Amygdala Abnormality and Its Role in Autistic Socio-emotional Impairment: A Proposed Study of Somatic Intervention Among Macaque Monkeys

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Autism is a neurodevelopmental disorder marked by pervasive impairments in behavior that manifest by three years of age, and sometimes earlier (American Psychological Association (APA), 1994). The characteristic behavioral impairments include hyperactivity, highly repetitive behaviors, emotional indifference, and lack of social engagement and reciprocity. Although affliction rates stand as high as 1 to 2 of every 1,000 individuals, and approximately $3 billion is expended yearly on research and social care, no definitive cause of the disorder has been identified (National Institute of Mental Health [NIMH], 2003). In recent years, however, neuroimaging and biochemical techniques have elucidated the pathophysiologic underpinnings that relate to the inability to exhibit typical socially- and emotionally-relevant behaviors (for review see Sweeten, Posey, Shekhar, & McDougle 2002). Face processing, when facial features are analyzed in order to extract social or emotional information, is one such behavior that is paramount to social interaction, and is impaired in autistics.

Humans have evolved mechanisms that allow them to perceptually analyze a face, particularly the expression it holds (Grelotti, Gaussier, & Schultz, 2002). During facial analysis, emotions and social signals are spontaneously extracted. Utilizing infrared corneal reflection techniques that follow eye movement as processing occurs, research has established that normal individuals use configural processing in face analysis (Pelphrey, Sasson, Reznick, Paul, Goldman, & Piven, 2002). Specifically, an individual directs most attention to the eyes, nose, and mouth, with approximately 70% of this attention focused on the eyes (Pelphrey et al., 2002). In contrast, visual scanpath observations during autistic face processing demonstrate a different analysis strategy. Little to no attention is directed toward the eyes or the nose; instead, autistics scan the mouth and lower, non-feature regions of the face. Consequently, when pictures are

presented depicting a facial expression of one of the six universally-basic human emotions (happiness, sadness, fear, anger, surprise, disgust) (Ekman, 1992), autistics engage in non-configural (or segmental) processing and misidentify the depicted emotion 30% of the time (note: 30% is seemingly low for misidentification; this issue will later be clarified) (Pelphrey et al., 2002). The percentage of misidentification increases in proportion to an increase in the demanding nature or emotional weight of the task (Grelotti et al., 2002). Further, autistics fixate on faces for significantly shorter periods of time in comparison to healthy individuals (Pelphrey et al., 2002). Interestingly, facial recognition is the only form of facial perception that shows little to no impairment among autistics.

The crucial factor that impairs the face-processing strategy in autism, while leaving facial recognition abilities intact, is the presence of emotional and social information (Grelotti et al., 2001). In recent years, functional neuroimaging and biochemical studies have elucidated the neuroanatomical structures active during emotional and social perception (Davidson and Slagter, 2000; Haznedar, Buchsbaum, Wei, Hof, Cartwright, & Bienstock, 2000). Moreover, differences have been localized and qualified that distinguish healthy from autistic participants (Narumoto, Okada, Sadato, Fukui, & Yonekura, 2001; Sweeten et al., 2002). The neural substrates of emotion have been identified by presenting affective stimuli while neuroimaging techniques record regional activity (Hall, Szechtman, & Nahmias, 2003). Facial expressions are the most widely utilized form of affective stimuli, for they arguably carry the greatest social and emotional import (Davidson et al., 2000; Narumoto et al., 2001; Hall et al., 2003; Fudge and Emiliano, 2003).

An added benefit of using facial presentation arises from the fact that different facial expressions can impart strong, moderate, or absent emotional information. Therefore, specific regions have been established as the substrates of emotional processing by comparing two forms of task-related neuroimaging scans: those that show neural activity during the presentation of emotional expressions, and those that show neural activity during the presentation of neutral faces (Narumoto et al., 2001). These scans have implicated an area of the cerebral cortex, the right superior temporal sulcus (STS), as the region active during emotional processing of faces (Narumoto et al., 2001; Hall et al., 2003). Activity is enhanced in the STS in response to the selective attention of healthy participants to facial emotion. In contrast, non-emotional attention elicits no response from the STS.

