## ABSTRACT

## CORTICAL RESPONSE TO FACIAL EXPRESSIONS OF YOUNG ADULT MALES WITH AUTISM SPECTRUM DISORDERS AND CONTROLS USING FUNCTIONAL MAGNETIC RESONANCE IMAGING (fMRI)

by
**Jagriti Arora**

Autism is a neurodevelopmental disorder that is defined by deficits in social and emotional impairments and this study aims to identify specific brain regions involved during facial processing. The simple task of focusing on the face during social interactions for the normal group is found difficult by the autistic group.

In this study, functional magnetic resonance imaging (fMRI) was used as subjects performed two experimental tasks (EXPLICIT and IMPLICIT) in which a series of photographs of nine males and nine females displaying three affective states (6 fear, 6 happy and 6 neutral) and six scrambled-face control stimuli were presented to the subjects. Subjects were required to attend to and recognize the emotional content of the face (explicit task) or recognize the gender of the face (implicit task).

The autistic as well as the control group showed activation in the temporal lobe (middle and superior temporal gyrus) during explicit processing of facial expressions. Implicit processing of faces found that the autistic group showed significantly more activation in the left middle temporal gyrus, bilateral superior temporal gyrus than the control group.

The differences in face processing between the normal and autistic group probably arise out of the fact that autistic individuals have reduced social interest and do not regard the face as socially important.

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by
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## APPROVAL PAGE

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This thesis is dedicated to my family and close friends who have always encouraged and supported me in whatever path I have choosen. I appreciate their love and understanding.

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# CHAPTER 1

# INTRODUCTION

Autism is a neurodevelopmental disorder that is defined by deficits in social and communication impairment as well as restricted interests. Autism is a disorder that usually begins at infancy, or within, in the first three years of life [1]. Due to its links to genetics, neural development and severe abnormalities in social interaction, autism offers the opportunity for scientists to study the neurobiological origins of social communication skills basic to human behavior.

The superior and middle temporal gyrus are linked to recognition of faces, interpretation of biological movement, the fusiform gyrus to face recognition and the amygdala to recognition of emotion as well as social orientation [2]. Therefore, it is useful to study these brain regions in autistic individuals to define brain regions that are severely affected. Once the affected brain regions are identified and understood, strategies can be developed for prevention, early diagnosis and treatment of the disease.

All persons with autism have difficulties with normal social behavior. These range from social interaction with others, the use of eye contact and facial expressions. Therefore, the understanding of the brain regions responsible for the interpretation of facial expressions might be predictive of the regions that cause brain dysfunction in autism.

In most social interactions, the focal point is the human face but this simple task for normals is difficult for individuals with autism. Thus, the study of face processing in autistic individuals is important to understand the social deficits of this disorder.

In a neuroimaging study performed by Karen Pierce et al. [3], it was found that as compared to normal individuals, autistic individuals see faces utilizing different neural systems, with each patient doing so via unique neural circuitry. Recent studies carried out by Cohen et al. [4] found reduced amgydala activation in response to social intelligence whereas Critchley et al. [5] found reduced amgydala activation in response to emotionally processing task. Schultz and colleagues [2] have found reduced activity in the fusiform gyrus (FG) and increased activity in the inferior temporal gyrus (ITG) during a face-processing task.

It has been found that autistic individuals have some ability in face identification (i.e. distinguishing female faces from male faces (Teunisse et al. [6]) but cannot distinguish between normally oriented faces and inverted faces (Hobsen et al. [7]).

The primary goal of this study was to use functional magnetic resonance imaging (fMRI) to investigate face processing in autistic individuals as compared to normal controls so as to get an insight in the neural circuitry of autistic individuals.

### 1.1 Specific Aims/Hypothesis

(1) It was hypothesized that the normal controls would demonstrate significantly more activity in the temporal lobe while involved in explicit processing of facial expressions.

(2) It was also hypothesized that young adults with autism or Asperger's Disorder would demonstrate a similar pattern of activity as normal controls while involved in explicit processing of facial expressions

(3) The control group would show activation in the amygdala region while involved in the implicit processing of facial expressions.

(4) The autistic group would demonstrate significantly less activity than normal controls in the amygdala region while involved in implicit processing of facial expressions.

## 1.2 Background

Autism is a neurodevelopmental disorder characterized by impairments in social interaction, communication and imaginative activities. In the majority of cases, it is associated with mental retardation. However, studies of cognitive impairments in autism have focused on high-functioning individuals who have largely intact intellectual and linguistic functions (Happe et al [9]). These high-functioning individuals appear to suffer from relatively more subtle cognitive deficits in processing social and emotional information. However, these deficits also underline the more obvious social impairments of individuals who have severe autism.

Histopathological studies suggest that both low and high functioning individuals with autism exhibit abnormalities in the limbic system and cerebellum (Bauman & Kemper [10], Courchene et al. [11]). Reduced number of Purkinje cells in the cerebellum, reduced neuronal cell size and increased cell packing density have been identified in the hippocampal complex, subiculum, entorhinal cortex, amygdala, mamillary body, medial septal nucleus and anterior cingulated gyrus (Bauman & Kemper [10]).

The amygdala is an area of the brain situated within the medial temporal lobe that has been shown to process affective or emotional stimuli (Whalen et al. [12]). In animal

studies, removal of the amygdala in primates has been found to result in decreased affiliative behavior, social communication and emotional reaction to other animals (Kling and Brothers [13]). Lesions of the amygdala in newborn monkeys have been shown to produce patterns of social withdrawal; these findings led to the conceptualization of an animal model for autism (Bachevalier [14]). In human studies, amygdala damage has been linked to abnormalities of affect; poor face recognition and impaired memory for the emotional content of stories (Cahill et al. [15]). SPECT studies in patients with autism spectrum disorders have shown decreased blood flow in the temporal lobe (Gillberg et al. [16]). In addition, patients with tuberous sclerosis who have lesions in the temporal lobe have been found to have distinct autistic features (Bolton and Griffiths [17]).

Abell et al. [18] in a structural MRI study of 15 high –functioning young adults (12 males, three females) with autism found that they had increased gray matter volume in the left amygdala/peri-amygaloid cortex as compared with matched controls. This finding was in keeping with the histopathological abnormalities found in other studies, as reported above. They proposed that a neural circuit that centers on the amygdala is essential to the awareness of self and others. The ventral temporal cortex sends connections to the amygdala and there are projections from the amygdala to the inferior pre-frontal area and anterior cingulated cortex. These connections are reciprocal. These connections with the temporal lobe enable visual stimuli to be imbued with emotional meaning and the connections with the frontal lobe provide pathways through which mental states such as emotions can be monitored and modulated. Friston et al. [19] proposed that the amygdala integrates highly processed perceptual inputs that have value or significance. The synthesis within the amygdala is then used to modulate and

consolidate adaptive changes in synaptic activity throughout the brain via projections to the ascending modulatory neurotransmitter systems. This theoretical model proposes that a neurodevelopmental abnormality involving the amygdala would explain the social and emotional impairments that exist in autism.

Courchesne et al. [11], in a review of limbic neuroanatomical abnormalities in autism, concluded that amygdala abnormalities were probably connected to the abnormalities of emotional expression and responsiveness shown by autistic individuals. In this regard, it was noted that direct experimental evidence for the brain-behavior link was needed. Abnormalities within the amygdala/peri-amygdaloid cortex might give rise to distinct abnormalities in the processing of fear. Abell et al. [18] proposed that fear conditioning may be more rapidly established and less amenable to extinction in autism.

Facial expressions are a mechanism through which internal emotional states become available as external signals; therefore, the face is a vital part of social cognition. Adolphs et al. [20] found that bilateral damage to the human amygdala was associated with abnormalities in the recognition of fear. In addition, patients in this study were shown to have difficulty identifying other subtle facial expressions.

An increasing number of neuroimaging studies are showing that the human amygdala is activated in response to emotional stimuli (Breiter et al. [21], Cahill et al. [22], Irwin et al. [23], Morris et al. [24]). These studies implicate the amygdala in the non-conscious monitoring of emotional stimuli and emotional facial expressions.

Whalen et al. [12] in a functional MRI study with 10 right-handed normal male subjects (ages 19-32) used backwardly masked facial expressions to ascertain whether activation of the amygdala occurred in humans in the absence of conscious knowledge. In

this procedure, the presentation of the fearful and happy faces was so brief that the subjects could not recall having seen them. They found that the amygdala was activated significantly more during the viewing of masked fearful faces than during the viewing of masked happy faces. This study found that backward masking of emotional facial expressions resulted in near total isolation of the amygdala. This was in contrast to a study by Morris et al. [24] which demonstrated activation of the amygdala and four additional brain regions to the presentation of nonmasked fearful faces versus happy faces.

Whalen et al. [12] found that the signal (blood oxygen level dependent fMRI signal) increases to masked fearful faces, which have been habituated. The process of habituation to emotional stimuli that have proven to be unimportant has been confirmed in human studies (Breiter et al. [21], Irwin et al. 23]). However, signal intensity reductions in response to happy faces continued throughout the task. It appears that both fearful and happy faces give humans important information about whether an environment is potentially threatening, and this impacts differentially on the level of activity in the amygdala.

A fMRI study of Baron-Cohen et al. [4] investigated social intelligence in 12 normal adults (six males, six females) and six high-functioning individuals with autism or Asperger's Disorder (four males, two females). The study provided support for Brothers' [25] 'social brain' theory of normal function (the network of neural regions that comprise the 'social brain' include orbito-frontal cortex, superior temporal gyrus and the amygdala) and proposed an amygdala theory of autism. Baron-Cohen's study predicted abnormal amygdala activation in the autism group.

The subjects were given a 'theory of mind' task and a gender recognition task (subjects were shown photographs of eyes and asked to choose between two words that best described the mental state of the photographed person-alternating with assessing the gender of the photographed person). When comparing the performance of the control and autism groups using fMRI, it was found that the normal controls activated fronto-temoporal neocortical regions as well as non-neocortical areas (particularly left amygdala). In contrast, the autism group activated the frontal areas less extensively than the control group, and did not activate the amygdala at all. Baron-Cohen et al. concluded that the left amygdala might be critically involved in the identification of mental state/emotional information from complex visual stimuli, for example the eye region. Subjects with autism, when performing the task, did not activate the amygdala but showed a significant response in the bilateral superior temporal gyrus. They concluded that individuals with autism might be compensating for this amygdala dysfunction by using language and facial memory functions. The authors acknowledged that further study is necessary to assess whether patients with autism have general hypo functioning of the amygdala or whether this is specific to tasks, which involve attributing mental state to others.

The distinction has been made between effortful 'explicit' versus automatic 'implicit' processing of facial expressions. New evidence confirms a neuroanatomical dissociation between conscious and unconscious processing of emotional information. Critchley et al. [5] reported on a fMRI study, which investigated whether distinct brain areas are activated by explicit as compared with implicit tasks. They studied nine right-handed normal, healthy young men (mean age +/- SD: 27 years +/- 7). The task involved

presenting subjects with photographs of people with mixed happy and angry facial expressions ("on" condition) alternating with neutral faces ("off" condition). The subject's explicit processing of expressions was assessed by asking them to attend to, and judge, the facial expressions. The implicit processing required the subjects to attend to the same series of photographs but in this instance, to judge the facial gender. The study found that explicit processing of facial expression activated the middle temporal gyrus significantly more than implicit processing. In contrast, implicit processing was found to activate the amygdala region more than occurred during explicit processing. The study concluded that there exists a functional dissociation between neuronal pathways for conscious (explicit) versus non-conscious (implicit) processing of facial expressions.

The study by Karen Pierce et al. [3], involved seven autistic and eight normal controls where changes in blood oxygen level were measured as subjects performed a face perception task (i.e. button press in response to female neutral face) alternating with a shape perception task (i.e. button press in response to circles). The results showed that while the autistic individuals could perform the face perception task, none of the regions supporting face processing in normals were found to be significantly active in the autistic subjects. She therefore suggested that as compared with normal controls, autistic individuals perceived faces utilizing a different neural system, with each autistic subject doing so via unique neural circuitry.

Canli et al. [22] carried out a fMRI study to find out whether a network of structures was involved in the encoding of emotional memory. Subjects (normal controls) viewed alternating blocks of negative and positive pictures that were similar in emotional arousal and were tested for long-term recognition memory a few months later.

Recognition memory for negative pictures were correlated with recognition memory for positive pictures across subjects, so subjects who remembered negative pictures well also remembered positive pictures well.

George, Driver and Dolan [26], in a fMRI experiment investigated how gaze direction influenced face processing as gaze contact is an essential part of social interaction. This study suggests that the network involved in face processing changes as a function of seen gaze direction with greater fusiform-amygdala activity for direct gaze, and greater fusiform-parietal activity for averted gaze.

In summary, a growing body of research supports the idea that different brain structures exist giving rise to the social deficits in autism. Dysfunction within the amygdala and related circuits appear to be linked to difficulties with the implicit (automatic, non-conscious) and explicit (effortful, conscious) processing of social and emotional information from facial expressions.

## 1.3 Functional Magnetic Resonance Imaging (fMRI)

Functional Magnetic Resonance Imaging (fMRI) is the use of MRI equipment to detect regional changes in cerebral metabolism or in blood flow, volume or oxygenation in response to task activation. The most popular technique utilizes blood oxygenation level dependent (BOLD) contrast, which is based on the differing magnetic properties of oxygenated (diamagnetic) and deoxygenated (paramagnetic) blood. That is, when an area of the brain becomes metabolically active, oxygen delivery to this site increases. The resulting oxygenated blood has different magnetic properties from deoxygenated blood. Since BOLD fMRI is sensitive to local blood oxygenation, the "activated" brain site can

be seen non-invasively and is reflected as an increase in signal intensity on the MRI scan. BOLD fMRI has been used extensively to indicate the anatomical site of neuronal activation in response to specific task demands. Thus, BOLD fMRI offers the ability to precisely map brain processing centers non-invasively during task execution [27].

### 1.3.1 Magnetic Resonance Signal Formation

Magnetic Resonance Imaging (MRI) is based on the physics of Nuclear Magnetic Resonance (NMR). For imaging in biological systems, hydrogen or "proton" NMR is the most common, primarily due to its high concentration in the human body and high sensitivity (it gives rise to large NMR signals).

