Distribution Agreement

The Role of the Amygdala and Orbital frontal Cortex in Processing Socially Relevant Crossmodal Signals

By

Christa Payne
Doctor of Philosophy

Psychology

Jocelyne Bachevalier, Ph.D.
Advisor

Lisa Parr, Ph.D.
Committee Member

Philippe Rochat, Ph.D.
Committee Member

Kim Wallen, Ph.D.
Committee Member

Stuart Zola, Ph.D.
Committee Member

Accepted:

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

Date

The Role of the Amygdala and Orbital frontal Cortex in Processing Socially Relevant Crossmodal Signals

By

Christa Payne
M.S., University of Texas at Houston, 2004
B.S., Texas A&M University - Commerce, 1999

Advisor: Jocelyne Bachevalier, Ph.D.

An abstract of a dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Psychology
2011

Abstract

The Role of the Amygdala and Orbital Frontal Cortex in Processing Socially Relevant Crossmodal Signals

By Christa Payne

Primate social success requires crossmodal integration of affective social signals from faces and voices, but little is known about the neural substrates supporting this ability. Hence, we assessed the contributions of amygdala and orbital frontal cortex, two structures known to process affect from faces and voices, in the acquisition of normal integration and affective processing abilities. Integration ability and scanning strategies of 16 adult rhesus macaques that received either lesions of the amygdala (Neo-A_{ibo}; n = 6) or the orbital frontal cortex (Neo-O_{asp}; n = 4) or sham operations (Neo-C; n = 6) as infants (males = females in each group) were quantified using eye-tracking. Four short (2-sec) video clips depicting novel male conspecifics producing one of four species-typical vocalizations (coo, grunt, scream, threat) were presented in a preferential viewing paradigm. Pairs of videos were played with the auditory component matching one of vocalizations in two conditions: Synchronized (simultaneous onset of audio and visual components); Desynchronized (delayed onset of audio component). Group Neo-C showed a preference for one of the two videos in both conditions, indicating integration ability. Scanning patterns of males and females differed, with females (eyes > mouth) attending to vocalization valence, but males (eyes = mouth) appeared to also attend to another stimulus feature, such as the inferred dominance status of the stimulus animals. Group Neo-A_{ibo} demonstrated normal integration ability, but the sex-specific scanning strategies exhibited by group Neo-C were completely disrupted. Neo-A_{ibo} males and females showed the exact opposite pattern of group Neo-C. By contrast, group Neo-O_{asp} showed weakened integration ability that was associated with increased looking to the eye regions. This increased salience of the eyes was attributed to higher aggression displayed by these monkeys in other laboratory measures. Collectively, our data show that both the amygdala and orbital frontal cortex are involved in the evaluation of socio-emotional audiovisual signals, albeit in slightly different ways. In line with current literature, the amygdala appears to contribute to sex-specific assessments of the social salience of the crossmodal stimuli, whereas the orbital frontal cortex is crucial for the appropriate regulation of responses to the stimuli.

The Role of the Amygdala and Orbital frontal Cortex in Processing Socially Relevant Crossmodal Signals

By

Christa Payne
M.S., University of Texas at Houston, 2004
B.S., Texas A&M University - Commerce, 1999

Advisor: Jocelyne Bachevalier, Ph.D.

A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Psychology
2011

Acknowledgments

The success of this project is indebted to the dedication of several individuals. First and foremost, my mentor, Jocelyne Bachevalier. Without the guidance, patience and freedom she gave me, this project would not have gotten off the ground. I would also like to thank my committee members, Lisa Parr, Philippe Rochat, Kim Wallen and Stuart Zola, for their input and service. I am grateful for Asif Ghazanfar for the use of his stimuli, Harold Gouzoules for his input on the design of the study, Nancy Bliwise for being willing to go into SPSS code to help me with the multivariate analyses, and Kati Gothard for her valuable comments.

I would also like to recognize the members of the Bachevalier laboratory, past and present. Without your support and friendship, these last several years would have been unbearable. A special thanks to Andy Kazama for his poise, athleticism and sense of humor, Jessica Raper for her musical knowledge, infectious laugh and unique clock chimes, Maria Alvarado for her mad craft skills and residing over the SBC, and Alyson Zeamer for being our resident farmer.

I honestly could not have done this without the undying love and encouragement of my husband, Matthew Payne, my mother, Tamara Getchell, and in-laws, Gary and Janet Payne. I am eternally grateful for your support and dedication.

Finally, I would be remiss not to recognize the 16 monkeys that made the ultimate sacrifice for the advancement of our understanding of the connection between the brain and behavior.

Table of Contents

List of Tables

MANUSCRIPT II: The Role of the Amygdala in Processing Bimodal Conspecific Vocalizations in Rhesus Macaques

MANUSCRIPT III: The Role of the Orbital Frontal Cortex in Processing Bimodal Conspecific Vocalizations in Rhesus Macaques

GENERAL DISCUSSION

List of Figures

MANUSCRIPT I: Crossmodal Integration of Conspecific Vocalizations in Rhesus Macaques

MANUSCRIPT II: The Role of the Amygdala in Processing Bimodal Conspecific Vocalizations in Rhesus Macaques

MANUSCRIPT III: The Role of the Orbital Frontal Cortex in Processing Bimodal Conspecific Vocalizations in Rhesus Macaques

GENERAL DISCUSSION

GENERAL INTRODUCTION

For the majority of the animal kingdom, social success depends upon effective communication. Even species that live in solitude must be able to transmit and perceive species-specific signals in order to procreate and defend their resources. Such signals are rarely limited to a single sensory modality. Animals, including primates, commonly use vocalizations, visual displays, olfactory cues, and somatosensory signals to convey their messages. The dynamic social environments in which human and nonhuman primates live require individuals to be able to recognize, manipulate, and behave according to the immediate social context. Thus, an individual must be able to construct representations of relations between self and others and to use those representations flexibly to guide social behavior (Adolphs, Sears, & Piven, 2001; Baron-Cohen et al., 1999). This relies upon the ability to distinguish and interpret a multitude of social cues, often broadcast over multiple sensory modalities. Hence, crossmodal integration has become a crucial component of social success in humans and nonhuman primates.

In primates, faces and voices are the dominant modalities of expression, especially emotional expression. Each conveys information about an individual's identity (i.e., idiosyncratic features that create unique identities) and affective state (e.g., emotional and motivational) (Darwin, 1872). The combination of channels can profoundly impact signal meaning and efficacy (Marler, 1965; Partan & Marler, 1999; Rowe, 1999). The importance of the reciprocal influence of facial movements and vocal expressions was first demonstrated with the McGurk effect (1976), illustrating that speech perception is affected by inconsistencies between signals. When presented with a video of someone producing a single phoneme (e.g., /ga/) dubbed with an audio recording of a different phoneme (e.g., /ba/), observers will often perceive a third, intermediate phoneme (e.g., /da/).

Whereas crossmodal integration has been extensively characterized in the context of visual speech, multisensory perception of emotion has only recently come into focus (reviewed by Campanella & Belin, 2007). Recent studies have demonstrated that, like the enhanced signal efficacy experienced in 'lip-reading', congruency between facial expressions and affective prosody optimizes behavioral responses to emotion-laden stimuli and that information from one sensory modality can influence the processing of another (Collignon et al., 2008; Collignon et al., 2010; de Gelder & Vroomen, 2000; Dolan, Morris, & de Gelder, 2001; Ethofer, Anders, Erb et al., 2006; Massaro & Egan, 1996). Notably, few studies have used dynamic stimuli, which have been shown to enhance neural responsiveness and emotion perception, and the neural correlates of emotional crossmodal integration have not been fully elucidated. Identifying the neuroanatomical substrates of crossmodal integration could provide valuable insight into human neuropsychological disorders that include deficits in emotional crossmodal integration (e.g., autism, schizophrenia, pervasive developmental disorder). The amygdala and orbital frontal cortex are two regions critically involved in processing socio-emotional information from the auditory and visual modalities. Thus, the purpose of the current project was to evaluate the contributions of the amygdala and orbital frontal cortex to crossmodal integration of complex social signals in rhesus macaques, a species of nonhuman primates that have been shown to spontaneously integrate the audio and visual components of their species-specific bimodal vocalizations in a laboratory setting.

1- Terminology

‘Sensory integration’ is frequently left without an exact definition. Different researchers tend to use the same terms, regardless of their level of analysis. That is, some investigators view sensory integration in the context of the specific response properties of neuronal activity, and others consider it as some higher level cognitive combination that merges different sensory information into a coherent percept (Kayser & Logothetis, 2007). To date, the literature is muddled by the use of multiple terms to describe sensory integration processes (i.e. heteromodal, multimodal, intersensory, polysensory, multisensory, amodal, supramodal, modality-specific, and unimodal). Thus, for clarification, the current discussion utilizes the following terminology: the terms ‘unisensory’ and ‘multisensory’ describe the activity of neurons that respond to one or multiple senses, respectively; ‘sensory-specific’ refers to neuroanatomical areas thought to process information from a single sensory-modality, whereas ‘heteromodal’ refers to those neuroanatomical areas that receive converging projections from different sensory systems; the terms ‘unimodal’ and ‘crossmodal’ refers to behavioral tasks or stimuli involving one or multiple senses, respectively. ‘Multimodal’ and ‘bimodal are also used interchangeably with ‘crossmodal’.

The study of sensory integration is heavily influenced by pioneering, electrophysiological studies in the superior colliculus, a subcortical convergence zone for sensory information (Stein & Meredith, 1993). Discussions of neuropsychological measures, such as reaction times, functional imaging activation and ERP responses, are predicated on the principles of neuronal responses. Thus, although the current project focuses on the behavioral and neuroanatomical aspects of sensory integration of emotional information, a review of concepts associated with the neuronal aspects within the context of behavior is warranted.

At the neuronal level, 'sensory convergence' occurs if a response is evoked either by stimuli from different modalities presented in isolation, or if the activity elicited by a stimulus of one modality is modulated from a stimulus of another modality. There are two characterized types of neuronal response modulation: 'enhancement' and 'depression'. Enhancement refers to the relative increase in response elicited by stimuli of multiple modalities as compared to that elicited by a single modality. The behavioral corollary to this phenomenon is quicker reaction times to the presentation of synchronized, redundant audiovisual stimuli as compared to the unimodal components. Conversely, response depression (or 'suppression') refers to a decrease in response to crossmodal stimuli compared to the unimodal response. The behavioral parallel to this would be slower reaction times when the modalities are discordant, as in a dubbed movie or when the audio and visual components are desynchronized. Finally, there are times when the crossmodal response is larger than the sum of responses elicited by each modality in isolation. At the neuronal level, this is referred to as 'superadditivity'. Behaviorally, when reaction times to the bimodal stimulus exceed the Race Model estimations (derived from reaction times to the unimodal components) (Miller, 1982), the auditory and visual cues are thought to interact.

The study of multisensory interactions has produced a number of important principles for sensory integration. The first principle pertains to the temporal arrangement of the stimuli. The principle of temporal coincidence posits that the sensitivity of neurons to multisensory enhancement is dependent upon the relative timing of the stimuli (Stein & Wallace, 1996). Only stimuli that occur in close temporal proximity cause response enhancement and stimuli that are sufficiently separated in time elicit their normal sensory-specific response.

The second principle concerns the spatial arrangement of the sensory stimuli within a neuron's receptive field. Neurons in the superior colliculus usually respond to stimulation within a restricted spatial region. Accordingly, the principle of spatial coincidence suggests that in multisensory neurons, the receptive fields of different modalities usually overlap and that enhancement is a result of crossmodal stimuli falling within this overlap. Conversely, stimuli falling outside of the overlap would cause a response depression (Stein, 1998). Moreover, response to unimodal stimuli can be substantially lessened or even eliminated by the presence of a spatially incongruent stimulus from another modality (Kadunce, Vaughan, Wallace, Benedek, & Stein, 1997).

Surround sound and ventriloquists take advantage of these principles. When auditory and visual information are presented simultaneously but from slightly different spatial locations, the location of the sound is shifted in the direction of the visual stimulus (Stein & Meredith, 1993). However, when the audio and visual components are sufficiently desynchronized either temporally or spatially, they are perceived as separate events. When the temporal coincidence of the components exceeds 250 msecs, they are perceived as separate events (Dixon & Spitz, 1980).

Lastly, the principle of inverse-effectiveness suggests that the degree of response modulation is dependent on the efficacies of the unimodal stimuli in driving the neuronal response (Perrault, Vaughan, Stein, & Wallace, 2003; Stanford, Quessy, & Stein, 2005; Stanford & Stein, 2007; Stein & Wallace, 1996). When a strong response is elicited by unimodal stimuli, the response is only minimally modulated by additional modalities. However, neurons that exhibit weak unimodal responses can show considerable modulation to crossmodal stimuli.

The principles of temporal and spatial coincidence imply that crossmodal interactions are specific to stimuli that could originate, or be perceived as originating, from the same source, and the principle of inverse-effectiveness provides a link between neuronal activity and the behavioral benefits of sensory integration (Kayser & Logothetis, 2007). These three principles have evolved into a set of criteria that are frequently used to determine whether an observed effect actually reflects sensory integration. They were derived from activity of individual neurons in the superior colliculus, yet they are often applied to other regions of the brain and to other measures of neuronal activity, such as functional imaging and ERP (Beauchamp, 2005; Calvert, 2001; Laurienti, Perrault, Stanford, Wallace, & Stein, 2005). Importantly, the adoption of dynamic stimuli has facilitated the characterization of these principles in emotional crossmodal integration, albeit most work in this area has been completed with human subjects.

2- Dynamic vs. Static Stimuli

The processing of emotional stimuli has historically been investigated in a single modality (i.e. emotional faces or emotional vocal expressions). Recently, a handful of studies have explored the multimodal nature of emotional expressions (reviewed by Campanella & Belin, 2007). Such research has confirmed that redundancy of information across facial expressions and affective prosody (an indicator of emotion in voices) facilitates reaction efficacy to emotional stimuli, as measured by reaction times and accuracy in emotion identification (Collignon et al., 2008; Collignon et al., 2010; de Gelder & Vroomen, 2000; Dolan et al., 2001; Ethofer, Anders, Erb et al., 2006; Kreifelts, Ethofer, Grodd, Erb, & Wildgruber, 2007; Pourtois, de Gelder, Bol, & Crommelinck, 2005). Additionally, information from one sensory modality can alter the perception of information from another modality (de Gelder & Vroomen, 2000; Ethofer, Anders, Erb et al., 2006; Massaro & Egan, 1996). For example, emotional prosody (vocal tone) can alter the perception of a facial expression (e.g., subjects rate fearful and neutral faces as more fearful when accompanied by fearful voices than when the same faces were presented in silence). Interestingly, this crossmodal bias persists even when instructed to ignore one of the modalities (Collignon et al., 2008; de Gelder & Vroomen, 2000; Ethofer, Anders, Erb et al., 2006; Kreifelts et al., 2007; Massaro & Egan, 1996).

However, it is important to note that the majority of investigations on crossmodal perception of emotion employed static images as visual stimuli. Multimodal emotional expression does not exist simply as snap-shots of prototypical emotional expressions with vocal accompaniment. It occurs with fluidity of motion and at varying intensities. Facial movements enrich emotion expression and facilitate emotion recognition and intensity perception (Ambadar, Schooler, & Cohn, 2005; Biele & Grabowska, 2006). These enhancements have also been reported in neurological cases with bilateral damage to the occipital and/or temporal lobes who are often impaired in emotion identification of static faces, but not in the recognition of dynamic expressions (Adolphs, Tranel, & Damasio, 2003; Humphreys, Donnelly, & Riddoch, 1993). Additionally, neuroimaging studies have shown that neuroanatomical regions that are responsive to facial affect in static images (i.e., posterior temporal sulcus, amygdala, and insula) show a greater hemodynamic response to dynamic emotional expressions (Haxby, Hoffman, & Gobbini, 2000, 2002; LaBar, Crupain, Voyvodic, & McCarthy, 2003). Finally, dynamic stimuli are more ethologically relevant than static images, a particularly important consideration when working with nonhuman primates. Together, these observations emphasize the importance of naturalistic, dynamic stimuli in the assessment of neuroanatomical substrates of emotional crossmodal integration.

3- Rhesus Macaque Vocalizations

The rhesus macaque communicative system is characterized by a relatively small repertoire of largely inflexible calls that are closely linked to particular social contexts. The acoustic qualities, facial characteristics, and social meanings of rhesus macaque vocalizations have been well characterized in both free-ranging and captive monkeys. The current project utilizes four species-specific calls that are distinguishable by all three factors: coo, grunt, scream and threat.

Coos are long, harmonically rich, affiliative calls produced with lip protrusion and a fairly wide separation between the lips and lower mandible (Hauser, Evans, & Marler, 1993; Partan, 2004). They are general contact calls often produced during troop movements, foraging in dense vegetation, or separation from social group, and rarely directed towards particular conspecifics. By contrast, grunts are affiliative calls that are directed toward specific individuals, often during social approaches (Rowell & Hinde, 1962). Acoustically, grunts are quiet, short, harsh calls associated with small mouth openings and minimal lip protrusion (Hauser et al., 1993; Partan, 2002).

Screams are produced within the context of submission with the presumed purpose to solicit aid from conspecifics (S. Gouzoules, Gouzoules, & Marler, 1984). Screams have been categorized by acoustic properties into five subclasses: arched, tonal, noisy, pulsed, and undulated. A noisy scream, used in the current project, is accompanied by partial teeth separation, retracted lips, and full baring of teeth (Hauser et al., 1993; Partan, 2002).

Threats are also produced during antagonistic encounters, but are emitted by the aggressor. Threats are noisy, short, pulsatile calls accompanied by a large mouth opening with slight lip protrusion resulting in little exposure of teeth (Hauser et al., 1993; Partan, 2002).

Research at various levels of analysis has demonstrated parallels between the vocal communication of humans and rhesus macaques. Playback experiments with female rhesus monkeys have established that they have vocal recognition of individuals and kin (Fugate, Gouzoules, & Nygaard, 2008; H. Gouzoules,

Gouzoules, & Marler, 1986; Rendall, Rodman, & Emond, 1996). Rhesus monkeys also exhibit crossmodal integration of the auditory and visual components of species vocalizations by spontaneously matching vocal calls with their associated facial postures (Ghazanfar & Logothetis, 2003). Furthermore, a recent neuroimaging investigation in rhesus macaques revealed that auditory presentation of species-specific calls activates neural networks associated with visual representations and affective processing. Thus, like humans, rhesus monkeys appear to possess cognitive representations of the affective content of species-specific calls (Gil-da-Costa et al., 2004). Finally, rhesus macaques also demonstrate crossmodal cognitive representations of familiar conspecifics by spontaneously matching the faces of familiar conspecifics to their voices (Sliwa, Duhamel, Pascalis, & Wirth, 2011). These behavioral and neuroanatomical similarities in the domain of emotional multimodal communication suggest that rhesus macaque vocalizations provide an appropriate model for investigating the neural substrates of emotional crossmodal integration. This is further illustrated in the following sections, examining the behavioral and neuroanatomical evidence in humans and rhesus macaques supporting the roles of the amygdala and orbital frontal cortex in emotional crossmodal integration.

4- Amygdala and Socio-emotional Processing

The amygdala has long been identified as a neural region critically involved in social cognition, especially processing socio-emotional signals. This heteromodal structure has been implicated in (a) coding and processing facial movements, eye-gaze directions, body postures, and gestures that are potent signals for the production and modulation of appropriate social and emotional responses towards other individuals (Adolphs, 1999, 2003; Bachevalier & Loveland, 2006; Bachevalier & Meunier, 2005; Bechara, Damasio, & Damasio, 2003); (b) linking discrete stimuli to their intrinsic motivational and socio-emotional significance (M. Baxter & Murray, 2000; Blundell, Hall, & Killcross, 2001; Morrison & Salzman, 2010; Murray, 2007; Rolls, 1992; Salzman & Fusi, 2010; Stefanacci, Clark, & Zola, 2003), such as associating a specific animal in a social troop and its level of agonistic behavior; (c) regulating motor, autonomic and endocrine manifestations of emotions through its connections with the striatum, brainstem and hypothalamus, respectively (Amaral, Price, Pitkanen, Carmichael, & Aggleton, 1992; Saunders & Rosene, 1988; Saunders, Rosene, & Van Hoesen, 1988). Although the role of the amygdala in processing emotion in faces has been extensively investigated (discussed below), the contribution of the amygdala in processing complex socio-emotional signals, such as bimodal, species-specific vocalizations, has yet to be fully explored.

Previous research in both humans and nonhuman primates has indicated that the amygdala is not critical for crossmodal integration (Goulet & Murray, 2001; Lee, Meador, Smith, Loring, & Flanigin, 1988; Nahm, Tranel, Damasio, & Damasio, 1993). Monkeys that received excitotoxic lesions of the amygdala in adulthood were not impaired in crossmodal integration as measured by a tactual-visual delayed-nonmatching-to-sample task (Goulet & Murray, 2001). Similarly, humans with bilateral damage to the amygdala were also not impaired in visual-tactual integration (Lee et al., 1988; Nahm et al., 1993). Based on these results, researchers concluded that the amygdala was not involved in crossmodal integration. However, the methodology of those investigations may have affected the ability to detect impairments following amygdala damage.

For example, the lesion studies employed matching paradigms that assessed acquisition and recall of crossmodal associations, and not crossmodal integration, per se. A common misconception within the crossmodal literature is that all paradigms utilizing the same combination of senses (e.g. vision and touch) rely on the same underlying mechanisms regardless of differences in parameters and design (Calvert, 2001). Despite this assumption, there is strong psychological and theoretical evidence to posit that different crossmodal tasks may rely upon different neuroanatomical processes (Calvert et al., 1997; Radeau, 1994; Stein & Meredith, 1993). Crossmodal matching tasks require the responder to determine whether previously associated features are congruent across *two distinct* objects. Conversely, tasks of crossmodal integration depend on the perception that two or more sensory cues (or components) are perceived to be originating from the *same* object (Radeau, 1994; Stein & Meredith, 1993).

Moreover, even if the same neural substrates subserve crossmodal matching and crossmodal integration, it seems premature to conclude that a neuroanatomical region is not involved in crossmodal integration based on negative results from a single pair of modalities (i.e., vision and touch). A lack of involvement of the amygdala in processing objects that could be identified by sight and touch does not preclude the region from being critically involved in the integration of complex socio-emotional stimuli that can be identified by sight and sound. Extensive research in both human and nonhuman primates has established a specific role of the amygdala in processing stimuli with socio-emotional significance, and not generalized object recognition (reviewed by Adolphs, 2010). The following sections review the neuroanatomical and behavioral contributions of the amygdala in processing bimodal and unimodal socio-emotional signals in humans and nonhuman primates.

A) Sensory Convergence in the Amygdala

The amygdala is a heteromodal association area that receives sensory inputs from virtually every sensory modality, and is reciprocally connected with those sensory cortical regions (reviewed by Amaral et al., 1992). Neural characterizations have revealed strong similarities between the amygdala of humans and nonhuman primates throughout development. The lateral nucleus receives a large array of highly processed sensory information, including visual information from faces and facial expressions, gaze direction, body postures, and movements, as well as auditory information from specific vocal sounds and intonations (McDonald, 1998; Yukie, 2002). The reciprocity of these projections back to the sensory areas via the basal nucleus provides a route by which the amygdala can modulate the cortical processing of sensory stimuli (Amaral, Behniea, & Kelly, 2003; McGaugh, Ferry, Vazdarjanova, & Roozendaal, 2000; Yukie, 2002). These feedback projections to cortical sensory areas are widespread and reach not only the higher-order areas from which it receives major inputs, but also the primary sensory areas. As a result, the amygdala is able to influence sensory inputs at very early stages in their processing, such as the fusiform gyrus, known to be involved in face processing. Whereas the basal and lateral nuclei serve as an interface between sensory-specific cortical inputs, the central nucleus constitutes a relay to the brainstem and hypothalamus through which the amygdala putatively influences the autonomic and endocrine manifestations of emotion. Efferent projections emanate from the basal and accessory basal nuclei to the ventral striatum, providing a potential access to subcortical elements of the motor system. These connections in turn facilitate the influence of the amygdala on actions, such as the modulation of facial and vocal expressions, body postures, and movements. The amygdala is also highly interconnected with the hippocampal formation, allowing for access to and modulation of stored information in cortical areas (such as past experience with an individual) (Amaral, 1992; Saunders & Rosene, 1988; Saunders, et al., 1988). This pattern of connectivity further supports a role of the amygdala in crossmodal integration of socio-emotional information, perhaps via modulatory processes.

B) Amygdala and Emotion

Efforts to discern the neurophysiological mechanisms associated with processing emotional social signals have been extensive. The evolution of functional imaging techniques has made the study of crossmodal integration of emotional social signals increasingly accessible. Nonetheless, this field of research is, as of yet, quite immature. Accordingly, it is important to consider data from investigations of unimodal emotional social signals before reviewing the data on crossmodal integration.

i) Amygdala and Facial Emotion

The role of the amygdala in processing emotional facial stimuli has been robustly characterized using a variety of techniques (Adolphs, 2002; Breiter et al., 1996; Kuraoka & Nakamura, 2007; Morris et al., 1996; Phelps, 2006; Rasia-Filho, Londero, & Achaval, 2000; Vuilleumier & Pourtois, 2007). Patients with selective damage to the amygdala are impaired in recognizing expressions of fear expressions. Such impairments appear to be largely gated by a lack of preferential scanning of the eye region (Adolphs et al., 2005). Consistent with this neuropsychological evidence, functional neuroimaging has repeatedly identified the amygdala as a neural substrate of fear expression processing (reviewed by Adolphs et al., 2001).

Interestingly, face processing impairments in autism have been associated with changes in activation of the amygdala (Baron-Cohen et al., 1999; Critchley et al., 2000; Pierce, Haist, Sedaghat, & Courchesne, 2004; R. T. Schultz, I. Gauthier et al., 2000; R. T. Schultz et al., 2003; R. T. Schultz, Romanski, & Tsatsanis, 2000). One particularly elegant design combined eye tracking techniques and fMRI to study facial discrimination in persons with autism (Dalton et al., 2005). By combining eye tracking with fMRI, the researchers were able to identify an increased activation in the amygdala that related to increased eye fixation. Further analysis revealed that the activation of the amygdala was associated with faces in general (rather than being specific to the emotional content of the stimuli).

Preliminary results from Bachevalier and colleagues further indicate that the amygdala is involved in processing socio-emotional signals. The visual scanning patterns of monkeys presented with static images of human and monkey neutral faces were assessed using eye-tracking techniques (Goursaud & Bachevalier, 2007; Goursaud, Bachevalier, Jones, & Klin, 2003). Consistent with previous findings (Guo, Robertson, Mahmoodi, Tadmor, & Young, 2003), normal-developing monkeys, like humans, preferentially looked towards the eye area of the face stimulus. Animals that received selective lesions of the amygdala in adulthood did not differ from sham-operated controls in the amount of time they spent scanning the full face and the nose/mouth region. However, like humans with damage to the amygdala (Adolphs et al., 2005) or autism (Dalton et al., 2005), animals with amygdala lesions spent less time scanning the eye region as compared to normal-developing monkeys. The inter-species correspondence highlights the appropriateness of investigating the neural substrates of socio-emotional processes in rhesus macaques.

ii) Amygdala and Vocal Emotion

In contrast to the role of the amygdala in processing facial emotion, the responsiveness of this region to vocal expressions of emotion remains unresolved. An fMRI investigation comparing facial and vocal expressions of fear and disgust demonstrated activation extending into the amygdala (Phillips et al., 1998). However, a PET study reported a deactivation of the amygdala in response to vocal emotion (Morris, Friston, & Dolan, 1998). Inconsistencies also extend into the human lesion literature, where some patients with bilateral amygdala damage display impairments in vocal emotion recognition (Scott et al., 1997), but other patients with comparable damage are unimpaired (S. W. Anderson, Bechara, Damasio, Tranel, & Damasio, 1999).

A more recent investigation explored the neural substrates of species-specific calls in rhesus macaques using PET imaging (Gil-da-Costa et al., 2004). As mentioned above, activation to vocal stimuli (coos and screams) was observed in higher-order visual areas as well as regions associated with the interpretation of highly salient and affective information, including the amygdala. The activation patterns suggest that rhesus monkeys possess a cognitive representation of species-specific calls. But the specific behavioral contributions of the amygdala in processing the affective calls could not be specifically addressed with the passive listening paradigm.

iii) Amygdala and Audiovisual Emotion

A few investigators have begun using bimodal socio-emotional stimuli to assess the neural correlates of crossmodal integration via electrophysiology and neuroimaging. One of the earliest explorations in humans used event-related fMRI to assess how the concurrent presentation of fearful voices facilitates recognition of fearful facial expressions and proposed that the amygdala is important for emotional crossmodal sensory convergence (Dolan et al., 2001). In this study, participants were presented with a static image facial expression of either fear or happiness simultaneously with an auditory recording of a short sentence uttered in either a happy or fearful tone. Increased activation within the amygdala was associated with congruency across modalities for the expression of fear (i.e., fear face and fearful tone), indicating that this region was involved in the crossmodal binding of the fearful face-voice pairing.

A follow-up investigation used PET and included both unimodal and bimodal stimuli to compare the difference between each single modality separately and in combination (Pourtois et al., 2005). The paradigm was modified to use a covert processing task in which subjects were not consciously attending to the emotional meaning of the stimuli (a gender decision task), but still provide evidence of audiovisual integration during the perception of emotion. Consistent with previous studies, amygdala activation was observed in response to fearful faces and fearful audio-visual pairs, but not for fearful voices. The activation to audio-visual pairs was greater than the activation to only faces, further indicating that affective information from face and voice converge in the amygdala (Pourtois et al., 2005).

As previously discussed, research has shown that affective auditory information modulates the perception of affective visual information (i.e., faces are rated as more fearful when presented in conjunction with fearful voices) (Massaro & Egan, 1996). Human neuroimaging has shown that difference between the amygdalar BOLD-response elicited by unimodal faces and the amygdalar BOLD-response elicited by bimodal face-voice pairings is correlated with the differences in valence ratings for the two conditions (Ethofer, Anders, Wiethoff et al., 2006). This relationship supports an involvement of the amygdala in modulation of cognitive evaluation of emotional facial expressions.

Notably, the above investigations used static depictions of prototypical facial expressions of fear and happiness and sentences spoken with different prosodies. Interestingly, the use of dynamic emotional stimuli in an event-related fMRI design failed to detect activation of the amygdala in the processing of emotional, multimodal stimuli (Kreifelts et al., 2007). Stimuli and task design may have contributed to the discrepancies with previous reports. Kreifelts and colleagues (2007) employed 2-sec video clips of professional actors speaking single words with either a neutral or one of six emotional intonations (alluring, angry, disgusted, fearful, happy or sad) with a congruent facial expression. Stimuli were presented both unimodally (audio or visual) and bimodally (audiovisual) and subjects were instructed to judge the stimuli according to the expressed emotional category using only non-verbal cues (i.e., prosody and facial expression) and completely disregard the semantic information. Half of the words used were neutral in word content and half had either a positive or negative valence. The inclusion of the neutral category and neutral words may have attenuated amygdala response. A recent fMRI investigation revealed that the amygdala was consistently activated when information from both modalities was emotional, but the BOLD response was significantly less when information from one of the modalities was neutral (Muller et al., 2011). Thus, although participants of Kreifelts and colleagues' study (2007) were instructed not to make judgments on the semantic content of words, the use of neutral words may have down-regulated amygdala activation across all stimulus types, resulting in no differences in activation between unimodal and bimodal conditions.

Collectively, neuroimaging work in humans is consistent with the amygdala being a region of sensory convergence and supports a putative role of this region in processing affective crossmodal information. To date, the limited work on the neural substrates of crossmodal integration of species-specific vocalizations in rhesus macaques have concentrated on developing an evolutionary basis of visual speech perception and, thus, has largely focused on primary sensory cortices (Ghazanfar, Chandrasekaran, & Logothetis, 2008; Ghazanfar & Lemus, 2010; Ghazanfar, Maier, Hoffman, & Logothetis, 2005), the superior temporal sulcus (a region involved in integrating audiovisual biological motion, including vocalizations) (Ghazanfar et al., 2008); and the ventrolateral prefrontal cortex (an area involved in audiovisual speech processing) (Sugihara, Diltz, Averbeck, & Romanski, 2006). However, one electrophysiological investigation explored the responses of single neurons in the rhesus macaque amygdala to affective information in faces and/or voices (Kuraoka & Nakamura, 2007). Researchers recorded neural activity while monkeys viewed video clips of conspecifics producing either a threat, coo or scream. Stimuli were presented in three conditions: visual (face only), auditory (voice only) and audiovisual (face and voice). The central nucleus of the amygdala contained multisensory neurons that responded to stimuli in the audiovisual condition, but varied in their response to the unimodal conditions. The majority of the multisensory neurons also responded to the visual element (audiovisual > visual), but not the auditory element. There were also multisensory neurons that responded only to the auditory element or did not respond to either the auditory or visual element, as well as multisensory neurons that responded to each element in isolation (Kuraoka & Nakamura, 2007). The results from this passive viewing paradigm provide clear support for a role of the amygdala in processing multimodal vocalizations in rhesus monkeys; however, further investigation is needed to elucidate the behavioral contributions of the amygdala in emotional crossmodal integration.

5- Orbital Frontal Cortex and Socio-emotional Processing

The orbital frontal cortex has also been implicated in social cognition, albeit in different domains than the amygdala. Whereas the amygdala is specifically involved in the processing of socio-emotional information, the orbital frontal cortex appears to use this information to guide and adjust behaviors appropriately in accordance with changing contexts and contributes to the anticipation of reward (Bechara et al., 2003; Clark, Cools, & Robbins, 2004; Holland & Gallagher, 2004; W. Schultz, Tremblay, & Hollerman, 2000). Although the contribution of the orbital frontal cortex in integrating gustatory, olfactory and texture (somatosensory) information to create a representation of flavor has been well characterized (O'Doherty et al., 2004), a role of this region in audiovisual integration of socio-emotional stimuli is yet to be investigated.

A) Sensory Convergence in the Orbital Frontal Cortex

Like the amygdala, the orbital frontal cortex receives highly processed information from all sensory modalities (visual, somatosensory, visceral, olfactory, and gustatory). The orbital frontal cortex receives visual inputs from the inferior temporal cortex and the cortex of the anterior superior temporal sulcus (Barbas, 1988, 1993; Barbas & Blatt, 1995; Pandya & Kuypers, 1969; Webster, Bachevalier, & Ungerleider, 1994). The region also receives auditory inputs from the auditory regions of the superior temporal cortex (Hackett, Stepniewska, & Kaas, 1999; Romanski & Goldman-Rakic, 2002; Romanski et al., 1999). Two distinct networks have been identified in the orbital frontal cortex. The medial network, comprised of area 14, is extensively connected to the hippocampus and associated areas, such as the cingulate, retrosplenial, parahippocampal and entorhinal cortices. In contrast, the lateral network, areas 11, 12 and 13, is most heavily linked with premotor and sensory areas, as well as the amygdala (Ghashghaei & Barbas, 2002; Ghashghaei, Hilgetag, & Barbas, 2007; Kondo, Saleem, & Price, 2003). The orbital frontal area receives information about all aspects of the external and internal environment through its connections with thalamic nuclei involved in associative aspects of memory, as well as the amygdala and temporopolar cortex, which have been implicated in the regulation of emotion. Thus, the orbital frontal cortex may serve as an integrative area using external and internal cues to facilitate the modulation and self-regulation of emotional behavior in relation to rapid changes in a social situation or context (as in dominance relationships and situational features).

B) Orbital Frontal Cortex and Emotion

Given the paucity of data on the putative role of the orbital frontal cortex in emotional crossmodal integration, the following sections will highlight the contributions of this region to processing affective information from faces and voices. The final subsection will review the literature supporting a role of the orbital frontal cortex in processing emotional audiovisual stimuli.

i) Orbital Frontal Cortex and Facial Emotion

The human orbital frontal cortex was first implicated in processing facial expressions of emotion by studies of patients with lesions of the ventral prefrontal cortex, including the orbital surface, who were impaired at identifying facial expressions (Hornak et al., 2003; Hornak, Rolls, & Wade, 1996). Patients with damage to this area are also impaired in the ability to discriminate eye direction, a skill linked to the assessment of affect (Broks et al., 1998; Young, Hellawell, Van De Wal, & Johnson, 1996). Activation within the orbital frontal cortex, as revealed by functional imaging techniques, has been associated with exposure to emotional facial expressions (Blair, Morris, Frith, Perrett, & Dolan, 1999; Gorno-Tempini et al., 2001; Nakamura et al., 1999; Sprengelmeyer, Rausch, Eysel, & Przuntek, 1998). Electrophysiological recordings in nonhuman primates have supported the human lesions and neuroimaging data concerning the role of the orbital frontal cortex in processing facial stimuli. Neurons in the inferior lateral convexity of the prefrontal cortex and orbital frontal cortex selectively respond to faces (Klopp, Marinkovic, Chauvel, Nenov, & Halgren, 2000; O'Scalaidhe, Wilson, & Goldman-Rakic, 1997, 1999; Rolls, 1999, 2000).

Furthermore, face processing impairments in autism have been associated with changes in activation of the orbital frontal cortex (Baron-Cohen et al., 1999; Critchley et al., 2000; Pierce et al., 2004; R. T. Schultz, I. Gauthier et al., 2000; R. T. Schultz et al., 2003; R. T. Schultz, L. Romanski et al., 2000). In the study discussed above which combined eye-tracking technology and fMRI in individuals with autism, Dalton and colleagues (2005), also indicated that increased activation of the orbital frontal cortex was also dependent upon eye fixation. However, unlike the amygdala that was responsive to faces in general, the orbital frontal cortex preferentially responds to the emotional content of the faces. Together, these findings suggest that for individuals with autism, eye fixation was associated with negatively valenced over-arousal mediated by activation in limbic regions, such as the amygdala and orbital frontal cortex.

The paradigm used to assess the effect of amygdala lesions on face processing in rhesus macaques discussed above (Goursaud & Bachevalier, 2007; Goursaud et al., 2003) was also used to assess the effect of orbitofrontal lesions. Whereas animals with lesions of the amygdala looked less to the eyes than normal controls, animals with orbitofrontal damage looked more to the eyes than normal controls. Interestingly, animals with lesions of the orbital frontal cortex also showed greater pupil dilation to faces than sham-operated controls, indicating a heightened arousal to the stimuli. These data suggest that the orbital frontal cortex is involved in the evaluation of socio-emotional information (Goursaud & Bachevalier, in preparation).

ii) Orbital Frontal Cortex and Auditory Emotion

Functional imaging has identified the orbital frontal cortex as a brain region that processes affective aspects of auditory stimuli (Blood, Zatorre, Bermudez, & Evans, 1999). Investigators presented subjects with a sequence of musical notes in which the consonance between the notes was systematically varied. The degree of consonance was directly related to the perceived pleasantness of the music. Activity in the medial orbital frontal cortex was positively correlated with the degree of consonance of the music, indicating that the more pleasant the music, the greater the activity in this region. Activity within the caudolateral orbital frontal cortex has also been reported in response to aversive music (Frey, Kostopoulos, & Petrides, 2000). Activation within area 11 or the orbital frontal cortex has also been reported to be associated with emotional judgments of human speech (Wildgruber et al., 2004; Wildgruber, Pihan, Ackermann, Erb, & Grodd, 2002). Consistent with neuropsychological evidence that indicates orbitofrontal damage produce deficits in the recognition of vocal emotion (Hornak et al., 1996), vocal expressions of emotion have also been shown to elicit activation in this region (Mitchell, Elliott, Barry, Cruttenden, & Woodruff, 2003; Morris, Friston, & Dolan, 1998). Finally, two studies in rhesus macaques have reported responses of the orbital frontal cortex to species-specific calls using electrophysiology (Rolls, Critchley, Browning, & Inoue, 2006) and 2-deoxyglucose (Poremba et al., 2003).

iii) Orbital Frontal Cortex and Audiovisual Emotion

To date, only two investigations have suggested an involvement of the orbital frontal cortex in processing emotional audiovisual stimuli. Dolan and colleagues (2001) reported that, along with the amygdala, the orbital frontal cortex exhibited greater activation to the congruent presentation of congruent fearful faces and voices than to the presentation of a fearful face and happy voice. This indicated a role of the orbital frontal cortex in processing affective crossmodal information, but does not delineate unique contributions of the orbital frontal cortex from those of the amygdala.

An investigation comparing the abilities of normally developing individuals to those of persons with autism has provided valuable insight into how humans process crossmodal socio-emotional stimuli. Loveland and colleagues (Loveland, Steinberg, Pearson, Mansour, & Reddoch, 2008) have begun to directly investigate auditory-visual processing of emotion in autism by combining fMRI techniques with a crossmodal-matching paradigm. Their emotional congruence task requires participants to make judgments about the congruence of auditory and visual sources of information for emotion. Participants were presented with a still picture depicting facial emotion (taken from the Ekman and Friesen (Ekman & Friesen, 1976) picture set), paired with a nonverbal vocalization that expresses an emotion. The auditory stimuli included nonverbal human female and male sounds of positive and negative valence. Two conditions of this task were utilized in a blocked fMRI design: (a) gender blocks, in which participants are asked to indicate whether the voice and face gender were the same or different; and (b) emotion blocks, in which participants are asked to indicate whether the voice and face affect were congruent or incongruent. In a pilot study of this paradigm, the non-autism group exhibited significantly greater activation in the middle frontal gyrus (BA 10 and 11) extending to the orbital frontal gyrus during the emotion condition, relative to the gender condition. Interestingly, unlike the non-autism controls, the autism group did not exhibit significant prefrontal activation during the emotion condition, but did have significant prefrontal activation during the gender condition. Whereas it is important to consider the behavioral paradigm employed in this investigation (crossmodal matching), these preliminary findings are consistent with a role of the orbital frontal cortex in processing socio-emotional information from faces and voices. Given the neuroanatomical characteristics of the orbital frontal cortex and the roles of this region in processing socio-emotional information from faces and voices, further exploration into the contributions of the orbital frontal cortex to emotional crossmodal integration is clearly warranted.

6- Specific Aims and Hypotheses

Despite the growing interest and understanding of the neural substrates of processing crossmodal socio-emotional signals, the unique contributions of structures such as the amygdala and orbital frontal cortex are not fully characterized. In light of the neuroanatomical and neuropsychological evidence reviewed above, **the general hypothesis upon which this project was predicated is that the amygdala and orbital frontal cortex are critically involved in integrating crossmodal socio-emotional stimuli**. To begin to test this hypothesis, we made use of a set of monkeys that had been prepared to investigate the role of the amygdala and orbital frontal cortex in the development of emotion and stress reactivity and decision-making abilities. Infant monkeys with selective neonatal insult to these two brain regions and sham-operated controls were tested at different time points during maturation in tasks measuring defensive and hostile behaviors as well as in tasks measuring their ability to switch behavioral strategies when reward contingencies were altered. Both types of lesions altered performance on these tasks (Kazama, Glavis-Bloom, & Bachevalier, 2008; Raper, Kazama, & Bachevalier, 2009; Raper, Wilson, Sanchez, & Bachevalier, 2011) and, importantly, the impairment seen after the neonatal amygdala and orbital frontal lesions was as severe as that reported when the same lesions are done in adulthood (Machado & Bachevalier, 2007; Machado, Kazama, & Bachevalier, 2009). These findings led to the conclusion that neonatal amygdala and orbital frontal lesions resulted in no recovery of functions, indicating that no other brain structures could adequately compensate for functions normally supported by the amygdala and orbital frontal cortex. Given these long-lasting effects of neonatal amygdala and orbital frontal cortex on emotion and decision-making, we reasoned that, if these two structures had a critical role to play in the ability to integrate crossmodal social cues, lesions of these structures in infancy will likewise result in significant impairment of this ability. This project had four specific aims:

(1) **The first aim was to characterize crossmodal integration of species-specific vocalizations in adult, nursery-reared rhesus macaques using dynamic stimuli in a previously validated preferential viewing paradigm** (Ghazanfar & Logothetis, 2003). (Manuscript I)

   a. We hypothesized that nursery-reared rhesus macaques would exhibit crossmodal integration abilities and that those abilities were not solely dependent upon the mechanical properties of the stimuli (i.e., the synchronization of the auditory element with the movements in the visual element).

(2) Given that cognitive theories of attention predict that attention plays a critical role in crossmodal integration (e.g., Treisman, 1996), **the second aim was to use eye-tracking technology to characterize the patterns of visual scanning of adult, nursery-reared, rhesus macaques as they processed dynamic, crossmodal species-specific vocalizations presented in a preferential viewing paradigm**. (Manuscript I)

   a. We hypothesized that nursery-reared rhesus macaques would preferentially attend to the eye regions over the mouth and rest of the stimulus videos.

(3) **The third aim was to assess the effect of selective, neonatal amygdala damage on crossmodal integration ability and scanning strategies of dynamic, species-specific vocalizations in adult rhesus macaques**. (Manuscript II)

   a. We hypothesized that adult rhesus macaques with neonatal damage of the amygdala would show impairments in crossmodal integration of species-specific vocalizations.

   b. We further hypothesized that the impairment would be associated with decreased scanning of the eye regions and increased scanning of the mouth regions.

(4) **The fourth aim was to assess the effect of neonatal orbitofrontal damage on crossmodal integration ability and scanning strategies of dynamic, species-specific vocalizations in adult rhesus macaques**. (Manuscript III)

   a. We hypothesized that adult rhesus macaques with selective neonatal lesions of the orbital frontal cortex would show impairments in crossmodal integration of species-specific vocalizations.
   b. We further hypothesized that the impairment would be associated with increased looking to the eye regions.

MANUSCRIPT I: Crossmodal Integration of Conspecific Vocalizations in Rhesus Macaques

Christa Payne[1,2]

Jocelyne Bachevalier[1,2]

[1]Department of Psychology, Emory University, Atlanta, GA 30062

[2]Division of Developmental and Cognitive Neuroscience, Yerkes National Primate Research Center, Atlanta, GA 30062

Abstract

Crossmodal integration of audio/visual information is vital for recognition, interpretation and appropriate reaction to social signals. Work in nonhuman primates has been largely limited to crossmodal matching tasks or focused on the evolutionary basis of audiovisual integration. Here, we examined how rhesus macaques process bimodal species-specific vocalizations using eye tracking. Six adult rhesus monkeys (3M, 3F) were presented two side-by-side videos of unknown male conspecifics emitting different vocalizations (i.e., coo, grunt, scream, threat), with the audio signal matching one of the videos. The percentage of time animals looked to the congruent videos was used to assess crossmodal integration ability and the percentages of time spent looking at each of the six a priori ROIs (eyes, mouth, and rest of each stimulus video) were used to characterize scanning patterns. Across all trials, animals looked more to the congruent video, confirming reports that rhesus monkeys spontaneously integrate conspecific vocalizations. Scanning patterns showed that, overall, monkeys looked more to the eye and mouth regions than the rest of the stimuli, and looked more to the eyes of the congruent than of the incongruent videos. Males and females displayed slightly different scanning patterns. For the congruent video, females looked longer to the eye than mouth regions, but males looked equally to the eyes and mouth. Males and females did not differ in their looking to the eye regions. Instead, the observed sex differences were reflective of males looking more to the mouth region than females. These results link to studies in humans indicating that when viewing videos of someone talking, people preferentially attend to the eyes over the mouth both during passive

viewing and when instructed to assess emotion-related. Thus, greater viewing of the eye as compared to the mouth regions in female monkeys may indicate heightened attentiveness to vocalization valence, whereas equal viewing of eye and mouth in male monkeys suggest that in addition to emotional valence, male monkeys may attend to other social cues (e.g. dominance status). This conclusion was further supported by post-hoc analyses that revealed that the scanning patterns of males were sensitive to the relative identity of the stimulus animals, but the scanning patterns of females were more sensitive to the relative valence of the vocalizations. Interestingly, the greater sensitivity to the emotional aspects of species-typical communication in female monkeys parallels recent findings in humans indicating that women process crossmodal emotion expressions more efficiently than men.

Introduction

Successful integration into a complex social environment requires humans and nonhuman primates to recognize, manipulate, and behave according to the immediate social context. Key elements of this task are building representations of relations between self and others and flexibly using these representations to guide social behavior (Adolphs et al., 2001; Baron-Cohen et al., 1999). This set of skills relies upon the ability to distinguish and interpret social cues in faces and voices that are often broadcast over multiple sensory modalities. Hence, crossmodal integration has become a crucial component of social success in humans and nonhuman primates.