Other lines of research converge on the STS as a substrate for social perception activities such as face-processing. Intercranial recordings of single cells located in the rostral/dorsal portion of the right STS highlight clusters of neurons that respond solely to expression (Narumoto et al., 2001). Related research demonstrated that electrical stimulation of these clustered neurons impairs the ability to accurately identify expressions. The expression-responsive neurons are therefore thought to comprise a

sensory processing area of the temporal lobe (Iacoboni, Koski, Brass, Bekkering, Woods, Dubeau, et al., 2001). This area essentially receives visual information, to which it then connects semantic meanings. The STS is also active during related forms of interpersonal communication such as following the eye gaze of others. It is therefore noteworthy that the activated cluster of neurons lies near the V5/MT brain region (Visual Area 5), which is responsible for the perception of moving visual stimuli (Iacoboni, 2001; Narumoto et al., 2001). Consequently, the ability to perceive emotional expressions as they change occurs due to axonal connections that allow for communication between the right STS and V5/MT brain regions.

The above neuroimaging studies on neural activity during facial presentation establish the conclusion that the right STS functions during fine-grained spatial processing. This is precisely the form of processing that is necessary for expert facial analysis. Moreover, the STS responds specifically to facial stimuli as the face imparts social and emotional information, usually through expressions. As discussed previously, autistics engage in segmental face-processing that ignores the eyes and nose, thereby extracting little socially- and emotionally-relevant cues from expressions (Pelphrey et al., 2002). Consequently, the finding is logical that autistics demonstrate impaired function in the right STS (for review see Davidson and Slagter, 2000). Research examining the functional and structural differences of the STS has continued to utilize facial presentations. Hall, Szechtman, and Nahmias (2003) examined levels of glucose metabolism using positron emission tomography (PET) in concordance with the presentation of facial emotions. PET scans record the amount of activity in a region based on glucose metabolism. A task involving the identification of sex from emotionally-neutral faces was included to establish which neural regions respond when visual stimuli provide no emotional information. As expected, significantly less activation was witnessed in the autistic STS in comparison to healthy participants. Instead, the thalamus within autistics functioned as greatly in response to facial presentations as did the STS in the control group.

The thalamus demonstrates increased activity due to the segmental face-processing approach utilized in autism. The segmental approach is neither effortless nor spontaneous (in comparison to the effortless configural approach of healthy individuals) (Grelotti et al., 2001). Instead, it assembles sensory information from numerous sources rather than from solely the eyes and nose. In turn, greater functional strain is imposed on the thalamus, which modulates the sensory processing system. As previously discussed, autistics presented with facial expressions depicting one of the six basic human emotions misidentify the emotion 30% of the time (Pelphrey et al., 2002). This figure appears low based on the characteristic inability of an autistic to retrieve facial emotional information. However, autism is not a degenerative disorder. Therefore, most autistic individuals are capable of learning compensatory strategies for their emotional and social deficits (NIMH, 2003). Segmental face-processing, in

concordance with greater thalamic activation, is one such strategy. This strategy is obviously not perfect, for autistics continue to demonstrate gross impairments in social and emotional interaction (Hall et al., 2003). However, with practice many are capable of distinguishing the most basic of emotions: happiness and sadness. Thus, a 30% misidentification rate denotes the underlying difficulties that autistics continue to have in deciphering more complex expressions such as surprise and disgust.

Within autism, the compensatory use of the thalamus suggests a malfunction in the emotion-linked sensory processing system on which normal individuals rely. Structural damage of the STS would seem the most logical conclusion. However, postmortem examinations and Magnetic Resonance Imaging (MRI) scans have determined that the volume and structure of the STS are not abnormal within autistic individuals (Rapin and Katzman, 1998; Sweeten et al., 2002). On the other hand, numerous studies utilizing neuroimaging techniques have discovered that the autistic amygdala and hippocampus are anatomically abnormal (Rapin and Katzman, 1998; Aylward, Minshew, Goldstein, Augustine, & Yates, 1999; Sweeten et al., 2002; Hall et al., 2003). The amygdala and hippocampus are two of several structures that comprise the limbic system, which is implicated in memory, learning, emotion, and motivation. MRI scans of the autistic amygdala displayed decreased neuronal size, increased neuronal packing, decreased dendritic extensions, and small cell bodies (Aylward et al., 1999). Consequently, amygdala volumes, both before and after correction for total brain volume, are greatly reduced in autism. The hippocampus has shown similar patterns of deformity to the amygdala. Interestingly, children with diseases that damage the limbic system, such as viral encephalitis, tuberous sclerosis, and cancer, exhibit autistic-like symptoms (Sweeten et al., 2002). Therefore, limbic system dysfunction is most likely at the root of autism.