The proton nuclei of the hydrogen atom possess a small magnetic moment. When placed within a magnetic field, a torque will be exerted upon them, resulting in a slight energetic advantage of one orientation (parallel to the field over another (the anti-parallel orientation). Over time, random atom collisions allow the complete system to reach a magnetic and thermal equilibrium with an excess of protons aligned with the magnetic field. The combined alignment of all of these protons results in a net magnetic moment; a subject placed within magnetic field thus, becomes "magnetized" [28]. In addition to their magnetic moment, atomic nuclei possess angular momentum –a quantum property known as "spin". Due to this angular momentum, rather than aligning simply with the magnetic fields, the individual nuclei precess about it (Figure1.1). The precessional rate or frequency is the characteristic of the atomic nucleus (eg. protons) and is proportional to the strength of the magnetic field, a property crucial to the process of image formation.

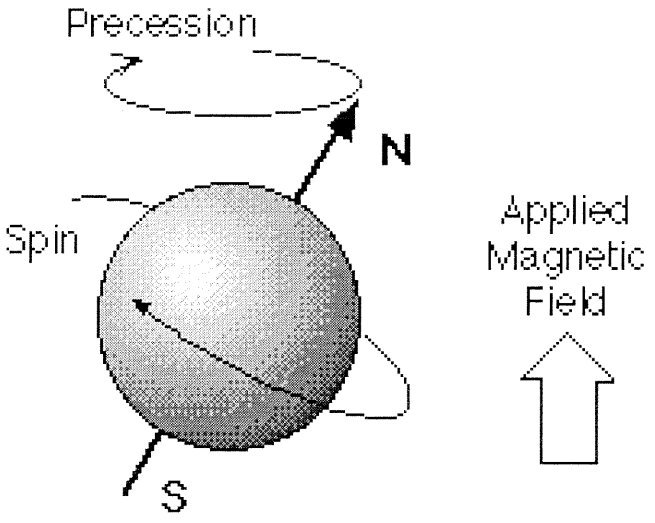



**Figure 1.1** Magnetic Properties of the proton nucleus of the Hydrogen atom [28].
(Left- The hydrogen proton possesses the quantum property of "spin" or angular momentum, and has a small magnetic dipole moment. When placed in a magnetic field, a torque is exerted on the particle, causing it to precess about the applied field. Right- The precessional frequency of the proton is directly proportional to the magnetic field strength).

Figure 1.2 shows that the proton magnetization can be decomposed into the sum of a stationary (longitudinal) and a rotating (transverse) component. Each proton nucleus within a magnetic field thus yields a tiny field that rotates about the applied field. The rotating field from the individual nuclei is generally aligned at random with respect to other protons in the subject.

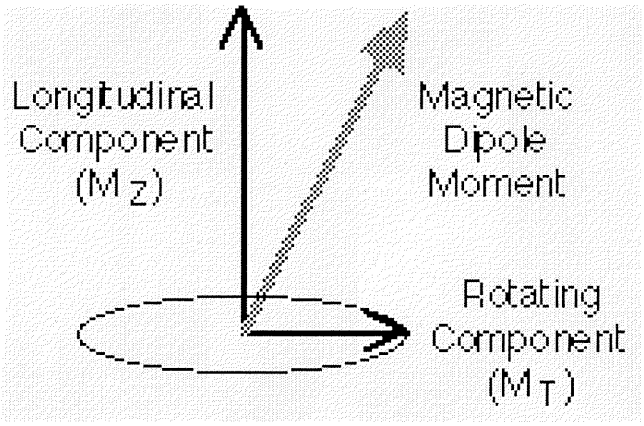



**Figure 1.2** Vector description of proton magnetization [28].
(The rotating magnetic moment of the proton can be decomposed into a longitudinal component, along the applied magnetic field, and a transverse component orthogonal to it and rotating (precessing) about it).

A coil placed near to the subject can detect this precessing magnetization as shown in Figure 1.3

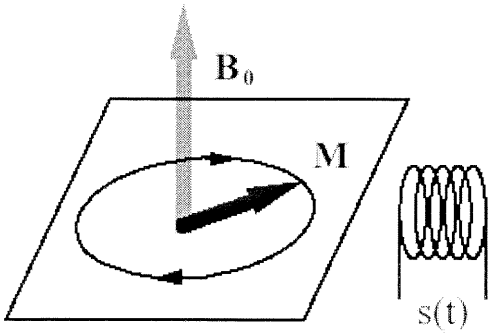



**Figure 1.3** Excited magnetization precessing around the static magnetic field thus, inducing a voltage, s (t), in a nearby coil [27].

A second magnetic field is applied which is orthogonal to the static field, and which rotates about the static field at the precessional frequency of the atomic nuclei. When the rotating field is present, the nuclei will precess about it, forcing the magnetization away from the equilibrium, and causing the ensemble of protons to precess together, or "in-phase". The combined rotating magnetic moment thus, produced by the ensemble of protons is observable as a time varying electromagnetic (radio) signal as the frequencies are similar to those used in radio transmisson. The second, rotating magnetic field is applied at radio frequencies and is therefore, known as an "RF" pulse [27].

### 1.3.2 Signal Characteristics

The contrast in a MR image is strongly dependent upon the way the image is acquired. By adding radio frequency or gradient pulses, and by careful choice of timings, it is possible to highlight different components in the object being imaged. The basics of contrast are the spin density throughout the object. If there are no spins present in a region it is not possible to get a signal at all. Proton spin densities depend upon the water content, typical values of which are given in Table 2.1 for various human tissues. The low proton spin density of bone makes MRI a less suitable choice for skeletal imaging. Since there is such a small difference in proton spin density between most other tissues in the body, other suitable contrast mechanisms must be employed. These are generally based on the variation in the values of T1 and T2 for different tissues.

**Table 2.1** Water Content of Various Human Tissues

| Tissue | % Water Content |
|---|---|
| Grey Matter | 70.6 |
| White Matter | 84.3 |
| Heart | 80.0 |
| Blood | 93.0 |
| Bone | 12.2 |

Two fundamental parameters are used to describe the MR signal. The magnetization, which has been created in the direction transverse to the steady magnetic field, induces a signal voltage. If the water protons are excited again before full recovery, a smaller signal is obtained. The rate of recovery is described by a 'longitudinal relaxation time' T1. Thus, the longitudinal relaxation rate, T1, is the rate at which nuclei, once placed in a magnetic field, exponentially approach thermal equilibrium, so that the magnetization (M) is described by the formula [28]

$$M = M0\,(1 - 2\exp(-t / T1)) \qquad (1.1)$$

where M0 is the equilibrium magnetization.

In biological tissues, T1 is quite long, from tens of milliseconds to seconds. Differences in T1's of tissues are one of the primary bases of contrast in clinical MRI. In the brain, there are three types of tissues of interest, gray matter, white matter and the cerebrospinal fluid (CSF). CSF has the longest T1 relaxation time while the white matter

has the shortest T1 and the gray matter's T1 relaxation time is closer to that of the white matter than CSF. An image whose signal intensity depends on the T1 relaxation time is called T1 weighted image [28].

A second parameter time constant describes the rate at which the MR signal decays. Once a MR signal is formed, i.e. after a RF pulse, it fades quickly. Small variations in the local magnetic field, for example those caused by neighboring magnetic nuclei, cause the protons to precess at slightly different frequencies, resulting in a dispersion of phase angles and hence a signal which decreases with time. The decay of the signal is generally exponential and can be characterized by the time constant T2, the 'transverse relaxation time'. In addition, if there are magnetic field variations across the subject caused by paramagnetic particles (deoxyhemoglobin in the body), there is a further phase dispersion causing a more rapid decay of the signal. This additional relaxation is denoted by T2'. Together the two effects result in a signal decaying with a time constant T2*, where $1/T2^* = 1/T2 + 1/T2'$. T2* relaxation time generally ranges from a few milliseconds to tens of milliseconds. An image whose signal intensity depends on the T2* relaxation time is called a T2 weighted image. CSF has the shortest T2* relaxation time while the white matter has the longest T2* and the gray matter is in the middle.

This study was carried out to detect blood oxygen level dependent (BOLD) changes in the different brain regions of the subjects. As the subjects performed the tasks (implicit and explicit), changes in blood flow to the brain were detected as an increase in the MRI signal.

# CHAPTER 2

# MATERIALS AND EXPERIMENTAL METHODS

## 2.1 Subjects

Two Groups (all subjects between ages 18 and 30) diagnosed with Autism or Aspergers's Disorder and controls w`ere recruited for the study. All subjects provided informed consent before participating in the study, according to guidelines established by the Institutional Review Board of the University of Medicine and Dentistry of New Jersey.

Group I : Seven male subjects diagnosed with autism or aspergers's disorder as measured on the ADI-R (Autism Diagnostic Interview-Revised [40]), the ADOS-G (Autistic Diagnostic Observation Schedule-Generic [41]) and the DSM-IV (Diagnostic and Statistical Manual [42]).

Many individuals with autism or aspergers's disorder were taking psychotropic medication (eg. selective serotonin reuptake inhibitors and atypical neuroleptics) for symptoms and behaviors related to these disorders. Subjects on medication were not required to discontinue these in order to qualify for inclusion in the study. This decision was made on the basis of (a) the ethical concerns about withdrawing people from medication (especially in a study which includes no direct therapeutic benefit to subjects) and (b) the pervasive social communication deficits that persist despite being on these medications.

Subjects suffered from no other major psychiatric disorder in the previous 12 months. Subjects had no history of seizures for at least the last two years prior to entering the study.

Group II : Eight normal, healthy male controls with no psychiatric diagnosis, as measured on the SCID I and II (Structured Clinical Interview for DSM-IV for both Axis I and Axis II disorders).

Subject groups were matched for mean age, handedness (as defined by the Edinburgh Inventory-Oldfiled, 1971), Verbal IQ, educational level and socio-economic status.

Patient Recruitment: Patients were recruited from the following sources:

(1) Dr. Cartwright's clinical consultations at the Autism and Anxiety Disorders Program (UMDNJ Newark)

(2) Autism advocacy groups (such as ASA, CAN, NAAR, COSAC and ASPEN)

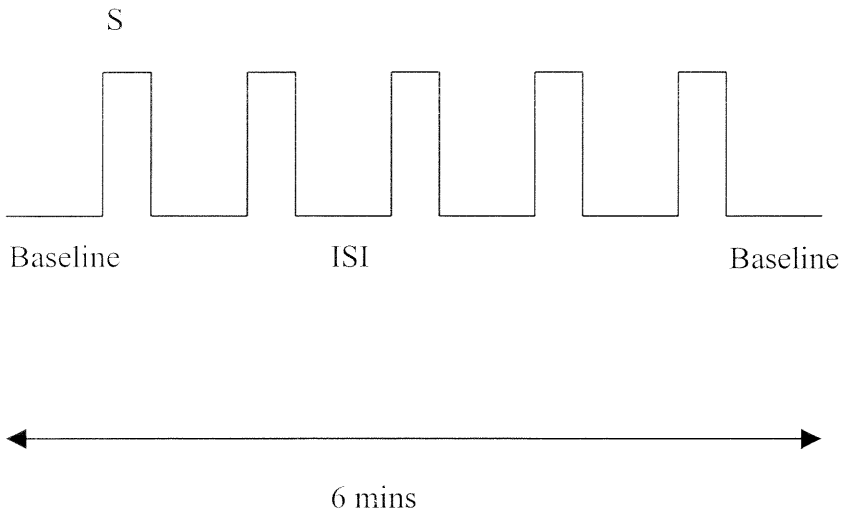
(3) Patients referred from the Seaver Autism Research center, Mount Sinai School of Medicine

(4) Advertising (newspapers, internet, etc)

A single gender cohort was studied in order to minimize heterogeneity and improve statistical power. The median male/female ratio of individuals with autism whose intellectual functioning falls within the normal range was 6:1 (Fombonne, 1999). Given these factors as well as the increased availability of males for recruitment, only males were studied. Future studies will include females to assess the generalizability of the current study's results. All subjects had a verbal IQ greater than 85, as measured on the Wechsler Adult Intelligence Scale (WAIS-III) (Wechler, 1997).

## 2.2 Experimental Task

Two experimental tasks (EXPLICIT and IMPLICIT) were carried out in which eighteen facial stimuli and six scrambled-face control stimuli were presented to the subjects with a baseline at the beginning and the end of the task as well as interscan intervals between each face stimulus (Figure 2.1).




Baseline - 30 secs, S - Face Stimuli - 4 secs, ISI - Interscan Interval - 5, 8, 11secs

**Figure 2.1** Experimental task.

Photographs of nine males and nine females displaying three affective states (6 fear, 6 happy and 6 neutral) were chosen from a published series ('Pictures of facial Affect,' Ekman and Friesen, 1976 [29]). Each stimulus displayed a happy, a fearful, a neutral or a scrambled facial expression as shown in Figure 2.2.