The remarkable behavioral (Brothers, 1995; Byrne & Whiten, 1988; Cheney & Seyfarth, 1990; de Waal, 1989) and neurobiological (Barton & Aggleton, 2000; Petrides, 1994) similarities between humans and nonhuman primates include the use of species-specific facial expressions and vocalization (MD. Hauser, 1993; Hinde & Roswell, 1962; Partan, 2004). In both species, decoding specific “message” of a social display relies on crossmodal integration. The rhesus communicative system is comprised of a small repertoire of relatively fixed calls that are associated with distinct facial expressions, postures, and gestures that are linked to particular social contexts. This repertoire has been successfully used to explore the evolutionary basis and neural mechanisms of visual speech perception (reviewed by (L. Romanski & Ghazanfar, 2010).

Recent studies have demonstrated that rhesus macaques spontaneously recognize the correspondence between the facial and vocal expressions (Ghazanfar & Logothetis, 2003). When pairs of videos depicting two different conspecific vocalizations (i.e., coo and threat) were presented simultaneously with the auditory track matching one of the videos, rhesus macaques looked longer to the congruent stimulus video. This was interpreted as spontaneous integration of the auditory and visual components of the stimuli. In this paradigm, however, the subject monkeys could rely on the temporal coincidence of facial movements with the onset of the vocal track. A subsequent electrophysiological experiment using the same videos presented one at a time and including a non-biological, mechanical control that mimicked the mouth movements of the videos (in space and time) indicated that integration of the bimodally presented vocalizations was not dependent upon temporal coincidence (Ghazanfar et al., 2005). However, given that the videos in the latter experiment were presented individually, the possibility remains that the preference for congruence observed in the preferential viewing paradigm was attributable to the mechanical or temporal coincidence of the auditory and visual components of the stimulus videos. Thus, the mechanisms underlying this spontaneous preference for congruence have yet to be systematically explored.

Eye-tracking technology has been a valuable tool for determining how humans and nonhuman primates process complex social signals, particularly the signals found in the faces of conspecifics. It has been shown, for example, that human subjects modify their scanning strategies of audiovisual stimuli based on the information they are instructed to extract and the efficacy of the social signals (Buchan, Pare, & Munhall, 2008; Lansing & McConkie, 1999, 2003; Vatikiotis-Bateson, Eigsti, Yano, & Munhall, 1998). However, little is known about the scanning strategies of comparable stimuli in nonhuman primates. To date, the only investigation to monitor how monkeys look at socially salient bimodal stimuli was designed to explore the evolutionary basis for the propensity of humans to use facial cues to enhance speech comprehension (Ghazanfar, Nielsen, & Logothetis, 2006). This report highlighted the importance of the eye region to rhesus monkeys, but does not directly identify the facial cues needed to support a preference for congruence.

Accordingly, the goals of the present investigation are to 1) assess integration ability in surrogate nursery-reared rhesus macaques using a preferential viewing paradigm, 2) determine whether spontaneous integration ability is solely dependent upon temporal or mechanical coincidence of the auditory and visual components of species-typical vocalizations using an ethologically relevant mechanical control, and 3) to characterize the scanning strategies during preferential viewing to determine what features the animals are using to process the stimuli.

Method

All procedures were approved by the Animal Care and Use Committee of the University of Texas Health Science Center at Houston in Houston, TX and of Emory University in Atlanta, GA and carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used, as well as any pain and suffering.

Subjects

Six adult rhesus monkeys (*Macaca mulatta*) aged 4-6 years (3 males, 3 females) were used in this investigation. These animals have served as sham-operated controls in a program of experiments designed to characterize the functional and neuroanatomical development of hippocampus, amygdala and orbital frontal cortex. Accordingly, they received sham operations at 10-12 days of age, which included small bilateral craniotomies with no penetration of the dura layer; underwent multiple magnetic resonance imaging (MRI) scans to assess gross neural development between 2 weeks and 2.5 years of age (Payne, Machado, Bliwise, & Bachevalier, 2010); and had repeated assessments of memory, emotional reactivity, social behavior, and reward appraisal throughout their lives. Animals were raised in a socially enriched nursery environment were raised in an enriched nursery environment (for details, see (Goursaud & Bachevalier, 2007).

Crossmodal integration task

A preferential viewing paradigm similar to that used by Ghazanfar and Logothetis (2003) was used in conjunction with eye-tracking techniques to 1) assess the animals' ability to integrate socially relevant crossmodal signals; and 2) identify the facial features used while processing these complex, social stimuli.

<u>Apparatus</u>: Testing was completed in a sound-attenuated room. Monkeys were seated in a primate chair in front of a 24-inch, flat panel LCD monitor with attached speaker and small eye-tracking camera (ISCAN, Inc.; Woburn, MA). Head movements were gently minimized with a device attached to the primate

chair. Ambient white noise was played to further dampen unrelated noises and a curtain concealed all additional equipment.

Stimuli: Animals were presented two side-by-side digital 2-sec videos of the facial gestures associated with species-typical calls (coo, grunt, scream and threat). The videos depicted two unknown rhesus ('stimulus animals') emitting the vocalizations. One stimulus animal generated the coo and threat vocalizations and the grunt and scream vocalizations were of the other stimulus animal (see Fig 1). Videos were 360 x 480 pixels and spaced apart maximally on a solid black background. The sound track corresponding with one of the presented facial gestures was heard through the speaker centered beneath the monitor. The auditory and visual components were played in a continuous loop for 10 sec (5 repetitions). Stimulus presentation was controlled using the Presentation software package (Neurobehavioral Systems, Inc; Albany, CA).

In pilot trials of crossmodal integration, stimuli were limited to pairs of vocalizations from the same stimulus animal (i.e., coo-threat and grunt-scream). These trials yielded results consistent with previous findings that rhesus monkeys spontaneously integrate bimodally presented conspecific vocalizations (Ghazanfar & Logothetis, 2003), but also indicated rapid habituation to the limited stimulus sets. Since previous results indicated that the relative identity of the stimulus animals had no effect on crossmodal integration ability (Ghazanfar & Logothetis, 2003), stimulus sets constructed from all combinations of stimulus video pairs were used to reduce habituation to the four videos. Inter-session-intervals of 4 – 7 days were also used for this purpose.

Task: Each trial began when the monkey fixated its gaze towards the camera.

The auditory component and the left-right position of the two facial gestures were counterbalanced. Stimuli were presented under two different conditions: Synchronized and Desynchronized. The Synchronized condition was used as the standard for integration assessment and was constructed so that the onsets of the auditory and visual components were simultaneous. A total of eight trials in the Synchronized condition were administered across four testing sessions (2 trials/day). The Desynchronized condition was employed to assess whether integration ability relied only upon the mechanical properties of the stimuli (i.e. the coincidence of mouth movements with the auditory component). Trials in the Desynchronized condition were constructed so that the onset of the auditory component was delayed 330 – 430 msec from the onset of the visual component, a delay range that has been shown to disrupt the perception of the stimuli as a single event (Dixon & Spitz, 1980). A total of eight trials in the Desynchronized condition were administered across two testing sessions (4 trials/day).

Measures

Integration Assessment: In a given trial, each video was either ‘congruent’ (i.e., depicted the facial gestures that matched the audio component) or ‘incongruent’ (i.e., facial gestures did not match the audio track). Crossmodal integration was determined by comparing the percent looking time to each video to the chance level of 50%. Integration of the audio and visual components was inferred when monkeys show a preference for one of the video clips (i.e., looks statistically more than chance (50%) to the either the congruent or incongruent stimulus video). Accordingly, an inability to integrate the complex social signals would be demonstrated by monkeys exhibiting equal looking times to each video in the pair.

Scanning Pattern Characterization: Percentages of looking time to *a priori* regions of interest (ROIs) of the video were recorded. Static ROIs of the 'Eyes' and 'Mouth' were created with the ISCAN P.O.R. Fixation Analysis software (v1.2, ISCAN, Inc., Fig 1) such that each ROI encapsulated the entire feature of interest throughout the entire 2sec video. The region of the video not included in either the 'Eyes' or 'Mouth' ROI was analyzed as the third ROI 'Other'. There were six ROIs in each trial: 'eyes', 'mouth', and 'other' for each the congruent and incongruent stimulus videos. Scanning patterns were characterized by comparing the amount of time animals spent looking at each ROI, which was calculated from the summation of the fixation durations in a given ROI. A fixation was defined as the eye gaze coordinates remaining within 1° x 1° visual angle for at least 100 msec. Fixation duration lasted until the eye gaze coordinates deviated more than 1° x 1° visual angle for more than 360 msec. Fixations were categorized by ROI using the ISCAN P.O.R. Fixation Analysis Software, and variability in looking time across both trials and animals was accounted for by expressing looking to each ROI as a percentage of total looking ((ROI/Total)*100).

Statistical Analyses

Assumptions of parametric statistics were met in all measures. Integration abilities were assessed separately for the Synchronized and Desynchronized conditions by comparing the percentage of looking to the congruent stimuli to the chance level of 50% using a one-sample t-test. Sex differences were evaluated via independent-samples t-tests and dependent-samples t-tests were used to compare the integration abilities across conditions. Given the purpose of the Desynchronized condition was to ensure that crossmodal integration of the complex social stimuli was not solely reliant upon the mechanical properties of the stimuli, only the scanning patterns of stimuli in the Synchronized condition were characterized. Scanning patterns of the ROIs of the congruent and incongruent stimulus videos was characterized using repeated measures MANOVA (stimulus video x ROI x sex) with planned comparisons.

Results

Overall Integration and Scanning Patterns

Integration Assessment: In the Synchronized condition (Fig 2), animals exhibited spontaneous integration of complex crossmodal social signals by looking significantly more than chance to the congruent stimulus video (t(5) = 2.674, *p* = 0.044), thus demonstrating a preference for congruence. Qualitatively this effect appears to be driven by the behavior of the females (see Fig 2A), but this apparent sex difference was not statistically significant (t(4) = -1.865, *p* = 0.136).

In the Desynchronized condition (Fig 2), animals did not show a preference for congruence (t(5) = -0.496, *p* = 0.641, Fig 2), most likely due to the individual variability in preference direction (i.e., three animals looked more to the congruent stimulus video and three animals looked more to the incongruent stimulus video) rather than to a sex difference (males = females; t(4) = -0.144, *p* = 0.892). Nevertheless, animals did show a general preference to one of the videos. When integration ability was assessed with the ‘preferred’ video (i.e., the video looked at most) of each animal, rhesus monkeys showed a statistically significant preference (t(5) = 5.611; *p* = 0.002; Fig 2B) with no difference between males and females (t(4) = -0.053, *p* = 0.960). The general preference in the Desynchronized condition indicates that de-synchronization of the audio and visual components did not impair animals’ ability to integrate the bimodal social signals.

Scanning Pattern Characterization: As illustrated in Figure 3, monkeys showed slight differences in their exploration of the congruent and incongruent stimulus videos. On the congruent stimulus video (Fig 3A), they spent more time looking to the eye region than the mouth region (eyes > mouth: F(1,4) = 14.740, *p* = 0.018), and looked longer to the eye and mouth regions than to the rest of the video (eyes > other F(1,4) = 14.740, *p* = 0.018; mouth > other F(1,4) = 271.710, *p* < 0.0001). On the incongruent stimulus video (Fig 3B), monkeys looked longer to the eyes and mouth regions than the rest of the video (eyes > other: F(1,4) = 11.294, *p* = 0.028; mouth > other: F(1,4) = 8.146, *p* = 0.046), but, unlike the congruent video, looked equally to the eyes and mouth (F(1,4) = 0.251, *p* = 0.643).

However, as shown in Figure 4, the relative looking to the eye and mouth regions tended to vary by sex in the congruent stimulus video (F(1,4) = 6.124, *p* = 0.069) (not observed in the incongruent video (F(1,4) = 0.013, *p* = 0.916)). Specifically, females differentiated the eye and mouth regions (eyes > mouth: F(1,4) = 19.933, *p* = 0.011), but males looked equally to both regions (eyes = mouth: F(1,4) = 0.931, *p* = 0.931). Differences in looking to the mouth region drive the observed sex difference, with males looking more to the mouth region than females (F(1,4) = 7.604, *p* = 0.051), whereas sexes looked equally to the eye region. Comparisons of the ROIs of congruent and incongruent stimulus videos revealed that the monkeys looked more to the eyes of the congruent stimulus video than the eyes of the incongruent stimulus video (F(1,4) = 10.214, *p* = 0.033), whereas time looking at the mouth or other regions did not differ between the congruent and incongruent stimulus videos and no sex effects were found.

Summary: The present results demonstrate that rhesus macaques spontaneously integrated bimodal social cues of novel conspecifics and are consistent with previous findings (Ghazanfar & Logothetis, 2003). The current investigation further supports the assumption that integration ability is not solely due to the mechanical properties of the vocalization (Ghazanfar et al., 2005) given that the animals exhibited a general preference to one of the videos in the Desynchronized condition. In addition, the differentiation of the congruent and incongruent eye regions indicates that looking to the eye regions drives the preference for congruence seen in the integration assessment. Even though monkeys overall relied on the eye regions to distinguish the two stimulus videos, males used the mouth regions more than females.

Despite consistencies between the present results and previous studies, the preference for congruence observed in the Synchronized condition of our study was relatively weak (mean = 53.3%) compared to that (67.4%) reported by Ghazanfar and Logothetis (2003). Although both investigations used the same general paradigm, several differences may account for the disparity. One obvious discrepancy is that the previous study used monkeys that were mother-reared in social groups, whereas those of the current study were surrogate-nursery-peer-reared animals. However, given that the scanning patterns exhibited in our investigation complement previous face processing studies reporting that rhesus preferentially attend to the socially salient features of conspecific faces (e.g. eyes and mouth; (Emery, Lorincz, Perrett, Oram, & Baker, 1997; Ghazanfar et al., 2006; Gothard, Erickson, & Amaral, 2004; Guo et al., 2003; Keating & Keating, 1982; F. Nahm, Parret, Amaral, & Albright, 1997; Sato & Nakamura, 2001), rearing condition does not likely contribute significantly to the comparatively weak preference for congruence.

The other important difference is that the previous study only used coo-threat pairings emitted from the same unfamiliar conspecific (different valence/same identity), whereas the present study created stimulus pairs from all possible combinations of videos of four different vocalizations from two unknown stimulus animals. Thus, stimulus pairs included not only different valence/same identity pairing, but also pairings of different valence/different identity, same valence/same identity, and same valence/different identity. This procedural change could potentially have impacted integration ability and/or preference direction. To test this hypothesis, post-hoc analyses of our data were performed on two putative factors, i.e. identity and valence.

Identity

The factor of 'Identity' refers to the identity of the stimulus animals presented on the two videos of a given trial. In 'Identity Same' trials, the same stimulus animal generated both vocalizations (i.e., the coo-threat pairings and grunt-scream pairings), whereas in 'Identity Different' trials two stimulus animals emitted both vocalizations (e.g., the coo-scream pairings or grunt-threat pairings). Thus, in Identity Different trials, the observer had the opportunity to make comparisons of and judgments about the two unfamiliar stimulus animals as it integrated the audio-visual stimuli. For example, in this trial type, the observer may have attempted to not only integrate the two sensory inputs but also make assessments of the dominance status of the conspecifics (both in relation to each other and to the observer). In contrast, in the Identity Same trials, when monkeys were shown just one unfamiliar conspecific making two different vocalizations, dominance assessments were restricted to comparisons between the stimulus animal and the observer, such that identity was essentially a null factor. In this post-hoc analysis, there were two 'Identity Same' stimulus-pairs and six 'Identity Different' stimulus-pairs within the Matched stimulus set.

Integration Assessment: Figure 5 shows that monkeys display preference differences across Identity Same and Identity Different trials. For trials in which the same conspecific emitted both vocalizations (Identity Same trials), animals exhibited a strong preference for congruence (t(5) = 3.530, *p* = 0.017, Fig 5A). When the vocalizations were produced by two different stimulus animals (Identity Different trials), there was more variability in preference direction, resulting in a lack of preference for the congruent stimulus video (t(5) = -0.649, *p* = 0.545, Fig 5A), although the animals showed a statistically significant general preference to one of the two videos (t(5) = 4.451, *p* = 0.007; Fig 4B). However, despite evidence of integration in both Identity trial types, the percentage of total looking to the congruent stimulus video was greater for the Identity Same trials than for the Identity Different trials (t(5) = 2.925, p = 0.033). There were no differences between males and females for either Identity trial type.

Scanning Pattern Characterization: Figure 6 illustrates marked differences in the exploration of Identity Same and Identity Different trials. In Identity Same trials (Fig 6A), rhesus monkeys looked equally to the eye and mouth regions, but longer to the eye region than to the rest of the congruent stimulus video (F(1,4) = 6.749, p = 0.060). By contrast, for the incongruent stimulus video, they looked equally to all regions. There were no sex differences in scanning patterns.

In Identity Different trials (Fig 6B), animals overall showed the same general pattern of scanning in both the congruent (eyes = mouth; eyes > other (F(1,4) = 44.709, p = 0.003); mouth = other) and incongruent stimulus videos (eyes = mouth; eyes > other (F(1,4) = 12.986, p = 0.023); mouth = other), but males and females differed in their relative scanning of the eyes and mouth regions of the congruent stimulus video (Fig 7). As in the analyses of all trials, males looked equally to the eye and mouth regions (F(1,4) = 0.075, p = 0.798), whereas females tended to look more to the eyes than the mouth (F(1,4) = 6.419, p = 0.064). As before, this distinction was characterized by males and females looking equally to the eye regions (F(1,4) = 3.21, p = 0.148), but differing in their exploration of the mouth regions (males > females: F(1,4) = 14.276, p = 0.019).

Summary: These findings suggest that, although the ability of crossmodal integration is retained regardless of the relative identity of the stimulus animal in the two videos, rhesus macaques appear to process the two stimulus types differently. Specifically, the strong preference for congruence reported in Identity

Same trials corresponds with a preference for the eye region in the congruent stimulus video, whereas the general preference seen in Identity Different trials corresponds with males attending more to the mouth region of the conspecific producing the vocalization than females. Thus, the looking behaviors associated with Identity Different trials, and not Identity Same trials, account for the differential patterns of looking exhibited by males and females reported in the analyses of all trials. Notably, the strong preference for congruence in Identity Same trials and the lack of preference for congruence in Identity Different trials could account for the weaker preference (as compared to Ghazanfar and Logothetis, 2003) we observed when all trials are considered.

Valence

Another way to partition the trials was by the factor Valence, referring to the emotional content of the stimulus vocalizations in a given trial. In 'Valence Same' trials, both vocalizations had either a positive (i.e., coo-grunt) or negative (i.e., threat-scream) valence. In 'Valence Different' trials, the two vocalizations had different valences (e.g., coo-threat or grunt-scream). As proposed in the Identity analyses, Valence was only a factor in Valence Different trials, since in these trials the two vocalizations were semantically inconsistent: one vocalization was naturally produced in a variety of affiliative contexts and the other naturally associated with aggressive encounters. By contrast, the vocalizations in Valence Same trials were semantically consistent (i.e., both vocalizations in a given trial were either affiliative or antagonistic), and thereby precluded the opportunity for the observer to make comparisons based on the relative general valence of the vocalizations. In this post-hoc analysis, there were four Valence Same stimulus-pairs and four Valence Different stimulus-pairs in the Matched stimulus set.

Integration Assessment: As shown in Figure 8, monkeys again show preference differences across Valence Same and Valence Different trials. When the stimulus vocalizations had the same general valence (Valence Same trials), rhesus monkeys did not show a preference for congruence ($t(5) = -1.368$, $p = 0.230$, Fig 8A), but did show a significant general preference for one of the two videos ($t(5) = 5.155$, $p = 0.004$, Fig 8C). In Valence Different trials, animals exhibited a tendency to look more to the congruent stimulus video ($t(5) = 2.327$, $p = 0.067$, Fig 8A), with a statistically significant general preference for one of the videos ($t(5) = 2.988$, $p = 0.031$, Fig 8C). Figure 8B illustrates that all six animals changed their preference across trial types, with five animals looking more to the incongruent stimulus videos (looking < 50%) in Valence Same trials and more to the congruent stimulus videos in Valence Different trials and one female exhibiting the opposite pattern. Exclusion of that female yielded significant differences in looking to Valence Same and Valence Different trials ($t(4) = -3.451$, $p = 0.026$). There were no differences between males and females at either valence parameter.

Scanning Pattern Characterization: As illustrated in Figure 9, there were distinctive differences in the exploration of the congruent stimulus video across Valence Same and Valence Different trials. In Valence Same trials (Fig 9A), rhesus macaques tended to look more to the eye regions than the rest of the congruent stimulus videos ($F(1,4) = 6.007$, $p = 0.070$), but overall, did not differentiate the eyes from mouth ($F(1,4) = 4.590$, $p = 0.099$) or the mouth from the rest of the video ($F(1,4) = 4.172$, $p = 0.111$). The same general pattern was seen in the scanning of the incongruent stimulus videos (eyes = mouth, eyes > other: $F(1,4) = 6.815$, $p = 0.059$; mouth = other). However, the lack of differentiation of the eye and mouth regions of the congruent stimulus video was attributable to sex differences in the relative looking of these regions (Fig 10). Like the analyses of all trials and Identity Different trials, males looked equally to the eye and mouth regions ($F(1,4) = 0.281$, $p = 0.624$), but females tended to look longer to the eyes than mouth regions ($F(1,4) = 6.249$, $p = 0.067$). This distinction was, again, driven by differences in looking to the mouth regions (males > females: $F(1,4) = 26.703$, $p = 0.007$), and not the eyes ($F(1,4) = 0.762$, $p = 0.432$).

In Valence Different trials (Fig 9B), animals exhibited the characteristic preference for the socially salient facial features (eyes and mouths) over the rest of the congruent stimulus video (eyes > other: 8.958, $p = 0.040$; mouth > other: $F(1,4) = 47.431$, $p = 0.002$), but only the differentiation between the mouth region and the rest of the video was seen in the incongruent stimulus video (eyes = other: $F(1,4) = 1.508$, $p = 0.287$; eyes > other: $F(1,4) = 19.400$, $p = 0.012$). Males and females had the same pattern of scanning in Valence Different trials.

Summary: As in the analyses of Identity, the results imply that, although manipulations of the valence parameter did not impact integration ability, per se, the relative valence of the vocalizations did influence the manner in which observers process these complex social cues. In particular, rhesus monkeys tend to exhibit a preference for congruence in Valence Different trials (when valence was a factor) but not in Valence Same trials (when valence was a null-factor).

This divergence suggests that they are attending to the valence of the vocalizations in their assessments of the two videos. As in the analyses of Identity, the inconsistency in preference direction and strength across Valence Same and Valence Different trials could account for the weak preference (as compared to the Ghazanfar and Logothetis study) observed when all trials are considered.

Furthermore, the characterizations of the scanning patterns intimate that males and females are differentially sensitive to the parameter of valence. When the two stimulus vocalizations carry the same relative valence, females attended to the mouth of the vocalizing stimulus animal (i.e., congruent video) less than males. However, when the vocalizations have different valences, males and females showed similar exploration of the mouths of vocalizing animals. Accordingly, whereas males looked equally to the eye and mouth regions irrespective of the relative valence of the vocalizations (i.e., in both Valence Same and Valence Different trials), the relative scanning of the eyes and mouths of the vocalizing animals exhibited by females appeared to be sensitive to the relative valence of the vocalizations (i.e., females looked more to the eyes in Valence Same trials, but equally to the eyes and mouths in Valence Different trials). Thus, the sex differences reported in the analyses when all trials were considered could also be attributed to Valence Same trials, and not Valence Different trials.

One potential limitation of the current post-hoc analysis is that, due to the limited number of videos used in the current investigation, all the stimulus-pairs in Valence Same trials also met the criteria of Identity Different trials (i.e., every pair of vocalizations used in Valence Same trials was comprised of videos of different stimulus animals). Conversely, not all Identity Different trials were Valence Same trials (i.e., Identity Different trials were comprised of both Valence Same and Valence Different trials). Consequently, the males looking more to the mouth region in both Identity Different trials and Valence Same trials was likely driven by a similar process.

Discussion

We examined the scanning strategies rhesus macaques use as they process species-specific, bimodal social signals. The results confirmed that rhesus macaques spontaneously integrate the auditory and visual components of complex social cues emitted by novel conspecific males (Ghazanfar & Logothetis, 2003). They further verified that these abilities were not solely reliant upon the temporal coincidence of mouth movements with the rhythm of the acoustic cues. Finally, although monkeys looked at the eyes more than other facial cues, their scanning patterns to the different facial cues varied significantly according to the identity of the two stimulus-monkeys, the emotional valence of the vocalizations emitted by the two stimulus-monkeys, and the sex and the dominance status of the observer.

Viewing of eye regions

Characterization of the scanning patterns indicated that rhesus monkeys attended to the eye regions of the stimulus animals as they evaluated the dynamic, bimodally presented vocalizations. This interest in the eye region adds to a number of previous studies reporting that both humans and monkeys preferentially investigate the eye regions of conspecifics presented either in static images (Adolphs et al., 2005; Farzin, Rivera, & Hessl, 2009; Gamer & Buchel, 2009; Gothard, Brooks, & Peterson, 2009; Gothard et al., 2004; Guo et al., 2003; Kennedy & Adolphs, 2010; Machado & Nelson, 2011; Pelphrey et al., 2002; Riby & Hancock, 2008; Spezio, Huang, Castelli, & Adolphs, 2007) or dynamic, naturalistic videos (Buchan, Pare, & Munhall, 2007; Everdell, Marsh, Yurick, Munhall, & Pare, 2007; Ghazanfar et al., 2006; Klin, Jones, Schultz, Volkmar, & Cohen, 2002). Both humans and rhesus monkeys broadcast important socio-emotional information through their eyes (e.g., their emotional or mental state, social intentions, or focus of their attention), thus attending to the eye region provides the observer with a wealth of socially relevant information (Emery, 2000).

Notably, in the only other investigation to monitor how rhesus look at bimodally presented vocalizations, the variability in looking behavior seen in our study was not reported. The earlier study was designed to explore the evolutionary basis for the propensity of humans to use facial cues to enhance speech comprehension, especially in noisy environments (Ghazanfar et al., 2006). When four adult male rhesus macaques were presented with video sequences of conspecific vocalizations presented with varying levels of species-typical background noise, they maintained their preference for looking to the eye region regardless of ambient noise conditions. Two factors should be considered when comparing our results with the previous findings. First, in the investigation by Ghazanfar and colleagues (2006), videos were presented one at a time, in sequence, as opposed to video pairs. Their paradigm matches closely to the

Identity Same trials of our investigation. In both cases, males looked more to the eye region. Second, in the Ghazanfar study (Ghazanfar et al., 2006), the stimulus monkeys were familiar to the observer monkeys. Hence, the male observer monkeys may not have had the same internal motivation of dominance assessment as they passively viewed the videos. Given the possible contribution of observer dominance status on scanning strategies seen in our study (discussed below), the use of familiar conspecifics as stimulus animals in a preferential viewing paradigm may yield less variability in looking behavior and warrants further exploration.

Sex differences in scanning patterns

The current findings further demonstrated that, in addition to the attention to the eye region exhibited by males and females, males also focused on the mouth region more than females. Although differential scanning by male and female monkeys has not previously been empirically investigated, previous studies have shown that humans modify their gaze behavior based on the information they intend to extract. The differential scanning patterns reported in earlier studies might help explain the different scanning patterns exhibited by males and females in our study.

When instructed to focus on emotion-related cues (e.g., prosody) or make social judgments (Buchan et al., 2008; Lansing & McConkie, 1999), human subjects look more to the eye region than the mouth region. However, when attending to speech-specific aspects of the communication signal (e.g., phonetic details in high levels of ambient noise), they focus significantly more to the mouth region (Lansing & McConkie, 2003; Vatikiotis-Bateson et al., 1998). Interestingly, when allowed to passively view videos of vocalizing actors, human subjects also preferentially attend to the eye regions (Everdell et al., 2007; Klin et al., 2002). It can thereby be inferred that, during passive viewing, humans preferentially attend to the socio-emotional aspects of the stimuli.

A similar type of dissociation may explain the differences in the gaze behavior of male and female monkeys in this investigation. Given the descriptions of human looking behavior, preferential looking to the eyes over the mouth exhibited by females may signify that they were making judgments largely based on the socio-emotional content of the stimuli. By extension, given that males look to the eye region as much as females, the equal looking to the eye and mouth regions exhibited by males would suggest that, in addition to the socio-emotional content, they were likely attending to other aspects of the stimuli. An examination of rhesus macaque social structure may provide insight into what other aspects of stimuli influenced male looking behavior. Briefly, rhesus monkeys have a matriarchal hierarchy. Females acquire the rank from their mothers, but adult males have to earn (and maintain) their dominance status. Thus, male and female behavior is subject to different ethologic pressures that likely influence how each sex reacts when presented with videos of unfamiliar males emitting agonistic and affiliative social signals. When male rhesus macaques encounter novel conspecifics, their initial assessment may focus on the relative dominance status of the new individuals. In the videos used in our investigation, an estimation of the dominance status of the stimulus animals could be provided by the size of the canines, which are larger in high-ranking males. Males looking more to the mouth region may reflect this strategy. Hence, the current results suggest that, whereas female looking behavior was likely influenced by the valence of the vocalizations (inferred by the preferential looking to the eye region), male looking behavior was also sensitive to the rank of the vocalizers (inferred by the equal looking to the eye and mouth regions). This interpretation is further supported by the analyses of the impact of identity and valence on integration ability and looking behavior. Figure 11 illustrates the patterns of looking exhibited by males and females across the two factors and shows that, whereas males monitored the eyes differently across stimulus-pairs, females monitored the mouth differently. Representative scan paths are provided in Figure 12.

In the subsets that included trials in which the vocalizations were made by the same stimulus animal (Fig 11, SID and DVAL), males qualitatively looked more to the eye region than the mouth region (Fig 12A), suggesting that, when identity was not a factor, they were largely influenced by the emotional aspects of the stimuli. By comparison, in subsets that included trials with two stimulus animals (Fig 11, DID and SVAL), males looked equally to the eye and mouth regions (Fig 12B), suggesting that, when identity was a factor, they were also influenced by the relative identity (or dominance status) of the stimulus animals. The opposite pattern was seen in female looking behavior. In the subsets that contained trials with the same relative valence (Fig 11, DID and SVAL), females preferentially attended to the eye regions (Fig 12D). In the subsets that included trials with different relative valences (Fig 11, SID and DVAL) females looked equally to the eye and mouth regions (Fig 12C). Thus, female rhesus may have used the mouth region to confirm their valence-based judgments when the relative valences of the stimuli were disparate.

Collectively, the current results suggest that male rhesus monkeys were more attuned to the relative identity of the novel conspecifics, and females found the relative valences of the vocalizations more salient. Although further studies are needed to better understand the significance of this sex difference, our data parallel recent findings in humans indicating that women recognize crossmodal emotional expressions of fear and disgust strikingly better than men (Collignon et al., 2010).

Additional influences on looking behavior

Other factors that could impact looking behavior are the dominance rank or hormonal status of the observer. First, it is interesting to note that in the integration assessments across Identity Same and Identity Different trials, two males drastically changed their looking behavior across trial type (see Fig 5; filled square and filled triangle), but the other male did not (see Fig 5; filled circle). The animals that modified their overall looking to the congruent and incongruent stimuli exhibited similar scanning strategies characterized by a robust preference for the eye region in Identity Same trials and slightly greater looking to the mouth region in Identity Different trials (see Fig 11; filled square and triangle). Conversely, the animal that showed consistent looking to the congruent and incongruent stimuli across Identity Same and Identity Different trials exhibited the opposite pattern (greater looking to mouth region in Identity Same and slightly more looking to the eye region in Identity Different; see Fig 11; filled circle). Interestingly, although the three males belong to different cohorts, the two males with similar looking strategies are characterized as higher-ranking and the other male is considered mid- to low-ranking, as assessed developmentally by behavioral observations during assays of emotional reactivity and dyadic social interactions. Relationships between temperament and observer responsiveness and looking behavior have been described in both humans (Isaacowitz, 2005; Mogg & Bradley, 2002; Mogg, Millar, & Bradley, 2000; Perlman et al., 2009) and rhesus monkeys (Capitanio, 2002; Gibboni, Zimmerman, & Gothard, 2009; Watson, Ghodasra, & Platt, 2009). Recent investigations of the genetic contributions of the serotonin transporter polymorphism have shown that animals carrying the short allele (S/L) variant of 5-HTTLPR look less to the eye regions of static images of conspecific males than monkeys homozygous for the long allele (L/L) (Gibboni et al., 2009; Watson et al., 2009). Further investigation is needed to fully assess the putative contributions of genetics on processing of dynamic, socially relevant, bimodal stimuli.

A second factor that could affect scanning patterns is the hormonal status in female monkeys. As seen in Figure 8, two females (open circle and open triangle) and all the males (filled shapes) looked more to the incongruent stimulus video in Valence Same trials but more to the congruent stimulus video in Valence Different trials. The third female (open square) exhibited the opposite pattern of looking. There is increasing evidence that changes in women’s hormone levels during the menstrual cycle affect their social perceptions and preferences of men (reviewed by (Jones et al., 2008). Similar effects of ovarian hormones on looking to male faces have been reported in female rhesus macaques (Lacreuse &

Herndon, 2003; Lacreuse, Martin-Malivel, Lange, & Herndon, 2007; Wallen & Rupp, 2010; Wallen & Tannenbaum, 1997). Although each animal was tested over a time period (28-42 days) that spanned all phases of the rhesus menstrual cycle (typical duration of 28 days) (Hutz, Dierschke, & Wolf, 1988), we did not assess hormonal levels throughout testing, and therefore cannot exclude the possibility that hormonal fluctuations contributes to the variability in female looking behavior.

Conclusions

Humans and nonhuman primates live in complex social environments. In primates, social signals are primarily transmitted via faces and vocalizations. The ability to process audiovisual information is necessary for the recognition of individuals and their emotional states. Rhesus macaques possess the ability to integrate the audio and visual components of species-specific vocalizations. The present results intimate that, although this ability was maintained regardless of the relative identity of the stimulus animals or the relative valence of the vocalizations, these parameters influence the way monkeys process the stimuli. Analysis of scanning strategies further supports this proposition and indicates that males are more sensitive to the parameter of identity and females are more sensitive to valence. Although the design of the present study did not allow for the exhaustive characterization of the unique contributions of the factors of relative identity and relative valence, the concordance of overall looking behavior and the supplementary explorations with both natural rhesus and human behavior is compelling. The current results emphasize that subsequent investigations in nonhuman primates should consider identity and valence in the selection of stimuli, as well as the dominance status and sex of the observer.

Characterization of these types of naturally occurring behavioral differences in normal subjects and identification of the neural substrates of those differences are particularly important for research on disorders characterized by deficits in emotional crossmodal integration, such as autism spectrum disorder (Hobson, Ouston, & Lee, 1988; Loveland, 2005; Loveland, Pearson, & Reddoch, 2005; Loveland et al., 1995), pervasive developmental disorder (Magnee, de Gelder, van Engeland, & Kemner, 2007, 2008); and schizophrenia (de Gelder et al., 2005; de Jong, Hodiamont, & de Gelder, 2010; de Jong, Hodiamont, Van den Stock, & de Gelder, 2009). Only a few functional neuroimaging studies in humans have begun to identify neuroanatomical correlates of emotional crossmodal integration and have shown greater responses to bimodally presented emotional expressions (face and voice) than unimodal emotional expressions in the amygdala (Dolan et al., 2001), medial temporal gyrus (MTG), anterior fusiform gyrus (Pourtois et al., 2005), and posterior superior temporal gyrus (pSTG; (Kreifelts et al., 2007), as well as the thalamus (Kreifelts et al., 2007). None have documented sex differences in activation patterns. Although several investigations have empirically demonstrated emotional crossmodal integration abilities in nonhuman primates (e.g. (Izumi & Kojima, 2004; Martinez & Matsuzawa, 2009; Parr, 2004), to date, the neural substrates of these abilities in monkeys have yet to be investigated.

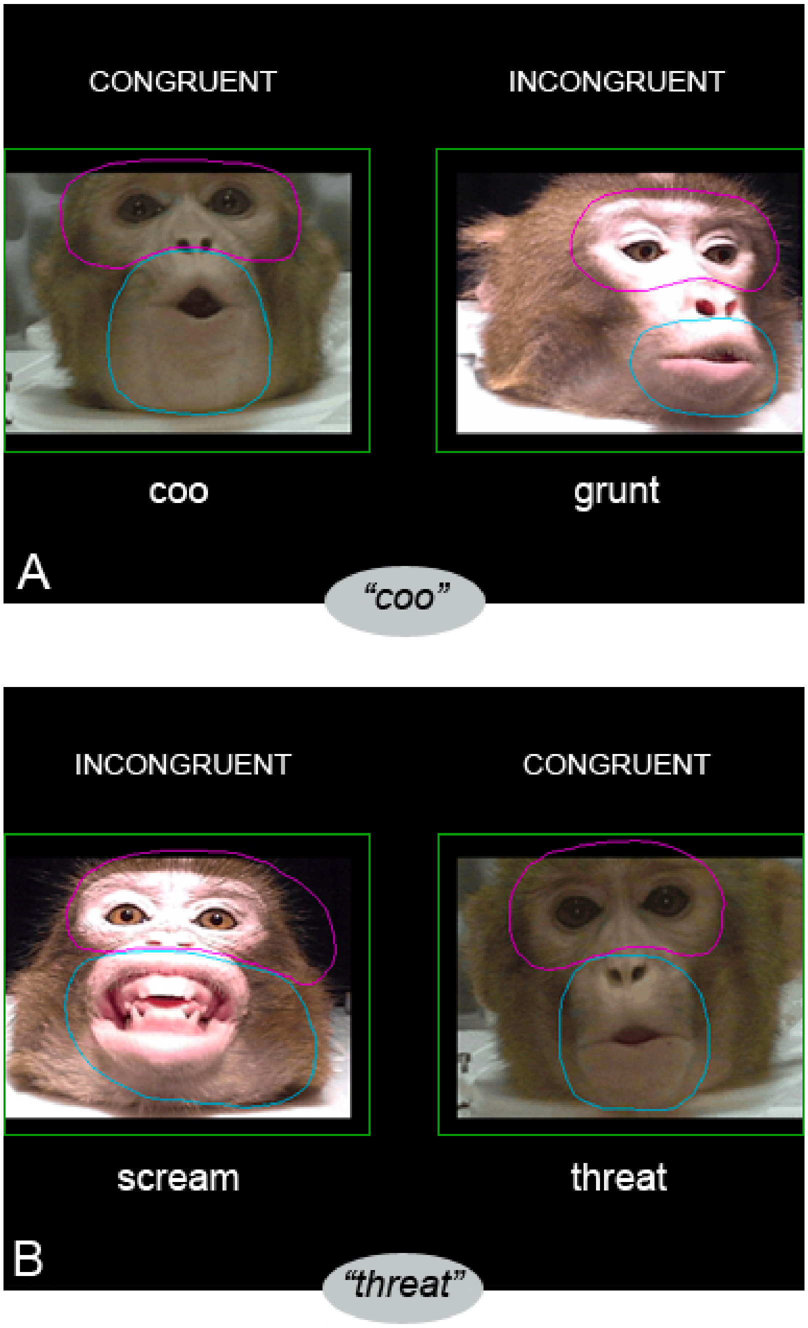
Acknowledgements

This work has been supported by NICHD 35471; NIMH 58846; Yerkes Base Grant NIH RR00165; Center for Behavioral Neuroscience grant NSF IBN-9876754; The Robert W. Woodruff Health Sciences Center Fund, Inc., Emory University; NIMH T32-MH0732505; Autism Speaks Mentor-Based Predoctoral Fellowship Grant: 1657. We thank Asif Ghazanfar, Princeton University Neuroscience Institute Departments of Psychology and Ecology & Evolutionary Biology, for generously allowing us to use his stimuli, and Nancy Bliwise, Emory University Psychology Department, for her guidance on data analysis. We also thank Lisa Parr and Harold Gouzoules for their contributions to the experimental design.

Figures

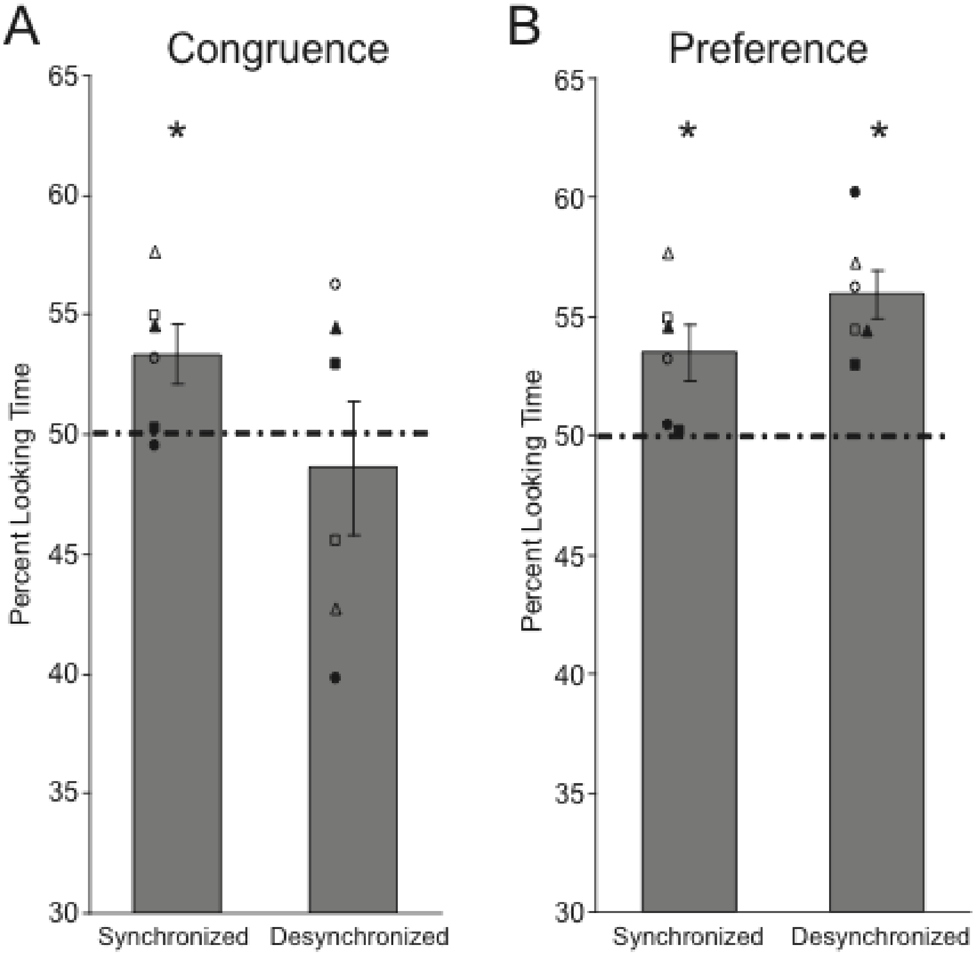
MS-I Figure 1: Schematic of Stimulus Presentation with ROIs.

Screen shots of (A) coo-grunt and (B) scream-threat pairings with ROIs. In (A), the vocalization was a “coo” and in (B), the vocalization was a “threat”. Stimulus sets were comprised of all possible combinations.



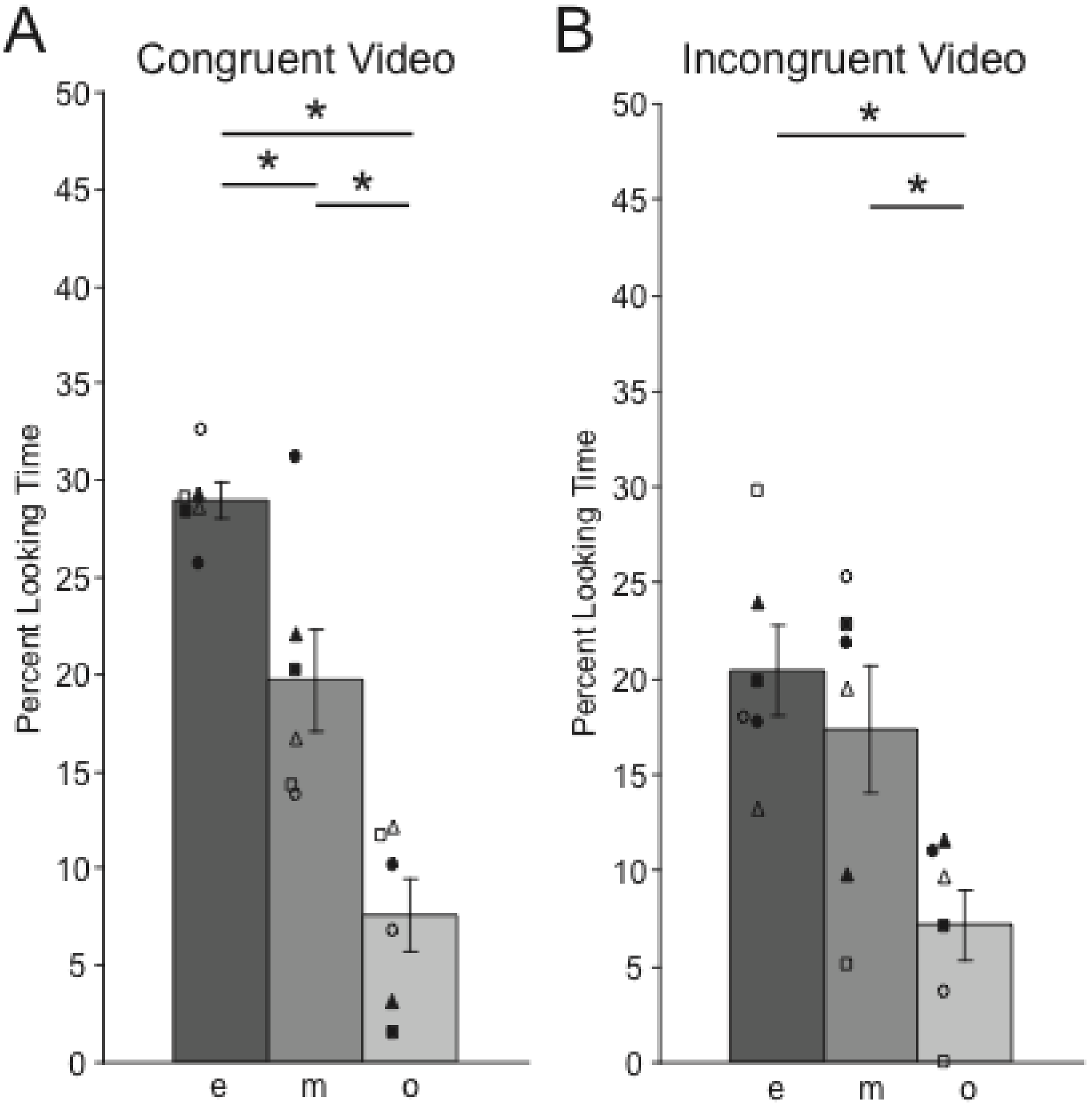
MS-I Figure 2: Integration Assessment – All Trials.

Percentages of looking time (± sem) to (A) the congruent stimulus video and (B) the 'preferred' stimulus video in the Synchronized and Desynchronized conditions across all trials. The dashed line represents chance level of 50%. In panel (A), values greater than 50% represent greater looking to the congruent video and values less than 50% represent greater looking to the incongruent video. Symbols represent individual data points for males (filled) and females (open). (*) $p < 0.05$.



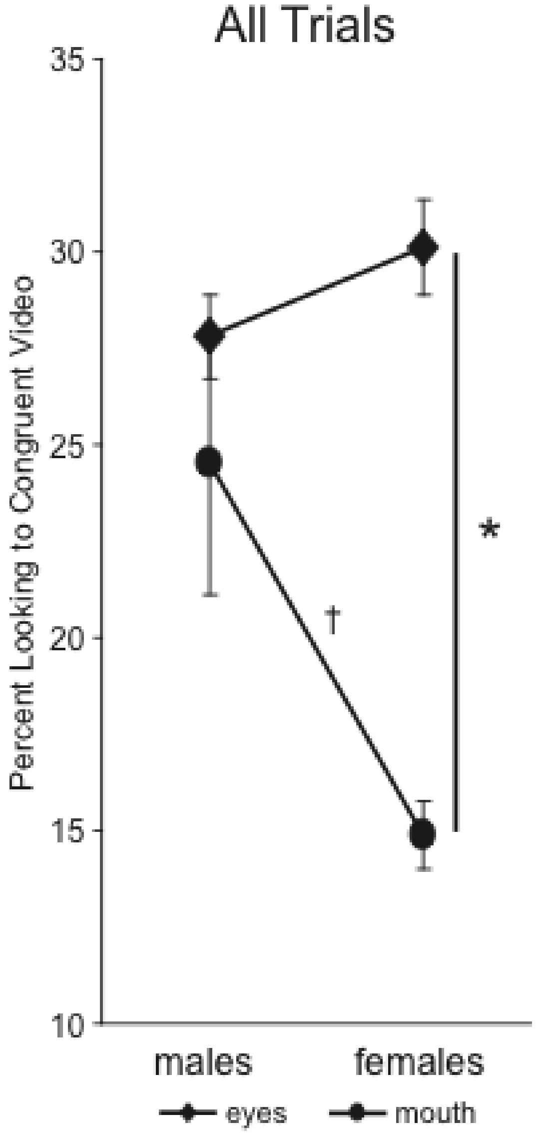
MS-I Figure 3: Scanning Patterns – All Trials.