Convergent research has further demonstrated that amygdala, rather than hippocampal, abnormality is responsible for emotionally-relevant face-processing deficits among autistic individuals. The hippocampus is active during tasks of learning and memory. On the other hand, motivation, emotional recognition, and displays of aggression elicit amygdala activity. However, PET scans tracing regional cerebral blood flow (rCBF) among autistics have failed to find increased amygdala activity in response to the presentation of facial expressions (Davidson and Slagter, 2000). In particular, healthy individuals demonstrate greatest activity in response to fear. However, in the study among autistic individuals who misidentified emotions 30% of the time, an average of 70% of those misidentifications were between fear and anger (Pelphrey et al., 2002). Another behavioral characteristic of autism is the bizarre, erratic display of aggression directed both inward and outward (APA, 1994). Thus, both the inability to detect fear in facial expressions and the characteristic random acts of aggression implicate amygdala abnormality as an underlying factor.

Amygdala response to motivation is also significant because the ability to

expertly discern the emotion of another individual is not innate. Face-processing, similar to any other task one seeks to master, requires extensive perceptual and cognitive attention and energy, and is motivated by the human drive for social interaction (Grelotti et al., 2002). In healthy individuals, the amygdala responds to motivating social interactions by releasing the feel-good neurotransmitter dopamine (DA) (Fudge and Emiliano, 2003). DA reinforces behaviors; consequently, healthy individuals strive to become experts in face-processing. This motivational drive is largely absent among autistics. One could argue that segmental face-processing, and thus use of the thalamus, demonstrates an autistic′s motivation for social interaction. However, as previously described, segmental face-processing is significantly shorter in duration than configural-processing. PET scans have found little to no anatomical abnormalities apparent in autistic attentional systems (Haznedar et al., 2000). This has led to the conclusion that lack of motivation, rather than a physical inability to attend, results in shorter processing during facial presentation (Pelphrey et al., 2002). One theory has consequently linked autistic lack of motivation to functional impairments of the DA system (Buitelaar, 2003). However, investigations into DA levels in autism have demonstrated few consistent results. Some studies examined DA levels in response to repetitive behaviors that an autistic individual finds pleasurable. The levels were no different in autism than in normal individuals, discrediting the possibility of autistic DA dysfunction. Findings, therefore, are consistent with the idea that amygdala abnormality underlies impairments in the connection of motivation to social interactions.

The theory that amygdala deformity subsumes social deficits in autism is further evidenced by research examining the effects of experimental manipulations, natural diseases, and injuries (Sweeten et al., 2002; Fudge and Emiliano, 2003). Monkeys are commonly utilized in experimental research due to the similarity of their neural system to that of humans. One study elicited over-stimulation in the basolateral nucleus of the amygdala (BLA) using stress-associated peptide corticotropin-releasing factor (CRF) (Sweeten, et al., 2002). In normal individuals, as CRF levels rise, the BLA is excited, and a deficit in typical social behaviors is demonstrated. Likewise, in this study intentional over-stimulation resulted in severe and chronic disruption of social interaction behaviors. Another study examined the effects of hippocampal versus amygdala lesions, with striking support for amygdala damage in concordance with autistic symptoms (Sweeten et al., 2002). Lesions of the monkey amygdala by two months of age produced disturbances in social and emotional functioning similar to the patterns seen in autism. Hippocampal lesions resulted in similar disturbances; however, by six months of age any deficits had disappeared. This occurrence conflicts with the stable pattern of deficits in behavior that are observed among autistic individuals. Thus, the theory that hippocampal abnormalities result in autistic social dysfunction has been discredited.