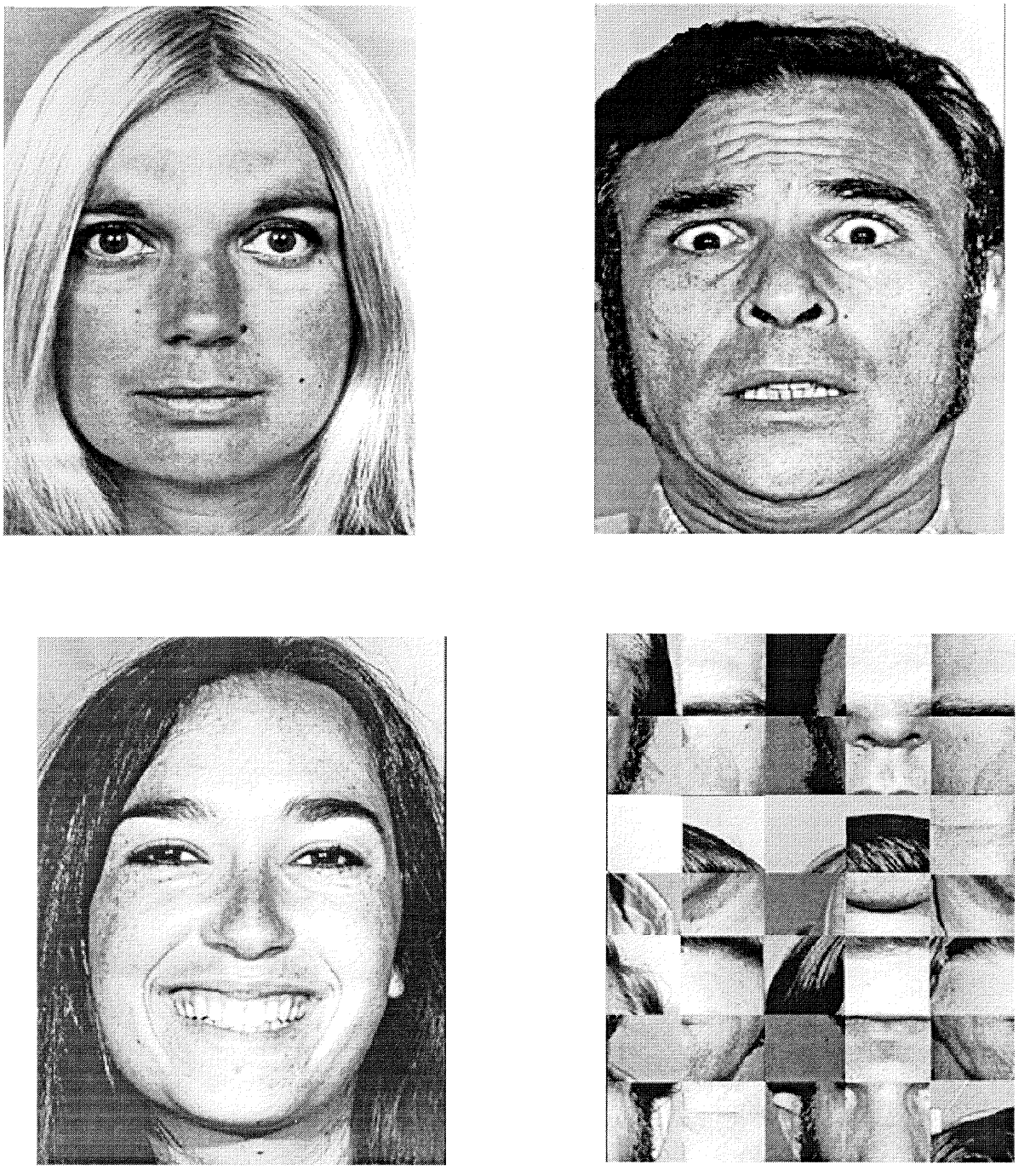


**Figure 2.2** Examples of faces taken from Ekman and Friesen series [29].

Happy and fearful expressions were chosen to represent powerful signals of social acceptance or rejection, and neutral facial expression was chosen for the control stimuli because it contains similar visual complexity, but conveys less information of immediate social valence.

*Explicit task* : Subjects viewed six different individuals with three facial expressions, and six scrambled-face control stimuli (total of twenty four faces), with presentation in a continuous pseudorandom order. Each photograph presentation lasted 4 seconds with an interscan interval (ISI) of 5, 8 or 11 seconds between each presentation. The ISI was varied over a range of values to increase the power of the design and also for behavioral reasons. It is critically important that the subjects are unable to predict a trial. The subjects were instructed to judge the emotional content of the face, distinguishing neutral faces from those displaying affect (fearful or happy), the response was collected using a fiber optic button (i.e. if the subject saw a fearful face, to press the button in the right hand and if the subject saw a happy or neutral face, to press the button in the left hand). Subjects were instructed not to press a button for the scrambled-face stimuli. This task lasted for six minutes.

*Implicit task:* Using the same paradigm for the implicit task, the subjects made a different response. The subjects responded to gender using the button pad, to press the button in the right hand for male faces and to press the button in the left hand for female faces. Again subjects were instructed not to press any button in the scrambled-face trials. This task lasted for six minutes.

*Design of the Paradigm:* The paradigm was designed using E-Prime software. There are basically two types of methods used to setup the paradigms for fMRI studies. They are

block trial and event related designs. In block task paradigms, multiple trials of a particular condition are grouped together in blocks and are presented in sequences (figure 2.3a) whereas event related paradigms allow different trials or stimuli to be presented in arbitrary sequences, Thus, eliminating potential confounds such as habituation, anticipation and other strategy effects (figure 2.3b).

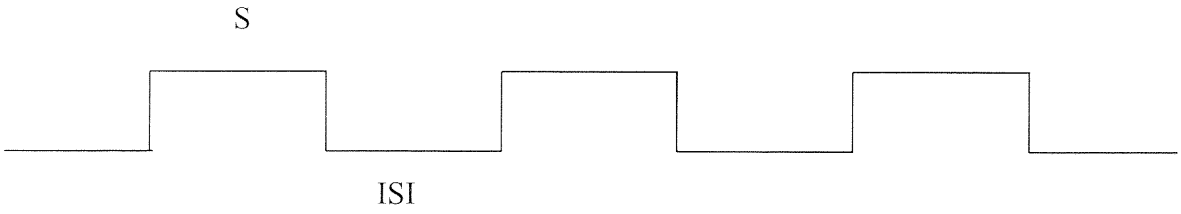
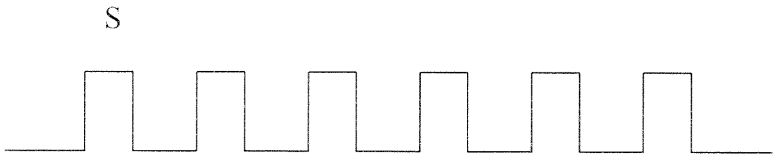



**Figure 2.3a** Block Design.




S - Stimuli, ISI – Interscan Interval

**Figure 2.3b** Event Related Design.

In a blocked design, the analysis proceeds by examining the signal change averaged across the entire block whereas in event related design, the analysis focuses on responses to single events Thus, providing a means of examining questions regarding the dynamics and time course of neural activity under various conditions. In other words, event related design allows detection of the Blood Oxygen Level Dependent (BOLD) hemodynamic response to neural activity. For the current study, event related design was selected as it best fitted to the requirements as explained above. The paradigm was counterbalanced such that tasks (explicit and implicit) were repeated an equal number of times.

### 2.3 fMRI Image Acquisition

All functional MRI studies were performed on a 1.5 Tesla GE Scanner using a whole body echo planar imaging (EPI) gradient system with a whole-head transmit-receive coil. Echo planar Imaging (EPI) is a technically demanding form of MRI, usually requiring specialized hardware. However, it has the advantage of being a very rapid imaging technique capable of capturing moving organs like the heart and dynamically imaging brain activation. Foam cushions were utilized to immobilize the head within the coil to minimize motion degradation and subjects wore MRI compatible earmuffs, fitted to a speaker for presentation of auditory stimuli, and for protection against scanner noise during acquisition. Stimuli were presented using AVOTEC goggles.

Initially, T1 anatomical images were obtained for overlay on functional data. The acquisition matrix was 256 x 256mm, the field of view (FOV) 240 x 240 mm with 28 slices at 5mm thickness. The functional MRI images were collected using T2* weighted multislice gradient echo EPI, the acquisition matrix was 64 x 64 mm, the field of view

(FOV) 240 x 240 mm with 28 slices at 5mm thickness delivering a voxel resolution of 3.75 x 3.75 x 5mm. The total experimental session did not exceed one hour.

## 2.4 Data Analysis

The data were analyzed using Statistical Parametric Mapping (SPM99) software developed by Department of Cognitive Neurology, London, UK [30]. Statistical Parametric Mapping refers to the construction of spatially statistical processes to test hypotheses about regionally specific effects. Statistical parametric maps (SPMs) are image processes with voxel values that are, under the null hypotheses, distributed according to a known probability density function (usually Gaussian). Statistical parametric mapping is a framework, subsuming the general linear model and the theory of Gaussian fields, that allows for a diverse interrogation of the functional imaging data.

### 2.4.1 Spatial Pre-processing Steps

The steps are setting the origin, slice timing, realignment, coregistration, normalization and smoothing.

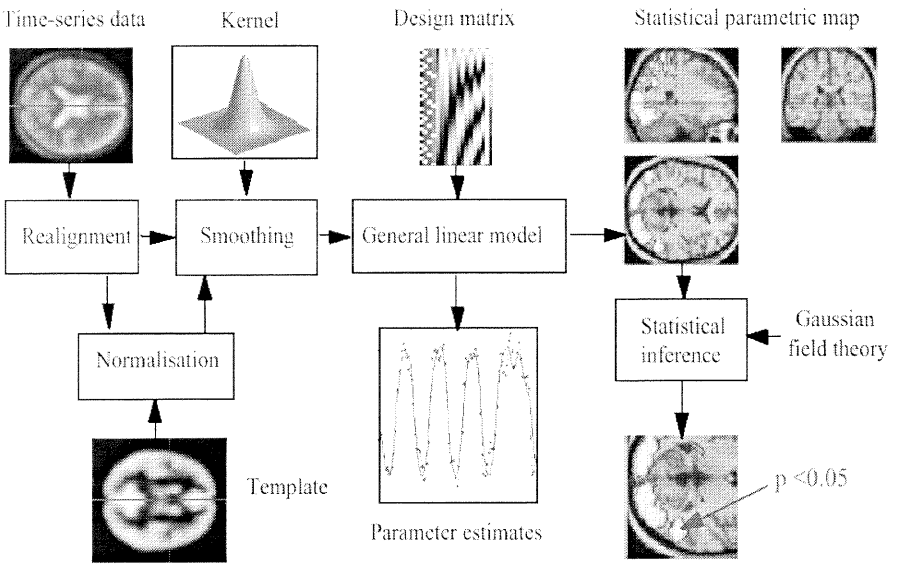



**Figure 2.4** Basic concepts and overview of SPM [19].

*Setting the origin:*

This step involves setting the origin of all images. The origin of the images is the reference point from which all locations are measured in millimeters. In order to make inferences regarding patterns and areas of activation between subjects, data is standardized to an anatomic atlas. The Talairach and Tournoux Atlas [31] provides such a brain standardization map, and is a commonly used coordinate system for functional brain studies. The origin of the standard space was chosen to be an anatomical landmark called the Anterior Comissure (AC) and so the origin of all the images was set to this same anatomical location.

*Slice timing:*

This adjusts for the timing differences during multi-slice image acquisition (fMRI) and therefore, corrects for timing differences between slices.

*Realignment:*

Subject head movement during the experiment is a major source of artifacts in fMRI data. It is common therefore, in fMRI data analysis to perform some motion correction, which reduces this effect.

The approach taken here is to correct only for in-plane translations and rotations of the head within the image. To realign the images, the brain was considered a rigid body, which means that six parameters are necessary to transform the rigid body into the space of another. The six parameters are x, y, z translations (mm) and pitch (x rotation in radians), roll (y rotation in radians) and yaw (z rotation in radians) as shown in figure (2.5). Working on a slice-by-slice basis, the first image is taken to be the reference image, to which all other images of that slice are to be aligned. Two-dimensional rotations and translations are applied to the second image, and the sum of the squares of the difference (ssd) between pixels in the first and second image is calculated. Further translations and rotations are applied to the image until the ssd is minimized. All subsequent images are realigned in the same way.

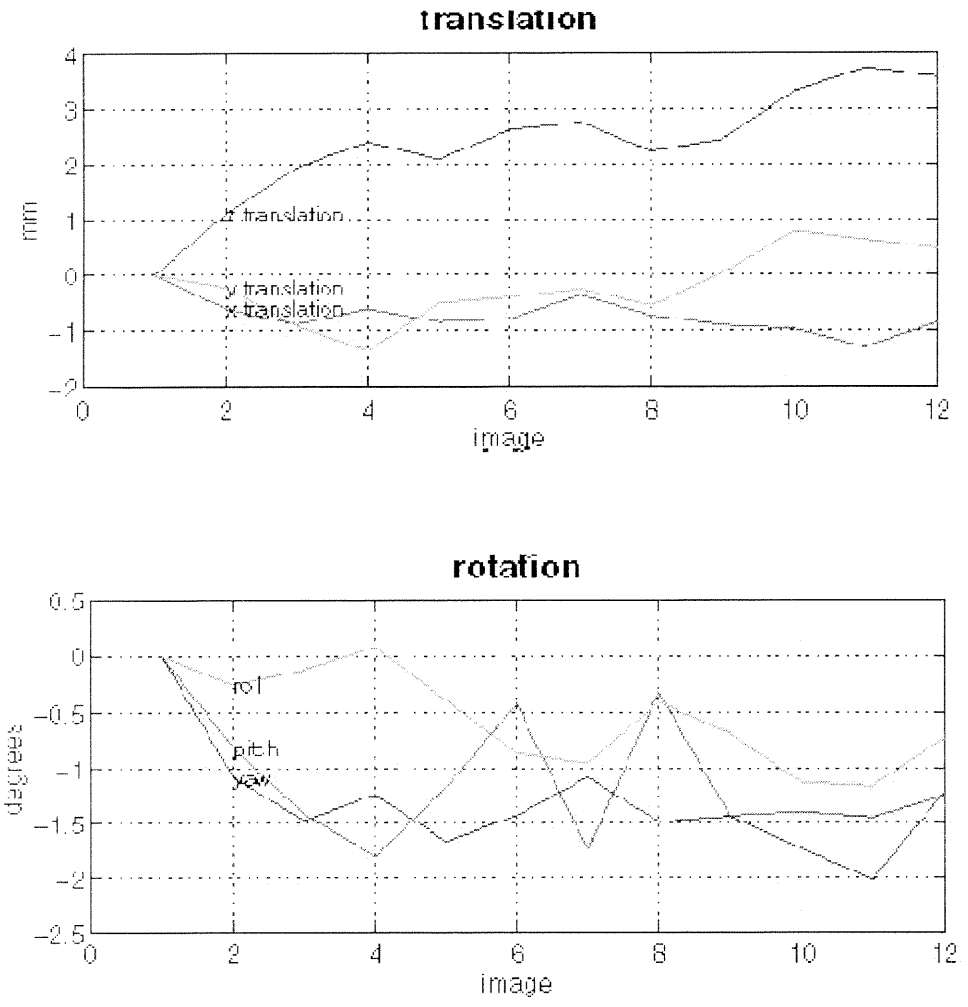


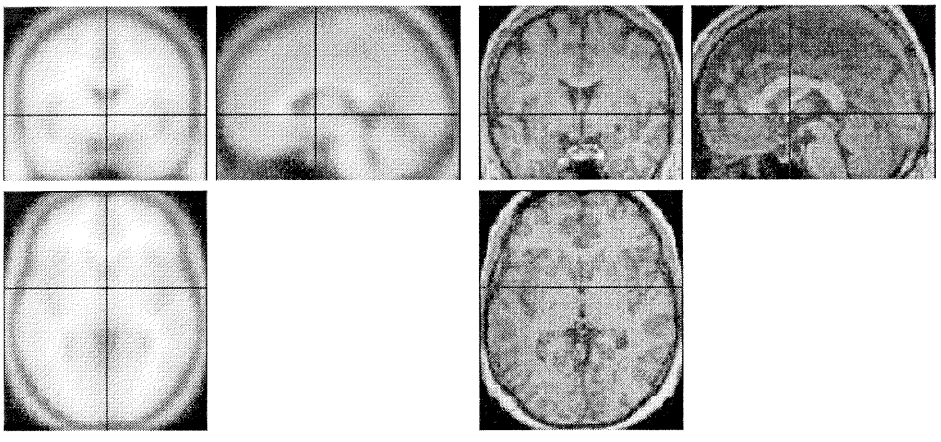

**Figure 2.5** Image translation and rotation [30].

*Coregistration:*

Coregistration allows for a valid overlay of the functional data onto the anatomical acquisition by registering functional acquisitions to the high-resolution anatomic acquisition.