Percentages of looking time (± sem) to the eyes (e), mouth (m), and other (o) of (A) the congruent stimulus video, and (B) the incongruent stimulus video. Symbols represent individual data points for males (filled) and females (open). (*) $p \leq 0.05$.



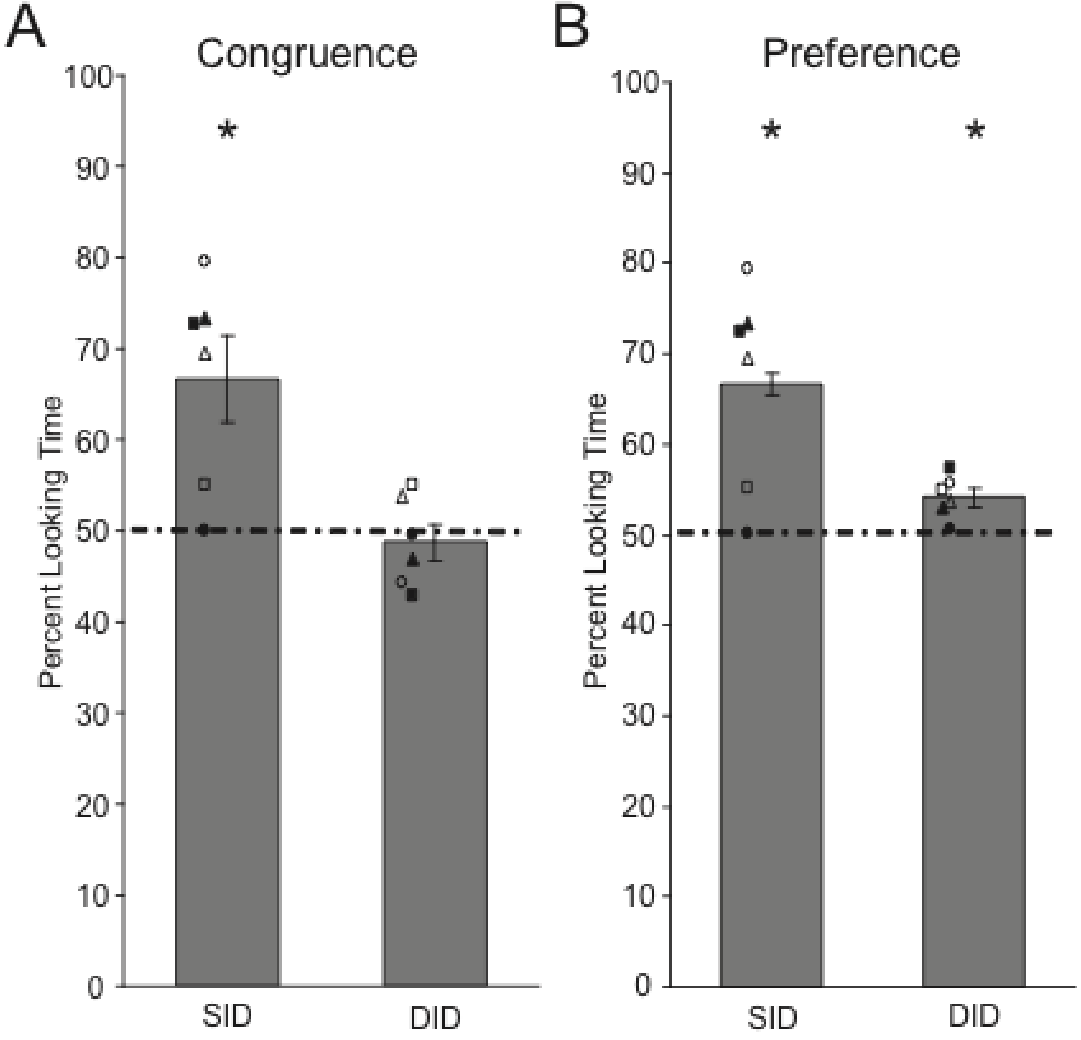
MS-I Figure 4: Sex Differences – All Trials.

Percentages of looking time (± sem) to the eyes (diamonds) and mouth (circles) of the congruent stimulus video for males and females. (*) $p \leq 0.05$; (†) $p \leq 0.075$.



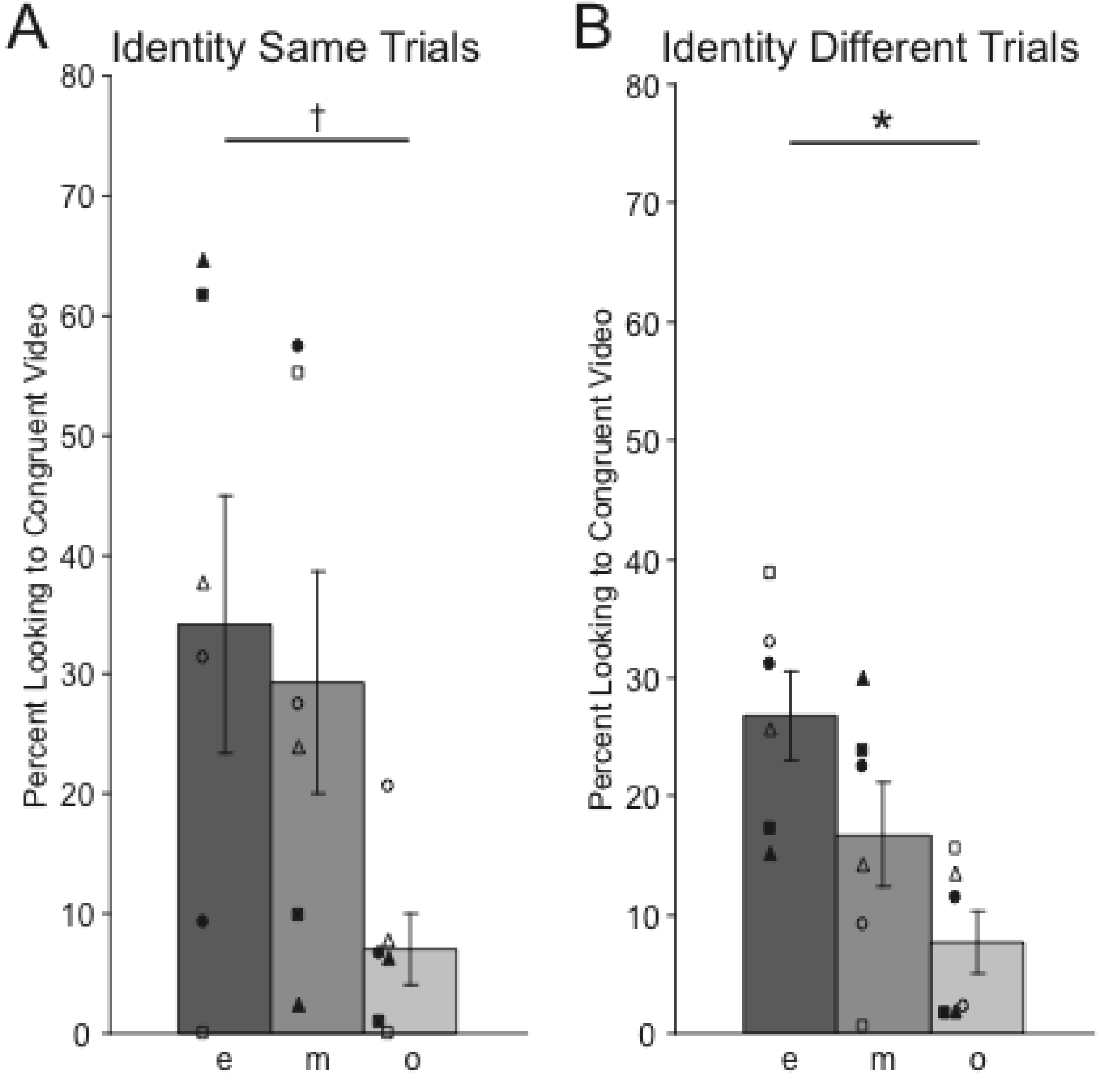
MS-I Figure 5: Integration Assessment – Relative Identity.

Percentages of looking time (± sem) to (A) the congruent stimulus video and (B) the 'preferred' stimulus video in the Synchronized condition across Identity Same (SID) and Identity Different (DID) trials. The dashed line represents chance level of 50%. In panel (A), values greater than 50% represent greater looking to the congruent video and values less than 50% represent greater looking to the incongruent video. Symbols represent individual data points for males (filled) and females (open). (*) $p < 0.05$.



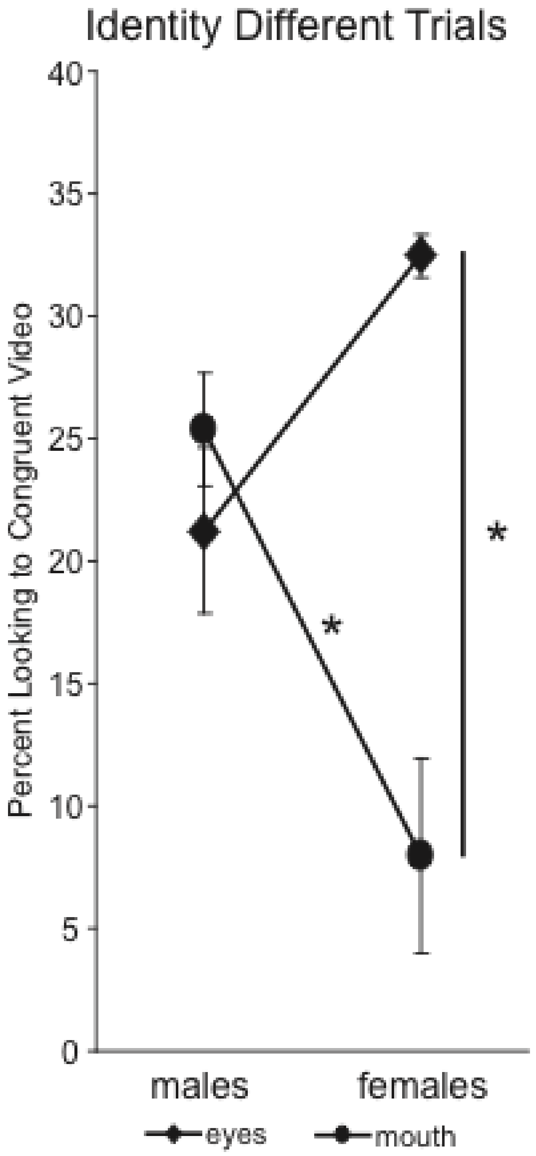
MS-I Figure 6: Scanning Patterns – Identity Trials.

Percentages of looking time (± sem) to the eyes (e), mouth (m), and other (o) of the congruent stimulus video in (A) Identity Same trials and (B) Identity Different trials. Symbols represent individual data points for males (filled) and females (open). (*) $p \leq 0.05$; (†) $p \leq 0.075$.



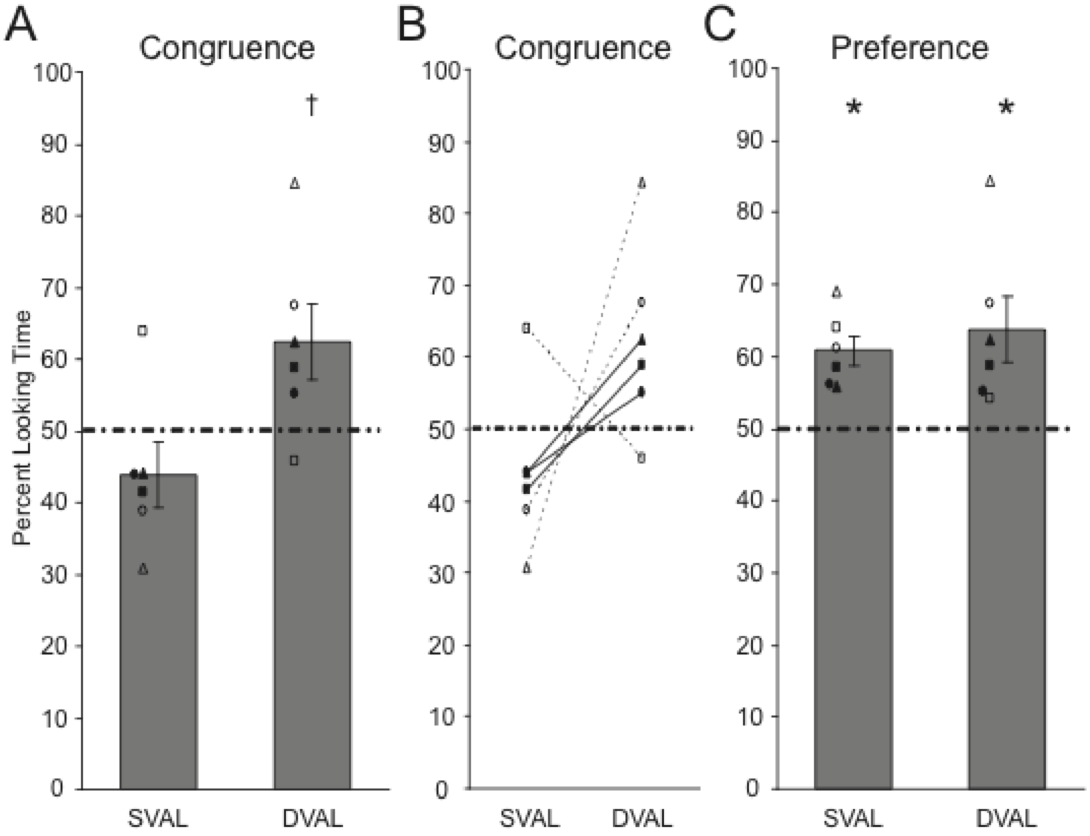
MS-I Figure 7: Sex Differences – Identity Different Trials.

Percentages of looking time (± sem) to the eyes (diamonds) and mouth (circles) of the congruent stimulus video in Identity Different trials for males and females. (*) *p* ≤ 0.05.



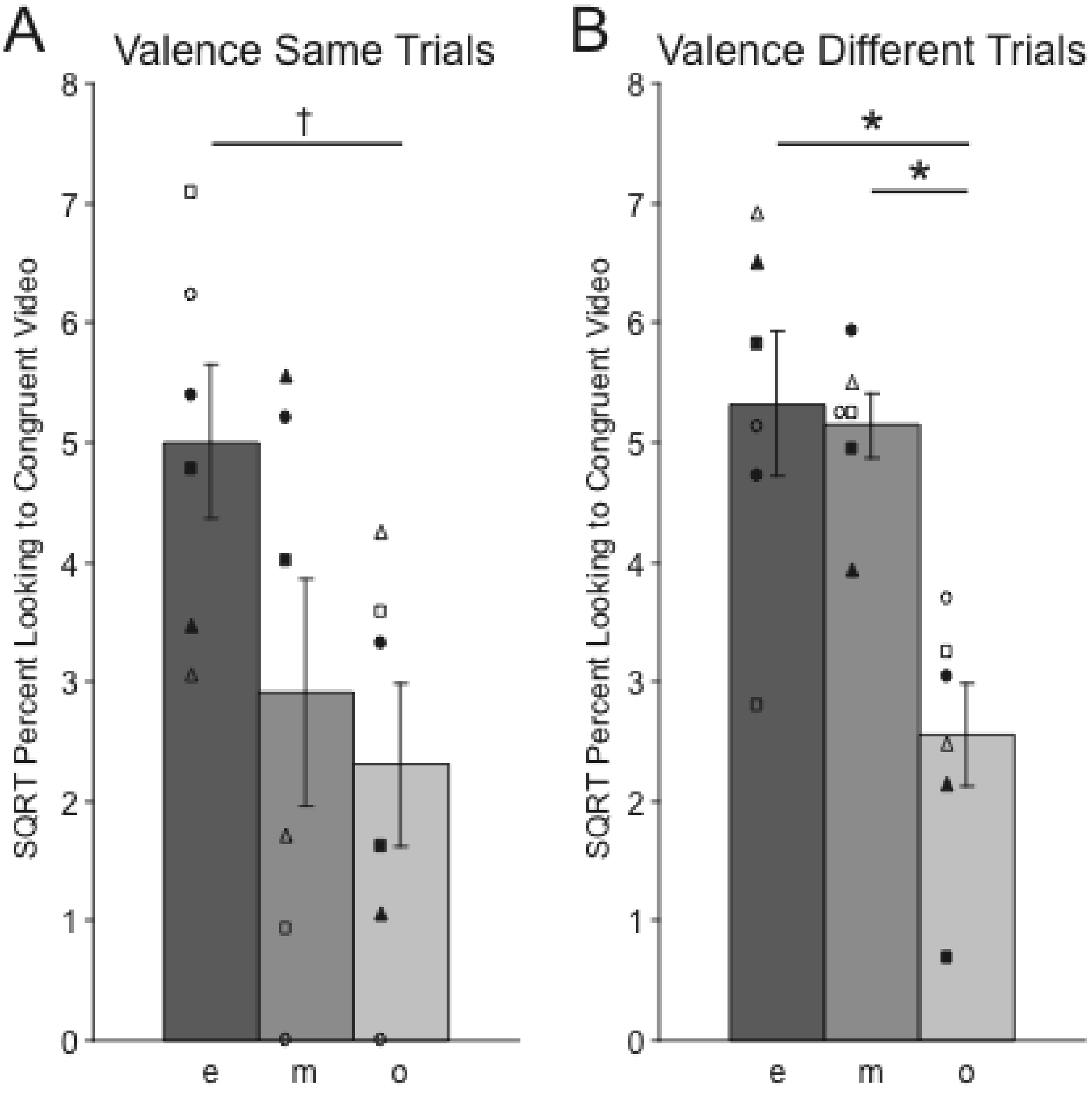
MS-I Figure 8: Integration Assessment – Relative Valence.

Percentages of looking time (± sem) to (A) the congruent stimulus video and (C) the 'preferred' stimulus video in the Synchronized condition across Valence Same (SVAL) and Valence Different (DVAL) trials. (B) Individual data points for percent looking to the congruent stimulus video in Valence Same and Valence Different trials with lines connecting animals across trials (solid lines = males, dotted lines = females). The dashed line represents chance level of 50%. In panels (A) and (B), values greater than 50% represent greater looking to the congruent video and values less than 50% represent greater looking to the incongruent video. Symbols represent individual data points for males (filled) and females (open). (*) $p < 0.05$; (†) $p \leq 0.075$.



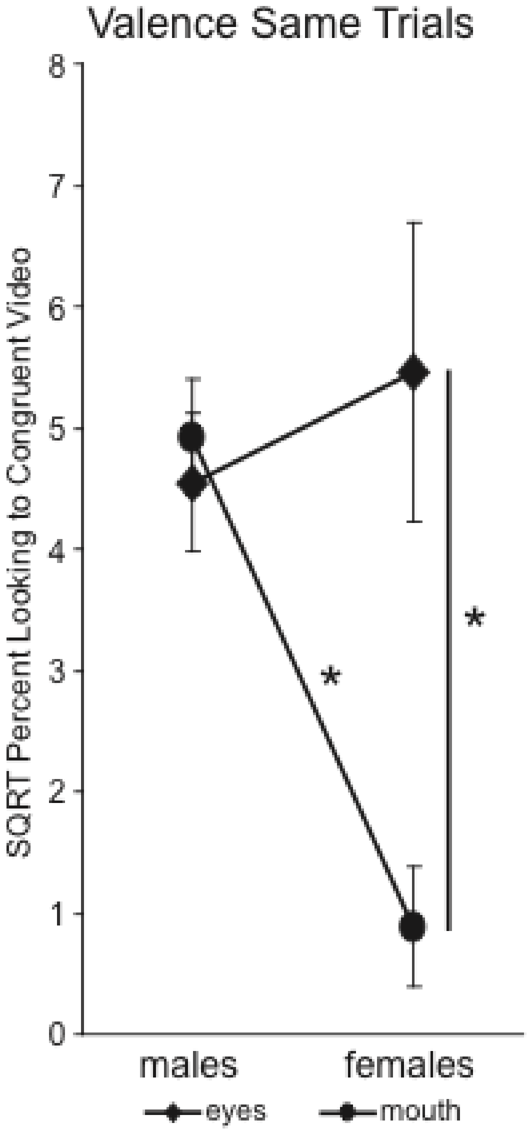
MS-I Figure 9: Scanning Pattern – Valence Trials.

Percentages of looking time (± sem) to the eyes (e), mouth (m), and other (o) of the congruent stimulus video in (A) Valence Same trials and (B) Valence Different trials. Analyses completed on square-root-transformed data to correct for inequality of error variances between variables. Symbols represent individual data points for males (filled) and females (open). (*) $p \leq 0.05$; (†) $p \leq 0.075$.



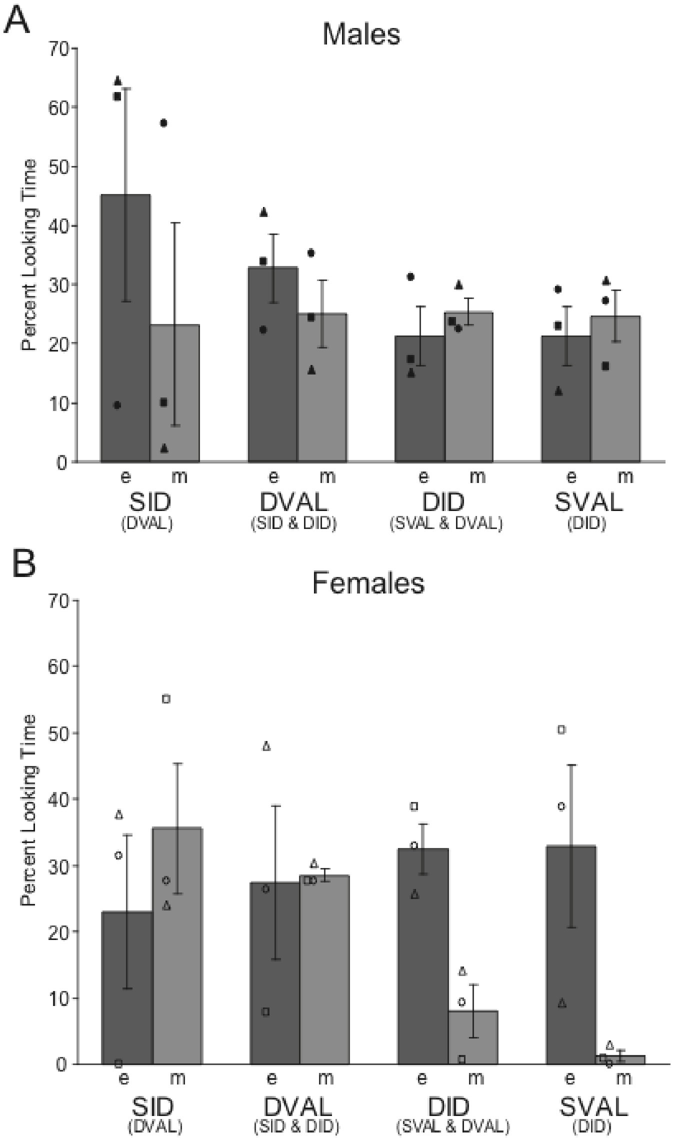
MS-I Figure 10: Sex Differences – Valence Same Trials.

Percentages of looking time (± sem) to the eyes (diamonds) and mouth (circles) of the congruent stimulus video in Valence Same trials for males and females. Analyses completed on square-root-transformed data to correct for inequality of error variances between variables. (*) $p \leq 0.05$.



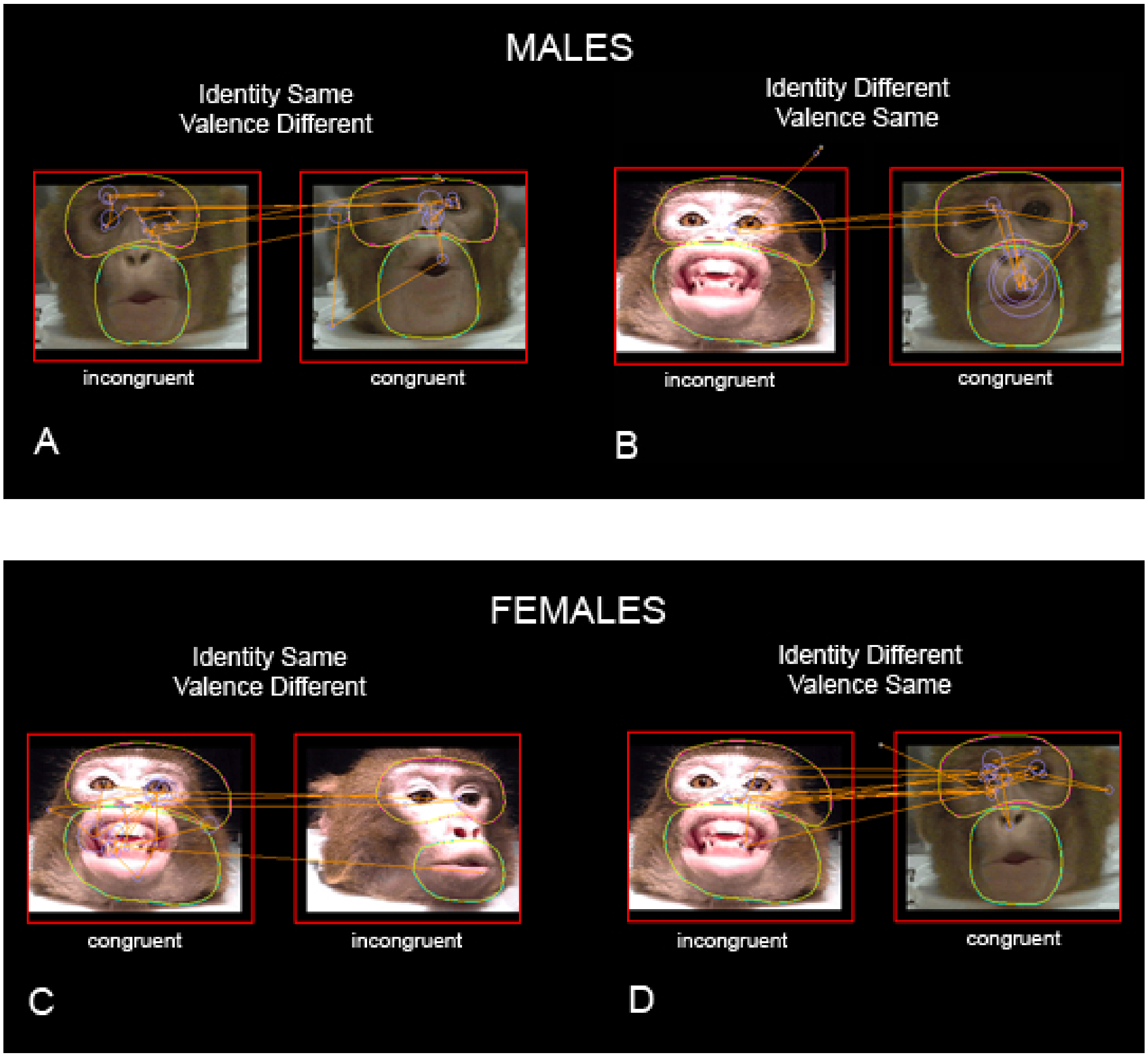
MS-I Figure 11: Relative Looking to Eyes and Mouths by Males and Females.

Percentages of looking time (± sem) to the eyes and mouth of the congruent stimulus video exhibited by (A) males and (B) females in Identity Same (SID), Valence Different (DVAL), Identity Different (DID) and Valence Same (SVAL) trials. Symbols represent individual data points for males (filled) and females (open). (*) $p \leq 0.05$; (†) $p \leq 0.075$.



MS-I Figure 11: Representative Scan Paths for Males and Females.

Male scan path on an Identity Same / Valence Different Trial (A) and on an Identity Different / Valence Same Trial (B). Female scan path on an Identity Same / Valence Different Trial (C) and on an Identity Different / Valence Same Trial (D). Circles represent fixation points with the size corresponding with fixation duration.



References

Adolphs, R., Gosselin, F., Buchanan, T. W., Tranel, D., Schyns, P., & Damasio, A. R. (2005). A mechanism for impaired fear recognition after amygdala damage. *Nature, 433,* 68-72.

Adolphs, R., Sears, L., & Piven, J. (2001). Abnormal processing of social information from faces in autism. *J.Cogn Neurosci., 13,* 232-240.

Baron-Cohen, S., Ring, H. A., Wheelwright, S., Bullmore, E. T., Brammer, M. J., Simmons, A., et al. (1999). Social intelligence in the normal and autistic brain: an fMRI study. *Eur.J Neurosci., 11*(6), 1891-1898.

Barton, R., & Aggleton, J. (2000). Primate evolution and the amygdala. In J. Aggleton (Ed.), *The Amygdala* (2nd ed., pp. 479-508). New York: Oxford University Press.

Brothers, L. (1995). Neurophysiology of the perception of intention by primates. In M. Gazzaniga (Ed.), *The Cognitive Neurosciences* (pp. 1107). Cambridge, MA: The MIT Press.

Buchan, J. N., Pare, M., & Munhall, K. G. (2007). Spatial statistics of gaze fixations during dynamic face processing. *Soc Neurosci, 2*(1), 1-13.

Buchan, J. N., Pare, M., & Munhall, K. G. (2008). The effect of varying talker identity and listening conditions on gaze behavior during audiovisual speech perception. *Brain Res, 1242*, 162-171.

Byrne, R., & Whiten, A. (1988). *Machiavellian intelligence: Social expertise and the evolution of intellect in monkeys, apes and humans*. Oxford: Clarendon Press.

Capitanio, J. P. (2002). Sociability and responses to video playbacks in adult male rhesus monkeys (Macaca mulatta). *Primates, 43*(3), 169-177.

Cheney, D., & Seyfarth, R. (1990). *How Monkeys See the World.* Chicago: University of Chicago Press.

Collignon, O., Girard, S., Gosselin, F., Saint-Amour, D., Lepore, F., & Lassonde, M. (2010). Women process multisensory emotion expressions more efficiently than men. *Neuropsychologia, 48*(1), 220-225.

de Gelder, B., Vroomen, J., de Jong, S. J., Masthoff, E. D., Trompenaars, F. J., & Hodiamont, P. (2005). Multisensory integration of emotional faces and voices in schizophrenics. *Schizophr Res, 72*(2-3), 195-203.

de Jong, J. J., Hodiamont, P. P., & de Gelder, B. (2010). Modality-specific attention and multisensory integration of emotions in schizophrenia: reduced regulatory effects. *Schizophr Res, 122*(1-3), 136-143.

de Jong, J. J., Hodiamont, P. P., Van den Stock, J., & de Gelder, B. (2009). Audiovisual emotion recognition in schizophrenia: reduced integration of facial and vocal affect. *Schizophr Res, 107*(2-3), 286-293.

de Waal, F. (1989). *Peacemaking among primates*. Boston: Harvard University Press.

Dixon, N. F., & Spitz, L. (1980). The detection of auditory visual desynchrony. *Perception, 9*(6), 719-721.

Dolan, R. J., Morris, J. S., & de Gelder, B. (2001). Crossmodal binding of fear in voice and face. *Proc.Natl.Acad.Sci.U.S.A, 98*(17), 10006-10010.

Emery, N. J. (2000). The eyes have it: the neuroethology, function and evolution of social gaze. *Neurosci Biobehav Rev, 24*(6), 581-604.

Emery, N. J., Lorincz, E. N., Perrett, D. I., Oram, M. W., & Baker, C. I. (1997). Gaze following and joint attention in rhesus monkeys (Macaca mulatta). *J.Comp Psychol., 111*(3), 286-293.

Everdell, I. T., Marsh, H. O., Yurick, M. D., Munhall, K. G., & Pare, M. (2007). Gaze behaviour in audiovisual speech perception: asymmetrical distribution of face-directed fixations. *Perception, 36*(10), 1535-1545.

Farzin, F., Rivera, S. M., & Hessl, D. (2009). Brief report: Visual processing of faces in individuals with fragile X syndrome: an eye tracking study. *J Autism Dev Disord, 39*(6), 946-952.

Gamer, M., & Buchel, C. (2009). Amygdala activation predicts gaze toward fearful eyes. *J Neurosci, 29*(28), 9123-9126.

Ghazanfar, A. A., & Logothetis, N. K. (2003). Neuroperception: facial expressions linked to monkey calls. *Nature, 423*(6943), 937-938.

Ghazanfar, A. A., Maier, J. X., Hoffman, K. L., & Logothetis, N. K. (2005). Multisensory integration of dynamic faces and voices in rhesus monkey auditory cortex. *Journal of Neuroscience, 25*(20), 5004-5012.

Ghazanfar, A. A., Nielsen, K., & Logothetis, N. K. (2006). Eye movements of monkey observers viewing vocalizing conspecifics. *Cognition*.

Gibboni, R. R., Zimmerman, P. E., & Gothard, K. M. (2009). Individual differences in Scanpaths correspond with serotonin transporter genotype and behavioral phenotype in rhesus monkeys (Macaca mulatta). *Front Behav Neurosci, 3,* 50.

Gothard, K. M., Brooks, K. N., & Peterson, M. A. (2009). Multiple perceptual strategies used by macaque monkeys for face recognition. *Anim Cogn, 12*(1), 155-167.

Gothard, K. M., Erickson, C. A., & Amaral, D. G. (2004). How do rhesus monkeys (Macaca mulatta) scan faces in a visual paired comparison task? *Anim Cogn, 7*(1), 25-36.

Goursaud, A. P., & Bachevalier, J. (2007). Social attachment in juvenile monkeys with neonatal lesion of the hippocampus, amygdala and orbital frontal cortex. *Behav Brain Res, 176*(1), 75-93.

Guo, K., Robertson, R. G., Mahmoodi, S., Tadmor, Y., & Young, M. P. (2003). How do monkeys view faces?--A study of eye movements. *Experimental Brain Research, 150*(3), 363-374.

Hauser, M. (1993). Right hemisphere dominance for the production of facial expression in monkeys. *Science, 261*(5120), 475-477.

Hinde, R., & Roswell, T. (1962). Communication by postures and facial expressions in the rhesus monkey (Macaca mulatta). *Proc Roy Soc Lon B, 138*.

Hobson, R. P., Ouston, J., & Lee, A. (1988). Emotion recognition in autism: coordinating faces and voices. *Psychol.Med., 18*(4), 911-923.

Hutz, R., Dierschke, D., & Wolf, R. (1988). Induction of atresia of the dominant follicle in rhesus monkeys (Macaca mulatta) by the local application of estradiol-17B. *Americal Journal of Primatology, 15*(1), 69-77.

Isaacowitz, D. M. (2005). The gaze of the optimist. *Pers Soc Psychol Bull, 31*(3), 407-415.

Izumi, A., & Kojima, S. (2004). Matching vocalizations to vocalizing faces in a chimpanzee (Pan troglodytes). *Anim Cogn, 7*(3), 179-184.

Jones, B. C., DeBruine, L. M., Perrett, D. I., Little, A. C., Feinberg, D. R., & Law Smith, M. J. (2008). Effects of menstrual cycle phase on face preferences. *Arch Sex Behav, 37*(1), 78-84.

Keating, C. F., & Keating, E. G. (1982). Visual scan patterns of rhesus monkeys viewing faces. *Perception, 11*(2), 211-219.

Kennedy, D. P., & Adolphs, R. (2010). Impaired fixation to eyes following amygdala damage arises from abnormal bottom-up attention. *Neuropsychologia, 48*(12), 3392-3398.

Klin, A., Jones, W., Schultz, R., Volkmar, F., & Cohen, D. (2002). Visual fixation patterns during viewing of naturalistic social situations as predictors of social competence in individuals with autism. *Archives of General Psychiatry, 59*(9), 809-816.

Kreifelts, B., Ethofer, T., Grodd, W., Erb, M., & Wildgruber, D. (2007). Audiovisual integration of emotional signals in voice and face: an event-related fMRI study. *Neuroimage, 37*(4), 1445-1456.

Lacreuse, A., & Herndon, J. G. (2003). Estradiol selectively affects processing of conspecifics' faces in female rhesus monkeys. *Psychoneuroendocrinology, 28*(7), 885-905.

Lacreuse, A., Martin-Malivel, J., Lange, H. S., & Herndon, J. G. (2007). Effects of the menstrual cycle on looking preferences for faces in female rhesus monkeys. *Anim Cogn, 10*(2), 105-115.

Lansing, C. R., & McConkie, G. W. (1999). Attention to facial regions in segmental and prosodic visual speech perception tasks. *J Speech Lang Hear Res, 42*(3), 526-539.

Lansing, C. R., & McConkie, G. W. (2003). Word identification and eye fixation locations in visual and visual-plus-auditory presentations of spoken sentences. *Percept Psychophys, 65*(4), 536-552.

Loveland, K. (2005). Social-emotional impairment and self-regulation in Autism spectrum disorders. In J. Nadel & D. Muir (Eds.), *Typical and impaired emotional development* (pp. 365-382). Oxford: Oxford University Press.

Loveland, K., Pearson, D., & Reddoch, S. (2005). *Anxiety symptoms in childrem and adolescents with autism*. Paper presented at the International Meeting for Autism Research.

Loveland, K., Tunali-Kotoski, B., Chen, R., Brelsford, K., Ortegon, J., & Pearson, D. (1995). Intermodal perception of affect in persons with autism or Down syndrome. *Develop Psychopath, 7*.

Machado, C. J., & Nelson, E. E. (2011). Eye-tracking with nonhuman primates is now more accessible than ever before. *Am J Primatol, 73*(6), 562-569.

Magnee, M. J., de Gelder, B., van Engeland, H., & Kemner, C. (2007). Facial electromyographic responses to emotional information from faces and voices in individuals with pervasive developmental disorder. *J Child Psychol Psychiatry, 48*(11), 1122-1130.

Magnee, M. J., de Gelder, B., van Engeland, H., & Kemner, C. (2008). Audiovisual speech integration in pervasive developmental disorder:

evidence from event-related potentials. *J Child Psychol Psychiatry, 49*(9), 995-1000.

Martinez, L., & Matsuzawa, T. (2009). Auditory-visual intermodal matching based on individual recognition in a chimpanzee (Pan troglodytes). *Anim Cogn, 12 Suppl 1*, S71-85.

Mogg, K., & Bradley, B. P. (2002). Selective orienting of attention to masked threat faces in social anxiety. *Behav Res Ther, 40*(12), 1403-1414.

Mogg, K., Millar, N., & Bradley, B. P. (2000). Biases in eye movements to threatening facial expressions in generalized anxiety disorder and depressive disorder. *J Abnorm Psychol, 109*(4), 695-704.

Nahm, F., Parret, A., Amaral, D., & Albright, T. (1997). How do monkeys look at a face? *Journal of Cognitive Neuroscience, 9*(5), 611-623.

Parr, L. A. (2004). Perceptual biases for multimodal cues in chimpanzee (Pan troglodytes) affect recognition. *Anim Cogn, 7*(3), 171-178.

Partan, S. (2004). Multisensoty animal communication. In G. Calvert, C. Spence & B. Stein (Eds.), *The Handbook of Multisensory Processes* (pp. 225-242). Cambridge, MA: The MIT Press.

Payne, C., Machado, C. J., Bliwise, N. G., & Bachevalier, J. (2010). Maturation of the hippocampal formation and amygdala in Macaca mulatta: a volumetric magnetic resonance imaging study. *Hippocampus, 20*(8), 922-935.

Pelphrey, K. A., Sasson, N. J., Reznick, J. S., Paul, G., Goldman, B. D., & Piven, J. (2002). Visual scanning of faces in autism. *J.Autism Dev.Disord., 32*(4), 249-261.

Perlman, S. B., Morris, J. P., Vander Wyk, B. C., Green, S. R., Doyle, J. L., & Pelphrey, K. A. (2009). Individual differences in personality predict how people look at faces. *PLoS One, 4*(6), e5952.

Petrides, M. (1994). Frontal lobes and behaviour. *Curr Opin Neurobiol, 4*(2), 207-211.

Pourtois, G., de Gelder, B., Bol, A., & Crommelinck, M. (2005). Perception of facial expressions and voices and of their combination in the human brain. *Cortex, 41*(1), 49-59.

Riby, D. M., & Hancock, P. J. (2008). Viewing it differently: social scene perception in Williams syndrome and autism. *Neuropsychologia, 46*(11), 2855-2860.

Romanski, L., & Ghazanfar, A. (2010). The primate frontal and temporal lobes and their tole in multisensory vocal communication. In M. Platt & A. Ghazanfar (Eds.), *Primate Neuroethology* (pp. 500-524). New York: Oxford University Press.

Sato, N., & Nakamura, K. (2001). Detection of directed gaze in rhesus monkeys (Macaca mulatta). *J.Comp Psychol., 115*(2), 115-121.

Spezio, M. L., Huang, P. Y., Castelli, F., & Adolphs, R. (2007). Amygdala damage impairs eye contact during conversations with real people. *Journal of Neuroscience, 27*(15), 3994-3997.

Vatikiotis-Bateson, E., Eigsti, I. M., Yano, S., & Munhall, K. G. (1998). Eye movement of perceivers during audiovisual speech perception. *Percept Psychophys, 60*(6), 926-940.

Wallen, K., & Rupp, H. A. (2010). Women's interest in visual sexual stimuli varies with menstrual cycle phase at first exposure and predicts later interest. *Horm Behav, 57*(2), 263-268.

Wallen, K., & Tannenbaum, P. L. (1997). Hormonal modulation of sexual behavior and affiliation in rhesus monkeys. *Ann N Y Acad Sci, 807*, 185-202.

Watson, K. K., Ghodasra, J. H., & Platt, M. L. (2009). Serotonin transporter genotype modulates social reward and punishment in rhesus macaques. *PLoS One, 4*(1), e4156.

MANUSCRIPT II: The Role of the Amygdala in Processing Bimodal Conspecific Vocalizations in Rhesus Macaques

Christa Payne[1,2]

Jocelyne Bachevalier[1,2]

[1]Department of Psychology, Emory University, Atlanta, GA 30062

[2]Division of Developmental and Cognitive Neuroscience, Yerkes National Primate Research Center, Atlanta, GA 30062

Abstract

In primate societies, social cues are primarily transmitted via faces and voices. Recognizing, integrating and interpreting multimodal signals are essential for social success, but the neural substrates mediating these functions are not fully understood. Here, we examined the role of the amygdala in processing bimodal species-specific vocalizations using eye tracking in rhesus macaques. The looking behaviors of six adult rhesus monkeys that received neonatal lesions of the amygdala (3M, 3F) were compared to the previously characterized looking behaviors of six adult, sham-operated rhesus macaques. Two side-by-side videos of unknown male conspecifics emitting different vocalizations (i.e., coo, grunt, scream, threat) were presented with the audio signal matching one of the videos. The percentage of time animals looked to the congruent videos was used to assess crossmodal integration ability and the percentages of time spent looking at each of the six a priori ROIs (eyes, mouth, and rest of each stimulus video) were used to characterize scanning patterns. Both groups looked more to one of the videos, indicating that early damage to the amygdala does not impair crossmodal integration of complex social signals. Scanning patterns showed that damage to the amygdala resulted in greater use of the mouth region to dissociate the two videos and a loss of the sex difference in stimulus feature salience seen in sham-operated animals. Unlike sham-operated males, males with neonatal amygdala lesions did not attend to the identity-related features that may indicate dominance. Similarly, unlike sham-operated females, the scanning strategies of females with neonatal amygdala lesions were not sensitive to the relative valence of the vocalizations. The results suggest that animals with damage to the

amygdala did not recognize the social relevance of stimulus features such as dominance status of novel male conspecifics or the valence of the vocalizations emitted by those individuals, and is consistent with a role of the amygdala in the recognition of the social salience of complex cues.

Introduction

The complex social structures of human and nonhuman primates mandates that individuals possess the capacity to recognize and interpret socially relevant signals and mount the appropriate, species-specific responses to those signals. The amygdala has long been identified as a neural substrate of these socio-emotional abilities (recently reviewed by Adolphs, 2010). In particular, the amygdala appears to (a) code and process facial movements, eye-gaze directions, body postures, and gestures that are potent signals for the production and modulation of appropriate social and emotional responses towards other individuals (Adolphs, 1999, 2003; Bachevalier & Loveland, 2006; Bachevalier & Meunier, 2005; Bechara et al., 2003); (b) link discrete stimuli to their intrinsic motivational and socio-emotional significance (M. G. Baxter, Parker, Lindner, Izquierdo, & Murray, 2000; Blundell et al., 2001; Morrison & Salzman, 2010; Murray, 2007; Rolls, 1992; Salzman & Fusi, 2010; Stefanacci et al., 2003), such as associating a specific animal in a social troop and its level of agonistic behavior; (c) regulate motor, autonomic and endocrine manifestations of emotions through its connections with the striatum, brainstem and hypothalamus, respectively (Amaral et al., 1992; Saunders & Rosene, 1988; Saunders et al., 1988). Although the role of the amygdala in processing emotion in faces has been extensively investigated (see Adolphs & Spezio, 2006), the contribution of the amygdala in processing complex socio-emotional signals, such as bimodal, species-specific vocalizations, has yet to be fully elucidated.

Previous research in both humans and nonhuman primates has indicated that the amygdala is not critical for crossmodal integration (Goulet & Murray, 2001; Lee et al., 1988; F. K. Nahm et al., 1993). Monkeys that received excitotoxic lesions of the amygdala in adulthood were not impaired in crossmodal integration as measured by a tactual-visual delayed-nonmatching-to-sample task (Goulet & Murray, 2001). Similarly, humans with bilateral damage to the amygdala were also not impaired in visual-tactual integration (Lee et al., 1988; F. K. Nahm et al., 1993). Based on these results, researchers concluded that the amygdala was not involved in crossmodal integration. However, the methodology of those investigations may have affected the ability to detect impairments following amygdala damage. Specifically, they employed matching paradigms that assessed acquisition and recall of crossmodal associations, and not crossmodal integration, per se, and the stimuli were objects that could be identified by sight and touch, and were inherently non-social.

Notably, recent human neuroimaging studies have begun to implicate the amygdala in processing bimodal socio-emotional stimuli (Dolan et al., 2001; Ethofer, Anders, Erb et al., 2006; Muller et al., 2011; Pourtois et al., 2005). Additionally, an electrophysiology investigation in rhesus macaques identified multisensory neurons that responded to species-specific vocalizations (Kuraoka & Nakamura, 2007). Such results are consistent with the heteromodal anatomical organization of the amygdala (Amaral et al., 1992; Saunders & Rosene, 1988; Saunders et al., 1988), and suggest that dysfunction within the amygdala may subserve the deficits in emotional crossmodal integration seen in human developmental neuropsychological disorders, such as autism (Hobson et al., 1988; Loveland et al., 2008), pervasive developmental disorder (Magnee et al., 2008), and schizophrenia (de Gelder, Vroomen, Annen, Masthof, & Hodiamont, 2003; de Jong et al., 2010).

The rhesus communicative system is comprised of a small repertoire of relatively fixed calls associated with distinguishable facial gestures and linked to particular social contexts. In addition, nonhuman primates, such as rhesus macaques, recognize the correspondence between facial and vocal expressions (Ghazanfar & Logothetis, 2003; Payne & Bachevalier, in preparation). Thus, they provide an ideal animal model to investigate the putative contributions of the amygdala in the emotional crossmodal integration abilities.

Recent studies from our laboratory have shown that selective neonatal lesions of the amygdala yielded significant changes in emotional and stress reactivity as well as in the ability to flexibly alter behavioral responses when context changed (Kazama et al., 2008; Raper et al., 2009; Raper et al., 2011). These behavioral and cognitive changes not only persisted when the animals reached adulthood but also were as severe as the behavioral changes reported in monkeys that had received similar amygdala lesions in adulthood (Bachevalier, Machado, & Kazama, 2011). Given these long-lasting effects of neonatal amygdala lesions on emotion regulation and decision-making, we conjectured that, if the amygdala had a critical role to play in the ability to integrate crossmodal social cues, lesions of this region in infancy would result in significant impairment of this ability. To test this proposal, the goals of the present investigation were to 1) determine if neonatal lesions of the amygdala disrupt integration ability in rhesus macaques using a preferential viewing paradigm, 2) characterize the scanning strategies of adult rhesus macaques with neonatal lesions of the amygdala using eye-tracking, and 3) compare the looking behavior of monkeys with neonatal amygdala lesions with those of previously characterized sham-operated controls (Payne & Bachevalier, in prep).