In light of the aforementioned evidence, it is clear that structural abnormality within the amygdala is the most likely source of social impairments in autism. It must be noted, however, that not all research has found the presence of amygdala deformities (Haznedar et al., 2000). MRI scans compared limbic structure volumes within 17 autistic and 15 control participants. No differences were noted. Also, a PET scan of rCBF during a verbal learning test found little to no difference among structural functioning. In comparison to numerous other studies documenting amygdala abnormality, the MRI results cannot be explained. The counterfactual PET results, however, appear to be due to the nature of the task. A verbal learning test would not ordinarily activate the amygdala (unless, possibly, some emotionally-relevant words are selected). If anything, previous findings suggest that the hippocampus instead would be activated, yet in this study it is not. The present research seeks to produce anatomical abnormalities in the amygdala, and to then record rCBF during the viewing of facial expressions. By doing so, a direct connection can be established between the sole presence of amygdala abnormalities and the impairment in social activities such as facial analysis.

Anatomic organization between the socio-emotional and the sensory system places the amygdala at the center of emotional perception in both humans and primates (Lane and Nadel, 2000; Fudge and Emiliano, 2003). The basolateral nuclear group (BLNG), located within the BLA, is the primary receiving portion of the amygdala. It is connected to axonal projections from the temporal cortex, including the STS, the orbital and medial prefrontal cortex (OMPFC), and the hippocampus. Initial perception of higher-order stimuli, such as facial expression, activates the STS. The OMPFC determines the relative reward value of the visual stimuli, with the hippocampus recording this value for future reference. Any emotionally-relevant information regarding the facial expression is then projected by each structure to the BLNG. Finally, the BLNG engages in the deepest socio-affective processing of the facial expression by integration of the information received.

As discussed previously, MRI and postmortem examinations have found moderate to severe structural abnormalities in autistic amygdala (Aylward et al., 1999). Due to the role that the amygdala is evidenced to play in social and emotional engagement, the next crucial step is to determine the connection between its structural abnormality and autistic impairments. Deformity includes dense neuronal packing, which appears to explain the other deformities of decreased dendritic complexity, small cell bodies, and highly complicated axonal pathways. Consequently, the prevailing theory for autistic amygdala deformity proposes a deficit in neuronal pruning during early development (Rapin and Katzman, 1998; Aylward et al., 1999). At birth, approximately 20-80% of neurons in each region are excessive. Neurons compete for synaptic targets, through which they receive nourishing neurotrophic factors. Neurons that either receive inadequate amounts of neurotrophic factor, or are useless due to a

lack of synaptic connections, engage in programmed cell death (apoptosis). During apoptosis, the neuron activates death genes, which allow caspases to dissolve the chromatin composition of the neuron. The excessive 20-80% of neurons in each region engage in programmed cell death. This process is referred to as neural pruning, the majority of which occurs during the first 10 to 35 weeks of life (for review see Jacobson, 1991).

The result of dysfunctional apoptosis mirrors the structural abnormality displayed in the autistic amygdala. In particular, neuronal packing produces stunted dendritic arborization, where the dendrites projecting from the end of axons are too crowded to properly expand. The theoretical consequence of stunted dendritic arborization is an interference in messages relayed from connected regions, including the STS, hippocampus, and OMPFC. This is consistent with previously-described research that the autistic BLNG fails to integrate emotionally-relevant information received from other structures. Further, the chronological pattern of deficit appearance in autism begins as early as 12 months, as evidenced by impairments in gaze following (Grelotti et al., 2002). This pattern coincides with the timeline of apoptosis that normally allows proper, functional synapses to be established by 12 months.