*Normalization:*

MRI scans from multiple individuals vary greatly due to differences in brain features (i.e. brain size and shapes varies across individuals). Therefore, it is generally useful to 'normalize' scans to a standard template in order to make inferences regarding patterns and areas of functional activation between subjects as shown in figure2.6. Normalization is the process of translating, rotating, scaling and warping a brain to roughly match a standard template image. At the time of each functional study, a high resolution T1 weighted MRI acquisition was also be performed for anatomical localization. This high-resolution anatomical scan was used in combination with the functional data sets for normalization to standard coordinates. After normalization, locations are reported using stereotaxic ("Talairach") coordinates. This format uses three numbers (X, Y, Z) to describe the distance from the Anterior Commissure (the 'origin' of Talairach space). The X, Y, Z dimensions refer left right, posterior-anterior, and ventral-dorsal, respectively.



(a) Image after Normalization

(b) Image before Normalization

**Figure 2.6** Spatial normalization of the image [30].

Normalization consists of two steps: first the determination of an optimum 12-parameter (three translations, three rotations, three zooms, three shears) affine transformation (defined below) (from an image to a template), followed by a nonlinear estimation of deformation. Thus, the affine spatial normalization [32] describes the first step involved in registering images of different subjects in to roughly the same coordinate system. The additional zooms and shears are needed to register heads of different shapes and sizes. This 12-parameter transformation corrects for the variation in position and size of the image, before more subtle differences are corrected by a non-linear registration. Non-linear Spatial Normalization corrects for gross differences in head shapes that cannot be accounted for by affine normalization alone.

*Affine Spatial Normalization:*

In order to average signals from functional brain images of different subjects, it is necessary to register the images together. This is often done by mapping all the images into the same standard space (Talairach and Tournoux [31]). All between subject coregistration or spatial normalization methods for brain images begin with determining the optimal 12 parameter affine transformation that registers the images together.

The objective is to fit the image to a template, using a 12 parameter affine transformation (parameter p1 to p12). The images may be scaled quite differently, so an additional intensity scaling parameter (p13) is included in the model. An affine transformation mapping (via matrix M, where the matrix elements are the parameter p1 to p12) from position x in one image to position y in an another is defined by [32]:

$$\begin{pmatrix} y_1 \\ y_2 \\ y_3 \\ 1 \end{pmatrix} = \begin{pmatrix} p_1 & p_4 & p_7 & p_{10} \\ p_2 & p_5 & p_8 & p_{11} \\ p_3 & p_6 & p_9 & p_{12} \\ 0 & 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} x_1 \\ x_2 \\ x_3 \\ 1 \end{pmatrix} \tag{2.1}$$

This mapping is often expressed as a simple matrix multiplication

$$y = Mx \qquad (2.2)$$

The elegance of formulating these transformations in terms of matrices is that several transformations can be combined by simply multiplying the matrices together to form a single matrix.

*Non-linear Spatial Normalization:*

Statistical Parametric Mapping using functional magnetic resonance images (fMRI) necessitates the transformation of images form several subjects into the same anatomical space. The basic idea is to use a target (template) image to define the standard space into which the different subjects are warped. By using a template, which conforms to the space of a standard coordinate system, such as that defined by Talairach and Tournoux (1988), it is possible to report anatomical positions in terms of Cartesian coordinates, relative to some reference (Anterior Commissure).

*Smoothing:*

Smoothing has three important objectives:

a) It increases the signal to noise ratio.

The neurophysiological effects of interest are produced by hemodynamic changes that are expressed over spatial scales of several millimeters, whereas noise usually has higher spatial frequencies. In fMRI, the noise can be regarded as independent for each voxel and has therefore, very high spatial frequency components.

Any reduction in the random noise in the image will improve the ability of a statistical technique to detect true activations. Smoothing each of the images improves the signal to noise ratio (SNR), but will reduce the resolution in each image, and so a balance must be found between improving the SNR and maintaining the resolution of the functional image. To convolve an image with a two dimensional Gaussian, the image is first convolved in one direction (horizontal) and then the image is convolved in the other direction (vertical) as shown in figure (2.7). A three dimensional convolution is the same, except for an additional convolution in the third direction which is of the form [32]

$$f(x,y,z) = \exp\{-(\frac{x^2}{2sx^2} + \frac{y^2}{2sy^2} + \frac{z^2}{2sz^2})\} \qquad (2.3)$$

where sx, sy and sz are the standard deviations of the gaussian in each direction.

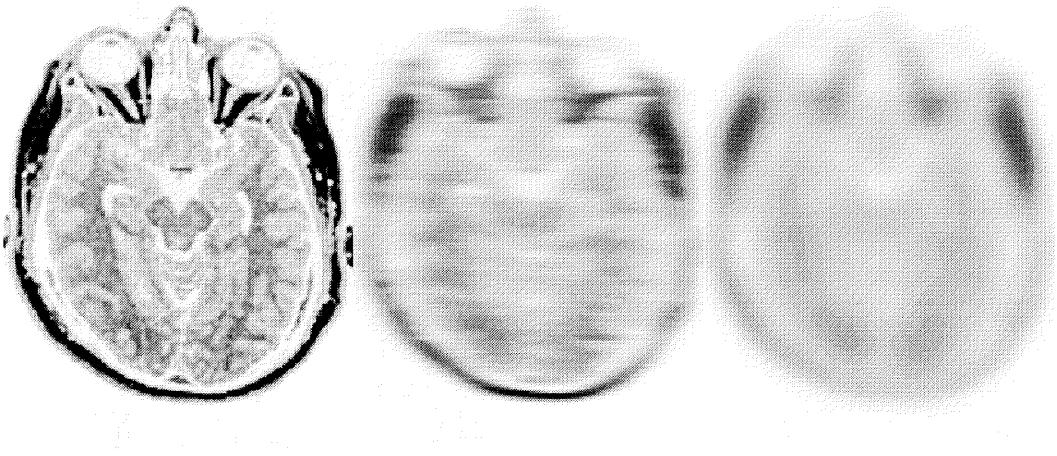


**Figure 2.7** Convolution with a two dimensional Guassian. The original image (left) is convolved horizontally (center), and then this image is convolved vertically (right) [32].

A good estimate of the extent of such a smoothing is given by the full width at half maximum (FWHM) of the gaussian kernel. The relationship between standard deviation and FWHM is

$$FWHM = 2.35\ s \tag{2.4}$$

b) It conditions the data so that they conform more closely to a Gaussian field model. This is important if the gaussian field theory is used to make statistical inferences about regionally specific effects (i.e. assign p-values).

c) Is specific to intersubject averaging. It ensures the hemodynamic changes from subject to subject are assessed on a spatial scale at which homologies in functional anatomy are typically expressed.

Finally, the data was filtered using a band pass filter. A high pass filter was used to filter out the low frequency components in the fMRI signal, which contained noise. A low pass filter that would attenuate the high frequency components was also used to smooth the data. The shape of this filter was the hemodynamic response function.

During event related statistical analysis, SPM uses a hemodynamic response function to model the occurrence of each event. Therefore, in order to map the brain activity on the transient fMRI signal, it is important to understand the basic nature of the BOLD contrast hemodynamic response. The ideal hemodynamic response function [33]

consists of two gamma functions, one positive and the other negative as shown in figure 2.8 below.

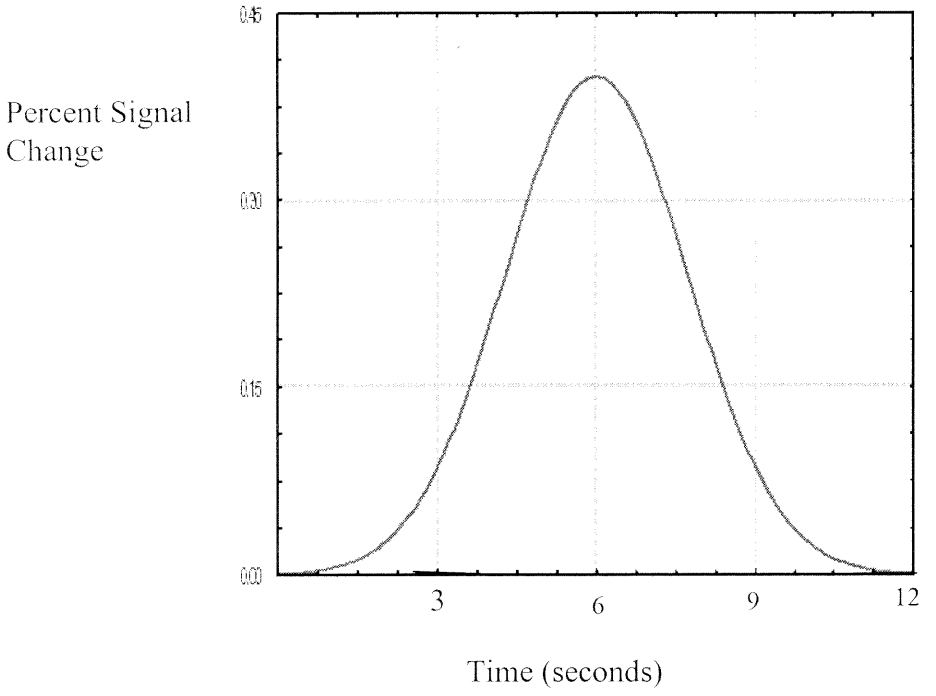



**Figure 2.8** The hemodynamic response [33].

In reality, the hemodynamic response is delayed in onset occurring about two seconds after neuronal activity, followed by a rapid rise in signal strength peaking at 5-7 seconds. A slow return to the baseline is completed by about 12 seconds.

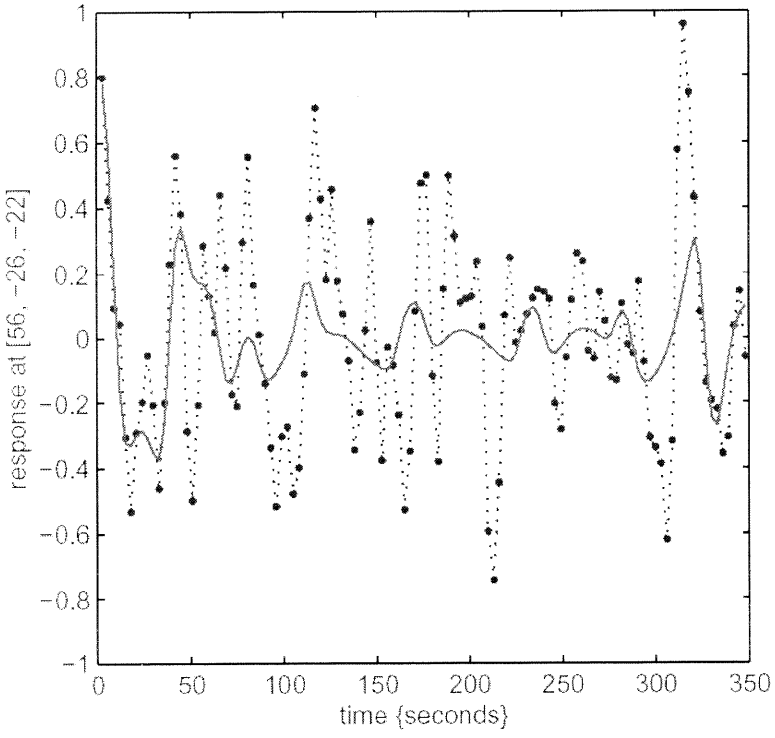



**Figure 2.9** The visual stimulus is shown as a dotted line together with data (shown as dots) and their modeled response using a gaussian function-hemodynamic response (solid line).

### 2.4.2 Statistical Analysis

The first step in the statistical analysis is the creation of the design matrix. A design matrix is made up of column vectors, which predicts the physiological responses to the changing task conditions. Hence, the design matrix defines the experimental design and nature of hypothesis testing.

The design matrix consists of two conditions i.e. face (fearful, happy, neutral) stimuli and baseline stimuli (Figure 2.10). The columns of the design matrix represent each of these conditions of the experiment and the rows of the design matrix represent the

time points of the experiment. Contrast weights are requested only for parameters of interest, weights for other parameters are set to zero. It is a good idea to keep these weights orthogonal (i.e. sum to zero). For example, in this study, the effect of face stimuli to baseline stimuli was assessed using a contrast that was 1 in all the face conditions, -1 in the baseline conditions and 0 elsewhere (1, -1, 0). This contrast asks the question, what voxels showed increases in the first condition relative to the second i.e. the second column is subtracted from the first column.

