy expressions (Nelson and De Haan, 1997). Furthermore, there is evidence showing infants are able to form categories of happy expressions (Barrera and Maurer, 1981; Striano et al., 2002) earlier than they are able to form categories of other expressions such as fear (Grossmann, 2010; Nelson and Dolgin, 1985).

Although emotional expressions can be distinguished as early as infancy, the ability to recognise different expressions remains rudimentary throughout early childhood; it follows a slow and protracted developmental course that continues into adolescence.
Preschoolers as young as 2.5 years of age are able to use some emotion labels to categorize different facial emotions (Widen and Russel, 2010) and the recognition and decoding of emotional expressions improves with age through the preschool years (Boyatzis, Chazan, and Ting, 1993; Philippot and Feldman, 1990).

In children at school age (> 6 yrs) recognition of facial expression becomes more rapid and is associated with a general increase in efficiency in encoding the faces (Chung and Thomson, 1995; De Sonneville et al., 2002). In a task that required children to match photographs of facial expressions with emotion, Kolb et al. (1992) demonstrated that improvement in facial expression recognition continues between the age of 10 and 14 years. Tonks et al. (2007) investigated the perception of facial expression under a variety of task demands, including affect discrimination, selection, matching and naming, and identity discrimination (control task) in children aged 9 – 15 years. Children under age 11 made significantly more errors than children aged over 12 years across tasks. These developmental changes are close to the periods of maturation associated with brain growth spurts and Piagetian periods of cognitive development (Kolb and Whishaw, 2003).

Studies have shown that the abilities of recognising and discriminating different emotions do not emerge at one specific stage or age, but they have differing developmental trajectories; maturation of the processing of some emotions precedes others (Camras and Allison, 1985; Gross and Ballif, 1991; Smith and Walden, 1998; Vicari et al., 2000). Happy faces appear to be the easiest for young children to discriminate and the high accuracy of this emotion shows little change with age. The ability to recognise negative emotions appears to develop later (Izard, 1971; Labarbera et al., 1976; Markham and Adams, 1992; Vicari et al., 2000) and follows a more protracted course that continues throughout childhood and early adolescence. Preschoolers’ explicit recognition of emotional expressions emerge gradually over development with age, with positive emotions such as happiness learned earliest and with the greatest accuracy (Boyatzis et al., 1993; Camras and Allison, 1985;
Michalson and Lewis, 1985), followed by negative emotions such as sad or angry expressions, then lastly by the fearful emotion (Camras and Allison, 1985).

Following the pre-school period, Vicari et al. (2000) measured emotional facial recognition (matching and memory) in 120 school-age children between age 5 to 10 years using emotions of happiness, surprise, sadness, anger, disgust and fear. They reported that, overall, children's performance improved with age for each emotion; the performance for happiness was the highest and changes for this affect across age were the least among all emotions tested. Furthermore, they found that the age-related improvement was especially evident for negative emotions including sadness, anger and fear (Vicari et al., 2000). Another study also found that from 7 to 10 years of age, children's recognition of emotional expression increased significantly, and this improvement was particularly evident for negative emotions (De Sonneville et al., 2002). However, Herba et al. (2006), using emotion-matching tasks, showed that the recognition of emotional faces in healthy children between age 4 and 15 improved with age and was particularly sensitive to happy and fearful emotions.

The increasingly complex social interaction in later childhood may place greater demands upon the brain systems responsible for the sensitivity to certain emotional expressions, such as the negative emotions. It can be postulated that the developmental pattern may be closely related to the development of higher cognitive skills associated with growth of the pre-frontal cortex (Tonks et al., 2007).

1.2 Structural brain development

Over the past decade, normal brain development has been well described by magnetic resonance imaging (MRI) research. Although total brain size is largely mature by five years of age, white matter volume continues to increase significantly from childhood to adulthood, whereas grey matter volume appears to decrease over this period (Durston et al., 2001).

During childhood and adolescence, the signal intensity of white matter increases with age in regions such as the ventral visual pathways, the prefrontal area, basal ganglia
and thalamic pathways, regions that are important for attention, emotion and motor development (Barnea-Goraly, et al., 2005). Cross-sectional studies have identified linear decreases in cortical gray matter and increases in white matter across ages 4 to 20 (Giedd et al., 1996; Reiss et al., 1996). A longitudinal MRI study of brain development has also observed these patterns: grey matter volume increased in the frontal and parietal lobes at preadolescent period, peaking around age 12, followed by a post-adolescent decrease in these two areas; the temporal lobes peaked about age 16, and the occipital areas continued to increase through age 20 (Giedd et al., 1999).

Relative to other brain areas, the frontal lobes appear to have a longer and more protracted development that continues into the third decade of life. White matter myelination follows inferior-to-superior and posterior-to-anterior progression in the brain and the frontal lobes are the last to myelinate (Yakovlev and Lecours, 1967). The volume of the prefrontal lobes increases slowly until 8 years of age and then undergoes rapid growth between 8 and 14 years (Kanemura et al., 2003). Sowell et al. (2001) reported a decrease of gray matter density over the frontal and parietal lobes between childhood (7–11 years) and adolescence (12–16 years), which was accelerated further – but only in the frontal cortex – from adolescence to adulthood (23–30 years). Figure 1 shows the observations summarized by Casey et al. (2005b) of the later and differential development of the prefrontal cortex relative to other brain areas, from data including proliferation and migration of cells (Jacobson, 1991; Rabinowicz, 1986), regional changes in synaptic density (Bourgeois et al., 1994; Huttenlocher, 1979; Huttenlocher, 1990) and protracted development of myelination into adulthood (Yakovlev and Lecours, 1967).
The data demonstrate that different brain areas differ in their time course of neural maturation over development. The sensory and motor areas of the brain mature relatively early in life, whereas the higher-order association regions such as the prefrontal cortices, critical for cognitive functions, mature later, throughout childhood and adolescence (Casey, et al., 2005a; Tsujimoto et al., 2008). In a study combining longitudinal and cross-sectional data that assessed changes in cortical thickness from 764 neuroanatomic MRIs acquired from 375 typically developing children and young adults aged from 3.5 to 33 years old, differing levels of cortical growth across different brain areas were found (Shaw et al., 2008). Cortical regions, including limbic areas, show predominantly simple and linear growth trajectories, where the peak development is achieved early in life, followed by a gradual decline in cortical thickness into adulthood. In contrast, polysensory and high-order association areas of the cortex have more complex developmental trajectories. Development in the lateral frontal, temporal, parietal and occipital cortical regions follows a cubic growth curve, with an increase in early childhood that reaches a peak in development at around 10 years old, followed by a decline in adolescence and stabilization in adulthood. The development of the anterior cingulate areas follow a quadratic curve, showing a stable increase and then decrease, with the peak in development occurring from the
adolescence period into early adulthood (after 10 years of age and continuing through adolescence) (Shaw 2008) (Figure 2).

**Figure 2:** Developmental trajectories of cortical thickness within divisions of the cerebral cortex.

The medial view of the brain map shows that most of the lateral frontal and parietal regions have a cubic (in red) developmental trajectory, with a period of initial childhood increase followed by adolescent decline and then stabilization. The graphs show the growth patterns for each of these divisions. Growth in the anterior cingulate and insula areas are characterised by a quadratic (in green) pattern with increase and decrease within the first three decades of life and lacking the phase of stabilization. A linear trajectory is seen in areas including the medial temporal cortex, posterior orbitofrontal, frontal operculum and medial occipitotemporal cortex. Adapted from Shaw et al. (2008), reprinted with permission of the Society for Neuroscience.

It has been suggested that changes in the structure of the brain are paralleled by increases in skill acquisition during development (Durston et al., 2006, Johnson, 2005). To measure how changes in brain activity reflect behavioural changes during learning and development, functional neuroimaging research has provided an opportunity to track cognitive and neural processes underlying cognitive maturation.
1.3 Electrophysiological and neural magnetic measures

Historically, research in cognitive development has relied on the measurement of observable behaviour; the efforts to link behavioural changes related to cognitive development to changes in brain development are more recent. With technological advances in the past three decades, a number of brain imaging methods are available for examination of brain structure and function. These technologies have provided a number of refined or new methods for imaging brain anatomy and ongoing brain activity in human subjects, including the classic electroencephalography (EEG), specifically event-related potentials (ERPs), structural and functional magnetic resonance imaging (MRI and fMRI), magnetoencephalography (MEG) and others such as computed tomography (CT), positron emission tomography (PET) and single photon emission computed tomography (SPECT).

Neuroimaging research on the developing brain emerged most rapidly with the ready availability of non-invasive techniques that could be used with young populations. This section introduces the neurophysiological measures of ERPs and EEG and particularly the neural magnetic recording of the magnetoencephalography (MEG), as well as relevant task designs important for developmental research and the current thesis studies. Findings from emotional face studies are described to illustrate the range of questions that can be addressed using these neuroimaging methods; this section does not attempt to review the large amount of literature involving all methods employed in developmental research.

1.3.1 The electroencephalogram (EEG) and event-related potentials (ERPs)

Neurophysiological measures are readily used in infants and children and provide information about the brain areas implicated in cognitive function and, more importantly, precise temporal information about the neural activity related to the
cognitive tasks. The most widely used methods in developmental studies are the electroencephalogram (EEG) and event-related potentials (ERPs) (Taylor and Baldeweg, 2002). The EEG is the more traditional brain imaging technique and remains a useful tool in developmental science with its non-invasive and painless recording of brain electrical activity measured from the scalp surface. The ease of recording and relatively robust signal makes this method preferable for children and infants, for whom the demands of other imaging techniques may be too great (e.g. PET, which requires exposure to radiation, or MRI, where head motion degrades the measurable signal). EEG measures ongoing electrical activity of the brain recorded from electrodes placed on the scalp. It can be used to assess brain maturation as EEG patterns change with age and reflect cortical development. EEG is also widely used in clinical settings, as it is a critical investigation tool for the assessment of epilepsy, the most common reason for referral to neurology in childhood.

By presenting subjects with repeating stimuli and averaging the EEG responses to the stimuli, averaged and time-locked waveforms are created, referred to as ERPs, that reflect the neural responses to a particular stimulus or response event. The recorded ERPs reflect the discharges from large populations of cortical neurons responding to the presented sensory stimuli associated with specific aspects of sensory and cognitive processing. ERP data have excellent temporal resolution, which allows mapping of the precise time course of brain activity within milliseconds after the onset of the presentation of a stimulus. ERPs are easily recorded in children and are valuable in studying a range of cognitive processes in children (Friedman, 1991; Taylor, 1995).

ERP research has shown neurophysiological responses associated with the emotional face processing in children at latencies within 300 ms (P1 and N/M170) where young children’s responses are observed that are somewhat similar to adults in form and sensitivities, albeit at much longer latencies; significant age-related changes in latencies and amplitudes occur in childhood and into adolescence (Taylor et al., 2001; 2004). Young children (4-7 years of age) showed latency effects at the early component P1, at around 110 ms, associated with implicit processing of emotions. The P1 was delayed in response to fearful and angry faces and had shorter latencies
for happy faces. This effect was not seen in older children. The adult findings of emotional sensitivities on the N170 were only seen in adolescents at 14-15 years of age (Batty & Taylor, 2006). These age-related emotion effects suggest that the processing of emotions in faces evolves gradually, with distinct types of processing emerging at different developmental periods.

In addition, it was also found that ERP responses in 7-month-old infants (at around 400 ms) can differentiate emotional from the neutral faces, and happy from fearful faces (Nelson & de Haan, 1996). These later-peaking sensory and cognitive components show developmental changes in terms of amplitude, latency of the peak, and overall pattern until early adolescence or later (Courchesne, 1978; Thomas & Nelson, 1996).

It was demonstrated that the assessment of infants' ERPs in facial recognition was more sensitive than the traditional behavioural measure of looking time. For example, it was found that 6-month-olds could recognise their mothers’ pictures; ERPs showed discrimination between their own mother's face and the face of another child's mother (de Haan & Nelson, 1997). Adult ERP research has demonstrated that the face-sensitive component (N170, a negative component peaking around 170 ms) is largest at posterior temporal leads to various types of facial stimuli (Bentin et al., 1996; George et al., 1996). This activation has been well verified through fMRI and intracranial studies (e.g., Allison et al., 1994; Puce et al., 1996). This component was not found in 6-month old infants in response to human faces but a later positive peak was sensitive to the species of the face (de Haan & Nelson, 1999) and at 12 months was also sensitive to face inversion, suggesting gradual specialisation of cortical face processing systems during postnatal development (de Haan et al., 2002).

Taylor et al. (1999) first demonstrated that the N170 was sensitive to faces in children (from 4 years of age), as seen in adults, with gradual, but marked latency and distributional changes found with age. The latencies and amplitudes of ERP components change with age, reflecting changes in cognitive processes, particularly over the childhood period when important cognitive and behavioural changes occur.
Some of the earlier components reach adult latencies in early childhood, while others continue to decrease in latency until adulthood. Amplitudes, which traditionally are thought to reflect neuronal recruitment, usually change throughout childhood, reflecting the increasing efficiency of perceptual and cognitive processes with age.

These studies illustrate that ERPs can provide critical information on cognitive processes in children and infants, and when and how various aspects of visual processing and recognition develop. They demonstrate that emotional face processing in childhood shows a prolonged maturational course before reaching adult maturity and specialisation. A weakness in the ERP studies, however, is in their poor spatial localisation. Although some studies suggest that face processing implicates different posterior ventral sources in childhood (e.g., Taylor et al., 2004), source localisation is considerably more accurate with MEG.

1.3.2 The magnetoencephalography (MEG)

Research in magnetoencephalography (MEG) emerged as a valuable tool in functional neuroimaging of the brain and has largely grown in recent years. Clinicians and researchers have successfully used MEG to localise pathological, active regions in neurological disease and assess patterns of neuronal activity engaged in various cognitive processes in both patient and healthy populations (Stam 2010). MEG provides complementary information to other neuroimaging methods such as the EEG/ERP and fMRI, as it is able to provide precise localisation of neural activity and yield important applications for clinical use (Taylor et al., 2008). Using novel and sophisticated source analysis techniques, MEG has led to exciting new insights into the neuroimaging field, as it has accurate spatial localisation favorably comparable with that of fMRI, and, unlike the fMRI, offers excellent real-time temporal resolution that captures fast neuronal firings with rapid-changing neuromagnetic fields in milliseconds, permitting more direct measures of neural
activity. Thus, MEG has proven a most valuable tool for research in neurocognitive processes and in clinical settings (Williams & Sachdev, 2010).

MEG is the noninvasive recording of magnetic fields generated from the electromagnetic activity within the human brain. It was pioneered by Cohen (1968), who demonstrated the viability of the noninvasive recording of MEG brain signals, although only one sensor was used. Recent technological advances have enabled MEG scientists to build systems allowing for recording of magnetic fields over the whole head and sophisticated data analyses. Modern MEG systems have numbers of recording sensors up to 300 channels, covering the whole head, which improves the sensitivity of localisation.