Method

All procedures were approved by the Animal Care and Use Committee of the University of Texas Health Science Center at Houston in Houston, TX and of Emory University in Atlanta, GA and carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used, as well as any pain and suffering.

Subjects

Six adult rhesus monkeys (*Macaca mulatta*) aged 4-6 years were used in this investigation. Each animal received MRI-guided ibotenic acid amygdala lesions (group Neo-A$_{ibo}$; 3 males, 3 females) at 7-17 days of age. Animals were raised in a socially enriched nursery environment that promoted the development of species-specific social skills (detailed in Goursaud & Bachevalier, 2007), and underwent repeated assessments of memory, emotional reactivity, social behavior, and reward appraisal throughout development. Their ability to integrate auditory-visual social information was compared to that of sham-operated adult monkeys (group Neo-C, 3 males and 3 females) that had received their sham-operations at 8-12 days of age, were reared in exactly the same way and had received identical behavioral and cognitive training throughout their life (Payne & Bachevalier, in preparation).

Magnetic Resonance Imaging Procedures

Animals of group Neo-A_{ibo} underwent two MRI scanning sessions according to procedures previously described (Saunders, Aigner, & Frank, 1990). To maintain experiential consistency across groups, the same pre-surgical scanning procedures were completed in animals of group Neo-C (Payne & Bachevalier, in preparation). Given the individual variability observed in the neuroanatomy of rhesus macaques, MRI scans were acquired pre-surgically to facilitate the production of subject-specific lesions. A second MRI session was performed to evaluate the extent of lesion using previously validated techniques (Malkova, Lex, Mishkin, & Saunders, 2001; Nemanic, Alvarado, Price, Jackson, & Bachevalier, 2002). For this session, an identical series of MRI scans were obtained one week after surgery for group Neo-A_{ibo}.

Neonates were removed from their home cage and lightly sedated with Isoflurane inhalation (1.0 – 3.0% to effect) to allow for intubation with an endotracheal tube, which provided constant Isoflurane sedation and respiration assistance throughout scanning and surgery. Heart rate, body temperature, and SPO_2 levels were monitored throughout the entire procedure. Neonates were transported to the University of Texas M.D. Anderson Cancer Center MRI facility in a temperature and humidity controlled incubator. Upon arrival, animals were secured in a non-ferromagnetic stereotaxic apparatus (Crist Instruments Co., Inc., Damascus, MD), aligned within the MRI scanner and had a 3” surface coil placed on its head to enhance the resolution of the MR images.

Three MRI sequences were obtained with a GE Signa 1.5 Tesla Echo Speed scanner (GE Medical Systems, Milwaukee, WI). The first series, a T1-weighted spin-echo sequence (echo time (TE) = 11 ms, repetition time (TR) = 450 ms, contiguous 4 mm sections, 12 cm field of view (FOV), 256 x 256 matrix), was acquired in the sagittal plane and used to align the two subsequent series. The second sequence (3D T1-weighted fast spoiled gradient (FSPGR)-echo sequence, TE = 2.6 ms, TR = 10.2 ms, 25° flip angle, contiguous 1 mm sections, 12 cm FOV, 256 x 256 matrix) was used to determine the three-dimensional coordinates for each neurotoxin injection site in the amygdala for group Neo-A$_{ibo}$. The final series of images acquired varied according to group. Animals in group Neo-A$_{ibo}$ received a Fluid Attenuated Inversion Recovery (FLAIR) sequence (TE = 140 msec, TR = 10000 msec, inversion time (TI) = 2200, contiguous 3 mm sections, 12 cm FOV, 256 x 256 matrix), which accurately indicates localized areas of edema, an indicator of neurotoxin-induced cell death, and was subsequently used to assess extent of damage for this group. Three FLAIR series were acquired for each monkey, offset posteriorly by 1 mm. After the pre-surgical scanning session, the animals were transported to the surgical suite.

Determination of Neonatal Amygdala Injection Coordinates

For each animal, the image representing the mid-portion of the amygdala was identified on the high-resolution FSPGR series and 4-6 injection targets were chosen from this image. To facilitate equitable dispersion of the ibotenic acid across all amygdaloid nuclei, the injection targets were spaced 2 mm apart in the M/L and 1 mm apart in the D/V directions. The three-dimensional (A/P, M/L and D/V) MRI coordinates were recorded and later converted into stereotaxic space. The intended lesions are illustrated in Figure 1 (left column) and included all amygdaloid nuclei while sparing of the adjacent cortical areas.

Surgical Procedures

Animals remained on Isoflurane sedation for the duration of surgery and received an intravenous drip solution of 5% dextrose and 0.45% sodium chloride to maintain hydration. Heart rate, respiration rate, blood pressure, expired CO_2 and body temperature were monitored throughout the entire procedure. The animal's head was shaved and the skin was disinfected with Nolvasan solution. To minimize the pain and inflammation associated with the incision, a long lasting local anesthetic (Marcaine 25%, 1.5 ml, s.c.) was injected subcutaneously along the incision line. Following midline incision, the skin and connective tissue (galea) were gently retracted to expose the skull.

Small craniotomies were made above the injection targets and small incisions were cut in the dura to allow a needle to be lowered to the appropriate injection coordinates. Injections of ibotenic acid (Biosearch Technologies, Novato, CA; 10mg/ml in phosphate buffered saline, pH 7.0) were administered simultaneously in both hemispheres. Needles attached to 10 µl Hamilton syringes (Drummond Scientific Company, Broomall, PA) held by a Kopf electrode manipulators (David Kopf Instruments, Tujunga, CA) were slowly lowered to each of the 4-6 injection sites and the neurotoxin was slowly injected (total of 0.8-1.6 µl, rate of 0.2 µl/30sec). After each injection, the needles remained in place for three minutes to allow for diffusion of the neurotoxin and to prevent the drug from spreading into the needle track during retraction.

At completion of the surgical surgeries, the opening was closed in neuroanatomical layers. Animals were then removed from the Isoflurane gas anesthesia and the stereotaxic apparatus and allowed to recover in an incubator ventilated with oxygen. All animals received pre- and post-surgical treatments to minimize risk of infection (Cephazolin, 25 mg/kg, per os) and control swelling (dexamethazone sodium phosphate, 0.4 mg/kg, s.c.). Treatments began twelve hours before and continued seven days after surgery. A topical antibiotic ointment (bacitracin-neomycin-polymyxin) was applied to the wound daily and acetaminophen (10mg/kg, p.o.) was given four times a day for three days after surgery to relieve pain.

Lesion Verification

Comparison of pre- and post- surgical MRI scans was used to estimate the extent of lesion (Malkova et al., 2001; Nemanic et al., 2002). In particular, the T1 FSPGR and FLAIR sequences were used to assess the extent of damage from neurotoxic injections into the amygdala. The amygdala was identified on the T1 images, whereas the FLAIR sequence reveals areas of intense fluid resulting from cell death. For each animal of group Neo-A_{ibo}, each coronal FLAIR image was matched to a series of drawings of coronal histological sections of a one-week-old normal rhesus monkey brain (J. Bachevalier, unpublished data), acquired at 1 mm interval. The extent of hyper-signal was visually identified on each MR image and plotted onto the corresponding drawings of the brain of the normal infant monkey. The surface area (in pixels) of damage to both the amygdala and adjacent areas (ento- and perirhinal cortices, hippocampus) was measured using

ImageJ® software (http://rsb.info.nih.gov/ij/). The total volume of damage for each structure was calculated from the measured surface areas in each hemisphere (Gundersen & Jensen, 1987). The volume of damage to each structure was then expressed as a percentage of the normal volume for that structure, which was previously estimated from the normal infant rhesus monkey brain, using similar method (detailed in Nemanic et al., 2002).

As shown in Table 1, the extent of bilateral amygdala damage in all cases averaged 62.5 %. For three cases (Neo-A_{ibo}-1, -4 and -6), the damage was substantial and symmetrical and the remaining three cases (Neo-A_{ibo}-2, -3 and -5) had moderate and asymmetrical amygdala damage, ranging from 33.0 % to 42.0 % in the left side and 61.1 % to 77.6 % in the right side. The extent of unintended damage to the adjacent cortical areas and the anterior portion of the hippocampus were negligible for all cases.

Crossmodal integration task

A passive preferential viewing paradigm was used to determine if the amygdala is required for the spontaneous ability of rhesus macaques to integrate the acoustic and visual components of crossmodally presented conspecific vocalizations (Ghazanfar & Logothetis, 2003). The impact of neonatal lesions of the amygdala on scanning patterns of these complex social signals was also assessed using eye-tracking technology. Behavioral testing of the animals with neonatal amygdala lesions was, in all respects, identical to that given earlier to the sham-operated monkeys.

Apparatus: Monkeys were seated in a primate chair fitted with a head-restraint device designed to gently inhibit head movements. Animals were placed in front of a 24-inch, flat panel LCD monitor with centered speaker and eye-tracking camera (ISCAN, Inc.; Woburn, MA). A curtain concealed all other equipment within the sound attenuated testing room and ambient white noise was played to further minimize any extraneous sounds.

Stimuli: Four videos of the facial postures associated with species-typical calls (coo, grunt, scream and threat) were used to construct two stimulus sets. Each trial consisted of two videos spaced apart maximally within the same visual plane (side-by-side) on a solid black background and the audio track corresponding to one of the videos. Videos were two unknown rhesus monkeys ('stimulus animals') and were restricted to the animals' heads. The coo and threat vocalizations came from one stimulus animal and the grunt and scream vocalizations were from the other. Trial duration was 10 seconds (2-sec video looped 5 times). Stimulus presentation was controlled using the Presentation software package (Neurobehavioral Systems, Inc; Albany, CA).

Pilot trials were constructed from pairs of vocalizations emitted by the same stimulus animal (i.e. coo-threat and grunt-scream pairs) and indicated rapid habituation of the limited stimuli. Previous results indicated that the relative identity of the stimulus animals had no effect on crossmodal integration ability (Ghazanfar & Logothetis, 2003). Thus, to reduce habituation, all combinations of stimulus video pairs were used to construct the stimulus sets. Allowing 4 – 7 days between testing sessions also minimized habituation. The left-right position of each video was counter-balanced within each stimulus set.

Task: In the first stimulus set, the onsets of the auditory and visual components were synchronized (Synchronized condition). This set was used as the standard for integration assessment and was comprised of eight trials administered over four testing sessions. The second stimulus set was used to assess whether integration of the aural and visual components relied upon cues that were redundant between modalities (i.e. the coincidence of mouth movements with the vocal element). Thus, in this stimulus set consisting of eight unique trials presented across two testing sessions, the onset of the audio track was delayed 330 – 430 msec from the start of the video (Desynchronized condition), a delay range that has been shown to disrupt the perception of the sensory elements as a single event (Dixon & Spitz, 1980).

Measures

Integration Assessment: In a given trial, each video was either 'congruent' (i.e., matching the audio component) or 'incongruent' (i.e., discordant with the audio track). An ability to integrate across modalities was determined by comparing the percent looking time to each video to the chance level of 50%. Integration was inferred when monkeys show a preference for one of the video clips (i.e., looks statistically more than chance to either the congruent or incongruent stimulus video). Consequently, animals looking equally to each stimulus video demonstrated an inability to integrate the complex social signals.

Scanning Pattern Characterization: Static *a priori* regions of interest (ROIs) of the 'Eyes' and 'Mouth' were created with the ISCAN P.O.R. Fixation Analysis software (v1.20, ISCAN, Inc., Fig 2) such that each ROI included the entire feature of interest throughout the entire 2sec video. A third ROI included the area of the video not encapsulated by either the 'Eyes' or 'Mouth' ROI and was referred to as 'Other'. Fixations were defined as eye gaze coordinates remaining within $1^\circ \times 1^\circ$ visual angle for at least 100 msec, and lasted until the eye gaze coordinates deviated more than $1^\circ \times 1^\circ$ visual angle for more than 360 msec. The ISCAN P.O.R. Fixation Analysis Software was used to group fixations into ROIs.

Each trial had six ROIs: 'eyes', 'mouth', and 'other' for each the congruent and incongruent stimulus videos. The duration of total looking in a trial was the accumulation of fixation durations across all ROIs. To account for variability in looking time across trials and animals, looking to each ROI was analyzed in terms of the percentage of total looking for each trial ((ROI/Total)*100).

Statistical Analyses

Assumptions of parametric statistics were met in all measures. Integration abilities were assessed separately for the Synchronized and Desynchronized conditions with one-sample t-tests to determine if the percentage of looking to the congruent stimuli was different than chance (50%). Integration ability and scanning strategies of group Neo-A_{ibo} were analyzed identically to the previous characterization of the looking behavior of group Neo-C (Payne & Bachevalier, in prep). Integration abilities across conditions were compared separately via dependent-samples t-tests, and sex differences were evaluated separately via independent-samples t-tests. Group differences in integration ability were assessed via ANOVA (percent looking x group x sex) with Bonferroni corrected post-hoc comparisons. Since the purpose of the Desynchronized condition was to ensure that integration ability was not only reliant upon the temporal coincidence of the audio and visual elements, characterization of scanning patterns of the stimuli was only assessed in the Synchronized condition. As in the integration assessment, scanning strategies of groups Neo-C and Neo-A$_{ibo}$ were compared using repeated measures MANOVA (Stimulus Video x ROI x Group x Sex) with planned comparisons.

Results

Overall Integration and Scanning Patterns

Integration Assessment after Neonatal Amygdala Lesions: Figure 3 illustrates that groups Neo-A$_{ibo}$ and Neo-C exhibited similar looking time for the congruent and incongruent videos across both the Synchronized and Desynchronized conditions. In the Synchronized condition, both groups looked more than chance (50%) to the congruent stimulus (Fig 3A), although this preference reached significance for group Neo-C but not for group Neo-A$_{ibo}$ ($t(5) = 0.921$, $p = 0.399$). The lack of statistical significance within group Neo-A$_{ibo}$ was likely due to individual differences in preference direction (i.e., four animals looked more to the congruent stimulus video and two animals looked more to the incongruent stimulus video). When preference direction was disregarded (Fig 3B), like group Neo-C, group Neo-A$_{ibo}$ showed a significant preference for one of the two stimulus videos ($t(5) = 5.073$; $p = 0.004$), demonstrating intact integration ability. Direct group comparisons further confirmed the similarities in integration abilities (Neo-A$_{ibo}$ = Neo-C: congruent stimulus video: $F(1,8) = 0.083$, $p = 0.781$, Fig 3A; ‘preferred’ stimulus video: $F(1,8) = 2.489$, $p = 0.153$, Fig 3B).

In the Desynchronized condition, like group Neo-C, group Neo-A$_{ibo}$ did not exhibit a preference for congruence (t(5) = -0.296, p = 0.779, Fig 3A), but did show a significant general preference for one of the two stimulus videos (Neo-A$_{ibo}$: t(5) = 4.978, p = 0.004, Fig 3B). Thus, again, there were no group differences for the measures of congruence (F(1,8) = 0.004, p = 0.949) or general preference (F(1,8) = 0.683, p = 0.433). The general preference in the Desynchronized condition indicates that, as seen in group Neo-C, the integration ability of group Neo-A$_{ibo}$ did not depend upon the synchronization of the audio and visual components.

Finally, as observed in group Neo-C, males and females of group Neo-A$_{ibo}$ did not differ in their integration ability for either the Synchronized (t(4) = - 0.307, p = 0.774) or Desynchronized conditions (t(4) = - 0.562, p = 0.604).

Scanning Patterns across All Trials: As shown in Figure 4, group Neo-A$_{ibo}$ exhibited general scanning strategies similar to those observed within group Neo-C. Overall, group Neo-A$_{ibo}$ preferentially attended to the eye and mouth regions of the congruent and incongruent stimulus videos, although this effect was stronger in the congruent video. In the congruent stimulus video (Fig 4A), group Neo-A$_{ibo}$ looked equally to the eye and mouth regions (F(1,4) = 3.378, p = 0.140), but more to each of these regions than to the rest of the congruent stimulus video (eyes > other: F(1,4) = 163.738, p < 0.0001; mouth > other: F(1,4) = 11.799, p = 0.026). In the incongruent stimulus video (Fig 4B), group Neo-A$_{ibo}$ showed the same basic scanning pattern, but the preferences for the eye and mouth regions over the rest of the congruent stimulus were less robust (eye ≥ other: F(1,4) = 5.575, p = 0.078; mouth = other: F(1,4) = 3.225, p = 0.147).

Despite the similarities in overall scanning patterns across groups, a significant ROI x Group x Sex interaction within the congruent stimulus video (F(1,8) = 6.894, *p* = 0.030) revealed that males and females of groups Neo- A$_{ibo}$ and Neo-C exhibited differential patterns of looking to the eye and mouth regions of the congruent stimulus video (Fig 5). As previously reported (Payne & Bachevalier, in prep), Neo-C males looked equally to the eye and mouth regions, and Neo-C females distinguished the two regions, looking more to the eyes than the mouth region. Group Neo-A$_{ibo}$ showed the opposite pattern of looking: Neo-A$_{ibo}$ males looked more to the eye region than the mouth region (F(1,8) = 7.268, *p* = 0.027), and Neo-A$_{ibo}$ females looked equally to the two regions (F(1,8) = 0.093, *p* = 0.769). Additionally, Neo-A$_{ibo}$ females looked less to the eye region of the congruent stimulus video than Neo-C females (F(1,8) = 7.532, *p* = 0.025).

Summary: The present results demonstrate that, like control monkeys, adult monkeys with neonatal amygdala lesions integrate bimodal social cues produced by novel conspecifics, and that ability was not solely reliant upon the mechanical properties of the vocalization. Despite unperturbed integration ability, monkeys with neonatal amygdala lesions did exhibit important differences in passive viewing of these complex social signals compared to sham-operated controls. Specifically, males and females with neonatal amygdala lesions had spontaneous viewing patterns directly opposite to those earlier described for male and female controls. None of the measurements for group Neo-A$_{ibo}$ correlated significantly with extent of damage to the amygdala.

Previous characterization of the looking behavior of sham-operated controls suggested that the differences in passive scanning patterns of males and females might reflect differences in the features to which the two sexes preferentially attend (Payne & Bachevalier, in prep). In particular, females appeared to focus primarily on the relative valence of the vocalizations (demonstrated by Neo-C females preferentially looking to the eye region over the mouth, see Fig 5), whereas males appeared to also be sensitive to features associated with the identity of the vocalizer, such as dominance (demonstrated by Neo-C males looking equally to the eye and mouth regions, see Fig 5). The reversed sex difference in spontaneous scanning strategies of group Neo-A_{ibo} (see Fig 5) suggests that neonatal amygdala lesions impact the recognition of the social salience of the stimulus videos. To further explore this hypothesis, we performed post-hoc analyses on the factors of identity and valence.

Identity

The factor of 'Identity' refers to the identity of the stimulus animals presented on the two videos of a given trial. In 'Identity Same' trials (2 trials), the same stimulus animal generated both vocalizations (i.e., the coo-threat pairings and grunt-scream pairings), whereas in 'Identity Different' trials (6 trials) two stimulus animals each emitted one vocalization (e.g., the coo-scream pairings or grunt-threat pairings).

<u>Integration Assessment after Neonatal Amygdala Lesions</u>: Groups Neo-A_{ibo} and Neo-C displayed nearly identical preference patterns across Identity Same and Identity Different trials (Fig 6). When the same conspecific emitted both vocalizations (Identity Same trials), groups exhibited a strong preference for congruence (Neo-A_{ibo}: $t(5) = 4.385$, $p = 0.007$; Neo-C: $t(5) = 3.530$, $p = 0.017$). In contrast, when the vocalizations were produced by two different stimulus animals

(Identity Different trials), both groups showed a preference (Neo-A_{ibo} (incongruent): t(5) = -2.631, p = 0.046; Neo-C (general): t(5) = 4.451, p = 0.007). Accordingly, like group Neo-C, the percentage of total looking to the congruent stimulus video was greater for the Identity Same trials than for the Identity Different trials (t(5) = 5.434, p = 0.003, Fig 6A). This difference persisted when preference for one of the two videos was analyzed (t(5) = 2.987, p = 0.031, Fig 6B). This pattern, also displayed by group Neo-C, indicated that both groups showed a stronger preference in Identity Same trials than in Identity Different trials. Moreover, like Neo-C monkeys, there were no differences between Neo-A_{ibo} males and females for either Identity trial type. The similarities between groups were confirmed statistically in that the percent looking to the congruent stimulus video did not vary across groups for either Identity Same (F(1,8) = 2.068, p = 0.188) or Identity Different trials (F(1,8) = 2.724, p = 0.137).

Scanning Pattern for Identity Same Trials: As shown on Figure 7, the general scanning patterns for Identity Same and Identity Different trials by animals with neonatal amygdala lesions appeared slightly different from those described for the sham-operated controls. However, within-group deviation prevented these qualitative differences from reaching statistical significance. In Identity Same trials, group Neo-A_{ibo} scanned the congruent and incongruent stimulus videos somewhat differently.

In the congruent stimulus video (Fig 7A), group Neo-A_{ibo} looked qualitatively more to the eye region than the rest of the stimulus video and qualitatively more to the mouth than the eye region, but these differences did not reach statistical significance (eyes = mouth: F(1,4) = 1.084, p = 0.357; eyes = other: F(1,4) =

5.024, p = 0.088). However, there was a robust preference for the mouth region over the rest of the stimulus video (F(1,4) = 13.224, p = 0.022). By contrast, in the incongruent stimulus video, group Neo-A$_{ibo}$ showed little differentiation between the three regions of interest, with only a tendency to look more to the eyes than the rest of the stimulus video (F(1,4) = 6.071, p = 0.069). There were no differences in the scanning patterns of Neo-A$_{ibo}$ males and females (F(2,8) = 2.762, p = 0.122).

In addition, group Neo-A$_{ibo}$ looked more to the mouth region of the congruent stimulus video than the mouth region of the incongruent stimulus video (F(1,4) = 14.853, p = 0.018), indicating that they used the mouths to distinguish the congruent and incongruent stimulus videos. This pattern was not observed for group Neo-C. However, this group difference was not statistically significant (Stimulus Video x ROI x Group: F(2,8) = 0.698, p = 0.512).

Scanning Pattern for Identity Different Trials: Figure 7 also illustrates that, when the vocalizations were emitted by two different conspecifics, the general scanning patterns of group Neo-A$_{ibo}$ were consistent with those reported for group Neo-C. Thus, like group Neo-C, Neo-A$_{ibo}$ monkeys displayed greater looking to the eyes than to the rest of the congruent stimulus video (F(1,4) = 26.483, p = 0.007), but looked equally to all three regions of the incongruent stimulus video. As in Identity Same trials, Neo-A$_{ibo}$ monkeys seemed to use the mouth regions to distinguish the congruent and incongruent stimulus videos, albeit in the opposite direction (incongruent mouth > congruent mouth: F(1,4) = 8.148, p = 0.046), a pattern not observed for group Neo-C.

There were no observable sex differences within group Neo-A$_{ibo}$ (F(2,8) =

0.776, $p = 0.492$, Fig 8). And, although there were no statistically significant Group x Sex interactions at any of the regions of interest, it is notable that the sex differences observed within group Neo-C were not exhibited by group Neo-A$_{ibo}$ (Fig 8). Whereas Neo-C males looked equally to the eye and mouth regions, and Neo-C females looked more to the eye region than the mouth region, Neo-A$_{ibo}$ males and females did not differ in their scanning of the eye and mouth regions. These qualitative differences were reflected in Neo-C males looking more to the mouth than Neo-A$_{ibo}$ males ($F(1,8) = 7.823$, $p = 0.023$).

Summary: Consistent with the analyses of all trials, neonatal lesions of the amygdala did not affect integration ability regardless of the relative identity of the vocalizer. The difference in preference direction and preference strength across trial type indicates that, like group Neo-C, animals in Neo-A$_{ibo}$ processed Identity Same and Identity Different trials somewhat differently. Although there were only minimal statistical differences between the looking behavior of groups Neo-C and Neo-A$_{ibo}$, there were some interesting qualitative differences that are illustrated in Figures 12 and 13.

First, in both Identity Same and Identity Different trials, Neo-A$_{ibo}$ monkeys looked longer to the mouth region of their preferred stimulus video (see congruent video in Identity Same and incongruent video in Identity Different; Figs 12, 13 bottom panels) than to the mouth region of the non-preferred video. This suggests that animals with neonatal amygdala lesions consistently used the mouth region to distinguish the two stimulus videos and explains the comparable percentages of looking to the mouth regions of the congruent and incongruent stimulus videos when all trials were considered (see Fig 4).

A second qualitative difference was the lack of the sex differences within the looking behavior of group Neo-A_{ibo}. Whereas Neo-C females exhibited similar looking to the eye and mouth regions across trial parameters (Fig 13A,B), the scanning strategies of Neo-C males varied across Identity Same and Identity Different trials (Fig 12A,B). That is, in Identity Same trials, Neo-C males looked more to the eye region than the mouth, but looked equally to these two regions in Identity Different trials. Thus, as discussed above, when the two videos were of the same stimulus animal, males appeared to attend to the valence of the vocalizations; and when the videos were of different monkeys, Neo-C males also seemed to attend to facial features that may be associated with the dominance status of the stimulus monkeys (i.e. canine size). By contrast, Neo-A_{ibo} males and females exhibited consistent relative looking to the eye and mouth regions across parameters (Fig 12 and Fig 13, bottom panels).

Finally, none of the measurements for group Neo-A_{ibo} correlated significantly with the extent of amygdala lesions.

Valence

A second set of analyses was performed on the factor of 'Valence', which refers to the emotional content of the stimulus vocalizations in a given trial. In 'Valence Same' trials, both vocalizations had either a positive (i.e., coo-grunt) or negative (i.e., threat-scream) valence. In 'Valence Different' trials, the two vocalizations had different valences (e.g., coo-threat or grunt-scream).

<u>Integration Assessment after Neonatal Amygdala Lesions</u>: Group Neo-A_{ibo} exhibited the same preference patterns as group Neo-C in both Valence Same and

Valence Different trials (Fig 9). In Valence Same trials, both groups looked more to the incongruent than the congruent stimulus videos, although this difference reached significance only for Neo-A$_{\text{ibo}}$ (t(5) = -3.204, *p* = 0.024) and both groups showed a significant general preference. In Valence Different trials, both groups tended to look more towards the congruent stimulus videos (Neo-A$_{\text{ibo}}$: t(5) = 2.219, *p* = 0.077; Neo-C: t(5) = 2.327, *p* = 0.067), and showed significant general preferences for one of the stimulus videos (Neo-A$_{\text{ibo}}$: t(5) = 3.561, *p* = 0.016; Neo-C: t(5) = 2.988, *p* = 0.031). Thus, as shown in Figure 9A, both groups looked qualitatively less to the congruent stimulus video in Valence Same trials than in Valence Different trials, but this difference was statistically significant only for group Neo-A$_{\text{ibo}}$ (t(5) = -2.996, *p* = 0.030). As in group Neo-C, there were no differences between Neo-A$_{\text{ibo}}$ males and females at either valence parameter. The consistencies in preference patterns between the two groups were statistically confirmed for both Valence Same (F(1,8) = 0.080, *p* = 0.784) and Valence Different (F(1,8) = 0.001, *p* = 0.979) trials.

<u>Scanning Pattern for Valence Same Trials</u>: As illustrated in Figure 10, when the vocalizations had the same general valence, the general scanning patterns of groups Neo-A$_{\text{ibo}}$ and Neo-C were virtually the same. In congruent stimulus videos (Fig 10A), group Neo-A$_{\text{ibo}}$ tended to look to the eye regions more than the mouth (F(1,4) = 6.487, *p* = 0.064), looked more to the eyes than the rest of the video (F(1,4) = 11.879, *p* = 0.026), but looked equally to the mouth region and the rest of the congruent stimulus video (F(1,4) = 1.245, *p* = 0.327). Group Neo-C displayed the same basic pattern. Also like group Neo-C, the only differentiation in the regions of the incongruent stimulus videos was between the eyes and the

rest of the video (eyes > other: F(1,4) = 7.727, *p* = 0.050).

There were no discernable differences in the looking behavior of the males and females of group Neo-A_{ibo} (F(2,8) = 0.265, *p* = 0.774). However, Figure 11 illustrates the significant Group x Sex interaction in the scanning of the mouth region of the congruent stimulus video (F(1,8) = 10.339, *p* = 0.012). Whereas males and females of group Neo-A_{ibo} exhibited similar exploration of the eye and mouth regions, with each sex looking relatively more to the eyes than to the mouth, Neo-C males and females explored the eye and the mouth regions differently, with males looking equally to the two regions and females looking much more to the eyes than mouth. Thus, the interaction was characterized by Neo-A_{ibo} males looking less to the mouth region than Neo-C males (F(1,8) = 5.209, *p* = 0.052) and Neo-A_{ibo} females looking more to the mouth region than Neo-C females (F(1,8) = 5.130, *p* = 0.053).

Scanning Pattern for Valence Different Trials: When the vocalizations carried disparate valences (Fig 10B), group Neo-A_{ibo} showed little difference from group Neo-C in their general scanning patterns, but did exhibit differences in their scanning of the congruent and incongruent stimulus video. Consistent with the scanning strategies of group Neo-C (Fig 10B), group Neo-A_{ibo} did not differentiate the eye and mouth regions of the congruent videos (F(1,4) = 0.126, *p* = 0.741), but did show the characteristic preference for the socially salient facial features (eyes and mouths) over the rest of the congruent stimulus videos (eyes > other: F(1,4) = 79.561, *p* = 0.001; mouth > other: F(1,4) = 12.497, *p* = 0.024). In the incongruent stimulus video, group Neo-A_{ibo} looked equally to all three regions of the incongruent stimulus videos. Also, like group Neo-C, there were no discernable sex differences within group Neo-A_{ibo} (F(2,8) = 0.629, p = 0.557).

As in the Identity Same trials, group Neo-A_{ibo} appeared to look more to the mouth region of the congruent stimulus video than the mouth region of the incongruent stimulus video, but this difference (not observed in group Neo-C) was not statistically significant (F(1,4) = 4.669, p = 0.097). The similarities in the basic scanning strategies of groups Neo-A_{ibo} and Neo-C were validated by the lack of a statistically significant Stimulus Video x ROI x Group interaction (F(2,8) = 0.355, p = 0.706).

Summary: Neonatal lesions of the amygdala did not impair integration ability irrespective of the relative valence of the vocalization. Differences in preference direction across Valence Same and Valence Different trials suggest that animals of group Neo-A_{ibo} distinguished the two trial types; yet, the scanning strategies of group Neo-A_{ibo} were largely consistent across trial types (Fig 12 and Fig 13, bottom panels). However, two important distinctions between groups Neo-A_{ibo} and Neo-C were observed in the looking to the mouth regions.

First, only Neo-A_{ibo} animals appeared to use the mouth region to discriminate the stimulus videos in Valence Different Trials. Secondly, whereas males and females of group Neo-C exhibited differential scanning strategies in Valence Same trials, there were no discernable sex differences in scanning strategies of group Neo-A_{ibo}. Hence, Neo-A_{ibo} males looked less to the mouth region of the congruent stimulus video than Neo-C males (Fig 11 and Fig 12, right panels), and Neo-A_{ibo} females looked more to the mouth region than Neo-C females (Fig 11 and Fig 13, right panels). A potential limitation of the post-hoc analyses is that all the stimulus-pairs in Valence Same trials also met the criteria of Identity Different trials (i.e., every pair of vocalizations used in Valence Same trials is comprised of videos of different stimulus animals). Conversely, not all Identity Different trials are Valence Same trials (i.e., Identity Different trials are comprised of both Valence Same and Valence Different trials). This was a factor of the limited stimulus set. Therefore, the sex differences in scanning patterns of Identity Different and Valence Same trials exhibited by group Neo-C but not group Neo-A$_{\text{ibo}}$ are likely driven by a similar process.

Finally, none of the measures for group Neo-A$_{\text{ibo}}$ correlated significantly with the extent of amygdala damage.

Discussion

We examined the effects of neonatal amygdala lesions on the scanning strategies of rhesus macaques as they process species-specific, bimodal social signals. The results indicate that, although early damage to the amygdala did not impair integration ability, such damage did affect the passive viewing of these complex social cues emitted by novel conspecific males. In particular, the putative species-typical male response to monitor identity-related features of novel male conspecifics, such as dominance, appears to be absent in males with neonatal lesions of the amygdala. Similarly, the suggested species-typical female response to preferentially attend to the valence of the vocalization emitted by novel male conspecifics was likewise absent in females with neonatal lesions of the amygdala. These results support the proposal that damage to the amygdala affects the ability to recognize or spontaneously attend to the species-specific salient aspects of complex social signals (Adolphs, 2010).

Retained Integration Ability

Neonatal lesions of the amygdala did not impair integration ability, as evidenced by animals expressing an overall preference for one of the videos across all analyses. Our results parallel previous lesion studies in adult humans and rhesus macaques using crossmodal associations of non-social stimuli (Goulet & Murray, 2001; Lee et al., 1988; F. K. Nahm et al., 1993), and suggest that having an intact amygdala throughout maturation is not required for the development of an ability to integrate crossmodal social signals. The lack of impaired crossmodal integration of social signals after neonatal amygdala lesions seems to be at odds with the findings with human neuroimaging studies that have identified the amygdala as a neural correlate of emotional crossmodal integration (Dolan et al., 2001). One explanation for this difference may relate to the timing of the lesion.

Like humans, the rhesus macaque brain undergoes protracted postnatal development and is not fully mature until 3-4 years of age (Knickmeyer et al., 2010; Malkova, Heuer, & Saunders, 2006; Payne et al., 2010). Thus, the early timing of the lesions in our study (approximately 2 weeks of age) allows for the possibility of functional compensation by other brain regions. However, this putative compensation of amygdala functions seems unlikely given that these same monkeys with neonatal amygdala lesions have long-lasting impairments in emotional, social and cognitive domains, as well as aberrant stress-related hormonal responses (Kazama et al., 2008; Raper et al., 2009; Raper et al., 2011). Although additional studies are required to determine whether damage to the amygdala in adulthood would impair crossmodal integration of socio-emotional signals, the present findings suggest that, in the absence of a functional amygdala, integration ability may be supported by other brain structures. Neuroimaging and electrophysiolgical studies have begun to characterize a network of neural regions involved in emotional crossmodal integration, including the amygdala, middle and superior temporal regions, fusiform gyrus, and orbital frontal cortex (reviewed by Campanella & Belin, 2007; de Gelder et al., 2004).

The present findings may provide insight into the neural substrate associated with deficits in emotional crossmodal integration characteristic of several human disorders, such as autism spectrum disorder (Hobson et al., 1988; Loveland, 2005; Loveland et al., 2005; Loveland et al., 1995), pervasive developmental disorder (Magnee et al., 2007, 2008); and schizophrenia (de Gelder et al., 2005; de Jong et al., 2010; de Jong et al., 2009). Our results suggest that perturbed integration ability in these neurodevelopmental disorders may not be associated with isolated dysfunction of the amygdala, and may reflect dysfunction at other loci within the neural network subserving crossmodal integration of socio-emotional signals, such as the middle and superior temporal regions, the fusiform gyrus, and/or orbital frontal cortex. Thus, the array of neural structures implicated in autism spectrum disorder, pervasive developmental disorder, and schizophrenia indicate that widespread dysfunction of the neural network supporting emotional crossmodal integration may have precluded functional compensation and resulted in integration impairments.

Disruption of scanning strategies

Neonatal lesions of the amygdala appear to disrupt the normal preference for the eye region exhibited by the sham-operated controls (Payne & Bachevalier, in preparation). Whereas sham-operated monkeys predominantly relied upon information from the eye regions to discriminate the congruent and incongruent stimulus videos, monkeys with amygdala lesions differentiated the mouth regions of the videos (see Figs 12 and 13, bottom panels). Although this pattern demonstrates that animals with neonatal amygdala lesions found the dynamic movements of the mouth regions to be more informative in their valuations of the stimulus videos, lesions of the amygdala did not produce the same patterns of looking in humans with either damage to the amygdala (Adolphs et al., 2005) or autism spectrum disorder (Corden, Chilvers, & Skuse, 2008; Dalton et al., 2005; Neumann, Spezio, Piven, & Adolphs, 2006; Pelphrey et al., 2002; Spezio et al., 2007), i.e., decreased looking to eyes and increased looking to mouths. Distinctions in methodology, in addition to differences in species-typical responses, likely contribute to this subtle divergence.

The previous studies in humans employed emotion identification paradigms with either static, unimodal images presented one-at-a-time (Adolphs et al., 2005; Dalton et al., 2005; Neumann et al., 2006; Spezio et al., 2007) or videos of complex social interactions (Jones et al., 2008; Klin et al., 2002). None of the designs had the same dynamics of the passive preferential viewing paradigm used here, which included direct comparisons between two simultaneously emoting conspecifics. Moreover, we have suggested that looking behaviors of male and female rhesus macaques in response to this preferential viewing paradigm may be subject to different societal pressures that manifest as distinctive relative looking to the eyes and mouth regions (Payne & Bachevalier, in preparation). Hence, a disruption of scanning strategies may look different in male and female rhesus macaques than it does in men and women (see discussion below).

Interestingly, one Neo-A $_{ibo}$ female did exhibit greater looking to the mouth than eye regions, a pattern of looking commonly reported in clinical investigations (Adolphs et al., 2005; Neumann et al., 2006). This female (case Neo-A_{ibo}-1) consistently spent more time looking to the mouth region than the eye or rest of the stimulus videos. This preference of the mouth region was consistent across all trial types and was not associated with which stimulus video the animal looked more towards. Although there was no overall correlation between lesion extent and looking to the mouth region ($r = -0.663$, $p = 0.152$), this pattern may be reflective of the larger extent of lesion (74.7%, see Table 1) in this animal compared to the other females (47.1% and 53.9%). The observation that the male with comparable damage to the amygdala (76.0%; case Neo-A_{ibo}-4) does not show the same pattern further supports the contribution of the purported sex differences in species-typical scanning strategies.

Disruption of sex-specific responses

Neonatal lesions of the amygdala appear to perturb the sex differences displayed by the sham-operated controls (Payne & Bachevalier, in preparation). Those sex differences were purportedly reflective of males and females attending to different properties of the stimulus vocalizations. In sham-operated females, the preferential looking to the eye region over the mouth (Fig 5) paralleled the looking strategies humans employ when instructed to attend to emotion-related cues or make social judgments of human facial expressions (Buchan et al., 2008; Lansing & McConkie, 1999), indicating that species-typical response for female rhesus macaques was to attend to the relative valence of the stimulus videos. This interpretation was further supported through post-hoc analyses of the effect of relative valence on looking behavior. In contrast, sham-operated males looked more to the mouths, without changing their scanning of the eye regions (Fig 5). Moreover, post-hoc analyses revealed that sham-operated males preferentially scanned the eye regions only when one novel conspecific produced both vocalizations, and not when different stimulus animals emitted the vocalizations. This pattern signified that, in addition to attending to the valence of the vocalizations, the species-typical response for male rhesus macaques was to also attend to identity-specific features that may signify the dominance status of the novel conspecific, such as canine size.

When all trials were considered, monkeys with neonatal amygdala lesions displayed the opposite pattern in their relative looking to the eye and mouth regions. Males preferentially attended to the eyes over the mouth regions, and females looked equally to the eye and mouth regions (Fig 5). However, sex differences were not seen in the post-hoc analyses. Interestingly, the monkeys with neonatal amygdala lesions showed the same differences in preference direction across trial types as the sham-operated animals did. Nonetheless, unlike sham-operated animals, the differences in preference direction seen following damage to the amygdala were not associated with differences in their scanning strategies across trial parameters. Together, these results indicate that, although monkeys with neonatal amygdala lesions detected the differences in trial parameters, they did not recognize the social relevance of the distinctions.

Interestingly, these animals with neonatal amygdala lesions have exhibited deficits in assessing the social relevance of stimuli in other experimental paradigms. In a social approach/avoidance paradigm, animals were presented with the opportunity to retrieve a desirable food item that was placed in front of either a socially neutral object (e.g., clear plastic bottle) or a socially fearful object (e.g. doll with large eyes) (Raper et al., 2009). Compared to sham-operated controls, animals with damage to the amygdala were faster to retrieve the food item placed in front of a fearful object, indicating that, unlike control animals, they did not recognize the objects as being socially threatening.

Our findings are consistent with a role of the amygdala in the ability to recognize the social salience of conspecific cues (Adolphs, 2010). Deficits in social relevance assessment have also been implicated in the aberrant scanning strategies observed in individuals with neurodevelopmental disorders such as autism spectrum disorder (reviewed by Falck-Ytter & von Hofsten, 2011). Essentially, people with autism do not spontaneously look at socially salient features, such as the eyes, because they do not understand the need to do so. The current results further suggest that dysfunction within the amygdala of persons afflicted with these disorders underlies their deficits in recognizing the social relevance of socio-emotional cues.

Additional influences on looking behavior

In addition to a decreased sensitivity to the relevance of monitoring features associated with the dominance status of the stimulus animals, neonatal lesions of the amygdala may also affect the putative contributions of their own dominance rank. In our previous characterization of the sham-operated controls, we noted patterns within the individual variability that suggested the observers' dominance rank impacted their scanning strategies, with higher ranking males appearing to be more attuned to the dominance status of the stimulus animals than lower ranking males. Although there was also considerable variability in the looking behavior of males with neonatal lesions of the amygdala, the variability was not consistent within individuals and could not be linked to any behavioral characteristic of the individual animals, such as dominance status. The apparent minimization of the relevance of self-dominance status by males with neonatal amygdala lesions is consistent with the role of the amygdala in the assessment of the social relevance of non-facial cues, such as dominance status.

The only observable pattern within the variability observed in the looking behavior of females with neonatal lesions of the amygdala was the one female that consistently looked more to the mouth region (described above). This highly consistent pattern was not linked to any behavioral characteristic of the animal. That is, the greater looking to the mouth region did not vary across trial types and was not related to the animals' preferred stimulus video. Although it seems likely that this females' global preference for the mouth region was a product of the surgical manipulation, we did not assess hormonal levels throughout testing, and therefore cannot exclude the possibility that hormonal fluctuations contributed to the variability in the looking behavior of any of the females (Jones et al., 2008; Lacreuse & Herndon, 2003; Lacreuse et al., 2007; Wallen & Rupp, 2010; Wallen

& Tannenbaum, 1997). The effect of neonatal amygdala lesion on hormonal levels has yet to be systematically assessed. Thus, we cannot rule out the possibility that differences in looking strategies between males and females across groups is associated with differential effects of neonatal amygdala lesions on hormonal systems.

Conclusions

Human and nonhuman primates live in complex social environments, in which faces and voices are the primary mode for transmission of social signals. Therefore, the capacity to recognize and appropriately respond to the social cues within one's community is heavily reliant upon the ability to process audiovisual information. The present investigation does not indicate that the amygdala is necessary for the integration of the auditory and visual components of complex social signals, but does support a critical role of the amygdala in the recognition of the social relevance of the features within those complex social signals.

Acknowledgements

This work has been supported by NICHD 35471; NIMH 58846; Yerkes Base Grant NIH RR00165; Center for Behavioral Neuroscience grant NSF IBN-9876754; The Robert W. Woodruff Health Sciences Center Fund, Inc., Emory University; NIMH T32-MH0732505; Autism Speaks Mentor-Based Predoctoral Fellowship Grant: 1657. We thank Asif Ghazanfar, Princeton University Neuroscience Institute Departments of Psychology and Ecology & Evolutionary

Biology, for generously allowing us to use his stimuli, and Nancy Bliwise, Emory University Psychology Department, for her guidance on data analysis. We also thank Lisa Parr and Harold Gouzoules for their contributions to the experimental design.

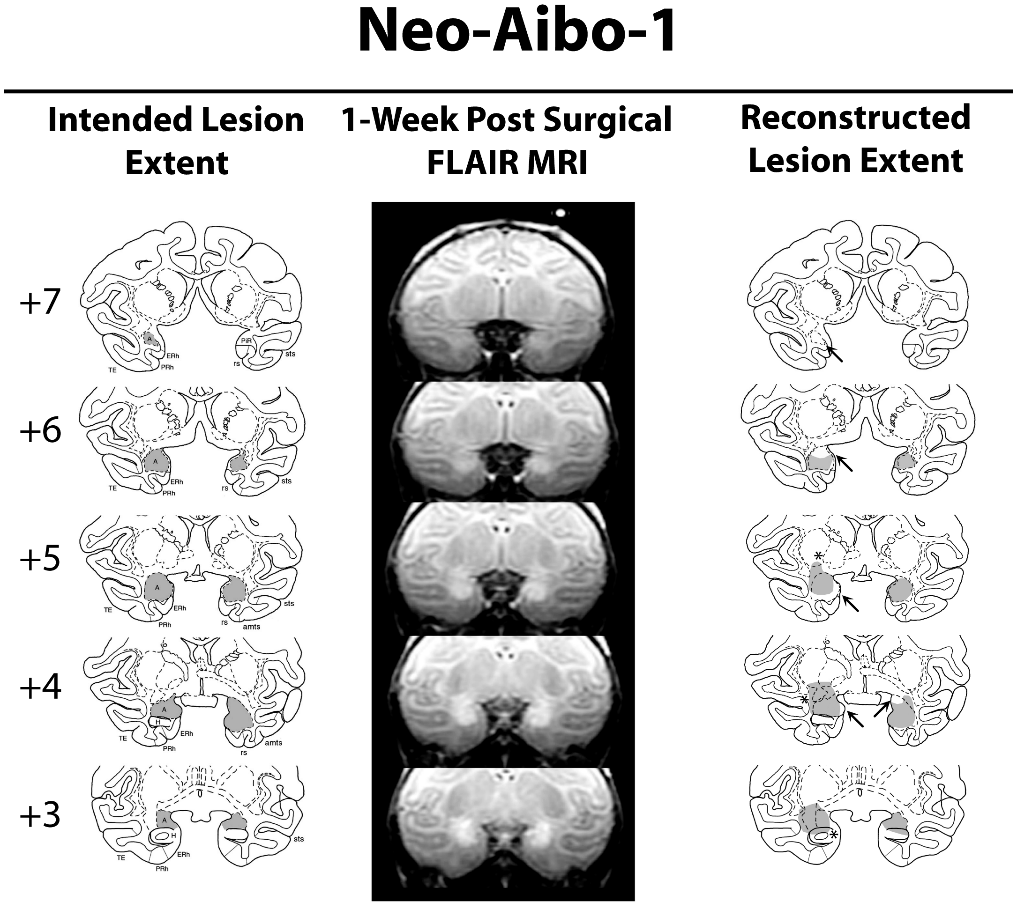
Tables & Figures.

MS-II Table 1: Extent of Lesion Assessment.

Intended ibotenic acid damage to the amygdala (Neo-A_{ibo}) and unintended damage to adjacent areas. Mean: average damage per group; L%: percent damage in the left hemisphere; R%: percent damage in the right hemisphere; X%: average damage in both hemispheres; W%: weighted average damage to both hemispheres (W% = (L% * R%) / 100, Hodos and Bobko, 1984); ERh: entorhinal cortex; PRh: perirhinal cortex; TE and TG: temporal cortical areas as defined by von Bonin and Bailey (1947).