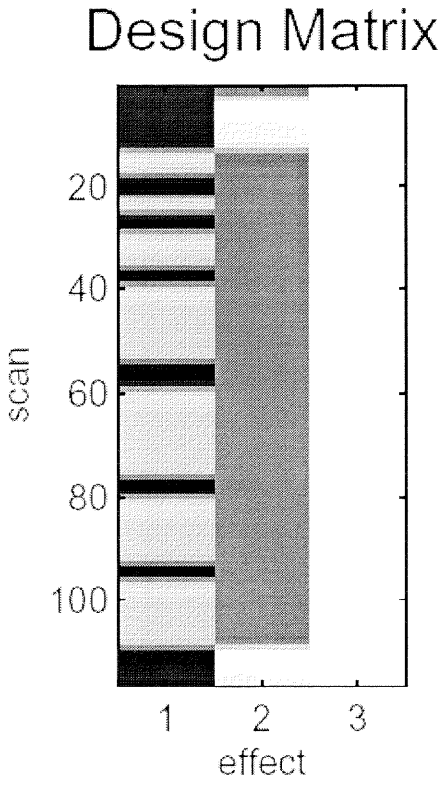



**Figure 2.10** Design Matrix.

The analysis uses the general linear model to assess the contribution or differences among parameter estimates (specified by a contrast).

*The General Linear Model:*

The general linear model is simply an equation that relates what one observes, to what one is expected to see. This is a standard statistical technique that is used in mostly all commercial statistics packages. The aim of the general linear model is to explain the variation of the time course y1...yi...yn, in terms of a linear combination of expected components (or explanatory variables) and an error term.

For a simple model with explanatory variable x1...xi...xn, the general linear model can be written as $yi = xiB + ei$

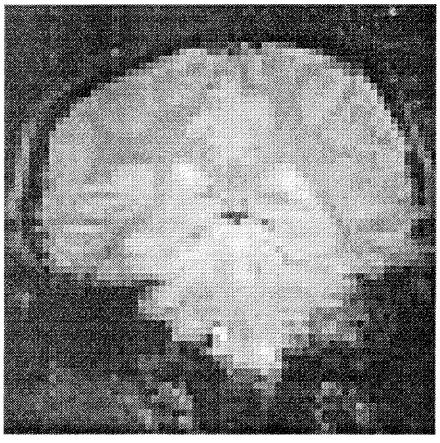
where B is the scaling or slope parameter, and ei is the error term. If the model has more variables it is convenient to write the general linear model in the matrix form

$$\mathbf{Y = XB + e}$$

where now Y is the vector of observed pixel values, B is the vector of parameter estimates (regression weights) and e is the vector of error terms. The matrix X is known as the design matrix. It has one row for every point in the original data, and one column for every explanatory variable in the model. In analyzing an fMRI experiment, the columns of X contain vectors corresponding to the 'on' and 'off' elements of the stimulus presented. By finding the magnitude of the parameter in B corresponding to these vectors, the presence or absence of activation can be detected.

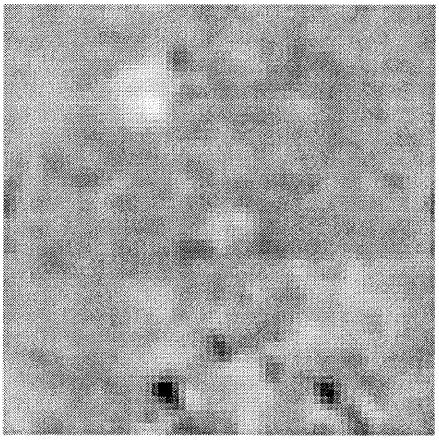
The contrasts of the parameter estimates in the design matrix are assessed. The result is a statistic t distribution in terms of a t value for each and every voxel (i.e. SPM {t}). The t-tests either look at positive or negative differences between parameter estimates. After calculating the t statistic, SPM converts the t statistic to Z scores i.e. SPM {Z}. Z scores are a way that SPM uses to display and analyze the p values (threshold) for the t statistic. The Z scores are the numbers from the unit normal distribution that would give the same p value as the t statistic. One of the simplest methods for obtaining a result from a fMRI experiment is to perform a simple subtraction. This is carried out by averaging together all the images acquired during the 'on' phase of the task, and subtracting the average of all the 'off' images. The disadvantage of such a technique is that it is extremely sensitive to head motion, leading to large amounts of artifact in the image. This can give rise to a ring of apparent activation near the brain boundaries. Figure 2.11a shows single slice through the motor cortex and figure 2.11b shows the result of subtracting the 'off' images from the 'on' images. Although signal increase can be seen in the primary motor cortex, there is also a large amount of artifact, particularly at the boundaries in the image. To reduce the effect of motion artifact, and to give a statistic of known distribution, a t-test can be used. This weights the differences in means, by the standard deviation in 'off' or 'on' values, giving high t-scores to large differences with small standard deviation, and low t-scores to small differences with large standard deviations. An image where each pixel is assigned a value based on the output of a statistical test is commonly known as a statistical parametric map. Figure 2.11c shows the statistical parametric map of t-scores for the sample data set.

Again, motor cortex activation is clearly seen, but the movement artifact is reduced compared to the subtraction technique.



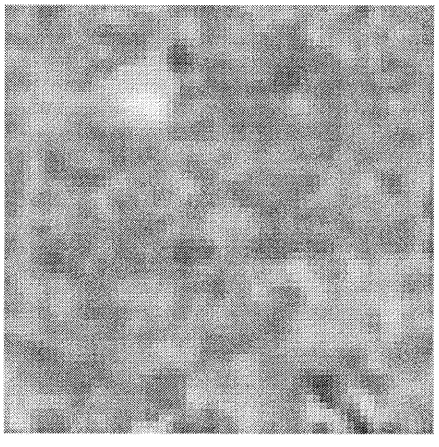
(a)

A single slice coronal EPI image through the primary motor cortex.



(b)

The mean of the images acquired during the 'off' period of the fMRI experiment subtracted from the mean of the imaged acquired during the 'on' period.



(c)

The t-statistical parametric map corresponding to image

**Figure 2.11** Use of subtraction techniques to analyze fMRI data [30].

The basics of the t-test is to define a resting state baseline, and compare the images acquired at each time point before, during and after the task with this baseline. Figure (2.12) illustrates the technique. For each time point following the stimulus, a mean and standard deviation image is constructed, as is a baseline mean and standard deviation image. Then a set of t-statistical parametric maps are formed by calculating, on a pixel by pixel basics, the t-score for the difference between mean image one and mean baseline image, mean image two and baseline, and so on.

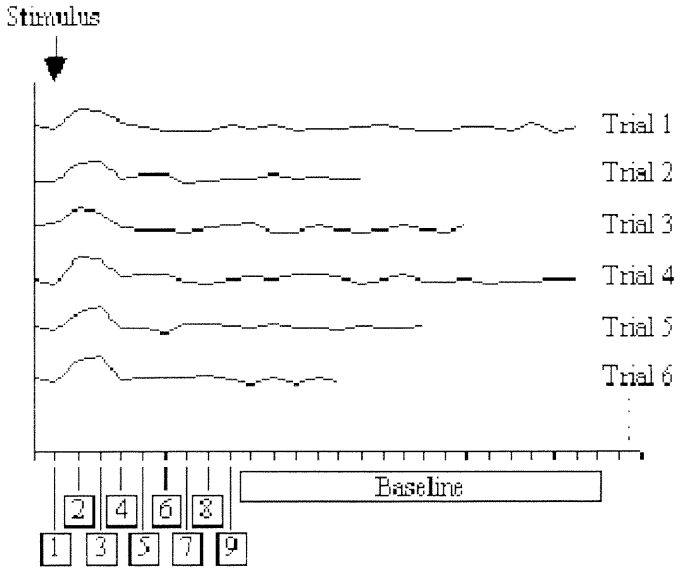



**Figure 2.12** The result of analyzing the data set using t-test [30].

### 2.4.3 Statistical Inference

The statistical analysis results in a statistical parametric map (SPM), which is spatially extended statistical processes that are used to test hypotheses about regionally specific effects in neuroimaging data. However, for localization and comparison of brain function, what is required is an image, which only highlights those pixels, which can be confidently labeled as active. This is done by thresholding the statistical parametric map. The threshold can be of two types (i) a critical height that the region has to reach or (ii) a critical size (above some threshold) that the region must exceed before it is considered significant.

To determine the threshold value, the distribution under the null hypothesis of the statistic being considered has to be understood. For example, consider a statistic that had a normal distribution as shown in Figure (2.13).

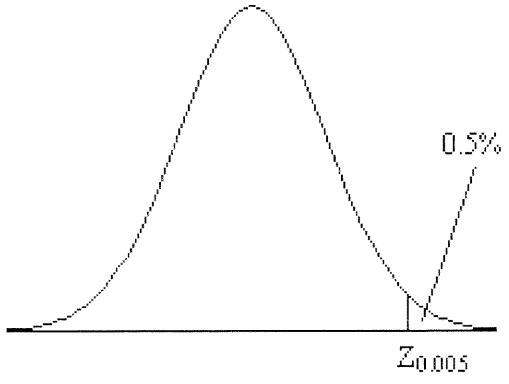



**Figure 2.13** The area under the normal distribution between Z0.005 and Zinf is 0.5% that of the total area under the curve [30].

This means if the value of Z obtained in the statistical calculation is greater than 0.005, we can be 99.5% confident the null hypothesis is false. The number 0.005 in such a case is said to be the p-value for that particular test. Such values are tabulated in standard statistical texts, or can be obtained directly from expressions for the distributions themselves. For our study a p-value of 0.01 was selected.

The correlation coefficient, r, can be transformed so that it has a Z distribution (i.e. is a Gaussian distribution with zero mean and unit variance), by applying the Fisher Z transform [30]

$$z = \frac{\sqrt{n-3}}{2} \cdot \ln \frac{1+r}{1-r} \tag{2.5}$$

This transform may therefore, be applied to the SPM of correlation coefficients, yielding a SPM of z scores. The theory of Gaussian random fields applies to images of z scores. Therefore, it is necessary to transform the t scores to z scores. This is done by calculating the area under the distribution (p value) between t and infinity and choosing an appropriate value of such that the area under the Z distribution between z and infinity is the same.

The final goal of the study is to have knowledge about the functional anatomy that generalizes across individuals and in order to determine so, it is necessary to perform within (intra) subject analysis. This analysis assumes that each subject makes the same, fixed contribution to the observed activation. The activation effect is assessed by comparing the contribution of the explanatory (expected) variable in terms of a contrast of the associated parameter estimates (regression weights) and error variance to produce a

t-statistic as fitted by the general linear model [34]. In intrasubject analysis, the inferences made are limited to the specific set of subjects included in the study as it gives the areas that are activated on the average across the subjects [35]. This analysis can be used to make inferences about typical characteristics at a population level while allowing for the fact that some subjects may not show this effect. This sort of inference may be entirely sufficient when trying to characterize generic aspects of human functional brain architectures, sufficient in the sense that knowing a particular characteristic is typically more useful than not knowing this fact. This is good if one wants to report the results qualitatively or as a case study of the specific subjects involved in the study. In this study, seven autistic and eight controls formed the sample of subjects. In order to make conclusions about the general population from which the subjects are drawn, between subject analysis also known as group analysis needs to be performed. The intrasubject analysis results in contrast parameter estimate map for each subject. A contrast image summarizes the activation (response) for each subject, which is then fed as input for the group analysis. Group analysis requires a great number of subjects to find a particular effect compared to within subject analysis. In general one needs at least 8 subjects and preferably 12 subjects for the group analysis to have sufficient power [36].

Appendix A.1, A.2, A.3 contains programs written by Jason Steffener [37] in Matlab used to perform the above steps. Appendix A.1 has the program jrs_extractTAL.m, which takes the coordinates of voxels in the SPM map and writes them to a file or files. The program jrs_sum_tal.m in Appendix A.2 takes the Talairach results file and summarizes it to give total voxels for each different brain region. Appendix A.3 has the program jrs_merge.m, which takes two output files from

jrs_sum_tal and creates a summary table of them. The result is a file listing all the brain regions and the total voxels in each for each of the two files.

Having thresholded the SPM to display on the 'active' regions, the data is fed to the atlas of Talairach and Tournoux [31], which is commonly used in functional imaging studies. The template image in SPM is based on 152 brains from the Montreal Neurological Institute [38].

The Talairach and Tournoux atlas contained three important innovations:

a) a brain coordinate system ("the Talairach coordinate system") defining an origin and X, Y and Z planes. In their system, the brain is first oriented so that the line joining the Anterior Commissure (AC) and Posterior Commissure (PC) is horizontal. The AC is the origin (X=0, Y=0, Z=0). The same rotations are performed on any 'activation' region and the coordinates are then scaled to give a distance in millimeters from the AC in each dimension (figure 2.14).

b) a spatial transform ("the Talairach transform") to match brains of different shape and size, using quadrant by quadrant linear scaling

c) an atlas of an individual brain ("the Talairach brain"), oriented according to the coordinate system (figure 2.15).

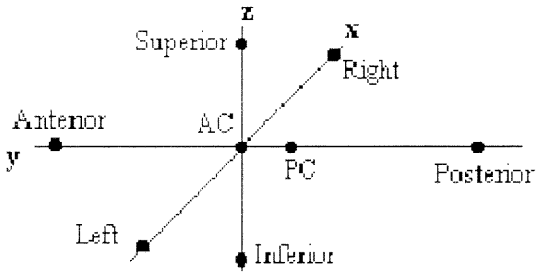



**Figure 2.14** Co-ordinate system in the transformation to Talairach space [31].

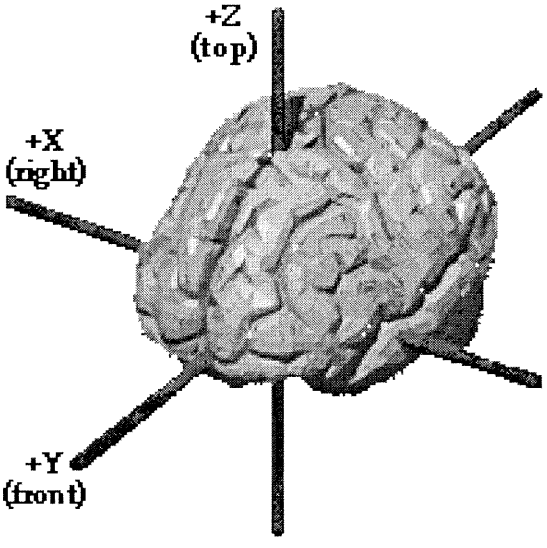



**Figure 2.15** The Talairach Brain showing the X, Y, Z co-ordinates [31].

# CHAPTER 3

# RESULTS

## 3.1 Total Explicit and Implicit Task Combined

The two sample t–test for the explicit and implicit task combined was done for the autistic as well as the control group.