MEG signals are recorded over the scalp from synchronous activity of populations (tens of thousands) of neurons and reflect currents generated by postsynaptic potentials (PSPs) in neurons at dendrites (Sato et al., 1991). Detection of the weak MEG signal is only made possible with the superconducting quantum interference devices (SQUIDs), which are extremely sensitive to rapidly-changing magnetic fields. In order to super-conduct, SQUIDs must be maintained at extremely low temperatures, typically around 4°K in liquid helium baths. Because neuromagnetic signals are very weak – on the order 1/10^{12} the strength of the earth’s static field – MEG recording must be carried out in rooms that have been carefully shielded from external magnetic sources. MEG measurement is highly sensitive to currents tangential to the skull, which originate in the cortical sulci. MEG and EEG recordings differ in terms of signal source. EEG measures cerebral electrical potentials at the scalp, with preference for radially oriented cortical sources (perpendicular to the surface of the skull and scalp) (reviewed in Sato et al., 1991; see also, Lopes da Silva, 1991), and thus it primarily measures extracellular volume potentials and not the primary currents. In contrast, MEG measures mostly tangential currents that generate magnetic fields perpendicularly through the skull, mainly those produced by post synaptic pyramidal cells within the cortical sulci that contain approximately two thirds of cortical neurons, and is less sensitive to radially oriented sources. Unlike EEG, MEG fields directly reflect the primary neuronal currents with minimum
distortion from different layers such as brain tissue, skull, and scalp. Unlike PET or fMRI that measure the slow blood-flow based activity in the brain usually at the order of seconds and greater, MEG and EEG detect neuronal discharges in the order of milliseconds. The MEG results are best visualized on anatomical MRIs, which require bringing the two modalities into the same coordinate system. Pre-processing techniques such as data filtering, noise detection and removal, and data averaging in time and space are often applied to improve the signal-to-noise ratio of MEG. These all facilitate MEG’s excellent sensitivity to detecting brain activity.

Source localisation and modeling

The neuronal signals recorded by MEG are obtained from the scalp, and there is no unique solution to the problem of reconstructing the exact location of the sources of the signals within the brain. This limitation is referred to as the inverse problem. The standard procedure to overcome the inverse problem in MEG is to introduce constraints on the possible solutions in order to exclude improbable solutions, leaving the one that is most suitable to describe the data. Therefore the functional localisation of brain sources of MEG signals depends to some degree on the models used and the corresponding assumptions. In what follows, some of the means of completing source analyses in MEG, and the issues related to these analyses are presented.

Electrical current flowing over a short distance in space, such as those generated in neurons in the brain, can be modeled as individual current elements or "dipoles". Neurons receive excitatory or inhibitory synaptic input at the dendrites. This electrical input is sent via electric conduction to the soma for integration. Each dendritic process thus creates small intracellular currents. The magnetic field resulting from synchronous dendritic post-synaptic potentials (PSPs) summate to produce a global magnetic signal that can be measured outside the head, given the cortical neurons have their main dendritic arbores organized in a parallel manner. The small individual dipoles can be represented by their total sum, which is often referred to as the "equivalent current dipole (ECD)" model. It is noted that action potentials traveling along the axons do not contribute significantly to the MEG signal
because of the nature of axonal conduction. An action potential in the axon creates a wave of depolarization, which is immediately followed by a wave of repolarisation that propagates down the axon. Magnetic fields created by these adjacent polarizations are opposing and thus result in minimal magnetic fields measured at a distance. Therefore MEG measures mainly the intracellular PSPs traveling along the dendrites of approximately 10,000 to 100,000 synchronously active pyramidal neurons (Hamalainen et al., 1993).

The ECD is historically the most commonly used model for brain source activity. It is based on the assumption that activation of a specific cortical region involves populations of functionally interconnected neurons within a relatively small area (Nunez, 1981). Given that the currents generated by these neuronal populations are flowing inside the head, which is roughly in spherical shape, the source of the brain's magnetic field can be mathematically modeled as dipoles in a sphere. However, it is more likely that neural activity is like a sheet of distributed dipoles (Baillet et al., 2001). Thus, dipole models may not be appropriate representations of the data in some instances. Traditionally, ECD analysis proceeds by estimating \textit{a priori} the number of equivalent dipoles and their approximate locations, and then adjusting the dipole parameters, such as the location and orientation, by a nonlinear search that minimizes differences between the fields computed from the dipole model and the measured fields. This procedure has proven useful for modeling simple configurations of focal brain activity, but is compromised by interactions between closely spaced and unknown sources that may produce incorrect solutions. Overlapping fields generated from spatially adjacent or simultaneously active neural populations create different magnetic field distributions compared to the non-overlapping fields from single dipoles, which make it difficult to interpret the underlying MEG generators with multiple simultaneously active neural populations. Thus, localisation of the distributed and complex patterns of brain activity involved in higher cognitive functions that usually engage multiple active brain regions are prone to error; other means are needed to estimate the neural sources underlying MEG in cognitive tasks.
Distributed current models based on the minimum-norm method, first proposed by Hämäläinen and Ilmoniemi (1984), can overcome some of these limitations, by modeling extended continuous patterns of brain activity. Another advantage of such linear source estimation techniques is that they eliminate the need for performing nonlinear fitting of user-defined models to the data required by dipole modeling approaches. However, minimum-norm based methods are inherently biased toward solutions close to the sensors and thus require additional constraints to prevent biased estimates of source location, particularly in the presence of noise.

A spatial filtering approach is a recent technique introduced to the neuromagnetic source modeling literature since early 1990s, based on the array processing technique known as beamforming. The beamforming method reduces the influence of unknown sources by removing spatial correlations in the data that do not correspond to source activity at the location of interest. This technique can be used effectively as a method to scan through source space and produce accurate 3-D volumetric images of source activity. Various beamforming algorithms have been introduced in the last decade that have been successfully applied to imaging both focal and distributed brain activity without the requirement for a priori source models. More recent techniques based on the minimum-variance beamforming algorithm have shown great promise as flexible and accurate approaches to source localisation in MEG. Several recent studies have applied the synthetic aperture magnetometry (SAM) beamformer algorithm described by Robinson and Verba (1999) to the localisation of MEG activity (Robinson & Vrba, 1999; Seikihara et al., 2000). This method constructs optimal spatial filters with the output of a weighted sum of the recorded MEG signals; the advantage of this approach is that it does not require a priori assumptions regarding active sources. These methods are computationally simpler but highly data-dependent. Thus caution should be taken in the selection of data to avoid failure to accurately reconstruct all sources due to insufficient signal-to-noise or excessive source correlation (Brookes et al., 2007). However, even under these circumstances, beamforming methods tend to be biased toward false negatives and less likely to produce false solutions in comparison to conventional dipole modeling techniques,
and it has been demonstrated that beamformers performed better and have higher sensitivity than other methods (Darvas et al., 2004).

An event-related beamformer (ERB), which is also used in the current studies, estimates weights from one time interval across a broad frequency (Cheyne et al., 2006). The ERB applies the minimum-variance beamforming equations and a scalar beamforming approach (single rather than multiple orthogonal sources at each brain location) by searching for the optimal source orientation. These weights are then applied to the averaged MEG data to calculate the total source power at a given time point and voxel location. This ERB procedure derives 4-D images (volume by time) of the event-related activity. A normalized value such as the pseudo-Z value (Robinson and Vrba, 1999) is used to make volumetric images of source power throughout the brain.

**Data Analyses**

Most MEG studies to date have focused on evoked responses, which are activations occurring at the same time, phase-locked with respect to stimulus or task onset from trial to trial. Typically, MEG processes require averaging across multiple trials of neuromagnetic responses. Good or clean MEG data should be able to replicate between and within subjects. The earliest salient activations, for example, those of shortest latencies (e.g., within the first hundred milliseconds), are typically tightly locked to the stimulus and rapid (short-lasting), usually generated from primary sensory cortical areas (e.g., visual cortex and association cortices) or subcortical regions (e.g., limbic regions) in processing pathways. These early responses usually yield sharp responses even when averaged across multiple trials. Short latency responses are also seen in frontal regions (Bayle & Taylor, 2010) helping define rapid neural processing seen with salient cognitive stimuli. The longer-latency responses increase progressively in duration and exhibit more jitter in relation to the stimulus onset. With averaging, they appear as sustained responses with slow rise and fall times and are usually linked to higher cognitive processes.
When responses are averaged over many trials, consistent spatial-temporal effects will summate, and random neuromagnetic changes, presumably irrelevant to the task processes, tend to cancel each other. Thus, averaging can improve the signal-to-noise ratio and provide spatially and temporally consistent findings over trials. In order to group-average the beamformer images, transforming each subject's functional maps into a common anatomical space is necessary (Barnes and Hillebrand, 2003). A person’s head and brain structure are unique but relatively consistent across individuals of similar age. A template brain is defined as a common anatomical space; those most commonly used are the Talairach-Tourneoux (Talairach and Tournoux, 1988) or MNI (Montreal Neurological Institute; www.bic.mni.mcgill.ca) template brains, allowing comparisons across studies and labs. Each individual's structural data are normalized to the common anatomical space. The beamformer images are then normalized to the common space. Averaging and statistical analyses can then be applied on these normalized beamformer images.

Subtraction of responses between conditions is a common procedure in neuroimaging (fMRI, ERPs and MEG) for identifying underlying processes seen in the experimental but not the control condition. In the current studies, the subtraction approach was used: the ERB weights were calculated for each condition individually and then the rectified ERB outputs were subtracted. However, if the noise levels are different between conditions, this procedure may lead to finding false sources. Because beamformer outputs are estimates of the SNR, differences could result from signal or noise differences between conditions. Therefore one needs to ensure that the noise levels are similar across conditions. Generally, subtracting trial conditions within the same experimental block, such as used in the current study paradigm, instead of between blocks, also helps reduce the noise floor, thus increasing the sensitivity of this image subtraction method. Another method is to subtract the single-trial sensor data of condition B from condition A with equal number of trials per condition, and then calculate the ERB on the difference data. This ensures that the magnitude and phase differences between conditions remain in the data, and the ERB calculations are performed on the data set of the difference waveforms.
To obtain a confidence level or threshold that determines which source locations have activity greater than noise, statistical analyses of beamformer images need to be performed. To achieve this, a null distribution of the noise is estimated to determine if each beamformer voxel's magnitude exceeds a given threshold decided by the user-defined alpha level (e.g. P < 0.01 or 0.05). One way to estimate noise distribution is using permutation analyses (Chau et al., 2004). Comparing the means and variances of the beamformer output statistically within a baseline time interval (control) can also determine if significant differences exist in the noise levels between conditions. Values from the permutations across all voxels and participants can be used to construct the null distribution. The confidence levels are then estimated from this distribution and are used to threshold beamforming images. Voxels with beamformer outputs that exceed the threshold are then considered significant. For the ERB method, the null distribution can be calculated from the images of a prestimulus time interval. The assumption for this is that the prestimulus interval contains only noise, and therefore the poststimulus beamformer outputs images are considered significant if they exceed the threshold determined from the prestimulus noise distribution. Once the beamformer images are thresholded, locations of the maximal ERB output can be identified by finding the peaks that are spatially separated by a preset amount (e.g. 1-5 cm). These images can then be visualized using one of many commercial or custom software packages for visualizing functional and structural brain images. The identified peak location that has the highest probability of being the generator or source can then be used as a location to extract the beamformer-output time courses for single or averaged trial data. The time course data from the peak ERB locations can also be analysed. Further information regarding the details and applications of MEG and beamforming can be found in Herdman and Cheyne (2009), and Hansen et al. (2010).

The superior temporal-spatial resolution of MEG has allowed confirmation of brain sources from findings of the event-related potentials (ERPs), particularly in children, as the majority of existing MEG studies have focused on the adult populations and have only slowly extended this powerful technique to the understanding of neurocognitive development. Given the highly sensitive aspects of MEG, the
combination of proper instructions to reduce head movement, well-designed experimental tasks and careful analyses are critical for good-quality data and can facilitate data analyses.

With these techniques, MEG allows precise localisation of cortical and subcortical sources related to task performance. Previous MEG studies have demonstrated that MEG is capable of detecting evoked responses in deep grey matter areas, including the hippocampus (Moses et al., 2009; Riggs et al., 2009), thalamus (Rogers et al., 1991) and amygdala (Ioannides et al., 1995). The current studies used MEG and focused on providing precise timing information of neural activities. The paradigm and MEG techniques allowed us to compare processing between different trial types within each experimental block in the current thesis studies. We measured the event-related MEG brain activations that offer precise temporal information of 'on-line' brain function (Hari et al., 2000) and applied beamforming source analyses that allow precise localisation of activation sources (Cheyne et al., 2006) including deep brain areas within the limbic system (Quraan et al., 2011).

1.3.3 Selection and design of the task

The electrophysiological and neural magnetic measures are highly sensitive to aspects related to the perception of the stimulus, such as the stimulus size and colour, duration of stimulus presentation and its spatial position etc. Careful selection with the tasks has tremendous impact on the sensitivity of neuroimaging data to brain-behaviour relations. Furthermore, tasks used in adult studies may not be as sensitive and applicable to children. For example, tasks that require explicit processing of and response to emotions may produce diverse and confounded results in children due to the children’s varying verbal or visuo-spatial abilities (Phan et al., 2002; Vicari et al., 2000), their understanding of the emotions or other cognitive skills such as visual-motor coordination. On the other hand, in tasks of passive viewing of stimuli children
may well lose attentional focus and thus create task-irrelevant and unwanted noise in the data.

Using implicit tasks to approach children's cognitive and emotion processing has the advantage of reducing these confounding variables. A developmentally feasible approach is the use of implicit emotion-processing tasks, which generally require participants to attend to an emotion-irrelevant aspect of the presented face stimuli, such as facial identity, gender or location (Herba and Phillips, 2004; Lobaugh et al., 2006). If the stimuli are presented at a rapid rate, it would reduce secondary processing of the stimuli such as verbal elaboration, which may also help equate the task for use between adults and children.

It has been proposed that as attentional demand of tasks increases, the frontal cortical regions are recruited to a greater extent and the subcortical regions (including the amygdalae) to a decreasing extent (e.g., Lange et al., 2003; Phan et al., 2002), which may be related to a top-down cortical-to-subcortical inhibition. This gradient implicating cortical versus subcortical areas would differ however, in children, as brain regions have differing maturational development; frontal regions have the slowest and most protracted developmental course whereas the amygdalae have an earlier and more rapid neuroanatomical maturation (Casey et al., 2005a, b; Shaw et al., 2008; Taylor, 2006). These considerations would make the interpretation of attentionally demanding emotional tasks used across children and adults complex and difficult. Thus, as the level of the task demand to the emotional stimuli employed in children can have a significant impact on their performance and the patterns of brain responses, tasks with low attentional demand may be more suitable for studying neural processing in children.
Chapter 2: The development of amygdalae and anterior cingulate cortices in emotion processing

Animal, human lesion and neuroimaging studies have consistently implicated specific brain regions in emotion processing (Aggleton, 1992; Milner, 1980). The processing of emotions appears to engage two networks, a ventral and a dorsal network; the dorsal system includes the frontal and anterior cingulate regions, and the ventral system includes areas in the limbic regions (Phillips et al., 2003). As key components of the limbic system, the amygdalae in the medial temporal region are established as an emotional gateway in the filtering and identifying emotionally salient stimuli and the generation of emotional states. The frontal cortical areas, especially the anterior cingulate cortices (ACC) in the inferior, midline frontal areas of the brain, are important for cognitive-emotion processing and regulation of emotions and subsequent behaviour.

Structural findings have demonstrated a differential rate of maturation between the ventral and the dorsal brain systems implicated in emotion processing. The limbic system matures relatively early in life (Shaw et al., 2008) whereas the frontal system has a later and slower development that occurs throughout childhood, adolescence and into adulthood (Casey 2005a, b; Herba 2004; Kanemura 2003; Tsujimoto 2008). In this chapter I review evidence of the role of the amygdalae and ACC in emotion processing.