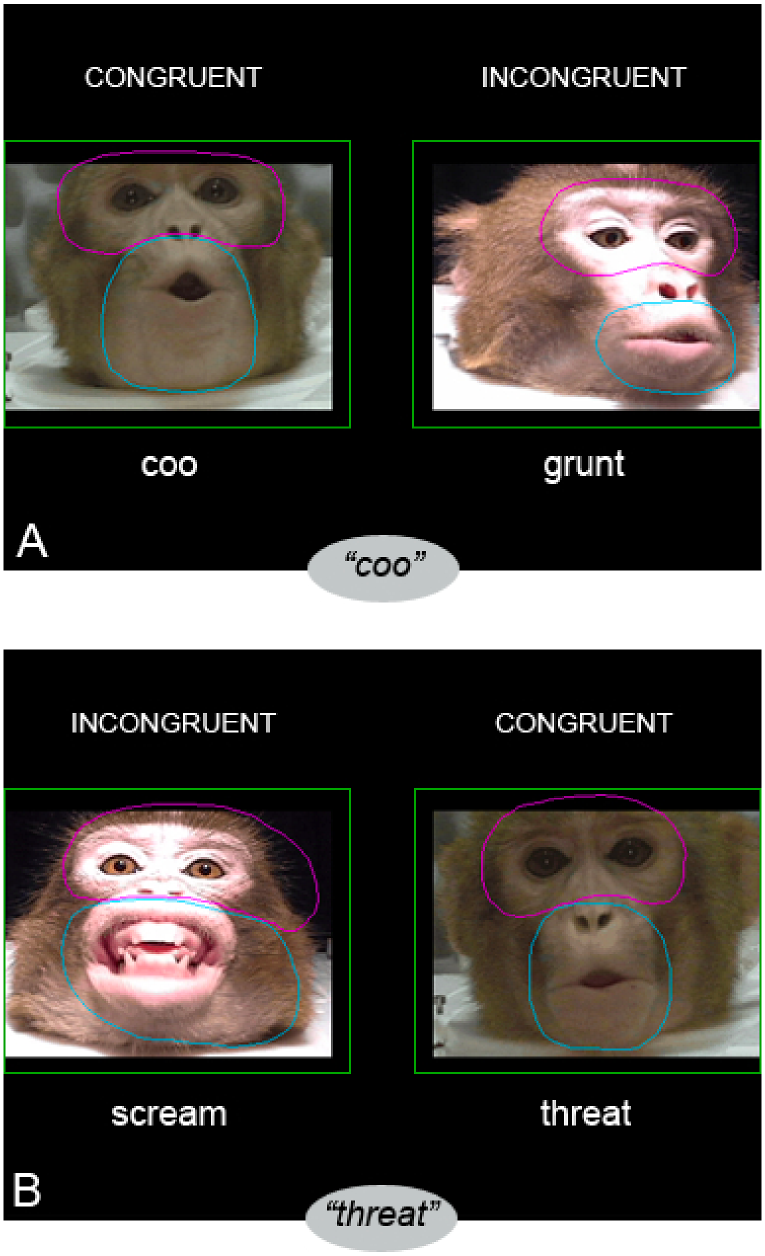
Cases	**Amygdala**				**Hippocampus**			
	L	R	X%	W	L	R	X%	W
Neo-Aibo-1	89.0	59.8	74.4	53.2	5.1	3.1	4.1	0.2
Neo-Aibo-2	42.0	77.6	59.8	32.6	0.0	0.8	0.4	0.0
Neo-Aibo-3	33.0	61.1	47.1	20.2	0.0	0.0	0.0	0.0
Neo-Aibo-4	62.1	90.0	76.0	55.9	1.9	3.0	2.4	0.1
Neo-Aibo-5	41.2	66.6	53.9	27.5	0.0	0.0	0.0	0.0
Neo-Aibo-6	52.1	75.6	63.8	39.3	5.6	10.3	8.0	0.6
Mean	**53.2**	**71.8**	**62.5**	**38.1**	**2.1**	**2.9**	**2.5**	**0.1**
Cases	**Erh**				**PRh**			
	L	R	X%	W	L	R	X%	W
Neo-Aibo-1	0.1	4.7	2.4	0.0	2.0	10.1	6.0	0.2
Neo-Aibo-2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neo-Aibo-3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neo-Aibo-4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neo-Aibo-5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neo-Aibo-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	**0.0**	**0.8**	**0.4**	**0.0**	**0.3**	**1.7**	**1.0**	**0.0**
Cases	**TE**				**TG**			
	L	R	X%	W	L	R	X%	W
Neo-Aibo-1	0.0	1.8	0.9	0.0	6.5	35.0	20.7	2.3
Neo-Aibo-2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neo-Aibo-3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neo-Aibo-4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neo-Aibo-5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neo-Aibo-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	**0.0**	**0.3**	**0.2**	**0.0**	**1.1**	**5.8**	**3.5**	**0.4**

MS-II Figure 1. Intended lesion and representative case for Neo-A_{ibo}. Intended damage is shown in gray on coronal sections through the amygdala of an infant macaque brain atlas (left column). FLAIR MR images (middle column) reveal hypersignals caused by cell death (white areas within and around the amygdala). The estimated lesion extent is reconstructed on matched coronal sections of an infant brain (right column). Arrows point to areas of unintended damage or sparing. Abbreviations: ls – lateral sulcus; sts – superior temporal sulcus; ots – occipital temporal sulcus; ERh – entorhinal cortex; PRh – perirhinal cotex; TE, temporal cortical area and TH/TF – cytoarchitectonic fields of the parahippocampal gyrus as defined by von Bonin and Bailey (1947). Reproduced with permission (Kazama & Bachevalier, in preparation).



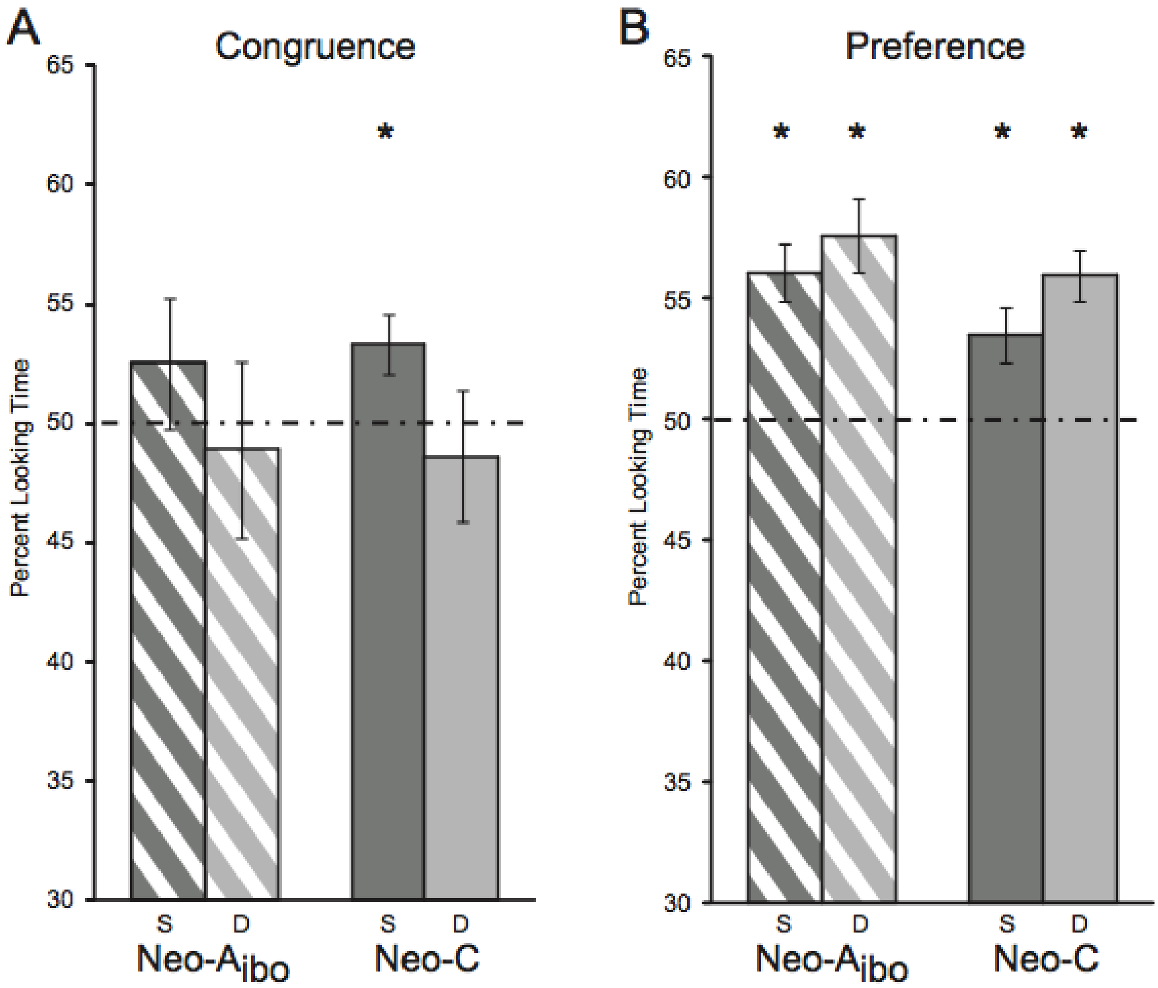
MS-II Figure 2: Schematic of Stimulus Presentation with ROIs.

Screen shots of (A) coo-grunt and (B) scream-threat pairings with ROIs. In (A), the vocalization was a “coo” and in (B), the vocalization was a “threat”. Stimulus sets were comprised of all possible combinations. Reproduced with permission (Payne & Bachevalier, in preparation).



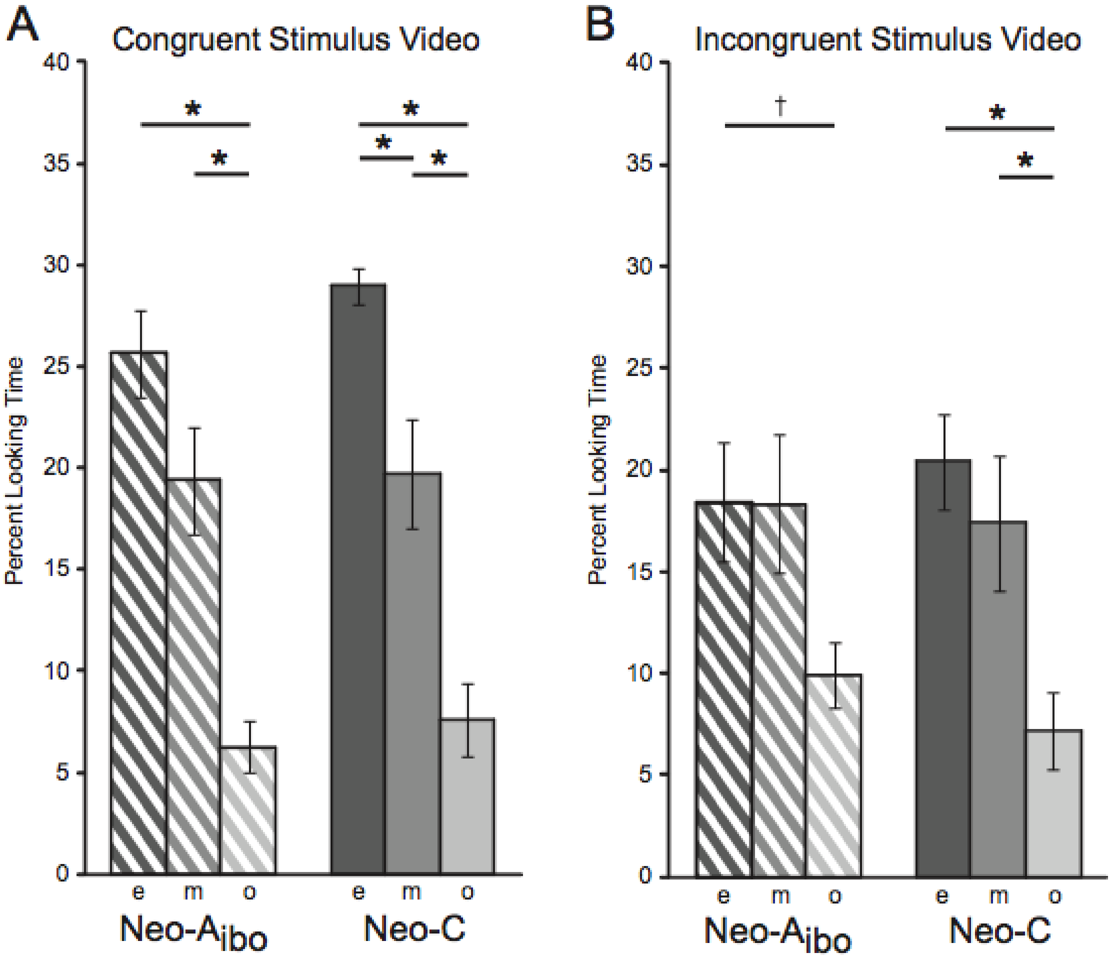
MS-II Figure 3: Integration Assessment – All Trials.

Percentages of looking time (± sem) to (A) the congruent stimulus video and (B) the 'preferred' stimulus video in the Synchronized (S) and Desynchronized (D) conditions across all trials for group Neo-A$_{ibo}$ (striped bars) and group Neo-C (solid bars). The dashed line represents chance level of 50%. In panel (A), values greater than 50% represent greater looking to the congruent video and values less than 50% represent greater looking to the incongruent video. (*) $p < 0.05$.



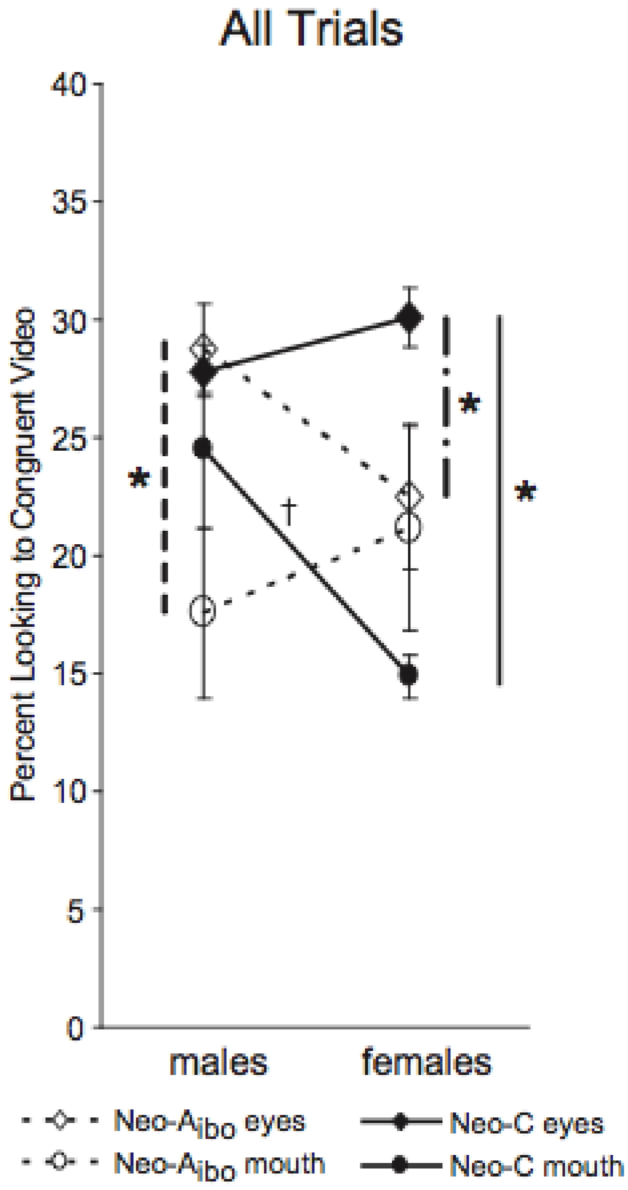
MS-II Figure 4: Scanning Patterns – All Trials.

Percentages of looking time (± sem) to the eyes (e), mouth (m), and other (o) of (A) the congruent stimulus video, and (B) the incongruent stimulus video for groups Neo-A$_{ibo}$ (striped bars) and Neo-C (solid bars). (*) $p \leq 0.05$; (†) $p \leq 0.08$.



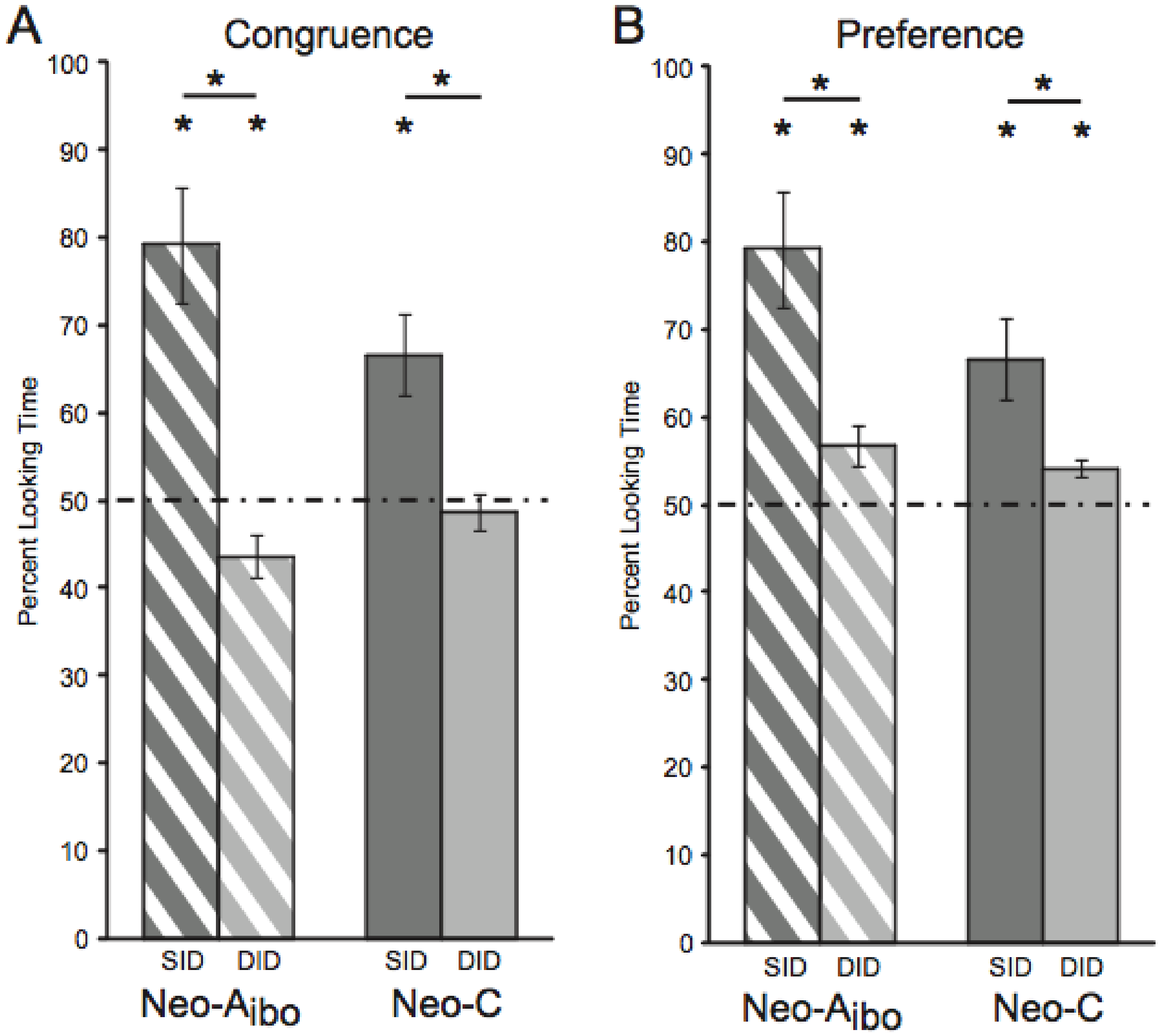
MS-II Figure 5: Sex Differences – All Trials.

Percentages of looking time (± sem) to the eyes (diamonds) and mouth (circles) of the congruent stimulus video for males and females of groups Neo-A_{ibo} (dashed lines, open symbols) and Neo-C (solid lines, filled symbols). (*) $p \leq 0.05$; (†) $p \leq 0.08$.



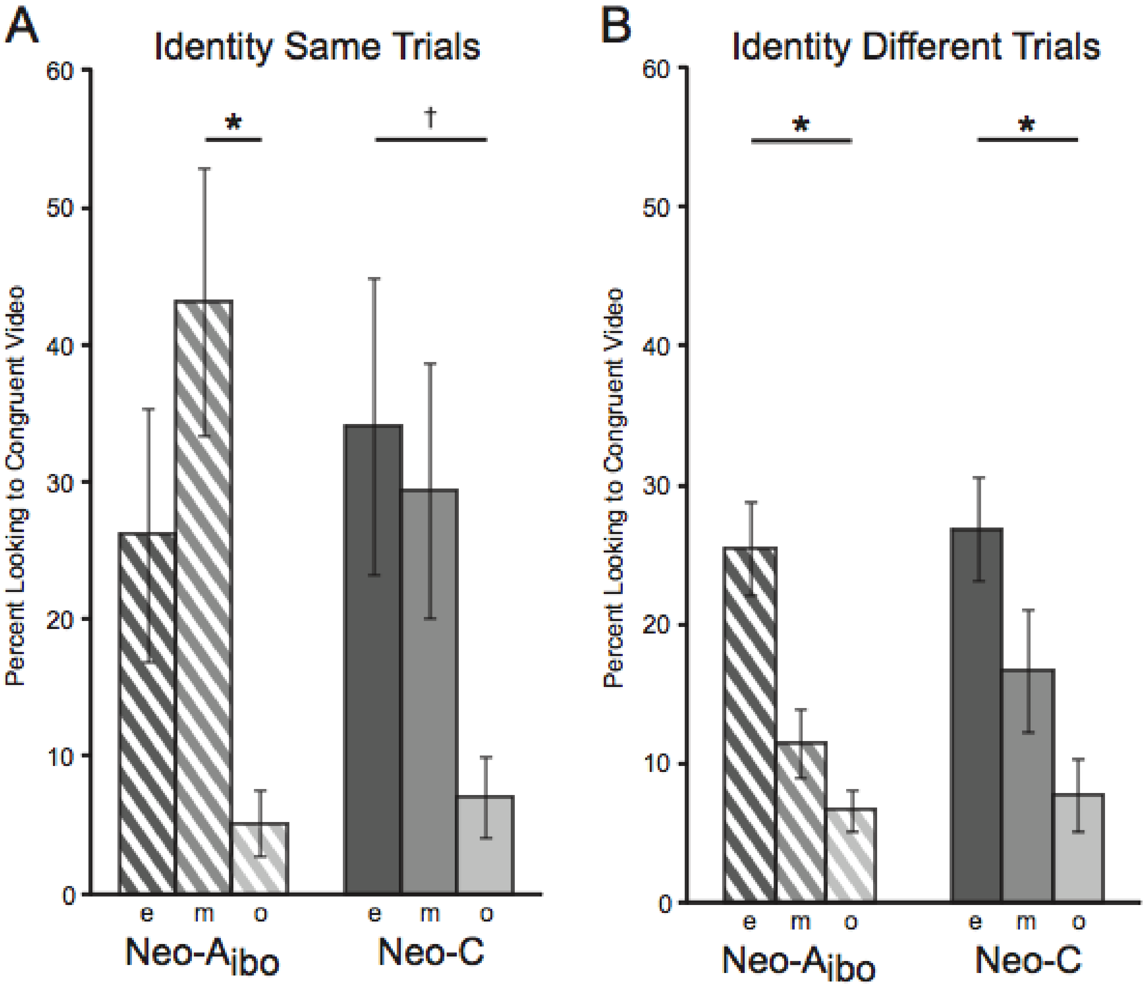
MS-II Figure 6: Integration Assessment – Relative Identity.

Percentages of looking time (± sem) to (A) the congruent stimulus video and (B) the 'preferred' stimulus video in the Synchronized condition across Identity Same (SID) and Identity Different (DID) trials for group Neo-A$_{ibo}$ (striped bars) and group Neo-C (solid bars). The dashed line represents chance level of 50%. In panel (A), values greater than 50% represent greater looking to the congruent video and values less than 50% represent greater looking to the incongruent video. (*) *p* < 0.05.



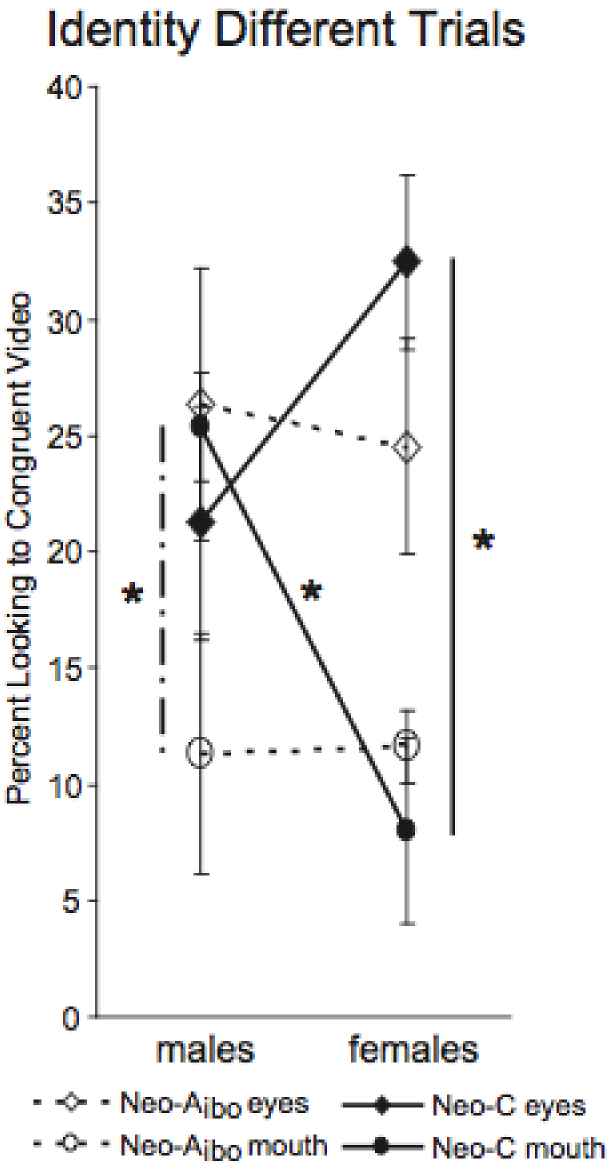
MS-II Figure 7: Scanning Patterns – Identity Trials.

Percentages of looking time (± sem) to the eyes (e), mouth (m), and other (o) of the congruent stimulus video in (A) Identity Same trials and (B) Identity Different trials for groups Neo-A_{ibo} (striped bars) and Neo-C (solid bars). (*) *p* ≤ 0.05; (†) *p* ≤ 0.08.



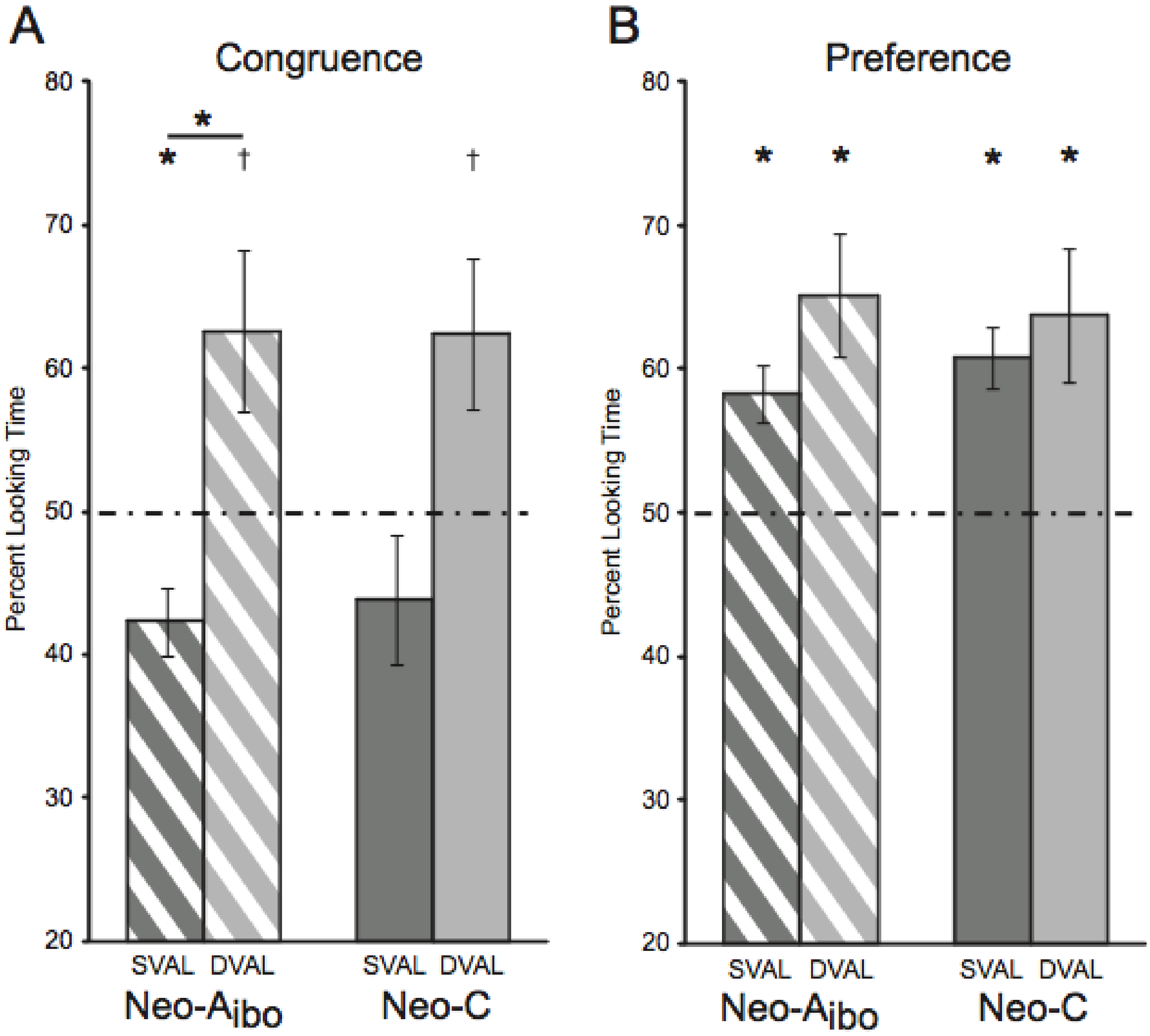
MS-II Figure 8: Sex Differences – Identity Different Trials.

Percentages of looking time (± sem) to the eyes (diamonds) and mouth (circles) of the congruent stimulus video in Identity Different trials for males and females of groups Neo-A$_{ibo}$ (dashed lines, open symbols) and Neo-C (solid lines, filled symbols). (*) $p \leq 0.05$; (†) $p \leq 0.08$.



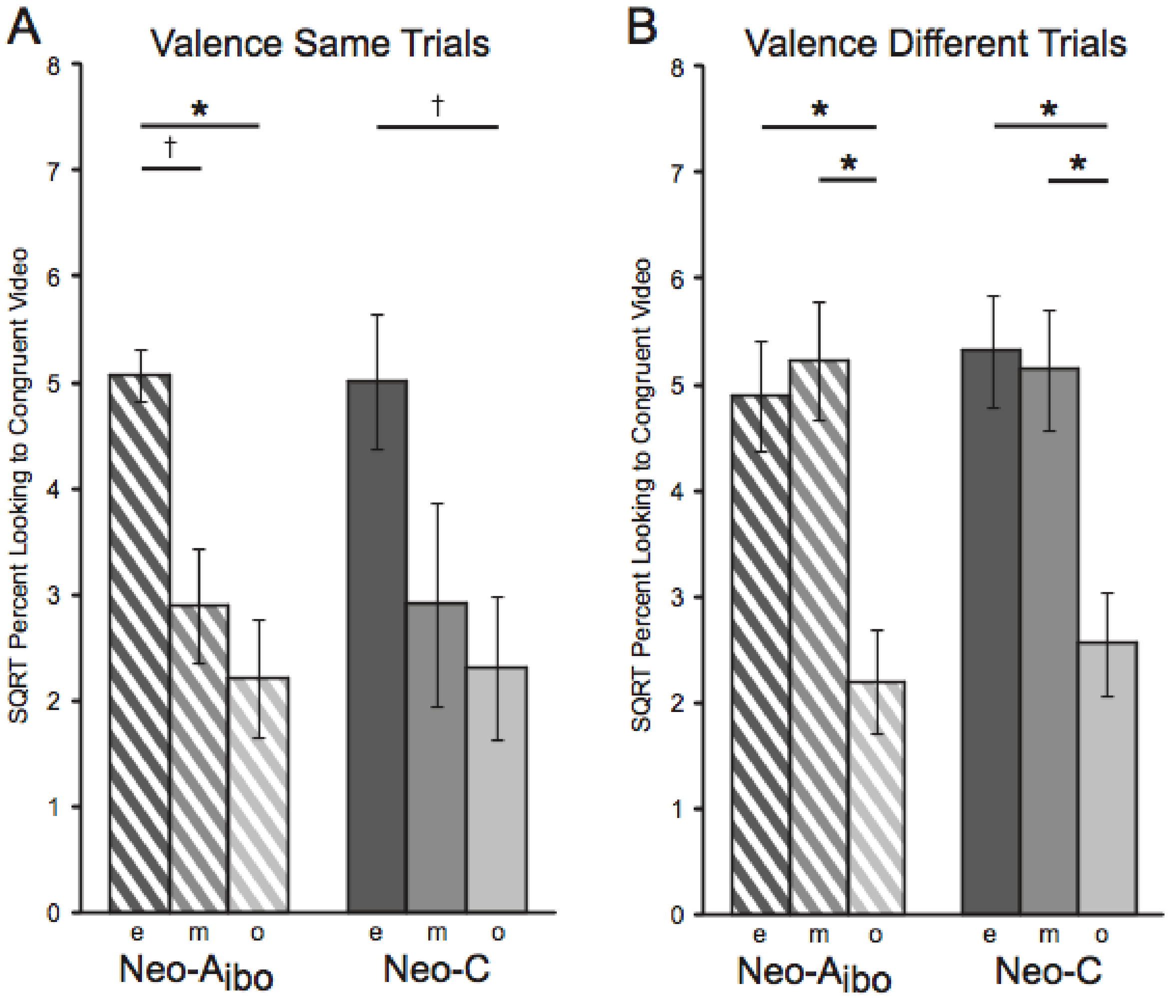
MS-II Figure 9: Integration Assessment – Relative Valence.

Percentages of looking time (± sem) to (A) the congruent stimulus video and (B) the 'preferred' stimulus video in the Synchronized condition across Valence Same (SVAL) and Valence Different (DVAL) trials for group Neo-A$_{\text{ibo}}$ (striped bars) and group Neo-C (solid bars). The dashed line represents chance level of 50%. In panel (A), values greater than 50% represent greater looking to the congruent video and values less than 50% represent greater looking to the incongruent video. (*) $p < 0.05$.



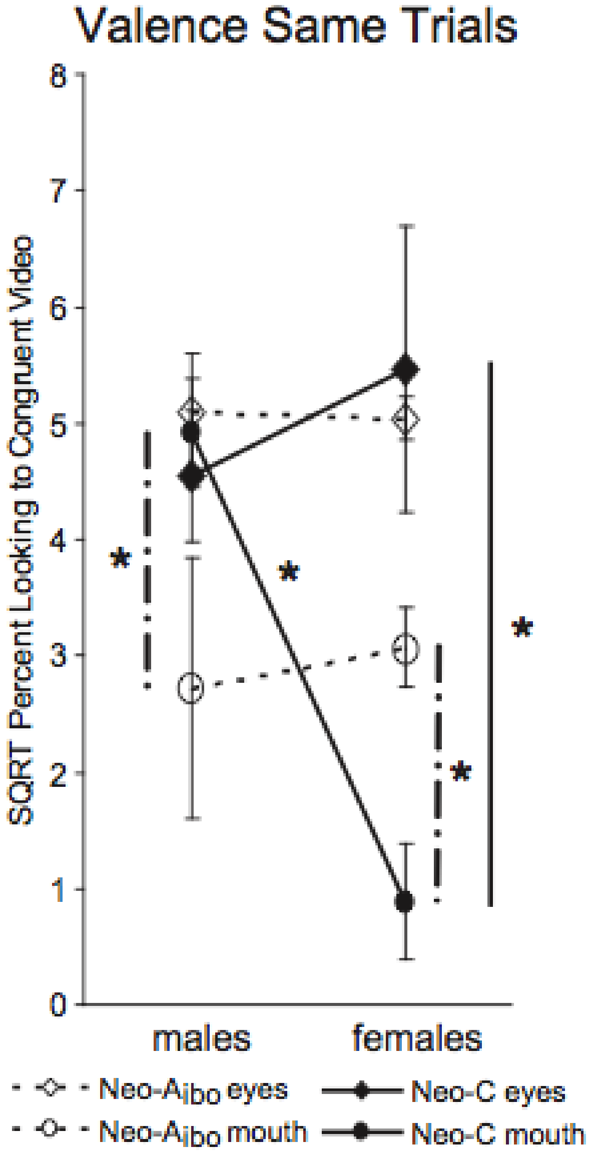
MS-II Figure 10: Scanning Pattern – Valence Trials.

Percentages of looking time (± sem) to the eyes (e), mouth (m), and other (o) of the congruent stimulus video in (A) Valence Same trials and (B) Valence Different trials for groups Neo-A$_{ibo}$ (striped bars) and Neo-C (solid bars). Analyses completed on square-root-transformed data to correct for inequality of error variances between variables. (*) $p \leq 0.05$; (†) $p \leq 0.08$.



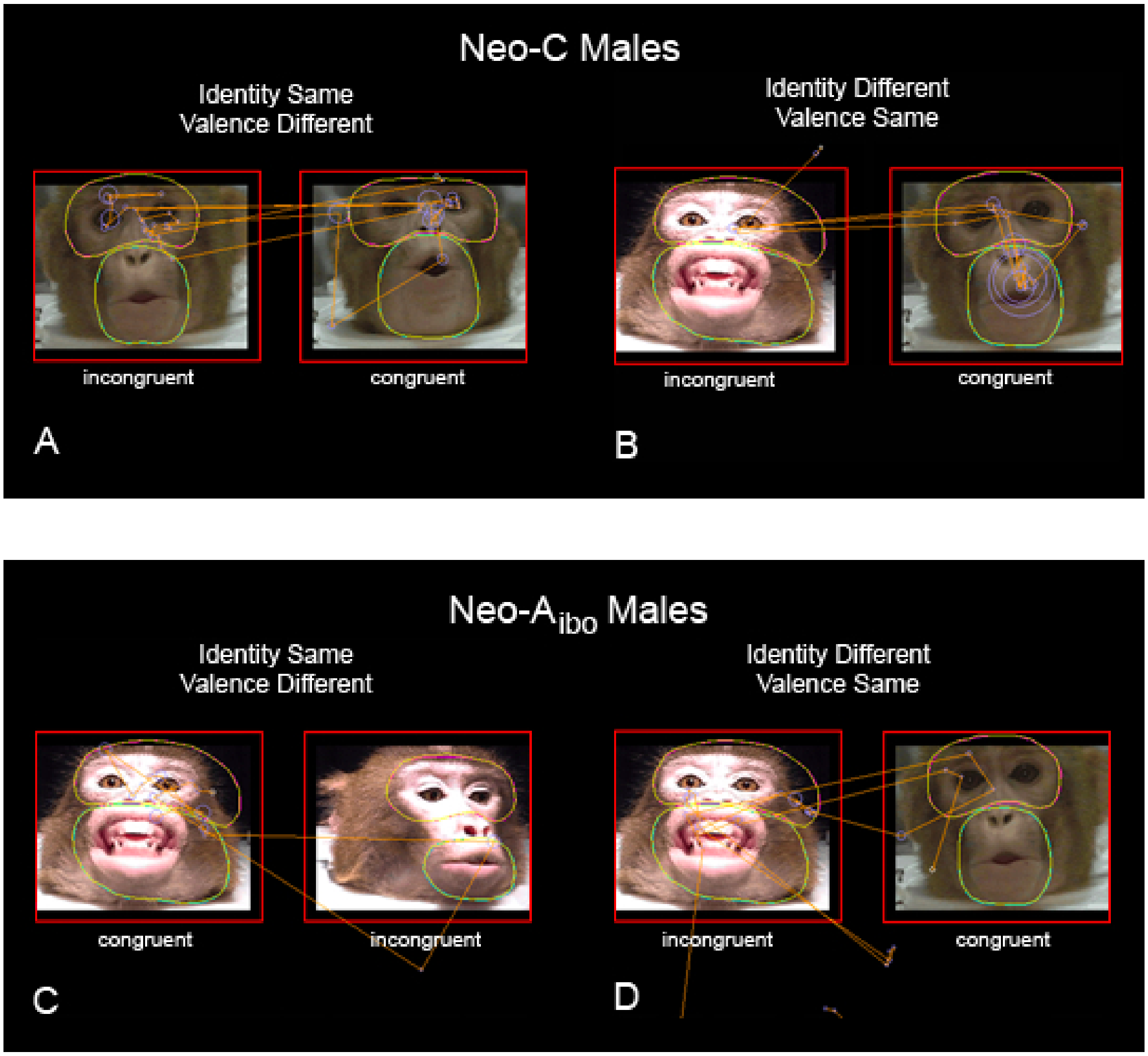
MS-II Figure 11: Sex Differences – Valence Same Trials.

Percentages of looking time (± sem) to the eyes (diamonds) and mouth (circles) of the congruent stimulus video in Valence Same trials for males and females of groups Neo-A$_{ibo}$ (dashed lines, open symbols) and Neo-C (solid lines, filled symbols). Analyses completed on square-root-transformed data to correct for inequality of error variances between variables. (*) $p \leq 0.05$.



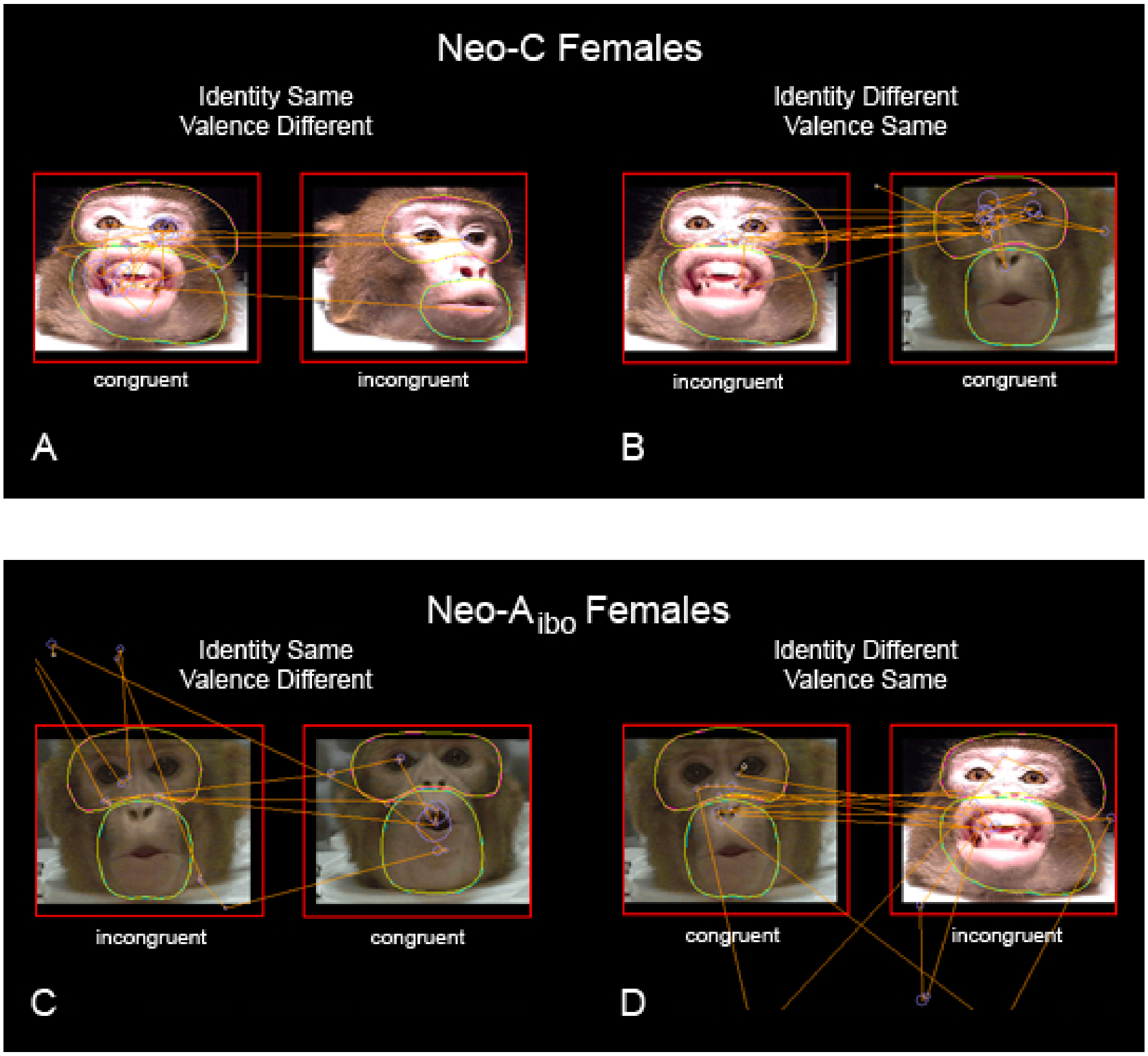
MS-II Figure 12: Representative Scan Paths – Males.

Neo-C male scan path on an Identity Same / Valence Different Trial (A) and on an Identity Different / Valence Same Trial (B). Neo-A$_{ibo}$ male scan path on an Identity Same / Valence Different Trial (C) and on an Identity Different / Valence Same Trial (D). Circles represent fixation points with the size corresponding with fixation duration. Note: Panels (A) and (B) were reproduced with permission (from Fig 11 in Payne & Bachevalier, in preparation).



MS-II Figure 13: Representative Scan Paths – Females.

Neo-C female scan path on an Identity Same / Valence Different Trial (A) and on an Identity Different / Valence Same Trial (B). Neo-A$_{\text{ibo}}$ female scan path on an Identity Same / Valence Different Trial (C) and on an Identity Different / Valence Same Trial (D). Circles represent fixation points with the size corresponding with fixation duration. Note: Panels (A) and (B) were reproduced with permission (from Fig 11 in Payne & Bachevalier, in preparation).



References

Adolphs, R. (1999). Social cognition and the human brain. *Trends Cogn Sci., 3*(12), 469-479.

Adolphs, R. (2003). Is the human amygdala specialized for processing social information? *Ann.N.Y.Acad.Sci., 985*, 326-340.

Adolphs, R. (2010). What does the amygdala contribute to social cognition? *Ann N Y Acad Sci, 1191*, 42-61.

Adolphs, R., Gosselin, F., Buchanan, T. W., Tranel, D., Schyns, P., & Damasio, A. R. (2005). A mechanism for impaired fear recognition after amygdala damage. *Nature, 433*, 68-72.

Adolphs, R., & Spezio, M. (2006). Role of the amygdala in processing visual social stimuli. *Prog Brain Res, 156*, 363-378.

Amaral, D. G., Price, J. L., Pitkanen, A., Carmichael, S. T., & Aggleton, J. P. (1992). Anatomical organization of the primate amygdaloid complex. In *The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction* (pp. 1-66). New York: Wiley-Liss, Inc.

Bachevalier, J., & Loveland, K. A. (2006). The orbitofrontal-amygdala circuit and self-regulation of social-emotional behavior in autism. *Neurosci.Biobehav.Rev., 30*(1), 97-117.

Bachevalier, J., Machado, C. J., & Kazama, A. M. (2011). *Behavioral outcomes of late-onset or early-onset orbital frontal cortex (areas 11/13) lesions in rhesus monkeys*. Paper presented at the New York Acadamy of Sciences.

Bachevalier, J., & Meunier, M. (2005). The neurobiology of social-emotional cognition in nonhuman primates. In A. Easton & N. J. Emery (Eds.), *The*

Cognitive Neuroscience of Social Behavior (pp. 19-57). East Sussex: Psychology Press.

Baxter, M. G., Parker, A., Lindner, C. C., Izquierdo, A. D., & Murray, E. A. (2000). Control of response selection by reinforcer value requires interaction of amygdala and orbital prefrontal cortex. *Journal of Neuroscience, 20*, 4311-4319.

Bechara, A., Damasio, H., & Damasio, A. R. (2003). Role of the amygdala in decision-making. *Ann N Y Acad Sci, 985*, 356-369.

Blundell, P., Hall, G., & Killcross, S. (2001). Lesions of the basolateral amygdala disrupt selective aspects of reinforcer representation in rats. *J Neurosci, 21*(22), 9018-9026.

Buchan, J. N., Pare, M., & Munhall, K. G. (2008). The effect of varying talker identity and listening conditions on gaze behavior during audiovisual speech perception. *Brain Res, 1242*, 162-171.

Campanella, S., & Belin, P. (2007). Integrating face and voice in person perception. *Trends Cogn Sci, 11*(12), 535-543.

Corden, B., Chilvers, R., & Skuse, D. (2008). Avoidance of emotionally arousing stimuli predicts social-perceptual impairment in Asperger's syndrome. *Neuropsychologia, 46*(1), 137-147.