Null Hypothesis – Ho : mean (autistic group) – mean (control group) = difference = 0

Alternative Hypothesis- Ha :

If diff < 0, the autistic group has significantly less activity than the control group.

If diff > 0, the autistic group has significantly more activity than the control group.

**Table 3.1** T – Test of the Different Brain Regions in the Autistic and the Control Group During Both the Explicit and the Implicit Task Combined

| Brain Region | Group | Mean | Standard Deviation | Ha: difference< 0 | Ha: difference> 0 |
|---|---|---|---|---|---|
| Left Fusiform Gyrus | Autism | 17.36 | 16.32 | p = 0.3183 | p = 0.6817 |
| | Control | 21.25 | 6.87 | | |
| Right Fusiform Gyrus | Autism | 7.36 | 13.22 | p = 0.2406 | p = 0.7596 |
| | Control | 12.57 | 25.42 | | |
| Left Amygdala | Autism | 2.71 | 7.17 | p = 0.4960 | p = 0.5060 |
| | Control | 2.75 | 5.36 | | |
| Right Amygdala | Autism | 0 | 0 | p = 0.1314 | p = 0.8686 |
| | Control | 2.63 | 9.02 | | |
| Left Inferior Temporal Gyrus | Autism | 30.14 | 40.14 | p = 0.8830 | p = 0.1170 |
| | Control | 15.38 | 21.72 | | |

| Brain Region | Group | Mean | Standard Deviation | Ha: difference < 0 | Ha: difference > 0 |
|---|---|---|---|---|---|
| Right Inferior Temporal Gyrus | Autism | 15.71 | 27.11 | p = 0.7481 | p = 0.2519 |
| | Control | 10.36 | 11.79 | | |
| Left Middle Temporal Gyrus | Autism | 55.5 | 55.59 | p = 0.9669 | **p = 0.0331** |
| | Control | 23.13 | 29.91 | | |
| Right Middle Temporal Gyrus | Autism | 25.43 | 36.47 | p = 0.8412 | p = 0.1558 |
| | Control | 14.31 | 18.89 | | |
| Left Superior Temporal Gyrus | Autism | 58.92 | 53.05 | p = 0.9952 | **p = 0.0048** |
| | Control | 14.38 | 22.58 | | |
| Right Superior Temporal Gyrus | Autism | 35.43 | 44.18 | p = 0.6983 | p = 0.3017 |
| | Control | 26.75 | 46.19 | | |

If the p value was less than or equal to 0.05, the test was considered significant. As shown in Table 3.1 above, the autistic group showed significant activity during the explicit and implicit task combined in the left Middle Temporal Gyrus (MTG) as well as the left Superior Temporal Gyrus (STG) as compared to the control group.

**Table 3.2** Brain Regions which showed Significant Activation in the Autistic and the Control Group During Both the Explicit and the Implicit Task Combined

| Brain Region | Ha: difference < 0 | Ha: difference > 0 |
|---|---|---|
| Left Middle Temporal Gyrus | No significant activation | Significant activation |
| Left Superior Temporal Gyrus | No significant activation | Significant activation |

### 3.2 Total Explicit Task Only

Two sample t–test for the total explicit task was done for the autistic as well as the control group.

**Table 3.3** T – Test of the Different Brain Regions in the Autistic and the Control Group During the Explicit Task

| Brain Regions | Group | Mean | Standard Deviation | Ha: difference < 0 | Ha: difference > 0 |
|---|---|---|---|---|---|
| Left Fusiform Gyrus | Autism | 17.29 | 17.49 | p = 0.4505 | p = 0.5495 |
| | Control | 19 | 31.66 | | |
| Right Fusiform Gyrus | Autism | 1.43 | 2.50 | p = 0.1097 | p = 0.8903 |
| | Control | 6.5 | 10.08 | | |
| Left Amygdala | Autism | 0 | 0 | - | - |
| | Control | 0 | 0 | | |

| Brain Region | Group | Mean | Standard Deviation | Ha: difference < 0 | Ha: difference > 0 |
|---|---|---|---|---|---|
| Right Amygdala | Autism | 0 | 0 | - | - |
| | Control | 0 | 0 | | |
| Left Inferior Temporal Gyrus | Autism | 26.57 | 46.87 | p = 0.6510 | p = 0.3490 |
| | Control | 19 | 25.37 | | |
| Right Inferior Temporal Gyrus | Autism | 14.43 | 10.69 | p = 0.8171 | p = 0.1829 |
| | Control | 9.36 | 10.18 | | |
| Left Middle Temporal Gyrus | Autism | 52.29 | 60.56 | p = 0.8886 | p = 0.1114 |
| | Control | 20.63 | 48.86 | | |
| Right Middle Temporal Gyrus | Autism | 32 | 47.57 | p = 0.8551 | p = 0.1449 |
| | Control | 12.88 | 11.92 | | |
| Left Superior Temporal Gyrus | Autism | 53.86 | 60.47 | p = 0.9645 | **p = 0.0355** |
| | Control | 11.36 | 10.14 | | |
| Right Superior Temporal Gyrus | Autism | 45.86 | 58.06 | p = 0.8984 | p = 0.1016 |
| | Control | 17 | 18.07 | | |

If the p value was found to be less than or equal to 0.05, the test was considered significant. The t–test showed (Table 3.3 above) that the autistic group showed significant activity in the left Superior Temporal Gyrus (STG) as compared to the control group for the total explicit task.

**Table 3.4** Brain Regions which showed Significant Activation in the Autistic and the Control Group During the Explicit Task

| Brain Region | Ha: difference < 0 | Ha: difference > 0 |
|---|---|---|
| Left Superior Temporal Gyrus | No significant activation | Significant activation |

### 3.3 Total Implicit Task Only

Two sample t–test for the total implicit task was done for the autistic as well as the control group.

**Table 3.5** T – Test of the Different Brain Regions in the Autistic and the Control Group During the Implicit Task

| Brain Region | Group | Mean | Standard Deviation | Ha: difference < 0 | Ha: difference > 0 |
|---|---|---|---|---|---|
| Left Fusiform Gyrus | Autism | 17.43 | 16.48 | p = 0.2948 | p = 0.7052 |
| | Control | 23.5 | 24.56 | | |
| Right Fusiform Gyrus | Autism | 13.29 | 17.03 | p = 0.3589 | p = 0.6411 |
| | Control | 18.63 | 34.63 | | |

| Brain Region | Group | Mean | Standard Deviataion | Ha: difference < 0 | Ha: difference > 0 |
|---|---|---|---|---|---|
| Left Amygdala | Autism | 5.43 | 9.71 | p = 0.4943 | p = 0.5066 |
| | Control | 5.5 | 6.65 | | |
| Right Amygdala | Autism | 0 | 0 | p = 0.1463 | p = 0.8537 |
| | Control | 5.25 | 12.60 | | |
| Left Inferior Temporal Gyrus | Autism | 33.71 | 35.55 | p = 0.9256 | p = 0.0744 |
| | Control | 11.75 | 18.35 | | |
| Right Inferior Temporal Gyrus | Autism | 17 | 38.39 | p = 0.6480 | p = 0.3520 |
| | Control | 11.36 | 13.86 | | |
| Left Middle Temporal Gyrus | Autism | 58.71 | 54.79 | p = 0.9210 | p = 0.0790 |
| | Control | 25.63 | 28.44 | | |
| Right Middle Temporal Gyrus | Autism | 18.86 | 22.76 | p = 0.5972 | p = 0.4028 |
| | Control | 15.75 | 24.85 | | |
| Left Superior Temporal Gyrus | Autism | 64 | 48.80 | p = 0.9783 | **p = 0.0217** |
| | Control | 17.36 | 31.13 | | |
| Right Superior Temporal Gyrus | Autism | 25 | 24.59 | p = 0.3304 | p = 0.6696 |
| | Control | 36.5 | 63.46 | | |

Once again, if the p value was less than or equal to 0.05, the test was considered significant. The t–test showed (Table 3.5 above) that the autistic group showed

significant activity in the the left Superior Temporal Gyrus (STG) as compared to the control group for the total implicit task.

**Table 3.6** Brain Regions which showed Significant Activation in the Autistic and the Control Group During the Implicit Task

| Brain Region | Ha: difference < 0 | Ha: difference > 0 |
|---|---|---|
| Left Superior Temporal Gyrus | No significant activation | Significant activation |

## 3.4 Group Analysis

### 3.4.1 Total Explicit Only

**Table 3.7** Total Number of Voxels Activated in the Different Brain Regions in the Autistic and the Control Group During the Explicit Task

| Brain Regions | Autism Group (Total number of voxels activated) | Control Group (Total number of voxels activated) |
|---|---|---|
| Left Fusiform Gyrus | 7 | 7 |
| Right Fusiform Gyrus | - | - |
| Left Amygdala | - | - |
| Right Amygdala | 15 | 15 |
| Left Inferior Temporal Gyrus | - | - |
| Right Inferior Temporal Gyrus | - | - |
| Left Middle Temporal Gyrus | 4 | - |
| Right Middle Temporal Gyrus | - | - |

| Brain Regions | Autism Group<br><br>(Total number of voxels activated) | Control Group<br><br>(Total number of voxels activated) |
|---|---|---|
| Left Superior Temporal Gyrus | 5 | 7 |
| Right Superior Temporal Gyrus | 8 | 8 |

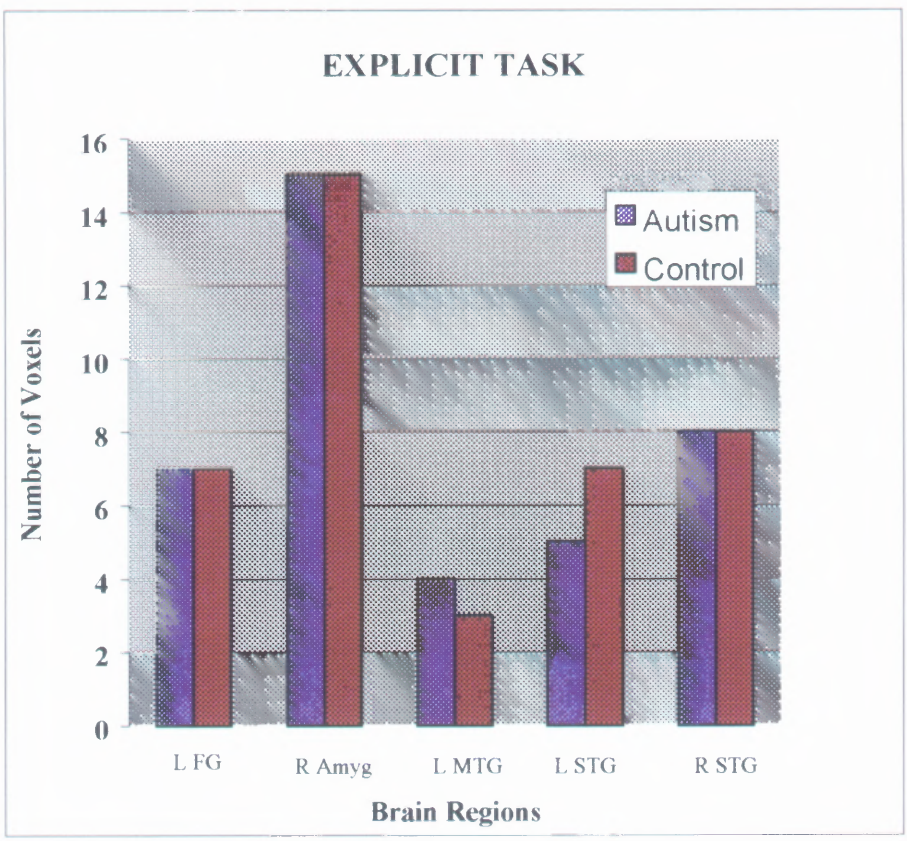



**Figure 3.1** Brain regions showing significant activation in the autistic and control group during the explicit task

The group analysis for the total explicit task found identical activation in the left fusiform gyrus (FG), right superior temporal gyrus (STG) as well as the right amygdala in both the autistic as well as the control group. However, both groups activated the left middle temporal gyrus (MTG) with the autistic group showing more activation as compared to the control group. The left superior gyrus (STG) was activated by both the autistic as well as the control group, with the autistic group showing less activation in this region compared to the control group (Table 3.7).