2.1 Specialisation of amygdalae in emotion processing

The amygdalae have been shown to play a key role in emotion processing (Breiter et al., 1996; Calder et al., 2001; LeDoux, 1996; Phan et al., 2002; Phillips et al., 1998, 2003; Wright et al., 2001). It has been well established that these structures are responsive to emotions, and particularly to fearful emotions, as expressed in faces (Cornwell et al., 2008; Davis and Whalen, 2001; LeDoux, 1996; Morris et al., 1996), eyes (Hardee et al., 2008; Whalen et al., 2004) or vocalisations (Davis and Whalen,
Morris et al. (1996) measured neural responses using positron-emission tomography (PET) in normal adults while subjects viewed photographs of fearful or happy faces. They found amygdala responses that were greater to the fearful than happy expressions. Cornwell et al. (2008) reported greater amygdala activity using MEG in healthy adults when subjects were performing a perceptual matching task of negative emotional faces compared with geometric shapes. In addition, lesions in the amygdalae can lead to deficits in the recognition of fearful facial expressions (Adolphs et al., 1994; Calder et al., 1996) and in fear conditioning (Bechara et al., 1995; LaBar et al., 1995).

Existing data have further revealed that the responses of the amygdalae to fearful faces are sensitive particularly when the faces were presented subliminally (Luo et al., 2009) or in the unattended peripheral visual fields (Ewbank et al., 2009).

In general, it appears that the right hemisphere has a greater role than the left in emotion processing (Gläscher et al., 2003; Noesselt et al., 2005). Research has demonstrated that the right amygdala is more sensitive than the left amygdala to implicit and automatic processing of emotional stimuli (Funayama et al., 2001; Morris et al., 1998, 1999; Whalen et al., 2004), whereas the left amygdala is linked to explicit and intentional, directed processing of emotional stimuli (Funayama et al., 2001; Hardee et al., 2008; Morris et al., 1996). This model of functional specialisation is supported by evidence of the right-lateralised amygdala activation related to implicit processing during masked processing of emotions in adults (Morris 1998), as well as findings of the left-lateralised amygdala activation in face matching tasks that explicitly compared and identified emotional faces (Cornwell et al., 2008).

Only a small number of neuroimaging studies have examined emotional processing in children. These studies have focussed predominantly on the nature of amygdala activity in response to fearful facial expressions (Baird et al., 1999; Thomas et al., 2001). These studies have yielded contradictory findings regarding the lateralisation of amygdala activity elicited by fear in faces and there is no coherent model to explain these discrepant findings.
Baird et al. (1999) studied twelve healthy adolescents aged 12 to 17 years old with fMRI using a task of facial affect recognition. The subjects were asked to either label the expression on the faces or to passively view the stimuli. Only fearful expressions were chosen in this study on the basis of previous work that showed amygdala-related responses to fearful faces (Adolphs et al., 1994, 1995); non-face stimuli were also used as the visual control. Their results showed a significant increase in the signal intensity in the amygdala regions in response to the recognition of the facial expression compared with viewing the fixation point. They did not find significant effects for the passive viewing of the emotional faces. They concluded that this was evidence that in the adolescent population the amygdalae activate during processing fearful faces. They only studied adolescents and did not explore how age might affect the amygdala responses across the age range included.

Thomas et al. (2001) compared children with mean age of 11 years and adults on amygdala responses in fMRI during passive viewing of fearful and neutral faces. They found amygdala activation during the presentation of the fearful faces relative to fixation in both children and adults. However, while the adults showed greater activity for the fearful faces, the children showed decreased activity for the fearful faces relative to the neutral expressions. The authors interpreted this finding as indicating differences in the significance of the expressions across age. While the fearful faces are more ambiguous and biologically relevant to adults, the neutral faces may be perceived as more ambiguous than the fearful faces to children. Children may read the emotional signals as a cue to the safety and nature of a new environment in a way that may be different from that of adults. Thomas et al. (2001) suggested for future research that investigators consider tasks that are unrelated to the emotional valence of the stimulus to avoid possible regulatory processes in children, such as studying the implicit processing of emotions.

Lobaugh et al. (2006) studied a group of 10 to 12 year-old children using an implicit face-processing task in which children made decisions based on the emotion-irrelevant aspects of the facial stimuli (gender discrimination). Six emotions were included in the study (disgust, fear, anger, sadness, surprise and happiness). They
found right amygdala dominance related to the implicit discrimination of the fearful and disgustful faces, which is consistent with findings in adults, and they concluded that the neural networks for emotion specific processing are present before adolescence.

In a recent fMRI study, Todd et al. (2011) examined bilateral amygdala activity in young children 4 – 8 years of age during processing of happy and angry faces. They found greater amygdala responses in children to happy faces than to the negative, angry faces, compared to the adults. The adults, in contrast, showed larger activation to the angry faces than to the happy ones. They also observed that responses to the negative emotion (angry) but not the happy ones increased and had a linear relation with age (Todd et al., 2011).

2.2 Emotion processing in the anterior cingulate cortices

The anterior cingulate cortex (ACC), located on the medial surface between two hemispheres, is a region of primitive prefrontal cortex that has strong links to the amygdalae and to other prefrontal regions. This area sometimes co-activates with the amygdala in emotion processing (Baird et al., 1999; Thomas et al., 2001). A meta-analysis conducted by Phan et al. (2002) summarizing 55 MRI and PET studies found that the ACC was consistently activated by a number of different emotional stimuli. As the ACC is positioned between the emotional subcortical areas and the cognitive prefrontal systems, the ACC is believed to have heterogeneous functions involving cognitive-emotional processes (Bush et al., 2000). For example, this structure has a top-down, regulatory role in emotion processing via its putative function in attention to emotionally salient information (Phan et al., 2002; Phillips et al., 2003).
The dorsal-cognitive versus ventral-emotional divisions in the ACC

Functional neuroimaging research in adults has identified the ACC as having multiple functions important for both cognitive and emotional processing (Bush et al., 2000; Colby, 1991; Devinsky et al., 1995; Posner and Petersen, 1990). According to a variety of hemodynamic neuroimaging studies in adults, the ACC has been divided into the cognitive, dorsal/caudal and the affective, rostral/ventral subdivisions (e.g. Bush et al., 2000). Adult literature has identified that the dorsal ACC (Figure 3a, ‘cognitive division’) has extensive anatomical connections with prefrontal areas such as the dorsolateral prefrontal cortex, as well as with the primary, premotor and supplementary motor areas, making it an ideal candidate for cognitive-response processing (Bush et al., 2000; Paus et al., 2001). It is believed that this region recruits cognitive resources related to attentional control via connections with other frontal regions. Activity in the dorsal ACC is found in various cognitive-related tasks; this ‘cognitive division’ is activated (Fig.3a, in red) by Stroop-related tasks, selective attention and response inhibition tasks that involve stimulus–response selection in the face of competing information (e.g. Bush et al., 2000; Herd et al., 2006). These types of tasks usually consist of stimuli such as colour-words, naming the same colour as its printed colour (congruent), compared to naming a different colour (incongruent), or, in some studies consist of emotional compared to neutral words (Emotional-Stroop paradigm). Typically, subjects are instructed to respond to the colour of the word and ignore the content. Findings have consistently shown an interference effect in the ACC or delay in reaction times for the colour-incongruent and for the emotional words, which has been established as resulting from the automatic processing of the task-irrelevant aspect (word content) of the stimuli that interferes with the task response.

The ventral ACC (Figure 3a, ‘affective division’), in contrast, has direct projections to the amygdalae, nucleus accumbens, hypothalamus, hippocampi and orbitofrontal cortices, and is more involved in the emotional component of tasks (Drevets and Raichle, 1998; Herd et al., 2006), related to cognitive evaluation of emotions (Bush et al., 2000; Devinsky et al., 1995) and regulation of emotional responses (Ochsner et al.,
For example, the ventral ACC is activated by affect-related tasks (Fig. 3a, in blue); activity in this area was found during automatic orienting to emotional salient and novel stimuli (Berns et al., 1997; Ranganath and Rainer, 2003), during symptom-related processing in affective disorders (Amir et al., 2005; Bush et al., 2000; Devinsky et al., 1995; Drevets and Raichle, 1998; Pissiota et al., 2003; Whalen et al., 1998), as well as during induced sadness in normal subjects and subjects with major depression (Bush et al., 2000).

Figure 3: Meta-analyses of activations and deactivations during cognitive and emotional studies.

The spatial coordinates show findings of activations (a) and deactivations (b) during these two categories of tasks. The red circles show areas that during cognitive-related task (a) the dorsal (cognitive) division was activated and (b) the ventral area was deactivated. The blue squares show regions that during emotional tasks (a) the ventral (affective) division was activated and (b) the dorsal area was deactivated.

Summarized and adapted from Bush et al. (2000), reprinted with permission of Elsevier.

However, the distinct cognitive versus emotional differentiation between the dorsal and ventral ACC has been challenged, as it appeared insufficient to explain evidence of the dorsal ACC activations in other emotional task conditions; neither could it explain the absence of the ventral ACC activation in a number of emotional task conditions. For example, the dorsal ACC was found to be activated in the emotional Stroop conditions compared with the neutral conditions (Haas et al., 2006; Mohany et
al., 2007), even when there was no conflicting information between the task-irrelevant emotional information and the task-related information. In addition, it was also reported that attention demanding tasks with emotional content could change the firing rate of dorsal ACC neurons (Davis et al., 2005).

Other researchers have proposed that the critical feature that dissociates the dorsal versus ventral ACC processing is the type of processing, rather than the content/conflict of processing (Herd et al., 2006; Milham and Banich, 2005; Pessoa, 2005). This integrative model can successfully explain the activation of the dorsal ACC seen in both the colour Stroop and emotional Stroop tasks. It is likely that activity in the dorsal ACC area in emotional tasks may result from the distracting nature of the ‘task-irrelevant’ attribute of the stimuli (the emotional information) that competes with the task-relevant response, thus requiring cognitive effort to maintain correct performance (Milhan and Banich, 2005; Mohanty et al., 2007).

Therefore a more precise view emphasizes the role of the dorsal ACC in processing distraction, or 'competition' of processing, in that the dorsal ACC is involved in cognitive control/inhibition of attention to distracting information, including task-irrelevant emotional stimuli that interfere and compete with task performance. Recent reviews also suggested that the dorsal ACC area is engaged in the integration of negative affect and cognitive control (Shackman et al., 2011). In contrast, the ventral ACC is associated with the attention to or the generation of states of emotions (Allman et al., 2001; Devinsky et al., 1995; Drevets and Raichle, 1998; Posner and Rothbart, 1998).

There are limited data concerning the role of the ACC in processing emotions in children. Unlike the adult literature, developmental studies predominantly describe the ACC structure as a whole yielding limited data on development of this brain region. One ERP study on emotional processing and the ACC, assessed ERP responses in six age groups (5-6, 7-8, 9-10, 11-12, 13-14, 15-16 years) during a go/no-go task with induced negative emotions (e.g. anxiety, anger, and/or distress) (Lewis et al., 2006). In this task, points for successful performance earned a valued
prize, and temporary loss of all points induced negative emotions that were confirmed by self-report scales. The emotion induction produced higher ERP amplitudes during the response inhibition task condition. Only the two oldest groups showed a medial-frontal source suggestive of the dorsal ACC region during conditions of negative emotions. Inspection of the source models across age groups showed a developmental increase in activation in the frontal midline regions indicating the anterior cingulate generator (Lewis et al., 2006), which has been frequently reported for adults (e.g., Nieuwenhuis et al., 2003; Tucker et al., 2003; van Veen and Carter, 2002).

Studies using fMRI have also observed ACC activity in children, although the studies focused mostly on the amygdala activations. It appears that the ACC activity is not evident until later childhood, as observations of the ACC activity were only reported for children above 9 years of age or in adolescents (Baird et al., 1999; Monk et al., 2003; Thomas et al., 2001). This may be related to the later-developing frontal-lobe functions that continue to mature into adolescence and early adulthood (Stuss, 2002; Taylor, 2006), consistent with the protracted development of the ACC and other frontal structures (Shaw et al., 2008).
Chapter 3: Rationale for the studies

The behavioural research reviewed in Chapter 1 has suggested a continued development of emotion processing throughout childhood and adolescence. However, many of the studies that explored normal emotional development in childhood have focused predominantly on narrow age ranges, mostly on the infant and preschool periods or early childhood (e.g., before the age of 10 years). The development of emotion processing from the school-age years into adolescence is under-studied. Existing data have differentiated development associated with processing different facial emotions, particularly happy versus fearful emotions, with the former usually showing early development and the latter showing later and protracted maturation. This observation regarding the differential developmental trajectory associated with processing these emotions suggests that dissociated neural processes may be involved.

Current models in the literature propose two parallel neural systems of emotion processing: (1) a system that includes subcortical regions such as the amygdalae and the ventral frontal areas, critical for the identification of emotionally salient stimuli and the generation of emotional states, and (2) a system that includes the dorsal frontal cortical areas, important for cognitive processing and regulation of emotions and subsequent behaviour (Phillips et al., 2003).

Functional neuroimaging research has highlighted the special roles of the amygdalae and the anterior cingulate cortices (ACC) in the processing of emotional stimuli such as emotional faces. However, existing developmental data are very limited, and research has only provided fragmentary data regarding the amygdala-ACC systems, as studies usually focused on a single structure such as the amygdalae or on narrow age range in children compared with adults.

In Chapter two I reviewed studies that showed neural activities of the amygdalae and ACC in children, adolescents and adults associated with the processing of emotional stimuli; these regions are implicated particularly when fearful expressions are presented, and are activated even when the emotions of the faces are not directly
attended (e.g., Vuilleumier 2001). This fear sensitivity may be related to the ability to
detect potential danger and involve specialised neural pathways that can facilitate
rapid responses and adaptive behaviour. The interest of the present thesis lies in
discovering how these specialised neural systems seen in adults are manifested in
younger children: whether they exhibit different activity patterns or whether there are
qualitatively differing processing systems accounting for the developing abilities.

Furthermore, given the dissociated anatomical development of the limbic versus ACC
brain structures important for emotional functions, one can speculate that functional
measures of brain activity of these two regions should follow similar differentiated
developmental patterns in children. A developmental model regarding these two
systems associated with emotion processing can therefore be proposed: the frontal
cortical regions such as the ACC may become involved to a greater extent from
younger to older age in children, whereas the subcortical areas, such as the amygdalae,
may be engaged to a decreasing extent (Herba and Phillips, 2004) or are adult-like
relatively earlier in life.

However, the developmental patterns of neural activations concerning the amygdalae
and the ACC associated with the functional specialisations in processing emotions
through childhood into adolescence remain undetermined. The limited data,
confounded with the varied age ranges, methodologies and task protocols in the
developmental neuroimaging literature, make it difficult to integrate the disparate
findings.

Critical factors that may have contributed to the diverse developmental findings can
be related to children's varying cognitive abilities. Tasks relying on children's explicit
processing of the emotional stimuli may be confounded by children's differing verbal
abilities or strategies used during the task. On the other hand, passive viewing of
stimuli does not control for children losing attentional focus. Furthermore, there are
certainly age-related neurodevelopmental and functional changes in the brain regions
involved in emotional processing, and studies have often averaged across a wide age
range which would not allow examination of age-related effects.
In addition, previous findings regarding the structural or functional development of the ACC have predominantly addressed the cognitive characteristics of the ACC, such as the inhibition during Stroop-like or go/no-go tasks. Relative to cognitive processing (e.g. during inhibition tasks), the development of emotion processing of the ACC during childhood is less described; little is known about the development of functional ACC activity over childhood associated with emotion processing. Emotion-recognition abilities undergo periods of development as the demands of the social environment increase with age. With the onset of adolescence, there are more adult-like demands in social interactions, and previous studies have identified this stage as a significant period of improvement in emotion recognition and social-cognitive abilities. We investigated the neurodevelopmental changes in processing facial emotions from childhood to early adolescence compared with that in adults using the magnetoencephalography (MEG) with the beamforming source analyses that allow precise localisation of brain activations (Robinson & Vrba, 1999; Cheyne et al., 2006) especially for deep-brain areas such as the limbic system (Quraan et al., 2011). To measure changes from childhood through to adulthood, the studies in this thesis investigated normal children from two age groups (7 – 10 years, mean age 8.8 years and 12 - 15 years, mean age 13.5 years) and one adult group (mean age 24.4 years).