It must be noted that the present hypothesis does not attempt to explain all the socio-emotional deficits within autism as arising from amygdala dysfunction. However, the neural system is integrated in such a manner that the more diffusely abnormal a structure, the greater the malfunction of connected systems (Rapin and Katzman, 1998). Therefore, the dysfunction of even one integral structure (in this case, the amygdala) can have profound negative effects on activity in the rest of the brain. As discussed, numerous lines of research support the theory that amygdala abnormality due to faulty neuronal pruning subsumes autistic impairments in social interaction and emotion. However, current neuroimaging studies that reveal autistic amygdala abnormality do so in hindsight of the appearance of deficits (Haznedar et al., 2000). Thus, a crucial piece of the puzzle is to determine whether structural changes in the amygdala elicit socio-emotional impairments. The alternative theory (which receives little support) is that behavioral deficits alter the amygdala structure. However, experimental manipulations of this theory have yet to be conducted for ethical and practical reasons. No genetic or environmental marker has been discovered that predicts the onset of autism. Thus, it has been unethical to clinically interfere with the development of the amygdala within human subjects. However, neuronal packing in the amygdala will only be definitively proven a substrate of autism once science is given the opportunity to interfere. Interestingly, the application of neurotrophic factor has been demonstrated to significantly decrease apoptosis (Jacobson, 1991). This crucial point provides a means through which amygdala abnormality can be experimentally induced among non-human subjects.

In light of the aforementioned practical and ethical concerns, experimental

manipulation of the macaque monkey (*Macaca fuscata*) is the next necessary step. The use of non-human subjects removes numerous extraneous variables that normally must be considered within theories of autism. Among humans, such variables as IQ, drug treatment, and varied environments can affect the level of autistic deficits or neural structures. Manipulations in the macaque are generalizeable to humans due to the fact that the primate neural structure highly mirrors that of humans (Lane and Nadel, 2000). Specifically, the STS processes socially-relevant information, which is then communicated through axonal projections to the lateral amygdala. The amygdala in turn engages in deep emotional processing of sensory stimuli. Possibly due to the parallel organization of human and macaque structures, macaques demonstrate numerous socially-interactive behaviors. They exhibit and act on fear (Lane and Nadel, 2000), respond to others, engage in gaze-following (Ferrari, Kohler, Fogassi, & Gallese, 2000), and most importantly, discern facial expressions through configural-processing (Sweeten et al.; 2002). Similar to humans, gaze following and configural-processing abilities increase with age as socially-interactive behaviors are reinforced (Ferrari et al., 2000). Lesion studies of monkeys, which examine intentional or incidental abnormalities in structure, have consequently facilitated an understanding of the role of the amygdala in normal social and emotional processing (Lane and Nadel, 2000; Sweeten et al., 2002). Amygdala lesions early in life elicited autistic-like characteristics among macaques, including little eye contact, lack of interest in face-processing, and decreased display of facial expressions.

These findings are consistent with the theory that amygdala abnormality among both humans and monkeys subsumes socio-emotional disturbances in autism. Consequently, the present research seeks to mimic autistic neuronal packing in the BLA portion of the amygdala through the application of neurotrophic factor. As described previously, neurons compete early in life for nourishing neurotrophic factor (for review see Jacobson, 1991). Those that receive it fail to engage in apoptosis. Therefore, application of neurotrophic factor greatly increases the number of surviving neurons, producing dense neuronal packing. It is hypothesized that later presentations of facial expressions will result in impaired face-processing, and that the impaired amygdala will display decreased activation in comparison to control monkeys. It is further hypothesized that no other structure will display impaired activity because neuronal packing will be manipulated solely in the amygdala. Should face-processing be impaired in accordance with only amygdala impairment, evidence will be established that a lack of neuronal pruning in the amygdala is responsible for social and emotional deficits in autism.

Methods

Approximately 8 macaque monkeys (7 weeks of age) will be used in the

proposed experiment. Of the 8, 4 will be experimental and 4 will be control, with an even number of males and females in each group. From the outset of the research, guidelines set forth by the Institutional Animal Use and Care Committee (IAUCC) will be followed for promoting the psychological well-being of the primates. They will be housed among non-experimental macaques in a mock-natural environment with the presence of natural visual, auditory, olfactory, and tactile stimuli. Further, decisions about combining or separating monkeys will be based on reducing territorial anxiety. All handlers will be selected based on prior training with primates, as established by the IAUCC. This enriched mock-environment will ensure that all 8 subjects are provided the opportunity to develop healthy social and emotional interactive behaviors.