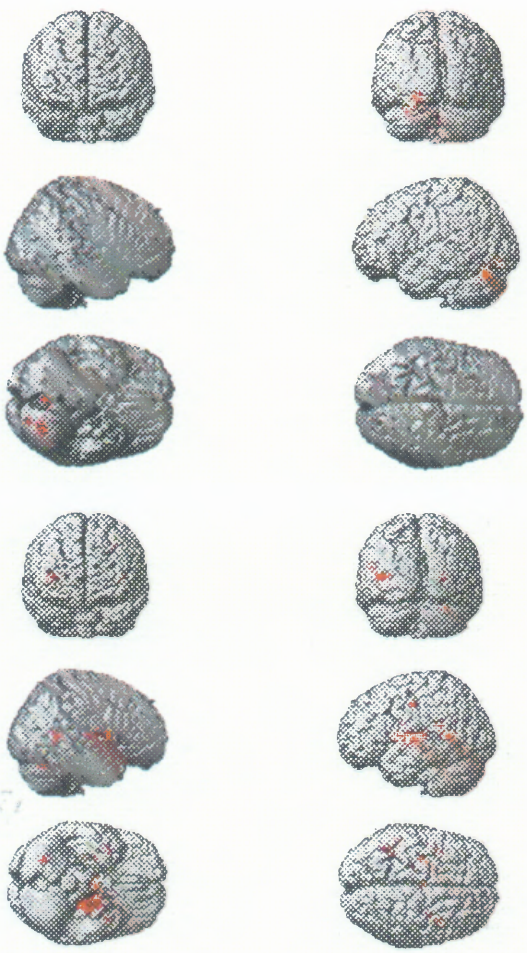


**Figure 3.2** Brain regions showing activation in the autistic group during the explicit task.

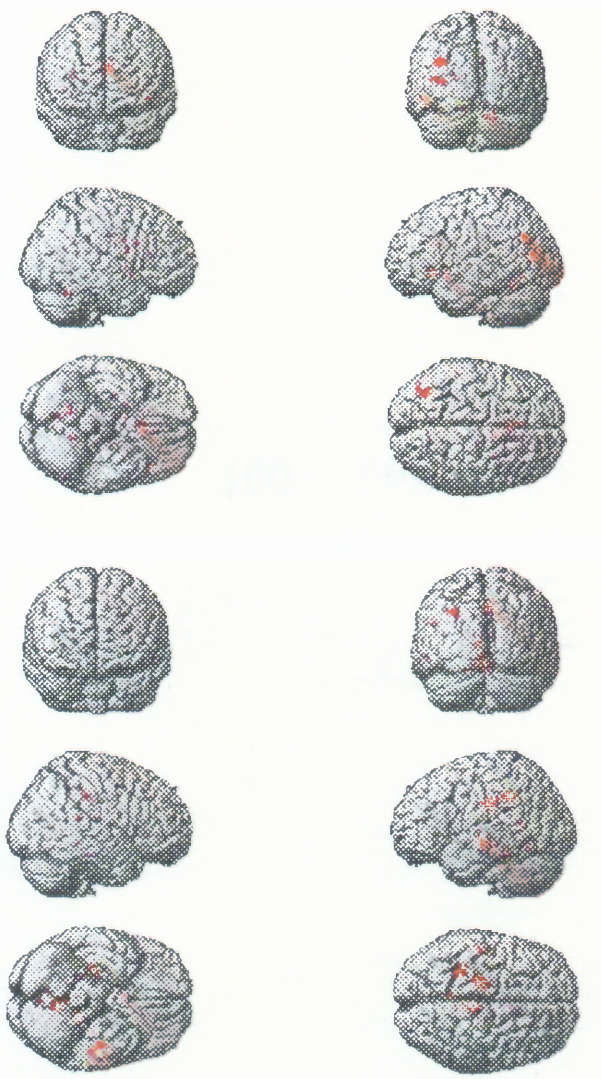


**Figure 3.3** Brain regions showing activation in the control group during the explicit task.

### 3.4.2 Total Implicit Only

**Table 3.8** Total Number of Voxels Activated in the Different Brain Regions in the Autistic and the Control Group During the Implicit Task

| **Brain Regions** | **Autism Group** (Total number of voxels activated) | **Control Group** (Total number of voxels activated) |
|---|---|---|
| Left Fusiform Gyrus | - | - |
| Right Fusiform Gyrus | - | - |
| Left Amygdala | - | - |
| Right Amygdala | - | - |
| Left Inferior Temporal Gyrus | - | - |
| Right Inferior Temporal Gyrus | - | - |
| Left Middle Temporal Gyrus | 4 | - |
| Right Middle Temporal Gyrus | - | - |
| Left Superior Temporal Gyrus | 255 | - |
| Right Superior Temporal Gyrus | 5 | - |

The group analysis for the total implicit task showed activation in the left as well as the right superior temporal gyrus (STG) in both the autistic as well as the control group with more activation in the autistic group. Activation was also found in the left middle temporal gyrus (MTG) in both groups, but there was more activation in the autistic group as compared to the control group (Table 3.8).

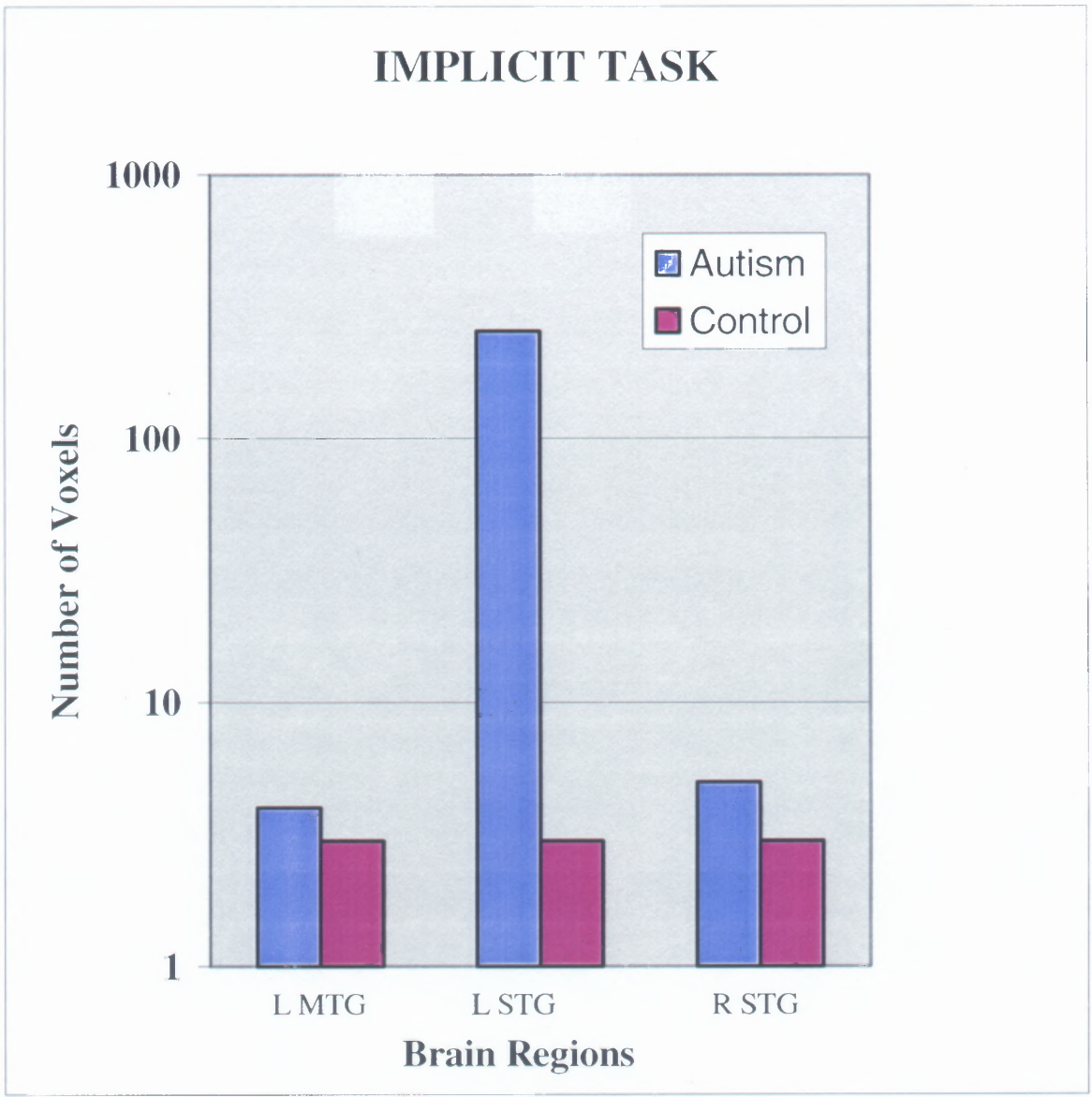



**Figure 3.4** Brain regions showing significant activation in the autistic and the control Group during the implicit task.

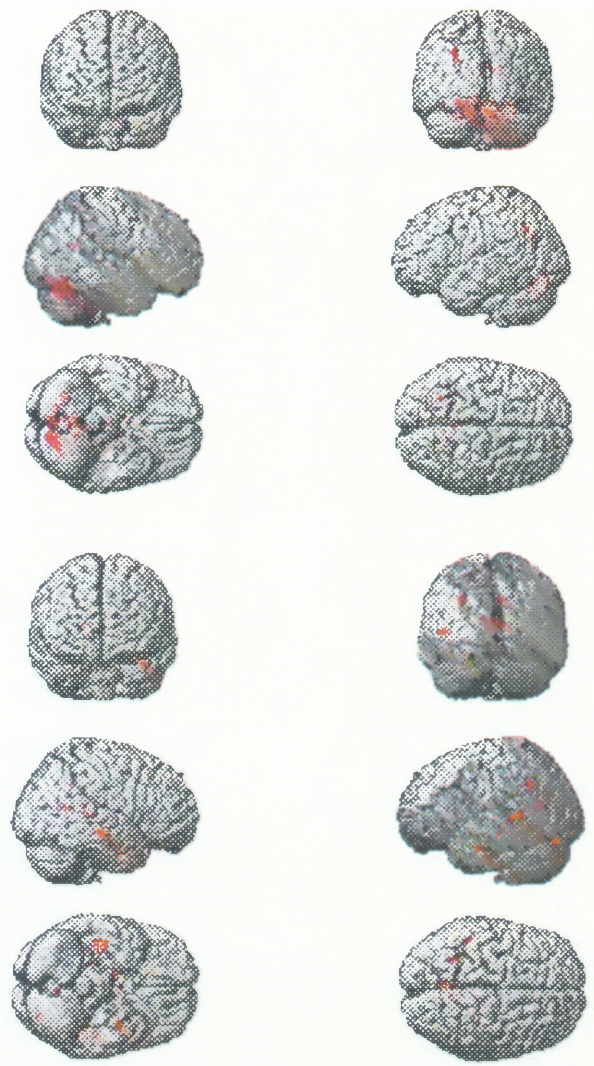


**Figure 3.5** Brain regions showing activation in the autistic group during the implicit task.

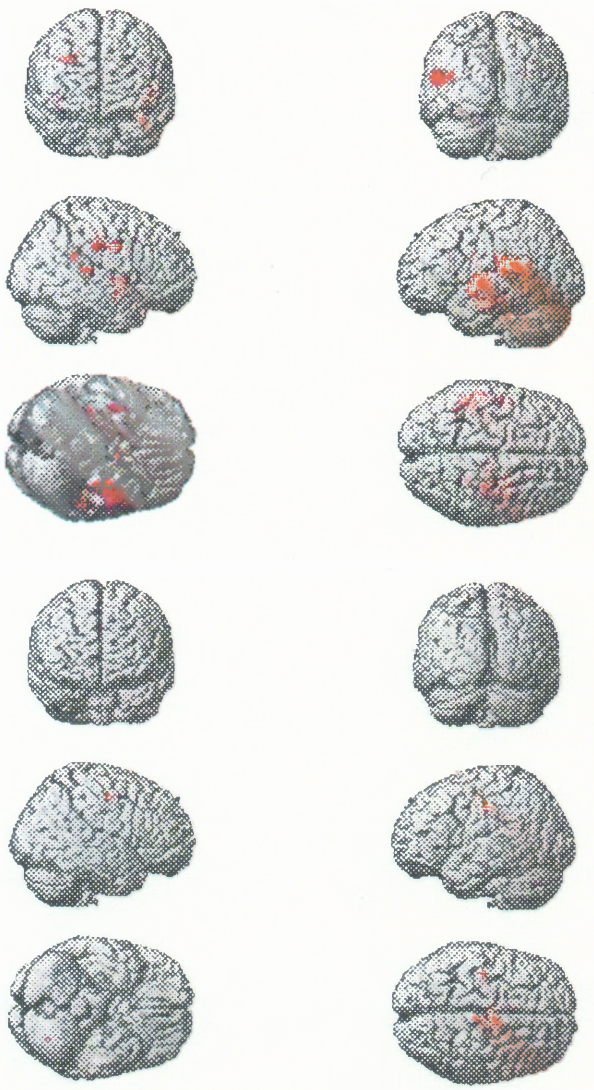


**Figure 3.6** Brain regions showing activation in the control group during the implicit task.

# CHAPTER 4

## DISCUSSION AND CONCLUSION

The study of face perception is important as it provides information about another person's mood, level of interest, intentions and is an essential part of social communication. It has been shown that the fusiform gyrus which is an important region for face recognition is related to the perception of unique identity whereas the superior temporal gyrus, also an important region in face recognition is related to interpretation of biological movements like perception of eye gaze, expression and lip movement. The amygdala provides information about the emotional significance of the perceived stimuli. The manner in which these regions interact to guide social behavior is of interest as it can provide insight into the working of different regions of the brain. Thus, it can help us to answer some questions related to whether autistic individuals process facial expressions in a manner different from normal individuals. Since impairment in social communication is a fundamental deficit in autistic individuals, the identification of their valid neural network would be extremely valuable for treatments more specifically tailored to this group.

Brothers' [25] proposed a network of neural regions, which includes the superior temporal gyrus (STG) and amygdala to comprise the 'social brain'. Damage to the amygdala impairs judgment of emotion and damage to the STG impairs face perception. Social intelligence is our ability to understand and interpret other peoples' behavior in terms of mental states to predict how others feel, think and behave. Brain damage can cause selective impairment in social judgment without any loss to general intelligence,

and thus, the idea that social intelligence might be independent from general intelligence. The study by Baron Cohen et al. [4] shows that the autism group had significantly more response in the superior temporal gyrus and did not activate the amygdala at all confirming Brothers' theory that extracting socially relevant information from visual stimuli is associated with activation of the STG. The autism group appears not to use the amygdala but places greater processing load on the temporal lobe structure, which is specialized in the recognition of facial expression in order to compensate for amygdala abnormality. The present study agrees with Cohen et al. as there was activation in the STG during the presentation of visual stimuli and no amygdala activation.

Critchley et al. [5] showed that during the explicit task, the autistic individuals had greater activity than controls in the left superior temporal gyrus whereas the normal controls had significantly more activity in the right fusiform region, which plays an important role in face processing as well as representing knowledge about people. Implicit processing of facial expressions in autistic individuals showed significant activation in the left superior and middle temporal gyrus whereas the normal controls showed greater activation in the amygdala. The present study shows similar results as Critchley et al. The amygdala is important in normal social and emotional behavior as well as learning and representing the motivational meaning of stimuli and this dysfunction of the amygdala region may partially explain some of the social deficits in autistic people. Critchley et al.[5] hypothesize that social deficits of autism may arise from impaired learning and representation of the motivational meaning of social stimuli during a critical period of early brain development.