It is strongly recommended to use tasks where the neural responses are well understood in adults to readily allow for comparisons across populations (Luna et al. 2010). Therefore, in the first study, involving only adults, we developed a simple target-detection task that directed attention away from emotional faces to avoid explicit processing of emotions. The focus of this first study is to probe and understand the neural correlates in healthy adults in response to the task-irrelevant, unattended emotional faces in MEG. The study investigated the early and implicit processing of facial emotions, an approach that can reduce secondary processing such as linguistic elaboration, and may increase the sensitivity of MEG in detecting rapid neural responses, especially in the ventral stream of the emotion-processing systems particularly the amygdala and ACC. The results showed significant activations in the
amygdala and ACC, and based on the findings of the latencies where significant sources were identified in the first study, the second study focused on the differences between different age groups for these two source areas as well as on the identification of the age-related developmental patterns.

It is important to apply methodological approaches favoured for developmental neuroimaging to minimize extraneous developmentally confounding factors (e.g., Gaillard et al. 2001). The first study thus established a simple and short paradigm (target-detection) and avoided explicit processing of emotions, suitable for testing children. In the second study, the hypothesis was that, based on the proposed models and previous review, neural activities of the amygdalae and ACC in children would show differential patterns: activities of the amygdalae would be involved from early childhood and may show developmental changes related to emotion sensitivity, while the ACC activity would mature later in childhood, possibly seen in the older subjects. In addition, we expected to identify effects related to the processing of different emotions (happy and fear) dissociated across groups.

The present studies measured event-related MEG responses providing real-time activity of the brain functions in milliseconds. The studies investigated fearful and happy faces (compared with neutral faces within the same experimental block, e.g. fearful versus neutral, happy versus neutral) to determine the developmental patterns of the underlying brain mechanisms related to processing these two facial emotions.
Chapter 4: Methods

This section describes the methods used in the two studies in the thesis. Specific details for the individual studies can be found within Chapters 5 and 6.

4.1 Task

The task contained 300 trials of an emotional face presented simultaneously with a scrambled pattern, each on either the left or the right side of a central fixation cross (Fig. 4). Twenty-five gray-scale photographs of different faces were used for each of three emotions (fearful, happy or neutral), subtending 4° of visual angle. Each emotional face was presented over trials in each hemifield, and the type and location of the face stimuli were equally distributed for the three expressions and two locations (left or right hemifield). The emotions were irrelevant to the task, as participants fixated the central cross and were instructed to respond to the scrambled pattern (the target) by pressing the button corresponding to the target location (left or right) with their index fingers. The stimuli in each trial were presented for 150 ms, with an ISI varying randomly between 1100 ms and 1300 ms. The duration of the task was about 7 minutes and subjects were given practice trials prior to starting the task, to ensure that they understood the task and were comfortable with the instructions and timing. The stimuli were displayed at a viewing distance of 50 cm on a black-background screen with a 4° visual angle inter-stimulus distance.
Figure 4: Target detection task.

![Figure 4: Target detection task. The target is the scrambled pattern, located randomly in the left or right hemifield, accompanied by an emotional face in the other hemifield. The subjects fixated at the central cross and responded to the target by pressing the button corresponding to the side of the target. Each trial was presented for 150 ms with the interstimulus interval varying between 1100 and 1300 ms.]

4.2 Subjects in study 1 and study 2

For study 1, fourteen healthy and right-handed adults (mean age, 27 years; seven females) were recruited. For study 2, twelve children 7 – 10 years of age (mean age 8.8 years), twelve adolescents 12 – 15 years of age (mean age 13.5 years) and twelve young adults (mean age 24.4 years) were tested (six males and six females in each group).

All participants had no history of neurological or psychiatric disorders or learning disabilities and had normal or corrected to normal vision. All participants and the parents of the child subjects provided informed written consent. The studies were approved by the Research Ethics Board of the Hospital for Sick Children.

4.3 Neuroimaging

Neuromagnetic activity was recorded using a 151-channel CTF MEG system (MISL Ltd., Canada) in a magnetically shielded room; participants lay comfortably with their
heads in the MEG helmet during the experiment. Head position relative to MEG sensors was determined by three reference sensors attached to the nasion, left and right pre-auricular points; head movement during the recording was less than 5 mm. The reference sensors were replaced with contrast markers for co-registration of each subject’s MEG recordings with their anatomical MR image. A T1-weighted MRI of the brain was obtained (3D SPGR, 116 slices; TR/TE/FA = 9ms/4.2 ms/15°; voxel size = 0.9375 x 0.9375 x 1.5 mm) using a 1.5T GE MR scanner (Signa Advantage System) and a product 8-channel head coil. Each subject’s inner skull surface was derived from the MRI using the BrainSuite software (Shattuck & Leahy, 2002) and a multisphere headmodel was fit to this surface for use in computing the forward solution.

MEG data were recorded at 625 samples/s with a bandpass of 0 to 100Hz and filtered off-line with a bandpass of 1 to 50 Hz. Data were time-locked to trial onset and averaged by emotion type across subjects. We localised sources across subjects by trial types for each component using an event-related minimum variance beamforming (ERB) method (Cheyne et al., 2006). Images of volumetric brain activity were normalized into Talairach space using both linear and nonlinear warping parameters obtained from each individual’s MRI using SPM2 (http://www.fil.ion.ucl.ac.uk/spm/software/spm2), producing a 5-mm voxel-grid of source power in standardized stereotaxic space (Chau et al., 2004; Singh et al., 2003).

4.4 Data analyses

We focused on brain activity related to the task-irrelevant fearful and happy faces relative to the neutral faces. To identify these emotion-sensitive brain activations, we subtracted the averaged ERB responses of neutral face trials from those of emotional faces for each group (e.g. fearful minus neutral, happy minus neutral). This contrast with the neutral trials effectively cancels out the large and usually dominant visual activations and controls for the effects of face processing, allowing for accurate localisation of smaller components (Quraan et al., 2011) related to the emotions of the
faces. The image threshold was determined using a non-parametric omnibus permutation test on individual whole-head images of mean activity power during the baseline period (between –100 and 0 ms prior to stimulus onset) to create a null distribution, from which a threshold pseudo-Z value was determined for group-averaged results corresponding to $P < 0.05$ (Chau et al., 2004; Cheyne et al., 2006). We focused on early processing stages and analysed MEG components that occurred between 100 ms to 300 ms (Fig. 1b), before behavioural responses were made, and identified significant activations in temporal and frontal lobe regions. We calculated the time courses for the significant and group-sensitive peak voxels of the amygdala and ACC activations for comparisons between groups using the rectified averaged time series output of the beamformer spatial filter (Cheyne et al., 2006; Robinson & Vrba, 1999). To identify the timing where significant differences in activity occurred between trial conditions and subject groups, we performed a sample-wise non-parametric permutation test on each time point.
Chapter 5: Study 1

In this study of adults, we focus on understanding and establishing the neural correlates involved in the processing of the task irrelevant and unattended emotional faces in MEG. The results from this study serve for the following second study for comparing different age groups in the identified latency window and source space.

Unattended emotional faces elicit early lateralized amygdala–frontal and fusiform activations.

Yuwen Hung\textsuperscript{1,3}, Mary Lou Smith\textsuperscript{2,3}, Dimitri J. Bayle\textsuperscript{1,4}, Travis Mills\textsuperscript{1}, Douglas Cheyne\textsuperscript{2}, Margot J. Taylor\textsuperscript{1,2*}

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1. Diagnostic Imaging, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G1X8

2. Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G1X8

3. Department of Psychology, University of Toronto Mississauga, Mississauga, Canada

4. Brain Dynamics and Cognition, INSERM, U821, and Université Lyon 1, Lyon, France

* Corresponding author
Abstract

Human adaptive behaviour to potential threats involves specialized brain responses allowing rapid and reflexive processing of the sensory input and a more directed processing for later evaluation of the nature of the threat. The amygdalae are known to play a key role in emotion processing. It is suggested that the amygdalae process threat-related information through a fast subcortical route and slower cortical feedback. Evidence from human data supporting this hypothesis is lacking. The present study investigated event related neural responses during processing of facial emotions in the unattended hemifield using magnetoencephalography (MEG) and found activations of the amygdala and anterior cingulate cortex to fear as early as 100 ms. The right amygdala exhibited temporally dissociated activations to input from different visual fields, suggesting early subcortical versus later cortical processing of fear. We also observed asymmetrical fusiform activity related to lateralized feed-forward processing of the faces in the visual–ventral stream. Results demonstrate fast, automatic, and parallel processing of unattended emotional faces, providing important insights into the specific and dissociated neural pathways in emotion and face perception.
Introduction

The ability to detect potential threats involves specialized neural systems that can facilitate fast responses to allow adaptive behaviour. The amygdalae are known to play a key role in emotion processing (LeDoux, 1996) and are responsive to stimuli such as fearful faces (Cornwell et al., 2008; Morris et al., 1996; LeDoux, 1996) or fearful eyes (Whalen et al., 2004; Hardee et al., 2008). It is proposed, based on animal research (LeDoux, 1996), that the amygdalae process threat related information through two routes: a fast subcortical route (thalamus–amygdala) and a slower cortical route (thalamus–sensory cortex–amygdala). The subcortical pathway has an evolutionary value that allows quick and automatic responses to potential danger. The cortical pathway provides feedback processing from the sensory afferent input for cortical evaluation (LeDoux, 1996; Johnson, 2005). However, direct evidence with human data supporting these two levels of processing is lacking, as the timing of brain activity is crucial to understanding the nature of neural events underlying rapid processing of emotional stimuli.

Neurophysiological, event-related potential (ERP) research has reported an early emotion effect that modulates the amplitude of neural responses at the latency of 100 ms in posterior regions and 120 ms in fronto-central areas for fearful expressions (Eimer and Holmes, 2002; Batty and Taylor, 2003; Holmes et al., 2003). It is hypothesized that this early emotion effect reflects fast and spontaneous extraction of affective information from the faces and is generated from the amygdala and interconnected cortical regions such as the anterior cingulate cortex (ACC) (Eimer and Holmes, 2007; Vuilleumier and Pourtois, 2007). Recent MEG studies have reported increases in gamma-band synchronization in the amygdala related to processing emotional stimuli at early latencies between 20 and 170 ms, which suggested processing in the subcortical pathway (Luo et al., 2007, 2009). However, there is a paucity of data from humans that differentiate the cortical versus subcortical processing of emotions through the amygdala.
Studies have found that the amygdala responses to fearful faces are sensitive when the faces were of low visual spatial frequency (Vuilleumier et al., 2003; Johnson, 2005), presented subliminally (Luo et al., 2009), or in the unattended peripheral visual fields (Ewbank et al., 2009). The possible common mechanism of these studies is that these coarse, less detailed visual signals can activate fast and automatic neural responses through a subcortical pathway in the amygdala. Asymmetries in amygdala responses have also been reported in a number of fMRI and PET studies, with explicit processing of fearful faces, eyes or negative pictures activating the left amygdala (Funayama et al., 2001; Hardee et al., 2008; Morris et al., 1996), whereas implicit processing of these stimuli activated the right amygdala (Morris et al., 1998, 1999; Funayama et al., 2001; Funayama et al., 2001, but see Whalen et al., 2004).

Additional timing information of such activity would contribute to our understanding of this subcortical processing. Converging observations have further suggested that emotional stimuli perceived in the left, rather than the right, visual field are more related to emotion processing implicating areas including the amygdala, particularly in the right hemisphere (Glascher and Adolphs, 2003; Noesselt et al., 2005). These findings may be related to a right-hemisphere specialization in emotion processing and its sensitivity to the afferent visual input from the contralateral visual field. Here we characterized the time course of emotion processing in the amygdala using emotional faces with hemifield presentation. With the spatiotemporal sensitivity of Magnetoencephalography (MEG), we expected to see a visual-field sensitive processing in the amygdala related to cortical feedback of the fearful stimuli, differentiated in time from early subcortical processing.

The ACC in the frontal lobes of the brain is involved in recruiting frontal, or executive, resources related to attentional control for task related responses, especially in the presence of distracting stimuli (Bush et al., 2000) such as task-irrelevant emotional information (Pessoa, 2005). fMRI studies have noted complex relations between the amygdala and the ACC during processing of emotions, as the two areas either co-activated (Petrovic et al., 2004; Mohanty et al., 2007; Banks et al., 2007; Haas et al., 2007) or de-activated with each other (Ochsner et al., 2002; Petrovic et al., 2004; Das et al., 2005; Felmingham et al., 2007). However, the limited temporal
resolution of fMRI makes it difficult to determine the real-time relation between these regions. Here we investigated the effect of emotional faces presented in the left or right hemifield on the time-locked neural activity focusing on areas implicated in the emotional face-processing network. We calculated the time courses of the neural sources engaged in processing the facial emotions. MEG has not been applied to research of cognitive neuroscience as extensively as other imaging techniques, yet it provides superior spatial localization than ERPs, approaching fMRI, and excellent temporal resolution, allowing us to tease apart early and rapid neural events.

Methods

Subjects. Fourteen healthy, right-handed subjects (mean age, 27 years; seven females) participated in the study. None had a history of neurological or psychiatric disorders and all had normal vision. All subjects provided informed written consent; the study was approved by the Hospital for Sick Children Research Ethics Board.

Task. We studied the effect of task-irrelevant emotional expressions and their location in the visual field (left or right hemifield). Stimuli were projected on a black-background screen at a viewing distance of 50 cm. Each trial contained a scrambled pattern and a face that were presented simultaneously and for the same duration, each on either the left or the right side of a central fixation cross (Fig. 1). Subjects were instructed to fixate the central cross while responding as quickly as possible to the scrambled pattern (the target) by pressing the left or right button corresponding to the target location with their index fingers. The stimuli in each trial were presented for 150 ms to avoid secondary elaboration or inhibition effects, with an ISI varying from 1100 to 1300 ms. The task contained 300 trials, including 150 trials for each of the left-visual-field (LVF) and the right-visual-field (RVF) face conditions with 50 trials of each of the three emotions in each hemifield. Twenty-five gray-scale photographs of different faces were randomly presented for each expression (75 faces were used in total). The visual angle of the stimuli and the inter-stimulus distance was 4°.
Neuroimaging. Neuromagnetic activity was recorded using a 151-channel CTF MEG system (VSM MedTech Ltd., Canada) in a magnetically shielded room at the Hospital for Sick Children in Toronto. During the experiment, participants lay comfortably with their head in the MEG helmet. Head position relative to the MEG sensors was determined by the use of three reference sensors attached at the nasion, left and right pre-auricular points. Head movement over the recording was less than 5 mm for all subjects. The reference sensors were replaced by contrast markers to allow co-registration with each subject's anatomical MR image. A T1-weighted MRI of the brain was obtained (3D SPGR, 116 slices; TR/TE/FA=9 ms/4.2 ms/15°; voxel size=0.9375x0.9375x1.5 mm) using a 1.5-T GE Excite MR scanner (Signa Advantage System) and an 8-channel head coil (GE Medical Systems, Milwaukee, WI). A multiple sphere model was used to fit to the inner skull surface derived from each subject's MRI using BrainSuite software (Shattuck and Leahy 2002).