Dalton, K. M., Nacewicz, B. M., Johnstone, T., Schaefer, H. S., Gernsbacher, M. A., Goldsmith, H. H., et al. (2005). Gaze fixation and the neural circuitry of face processing in autism. *Nat.Neurosci., 8*, 519-526.

de Gelder, B., Vroomen, J., Annen, L., Masthof, E., & Hodiamont, P. (2003). Audio-visual integration in schizophrenia. *Schizophr Res, 59*(2-3), 211-218.

de Gelder, B., Vroomen, J., de Jong, S. J., Masthoff, E. D., Trompenaars, F. J., & Hodiamont, P. (2005). Multisensory integration of emotional faces and voices in schizophrenics. *Schizophr Res, 72*(2-3), 195-203.

de Gelder, B., Vroomen, J., Pourtois, G., Calvert, G. A., Spence, C., & Stein, B. E. (2004). Multisensory perception of emotion, its time course, and its neural basis. In *The handbook of multisensory processes* (pp. 581-596). New York: Bradford Books.

de Jong, J. J., Hodiamont, P. P., & de Gelder, B. (2010). Modality-specific attention and multisensory integration of emotions in schizophrenia: reduced regulatory effects. *Schizophr Res, 122*(1-3), 136-143.

de Jong, J. J., Hodiamont, P. P., Van den Stock, J., & de Gelder, B. (2009). Audiovisual emotion recognition in schizophrenia: reduced integration of facial and vocal affect. *Schizophr Res, 107*(2-3), 286-293.

Dixon, N. F., & Spitz, L. (1980). The detection of auditory visual desynchrony. *Perception, 9*(6), 719-721.

Dolan, R. J., Morris, J. S., & de Gelder, B. (2001). Crossmodal binding of fear in voice and face. *Proc.Natl.Acad.Sci.U.S.A, 98*(17), 10006-10010.

Ethofer, T., Anders, S., Erb, M., Droll, C., Royen, L., Saur, R., et al. (2006). Impact of voice on emotional judgment of faces: an event-related fMRI study. *Hum Brain Mapp, 27*(9), 707-714.

Falck-Ytter, T., & von Hofsten, C. (2011). How special is social looking in ASD: a review. *Prog Brain Res, 189*, 209-222.

Ghazanfar, A. A., & Logothetis, N. K. (2003). Neuroperception: facial expressions linked to monkey calls. *Nature, 423*(6943), 937-938.

Goulet, S., & Murray, E. A. (2001). Neural substrates of crossmodal association memory in monkeys: the amygdala versus the anterior rhinal cortex. *Behav Neurosci, 115*(2), 271-284.

Goursaud, A. P., & Bachevalier, J. (2007). Social attachment in juvenile monkeys with neonatal lesion of the hippocampus, amygdala and orbital frontal cortex. *Behav Brain Res, 176*(1), 75-93.

Gundersen, H. J., & Jensen, E. B. (1987). The efficiency of systematic sampling in stereology and its prediction. *Journal of Microscopy, 147*, 229-263.

Hobson, R. P., Ouston, J., & Lee, A. (1988). Emotion recognition in autism: coordinating faces and voices. *Psychol.Med., 18*(4), 911-923.

Jones, B. C., DeBruine, L. M., Perrett, D. I., Little, A. C., Feinberg, D. R., & Law Smith, M. J. (2008). Effects of menstrual cycle phase on face preferences. *Arch Sex Behav, 37*(1), 78-84.

Kazama, A., Glavis-Bloom, C., & Bachevalier, J. (2008). *Neonatal amygdala and orbital frontal cortex lesions disrupt flexible decision-making in adult macaques*. Paper presented at the Society for Neuroscience.

Kazama, A. M., & Bachevalier, J. (in preparation). *Normal maturation of object reversal learning after selective neonatal damage of amygdala and orbital frontal areas 11 & 13 in monkeys*.Unpublished manuscript.

Klin, A., Jones, W., Schultz, R., Volkmar, F., & Cohen, D. (2002). Visual fixation patterns during viewing of naturalistic social situations as predictors of social competence in individuals with autism. *Archives of General Psychiatry, 59*(9), 809-816.

Knickmeyer, R. C., Styner, M., Short, S. J., Lubach, G. R., Kang, C., Hamer, R., et al. (2010). Maturational trajectories of cortical brain development through the pubertal transition: unique species and sex differences in the monkey revealed through structural magnetic resonance imaging. *Cereb Cortex, 20*(5), 1053-1063.

Kuraoka, K., & Nakamura, K. (2007). Responses of single neurons in monkey amygdala to facial and vocal emotions. *J Neurophysiol, 97*(2), 1379-1387.

Lacreuse, A., & Herndon, J. G. (2003). Estradiol selectively affects processing of conspecifics' faces in female rhesus monkeys. *Psychoneuroendocrinology, 28*(7), 885-905.

Lacreuse, A., Martin-Malivel, J., Lange, H. S., & Herndon, J. G. (2007). Effects of the menstrual cycle on looking preferences for faces in female rhesus monkeys. *Anim Cogn, 10*(2), 105-115.

Lansing, C. R., & McConkie, G. W. (1999). Attention to facial regions in segmental and prosodic visual speech perception tasks. *J Speech Lang Hear Res, 42*(3), 526-539.

Lee, G. P., Meador, K. J., Smith, J. R., Loring, D. W., & Flanigin, H. F. (1988). Preserved crossmodal association following bilateral amygdalotomy in man. *Int J Neurosci, 40*(1-2), 47-55.

Loveland, K. (2005). Social-emotional impairment and self-regulation in Autism spectrum disorders. In J. Nadel & D. Muir (Eds.), *Typical and impaired emotional development* (pp. 365-382). Oxford: Oxford University Press.

Loveland, K., Pearson, D., & Reddoch, S. (2005). *Anxiety symptoms in childrem and adolescents with autism*. Paper presented at the International Meeting for Autism Research.

Loveland, K., Tunali-Kotoski, B., Chen, R., Brelsford, K., Ortegon, J., & Pearson, D. (1995). Intermodal perception of affect in persons with autism or Down syndrome. *Develop Psychopath, 7*.

Loveland, K. A., Steinberg, J. L., Pearson, D. A., Mansour, R., & Reddoch, S. (2008). Judgments of auditory-visual affective congruence in adolescents with and without autism: a pilot study of a new task using fMRI. *Percept Mot Skills, 107*(2), 557-575.

Magnee, M. J., de Gelder, B., van Engeland, H., & Kemner, C. (2007). Facial electromyographic responses to emotional information from faces and voices in individuals with pervasive developmental disorder. *J Child Psychol Psychiatry, 48*(11), 1122-1130.

Magnee, M. J., de Gelder, B., van Engeland, H., & Kemner, C. (2008). Audiovisual speech integration in pervasive developmental disorder: evidence from event-related potentials. *J Child Psychol Psychiatry, 49*(9), 995-1000.

Malkova, L., Heuer, E., & Saunders, R. C. (2006). Longitudinal magnetic resonance imaging study of rhesus monkey brain development. *Eur J Neurosci, 24*(11), 3204-3212.

Malkova, L., Lex, C. K., Mishkin, M., & Saunders, R. C. (2001). MRI-Based evaluation of locus and extent of neurotoxic lesions in monkeys. *Hippocampus, 11*(4), 361-370.

Morrison, S. E., & Salzman, C. D. (2010). Re-valuing the amygdala. *Curr Opin Neurobiol, 20*(2), 221-230.

Muller, V. I., Habel, U., Derntl, B., Schneider, F., Zilles, K., Turetsky, B. I., et al. (2011). Incongruence effects in crossmodal emotional integration. *Neuroimage, 54*(3), 2257-2266.

Murray, E. A. (2007). The amygdala, reward and emotion. *Trends Cogn Sci, 11*(11), 489-497.

Nahm, F. K., Tranel, D., Damasio, H., & Damasio, A. R. (1993). Cross-modal associations and the human amygdala. *Neuropsychologia, 31*(8), 727-744.

Nemanic, S., Alvarado, M. C., Price, R. E., Jackson, E. F., & Bachevalier, J. (2002). Assessment of locus and extent of neurotoxic lesions in monkeys using neuroimaging techniques: a replication. *J Neurosci Methods, 121*(2), 199-209.

Neumann, D., Spezio, M. L., Piven, J., & Adolphs, R. (2006). Looking you in the mouth: abnormal gaze in autism resulting from impaired top-down modulation of visual attention. *Soc Cogn Affect Neurosci, 1*(3), 194-202.

Payne, C., & Bachevalier, J. (in preparation). *Crossmodal integration of conspecific vocalizations in rhesus macaques.*Unpublished manuscript.

Payne, C., Machado, C. J., Bliwise, N. G., & Bachevalier, J. (2010). Maturation of the hippocampal formation and amygdala in Macaca mulatta: a volumetric magnetic resonance imaging study. *Hippocampus, 20*(8), 922-935.

Pelphrey, K. A., Sasson, N. J., Reznick, J. S., Paul, G., Goldman, B. D., & Piven, J. (2002). Visual scanning of faces in autism. *J.Autism Dev.Disord., 32*(4), 249-261.

Pourtois, G., de Gelder, B., Bol, A., & Crommelinck, M. (2005). Perception of facial expressions and voices and of their combination in the human brain. *Cortex, 41*(1), 49-59.

Raper, J., Kazama, A., & Bachevalier, J. (2009). *Blunted fear reactivity after neonatal amygdala and orbital frontal lesions in rhesus monkeys*. Paper presented at the Society for Neuroscience.

Raper, J., Wilson, M., Sanchez, M., & Bachevalier, J. (2011). *Neonatal amygdala lesions alter emotional and neuroendocrine responses to an acute stressor in adult rhesus monkeys*. Paper presented at the Gordon Research Conference: Amygdala in Health and Disease.

Rolls, E. (1992). Neurophysiology and functions of the primate amygdala. In J. Aggleton (Ed.), *The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction* (pp. 143-165). New York, NY: Wiley-Liss.

Salzman, C. D., & Fusi, S. (2010). Emotion, cognition, and mental state representation in amygdala and prefrontal cortex. *Annu Rev Neurosci, 33*, 173-202.

Saunders, R. C., Aigner, T. G., & Frank, J. A. (1990). Magnetic resonance imaging of the rhesus monkey brain: use for stereotactic neurosurgery. *Exp Brain Res, 81*(2), 443-446.

Saunders, R. C., & Rosene, D. L. (1988). A comparison of the efferents of the amygdala and the hippocampal formation in the rhesus monkey: I. Convergence in the entorhinal, prorhinal, and perirhinal cortices. *J Comp Neurol, 271*(2), 153-184.

Saunders, R. C., Rosene, D. L., & Van Hoesen, G. W. (1988). Comparison of the efferents of the amygdala and the hippocampal formation in the rhesus monkey: II. Reciprocal and non-reciprocal connections. *J Comp Neurol, 271*(2), 185-207.

Spezio, M. L., Huang, P. Y., Castelli, F., & Adolphs, R. (2007). Amygdala damage impairs eye contact during conversations with real people. *Journal of Neuroscience, 27*(15), 3994-3997.

Stefanacci, L., Clark, R. E., & Zola, S. M. (2003). Selective neurotoxic amygdala lesions in monkeys disrupt reactivity to food and object stimuli and have limited effects on memory. *Behav Neurosci, 117*(5), 1029-1043.

von Bonin, G., & Bailey, P. (1947). *The neocortex of the Macaca mulatta.* Champaign, IL: University of Illinois Press.

Wallen, K., & Rupp, H. A. (2010). Women's interest in visual sexual stimuli varies with menstrual cycle phase at first exposure and predicts later interest. *Horm Behav, 57*(2), 263-268.

Wallen, K., & Tannenbaum, P. L. (1997). Hormonal modulation of sexual behavior and affiliation in rhesus monkeys. *Ann N Y Acad Sci, 807,* 185-202.

MANUSCRIPT III: The Role of the Orbital Frontal Cortex in Processing Bimodal Conspecific Vocalizations in Rhesus Macaques

Christa Payne[1,2]

Jocelyne Bachevalier[1,2]

[1]Department of Psychology, Emory University, Atlanta, GA 30062

[2]Division of Developmental and Cognitive Neuroscience, Yerkes National Primate Research Center, Atlanta, GA 30062

Abstract

The orbital frontal cortex is a heteromodal association area that receives converging projections from multiple sensory systems and has been implicated in processing affective information. Here, we examined the role of the orbital frontal cortex in encoding bimodal species-specific vocalizations using eye tracking in rhesus macaques. The looking behaviors of four adult rhesus monkeys that received neonatal lesions of the orbital frontal cortex (group Neo-O_{asp}; 2M, 2F) were compared to the previously characterized looking behaviors of six adult, sham-operated rhesus macaques (group Neo-C). Two side-by-side videos of unfamiliar male rhesus macaques (stimulus animals) emitting different vocalizations (i.e., coo, grunt, scream, threat) were presented with the audio tract from one of the videos. Crossmodal integration ability was inferred when the animals exhibited a preference (i.e., looked longer than 50% chance) for one of the two videos. Scanning patterns were characterized by comparing the percentages of time spent looking at each of the six a priori ROIs (eyes, mouth, and rest of each stimulus video). Group Neo-O_{asp} failed to show a preference for one of the videos when the onsets of the auditory and visual components were synchronized, indicating impairment in crossmodal integration. However, the percentage of time animals looked towards their preferred stimulus video did not vary across groups, and group Neo-O_{asp} did exhibit a preference when the onset of the auditory component was delayed relative to the visual component. Post-hoc analyses on the affect of trial parameters of Identity and Valence indicated that integration ability was sensitive to the relative identity of the stimulus animals, but not the relative valence of the vocalizations. Group Neo-O_{asp} showed

integration ability in trials comprised of two videos of the same stimulus animal (Identity Same), but not in trials with videos of two different stimulus animals (Identity Different). Analyses of scanning patterns revealed that both groups preferentially attended to the eyes over the mouths and rest of the stimulus videos in Identity Same and Identity Different trials, yet displayed striking differences in overall scanning strategies across trial types. Like group Neo-C, the preference observed in Identity Same trials was associated with a general preference for the eyes and discrimination between the eye regions of the two stimulus videos (congruent > incongruent). The lack of preference in Identity Different trials was characterized by a strong preference for the eye regions of both stimulus videos (congruent = incongruent), which resulted in Neo-O_{asp} monkeys looking more to the eye regions than group Neo-C. This greater salience for the eyes was consistent with increased aggression, a common trait following damage to the orbital frontal cortex, and indicated that group Neo-O_{asp} interpreted the stimuli differently than group Neo-C (e.g. more threatening). Their interpretation of and response to the complex social signals likely interfered with the expression of integration ability. The current results parallel the looking behavior of human children with high aggression, and are consistent with a role of the orbital frontal cortex in creating appropriate representations of complex social signals.

Introduction

The dynamic social environments in which human and nonhuman primates live require individuals to be able to recognize, manipulate, and behave according to the immediate social context. The orbital frontal cortex has been implicated in these socio-emotional abilities. In particular, the orbital frontal cortex appears to use this information to guide and adjust behaviors appropriately in accordance with changing contexts and contributes to the anticipation of reward (Murray, 2007; Schoenbaum & Esber, 2010).

Human patients with discrete lesions to the caudal orbital frontal cortex, including areas 11 and 13, are characterized by an array of socio-emotional deficits, ranging from behavioral changes, such as increased aggression (Bechara, Damasio, & Damasio, 2000; Hornak et al., 2003; Hornak et al., 1996), to cognitive changes, such as difficulties with identifying faces and/or voices (Hornak et al., 2003; Hornak et al., 1996; Rolls, 1999). A role of the orbital frontal cortex in processing emotional content from faces and voices has been suggested by electrophysiological studies in nonhuman primates (Klopp et al., 2000; O'Scalaidhe et al., 1997, 1999; Rolls, 1999, 2000; Rolls et al., 2006) and neuroimaging studies in humans (Blair et al., 1999; Dalton et al., 2005; Gorno-Tempini et al., 2001; Mitchell et al., 2003; Morris et al., 1998; Nakamura et al., 1999; Sprengelmeyer et al., 1998). One particularly compelling neuroimaging study linked dysfunction of the orbital frontal cortex with deficits in crossmodal perception of emotion exhibited by children with autism spectrum disorder (Loveland et al., 2008).

Such reports are consistent with the neuroanatomical characteristics of the primate orbital frontal cortex, a heteromodal association area that receives converging projections from multiple sensory systems, including visual (Barbas, 1988, 1993; Barbas & Blatt, 1995; Barbas & Pandya, 1989; Carmichael & Price, 1995; Morecraft, Geula, & Mesulam, 1992; Pandya & Kuypers, 1969; Seltzer & Pandya, 1989; Webster et al., 1994) and auditory inputs (Barbas, 1988, 1993; Hackett et al., 1999; L. M. Romanski & Goldman-Rakic, 2002; L. M. Romanski et al., 1999).

Despite a preponderance of evidence that the orbital frontal cortex is poised as a region involved in the integration and interpretation of socio-emotional information broadcast over multiple sensory modalities, to date, there have been no investigations directly assessing the role of this region in crossmodal integration of complex social signals. Recent reports from our laboratory have indicated that selective neonatal lesions of the orbital frontal cortex generated significant changes in the ability to flexibly alter behavior in response to contextual changes (Kazama et al., 2008) and in emotional and stress reactivity (Raper et al., 2009; Raper, Wilson, Sanchez, & Bachevalier, 2010). These deficits persisted into adulthood and were comparable to the behavioral changes reported in monkeys that had received similar lesions of the orbital frontal cortex in adulthood (Bachevalier et al., 2011). Given the enduring effects of neonatal orbitofrontal lesions on decision-making and emotion regulation, we speculated that, if the orbital frontal cortex plays a critical role in the integration of audiovisual social cues, lesions of this region in infancy would result in significant impairment of this ability.

To test this proposal, the goals of the present investigation are to 1) determine if neonatal lesions of the orbital frontal cortex disrupt integration ability in rhesus macaques using a preferential viewing paradigm, 2) characterize the scanning strategies of adult rhesus macaques with neonatal lesions of the orbital frontal cortex using eye-tracking, and 3) compare the looking behavior of monkeys with neonatal orbitofrontal lesions with those of previously characterized sham-operated controls (Payne & Bachevalier, in preparation).

Method

All procedures were approved by the Animal Care and Use Committee of the University of Texas Health Science Center at Houston in Houston, TX and of Emory University in Atlanta, GA and carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used, as well as any pain and suffering.

Subjects

Four adult rhesus monkeys (*Macaca mulatta*) aged 4-6 years were used in this investigation. Each animal received either visually guided aspiration lesions of the orbital frontal cortex (group Neo-O_{asp}; 2 males, 2 females) at 7-19 days of age. Animals were raised in a socially enriched nursery environment that promoted species-specific social skills (detailed in Goursaud & Bachevalier, 2007) and had repeated assessments of memory, emotional reactivity, social behavior, and reward appraisal throughout their lives. Their ability to process auditory-visual social information was compared to that of sham-operated adult monkeys (group Neo-C, 3 males and 3 females) that had received their sham-operations at 8-12 days of age, were raised in exactly the same way and received identical behavioral and cognitive training throughout their lives (Payne & Bachevalier, in preparation).

Magnetic Resonance Imaging Procedures

Animals of group Neo-O_{asp} underwent two surgery-related MRI scanning sessions according to procedures previously described (Saunders et al., 1990). Each animal received a pre-surgical MRI to facilitate the production of subject-specific lesions. MRIs obtained 7 – 10 days after surgery were used to evaluate the extent of lesion as previously described (Machado & Bachevalier, 2006).

Neonates were removed from their home cage and lightly sedated with Isoflurane inhalation (1.0 – 3.0% to effect) and intubated with an endotracheal tube to provide constant Isoflurane sedation and respiration assistance throughout surgical procedures. Vital signs were monitored throughout the entire procedure. Neonates were transported to the MRI facility at University of Texas M.D. Anderson Cancer Center in an incubator. Upon arrival, animals were placed in a non-ferromagnetic stereotaxic apparatus (Crist Instruments Co., Inc., Damascus, MD), aligned within the GE Signa 1.5 Tesla Echo Speed scanner (GE Medical Systems, Milwaukee, WI) with 3" surface coil.

Three MRI sequences were obtained. The first series was a T1-weighted spin-echo sequence (echo time (TE) = 11 ms, repetition time (TR) = 450 ms, contiguous 4 mm sections, 12 cm field of view (FOV), 256 x 256 matrix) acquired in the sagittal plane and was used to align the two subsequent series. The second was a 3D T1-weighted fast spoiled gradient (FSPGR)-echo sequence (TE = 2.6 ms, TR = 10.2 ms, 25° flip angle, contiguous 1 mm sections, 12 cm FOV, 256 x 256 matrix) used to visualize the sulcal patterns of the orbital frontal cortex for group Neo-O_{asp}. The final series of images acquired was a Fast Spin-Echo – Inversion Recovery (FSE-IR) series (TE = 20 ms, TR = 4500/250 ms, ETL = 6, BW = 32 kHz, contiguous 1.5 mm sections, 12 cm FOV, 256 x 256 matrix, 2NEX). This later series was used as part of another study to trace the developmental trajectory of several brain structures (Payne et al., 2010). Upon scanning completion, the animals were transported to the surgical suite.

Surgical Procedures

Animals received an intravenous drip solution of 5% dextrose and 0.45% sodium chloride to maintain hydration during surgery. A long lasting local anesthetic (Marcaine 25%, 1.5 ml) was injected subcutaneously along the incision line to minimize pain and inflammation. Following the midline incision, the skin, connective tissue (galea) and temporalis muscles were gently retracted to expose the skull. For animals of group Neo-O_{asp}, craniotomies were made directly above each orbit. To gain a full view of the ventral surface, the bone of the supra-orbital ridge was gently eroded with a hand drill and rongeurs. Incisions were made in the dura and the frontal lobe was gently elevated. A surgical microscope was used to visualize the medial and lateral orbital sulci and the olfactory stria, (Figure 2, left column). The pia was cauterized along the lateral border of the olfactory stria and along the medial lip of the lateral orbital sulcus, which provided the medial and lateral borders of the lesions. The anterior border was a line joining the rostral tips of the medial and lateral orbital sulci and the posterior border ended at a point where the olfactory stria turns laterally. Cytoarchitectonic areas 11 and 13 were contained within these borders and were aspirated with 21 & 23 gauge aspirating probes and an electro-cautery. Extreme care was provided to end the lesion before touching the white matter just below the cortical mantle. Animals were then taken off Isoflurane gas anesthesia, removed from the stereotaxic apparatus and allowed to recover in an incubator ventilated with oxygen.

All animals received pre- and post-surgical treatments to minimize risk of infection (Cephazolin, 25 mg/kg, per os) and control swelling (dexamethazone sodium phosphate, 0.4 mg/kg, s.c.). Treatments began twelve hours before and continued seven days after surgery. A topical antibiotic ointment (bacitracin-neomycin-polymyxin) was applied to the wound daily and acetaminophen (10mg/kg, p.o.) was given four times a day for three days after surgery to relieve pain.

Lesion Verification

The extent of cortical removal from the aspiration lesions of the orbital frontal cortex was evaluated using the pre- and post-surgical T1 FSPGR. Briefly, the extent of cortical damage was plotted onto a series of drawings of coronal histological sections of a one-week-old normal rhesus monkey brain (J. Bachevalier, unpublished data), acquired at 1 mm interval. The total volume of damage for each structure was calculated from surface area measurements (Gundersen & Jensen, 1987) taken for each hemisphere with ImageJ® software (http://rsb.info.nih.gov/ij/). The percentages of intended damage to cortical areas 11 and 13 and unintended damage to adjacent cortical fields (10, 14, 12, and Ia) were then expressed as a percentage of the normal volume for that structure, which was previously estimated from the normal infant rhesus monkey brain, using similar methods.

As shown in Table 1, all four cases with orbital frontal lesions had complete and symmetrical damage, ranging from 85.0 % to 94.3 %. This damage included area 11 (mean: 86.5%) and area 13 (mean: 92.8 %). Unintended damage to cortical areas 10, 12 and 14, including the white matter beneath the cortex, was relatively minor (means: 2.7%, 13.8% and 12.1%, respectively), but Ia received significant damage (mean: 56.7%).

Crossmodal integration task

A preferential viewing paradigm similar to that employed by Ghazanfar and Logothetis (Ghazanfar & Logothetis, 2003) was used in conjunction with eye-tracking technology to 1) determine whether the orbital frontal cortex is necessary for the spontaneous integration of the auditory and visual components of species-specific vocalizations seen in normal monkeys; and 2) to assess the impact of neonatal lesions of the orbital frontal cortex on monkeys scanning patterns of these complex social cues.

<u>Apparatus</u>: Monkeys were seated in a primate chair in a sound-attenuated room. Stimuli were presented on a 24-inch, flat panel LCD monitor with attached speaker and data was collected with an eye-tracking camera (ISCAN, Inc.; Woburn, MA) located between the stimulus screen and primate chair. A head-restraint device attached to the primate chair was used to gently reduce head

movements. A curtain concealed all supplementary equipment and ambient white noise was used to further muffle extraneous noise.

Stimuli: Stimuli were constructed from pairs of four videos depicting the facial postures associated with species-typical vocalizations (coo, grunt, scream and threat) emitted by two unknown conspecific males (‘stimulus animals’). Each stimulus animal produced two different calls. The coo and threat videos were of one stimulus animal and the videos of the grunt and scream were of the other stimulus animal. Each trial consisted of two videos (360 x 480 pixels) on a black background spaced apart maximally within the same visual plane and the corresponding sound track of one of the presented videos. In a given trial, the stimulus video that corresponded with audio track was referred to as the ‘congruent stimulus video’, and the video that did not match the audio track was referred to as the ‘incongruent stimulus video’ (Fig 2). Video clips were 2-sec long and each trial was looped 5 times for a total trial duration of 10 seconds. The left-right positioning of each vocalization was counter-balanced within each stimulus set. Stimulus presentation was controlled using the Presentation software package (Neurobehavioral Systems, Inc; Albany, CA).

In pilot trials of integration ability of normal monkeys, only vocalizations emitted by the same stimulus animal were used in a given trial (i.e. coo-threat and grunt-scream). This preliminary testing indicated that normal monkeys rapidly habituated to the limited stimulus sets. Given that previous results indicated that integration ability was not affected by the relative identity of the stimulus animals (Ghazanfar & Logothetis, 2003), stimulus sets were constructed from all possible permutations of stimulus video pairs to reduce habituation.

Task: Stimuli were presented under two conditions: Synchronized and Desynchronized. In the Synchronized condition, eight trials (2 trials/session) were administered such that the onsets of the auditory and visual components were simultaneous, and served as the standard for integration assessment. The Desynchronized condition was utilized to determine if integration of the crossmodal components relied upon the coincidence of mouth movements with the vocal element. This stimulus set was comprised of eight unique trials (4 trials/day) presented such that the sound track started 330 – 430 msec after the videos began, a delay range that has been shown to disrupt the perception of the stimuli as a single event (Dixon & Spitz, 1980).

Measures

Integration Assessment: For each trial, the percentages of time monkeys looked to the congruent (i.e., matching the audio component) and incongruent (i.e., different from the audio component) videos was calculated. Integration ability was inferred when monkeys looked statistically more than chance (50%) to either the congruent or incongruent stimulus video (i.e., exhibited a preference). Accordingly, an inability to integrate the bimodal social cues was demonstrated by animals looking equally to each stimulus video.

Scanning Pattern Characterization: Static *a priori* regions of interest (ROIs) of the 'Eyes' and 'Mouth' were created with the ISCAN P.O.R. Fixation Analysis software (v1.20, ISCAN, Inc., Fig 2). The boundaries of each ROI were drawn so the entire feature of interest was surrounded throughout the full 2sec video and there was no overlap between ROIs. A third ROI ('Other') included the area of the video not encapsulated by either the ‘Eyes’ or ‘Mouth’ ROI. Each trial had six ROIs: ‘eyes’, ‘mouth’, and ‘other’ for each the congruent and incongruent stimulus videos. Looking to each ROI was defined as the accumulation of fixation duration for a given ROI. Fixations were defined as eye gaze coordinates remaining within 1° x 1° visual angle for at least 100 msec, and lasted until the eye gaze coordinates deviated more than 1° x 1° visual angle for more than 360 msec. Fixations were categorized and quantified by ROI with the ISCAN P.O.R. Fixation Analysis Software. To account for variability in looking time across trials and animals, looking to each ROI was analyzed in terms of the percentage of total looking for each trial ((ROI/Total)*100).

Statistical Analyses

Assumptions of parametric statistics were met in all measures, but small and unequal group sizes mandated the use of nonparametric statistics for some analyses. Integration ability and group comparisons were assessed using the percentage of time groups spent looking towards the congruent stimulus video. The looking behavior of group Neo-C has been previously characterized (Payne & Bachevalier, in preparation), and the behavior of group Neo-O_{asp} was assessed in a comparable manner. Integration abilities were analyzed separately for the Synchronized and Desynchronized conditions using one-sample t-tests against the chance level of 50%. Differences between conditions were quantified via dependent-sample t-tests, and sex differences were evaluated separately with nonparametric Mann-Whitney tests. Given the unequal and small group sizes, group comparisons in integration ability were assessed with nonparametric

Kruskal-Wallis tests (percent looking to congruent stimulus video x group). The purpose of the Desynchronized condition was to ensure that integration ability was not purely a function of the audio and visual elements being temporally linked. Therefore, the characterization of scanning patterns was only assessed in the Synchronized condition. The percentages of time groups spent looking to the ROIs in the congruent and incongruent stimulus videos were compared using repeated measures MANOVA (stimulus video x ROI x group) with planned comparisons. Correlations between looking behavior and extent of lesion were assessed via Pearson product-moment correlation coefficients. Although it was not appropriate to assess sex differences statistically (2 males, 2 females), descriptive comparisons were provided.

Results

Integration Assessment after Neonatal Orbital Frontal Lesions

Figure 3 illustrates some notable differences between the preference patterns exhibited by groups Neo-O_{asp} and Neo-C in the Synchronized and Desynchronized conditions.

In the Synchronized condition (Fig 3A), unlike group Neo-C that showed a preference for the congruent stimulus videos, group Neo-O_{asp} looked equally to the congruent and incongruent stimulus videos ($t(3) = -0.041$, $p = 1.902$). Individual variability in preference direction (i.e., looking more towards the congruent or incongruent video) may have contributed to this null finding; therefore general preference (i.e., irrespective of direction) was compared to chance. When overall preference was investigated (Fig 3B), the same group differences occurred with group Neo-O_{asp}, again, showing no general preference (t(3) = 1.902; p = 0.153). There were no qualitative differences in preference between males and females of group Neo-O_{asp}, and no statistically significant correlations between the percentages of time an animal looked towards their 'preferred' stimulus video (preference strength) and the extent of damage to areas 11 and 13, either separately or combined (area 11: r = -0.794, p = 0.206; area 13: r = -0.254, p = 0.746; combined: r = -0.797, p = 0.203).

During the Desynchronized condition, groups Neo-O_{asp} and Neo-C looked equally to the congruent and incongruent stimulus video (Neo-O_{asp}: t(3) = 0.092, p = 0.933; Neo-C: t(5) = -0.496, p = 0.641); however, both groups showed a significant overall preference for one stimulus video (Neo-O_{asp}: t(3) = 3.181, p = 0.050; Neo-C: t(5) = 5.611, p = 0.002). Interestingly, in this condition, the two females looked more to the incongruent stimulus video but the two males looked more to the congruent stimulus video and preference strength correlated with the extent of damage to area 13 (r = 0.945, p = 0.055) but not to area 11 (r = -0.113, p = 0.887) or combined damage (r = 0.815, p = 0.185).

Although group Neo-C exhibited statistically significant preferences in both conditions and Neo-O_{asp} only exhibited a preference in the Desynchronized condition, the group differences did not reach significance in either condition (Synchronized: H = 1.136, d.f. = 1, p = 0.286; Desynchronized: H = 0.182, d.f. = 1, p = 0.670). The absence of group differences in conjunction with group Neo-O_{asp} showing a preference in the Desynchronized condition, suggests that crossmodal integration was disrupted, but not completely absent, after neonatal orbital frontal lesions. Instead, the lack of a statistically significant preference in the

Synchronized condition for group Neo-O_{asp} may have reflected the high individual variability in a small group of animals rather than an inability to integrate the bimodal social signals. Indeed, in this condition, two animals with neonatal orbital frontal lesions looked equally to both stimulus videos and two animals showed a preference for one of the videos. However, it is important to note that correlation analyses revealed a relationship between preference strength and extent of lesion, such that animals with greater damage to area 13 exhibited stronger preferences in the Desynchronized condition.

Given that group Neo-C showed variation in preference strength and direction associated with the relative identity of the stimulus animals and the relative valence of the vocalizations in a given trial (Payne & Bachevalier, in prep), post-hoc analyses on the factors of Identity and Valence were conducted to further assess the integration ability of group Neo-O_{asp}.

Relative Identity Integration Assessment: The factor 'Identity' refers to the stimulus animals in the two videos presented in a given trial. The two 'Identity Same' trials contained vocalizations produced by a single stimulus animal (i.e., the coo-threat pairings and grunt-scream pairings), and the six 'Identity Different' trials were comprised of vocalizations emitted by two different stimulus animals (e.g., the coo-scream pairings or grunt-threat pairings).

As shown in Figure 4, groups Neo-O_{asp} and Neo-C exhibited similar preference patterns in Identity Same and Identity Different trials with greater preferences in Identity Same trials than in Identity Different trials, although the difference between trial types only reached significance in the measure of congruence for group Neo-O_{asp} ($t(3) = 3.407$, $p = 0.042$, Fig. 4A). Like group

Neo-C, group Neo-O_{asp} exhibited a preference for congruence in Identity Same trials (t(3) = 3.093, p = 0.054, Fig 4A) but not Identity Different trials (t(3) = -1.192, p = 0.319). Also like group Neo-C, Neo-O_{asp} monkeys showed a general preference across both trial types (Fig 4B), although for group Neo-O_{asp} this preference reached significance only for Identity Same trials (t(3) = 3.093, p = 0.054).

There were no qualitative differences between Neo-O_{asp} males and females. Interestingly, preference assessments correlated with lesion extent in both trial parameters. In Identity Same trials, preference strength tended to be inversely correlated with the extent of damage to areas 11 and 13 (r = -0.930, p = 0.070), and in Identity Different trials, preference strength tended to be inversely related to damage to area 11 (r = -0.922, p = 0.078), indicating that greater damage to these areas resulted in weaker preferences.

Despite minor statistical discrepancies in the separate integration assessments of groups Neo-O_{asp} and Neo-C, looking to the congruent and preferred stimulus videos did not vary across groups for either Identity Same (congruent/preferred: H = 0.409, d.f. = 1, p = 0.522) or Identity Different (congruent: H = 0.409, d.f. = 1, p = 0.522; preferred: H = 1.136, d.f. = 1, p = 0.286) trials.

<u>Relative Valence Integration Assessment</u>: The factor of 'Valence' refers to the emotional content of the stimulus vocalizations in a given trial. In 'Valence Same' trials, both vocalizations had either a positive (i.e., coo-grunt) or negative (i.e., threat-scream) valence. In 'Valence Different' trials, the two vocalizations had different valences (e.g., coo-threat or grunt-scream).

Figure 5 illustrates the similarities in preference patterns of groups Neo-O_{asp} and Neo-C across Valence Same (H = 0.045, d.f. = 1, *p* = 0.831) and Valence Different (H = 1.636, d.f. = 1, *p* = 0.201) trials. Thus, like group Neo-C, group Neo-O_{asp} showed a significant general preference for one of the stimulus videos for both Valence Same (t(3) = 3.207, *p* = 0.049, Fig 5B) and Valence Different (t(3) = 3.113, *p* = 0.053, Fig 5B) trials. However, there were a couple qualitative differences between trial parameters. First, in Valence Same (but not Valence Different) trials, the males looked more to the incongruent stimulus video but the females looked more to the congruent stimulus video. Also, general preference strength was negatively correlated with the extent of damage to area 11 (r = -0.996, *p* = 0.004) in Valence Same trials, but not Valence Different trials.

Summary: Despite the lack of a statistically significant preference when all trials in the Synchronized condition were considered, groups Neo-C and Neo-O_{asp} did not differ in degree of preference. Likewise, when parameters of Identity and Valence were assessed, group Neo-O_{asp} displayed preference patterns similar to, albeit less robust than, group Neo-C. Moreover, the preference that group Neo-O_{asp} demonstrated in the Desynchronized condition indicates that animals with neonatal orbital frontal lesions could integrate the bimodal social cues in certain circumstances (Fig 3). Thus, the crossmodal integration ability of group Neo-O_{asp} was impaired, but not entirely absent.

Interestingly, despite the completeness of the orbital frontal lesions and the small variation in lesion extent between animals (see Table 1), preference strength showed by group Neo-O_{asp} in the Identity and Valence conditions was negatively correlated with the extent of lesion, indicating that the greater the damage to the regions of the orbital frontal cortex, the weaker the individual preference. The inverse relationships between extent of lesion and preference strength for trials in the Synchronized condition seems contradictory to the direct relationship seen between lesion extent and preference strength for trials in the Desynchronized condition. This apparent discrepancy has important implications for the interpretation of the data and will be fully considered in the discussion.

Scanning patterns were characterized across all trials, as well as for the different trial types, to further elucidate the impairment in bimodal integration of social cues exhibited by group Neo-O_{asp}.

Scanning Patterns after Neonatal Orbital Frontal Lesion

When all trials were considered, there was no Stimulus Video x ROI interaction ($F(2,6) = 2.392$, $p = 0.172$), indicating that group Neo-O_{asp} showed the same general patterns of looking in the congruent and incongruent stimulus videos. This was also the case for analyses of the parameters of Identity and Valence. Consequently, in all subsequent analyses, regions of interest were collapsed across stimulus videos (e.g., congruent eyes + incongruent eyes) to characterize overall scanning patterns of group Neo-O_{asp} (Fig 6A). However, since group Neo-C appeared to use the eye regions to discriminate between the congruent and incongruent videos (i.e., looked longer to congruent eyes than incongruent eyes, but looked equally to the congruent and incongruent mouths and to the rest of the two videos; Payne & Bachevalier, in prep), comparisons were also made for each region of interest across stimulus videos (e.g., congruent mouth vs. incongruent mouth) to identify the distinguishing region(s), if any, used by group Neo-O_{asp} during bimodal integration.

As shown in Figure 6A, group Neo-O_{asp} exhibited the same overall pattern of scanning as group Neo-C. Neo-O_{asp} monkeys looked more to the eye regions than either the mouth regions (F(1,3) = 68.009, *p* = 0.004) or the rest of the videos (F(1,3) = 93.008, *p* = 0.002), and tended to look more to the mouth regions than the rest of the videos (F(1,3) = 8.344, *p* = 0.063).

However, despite the similarities in general scanning patterns, there were several notable differences in the cues used by the two groups to discriminate between the two videos. First, whereas group Neo-C used the eye regions to differentiate the congruent from the incongruent videos (congruent > incongruent), group Neo-O_{asp} used the mouth regions (incongruent > congruent; F(1,3) = 12.662, *p* = 0.038). Secondly, a statistically significant ROI x Group interaction (F(1,8) = 5.654, *p* = 0.045) revealed that group Neo-O_{asp} looked more to the eye regions than group Neo-C (F(1,8) = 6.499, *p* = 0.034, Fig 6A). This difference was further supported by a significant correlation between the percentage of time group Neo-O_{asp} looked to the eyes and their total extent of lesion (r = 0.984, *p* = 0.016), with more extended lesions leading to greater looking to the eye region.

Finally, although Neo-C males looked more to the mouth region of the congruent stimulus videos than Neo-C females, there were no perceivable sex differences in the looking behavior of Neo-O_{asp} (Fig 6B), with both males and females looking more to the eye than to the mouth region of the congruent stimulus videos. Figure 6B clearly illustrates that Neo-O_{asp} males did not attend to the mouth region of the stimulus animal emitting the vocalization as much as

Neo-C males did (H = 3.000, d.f. = 1, p = 0.083).

Scanning Pattern for Identity Same Trials: Figure 7 shows that the general scanning of Identity Same trials were similar for groups Neo-O_{asp} and Neo-C. Consistent with the analyses of all trials, group Neo-O_{asp} looked more to the eye regions than either the mouth regions (F(1,3) = 13.102, p = 0.036) or the rest of the videos (F(1,3) = 49.997, p = 0.006), but looked equally to the mouth regions and the rest of the videos (F(1,3) = 1.299, p = 0.337). However, group Neo-O_{asp} tended to look more to the eye region of the congruent video than the eye region of the incongruent video (F(1,3) = 8.260, p = 0.064), indicating that, unlike the analyses of all trials, they used the eye regions to distinguish the congruent and incongruent stimulus videos. Group Neo-C also appeared to use the eye regions, but the difference did not approach statistical significance. There were no discernable sex differences in any region of either the congruent or incongruent stimulus video for either group.

There were no statistically significant group differences in the patterns of scanning in Identity Same trials (F(2,16) = 0.090, p = 0.914). There were also no correlations of scanning patterns with lesion extent. Thus, the preference for congruence exhibited by both groups was associated with an overall preference for the eye region and greater looking to the eye region of the congruent stimulus video than to the eye region of the incongruent stimulus video.

Scanning Pattern for Identity Different Trials: As above, group Neo-O_{asp} exhibited an overall pattern similar to group Neo-C when the vocalizations came from different monkeys (Fig 8A). They looked more to the eye regions than either the mouth regions (F(1,3) = 66.666, p = 0.004) or the rest of the videos (F(1,3) =

110.061, $p = 0.002$), but looked equally to the mouth regions and the rest of the videos ($F(1,3) = 4.388$, $p = 0.127$).

Again, despite the similarities between groups, there were some notable differences. As reported in the analyses of all trials, group Neo-O_{asp} looked more to the eye regions of the Identity Different trials than group Neo-C ($F(1,8) = 5.855$, $p = 0.042$, Fig 8A); and there was a significant correlation between the overall extent of damage and the percentage of time group Neo-O_{asp} looked to the eyes ($r = 0.989$, $p = 0.011$). Additionally, despite a robust preference for the eye regions, group Neo-O_{asp} used the mouth to discriminate the congruent and incongruent stimulus video (incongruent > congruent: $F(1,3) = 20.499$, $p = 0.020$). No such discrimination was observed in the scanning patterns of group Neo-C in Identity Different trials. In fact, even the Neo-C males, who expressed a greater interest in the mouth regions in Identity Different trials, did not discriminate the two mouth regions. Lastly, the sex differences reported of group Neo-C were not apparent within group Neo-O_{asp} (Fig 8B), revealing that both males and females of group Neo-O_{asp} looked more at the eye region that at the mouth region.

<u>Scanning Pattern for Valence Same Trials</u>: Figure 9A shows that group Neo-O_{asp} exhibits the same general scanning pattern as group Neo-C in Valence Same trials. Overall, Neo-O_{asp} monkeys looked more to the eye regions than either the mouth regions ($F(1,3) = 12.082$, $p = 0.040$) or the rest of the stimulus videos ($F(1,3) = 31.002$, $p = 0.011$), but did not differentiate the mouth regions from the rest of the videos ($F(1,3) = 0.530$, $p = 0.519$). There were no differences between the congruent and incongruent stimulus videos at any region of interest (eyes:

F(1,3) = 0.079, p = 0.797; mouth: F(1,3) = 5.895, p = 0.094; other: F(1,3) = 0.645, p = 0.481). There were no discernable differences in the looking behavior of the males and females of group Neo-O_{asp}.

Overall, the similarities between groups was confirmed in that there were no statistically significant differences between the scanning patterns of groups Neo-C and Neo-O_{asp} (F(2,16) = 0.418, p = 0.665). However, it is important to note that the sex differences exhibited by group Neo-C were not present within group Neo-O_{asp} (Fig 9B). Although ROI x Group x Sex could not be directly assessed, Figure 9B illustrates that, whereas Neo-C males looked more to the mouth region than Neo-C females, Neo-O_{asp} males and females looked equally to the mouth regions. This observation was indirectly supported by the negative correlation between the percentage of time group Neo-O_{asp} looked towards the mouth regions and the extent of damage to area 11 (r = -0.952, p = 0.048). That is, greater damage was associated with less looking to the mouth regions.

Scanning Pattern for Valence Different Trials: As seen in Figure 10, the general scanning patterns of Valence Different trials appeared to vary slightly across groups Neo-O_{asp} and Neo-C. Whereas group Neo-C only differentiated the socially salient regions (eyes and mouths) from the rest of the stimulus videos, Neo-O_{asp} monkeys differentiated all the regions from each other (eyes > mouth: F(1,3) = 31.254, p = 0.011; eyes > other: F(1,3) = 71.806, p = 0.003; mouth > other: F(1,3) = 8034.146, $p < 0.0001$). As in Valence Same trials, group Neo-O_{asp} did not differentiate the regions of interest of the congruent and incongruent stimulus videos (eyes: F(1,3) = 3.776, p = 0.147; mouth: F(1,3) = 0.042, p = 0.851; other: F(1,3) = 0.442, p = 0.554) and there were no discernable sex differences in any region of interest.

The qualitative group differences were confirmed with a significant ROI x Group interaction (F(2,16) = 4.020, p = 0.038, Fig 10), characterized by group Neo-O_{asp} looking more to the eyes (F(1,8) = 8.890, p = 0.018) but less to the mouth (F(1,8) = 6.247, p = 0.037) than group Neo-C. However, there were no significant correlations between group Neo-O_{asp} scanning and extent of lesion.

Summary: Although, initially, groups appeared to exhibit considerable consistency in their general scanning strategies, the above analyses demonstrate that neonatal lesions of the orbital frontal cortex resulted in marked perturbations of normal scanning strategies. Namely, whereas the scanning strategies of sham-operated controls appeared to be sensitive to the trial parameters of Identity and Valence, monkeys with damage to the orbital frontal cortex showed a robust preference for the eye region across all trial parameters and this preference was even stronger than that seen in sham-operated monkeys. Moreover, this preference was inversely correlated with extent of damage. Interestingly, although group Neo-O_{asp} appeared to use the eye regions to discriminate videos of the same stimulus animal, they seemed to use the mouth regions to differentiate the videos of different stimulus animals (despite a robust preference for the eye regions). Finally, it is also important to note that sex differences seen in sham-operated animals were not apparent in monkeys with lesions of the orbital frontal cortex. That is, although females with neonatal lesions of the orbital frontal cortex exhibited the same overall pattern of looking as control females (i.e., eyes > mouth), the males with neonatal orbital frontal cortex lesions did not show the robust looking to the mouth regions that was observed in control males.

Discussion

This was the first attempt to assess the effect of neonatal lesions of the orbital frontal cortex on crossmodal integration and visual attention during the encoding of social information from bimodal, species-specific cues in rhesus macaques. The results indicate that neonatal lesions of the orbital frontal cortex resulted in a weak ability to integrate bimodal social signals. This weaker integration of social signals was associated by substantial changes in viewing patterns that were characterized by greater attention to the eye regions across all trial types as compared to control animals. These results will be discussed in turn below.

Weaker ability to integrate bimodal social signals

The assessments of integration ability failed to provide conclusive evidence for an involvement of the orbital frontal cortex in crossmodal integration of complex social cues. Despite a lack of significant preference across all trials in the Synchronized condition, the strength of preference exhibited by adult monkeys with neonatal orbital frontal lesions. Additionally, the presence of a significant preference in the Desynchronized condition further evidenced some level of integration ability present after the neonatal orbital frontal lesions. However, it is important to note that integration ability was not consistent across trial types and that preference strength in the Synchronized trial types appeared to be inversely related to the extent of damage to the orbital frontal cortex. That is, the greater the damage, the weaker the preference, indicating that orbital frontal lesions did impair crossmodal integration of complex social signals. Although these findings suggest that an intact orbital frontal cortex is required for the normal development of bimodal integration of social signals in monkeys, additional research will be needed to confirm these findings and determine whether the same lesions acquired in adulthood would result in a similar deficit.

Nevertheless, the present results are conducive with recent investigations characterizing individuals with autism, a neurodevelopmental disorder associated with dysfunction of the orbital frontal cortex (reviewed by Bachevalier & Loveland, 2006). Investigators used a preferential viewing paradigm to assess the ability of children and adolescents with autism to detect emotion congruency across auditory and visual modalities (crossmodal emotion perception) (Loveland et al., 2005). Similar to the current design, subjects were presented with split-screen videos of the same person exhibiting two different negative emotions (anger, sadness, fear, or surprise) and an audio track of the person narrating a text that could match either emotion. Prosody of the centrally emitted sound track matched only one of the represented emotions. As compared to matched controls, individuals with autism looked less to the video congruent with the auditory emotional cues, indicating weaker bimodal integration ability than controls. Interestingly, a follow-up neuroimaging study by the same authors (Loveland et al., 2008) demonstrated that a dysfunction of the orbital frontal cortex might be responsible for this weaker bimodal integration. In this block fMRI design study, individuals with autism and matched controls were presented with human faces paired with wordless emotional vocalizations. Two conditions were presented in alternating blocks. In Gender blocks, participants were instructed to respond via button-press to indicate whether the ‘gender’ of the voice-face pairings were the same (congruent) or different (incongruent); and in Emotion blocks, they were asked to indicate whether the ‘affect’ of the voice-face pairing were congruent or incongruent. When the Emotion condition was compared to the Gender condition, matched controls exhibited significantly greater activity in the prefrontal and orbital frontal cortices than the autism group.