It can be seen from the results of this study that the autistic as well as the control group showed activation in the temporal lobe (middle and superior temporal gyrus) (refer to Figure 3.1 in the results section) during explicit processing of facial expressions. The superior temporal gyrus (STG) is involved in the recognition of faces and changeable aspects of the face such as expression, perception of eye gaze. During the explicit task, subjects were asked to judge the emotional content of the stimuli (face) being presented. The fact that the control group displayed activation in the temporal lobe during the explicit task supports hypothesis # 1 (see page 2). Hypothesis # 2 is supported by the result that the autistic group demonstrated similar pattern of activation as normal controls during the explicit task (see page 2).

During the implicit processing of facial expressions it was found that the autistic group showed significantly more activation in the left middle temporal gyrus (MTG), bilateral superior temporal gyrus (STG) than the control group as these regions are involved in the recognition of faces. No significant activation was found in the amygdala in the autistic group (refer to Figure 3.4 in the results section). This does not agree with hypothesis # 3 (see page 3). However, these results agree with the study by Cohen who suggested that in the autistic group extracting socially relevant information from visual stimuli is associated with the activation of the STG. Also, in a study performed by Critchley, implicit processing of facial expressions showed significant activation in the left MTG and STG in the autistic group whereas the control group showed activation in the amygdala suggesting that the dysfunction of the amygdala may partially explain some of the social deficits in autism. The control group did not show significant amygdala activation. This result does not support hypothesis # 4 (see page 3). This may be due to

the difficulty in imaging the amygdala because of its small size and also because of small sample size (the study included 7 autistic and 8 normal subjects). Another possible reason that amygdala activation was not found during implicit processing of faces is that the present study had only 18 photographs of faces which were presented as stimuli. This means that there were less number of trials, giving less data, resulting in a low signal to noise ratio with no significant amygdala activation.

This study also showed identical activation in the fusiform gyrus (FG) (refer to the graph in the results section) in the autistic and control group during explicit processing of facial expressions. The autistic individuals can recognize faces i.e. differentiate between male and female faces just like normal controls. The problem arises when the autistic individuals have to interpret the meaning of the faces. They have difficulty when emotion is added in the faces ie. to differentiate between fearful, happy, neutral faces. It is know that the fusiform gyrus is linked to the recognition of faces (representation of identity). Therefore, the results of this study showed activation in the FG in the autistic and control group during explicit processing of faces.

However, a study conducted by Karen Pierce et al. [3] found weak or no activation in the fusiform gyrus in autistic subjects whereas the normal subjects showed fusiform activation. She suggested that the fusiform gyrus is not necessary for face processing and that multiple regions may be capable of supporting face processing in the autistic individuals. Her study found that although the autistic subjects could perform the face perception task, none of the regions supporting face processing in normal controls were found to be significantly active in autistic individuals. Pierce also suggested that compared to normal controls, autistic individuals 'see' faces utilizing different neural

systems (frontal cortex, primary visual cortex, cerebellum) with each patient doing so via unique neural circuitry.

The difference in the results between the study by Pierce and the current study maybe due to a number of reasons. The current study had 18 facial stimuli to which the subjects responded whereas the study by Pierce included 60 facial stimuli, resulting in more data. Also, the tasks performed by the subjects in the two studies were different. Pierce asked the subjects to perform a face perception task (button press in response to a female neutral face) and a shape perception task (button press in response to a circle). She then used the face perception and shape perception task as the contrasts to be compared. The current study involved explicit and implicit processing of facial expressions. The response to faces as compared to the resting baseline was then used as contrasts to be compared.

Even at an early age, children with autism differ from normal children in interest in others and social behavior. It is evident from early on that children with autism do not value social stimuli like the face in the same way that typically developing children do (Cohen et al. [4]). This developmental abnormality is likely to place an obstacle in the developmental path of normal face processing strategies in these children.

The differences in face processing between normal and autistic individuals probably arise out of the fact that autistic individuals have reduced social interest and do not regard the face as socially important (Klin et al. [39]). Klin et al. also suggest that whereas most people attend to the eyes during social interactions, autistic individuals look at the mouth in order to obtain more verbal information about the interaction. The current study showed no activation in the fusiform gyrus (FG) and increased activity in

the superior temporal gyrus (STG) in the autism group indicating that the autistic individuals process faces in a different manner than normal controls relying more on feature based strategies that are more typical of nonface object perception. (Schultz et al. [2])

Autistic individuals have reduced social interest and may therefore, fail to develop the brain areas related to face specialization whereas face specialization may develop in normal individuals as they are socially motivated to regard faces and such motivation promotes expertise for faces. Therefore, autistic individuals may lack the expertise that typically developing individuals have with face recognition. This expertise model may provide an answer for the neural deficits that autistic individuals have for face processing.

### 4.1 Flaws in the Current Design

A 1.5 Tesla fMRI scanner was used for imaging the subjects. The strength of the signal obtained form such a scanner is of small amplitude and therefore, the signal to noise ratio is small. The brain regions are small and difficult to detect if the signal strength is not strong enough and so future studies should use a 3 Tesla scanner.

The template brain was used to standardize the brain in the Talairach Atlas in order to make inferences regarding the brain activities and patterns of a normal individual. This same standardized brain was used for the autistic individuals, which may not be the right thing to do as this group may have differences in brain anatomy as compared to normal individuals.

The paradigm used in the study consists of an event related design with only eighteen face stimuli in each task, which makes it difficult to activate the different brain

regions of interest especially the amygdala where habituation is known to be an issue and its small size adds to the problem of not being detected. Therefore, the paradigm should have an increase number of facial stimuli in each task or make use of a block design so that there are more facial stimuli to make the paradigm stronger. Seven autistic and eight normal controls were scanned. Studies have shown that there should be at least 10-12 subjects per group to increase the statistical power.

The contrast that we used compared faces to the baseline. The baseline had text, which read 'Please keep your head still'. This kind of baseline with text is not ideal as it may activate certain brain regions related to text, which may affect the results of the study. Instead an ideal baseline would be a blank screen or a screen with a '+" sign.

### 4.2 Future Directions

The current study shows that the autistic individuals process faces in a different manner than normal controls relying more on feature based strategies that are more typical of nonface object perception. Therefore, the next step would be to design a paradigm with facial stimuli as well as objects (Schultz et al. [2]) or shapes (Pierce et al. [3]).

Schultz et al. suggest that face perception in the autism group is like processing of objects in normal individuals who are free from social disability. Karen Pierce et al. conducted a study, which involved a face perception as well as a shape perception task. The study found that although the autistic individuals could perform the face perception task, none of the regions supporting face processing in normals were found to be significantly active in the autism group suggesting that the autistic individuals see faces utilizing different neural systems.

The comparison of whole faces to scrambled faces would be a suitable contrast to study.

Finally, it would be a good idea to incorporate eye tracking of subjects while scanning. This would provide a means to confirm that autistic individuals concentrate more on the mouth than the eyes as normal individuals do during face processing (Klin et al. [39]).

## 4.3 Conclusion

From an early age, children with autism do not show interest in social behavior. They do not regard the face as socially relevant in the same way that typically developing children do. This developmental abnormality is likely to place an obstacle in the developmental path of normal face processing in autistic individuals.

The differences in face perception between normal and autistic individuals arises out of the fact that the autistic group has social impairments and does not regard the face as socially important.

Normal individuals develop an expertise for faces, as they do not suffer from any social disability whereas the autistic group shows reduced social communication and lack of interest to faces hindering them to develop an expertise for faces. This expertise model is the key in understanding and gaining an insight in the neural network of autistic individuals.

# APPENDIX

## PROGRAMS USED IN THE ANALYSIS SECTION

The programs in this section were written by Jason Steffener [37] in Matlab. The first section A.1 contains the program jrs_extractTAL.m which takes the coordinates of the voxels in the SPM map and writes them to a file or files. The second section A.2 contains the program jrs_sum_tal.m which takes the Talairach results and summarizes it to give the total voxels for each different brain region. The final section A.3 contains the program jrs_merge.m which takes the two output files from the program jrs_sum_tal and creates a summary table of them. The result is a file listing all the brain regions and the total voxels in each for each of the two files.

**A.1** Program jrs_extractTAL.m takes the coordinates of the voxels in the SPM map and writes them to a file or files.

```
This is a script, which takes all the voxels activated in your glass
brain, and writes them out to a file or files. The purpose is for
preparing your data to be sent to the Talairach Daemon. It is for this
reason that there is a size limit on the extracted file size of 10000
entries. The Talairach Daemon program imposes this file size limit.
This script also employs the MNI to Talairach coordinates program of
Matt Brett.

a=SPM.XYZmm';
b=mni2tal(a);
n=size(b,1);
m=n/10000;
m=ceil(m);
P=SPM.title;
q=findstr(P,' ');
for i=1:length(q)
   P(q(i))='_';
end
for j=1:m
outfile=cat(2,P,'_k',num2str(SPM.k),'_T',num2str(SPM.u),'_',num2str(j),
'.txt')
fid=fopen(outfile,'a');
for i=(j-1)*10000+1:j*10000
   if i>n
      break
```

```
    else
        c=round(b(i,:));
            fprintf(fid,'%2.0f  %2.0f   %2.0f\n',c);
    end
    end
end
status=fclose(fid);
clear a b n P q c
```

**A.2** Program jrs_sum_tal.m takes the Talairach results file and summarizes it to give the total voxels for each different brain region.

```
This script opens a text file, which is the output of the Talairach
Deamon returned by the Deamon, and returns a comma deliminated text
file with each row being a different brain region and the final number
being the total number of activated voxels per brain region.


amount=[1];
in=spm_get(1,'*.txt','Choose the Talairach file')
file=textread(in,'%s','delimiter','\n','whitespace','');
final={'Level1   ,Level2   ,Level3   ,Level4   ,Level5     '};
[k,l]=size(file);
for i=1:k
   tmp=char(file(i));
        commas=findstr(',',tmp);
        [n,m]=size(tmp);
   tmp2=tmp(commas(4)+1:m);
   %tmp2 is the important part of line 'i' of the
   %input file
        bool=strcmp(final,tmp2);
   %this makes the comparison between the out file (final)
   %and the in final (tmp2)
if bool==0
   [o,p]=size(final);
   final{p+1}=tmp2;
   amount=[amount,1];
else
   position=find(bool);
```

```
    amount(position)=amount(position)+1;
end;
end
%slash=findstr('\',in);
%dot=findstr('.',in);
[PATH,NAME,EXT]=fileparts(in)


out=cat(2,'total_',NAME,'.txt')
fid=fopen(out,'a');

%UP to this point works!!!!
[m,n]=size(final);
for j=1:n
    temp=final{j};
    temp2=cat(2,temp,',',num2str(amount(j)));
    fprintf(fid,temp2);

    fprintf(fid,'\n');
end
status=fclose(fid);

clear
```

**A.3** Program jrs_merge.m takes the two output files from jrs_sum_tal and creates a summary table of them. The result is a file listing all the brain regions and the total voxels in each for each of the two files.

```
amount=[1];
in=spm_get(2,'*.txt','Choose Two Talairach files to merge')
in1=textread(in(1,:),'%s','delimiter','\n','whitespace','');
in2=textread(in(2,:),'%s','delimiter','\n','whitespace','');
%pick the larger input file
[a]=size(in1,1);
[b]=size(in2,1);
%The problem with arranging the files into which is largest
%is you may not know which is which then!
%if a>=b
   file1=in1;
        file2=in2;
%else
%   file2=in1;
%   file1=in2;
%end
%file1 is the larger file
[k1,l1]=size(file1);
[k2,l2]=size(file2);

f1sum=(zeros(k1,1));
f1=char(zeros(k1,100));
for i=1:k1      %create array of file1 sums
   tmp1=char(file1(i));%take line(i) of file
   if size(tmp1,2)~=0  %This checks for empty lines
```

```
            commas1=findstr(',',tmp1);
                [n1,m1]=size(tmp1);
             tmp1t=tmp1(max(commas1)+1:m1);
          t=size(tmp1t,2);
             f1sum(i)=str2num(tmp1t);%%%%%%%%
          tmp1s=tmp1(1:max(commas1)-1);
             s=size(tmp1s,2);
          f1(i,1:s)=(tmp1s);
   end
end
 %We no have 2 char arrays, one for the areas in the bigger TD file
 %and one containg the sums of the locations
f2sum=(zeros(k2,1));
f2=char(zeros(k2,100));
for i=1:k2      %create array of file1 sums
   tmp2=char(file2(i));%take line(i) of file
   if size(tmp2,2)~=0
            commas2=findstr(',',tmp2);
                [n2,m2]=size(tmp2);
             tmp2t=tmp2(max(commas2)+1:m2);
          t=size(tmp2t,2);
             f2sum(i)=str2num(tmp2(max(commas2)+1:m2));
           tmp2s=tmp2(1:max(commas2)-1);
           s=size(tmp2s,2);
           f2(i,1:s)=(tmp2s);
   end
end
 %This all works!!!
 %What we now have is four arrays, 2 for each input
```

```
%file.
count=k1;
outfile=f1;
outfilecount=f1sum;
for k=1:k2     %loop over file2
 for j=1:k1    %loop over file1
   bool=strcmp(f1(j,:),f2(k,:));
   if bool==1
      outfilecount(j,2)=f2sum(k);%this works
      break
      end
end
if bool==0
    count=count+1;
     outfile(count,:)=f2(k,:);
       outfilecount(count,2)=f2sum(k);
end
end

m=size(outfile,1);
out='mergeout.txt'
fid=fopen(out,'a');
fprintf(fid,',,,,,');
slash=findstr('\',in(1,:));
dot=findstr('.',in(1,:));
fprintf(fid,in(1,max(slash)+1:dot-1));

fprintf(fid,',');
```

```
slash=findstr('\',in(2,:));
dot=findstr('.',in(2,:));
fprintf(fid,in(2,max(slash)+1:dot-1));
fprintf(fid,'\n');

for j=1:m
   temp=deblank(outfile(j,:));
   temp3a=outfilecount(j,1);
      temp3b=outfilecount(j,2);
   temp2=cat(2,temp,',',num2str(temp3a),',',num2str(temp3b));
   fprintf(fid,temp2);

   fprintf(fid,'\n');
end

status=fclose(fid);
%disp(in);
disp('Done!!');
clear
```

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