Data. MEG was recorded at 625 samples/s with a bandpass of 0 to 100 Hz and filtered off-line with a bandpass of 1 to 50 Hz. MEG data were time-locked to trial onset and averaged by the trial type. We analyzed the first two MEG components (M1 and M2) that occurred at 100 and 150 ms (Fig. 2) before behavioural responses were made. We localized sources at each latency for each subject and condition using an event-related minimum variance beamforming (ERB) method (Cheyne et al., 2006, 2007). Volumetric images of brain activity were spatially normalized into Talairach space using SPM2 (http://www.fil.ion.ucl.ac.uk/spm/software/spm2) using both linear and nonlinear warping parameters obtained from each individual's MRI, producing a 5-mm voxel-grid of source power in standardized stereotaxic space (Singh et al., 2003; Chau et al., 2004). Normalized group-averaged images were superimposed on the 3D template brain using the MRICro program (http://www.sph.sc.edu/comd/rorden/mricro.html). Results showed significant activations in the amygdala, ACC, and fusiform areas (regions of interest). We then calculated the time courses for the peak voxels within these regions using the rectified averaged time series output of the beamformer spatial filter (Robinson and Vrba, 1999; Cheyne et al., 2006).
Statistical analysis. We focused on the first two MEG components occurring at 100 and 150 ms. To assess group-averaged activations for each trial type and component, the threshold was determined using a non-parametric omnibus permutation test (P < 0.01) on individual whole-head ERB images. This test corrects for multiple comparisons by using a threshold taken from a distribution of the global maximum brain values Nichols and Holmes, 2002; Singh et al., 2003). To identify emotion-related activations to the fearful and happy faces relative to the neutral faces, we calculated the differences of the source power between the emotional and neutral faces for each latency component for each subject. To assess the group-averaged contrast activations, the nonparametric omnibus permutation test was applied on the contrasted images of mean activity power during baseline period (between –100 and 0 ms prior to stimulus onset) to create a null distribution from which threshold pseudo-Z values were selected for each contrast corresponding to P<0.001 (Chau et al., 2004; Cheyne et al., 2006). For the significant voxels of peak amplitude in regions of interest identified from the averaged contrast results, we examined the time courses and computed a sample-wise parametric permutation test (uncorrected) on each time point across subjects to identify differences in activity over time between two conditions. RTs were analyzed in a 3×2 ANOVA (three emotions by two hemifields) with SPSS software.

Results

Reaction times

The reaction times (RTs) showed a significant interaction between emotion and the location of the face in the visual field (P < .001, two-way ANOVA): RTs to the target (the scrambled pattern) were the longest when the target was paired with a LVF fearful face (mean value=324 ms). These RTs were significantly longer than those when the target was presented with either a neutral (309 ms, P < .005) or a happy (317 ms, P < .05) LVF face.
**ACC and amygdala activations**

Contrasts between the fearful and neutral faces calculated from the first component at 100 ms (M1) across the visual fields showed significantly increased dorsal ACC and decreased ventral ACC activity bilaterally, but predominantly in the left hemisphere (P < .001; nonparametric permutation test; Fig. 3A). In the same fearful-neutral contrasts at M1, we also found a significant source in the right amygdala (P < .001; non-parametric permutation test; Fig. 3B), activating more in the fearful than in the neutral emotion condition across visual fields as shown in the time courses (P < .005, sample-wise permutation test; Fig. 3C). Separating LVF and RVF face presentations, the source activity of the right amygdala in the LVF fearful condition showed two peaks, significantly stronger than those in the LVF neutral condition, at 100 ms (P < .005; sample-wise permutation) and 165 ms (P < .01; Fig. 3D). In the RVF condition, the amygdala showed only a fearful-neutral difference at 100 ms (P < .005; Fig. 3E). We did not observe activation in the left amygdala in the fearful-neutral contrasts, and neutral-happy contrasts were not significant in either the ACC or the amygdala.

**Fusiform activations**

We found significant bilateral fusiform activations at the second MEG component at 150 ms across all conditions (P < .01; nonparametric permutation test), with a right hemispheric dominance (Fig. 4A). The time course of the right fusiform activity showed a larger amplitude to fearful than to neutral (P < .007; sample-wise permutation) and happy faces (P < .009) at 170 ms across the visual fields (Fig. 4B). Comparison of the time courses between the LVF and RVF face presentation across emotions showed dissociated fusiform activity. The right fusiform exhibited significantly higher activation to the LVF compared to the RVF face presentations during 150–170 ms (P < .005; Fig. 4C). In contrast, the left fusiform was activated
more strongly with the RVF than LVF face presentation in the latency range of 120–150 ms (P < .005; Fig. 4D); at the earlier peak in the left fusiform (85 ms), there was no significant difference between the two visual field conditions.

**Discussion**

The present study demonstrates that the ACC–amygdala regions are involved in the rapid processing of unattended fearful facial expressions. We found significant activations within the ACC and right amygdala at 100 ms that were stronger in response to the task irrelevant fearful, relative to the neutral, faces. The dorsal ACC showed increased activity in response to the fearful relative to the neutral faces, while the ventral ACC showed decreased activity in this comparison. Research has reported a heterogeneous division of function within the ACC (Bush et al., 2000) involving both cognitive and emotion processing. It has been posited that the dorsal ACC region is associated with top–down cognitive control of distracting stimuli, including emotional information, during attention-demanding tasks, whereas the ventral ACC region is associated with bottom–up, stimulus-driven processing of emotional events or during pathological or induced internal emotional states (Drevets and Raichle, 1998; Bush et al., 2000; Pessoa, 2005; Herd et al., 2006). Our data support a reciprocal suppression between these two functionally and anatomically connected areas (Drevets and Raichle, 1998; Margulies et al., 2007) with opposite and time-locked patterns of dorsal versus ventral ACC activity within a single task related to processing the task irrelevant fearful faces. These opposing activations may be related to early attentional control of the ACC to suppress the distracting fearful emotion of the faces, as they capture attention automatically and interfere with the task at hand. The co-activation of the ACC and the amygdala suggests a fear-related network in the two areas (Banks et al., 2007) and supports the anatomical connections between these two regions reported in animal studies (Conde et al., 1995; Sesack et al., 1989). These findings may contribute to understanding emotional disorders and
emotion regulation, where individuals have difficulties in suppressing irrelevant and negative emotional events that appear to occupy attention non-volitionally.

The amygdala responses to the task-irrelevant fearful faces add new data to literature demonstrating the specialized and implicit function of the amygdalae to fearful objects (Breiter et al., 1996; Morris et al., 1996). Previous research has suggested that the right amygdala is more engaged in a fast, implicit, and reflexive processing of stimuli that signal potential threat, while the left amygdala is more engaged in a sustained, explicit, linguistic-related evaluation of negative emotional events (Morris et al., 1998; Funayama et al., 2001; Wright et al., 2001; Gläscher and Adolphs, 2003; Hardee et al., 2008). Evidence consistent with this specialization includes recent MEG findings of left-lateralized amygdala activation in tasks that explicitly compared and identified emotional faces (face matching tasks) at time windows after 100 ms (Cornwell et al., 2008), along with the current data of right-lateralized amygdala activation at 100 ms indexing implicit processing of task-irrelevant emotional information of the faces. The timing of brain activation is crucial to allow specification of distinct neural events, which provide critical information to identify neurocognitive functions.

To date, studies have not been able to characterize the timing of early-subcortical versus later-cortical processing in fear perception. Here we provide evidence supporting the hypothesis regarding this two-level processing model. MEG allowed us to differentiate the temporal activity of the amygdala related to the visual-field sensitivity to the fearful emotion: the right amygdala showed a significant difference in fearful-neutral activity to both LVF and RVF face presentations at 100 ms, while this difference was present only at 165 ms to the LVF face presentation. Although larger right amygdala response to fearful faces in the LVF has been previously observed (Noesselt et al., 2005), the mechanism of this visual-field superiority of the amygdala was unknown. Here the data showed that the right amygdala activation sensitive to the LVF fearful emotions occurred at a later stage of processing, differentiating early visual-field-independent activation at 100 ms from the LVF-dependent activation at 165 ms. This dissociation in visual-field sensitivity suggests
that different neural inputs may be involved in the early versus late processing stages. Recent models have proposed that the amygdala receives visual input through both a fast, direct subcortical pathway (via retinal–collicular–pulvinar) (Linke et al., 1999; Morris et al., 1999; Williams et al., 2006) and a slow, indirect visual cortical pathway (via lateral geniculate nucleus–amygdala) (Lamme and Roelfsema, 2000; Williams et al., 2006). The early amygdala activation indicates a fast and automatic response, independent of the location of the fearful stimuli in the visual field. We speculate that this early and visual-field independent response to fear is mediated by the fast subcortical pathway; the later, visual-field dependent activation is mediated by the slower cortical pathway predominantly from the right hemisphere that preferential receives LVF visual input. The secondary amygdala response may also account for the behavioural findings in which the LVF fearful faces delayed the responses to the target in the current study. Studies regarding activity in the ACC and the amygdala in processing negative events (Banks et al., 2007; Ochsner et al., 2002) should consider the anatomically heterogeneous and temporally dynamic nature of activity in these areas in the future.

Fusiform activations at 150–200 ms (M170) during face processing have been well established (Barbeau et al., 2008; McCarthy et al., 1997; Puce et al., 1995, 1996), even when the faces were not directly attended (Cauquil et al., 2000; Furey et al., 2006), as we also observed. The current study showed fusiform sensitivity to facial emotions, as the right fusiform activated more to the fearful compared to other emotions, consistent with previous ERP results where fearful faces produced the largest and longest latency N170s (Batty and Taylor, 2003). The time course in the right fusiform showed higher activity to fearful faces at 170 ms, later than the peak response to all faces at 150 ms, suggesting that the emotional component may be differentially processed later in face perception. It has been argued that this delay in the M/N170 to fearful faces is due to incorporation of feedback from the rapid earlier processing for highly salient stimuli (Batty and Taylor, 2003).

In contrast to most previous studies, the faces in our task were presented in the hemifields maximizing laterality effects in the visual ventral stream (Boles, 1983;
Enns and Kingstone, 1997; Liu and Ioannides, 2006) as our subjects fixated the central cross and used peripheral vision to detect the targets. We observed that both the left and right fusiform regions showed higher amplitude activations with contralateral than ipsilateral faces between 120 and 170 ms (M170). These peripherally presented faces resulted in larger contralateral fusiform activations, dissociating the left and right fusiform responses. This contralateral visual-field superiority suggests that the fusiform receives visual input predominantly from visual areas in the same hemisphere, supporting the proposal of an occipital–fusiform feed-forward mode in face perception (Liu and Ioannides, 2006; Rossion et al., 2003).

Multiple stages of fusiform activation in response to faces have been reported (Barbeau et al., 2008; Liu et al., 2002), and it has recently been suggested that the left fusiform may account for an early stage of fusiform activation in response to faces (Cornwell et al., 2008; Rossion et al., 2003). The left fusiform in our data also showed two peaks of activation at 85 and 135 ms, both earlier than that of the right fusiform (150 ms), suggesting that the left fusiform may be responsible for an early phase while the right fusiform may be responsible for a later phase of face processing. In addition, the peak in the left fusiform at 85 ms, unlike the peak at 135 ms, did not show the contralateral visual-field superiority in processing faces, implying that different processing may be involved in the early stage of face perception in this region. This rapid face processing is likely related to subcortical processing of faces, which have lower spatial frequency when perceived peripherally (Johnson, 2005; Vuilleumier et al., 2003).

The slower behavioural responses in the presence of the fearful, compared with the neutral, faces indicate interference from the task irrelevant fearful emotion. The LVF effect of the fearful expression in delaying task responses is in accordance with previous observations of an advantage of fearful stimuli in LVF to impair task performance (Noesselt et al., 2005). The behavioural effect suggests that fear related information may be processed more automatically by the right hemisphere, which receives input from the left visual field more directly. This model of hemispheric
specialization from behavioural data was supported by our MEG results that showed the fear-sensitive activations in the right amygdala and right fusiform areas.

Conclusions

The present study provides novel timing information on early brain activations in the amygdala, ACC, and fusiform regions, adding to our knowledge of implicit processing of human facial emotions. The sensitivity to task-irrelevant fearful emotions suggests that the unattended information operates at a level where potential threat is automatically processed. The early timing of amygdala–ACC activations (at 100 ms) suggests a specialized frontal–limbic network that could facilitate fast reaction to potential threat. The dissociation of the amygdala fear processing into early, visual-field-independent versus later, left visual-field-dependent stages can help scientists and clinicians understand complex cognitive–neurological deficits linked to the amygdala. The double dissociation in fusiform laterality related to the sensitivity to the contralateral faces can help future neuroimaging studies probing lateralized processing in this region and may serve to differentiate neural deficits due to lateralized brain lesions from possible functional compensation. Future studies may include more emotion types, such as angry expressions, to the task to determine the amygdala's specificity to negative emotions other than fear. Future directions may also include assessing network connectivity between the amygdalae, ACC, and fusiform regions. Finally, the present study demonstrates that MEG with sophisticated temporal source analyses can provide detailed measures of both the location and time course of neurocognitive events in deep brain structures.

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Figure 5: Figure 1 of Study 1. Target detection task.

Figure 1 The target-detection task. Each trial contains a scrambled pattern (target) randomly located in either left or right hemifield, accompanied by an emotional face in the other hemifield. Subjects fixated the central cross and responded to the target by pressing buttons corresponding to the side of the target. Stimuli were presented for 150 ms with an interstimulus interval varying between 1100 and 1300 ms.
Figure 6: Figure 2 of Study 1. MEG waveforms.

**Study 1-Figure 2** The MEG waveforms averaged across subjects. Significant results were found from early latency components at 100 and 150 ms.
Figure 7: Figure 3 of Study 1. Amygdala-ACC activations in adults.

Figure 3 (A) Reciprocal responses to fear within the ACC. Significant activity increases (red) in dorsal ACC and decreases (blue) in ventral ACC were found to the fearful in contrast to the neutral faces at 100 ms across the visual fields. Peak Talairach coordinates of the dorsal ACC: −10, 30, 22; the ventral ACC: −10, 39, 3. (B) Right amygdala activation. Brain image of fearful versus neutral (F–N) difference at M1 showed the right amygdala source, averaged across the visual fields and all subjects. Peak Talairach coordinates: 30, −5, −12. (C) Averaged time series of the right amygdala peak source showed a higher peak amplitude in response to all fearful compared to all neutral conditions at 100 ms across visual fields. (D) With the LVF face presentation, the time course of the right amygdala showed a bifid activity with larger responses to the LVF fearful faces than to the LVF neutral faces at 100 and 165 ms. (E) With the RVF face presentation, the right amygdala only showed significant differences in activity, higher to fearful than to neutral faces, at 100 ms.
Figure 8: Figure 4 of Study 1. Fusiform responses.