The 8 macaques will be examined prior to commencement of the experiment using MRI to ensure normal structural development. Macaques displaying abnormal development of any structure will be rejected from the subject pool. At approximately 9.5 weeks of age, the BLA of 4 macaques will be outlined by 2 separate researchers through examination of the MRI scans. The 4 will be anesthetized. Guided by the location of the outlined amygdala, the macaques are to be injected with neurotrophic factor in order to suppress the occurrence of apoptosis. After a week has passed, an absolute and a relative amygdala volume will be calculated in cubic centimeters to determine the extent to which apoptosis has been suppressed. Whether further neurotrophic factor must be later administered (at the end of 15 weeks) will be determined by comparisons to the relative amygdala volume of controls. Also, other structural volumes will be compared to relative volumes in controls to ensure no abnormal neuronal changes, such as packing, have occurred. Subjects that demonstrate abnormal structural change other than in the amygdala will be removed from the subject pool.

The project intends to use two forms of data collection regarding behavioral manifestations of each monkey. The first form of data will be observational, collected for the subsequent 45 weeks (at the end of which the monkeys will be 1 year of age). Handlers, blind to the identity of manipulated versus control monkeys, will record levels of daily social interaction behaviors using a 5-point scale. For each of 12 behavioral questions, a score of 5 will represent the most active engagement in social behaviors. A score of 1 will represent no social behaviors. Points between 1 and 5 will represent a continuum of level of social behavior demonstrated. Recordings will be made for the dimensions of gaze following, empathy towards others, and helping behavior. Any stability or changes in social interaction behaviors will be identified by comparing weekly scores for each monkey to their scores from the prior week. Interrater reliability for each score will be assessed through use of the statistical program SPSS.

This phase of data collection is obviously prone to human bias and error.

Therefore, the resulting data will be used solely to create a socio-emotional profile of behavior for each monkey. Two new raters, blind to the hypothesis of the experiment as well as the identity of each group member, will compile the scores of all 45 weeks. Monkeys who score 0-1080 at the end of the 45 weeks will be classified displaying autistic characteristics. Scores of 1081-1485 will be deemed borderline autistic characteristics. Finally, those with a score of 1486-2700 will be labeled displaying normal characteristics. The classification for each monkey will then be compared with whether the monkey is a member of the experimental or the control group.

The second form of data collection will utilize neuroimaging and immunocytochemical techniques. At one year of age, MRI scans will demonstrate whether increased amygdala volume is still present among those monkeys treated with neurotrophic factor. Control and experimental subjects will then be presented 12 high-resolution, monochromatic pictures of monkey faces, each depicting 1 of the 6 different expressions. Each picture will remain for 7 seconds. The head and body of each monkey will be comfortably restrained so that only the eyes may move. Eye movement is monitored by an infrared corneal reflection technique to determine any differences in face-processing. For each picture, the direction of corneal attention on facial features will be recorded. Monkeys who demonstrate more than 50% fixation on non-configural features will be deemed segmental face-processors. Also, duration of facial-fixation will be recorded through the corneal reflection technique.

In concordance with this face-processing task, functional Magnetic Resonance Imaging (fMRI) scans will record which neural structures are hemodynamically active (receiving higher levels of rCBF) in response to the presented emotionally- and socially-relevant facial expressions. FMRI, like PET, records the changing activity of neural regions. However, the advantage of fMRI over PET is that no injection is required in order to witness differences in activity. During a second presentation of expressions to both groups, the anterograde tracer biotinylated dextran amine (BDA) will be injected into the STS, OMPFC, and hippocampus. Research has demonstrated BDA to provide excellent and abundant labeling of axons and terminals (Veenman, Reiner, & Honig, 1992). In other words, BDA, as it is transported along axons to other neurons, provides excellent contrast for microscopically viewing axonal pathways. In the experimental group, the structures to which the tracers are transported will be compared to the healthy control group to determine if impairments exist in axonal connections innervating the amygdala.

Findings and Significance

Social and emotional impairment in interactive behavior is the most characteristic manifestation of autism (APA, 1994). Numerous lines of research have suggested that structural abnormalities found in the amygdala, specifically among the

BLA, subsume this characteristic. However, to date studies have utilized powerful neuroimaging techniques to examine neural structures in hindsight of observed autistic impairments. The result of such techniques has demonstrated a strong correlation between autistic deficits and the presence of neural deformities. The present hypothesis proposes the crucial next step in determining whether amygdala abnormality due to faulty neuronal pruning subsumes autistic impairments in social interaction and emotion.