Inflexibility of Scanning Strategies

Neonatal lesions of the orbital frontal cortex affected the flexibility of species-typical scanning patterns of unfamiliar, vocalizing conspecifics. In particular, sham-operated monkeys appeared to scan the videos of emoting stimulus animal (i.e., congruent video) differently than the incongruent video, and males and females displayed differences in the way they scanned the emoting stimulus animal that were not observed in the monkeys with orbitofrontal damage. Sham-operated females looked more to the eyes than the mouth of the emoting stimulus animal (Fig 12, top panels), a pattern that paralleled the looking strategies humans employ when instructed to attend to emotion-related cues or make social judgments of human facial expressions (Buchan et al., 2008; Lansing & McConkie, 1999), and suggested that they attended to the valence of the vocalization. In contrast, sham-operated males looked to the eyes of the emoting stimulus animal as much as females, but also looked more to the mouths. Post-hoc analyses revealed that sham-operated males preferentially scanned the eye regions only when the same stimulus animal produced both vocalizations, and not when two different stimulus animals emitted the vocalizations (Fig 11, top panels). This pattern signified that, in addition to attending to the valence of the vocalizations (eyes), the male rhesus macaques also attended to identity-specific features that may signify the dominance status of the novel conspecific, such as canine size (Payne & Bachevalier, in preparation).

By contrast, the scanning strategies of monkeys with neonatal lesions of the orbital frontal cortex were inflexible and largely unaffected by the trial parameters of Identity and Valence. Whereas sham-operated controls only exhibited a preference for the eyes of the emoting stimulus animals, monkeys with orbitofrontal damage showed a robust preference for the eyes in both the congruent and incongruent stimulus videos (Fig 11 and Fig 12, bottom panels). This coincided with the observation that animals with lesions of the orbital frontal cortex looked more to the eyes than sham-operated controls. Furthermore, both males and females expressed this clear preference for the eyes across all trial types. Thus, lesions of the orbital frontal cortex appear to increase the salience of the eyes and effectively dampen the salience of features associated with the dominance status of the unfamiliar stimulus animals.

The general increased salience of the eyes may have also contributed to the inconsistencies in the assessments of integration ability. Just as the greater attentiveness to the eyes was accompanied by inattentiveness to other features of the novel males, it may have interfered with the salience of the auditory component, or perhaps even the perception of it. The expression of a preference in Identity Same trials, but not Identity Different trials, supports this possibility. Hence, when presented with the same stimulus animal, the repetition of the eyes across videos allowed the animals with orbitofrontal lesions to attend to the auditory component and exhibit a preference for the congruent stimulus video. This preference was characterized by a differentiation of the eye regions of the stimulus videos (congruent > incongruent; Fig 11C and Fig 12C), and by preference strength being related to extent of damage to the orbitofrontal cortex, such that the greater their damage, the weaker their preference.

In contrast, when presented with two unfamiliar males, their compulsion to look at the eyes of both animals may have overshadowed their attention to the auditory component, resulting in equal looking to the two stimulus videos (Fig 11D and Fig 12D). The expression of a preference in the Desynchronized condition further supports the notion that increased salience of the eyes interfered with the perception of, or attention to, the auditory component. Perhaps the desynchronization of the two components made them more aware of the auditory cue, resulting in the presence of crossmodal integration in this condition. The correlation between extent of damage and preference strength is consistent with this supposition. That is, animals with larger orbital frontal damage showed a) greater looking to the eye regions in the Synchronized condition, and b) longer looking to their preferred stimulus video in the Desynchronized condition.

The results from the integration assessment, combined with the characterization of the scanning patterns, suggest that neonatal lesions of the orbital frontal cortex disrupt the relative salience of the eyes and vocal cues, resulting in a weaker ability to integrate complex bimodal social signals. However, further investigation is needed to fully examine this possibility.

Increased Aggression

In our previous characterization of the looking behavior of the sham-operated controls, we argued that the females' preference for the eyes over the mouth of the emoting stimulus animal signified attentiveness to the valence of the stimulus (see above). This interpretation was further supported by post-hoc analyses on Identity and Valence, which revealed differential scanning of the eye and mouth regions across the different trial types (Payne & Bachevalier, in preparation). Although monkeys with neonatal orbitofrontal lesions showed a strong preference for the eye regions, the consistency of this preference across both stimulus videos regardless of trial type indicates that their preference for the eyes was not associated with attentiveness to vocalization valence. Instead, we hypothesize that the increased looking to the eye regions likely represented increased aggressivity.

In the rhesus macaque society, a direct stare to the eyes of a conspecific is a direct threat to that individual (Altmann, 1962; Hinde & Roswell, 1962). Thus, whereas the species-typical response to encounters with unfamiliar males appears to flexibly vary by the sex of the observer and the context of the encounter (e.g. nature/valence of the encounter or dominance status of the new monkey) (Payne & Bachevalier, in preparation), damage to the orbital frontal cortex results in increased aggressivity that manifests as an increased salience for the eyes. This aggression was seen in both males and females, did not vary across trial parameters (see Figs 9 and 10, bottom panels), and was related to the extent of orbital frontal damage, with greater damage being associated with greater looking to the eyes. This greater interest for the eye regions parallels recent findings from our lab indicating that animals that received aspiration lesions of areas 11 and 13 in adulthood looked more to the eyes of static faces than normal controls (Goursaud & Bachevalier, in preparation).

The inflexibility and heightened aggression evident in the looking behavior of monkeys with neonatal orbital frontal cortex damage is highly consistent with other assessments of their behavior. For example, when exposed to an unknown human staring at them in a Human Intruder paradigm, the same monkeys with neonatal damage to the orbital frontal cortex displayed hostile behaviors, including threats directed towards the unfamiliar intruder, significantly more than sham-operated controls (Raper et al., 2010). This increased aggression was apparent as early as six months of age when they directed more aggressive behaviors towards peers in dyadic social interactions than sham-operated controls (Payne, Goursaud & Bachevalier, unpublished findings). Increased aggression has also been reported in monkeys that received lesions of the orbital frontal cortex in adulthood (Izquierdo, Suda, & Murray, 2005; Machado & Bachevalier, 2006).

In humans, damage to the prefrontal cortex that includes the orbital surface, whether acquired in adulthood or infancy (Anderson et al., 2007; Anderson, Barrash, Bechara, & Tranel, 2006; Anderson et al., 1999; Eslinger, Flaherty-Craig, & Benton, 2004; Eslinger, Grattan, Damasio, & Damasio, 1992; Vargha-Khadem, 2006), also results in increases in impulsivity and aggressivity. In addition, increased aggression is a hallmark characteristic of human psychiatric disorders, such as intermittent explosive disorder, antisocial personality disorder, and behavioral variant frontotemporal dementia that are thought to involve malfunction of the orbital frontal cortex (recently reviewed by Blair, Peschardt, Budhani, Mitchell, & Pine, 2006; Gansler et al., 2009). Social information processing theories posit that the increased aggression observed in these human disorders is related to a hypersensitivity to cues of threat and hostility that others emit, as well as an overly hostile intent attribution (Crick & Dodge, 1994; Lemerise & Arsenio, 2000). However, little is known about the visual attention in encoding of social information. A recent investigation tested the hypothesis that aggressive children attend more towards hostile cues than non-hostile cues using eye-tracking technology (Horsley, de Castro, & Van der Schoot, 2010). Interestingly, compared to low-aggressive children, high-aggressive children looked more to non-hostile cues and less to hostile cues, but did attribute more hostility to non-hostile cues than normal controls.

Our results are consistent with the behavior of the high-aggressive children characterized by Horsely and colleagues (2010). The increased attention to the eye regions exhibited by animals with neonatal orbitofrontal lesions indicated that, although they scanned the same social cues as sham-operated controls, they responded with aggression, which could indicate that they attributed hostile intent to the stimulus animals (regardless of valence vocalization). Thus, our findings suggest that damage to the orbital frontal cortex did not impair the encoding of social information, but did affect the representation of the information, which manifested behaviorally as increased aggression.

Previous research has shown that aggression and scanning behavior varies with fluctuations of gonadal hormones (Kalin, 1999; Lacreuse & Herndon, 2003;

Lacreuse et al., 2007; Mehlman et al., 1997; Wallen & Rupp, 2010; Wallen & Tannenbaum, 1997). Although not monitored in the current investigation, the consistency in looking behavior of males and females with lesions of the orbital frontal cortex, as well as their consistency across trial types, suggests that such fluctuations did not appear to affect scanning strategies of animals with orbitofrontal damage. However, considerable variability was seen in the sham-operated controls (Payne & Bachevalier, in preparation). Even though the extended testing period (28-42 days) likely minimized the contribution of hormonal levels to the current findings, we cannot exclude the possibility that hormonal fluctuations contributed to the variability in the looking behavior of sham-operated monkeys. The effect of orbitofrontal damage on cyclical fluctuations of gonadal hormones and the putative behavioral outcomes warrants further investigation.

Conclusions

In primate societies, the capacity to recognize and appropriately respond to social cues relies upon the ability to process audiovisual information. The present results suggest that the orbital frontal cortex is involved in crossmodal integration by balancing attention across the auditory and visual components, as well as the interpretation of complex social signals. Damage to this region resulted in an increased salience to the visual cues, especially to the eyes and at the expense of the auditory cues, and an increased aggressivity, which manifested as inflexibility in scanning strategies. Together, the results are consistent with a role of the orbital frontal cortex in the integration and interpretation of complex social signals broadcast over multiple sensory modalities.

Acknowledgements

This work has been supported by NICHD 35471; NIMH 58846; Yerkes Base Grant NIH RR00165; Center for Behavioral Neuroscience grant NSF IBN-9876754; The Robert W. Woodruff Health Sciences Center Fund, Inc., Emory University; NIMH T32-MH0732505; Autism Speaks Mentor-Based Predoctoral Fellowship Grant: 1657. We thank Asif Ghazanfar, Princeton University Neuroscience Institute Departments of Psychology and Ecology & Evolutionary Biology, for generously allowing us to use his stimuli, and Nancy Bliwise, Emory University Psychology Department, for her guidance on data analysis. We also thank Lisa Parr and Harold Gouzoules for their contributions to the experimental design.

Tables & Figures.

MSIII – Table 1: Extent of Lesion Assessment.

Intended aspiration damage to the orbital frontal cortex (Neo-O_{asp}) and unintended damage to adjacent areas. Mean: average damage for group; L%: percent damage in the left hemisphere; R%: percent damage in the right hemisphere; X%: average damage in both hemispheres; W%: weighted average damage to both hemispheres (W% = (L% * R%) / 100) (Hodos & Bobko, 1984); cytoarchitectonic subregions of the macaque frontal lobe and Ia: agranular insular areas as defined by Carmichael and Price (1995).

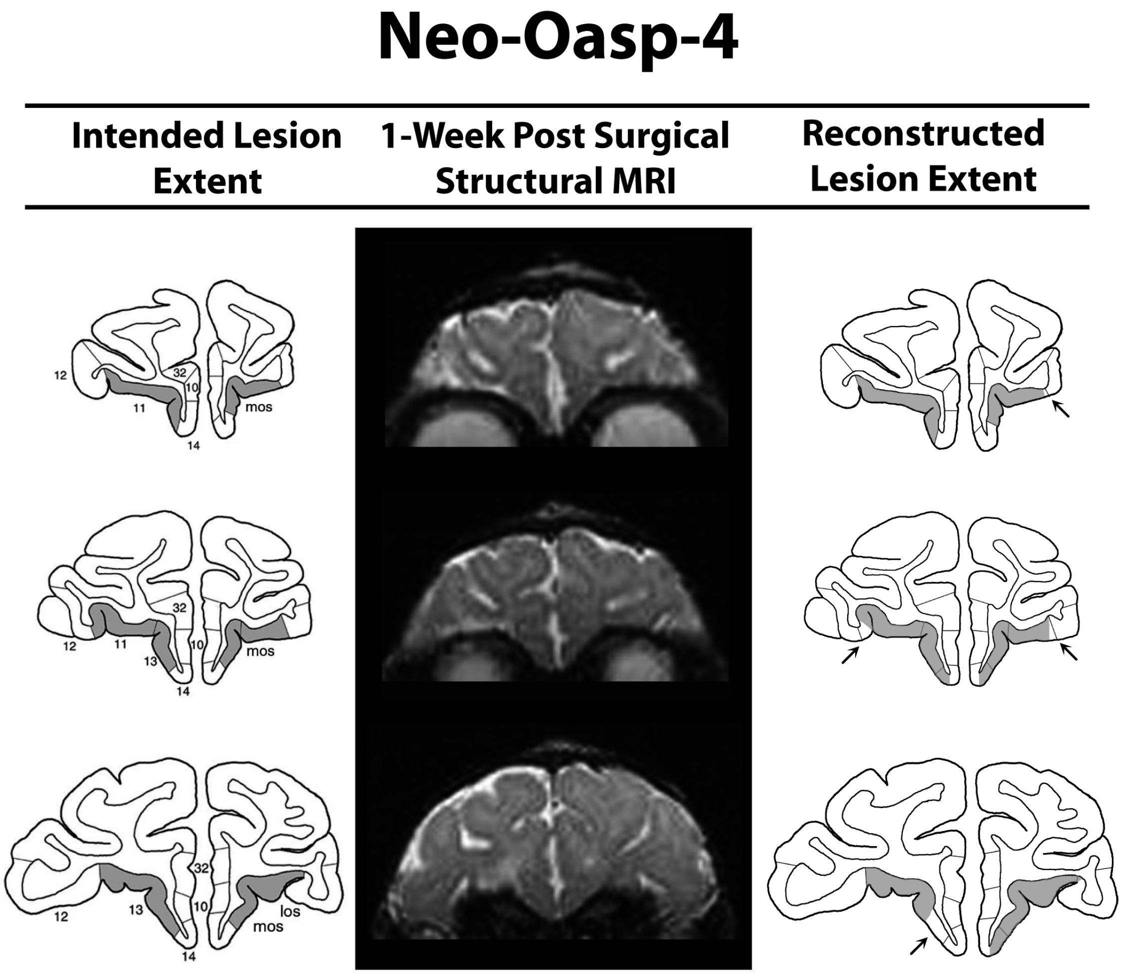
Cases	**Area 11**				**Area 13**			
	L	R	X%	W	L	R	X%	W
Neo-Oasp-1	80.5	92.7	86.6	74.7	93	73.5	83.3	68.4
Neo-Oasp-2	62.6	95.6	79.1	59.9	99.3	100	99.6	99.3
Neo-Oasp-3	84.1	93.9	89	79	87.3	95.6	91.4	83.4
Neo-Oasp-5	84	98.9	91.5	83.1	96.8	97.2	97	94.1
Mean	**77.8**	**95.3**	**86.5**	**74.2**	**94.1**	**91.6**	**92.8**	**86.3**

Cases	**Area 10**				Area 12			
	L	R	X%	W	L	R	X%	W
Neo-Oasp-1	0	0	0	0	40.2	11	25.6	4.4
Neo-Oasp-2	5.3	0	2.6	0	9.3	1.4	5.4	0.1
Neo-Oasp-3	0	0	0	0	22.3	21.6	22	4.8
Neo-Oasp-5	6.2	10.2	8.2	0.6	18.5	22.8	20.6	4.2
Mean	**2.9**	**2.6**	**2.7**	**0.2**	**18.6**	**12.2**	**15.4**	**2.7**

Cases	**Area 14**				**Ia**			
	L	R	X%	W	L	R	X%	W
Neo-Oasp-1	8	10.2	9.1	0.8	11.6	3.4	7.5	0.4
Neo-Oasp-2	31.9	6.8	19.4	2.2	78.5	57.7	68.1	45.3
Neo-Oasp-3	18.7	11.6	15.1	2.2	16.5	13.8	15.1	2.3
Neo-Oasp-5	6.5	11	8.5	0.7	87	67.8	77.4	59
Mean	**15**	**10.4**	**12.7**	**1.4**	**55.2**	**41.5**	**48.3**	**32.1**

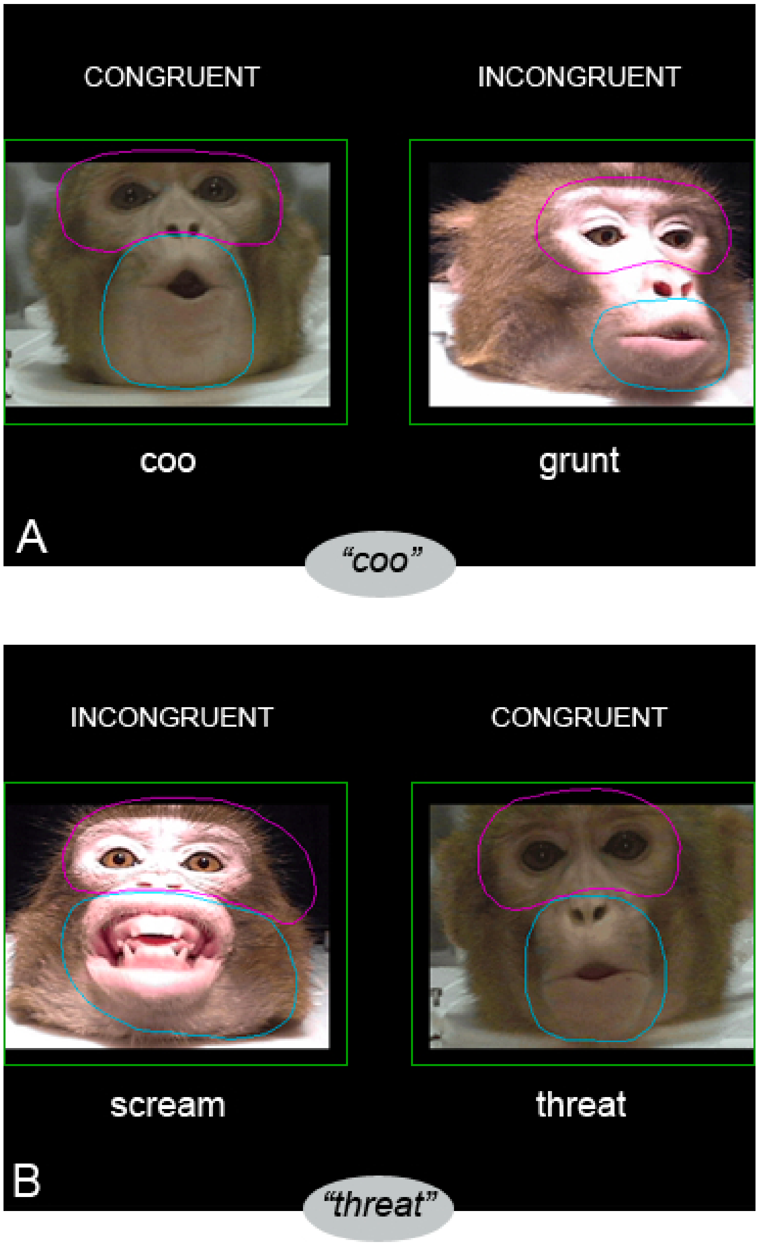
MSIII – Figure 1. Intended lesion and representative case.

Intended damage is shown in gray on coronal sections through the orbital frontal cortex of a normal infant macaque brain atlas (left column). Structural MR images (middle column) indicate extent of cortical damage. The estimated lesion extent is reconstructed on matched coronal sections of an infant brain (right column). Arrows point to areas of sparing of areas 11 and 13. Abbreviations: mos – medial orbital sulcus; los – lateral orbital sulcus; numbers refer to Brodmann areas (Brodmann, 1909/2006). Reproduced with permission (Kazama & Bachevalier, in preparation).



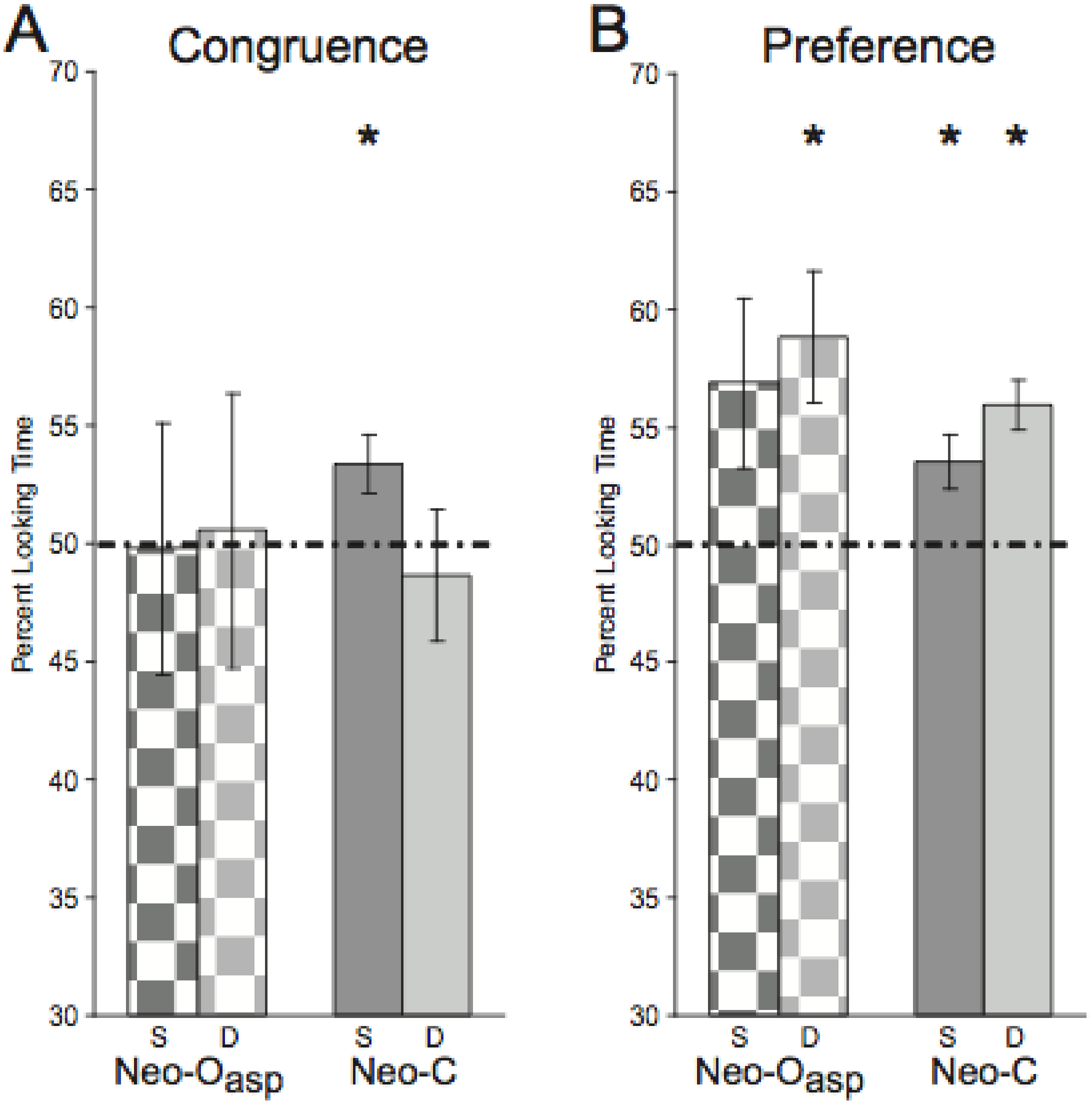
MSIII – Figure 2: Schematic of Stimulus Presentation with ROIs.

Screen shots of (A) coo-grunt and (B) scream-threat pairings with ROIs. In (A), the vocalization was a "coo" and in (B) the vocalization was a "threat". Stimulus sets were comprised of all possible combinations. Reproduced with permission (Payne & Bachevalier, in preparation).



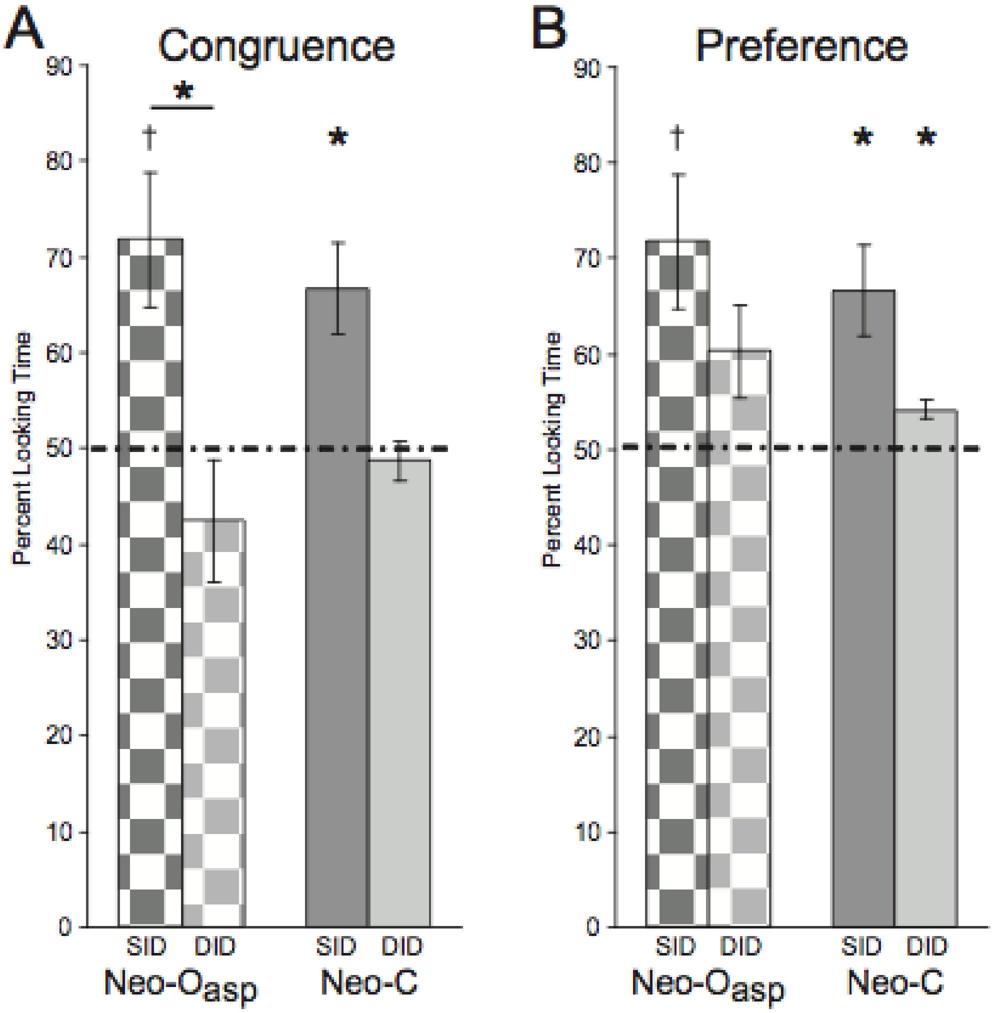
MSIII – Figure 3: Integration Assessment – All Trials.

Percentages of looking time (± sem) to (A) the congruent stimulus video and (B) the 'preferred' stimulus video in the Synchronized (S) and Desynchronized (D) conditions across all trials for group Neo-O_{asp} (checkered bars) and group Neo-C (solid bars). The dashed line represents chance level of 50%. In panel (A), values greater than 50% represent greater looking to the congruent video and values less than 50% represent greater looking to the incongruent video. (*) $p < 0.05$.



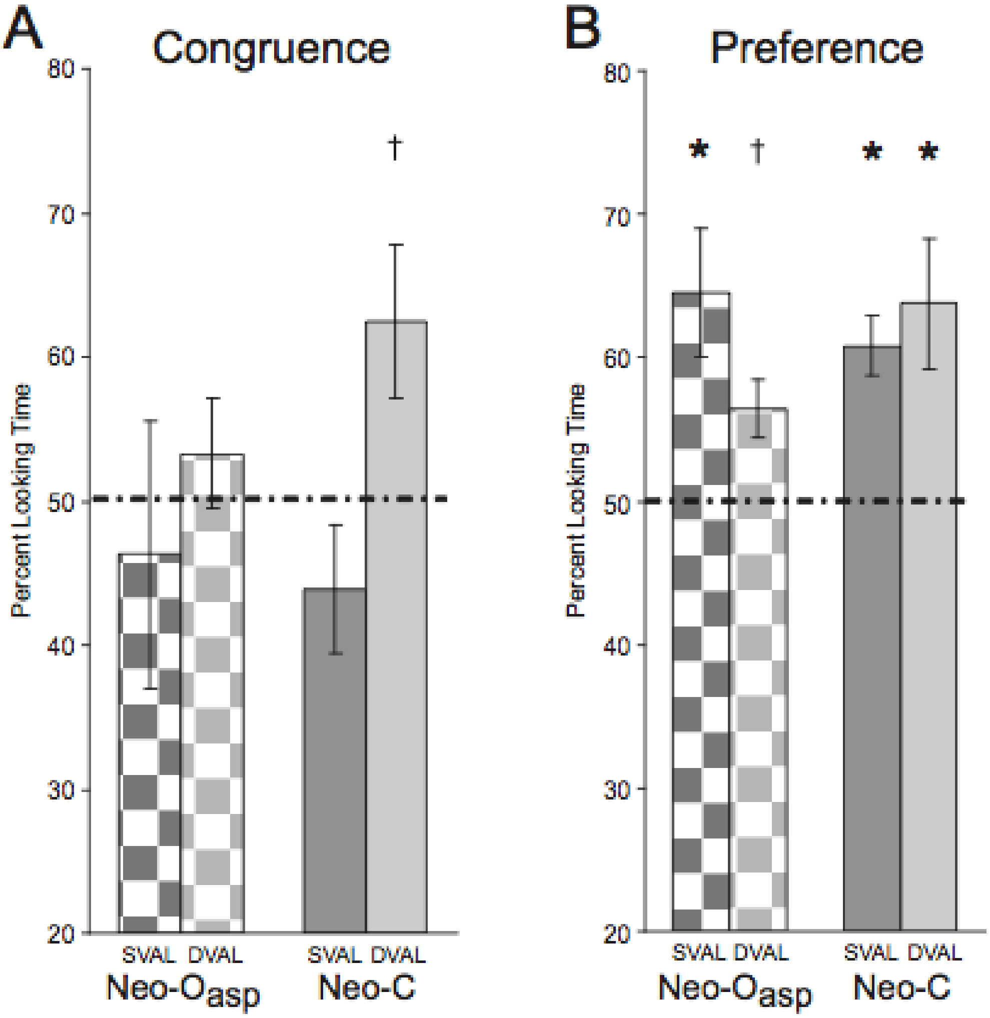
MSIII – Figure 4: Integration Assessment – Relative Identity.

Percentages of looking time (± sem) to (A) the congruent stimulus video and (B) the 'preferred' stimulus video in the Synchronized condition across Identity Same (SID) and Identity Different trials (DID) for group Neo-O$_{asp}$ (checkered bars) and group Neo-C (solid bars). The dashed line represents chance level of 50%. In panel (A), values greater than 50% represent greater looking to the congruent video and values less than 50% represent greater looking to the incongruent video. (*) $p < 0.05$; (†) $p \leq 0.075$.



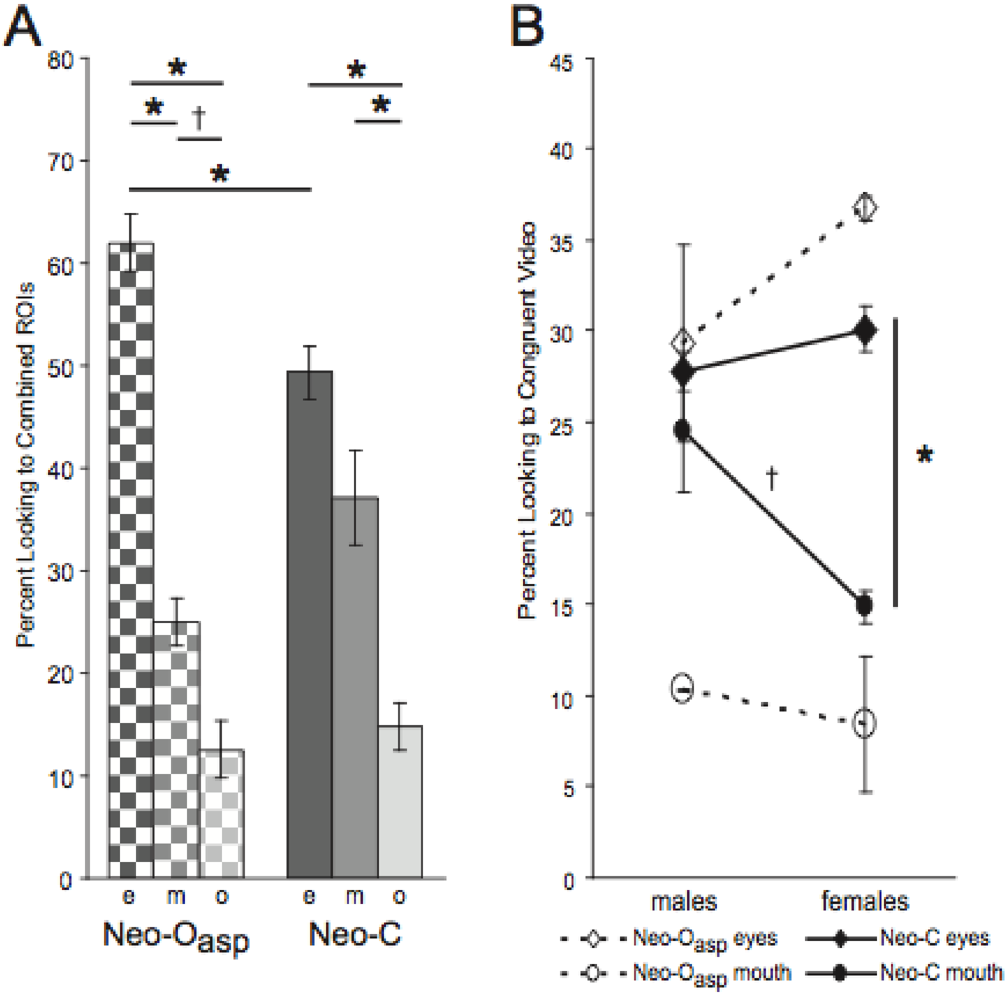
MSIII – Figure 5: Integration Assessment – Relative Valence.

Percentages of looking time (± sem) to (A) the congruent stimulus video and (B) the 'preferred' stimulus video in the Synchronized condition across Valence Same (SVAL) and Valence Different (DVAL) trials for group Neo-O$_{asp}$ (checkered bars) and group Neo-C (solid bars). The dashed line represents chance level of 50%. In panel (A), values greater than 50% represent greater looking to the congruent video and values less than 50% represent greater looking to the incongruent video. (*) $p < 0.05$; (†) $p \leq 0.075$.



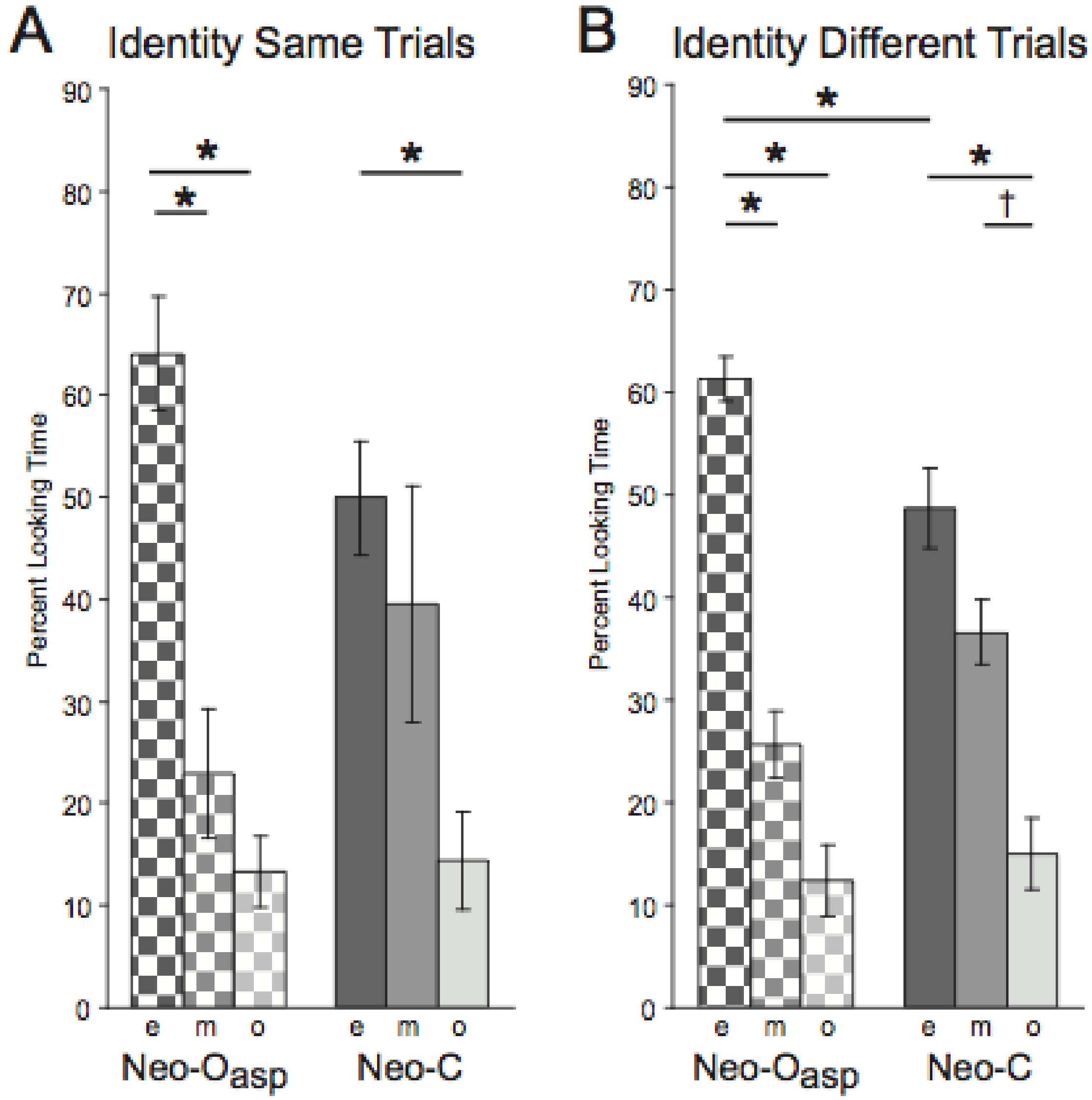
MSIII – Figure 6: Scanning Patterns – All Trials.

(A) Percentage of looking time (± sem) to the eyes (e), mouth (m), and other (o) for both the congruent and incongruent stimuli combined in groups Neo-O_{asp} (checkered bars) and Neo-C (solid bars). (B) Percentages of looking time (± sem) to the eyes (diamonds) and mouth (circles) of the congruent stimulus video for males and females of groups Neo-O_{asp} (dashed lines, open symbols) and Neo-C (solid lines, filled symbols). (*) $p \leq 0.05$; (†) $p \leq 0.08$.



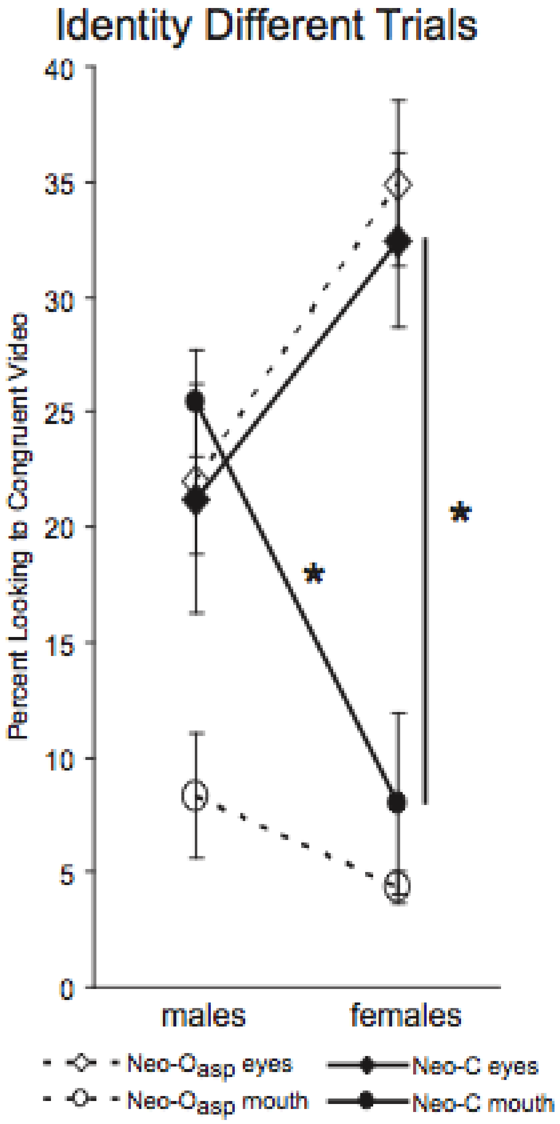
MSIII – Figure 7: Scanning Patterns – Identity Trials.

Percentages of looking time (± sem) to the eyes (e), mouth (m), and other (o) for the congruent and incongruent stimuli combined in (A) Identity Same trials and (B) Identity Different trials for groups Neo-O$_{asp}$ (checkered bars) and Neo-C (solid bars). (*) $p \leq 0.05$, $p \leq 0.08$.



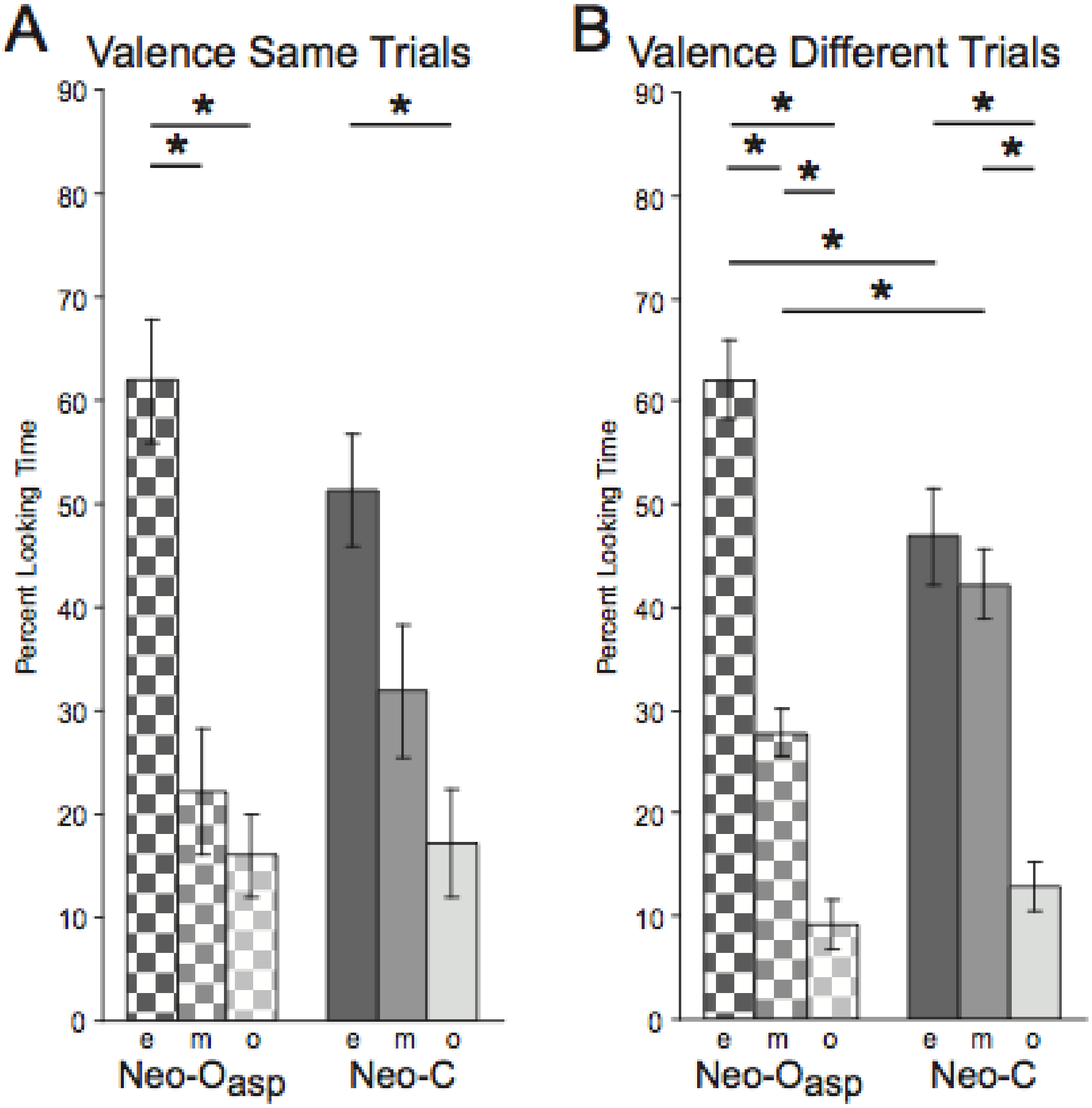
MSIII – Figure 8: Sex Differences – Identity Different Trials.

Percentages of looking time (± sem) to the eyes (diamonds) and mouth (circles) of the congruent stimulus video in Identity Different trials for males and females of groups Neo-O_{asp} (dashed lines, open symbols) and Neo-C (solid lines, filled symbols). (*) $p \leq 0.05$.



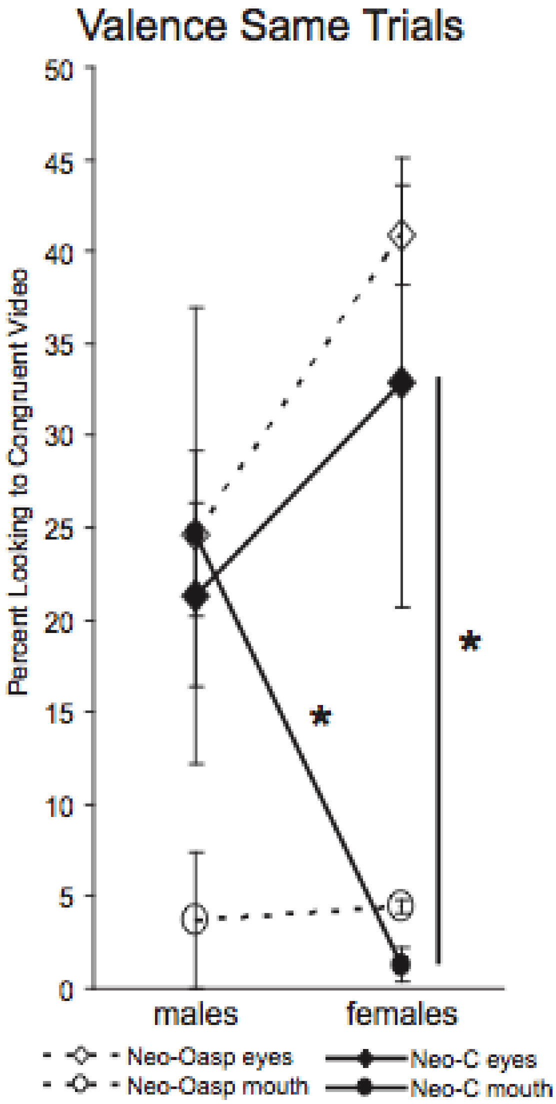
MSIII – Figure 9: Scanning Patterns – Valence Trials.

Percentage of looking time (± sem) to the eyes (e), mouth (m), and other (o) for congruent and incongruent stimuli combined in (A) Valence Same trials and (B) Valence Different trials for groups Neo-O$_{asp}$ (checkered bars) and Neo-C (solid bars). (*) $p \leq 0.05$.