Figure 4 Bilateral fusiform activations and double-dissociated time series. (A) Significant bilateral fusiform responses were observed in the averaged MEG response across conditions at 150 ms. (B) The right fusiform (30, –64, –9) showed greater activation in response to all fearful compared to all neutral faces at 170 ms across visual fields. (C) The right fusiform responded more to the LVF than to the RVF faces from 150–170 ms across emotions. (D) The left fusiform (–30, –68, –9), in contrast, responded more to the RVF rather than the LVF faces during the second peak, 120–150 ms, while at the first peak (85 ms), no significant difference was observed between the two conditions. Peak Talairach coordinates were used to calculate time courses.
Chapter 6: Study 2

Based on the latencies and findings in the first study, this study examined developmental patterns and age-related differences in brain responses related to the early and implicit processing of the facial emotions including child and adolescent subjects using the same task established in Study 1. The focus of this study was to identify the neural responses within each group first and then determine the differences between different age groups and the age-related developmental patterns.

Development of ACC-amygdala activations in processing unattended fear

Yuwen Hung\textsuperscript{1-3}, Mary Lou Smith\textsuperscript{2,3}, Margot J. Taylor\textsuperscript{1-3*}

\textsuperscript{1}Diagnostic Imaging,

\textsuperscript{2}Neurosciences & Mental Health, Research Institute,

Hospital for Sick Children,

\textsuperscript{3}Psychology, University of Toronto,

Toronto, Canada

* Corresponding author

Address: Diagnostic Imaging,

The Hospital for Sick Children,
Abstract

The ability to assess facial expressions of others involves specialised brain systems important for emotional and social learning, a skill that emerges over childhood. We investigated the development of neural responses associated with implicit processing of facial emotions using magnetoencephalography in children (7–10 yrs), adolescents (12–15 yrs) and adults. The results demonstrated distinct spatial-temporal activations in the ACC and amygdala emotion-processing systems. The processing of emotions first engaged the earlier-developing amygdala responses and then involved the later-maturing ACC system. With increasing age there was a shift in lateralisation of amygdala responses sensitive to the fearful faces. The findings contribute to a critical understanding of the development related to functional specialisation of fear perception in the frontal-limbic emotion systems. The present study offers important insights into the developmentally time-sensitive impact on the functioning of these brain regions.
Introduction

The development of emotional processing involves specialisation of neural systems allowing for fast and adaptive behaviour, and is important for the acquisition of effective social skills in children. An important aspect of normal development is the ability to understand the facial expressions of others as a cue to the nature and safety of the environment. Behavioural studies have found that children's ability to recognise emotional faces improves with age (Herba et al., 2006); recognising happiness matures earliest with fear being one of the more difficult to recognise in preschool and early school-aged children (Markham and Adams, 1992). Over the ages of 7 to 10 years, children's speed of recognition increases significantly, particularly to negative emotions (De Sonneville et al., 2002; Vicari et al., 2000). ERP studies showed that young children had shorter latencies for happy faces and delayed responses for fearful faces at early latencies (e.g. at P1 component, around 100 ms), while adolescents showed emotional sensitivity at longer latencies (at N170, around 150 – 200 ms) (Batty and Taylor, 2006). These observations of asynchronous development of emotion recognition suggest that different neural systems and mechanisms may be involved over childhood sensitive to the various emotions.

Functional neuroimaging studies have highlighted the involvement of the amygdalae and the anterior cingulate cortex (ACC) during emotion processing in response to stimuli such as faces with fearful expressions (LeDoux, 1996), even when the emotions are not directly attended (Vuilleumier et al., 2001). The challenge lies in understanding how the specialised neural systems are developed in children. A number of studies have reported activity in the amygdalae and ACC regions in childhood and adolescence related to processing emotional faces, including fearful expressions (Baird et al., 1999; Monk et al., 2003; Thomas et al., 2001), particularly when attention was not directed to the emotional faces (Monk et al., 2003). However, the observations of ACC activity were only reported for children in later childhood (over 9 years old) or adolescents (Baird et al., 1999; Lewis et al., 2006; Monk et al., 2003; Thomas et al., 2001), which may be associated with development of frontal-
lobe functions that continues into adolescence and early adulthood (Taylor, 2006).
Findings of amygdala activity in children were variable, with reports of bilateral, left or right lateralised activation (Baird et al., 1999; Lobaugh et al., 2006; Thomas et al., 2001; Todd et al., 2011).

Parallel neural systems of emotion processing have been proposed (Phillips et al., 2003) that implicate dorsal and ventral processing streams. The ventral/subcortical regions, such as the amygdalae, are critical for identification of emotionally salient stimuli and the generation of emotional states, while the dorsal/frontal cortical areas, such as the ACC, are important for the regulation of emotion processing and related behaviour. A developmental model of emotion-processing regarding these two systems has been proposed: the frontal cortical regions may be involved to a greater extent with increasing age over childhood, while subcortical areas such as the amygdalae may be engaged to a decreasing extent (Herba and Phillips, 2004), or are adult-like relatively earlier in life according to the structural development in these regions (Shaw et al., 2008). However, existing data have not characterised these possible developmentally differentiated patterns of brain activity between the amygdala and ACC regions during emotion processing within a single study. Published reports have focused either on activity of one particular structure, such as the amygdalae, or on a narrow age range in children, and were not able to provide the exact timing information of brain activations in these regions (Baird et al., 1999; Monk et al., 2003; Thomas et al., 2001; Todd et al., 2011). A range of methodologies and protocols also make it difficult to integrate findings across studies. Thus, the developmental patterns of emotion processing in the amygdala and ACC systems over childhood remain unclear. In addition, results from tasks that require explicit processing of emotions may be confounded by children’s varying verbal or visuo-spatial abilities (Phan et al., 2002; Vicari et al., 2000).

The current study used magnetoencephalography (MEG) with source analyses that allow precise localisation of brain activations (Cheyne et al., 2006) including deep brain areas such as the limbic system (Quraan et al., 2011) and provide real-time activity of brain function in milliseconds. To avoid explicit processing of emotions,
we directed attention away from briefly presented emotional faces (fearful, happy or neutral) in a simple target-detection task (Fig. 1) in two normal child groups (ages 7–10 and 12–15 yrs) and an adult group. We compared the processing between trials with emotional faces and assessed effects specific to each emotion (e.g. fearful versus neutral, happy versus neutral).

In our previous MEG work using this paradigm with normal adults, we identified early fear-sensitive brain activations within the amygdala and the ACC in response to the unattended fearful faces at 100 ms (Hung et al., 2010). The current study used the same paradigm to compare activation of the amygdalae and ACC across three age groups during the rapid and implicit processing of facial emotions. We expected to see activations of the amygdalae and ACC in children related to the processing of emotions, with adult-like patterns observed in the older child group. We hypothesised that, based on the proposed model and previous observations, neural activities of these two areas would show differential patterns over childhood: activations of the ACC would mature relatively later in childhood, while amygdala activities would be involved from earlier childhood but may exhibit changes in emotion sensitivity or laterality. Our results contribute critical insights into the complex but dissociable brain functions in emotion processing over development.

**Methods**

**Subjects.** Twelve children 7 – 10 years of age (mean age 8.8 years), twelve adolescents 12 – 15 years of age (mean age 13.5 years) and twelve young adults (mean age 24.4 years) participated in the study; each group had six males and six females. All participants had normal vision and none had a history of neurological or psychiatric disorders or learning disabilities. The study was approved by the Research Ethics Board of the Hospital for Sick Children; all participants and the parents of the children provided informed consent.
**Task.** The task contained 300 trials of an emotional face presented simultaneously with a scrambled pattern, each on either the left or the right side of a central fixation cross (Fig. 1). Twenty-five gray-scale photographs of different faces were used for each of three emotions (fearful, happy or neutral), subtending 4° of visual angle; each emotional face was presented twice in each hemifield, and the type and location of the face stimuli were equally distributed for the three expressions and two locations (left or right hemifield). The emotions were irrelevant to the task, as participants fixated the central cross and were instructed to respond to the scrambled pattern (the target) by pressing the button corresponding to the target location (left or right) with their index fingers. Stimuli in each trial were presented for 150 ms, with an ISI varying randomly between 1100 ms and 1300 ms. Duration of the task was about 7 minutes; subjects were given practice trials prior to starting the task, to ensure that they understood the task and were comfortable with the instructions and timing. The stimuli were displayed at a viewing distance of 50 cm on a black-background screen with a 4° visual angle inter-stimulus distance.

**Neuroimaging.** Neuromagnetic activity was recorded using a 151-channel CTF MEG system (MISL Ltd., Canada) in a magnetically shielded room; participants lay comfortably with their heads in the MEG helmet during the experiment. Head position relative to MEG sensors was determined by three reference sensors attached to the nasion, left and right pre-auricular points; head movement during the recording was less than 6 mm. The reference sensors were replaced with contrast markers for co-registration of each subject’s MEG recordings with their anatomical MR image. A T1-weighted MRI of the brain was obtained (3D SPGR, 116 slices; TR/TE/FA = 9ms/4.2 ms/15°; voxel size = 0.9375 x 0.9375 x 1.5 mm) using a 1.5T GE MR scanner (Signa Advantage System) and a product 8-channel head coil. Each subject’s inner skull surface was derived from the MRI and a multisphere head model was fit to this surface for use in computing the forward solution.

MEG activity was recorded at 625 samples/s with a bandpass of 0 to 100Hz and filtered off-line with a bandpass of 1 to 50 Hz. Data were time-locked to trial onset and averaged by emotion type across subjects. We localised sources across subjects
by trial type using an event-related minimum variance beamforming method (Cheyne et al., 2006; Robinson and Vrba, 1999). Volumetric brain activity was normalized into Talairach space using both linear and nonlinear warping parameters obtained from each individual’s MRI using SPM2 (http://www.fil.ion.ucl.ac.uk/spm/software/spm2), producing a 5-mm voxel-grid of source power in standardized stereotaxic space (pseudo-Z) (Singh et al., 2003).

Analyses. We were interested in brain activations sensitive to the task-irrelevant emotional facial expressions. To identify this emotion-related activity we subtracted MEG data of neutral face trials from those of emotional trials for each subject (fearful minus neutral, happy minus neutral) allowing for accurate localisation of smaller components (Quraan et al., 2011) related to the emotions of the faces. Based on our previous findings in adults where significant amygdala and ACC activations were found at early latencies (< 300 ms) before behavioural responses were made (Hung et al., 2010), we averaged MEG activities over every 30 ms between 90 ms to 300 ms (e.g. 90 – 120 ms, 120 – 150 ms etc.) within each group. The image threshold was determined using a non-parametric omnibus permutation test on whole-head activities across subjects during the baseline period (between −100 and 0 ms prior to stimulus onset) that created a null distribution, from which a threshold pseudo-Z value was selected for the group-averaged results corresponding to P < 0.05 (corrected).

Significant results were observed in the amygdalae and ACC areas in the early latency (90 – 120 ms) across the groups. To see the group-sensitive activity during each emotional trial type across time, we calculated the time course of the significant peak locations of the amygdala and ACC for each emotion using rectified averaged time series output of the beamformer spatial filter (Cheyne et al., 2006; Robinson and Vrba, 1999).

To substantiate the observed differences among age groups independently from the group-averaged whole-head results and examine how the effects of emotions impact the age groups differently, we conducted two-way analysis of covariance (ANCOVA, mixed design of emotion by group) using the individual peak magnitude in the
significant time window of the time-series output (in nano-ampere-metre/ n-A-m) from the amygdala and ACC locations. The two emotions (fearful, happy) were used for the comparisons across the three age groups; the activity in the neutral trials during the same time window and the activity before stimulus onset across all trials (baseline) were used as covariates to control for the confounding effects of general face processing and baseline differences between individuals, respectively. We then tested the relations between age and the ACC emotion activity by assessing partial correlations between the individual’s age (years) and their respective magnitude of ACC activation (n-A-m) in the fearful and happy trials, partialling out the effects in the neutral trials and the baseline period. Furthermore, for the activations in the left (L) and the right (R) amygdala, to see the relations between age and laterality in these regions, we calculated the laterality index (L–R/L+R X 100) and applied the same procedure of the partial correlation on age and laterality. Note that a positive laterality score reflects left lateralisation whereas a negative value reflects right lateralisation. Reaction times (RTs) and numbers of correct responses for each emotional trial type were also analysed with ANOVAs.

Results

Event-related emotion activations

The MEG results showed significant findings at 90 – 120 ms across groups. In the younger children, we found significantly increased activations in the left amygdala (peak Talairach coordinates: – 25, – 1, – 17) in both happy and fearful, minus neutral, face trials (P < .05; non-parametric permutation test; Fig. 2a). The older children showed significant activations in the ACC (peak Talairach coordinates: 5, 30, 22, at midline) only to the fearful emotions (P < .05; non-parametric permutation test; Fig. 2b) but not in the amygdalae; this ACC source was not observed in the young children for either emotion. In adults, significant ACC and right amygdala activations were observed in the fear-versus-neutral trials only (peak ACC location: – 5, 25 22, at midline; right amygdala: 30, – 5, – 12; Fig. 2c).
Time course of emotion activity

To identify the timing of significant emotion-related activity, we performed sample-wise non-parametric permutation tests on the normalized time course output (pseudo-Z) between trial types at each time point; differences passing the significance level of $P < .005$ (uncorrected) were considered significant. The same locations of the left and right amygdalae were analysed across the groups. Consistent with the beamforming results, in the young children, the time course of the left amygdala showed significantly greater activity to both happy and fearful faces from 100 to 150 ms, compared to the neutral faces (Fig. 3a1); no significant difference was observed between the fearful and happy trials. We also observed some fear-related activity in the right amygdala and the ACC of the young children, i.e., activation increased for the fearful expression, although it was not statistically significant (Fig. 3a2, 3a3). In the older children, the time course of the ACC showed significantly increased responses specific to the fearful faces at both 100–150 ms and 250–280 ms (Fig. 3b3), while the right amygdala showed activity that was not emotion sensitive. In adults, the significant right amygdala activation exhibited a rapid and fear-sensitive response at around 100 ms (Fig. 3c2), while the ACC had a more dispersed response to the fearful emotion between 100 – 150 ms (Fig. 3c3). The peak ACC locations from adults and the older children were seen as the same source, at midline within the dorsal ACC region.

Age-related development in fear processing

Direct comparisons were also made of the MEG activity across the three age groups. Peak magnitudes for the fearful and happy faces during the active time course of 90–150 ms were analysed for each source, with the activity in the neutral trials and the baseline (–100 to 0 ms) controlled as covariates and partialled out. The 2 x 3 ANCOVA showed a significant interaction between the emotion and group for the
ACC activation \( [F(2,31)=3.842, p<.05,] \). To investigate the effect for each emotion separately across the groups, one-way ANCOVA was conducted for the fearful and happy emotions (with the same covariates). A main effect of group was observed for the fearful faces \( [F(2,31)=4.28, p<.05] \) and not for the happy faces; the adults exhibited significantly greater ACC activity to fearful faces (mean value = 7.44 n-A-m) than either the younger (5.61) or the older (4.11) child groups \( [P<.05, \text{ Least Significant Difference (LSD) post-hoc tests}] \).

The ANCOVA for the left amygdala showed that the young children had significantly larger amplitudes in general (mean = 8.36 n-A-m) than both the adults (4.82) and the older children (5.00 n-A-m; \( P<.05, \text{ LSD tests} \)) \( [\text{group main effect: } F(2,31)=5.74, P<.05, \text{ two-way ANCOVA}] \). The right amygdala, in contrast, showed a main effect for emotion \( [F(1,31)=5.24, P<.05] \) and also for group \( [F(2,31)=4.78, P<.05, \text{ two-way ANCOVA}] \). Activity from the right amygdala was larger in adults (7.64) than the other two groups (older children=6.35, young children=5.44 n-A-m; \( P<.05, \text{ LSD} \)); the activity was the largest for the fearful emotion (7.68) compared to the happy emotion (4.91; \( P<.05, \text{ LSD} \)) in this location.