This study will induce structural abnormality therefore macaque monkeys will be used. Results from a sample of macaque monkeys are generalizeable to humans because their neural structure mirrors that of humans. Consequently, it is expected that the application of neurotrophic factor at 9.5 weeks of age will suppress apoptosis in macaque monkeys, as it is shown to do in human neurons. The consequence will be a packing of neurons within the amygdala that parallels the presence of amygdala deformity demonstrated among autistic individuals. This deformity is expected to be observable in MRI scans of amygdala volumes conducted a week after administration of the neurotrophic factor. It is, however, possible that apoptosis in the amygdala does not normally occur until the lapse of a specific period of weeks after birth. Therefore, should MRI scans find relative amygdala volumes to be equal to that of controls, more neurotrophic factor will be administered. It is further expected that suppression of apoptosis will *only* occur in the amygdala, for the proposed research seeks to eliminate abnormalities in other neural structures as an explanation for autistic characteristics.

The organization of the macaque emotional sensory processing system also mimics that of humans. Therefore, it is hypothesized that the induced structural abnormalities of macaques will elicit autistic-like impairments in social and emotional behavior. Scores obtained during the 45-week observational data collection period should reflect these impairments. Specifically, it is expected that experimental monkeys will demonstrate an initial decrease in scores from pre-manipulation levels, as well as in comparison to controls. Further, these monkeys should place into the displaying autistic characteristics classification. Each socio-emotional profile is expected to supplement the concrete data provided by corneal reflection techniques, and by fMRI scans of rCBF and microvascular oxygenation during facial expression presentations. During the exposure to faces, experimental macaques should show segmental facial analysis, as well as a decreased duration of fixation time. The fMRI scans should display relatively normal functioning among the affect-linked sensory system structures, including the STS, OMPFC, and hippocampus. Finally, it is expected that BDA tracers injected into the STS, OMPFC, and hippocampus will fail to properly follow the BLA axonal pathways utilized by controls.

The finding that normal sensory processing occurs in non-amygdala structures, in correspondence with the existence of autistic-like behavioral impairments, will provide evidence for the hypothesis that the pathophysiologic underpinning of autism

lies within the amygdala. An inability for the BLA to axonally receive messages from higher-order sensory systems, as evidenced by abnormal transportation of BDA tracers, supports the subsequent hypothesis that neuronal packing impairs the BLA in integrating information. Experimentally suppressing apoptosis in the macaque amygdala is the next crucial step in determining the pathologic underpinning of autism. Such a determination will subsequently give rise to research regarding the exact origin of autistic neuronal packing in the amygdala. Unfortunately, a lack of advancements in neuroimaging and biochemical techniques currently prevents research from determining whether a genetic abnormality for apoptosis exists within the amygdala. One alternative explanation is that an excessive level of neurotrophic factor is released during early development, suppressing programmed neuronal death. It is the hope of this proposal that progress in the understanding of autism will motivate future researchers to develop these more advanced techniques.

The present research will consequently play a significant role in the future of diagnosis, treatment, and possible preventative measures for autism. There is currently no gold standard with which to diagnose the presence of this disorder (Rapin and Katzman, 1998). A concrete understanding of its pathophysiology will allow such a standard to be created based on the presence of specific abnormalities, such as in the amygdala. Due to the fact that a diagnosis of autism often requires lifelong societal support, strenuous research has focused on curative treatments. Most medications have sought to alleviate the social and emotional impairments, such as segmental facial analysis, that hinder social reciprocity (Werry, 2001). Alternatives have targeted numerous sources such as neurotransmitter release, and the ability of the amygdala to integrate sensory information (Buitelaar, 2003). The latter treatment, a synthetic acetylcholine analogue called Org 2766, was found to facilitate significant improvements in eye-contact and social reciprocity. However, as with any other drug, Org 2766 has emotionally-aggravating side-effects such as agitation and irritability. It is clear that the future of research, including the present proposal, must elucidate the physiological abnormalities within autism. Only with such an understanding can preventive measures begin to fight such drastic and heartbreaking impairments as an inability to draw social and emotional clues from a facial expression.

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