The partial correlation tests showed a significant positive relation between age and the ACC activation only for the fearful faces \( (r = 0.45, \text{ df} = 32, P < .01, \text{ two-tailed}) \) not for the happy faces \( (r = 0.17, \text{ not significant}) \). To demonstrate this relation, Figure 4a shows the partial regression plot that reflects the relation between age and the peak amplitude of the ACC activation in the fearful trials. To illustrate the age-related effect Figure 4b shows greater ACC activation to fearful faces, in the contrast of adult versus young child groups.

For the lateralised amygdala sources, the partial correlation for amygdala laterality showed a significant negative correlation between age and laterality index only for the fearful faces \( (r = -0.39, \text{ df} = 32, P < .05, \text{ two-tailed}; \text{ Fig. 4c}) \), not for the happy faces \( (r = -0.29, \text{ n.s.}) \), reflecting laterality changes from more left to more right lateralised with increasing age.
**Behavioural results**

The task had a high performance rate, close to ceiling with 90 – 97% correct responses across groups. ANOVAs did not show an interaction effect between the emotion and group on either the RTs or correct responses; neither was there an effect for emotion or group for either measure (Mean RTs = 357 ms).

**Discussion**

The ability to efficiently process facial emotions has a critical role for emotional and social learning (LeDoux, 1994), and is important for developing subtle skills essential to social interactions. Evaluating emotions of others too slowly could impede social communication and its development. The detection of negative emotions has evolutionary significance and involves specialised neural systems allowing for rapid and adaptive responses. The present study determined the development of neural activations during implicit processing of facial emotions from early childhood into the adulthood, and provides new spatial-temporal evidence with MEG demonstrating protracted but differing development in two key brain regions: the ACC and amygdala systems.

Unlike the finding in adults and older children, the ACC was not activated significantly in the younger children, and showed the fear-specific and age-correlated increase with development. The amygdala responses to emotions, in contrast, were evident in the younger children, and showed an age-correlated laterality shift for processing fear from the left to the right lateralisation from younger children to the adults. The co-activation of the amygdala and ACC in adults reflects functional connections between these two regions in processing the fearful expressions (Banks et al., 2007; Bush et al., 2000), consistent with anatomical evidence of connections between these areas in human and animal studies (Conde et al., 1995; Shackman et al., 2011). The time course in children, however, showed patterns of this co-activity
which, unlike that in adults, was not fully sensitive to the processing of emotions, reflecting the immature function in the developing brain.

The results support the proposed model of the differential development in dorsal versus ventral emotion-processing systems, as emotional processing in the amygdalae in the ventral, subcortical system developed earlier in childhood, whereas the specialised processing of fear involving the dorsal cortical system in the ACC, appeared to have a later maturation (Herba and Phillips, 2004; Phillips et al., 2003). This differentiation is parallel to the differing anatomical development between these areas observed in structural imaging: the maturation rate in the limbic areas including the amygdalae reaches adult level earlier in life and follows a stable developmental course relative to other cortical regions, while the ACC and other frontal areas have a later developmental peak and continue to mature throughout adolescence (Casey et al., 2005a; Kanemura et al., 2003; Tsujimoto, 2008).

The dissociations in the early versus later amygdala-ACC functional development offer important insight into the developmental significance regarding the timing of brain damage in these regions. For example, lesion studies have observed that amygdala damage early in life was linked to severely impaired recognition of facial expressions of fear and profound behavioural disturbance, whereas damage in this structure in adulthood spared fear recognition (Adolphs et al., 1994; Hamann et al., 1996). Based on our data, early in childhood emotional processing relied on the early-developed amygdala functions; with increasing age, the processing of emotions relied more on the later-matured ACC function in adolescence and then on the ACC-amygdala system in early adulthood. This may explain the previous behavioural observations of a later development in recognition of negative emotions such as fear (De Sonneville et al., 2002; Markham and Adams, 1992; Vicari et al., 2000), and why the processing of the emotions continues despite amygdala dysfunction later in life. It can be inferred that the amygdala is crucial during early development in establishing specific processing of emotions with interconnected systems; once the networks develop, dysfunctions of the amygdala may be compensated by functions of other brain regions, potentially involving higher-level cortical areas, such as the ACC.
Age-related development in ACC fear processing

Functional data regarding the development of ACC in emotional processing, unlike the amygdalae, are less described. Studies have consistently observed ACC activation to a range of cognitive and emotional stimuli (Phan et al., 2002). It is established that the ACC has an integrative and regulatory role in processing emotions via its function in attention to emotionally salient information (Phan et al., 2002; Phillips et al., 2003). The dorsal ACC region, as observed in the current study, is one of the reliably activated structures in fear conditioning studies (Etkin and Wager, 2007). Recent reviews suggest that the dorsal ACC is involved in processing negative affect and cognitive control related to the expression of emotional responses and executing goal-directed behaviour (Etkin et al., 2011; Shackman et al., 2011).

Developmental researchers have observed a close relation between the maturation of the ACC structure and the ability to regulate emotions, cognitive and behavioural control with social demands or during tasks involving irrelevant distraction in infancy and early childhood (Posner, 2007) as the complexity of the social environment increases with age. We found in the older but not the younger children the adult-like ACC activations sensitive to the processing of the fearful expression. The results link with the limited reports of ACC activity seen only in children in either late childhood or adolescence (Baird et al., 1999; Lewis et al., 2006; Monk et al., 2003; Thomas et al., 2001). This finding may be associated with the social environmental change with the onset of adolescence, when children become more adult-like in their social behaviour.

The time course of the ACC activation of the older children showed temporally dispersed activities in the fear-sensitive processing (Fig. 3b3), unlike the more focal activation seen in adults (Fig. 3c3). This temporal information suggests that even until mid adolescence, the adult-like event-related ACC response was still undergoing qualitative changes. Although the time course of ACC in the young children exhibited some level of activity (Fig. 3a3), it was not emotion-sensitive.
Across all age groups, the results demonstrated that the fear-related response of the ACC gradually increased with age (Fig. 4a), suggesting a continuous functional development in this structure. This finding confirmed previous ERP data that suggested a developmental increase in activity in the frontal midline regions suggestive of the dorsal ACC during conditions of negative emotions (Lewis et al., 2006). This pattern of age-correlated increase is also consistent with data of anatomical growth which suggested that the ACC, similar to other frontal structures, undergoes a protracted maturation that continues into early adulthood (Shaw et al., 2008; Stuss and Knight, 2002; Taylor, 2006).

**Age-related laterality change in amygdala fear responses**

The amygdalae are well-established as part of the neural substrates that evolved to detect potential threats and respond to emotionally charged stimuli (Adolphs et al., 2005; LeDoux, 1996; Phillips et al., 2003; Sabatinelli et al., 2011), especially when the stimuli are not directly perceived (Adolphs et al., 2005; Morris et al., 1996). The current results for amygdala activations accord with previous findings of amygdala responses to emotional faces presented subliminally (Luo et al., 2009) or in unattended hemifields (Ewbank et al., 2009).

Recent meta-analyses have reported that the amygdalae have a higher probability of responding to fearful faces compared to happy or sad faces (Costafreda et al., 2008; Fusar-Poli et al., 2009). We found that the amygdala activations in the younger children were not emotion-specific, and that the fear-sensitive amygdala activation developed later and was present in adults. This non-specific to fear-specific pattern from the childhood to adulthood is in accord with the model that brain responses in children were less differentiated than seen in adults in emotion (Thomas et al., 2001) and memory processing (Bigel and Smith, 2001), and may reflect a process of functional specialisation in fear processing. The development of the fear sensitivity in the amygdalae is also accompanied with laterality change: the younger children exhibited the left-dominant, non-specific emotional responses, whereas the adults
showed the right-lateralised, fear-specific activation in the amygdala (Fig. 4c). In addition, the ANCOVA results showed that the right amygdala, in contrast to the left amygdala, had an additional emotion effect for fear across all groups, suggesting that in general the right amygdala is more sensitive to the fearful emotion during the implicit processing task that we used.

The observation of the right-lateralisation in the amygdalae responses is consistent with a previous observation of right-dominant amygdala response in 10–12 year-old children during an implicit emotional task (Lobaugh et al., 2006), and may be associated with the specialised role of the right hemisphere in the implicit processing of emotions during the current task. Specific views regarding laterality in the amygdalae suggest a relative right-lateralisation linked to implicit attention and reflexive processing of emotional stimuli (e.g. for masked emotional stimuli), in contrast to a relative left-lateralisation related to explicit or elaborative processing of the emotions (e.g. for linguistic-related stimuli and the experience of emotions) (Costafreda et al., 2008; Gläscher and Adolphs, 2003; Morris et al., 1999; Noesselt et al., 2005). In the current study, we dissociated the amygdala laterality in developmental periods, offering new insight on the developmental course of the implicit perception of fear. We demonstrate that the amygdalae, where structural changes are largely stabilized in childhood (Shaw et al., 2008), undergoes qualitative development. This complexity may help explain the diverse observations in children where amygdala activity varied in emotional sensitivity and laterality (Baird et al., 1999; Lobaugh et al., 2006; Thomas et al., 2001; Todd et al., 2011).

**Conclusion**

The development of an efficient processing system capable of fast detection of danger serves a protective function from early stages in development. While the adult-like emotion systems are in place in childhood, they operate in a qualitatively different fashion. We dissociated the development of emotion processing in the ACC-amygdala systems with MEG source analyses, which allow accurate source
localisation and precise timing of both cortical and subcortical activations. The findings from the non-specific emotion responses in the amygdala in early childhood to the fear-specialised activation in the ACC and amygdala systems in later childhood and in adulthood suggest that the sensitivity of fear perception is acquired over development. Although the amygdalae are crucial in emotion processing, with the development of neural systems such as the ACC, dysfunctions in the subsystem during development may have time-limited impact. In the presence of the irrelevant emotional faces, the unattended emotional information of the faces captured attention automatically; results can help scientists and clinicians identify the emergence of abnormal emotional processing.

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Author Contributions

Y.H. contributed to the study question and hypothesis, task design, data processing and analyses, interpretation of data, and writing the paper. M.L.S. and M.J.T. contributed to the study question, the writing of the paper, and supervised the project.
Figure 9: Figure 1 of Study 2. The target detection Task.

*Figure 1* The experimental task (target detection task). The target was the scrambled pattern located randomly either in the left or right hemifield, accompanied by an emotional face in the other hemifield. Participants fixated the central cross and responded to the target location by pressing corresponding buttons. Each trial was presented for 150 ms with an inter-stimulus-interval varying between 1100 and 1300 ms.
Figure 10: Figure 2 of Study 2. Amygdala and ACC activations in children.

Figure 2 Event-related amygdalae and ACC activations sensitive to the emotions of the unattended faces found in different age groups at early latency 90 – 120 ms. (a) Younger children showed increased activations in the left amygdala in response to the happy and the fearful relative to neutral emotions. (b) Older children showed midline ACC activations sensitive to the fearful emotion. (c) In adults, significant ACC and right amygdala activations were shown specific to the fearful emotion.
Figure 11: Figure 3 of Study 2. Time course of amygdala and ACC activity in children.

**Figure 3** Time course of the group-sensitive emotion activities in the amygdalae and ACC. (a) In the young children, the time course of the left amygdala showed the significantly increased activation to happy and fearful expressions across 100 to 150 ms, and some fear-related activity was observed in the right amygdala and ACC that was not emotion sensitive. (b) In the older children, the time course of the ACC showed multiple and significantly increased responses to the fearful emotion at 100–150 ms and 250–280 ms, and the right amygdala showed some level of activity that
was not emotion sensitive. (c) In adults, the significant right amygdala activation exhibited a rapid and fear-sensitive response around 100 ms, while the ACC had a more dispersed response to the fearful emotion between 100 – 150 ms.

**Age-related fear development**

![Partial regression plot](image)

**Figure 12:** Figure 4 of Study 2. Partial correlations between age and fear-related activity in children.

**Figure 4** Partial correlations between age and fear-sensitive activity. (a) The partial correlation test showed a significant positive relation between age and peak
amplitudes of the ACC activation during the fearful trials. The ACC fear activity increased with age. (b) For illustration purpose for the age-related effect, in the contrast of adults versus young children, greater ACC activation to the fearful faces (fearful minus neutral) was observed. (c) The partial correlation test showed a significant negative relation between age and the amygdalae laterality index (L–R/L+R X 100) during the fearful trials. The amygdalae laterality changed from more left (positive scores, L) to more right lateralised (negative scores, R) with increasing age related to processing the fearful emotion. * Residual values were plotted in the partial regression plot that reflects the pure relation between age and source activity by partialling out effects of activity during the neutral trials and in the baseline period (before stimulus onset).
Chapter 7: General discussion

The ability to process facial emotions is critical for emotional and social learning (LeDoux, 1994). Social functioning requires rapid processing of emotional expressions in ascertaining the intentions and emotional reactions of others. Skills utilised in recognising the emotional state of others are important to guide appropriate socio-emotional behaviour. A critical aspect of child development is the ability to read emotional signals of adults and peers as cues to the safety and nature of a new environment. The ability to detect negative emotions that signals potential threats, has evolutionary significance and involves specialised neural systems allowing for rapid and adaptive responses.

As the demands of the social environment increase with age, emotion-processing abilities undergo periods of development. An identifiable social environmental change occurs with the onset of adolescence, when children strive to become more adult-like in their social interactions (Baron-Cohen et al., 2001; Bowers, et al., 1999). Consistent with the developmental findings in the current thesis (Study 2), the few neuroimaging studies of adolescents and pre-adolescent children that assessed emotion processing suggested that age-related behavioural changes may depend on the maturation of certain brain regions involving the amygdala and ACC structures (Lobaugh et al., 2006; Monk et al., 2003). For example, Lobaugh et al (2006) demonstrated that neural activities of emotion-specific processing in the amygdalae and the cingulate gyrus that are typically engaged in adults during processing emotions were present in children aged 10-12 related to the processing of negative emotions such as fear, disgust and sadness in an implicit face-processing task. They demonstrated that distinct neural systems distinguished different types of emotions and that the implicit discrimination was attained by the age of 10. There are relatively fewer data in younger children. In addition, most other neuroimaging studies of processing emotional faces have focused on one particular brain region, mostly the amygdalae, and have not been able to provide the exact timing information of brain activations in these regions (Baird et al., 1999; Monk et al., 2003; Thomas et al., 2001; Todd et al., 2011).
The focus of the current thesis was the investigation of the age-related functional development of the spatial and temporal neural activations during implicit processing of facial emotions from childhood into adulthood using MEG. Study 1 established the understanding of the neural correlates in normal adults, showing that the ACC and right-lateralised amygdala were activated in response to rapid, implicit processing of the task-irrelevant facial emotions specifically for the processing of fear (Fig. 3; Hung et al., 2010). Study 2 demonstrated that the maturation of this emotion processing in these brain regions, with both quantitative and qualitative development from the school-age period (7-10 yrs) to adolescence (12-15 yrs) and adulthood.

Specifically, the results of Study 2 showed protracted but differing developmental patterns of fear processing in the amygdala and the ACC systems. We found that while the fully developed amygdala-ACC systems for emotion processing were activated in adults, the ACC was only activated significantly in the older but not the younger children. Furthermore, the ACC showed an age-related increase in activity with development. In contrast, the amygdala activation was evident in the younger children and showed an age-related laterality change, shifting from left to right lateralisation from the younger children into the adult group. These results provide new evidence characterising distinct spatial and temporal patterns related to age-related changes in the amygdala and ACC emotion-processing systems over childhood into adulthood.

We found that the functional responses related to the processing of facial emotions in the ACC and amygdalae developed in a dissociated fashion throughout childhood, with the adult pattern appearing only later in the childhood/early-adolescence period. This result supports the proposed model of the differential developmental patterns in dorsal versus ventral emotion-processing systems, as emotional processing in the amygdalae in the ventral, subcortical system developed earlier in childhood, whereas the processing of emotions in the dorsal cortical system implicating the ACC, appeared to have a later maturation (Phillips et al., 2003; Herba et al., 2004). This finding is consistent with evidence of the differing anatomical development in these areas. Structural imaging has shown different maturation rates between the limbic
areas and the ACC, as relative to the other cortical areas, the limbic regions including the amygdalae reach adult-levels earlier and remain relatively stable over childhood, while the ACC and other frontal regions have a later peak in development (~after 10 years old) and continue to mature throughout adolescence (Casey et al., 2005a, 2005b; Kanemura et al., 2003; Shaw et al., 2008; Tsujimoto, 2008).

This dissociation of neural activities in development offers important insight on the impact of the timing of brain damage involving these regions on subsequent behaviour, and may explain why early damage in the amygdala is related to more severe disturbance than damage later in life. Lesion studies have suggested that although the amygdala plays an important role during development for establishing the networks necessary for processing and recognising the emotional expressions, once the networks are established, they may function independently of the amygdala (Adolphs et al., 1994, 1995, 1996; Hamann et al., 1996). In the current study, the finding of the early versus later developed amygdala and ACC emotion systems provides neuroimaging evidence supporting this observation. Our findings suggest that early in childhood, emotional processing relied more on the early-developed amygdala function when the specialised processing for specific emotions such as fear is being developed and has not yet been established. With increasing age, emotional processing relies more on the later-maturing ACC function. This could explain why emotional processing continues despite amygdala dysfunction later in life, and why damage in the amygdala early in life has a more severe impact on the processing of fear. It can also be inferred that, once a connected neural system is developed, such as one involving the ACC or other frontal cortical regions, the dysfunctions of the amygdala may be compensated by these higher-level cortical functions. Accordingly, damage to the ACC or the connected frontal areas may have more negative impact if the damage occurs from later childhood to adolescence.

On the other hand, the significant and co-activated amygdala and ACC responses to fear in the whole-brain analyses reflect functional connections between these two regions (Banks et al., 2007; Bush et al., 2000) in processing the fearful information, and is consistent with anatomical evidence of connections between these areas in
animal and human studies (Conde et al., 1995; Sesack et al., 1989; Shackman et al., 2011). Within the first two years of life, there is a large increase in myelination that improves the connection and efficiency of the neural pathways between the cortical and subcortical areas (Herschkowitz, 2000), and this myelination continues to develop into adolescence (Yakolev and Lecours, 1967; Paus et al., 1999). With increasing age, connections between these ‘higher cortical’ areas and the subcortical structures become further refined. It is therefore suggested that once amygdalar function is developed, it is in part the continued development of white matter tracts between this structure and other cortical and frontal regions that provide the continuing development of emotion processing with age, including the meaning and context of the emotional experience (Herba and Phillips, 2004). Therefore, with increasing age the amygdala-ACC co-activations in the current studies may become more important than either specific region due to the stronger connections between the areas. In our child subjects in Study 2, the time course showed patterns of this co-activity which, unlike that seen in the adults, was not fully sensitive to emotions. Therefore it is likely that this observation is due to immature functional connections in response to fear between the amygdala and ACC areas. In addition, the older children did not exhibit the emotion-related activity either in the whole-brain localisation or in the time courses. This result may be related to the reciprocal (inhibitory) relations in activity between the ACC and the amygdala regions (Drevets and Raichle, 1998; Lewis and Todd, 2007). However, one limitation with the current study is the limited knowledge of the development of the functional connectivity between the ACC and amygdalae. Future studies assessing functional connectivity between these regions will help to clarify the nature of the development and may complement the findings of the current study.

**Age-related laterality change in amygdala fear responses**

The amygdalae are well-established as part of the neural substrates that evolved to detect potential threats and respond to emotionally charged stimuli (Adolphs et al.,
2005; LeDoux, 1996; Phillips et al., 2003; Sabatinelli et al., 2011), including when the stimuli are not directly perceived (Adolphs et al., 2005; Breiter et al., 1996; Morris et al., 1996). The current results for amygdala activations accord with previous findings of amygdala responses to emotional faces presented subliminally (Luo et al., 2009) or in unattended hemifields (Ewbank et al., 2009). Recent meta-analyses demonstrated that the amygdala has greater sensitivity to fearful faces compared to happy or sad faces (Costafreda et al., 2008; Fusar-Poli et al., 2009). In the current thesis, we found developmental patterns of the emotional sensitivity in the amygdala. The data showed that while the fear-specific amygdala activation is present in adults, the amygdala activations in the younger children were non-emotion-specific, as they were present for both happy and fearful faces. This non-specific to fear-specific pattern reflects the process of functional differentiation and specialisation, and supports the notion that brain responses in children are less differentiated than seen in adults (Thomas et al., 2001). These data are also consistent with studies in younger children where a positivity bias is seen: there are larger responses to positive than negative faces (e.g., Todd et al., 2011).

The current data further showed that the amygdales, where structural changes were thought to be stable in childhood (Shaw et al., 2008), undergo complex and qualitative development. The development of emotion sensitivity in the amygdales was also accompanied by a laterality change: with increasing age, the laterality in fear processing gradually changed from left-dominant in early childhood to right-dominant into adulthood (Fig. 4c, Study 2). We demonstrated the development of the amygdala fear specialisation and right lateralisation and highlighted the special role of the right hemisphere in these structures in processing fear.

Traditionally, the left and right hemispheres of the brain were characterized, in a simplified model, as engaged preferentially in language versus emotional or visuospatial functions, respectively. More current models regarding neural lateralisation have conceptualised the hemispheric differentiation in terms of the type of processing rather than the content processed by each hemisphere (Powell et al., 2004). A number of imaging studies have observed asymmetric amygdala activities,
and researchers have suggested a specialised and greater role of the right hemisphere in the implicit processing of emotions (Gläscher and Adolphs, 2003; Noesselt et al., 2005; Whalen et al., 1998). In contrast, the left hemisphere is believed to be more specialised at dealing with effortful and routine representations and strategies, which would include linguistic-related elaboration such as language (Powell et al., 2004). Our finding of the increasing right lateralisation of the amygdala activations in the studies is consistent with the classic view of this role of the right hemisphere in processing emotions, and is also consistent with a previous study using an implicit emotional task in children (Lobaugh et al., 2006), where the right dominant amygdala response was found. Other researchers have further suggested that the right amygdala is more involved in implicit perception and reflexive processing of the emotional stimuli (e.g. for masked emotional stimuli), whereas the left amygdala is more engaged in explicit or elaborative processing of emotional events (e.g. for linguistic-related stimuli and the experience of emotions) (Costafreda et al., 2008; Funayama et al., 2001; Hardee et al., 2008; Morris et al., 1996, 1999; Whalen et al., 2004). Thus the development of right-lateralised amygdala activations found in the current studies can be related to the implicit attention to the unattended, task-irrelevant fearful information of the faces in the task used in the current thesis.

Although several studies have reported amygdala responses to different emotions, this structure has a higher probability of responding to negative emotions in general (Costafreda et al., 2008; Fusar-Poli et al., 2009; Sergerie et al., 2008). However, several observations showed an effect for happy faces, particularly in children. For example, Todd et al. (2011) found greater bilateral amygdala activity to happy faces (relative to angry faces) in children aged 4 to 8 years compared to adults, whereas adults had larger responses to the negative, angry faces. ERP data also demonstrated that young children showed earlier latency effects for happy faces at P1 component (around 100 ms) (Batty & Taylor, 2006). This early effect for the happy emotion can be linked to behavioural findings in which the happy expression is the easiest for young children to discriminate and is recognised by children earlier in development, whereas the performance for negative emotions develops later (Boyatzis et al., 1993; Camras and Allison, 1985; Izard, 1971; Labarbera et al., 1976; Markham and Adams,
Similarly, in the results of Study 2 of the current thesis, only the young child group appeared to have the sensitivity and the higher responses to the happy faces. However, this higher activation did not statistically differ from that seen to the fearful emotion, perhaps due to the children being older than those in the Todd et al. (2011) study. Future studies addressing these dissociations over a wider age range may provide a more detailed understanding of the neural mechanisms associated with the early development of processing different emotions.

**Age-related development in ACC fear processing**

Studies have consistently observed ACC activation to a range of cognitive and emotional stimuli (Bush et al., 2000; Phan et al., 2002). It is suggested that the ACC has a multi-functional role involving the integration and regulation of emotional and cognitive information through appropriate selection and allocation of attention (Bush et al., 2000; Kern et al., 2004). This structure plays an important role integrating stimuli coming from a variety of sources associated with its anatomical location and connections between the limbic (emotion) and cortical (cognitive) regions. Thus, the ACC has a regulatory role in processing emotions via its function in attention to emotionally salient information (Phan et al., 2002; Phillips et al., 2003). Recent reviews suggested that the dorsal ACC is involved in processing negative affect and cognitive control related to the expression of emotional responses and executing goal-directed behaviour (Etkin et al., 2011; Shackman et al., 2011). The dorsal ACC region, as observed in the current study, is one of the reliably activated structures in fear conditioning studies (e.g. Etkin & Wager, 2007).

Functional data on the development of ACC and emotional processing are relatively less described in children. Researchers have observed a close relation between the maturation of ACC and the ability to regulate emotions, cognitive and behavioural control with social demands or during tasks involving irrelevant distraction in infancy and early childhood (Posner 1998, 2007). Functional ACC activity has been observed
in later childhood and adolescence related to processing emotional faces, especially with fearful expressions (Baird et al., 1999; Lewis et al., 2006; Monk et al., 2003; Thomas et al., 2001). In the present study, we assessed children from a younger age range and found the age-related increases in the ACC responses related to processing fear. The results support the study hypothesis and confirm the suggestion that it is not until mid-to-late childhood and adolescence that the development of the emotion sensitivity of the ACC is evident (Baird et al., 1999; Lewis et al., 2006; Monk et al., 2003; Thomas et al., 2001). This finding also confirmed ERP data showing a developmental increase in activity in the frontal midline regions suggestive of the dorsal ACC region during conditions of negative emotions (Lewis et al., 2006).

The age-correlated ACC activity also reflects a continuous functional development in this structure, and is consistent with the anatomical evidence suggesting that the ACC, like other frontal structures, undergoes a protracted maturation that continues into adolescence and early adulthood (Shaw et al., 2008; Stuss and Knight, 2002; Taylor, 2006). However, the time course of the ACC activation of the older children showed temporally dispersed activities (Fig. 3b3, Study2), unlike the more focal activation observed in adults (Fig. 3c3, Study 2), which may be related to immature neural activity in this region even in later childhood, consistent with the view of immature prefrontal activity in children (Bunge et al., 2002).

Summary and Conclusions

The findings in the current thesis are consistent with recent behavioural findings that suggested that the development of the ability to discriminate facial emotions develops later in childhood, relative to facial identity (Johnston et al., 2011). Studies in face processing have suggested that the discrimination of emotions continues to develop throughout childhood and early adolescence (Batty and Taylor 2006; Herba and Phillips, 2004; Kolb et al 1992). The present thesis offers new data on the neural underpinnings of this functional development with the results of the ACC-amygdala emotion-processing systems through childhood, adolescence and adulthood. Our
results showed that while the adult-like emotion systems are in place in childhood, they operate in a qualitatively differing fashion, with the adult-like patterns of neural responses still emerging in early adolescence. We demonstrated age-related and emotion-sensitive developmental patterns from the non-specific emotion responses in the amygdala during early childhood into the fear-specialised activation in the ACC and amygdala in later childhood and adulthood.

The patterns of the current data demonstrated that fear engaged differing subcortical-cortical regions as children matured. This emotion differentiation suggests that the perception of fear, rather than being innate, is acquired over development in humans (Adolphs et al., 1996; Adolphs, 1999).

MEG allows tracking the neural responses down to millisecond timescales. The MEG recording and sophisticated source analyses applied in the current thesis offer detailed measures for the location and time course of neurocognitive events in deep brain regions (Quraan et al., 2011). The use of the simple and implicit emotion-processing task in the present studies provides a better opportunity to examine the developmental patterns of neural cognitive responses in children, to probe the amygdala and related cortical responses in emotional processing (Ewbank et al., 2009; Johnson, 2005; Luo et al., 2009; Vuilleumier et al., 2003); this approach also avoids confounding effects related to the heterogeneity in cognitive development in children during explicit tasks. The event-related design also provides a suitable environment for the investigation with children, as it allows for the presentation of trials of interest and control trials within the same experimental block, thus reducing the testing time and noise effect from head movement, or possible habituation of amygdala responses seen in block designs (Herba and Phillips, 2004; Wright et al., 2001). The early MEG responses may be valuable for future clinical screening or diagnostic assessment, as the latencies of the early responses in MEG are stable across repeat testing and individuals and thus both the stimulus and response timing are known with reasonable precision. Our results of age-related differential patterns are attributed to developmental changes related to the automatic processing of facial emotions, as the task performance was equivalent across all subjects and the effect of neutral faces was controlled for
reducing possible age-related confounding variance related to face processing across the age groups.

The finding of the dissociation in the early versus later developed amygdala and ACC emotion systems suggests that the impact of regional brain damage in these areas during development may be time-sensitive and time-limited. This observation contributes to the understanding of the ‘critical timing’ of brain damage and its impact, as well as mechanisms of possible neural-cognitive compensations related to brain dysfunction during development. The findings may thus have implications for future assessment for children with neurocognitive abnormalities such as temporal lobe epilepsy or other brain dysfunction. In such children, the behavioural response to emotions may remain similar across task conditions as seen in normal children and adults in study 2. Therefore, their social-cognitive deficits associated with processing emotions may potentially be undetected until later in life (Turkstra, 2000; Turkstra et al., 2001). It is therefore important to establish new methods and efficient tests for screening purposes and for early identification of abnormalities in emotional processing.

A core component of many psychiatric illnesses is poor social and emotional functioning, which is associated with impaired regulation of emotional behaviour (Herba and Phillips, 2004). Abnormalities in the processing and recognition of emotional expressions have been associated with psychiatric disorders in adult (Phillips et al., 2003a) and child populations (Blair et al., 2003). In the presence of the unattended emotional faces in the current studies, the task-irrelevant emotional information of the faces captures attention automatically. The results can therefore be applied by scientists and clinicians to identify the emergence of abnormal processes and development in children with affective dysfunctions, such as emotional disorders or autism, where individuals have difficulties in regulating or inhibiting emotional responses. Phillips et al. (2003b) proposed that specific patterns of functional abnormalities in parallel neural systems engaged in the response and regulation of emotional stimuli, may be associated with the generation of different symptoms of psychiatric disorders. Findings in the current thesis thus will inform the
understanding and identification of the neural bases of normal and abnormal emotional development for future studies, and may aid the development of earlier interventions for children and adolescents with psychiatric disorders.

Future investigations may also consider including other types of negative facial expressions, such as anger, to determine whether the findings in the current study are fear-specific or a result of sensitivity to negative emotions in general.

Even though a network system is involved, different neural subsystems make distinct contributions and are differentially important for aspects of cognitive and emotional functions. Therefore using appropriate tasks and methods to dissociate the specialised neural responses and their developmental courses in various populations may be the key, in future research, to a greater understanding of mechanisms related to the neurocognitive development and functional specialisations of the brain.
References