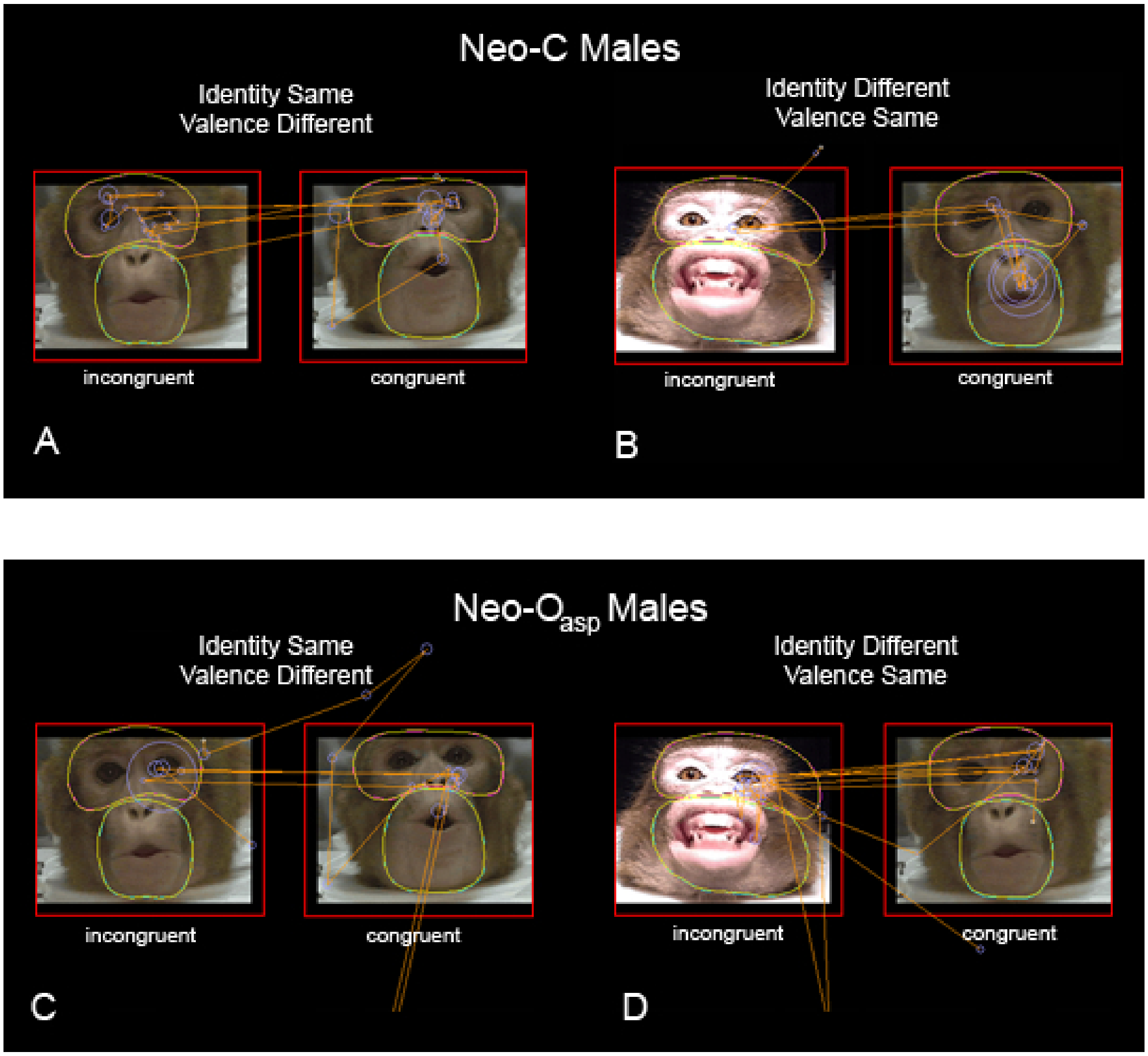
MSIII – Figure 10: Sex Differences – Valence Same Trials.

Percentages of looking time (± sem) to the eyes (diamonds) and mouth (circles) of the congruent stimulus video in Valence Same trials for males and females of groups Neo-O_{asp} (dashed lines, open symbols) and Neo-C (solid lines, filled symbols). (*) $p \le 0.05$.



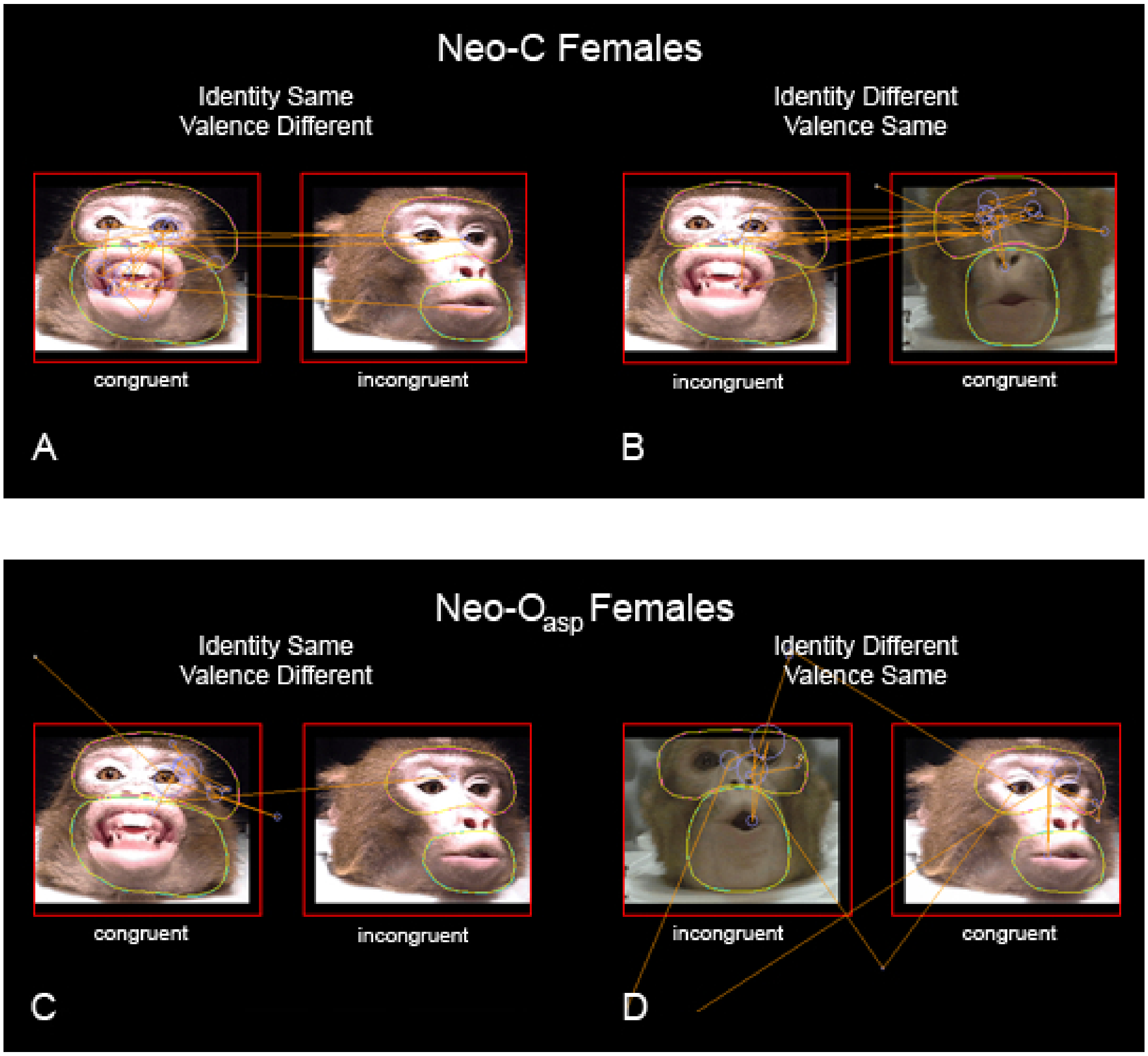
MSIII – Figure 11: Representative Scan Paths – Males.

Neo-C male scan path on an Identity Same / Valence Different Trial (A) and on an Identity Different / Valence Same Trial (B). Neo-O_{asp} male scan path on Identity Same / Valence Different Trial (C) and on Identity Different / Valence Same Trial (D). Circles represent fixation points with the size corresponding with fixation duration. Note: Panels (A) and (B) were reproduced with permission (from Fig 11 in Payne & Bachevalier, in preparation).



MSIII – Figure 12: Representative Scan Paths – Females.

Neo-C female scan path on (A) Identity Same / Valence Different Trial and on (B) Identity Different / Valence Same Trial. Neo-O$_{asp}$ female scan path on (C) Identity Same / Valence Different Trial and on (D) Identity Different / Valence Same Trial. Circles represent fixation points with the size corresponding with fixation duration. Note: Panels (A) and (B) were reproduced with permission (from Fig 11 in Payne & Bachevalier, in preparation).



References

Altmann, S. A. (1962). A field study of the sociobiology of rhesus monkeys, Macaca mulatta. *Ann N Y Acad Sci, 102*, 338-435.

Anderson, S. W., Aksan, N., Kochanska, G., Damasio, H., Wisnowski, J., & Afifi, A. (2007). The earliest behavioral expression of focal damage to human prefrontal cortex. *Cortex, 43*(6), 806-816.

Anderson, S. W., Barrash, J., Bechara, A., & Tranel, D. (2006). Impairments of emotion and real-world complex behavior following childhood- or adult-onset damage to ventromedial prefrontal cortex. *J Int Neuropsychol Soc, 12*(2), 224-235.

Anderson, S. W., Bechara, A., Damasio, H., Tranel, D., & Damasio, A. R. (1999). Impairment of social and moral behavior related to early damage in human prefrontal cortex. *Nat Neurosci, 2*(11), 1032-1037.

Bachevalier, J., & Loveland, K. A. (2006). The orbitofrontal-amygdala circuit and self-regulation of social-emotional behavior in autism. *Neurosci.Biobehav.Rev., 30*(1), 97-117.

Bachevalier, J., Machado, C. J., & Kazama, A. M. (2011). *Behavioral outcomes of late-onset or early-onset orbital frontal cortex (areas 11/13) lesions in rhesus monkeys*. Paper presented at the New York Acadamy of Sciences.

Barbas, H. (1988). Anatomic organization of basoventral and mediodorsal visual recipient prefrontal regions in the rhesus monkey. *J Comp Neurol, 276*(3), 313-342.

Barbas, H. (1993). Organization of cortical afferent input to orbitofrontal areas in the rhesus monkey. *Neuroscience, 56*(4), 841-864.

Barbas, H., & Blatt, G. J. (1995). Topographically specific hippocampal projections target functionally distinct prefrontal areas in the rhesus monkey. *Hippocampus, 5*(6), 511-533.

Barbas, H., & Pandya, D. N. (1989). Architecture and intrinsic connections of the prefrontal cortex in the rhesus monkey. *J Comp Neurol, 286*(3), 353-375.

Bechara, A., Damasio, H., & Damasio, A. R. (2000). Emotion, decision making and the orbitofrontal cortex. *Cereb Cortex, 10*(3), 295-307.

Blair, R. J., Morris, J. S., Frith, C. D., Perrett, D. I., & Dolan, R. J. (1999). Dissociable neural responses to facial expressions of sadness and anger. *Brain, 122 (Pt 5),* 883-893.

Blair, R. J., Peschardt, K. S., Budhani, S., Mitchell, D. G., & Pine, D. S. (2006). The development of psychopathy. *J Child Psychol Psychiatry, 47*(3-4), 262-276.

Brodmann, K. (1909/2006). *Brodmann's localisation in the cerebral cortex: The principles of comparative localisation in the cerebral cortex based on cytoarchitectonics* (L. Garey, Trans. 3rd ed.). New York: Springer Science + Business Media, Inc.

Buchan, J. N., Pare, M., & Munhall, K. G. (2008). The effect of varying talker identity and listening conditions on gaze behavior during audiovisual speech perception. *Brain Res, 1242*, 162-171.

Carmichael, S. T., & Price, J. L. (1995). Limbic connections of the orbital and medial prefrontal cortex in macaque monkeys. *J.Comp Neurol., 363*, 615-641.

Crick, N., & Dodge, K. (1994). A review and reformulation of social information-processing mechanisms in children's social adjustment. *Psychol Bull, 115*(1), 74-101.

Dalton, K. M., Nacewicz, B. M., Johnstone, T., Schaefer, H. S., Gernsbacher, M. A., Goldsmith, H. H., et al. (2005). Gaze fixation and the neural circuitry of face processing in autism. *Nat.Neurosci., 8*, 519-526.

Dixon, N. F., & Spitz, L. (1980). The detection of auditory visual desynchrony. *Perception, 9*(6), 719-721.

Eslinger, P. J., Flaherty-Craig, C. V., & Benton, A. L. (2004). Developmental outcomes after early prefrontal cortex damage. *Brain Cogn, 55*(1), 84-103.

Eslinger, P. J., Grattan, L. M., Damasio, H., & Damasio, A. R. (1992). Developmental consequences of childhood frontal lobe damage. *Arch Neurol, 49*(7), 764-769.

Gansler, D. A., McLaughlin, N. C., Iguchi, L., Jerram, M., Moore, D. W., Bhadelia, R., et al. (2009). A multivariate approach to aggression and the orbital frontal cortex in psychiatric patients. *Psychiatry Res, 171*(3), 145-154.

Ghazanfar, A. A., & Logothetis, N. K. (2003). Neuroperception: facial expressions linked to monkey calls. *Nature, 423*(6943), 937-938.

Gorno-Tempini, M. L., Pradelli, S., Serafini, M., Pagnoni, G., Baraldi, P., Porro, C., et al. (2001). Explicit and incidental facial expression processing: an fMRI study. *Neuroimage, 14*(2), 465-473.

Goursaud, A. P., & Bachevalier, J. (2007). Social attachment in juvenile monkeys with neonatal lesion of the hippocampus, amygdala and orbital frontal cortex. *Behav Brain Res, 176*(1), 75-93.

Goursaud, A. P., & Bachevalier, J. (in preparation). Face processing and emotion regulation are altered by orbitofrontal cortex lesion in adult monkeys (Macaca mulatta).

Gundersen, H. J., & Jensen, E. B. (1987). The efficiency of systematic sampling in stereology and its prediction. *Journal of Microscopy, 147*, 229-263.

Hackett, T. A., Stepniewska, I., & Kaas, J. H. (1999). Prefrontal connections of the parabelt auditory cortex in macaque monkeys. *Brain Res, 817*(1-2), 45-58.

Hinde, R., & Roswell, T. (1962). Communication by postures and facial expressions in the rhesus monkey (Macaca mulatta). *Proc Roy Soc Lon B, 138*.

Hodos, W., & Bobko, P. (1984). A weighted index of bilateral brain lesions. *J Neurosci Methods, 12*(1), 43-47.

Hornak, J., Bramham, J., Rolls, E. T., Morris, R. G., O'Doherty, J., Bullock, P. R., et al. (2003). Changes in emotion after circumscribed surgical lesions of the orbitofrontal and cingulate cortices. *Brain, 126*(Pt 7), 1691-1712.

Hornak, J., Rolls, E. T., & Wade, D. (1996). Face and voice expression identification in patients with emotional and behavioural changes following ventral frontal lobe damage. *Neuropsychologia, 34*(4), 247-261.

Horsley, T. A., de Castro, B. O., & Van der Schoot, M. (2010). In the eye of the beholder: eye-tracking assessment of social information processing in aggressive behavior. *J Abnorm Child Psychol, 38*(5), 587-599.

Izquierdo, A., Suda, R. K., & Murray, E. A. (2005). Comparison of the effects of bilateral orbital prefrontal cortex lesions and amygdala lesions on emotional responses in rhesus monkeys. *J Neurosci, 25*(37), 8534-8542.

Kalin, N. H. (1999). Primate models to understand human aggression. *J Clin Psychiatry, 60 Suppl 15*, 29-32.

Kazama, A., Glavis-Bloom, C., & Bachevalier, J. (2008). *Neonatal amygdala and orbital frontal cortex lesions disrupt flexible decision-making in adult macaques*. Paper presented at the Society for Neuroscience.

Kazama, A. M., & Bachevalier, J. (in preparation). Normal maturation of object reversal learning after selective neonatal damage of amygdala and orbital frontal areas 11 & 13 in monkeys.

Klopp, J., Marinkovic, K., Chauvel, P., Nenov, V., & Halgren, E. (2000). Early widespread cortical distribution of coherent fusiform face selective activity. *Hum Brain Mapp, 11*(4), 286-293.

Lacreuse, A., & Herndon, J. G. (2003). Estradiol selectively affects processing of conspecifics' faces in female rhesus monkeys. *Psychoneuroendocrinology, 28*(7), 885-905.

Lacreuse, A., Martin-Malivel, J., Lange, H. S., & Herndon, J. G. (2007). Effects of the menstrual cycle on looking preferences for faces in female rhesus monkeys. *Anim Cogn, 10*(2), 105-115.

Lansing, C. R., & McConkie, G. W. (1999). Attention to facial regions in segmental and prosodic visual speech perception tasks. *J Speech Lang Hear Res, 42*(3), 526-539.

Lemerise, E. A., & Arsenio, W. F. (2000). An integrated model of emotion processes and cognition in social information processing. *Child Dev, 71*(1), 107-118.

Loveland, K., Pearson, D., & Reddoch, S. (2005). *Anxiety symptoms in childrem and adolescents with autism*. Paper presented at the International Meeting for Autism Research.

Loveland, K. A., Steinberg, J. L., Pearson, D. A., Mansour, R., & Reddoch, S. (2008). Judgments of auditory-visual affective congruence in adolescents with and without autism: a pilot study of a new task using fMRI. *Percept Mot Skills, 107*(2), 557-575.

Machado, C. J., & Bachevalier, J. (2006). The impact of selective amygdala, orbital frontal cortex, or hippocampal formation lesions on established social relationships in rhesus monkeys (Macaca mulatta). *Behav Neurosci, 120*(4), 761-786.

Mehlman, P. T., Higley, J. D., Fernald, B. J., Sallee, F. R., Suomi, S. J., & Linnoila, M. (1997). CSF 5-HIAA, testosterone, and sociosexual behaviors in free-ranging male rhesus macaques in the mating season. *Psychiatry Res, 72*(2), 89-102.

Mitchell, R. L., Elliott, R., Barry, M., Cruttenden, A., & Woodruff, P. W. (2003). The neural response to emotional prosody, as revealed by functional magnetic resonance imaging. *Neuropsychologia, 41*(10), 1410-1421.

Morecraft, R. J., Geula, C., & Mesulam, M. M. (1992). Cytoarchitecture and neural afferents of orbitofrontal cortex in the brain of the monkey. *J Comp Neurol., 323*(3), 341-358.

Morris, J. S., Friston, K. J., & Dolan, R. J. (1998). Experience-dependent modulation of tonotopic neural responses in human auditory cortex. *Proc Biol Sci, 265*(1397), 649-657.

Murray, E. A. (2007). The amygdala, reward and emotion. *Trends Cogn Sci, 11*(11), 489-497.

Nakamura, K., Kawashima, R., Ito, K., Sugiura, M., Kato, T., Nakamura, A., et al. (1999). Activation of the right inferior frontal cortex during assessment of facial emotion. *J Neurophysiol, 82*(3), 1610-1614.

O'Scalaidhe, S., Wilson, F. A., & Goldman-Rakic, P. S. (1997). Areal segregation of face-processing neurons in prefrontal cortex. *Science, 278*(5340), 1135-1138.

O'Scalaidhe, S. P., Wilson, F. A., & Goldman-Rakic, P. S. (1999). Face-selective neurons during passive viewing and working memory performance of rhesus monkeys: evidence for intrinsic specialization of neuronal coding. *Cereb Cortex, 9*(5), 459-475.

Pandya, D. N., & Kuypers, H. G. (1969). Cortico-cortical connections in the rhesus monkey. *Brain Research, 13*(1), 13-36.

Payne, C., & Bachevalier, J. (in preparation). *Crossmodal integration of conspecific vocalizations in rhesus macaques.*Unpublished manuscript.

Raper, J., Kazama, A., & Bachevalier, J. (2009). *Blunted fear reactivity after neonatal amygdala and orbital frontal lesions in rhesus monkeys.* Paper presented at the Society for Neuroscience.

Raper, J., Wilson, M., Sanchez, M., & Bachevalier, J. (2010). *Neonatal orbital frontal lesions after basal and stress reactive cortisol levels in adult rhesus monkeys.* Paper presented at the Neurobiology of Stress Workshop.

Rolls, E. T. (1999). *The brain and emotion.* Oxford, England: Oxford University Press.

Rolls, E. T. (2000). The orbitofrontal cortex and reward. *Cereb.Cortex, 10*, 284-294.

Rolls, E. T., Critchley, H. D., Browning, A. S., & Inoue, K. (2006). Face-selective and auditory neurons in the primate orbitofrontal cortex. *Exp Brain Res, 170*(1), 74-87.

Romanski, L. M., & Goldman-Rakic, P. S. (2002). An auditory domain in primate prefrontal cortex. *Nat Neurosci, 5*(1), 15-16.

Romanski, L. M., Tian, B., Fritz, J., Mishkin, M., Goldman-Rakic, P. S., & Rauschecker, J. P. (1999). Dual streams of auditory afferents target multiple domains in the primate prefrontal cortex. *Nat.Neurosci., 2*(12), 1131-1136.

Saunders, R. C., Aigner, T. G., & Frank, J. A. (1990). Magnetic resonance imaging of the rhesus monkey brain: use for stereotactic neurosurgery. *Exp Brain Res, 81*(2), 443-446.

Schoenbaum, G., & Esber, G. R. (2010). How do you (estimate you will) like them apples? Integration as a defining trait of orbitofrontal function. *Curr Opin Neurobiol, 20*(2), 205-211.

Seltzer, B., & Pandya, D. N. (1989). Intrinsic connections and architectonics of the superior temporal sulcus in the rhesus monkey. *J.Comp Neurol., 290*(4), 451-471.

Sprengelmeyer, R., Rausch, M., Eysel, U. T., & Przuntek, H. (1998). Neural structures associated with recognition of facial expressions of basic emotions. *Proc Biol Sci, 265*(1409), 1927-1931.

Vargha-Khadem, F. (2006). Socio-emotional impairments following lesions of orbital frontal cortex in human infants. In J. Bachevalier (Ed.).

Wallen, K., & Rupp, H. A. (2010). Women's interest in visual sexual stimuli varies with menstrual cycle phase at first exposure and predicts later interest. *Horm Behav, 57*(2), 263-268.

Wallen, K., & Tannenbaum, P. L. (1997). Hormonal modulation of sexual behavior and affiliation in rhesus monkeys. *Ann N Y Acad Sci, 807*, 185-202.

Webster, M. J., Bachevalier, J., & Ungerleider, L. G. (1994). Connections of inferior temporal areas TEO and TE with parietal and frontal cortex in macaque monkeys. *Cereb.Cortex, 4*(5), 470-483.

GENERAL DISCUSSION

The primary goal of this project was to investigate the neural substrates of audiovisual integration of affective information in rhesus macaques. Primate societies, including humans and rhesus monkeys, depend upon complex signals, broadcast through multiple sensory modalities, the most common being vision (facial expressions) and audition (vocalizations). And although processing emotional content has been extensively research for each modality, individually, little is known about the neuroanatomical structures critical for the integration of affective information across modalities. Identifying the brain regions critical for emotional crossmodal integration would enhance our general understanding of primate social cognition, and provide valuable insights into human neuropsychological disorders such as autism, pervasive developmental disorder, and schizophrenia, all of which present with deficits in integrating affective information from multiple modalities (de Gelder, Vroomen, Annen, Masthof, & Hodiamont, 2003; de Jong, Hodiamont, & de Gelder, 2010; Hobson, Ouston, & Lee, 1988; Loveland et al., 2008; Magnee, de Gelder, van Engeland, & Kemner, 2008).

The amygdala and orbital frontal cortex are two heteromodal areas that receive emotional information from both auditory and visual modalities (Barbas, 1988, 1993; Barbas & Blatt, 1995; McDonald, 1998; Pandya & Kuypers, 1969; Webster et al., 1994; Yukie, 2002). Human neuroimaging studies have proposed involvement of the amygdala and orbital frontal cortex in emotional crossmodal integration. However, the unique roles of each region in processing affective, audiovisual signals are not fully understood. Thus, we used a nonhuman primate neonatal lesion model to characterize the distinctive contributions of the amygdala and orbital frontal cortex to the integration of multimodal socio-emotional information, and to determine whether either region is necessary for the development of normal, species-specific behavior.

Using dynamic stimuli in a previously validated preferential viewing paradigm and eye tracking technology, we predicted that a) nursery-reared rhesus macaques would demonstrate crossmodal integration of species-specific vocalizations that was not reliant on the mechanical properties of the auditory and visual components, and that was associated with a robust preference for the eye regions over the mouths and rest of stimulus videos; b) animals with neonatal damage to the amygdala would exhibit impairments in crossmodal integration associated with decreased attention to the eye regions and increased attention to the mouth regions; and c) animals with neonatal lesions of the orbital frontal cortex would show deficits in crossmodal integration associated with increased looking to the eye regions. As summarized in Table 1, the current project revealed some intriguing group differences, although not all were consistent with our predictions.

1- How do rhesus macaques integrate crossmodal social signals?

A) Integration and scanning patterns

First, consistent with our hypotheses, surrogate nursery-reared adult rhesus macaques spontaneously integrated the auditory and visual components of complex social cues emitted by novel conspecific males, demonstrated by a preference for congruence in the Synchronized condition. As expected, a preference was also observed in the Desynchronized condition, indicating that integration ability was not dependent on the temporal coincidence of mouth movements with the rhythm of the acoustic cues. Also as predicted (Gothard, Brooks, & Peterson, 2009; Gothard, Erickson, & Amaral, 2004; Guo et al., 2003; reviewed by Parr, 2011), preference in the Synchronized condition was associated with greater exploration of the eyes than either the mouth regions or the rest of the stimulus videos and animals used the eye regions to distinguish the two videos (i.e., congruent eyes > incongruent eyes). However, an interesting sex difference was noted in the relative looking to the eye and mouth regions of the vocalizing stimulus animal (i.e., congruent stimulus video). That is, whereas females showed the expected pattern of longer looking to the eyes than the mouth, males looked equally to the two regions. This distinction was attributed to differential looking to the mouth regions (males > females), and not to differences in looking to the eyes (males = females).

The pattern of attending more to the eyes than the mouth regions exhibited by female rhesus macaques paralleled the looking behavior of human subjects instructed to focus on emotion-related cues (e.g., prosody) or make social judgments (Buchan, Pare, & Munhall, 2008; Lansing & McConkie, 1999), suggesting that female rhesus macaques attended to the valence of the vocalizations. By contrast, the equal looking to the eye and mouth regions exhibited by male rhesus macaques, in conjunction with males attending to the eyes as much as females indicated that males appeared to be attending valence as well as to another stimulus factor. Post-hoc analyses on the stimulus parameters of Identity and Valence further elucidated this sex difference. Interestingly, the relative identity of the stimulus animals (same or different) influenced the scanning patterns of male rhesus macaques. Males looked equally to the eyes and mouths in trials comprised of two different stimulus animals but looked more to the eyes than the mouth when the videos were of the same stimulus monkey. By contrast, the relative valence of the vocalizations (same or different) influenced the scanning patterns of female rhesus macaques. Females differentiated the eyes and mouth when the vocalizations had the same general valences, but looked equally to the regions when the vocalizations carried disparate valence.

The distinctive scanning patterns of sham-operated males and females suggest that they may have attended to different aspects of the complex socio-emotional signals. Because such differences are important to consider when characterizing behavioral deficits within a population (whether it be human subjects with neuropsychiatric disorders or individuals with circumscribed neural damage), as well as when identifying neural substrates of social behavior, it is necessary to consider how these distinctive male and female patterns of assessing social signals may likely relate to rhesus macaque social structures.

B) Species-specific scanning patterns

Rhesus monkeys have a matriarchal hierarchy (de Waal, 1996). The status of an individual female is determined by the rank of her mother. Families (including pre-pubertal males) are organized into a stable, linear dominance hierarchy of matrilines that determines priority of access to resources. Whereas females acquire the rank from their mothers, adult males have to earn their dominance status. As a male matures into adulthood, he leaves his birth troop and temporarily joins a bachelor group comprised of males from other troops before integrating into another established troop. Accordingly, males must establish and maintain their rank within the hierarchy through encounters (both aggressive and affiliative) with other monkeys. For males, dominance status is largely associated with physical stature. High-ranking males tend to have larger, more muscular builds than lesser-ranked males. High-ranking males also tend to have larger canine teeth. Thus, an estimation of the dominance status of an individual can be deduced from visual cues such as musculature and canine size.

As described above, male and female behavior is subject to different ethologic pressures. These societal differences likely influence how males and females react when presented with videos of unfamiliar males emitting agonistic and affiliative social signals. When male rhesus macaques encounter novel males, their initial assessment may focus on the relative dominance status of the new individuals. However, when exposure of the unfamiliar conspecifics is limited to the head region, as in the current investigation, the observer could use information from the mouth regions to infer characteristics such as age and relative dominance. Accordingly, our findings suggest that when presented with unknown, male conspecifics emitting antagonistic and affiliative socio-emotional cues, the female rhesus macaques preferentially attended to valence of the social signal (demonstrated by greater looking to the eyes than the mouth). Conversely, the male rhesus macaques appeared to be more concerned with dominance status of the individual broadcasting the signal (demonstrated by equal looking to the eyes and mouth).

Obviously, these findings should be considered preliminary with regards to defining ‘species-typical’ behavior in processing audiovisual socio-emotional cues, in that they reflect the behavior of three male and three female nursery-reared monkeys. Nonetheless, they are ethologically compelling and indicate that further exploration of sex differences within species-typical behavior is warranted (if not required). Providing further support for the legitimacy of the observed sex differences is the observation, discussed in the succeeding section, that damage to neural structures implicated in the evaluation of socio-emotional stimuli appears to disrupt the apparent motivational differences across male and female scanning patterns.

2- Effects of neonatal amygdala and orbital frontal cortex

A) Amygdala lesions

Contrary to our hypothesis, animals with neonatal lesions of the amygdala were not impaired in emotional crossmodal integration. Nor was their integration ability dependent on the temporal coincidence of the auditory component with the movements of the mouth. Perhaps even more unexpected was the observation that when all trials were considered, animals with neonatal amygdala lesions exhibited the same general scanning patterns as sham-operated controls (i.e., preference for the eye regions). This characterization appears to contradict the extensive research on facial emotion perception in human and nonhuman primates that has repeatedly associated dysfunction of the amygdala with decreased looking to the eyes and increased looking to the mouth. However, a Group x Sex interaction in the scanning of the eyes and mouths revealed that neonatal lesions of the amygdala disrupted the sex-specific scanning behaviors.

Additionally, whereas sham-operated controls used the eye regions to distinguish the congruent and incongruent stimulus videos, monkeys with neonatal lesions of the amygdala used the mouth regions, indicating a general increased salience of the mouth regions for both males and females.

Collectively, these findings suggest that, although the amygdala is not required for the acquisition of the ability to integrate audiovisual social cues, this region is critically involved in processing these complex signals. The data speak to the putative role of the amygdala in recognizing the socio-emotional salience of social signals.

B) Effects of orbital frontal lesions

Consistent with our hypothesis, monkeys with neonatal lesions of the orbital frontal cortex were impaired in emotional crossmodal integration, but, contrary to our expectations, not entirely absent. Although monkeys with orbital frontal damage did not exhibit a preference when all trials of the Synchronized condition were considered, they did demonstrate integration ability in the Desynchronized condition. Analysis of scanning patterns provided further insight into the possible mechanisms subserving their impaired integration ability. As predicted, monkeys with damage to the orbital frontal cortex expressed an increased salience for the eye regions (i.e., looked more to the eye regions than sham-operated controls). This increased exploration of the eyes correlated with the extent of damage to the orbital frontal cortex (i.e., greater damage was associated with greater looking to the eyes).

This robust preference for the eye regions might have reflected increased aggression in these animals, which likely interfered with their attention to (or perception of) the auditory elements, resulting in impaired emotional crossmodal integration.

C) Comparisons of the neonatal amygdala and orbital frontal lesions.

Given that the amygdala and orbital frontal cortex appear to serve distinctive functions in processing audiovisual socio-emotional signals in rhesus macaques. Direct comparisons of the effects of amygdalar and orbitofrontal damage on scanning patterns are therefore necessary for a comprehensive discussion. Thus, the following section is devoted to statistical and qualitative comparisons of the looking behavior of adult monkeys with neonatal lesions of the either the amygdala (Neo-A_{ibo}) or orbital frontal cortex (Neo-O_{asp}), with references to the looking behavior of sham-operated controls (Neo-C).

<u>Integration Ability</u>: Figures D1 and D2 summarize the integration ability of all three groups across all trials (Fig D1) and the parameters of Identity and Valence (Fig D2). As detailed in Manuscript II, groups Neo-C and Neo-A_{ibo} exhibited general preferences in both the Synchronized and Desynchronized conditions and across all trial parameters. By contrast, Manuscript III revealed that, when all trials were considered, group Neo-O_{asp} showed a preference only in the Desynchronized condition. Post-hoc analyses revealed that group Neo-O_{asp} demonstrated integration ability in both Valence parameters and Identity Same trials, but not Identity Different trials. Thus, group Neo-O_{asp} not showing an overall preference in the Synchronized condition was driven by a disruption of

integration ability when presented with two different stimulus animals. The results together indicate that integration ability was spared for group Neo-A_{ibo}, but slightly impaired for group Neo-O_{asp}. Despite the qualitative differences between groups Neo-A_{ibo} and Neo-O_{asp}, there were no statistically significant group differences in preference strength for any trial parameter. However, comparisons of scanning strategies indicate some interesting differences in the ways groups Neo-A_{ibo} and Neo-O_{asp} explored the bimodal, socio-emotional stimuli.

Scanning Strategies: When all trials were considered, the scanning patterns of groups Neo-A_{ibo} and Neo-O_{asp} differed from group Neo-C, albeit in qualitatively different ways. Please note that, as in Manuscript III, there were no Stimulus Video x Group interactions at any region of interest. Hence, the following comparisons were between the collapsed regions of interest (e.g., congruent eyes + incongruent eyes). Figure D3 shows that all three groups exhibit the same general scanning pattern (eyes > mouth; eyes > other; mouth > other). Accordingly, the difference in attention for the eye regions reported between group Neo-C and Neo-O_{asp} (Neo-O_{asp} > Neo-C) was also observed between the two lesion groups. Group Neo-O_{asp} showed a significantly greater interest in the eye regions than group Neo-A_{ibo} ($F(1,8) = 9.063$, $p = 0.017$).

Similar distinctions in the general scanning patterns of groups Neo-A_{ibo} and Neo-O_{asp} were also seen in the post-hoc analyses on the parameters of Identity and Valence. As shown in Figure D4, group Neo-A_{ibo} looked less to the eyes than Neo-O_{asp} in all four trial types (Identity Same: $F(1,8) = 6.224$, $p = 0.037$; Identity Different: $F(1,8) = 5.235$, $p = 0.051$; Valence Same: $F(1,8) = 5.427$, $p = 0.048$;

Valence Different: F(1,8) = 13.594, *p* = 0.006). An additional distinction was seen in the mouth region for Identity Same trials, with group Neo-A$_{ibo}$ looking more to the mouth than group Neo-O$_{asp}$ (F(1,8) = 4.280, *p* = 0.072).

As detailed in Manuscripts II and III, neonatal lesions of the amygdala and orbital frontal cortex each disrupted the sex differences displayed by sham-operated controls. Although small group sizes preclude the direct of assessment of sex differences between groups Neo-A$_{ibo}$ and Neo-O$_{asp}$, Figure D5 illustrates striking variability in the relative looking to the eye and mouth regions across groups when all trials were considered. Comparisons between groups Neo-C and Neo-A$_{ibo}$ revealed differences in both males and females, with the sexes exhibiting the exact opposite viewing patterns across group (Neo-C males/Neo-A$_{ibo}$ females: eyes = mouth; Neo-C females/Neo-A$_{ibo}$ males: eyes > mouth). Yet, the comparisons of groups Neo-C and Neo-O$_{asp}$ showed a difference only between the males of the two groups (Neo-C males: eyes = mouth; Neo-O$_{asp}$ males: eyes > mouth; Neo-C/Neo-O$_{asp}$ females: eyes > mouth). As with the differences in general scanning patterns, the distinctions in the looking behavior of males and females were also apparent in the analyses of Identity and Valence (Fig D6).

<u>Summary of Lesion Comparisons</u>: In all, direct statistical comparisons of groups Neo-A$_{ibo}$ and Neo-O$_{asp}$ did not reveal any new distinctions between groups. Instead, they served as confirmation of the qualitative differences in the differential effects of neonatal lesions of the amygdala and orbital frontal cortex. Since neither lesion resulted in a complete failure to integrate or process social signals, the findings suggest that the amygdala and orbital frontal cortex are part of a network of neural regions that uniquely contribute to processing audiovisual

socio-emotional signals.

3- Neural network of Emotional Crossmodal Integration

Human neuroimaging studies have begun to identify a network of neuroanatomical regions involved in crossmodal integration of emotional information. As shown in Figure D7, the fusiform gyrus, middle and superior temporal regions, amygdala, and orbital frontal cortex work in concert to process affective information from faces and voices (reviewed by Campanella & Belin, 2007). The next sections include a brief overview of functional contributions of this network as suggested by the use of neuroimaging techniques in normal, healthy humans, followed by a discussion of our findings within the context of this putative network and the current understanding of the unique contributions of these structures.

A) Fusiform Gyrus

The fusiform gyrus, located in the ventral occipitotemporal region, is an extrastriate cortical area that largely receives visual inputs from the striate cortex and that has been associated with the visual processing of faces (reviewed by Kanwisher & Yovel, 2006). The first fMRI investigation of the neural correlates of emotional crossmodal integration reported activation within the cortex of the fusiform gyrus that was associated with the perceptual facilitation in processing audiovisual expressions of fear over unimodal expressions of fear (Dolan et al., 2001). Subsequent studies have replicated activation in the fusiform gyrus in response to audiovisual perception of emotion (Ethofer, Anders, Erb et al., 2006;

Pourtois et al., 2005; Robins, Hunyadi, & Schultz, 2009). These findings are somewhat curious considering that the fusiform gyrus is traditionally considered a visual area specifically involved in face processing (Kanwisher & Yovel, 2006). Interestingly, functional connectivity analyses indicate that the enhanced perception of bimodal fear compared to unimodal fear is also associated with enhanced connectivity between the fusiform gyrus and the amygdala (Ethofer, Anders, Erb et al., 2006). Thus, the crossmodal effects of on emotion detection in the fusiform gyrus do not likely reflect convergence of emotional information from faces and voices. They might, instead, be mediated by top-down control from heteromodal association areas, such as the amygdala.

B) Middle and Superior Temporal Areas

As compared to the unimodal fusiform gyrus, the middle and superior temporal areas are heteromodal convergence zones (E. G. Jones & Powell, 1970; Seltzer & Pandya, 1978) that show greater activation to non-verbal emotional information presented audiovisually than the same information presented in either modality alone (Ethofer, Pourtois, & Wildgruber, 2006; Pourtois et al., 2005). These activations correspond with reports of increased activation in the adjacent posterior superior temporal sulcus (pSTS) associated with audiovisual speech perception (Beauchamp, Lee, Argall, & Martin, 2004; Calvert & Campbell, 2003; J. A. Jones & Callan, 2003; Olson, Gatenby, & Gore, 2002; Saito et al., 2005; van Atteveldt, Formisano, Goebel, & Blomert, 2004; Wright, Pelphrey, Allison, McKeown, & McCarthy, 2003). This correspondence brings into question whether or not the activation in the middle and superior temporal areas is associated with emotional crossmodal integration or simply reflective of a general role in audiovisual speech perception. Unfortunately, the use of only affective (fearful and happy) stimuli severely limited the interpretation of the data with regards to the specific involvement of these regions in audiovisual integration of emotional information.

A recent event-related neuroimaging study further characterized the involvement of the posterior superior temporal regions in emotional crossmodal integration (Kreifelts et al., 2007). The reported activation was quite large and extended from the posterior temporal gyrus (pSTG) to the middle temporal gyrus (MTG) and including pSTS. In this study, participants were asked to identify the emotion portrayed in video clips of professional actors speaking single words using only the non-verbal cues. Importantly, stimuli included words spoken with both neutral and affective intonations, allowing investigators to more directly assess the neural correlates of emotional crossmodal integration. Consistent with previous reports, activation within the pSTG was greater to bimodal than to unimodal emotional stimuli. Activation in this region was also stronger in response to emotional stimuli than to neutral stimuli in every experimental condition (auditory, visual, audiovisual), and thus, demonstrated enhancement. Regression analysis indicated that pSTG activation was related to the greater accuracy in the classification of bimodal stimuli than either category of unimodal unimodal stimuli; and connectivity analysis revealed that the functional connectivity of pSTG with associative auditory and visual areas was enhanced during audiovisual integration. Another neuroimaging study found comparable activation in STG using semantic content that was emotionally ambiguous, as opposed to emotionally neutral (Robins et al., 2009), and an electrophysiological investigation in rhesus macaques revealed interactions between neurons of STS and auditory cortex (Ghazanfar et al., 2008). The combination of a) enhancement of neural activity to the bimodal as compared to the unimodal stimuli that was b) specific to the emotional content of the stimuli and c) related to the behavioral gain, in conjunction with d) enhanced functional connectivity with unimodal association areas provide strong evidence for the role of the superior temporal region in emotional crossmodal integration, perhaps related to the audiovisual identification of the emotion.

C) Amygdala

The multimodal afferents of amygdala suggest that this region may be in a position to integrate these crossmodal signals. In particular, the basolateral nuclei of the amygdala receives highly processed information from auditory and visual association cortices (McDonald, 1998), and electrophysiological studies in rhesus have identified multisensory neurons selectively responsive to the emotional content of audiovisual information. These qualities make the amygdala a potential locus of integration of socio-emotional auditory and visual information. Human neuroimaging data support this supposition.

The same study that proposed a role of the fusiform gyrus in emotional crossmodal integration also reported an association between audiovisual perception of emotion and amygdalar activity (Dolan et al., 2001). Later studies have replicated and expanded on the putative role of the amygdala in crossmodal integration of affective information (Ethofer, Anders, Erb et al., 2006; Muller et al., 2011; Pourtois et al., 2005). In particular, amygdala activation has been associated with the modulatory effect of valenced auditory information on affective ratings of visual information (Ethofer, Anders, Erb et al., 2006). Accordingly, the amygdala may contribute to emotional crossmodal integration not by directly integrating the different social signals, but, more importantly, by extracting the emotional valence of crossmodal social signals.

D) Orbital Frontal Cortex

The orbital frontal cortex, and more specifically areas 11 and 13, receives information from all sensory areas as well as autonomic signals from the body (Barbas, 1988, 1993; Barbas & Blatt, 1995; Hackett et al., 1999; Pandya & Kuypers, 1969; Romanski & Goldman-Rakic, 2002; Romanski et al., 1999; Webster et al., 1994). Thus, it is well suited not only for integrating sensory signals provided by the environment, but also for integrating these external signals with the internal signals generated by the body. In addition, the orbital frontal areas 11/13 have strong connections with the amygdala (Ghashghaei & Barbas, 2002; Ghashghaei et al., 2007; Kondo et al., 2003) and growing evidence from studies on appetitive learning has indicated that the cross talk between the amygdala and orbital frontal cortex are critical for flexible modulation of behavioral responses (M. G. Baxter, Parker, Lindner, Izquierdo, & Murray, 2000; Izquierdo & Murray, 2004, 2010). Thus, the orbital frontal cortex is thought to take information about external and internal stimuli, as well as the emotional valence of these stimuli provided by the amygdala, and integrate this information in relation with the context of a situation to select the most appropriate behavioral responses (Murray, 2007; Rolls & Grabenhorst, 2008; Schoenbaum & Esber, 2010; Schoenbaum, Roesch, Stalnaker, & Takahashi, 2009).

There is a wealth of evidence from research in both human and nonhuman primates implicating the orbital frontal cortex in processing emotional content from faces and voices (Blair et al., 1999; Dalton et al., 2005; Gorno-Tempini et al., 2001; Klopp et al., 2000; Mitchell et al., 2003; Morris, Friston, Buchel et al., 1998; Nakamura et al., 1999; O'Scalaidhe et al., 1997, 1999; Rolls, 1999, 2000; Rolls et al., 2006; Sprengelmeyer et al., 1998). Yet, as discussed above, the data directly supporting the involvement of the orbital frontal cortex in audiovisual crossmodal integration is sparse. In addition to activation in the fusiform gyrus and amygdala, Dolan and colleagues (2001) also noted increased BOLD response in the orbital frontal cortex to congruent audiovisual presentations of fearful emotional expressions (i.e., fearful face and fearful voice) as compared to incongruent expressions (i.e., fearful face and happy voice). These data suggested that the activation in the orbital frontal cortex was associated with assessments of emotional congruence. As detailed in the introduction, Loveland and colleagues (2008) also reported increased activation extending into the orbital frontal gyrus associated with the assessment of emotional congruence, as compared to assessment of gender congruence, between auditory and visual stimuli. These neuroimaging studies, coupled with the neuroanatomical organization and its known functional contributions in processing emotional information in the auditory and visual modalities, suggest that the orbital frontal cortex may be involved in crossmodal affect assessment of audiovisual signals and might contribute to emotional crossmodal integration via modulatory, top-down processes.

E) Summary

The above review demonstrates that integration of emotional content from faces and voices is a complex process that cannot be attributed to a single brain region, but rather requires an interactive network of structures working together. Although the functional contributions of the middle and superior temporal areas have been well characterized using elegant neuroimaging and electrophysiological designs, the preceding review highlights how little is known about the unique contributions of the other regions. The following sections are devoted to a discussion of how our data may strengthen, or when contrary refute, the proposed contributions of the amygdala and orbital frontal cortex to emotional and social behavior.

4- Does the Amygdala Modulate Evaluations of Socio-emotional Audiovisual Information?

A) Amygdala and integration of crossmodal social cues

As described above, the broad connectivity of the amygdala makes putative convergence zone for socio-emotional audiovisual information. Concomitantly, human neuroimaging studies have associated activation of the amygdala with the assessment of the emotional value of affective information from faces and voices (Dolan et al., 2001; Ethofer, Anders, Erb et al., 2006; Muller et al., 2011; Pourtois et al., 2005), as opposed to direct integration of the signals. The current findings are generally consistent with these observations.

First, our neonatal lesions of the amygdala did not impair emotional crossmodal integration in rhesus macaques, indicating that the amygdala is not a primary locus for sensory integration of socio-emotional information from conspecific faces and voices. Although the timing of the lesion (see Manuscript II) should be considered before concluding the amygdala is not critically involved in emotional crossmodal integration, the current findings are consistent both with other lesion studies that have examined the role of the amygdala in sensory integration of nonsocial stimuli (Goulet & Murray, 2001; Lee et al., 1988; Nahm et al., 1993), and electrophysiological data that have identified only sparse populations of multisensory neurons within the amygdala that are selective for audiovisual emotional content (1% of surveyed neurons) (Kuraoka & Nakamura, 2007). Our characterizations of the scanning patterns further complement the neuroimaging data on an evaluative role of the amygdala.

B) Amygdala and scanning patterns of faces

Based on the extensive literature implicating the amygdala of humans and nonhuman primates in the expression of spontaneous scanning of the eye regions of unimodal faces, we predicted that, compared to sham-operated controls, animals with neonatal damage to the amygdala would exhibit decreased exploration of the eyes and increased attentiveness to the mouth regions of the crossmodal stimuli. Quite unexpectedly, they displayed the same general preference for the eye region as sham-operated controls. Despite this observation, there was an indication of increased salience of the mouth regions. Whereas sham-operated monkeys used the eyes to differentiate the congruent and incongruent stimuli, animals with amygdala damage used the mouth regions. Additionally, there were striking differences in the way males and females of each group explored the eye and mouth regions of the vocalizing stimulus animal (i.e., congruent stimulus video), suggesting that neonatal lesions of the amygdala disrupted the species-typical scanning behaviors.

As described in Manuscript II and illustrated in Figure D5, unlike sham-operated males, males with neonatal amygdala lesions looked more to the eye than mouth regions of the vocalizing conspecific when all trials were considered. Similarly, unlike sham-operated females, females with amygdalar damage scanned the two regions equally. Post-hoc analyses on the parameters of Identity and Valence (see Fig D6) showed similar disturbances in the scanning patterns of males and females across the four trial types. That is, the scanning patterns of males with amygdalar damage did not evidence sensitivity to the identity of the stimulus monkeys, and the scanning patterns of females with lesions of the amygdala did not appear to be sensitive to the valence of the vocalizations. Thus, neonatal lesions of the amygdala perturbed the normal, spontaneous exploration of the eyes and mouths of conspecifics. These findings parallel characterizations of patients with circumscribed damage to the amygdala, as well patients with autistic spectrum disorder, who show less spontaneous exploration of socially relevant information, including the eyes, because they do not understand the need to do so (reviewed by Adolphs, 2010; Falck-Ytter & von Hofsten, 2011). Such deficits together with the aberrant relative looking to the eye and mouth regions displayed by males and females with neonatal amygdala damage suggest that the amygdala may facilitate attention towards important social cues to abstract knowledge about emotional content of these cues (A. K. Anderson & Phelps, 2001; Morris, Friston, & Dolan, 1998). Thus, as already posited by others, the amygdala receives information pertaining to the socio-emotional significance early in stimulus processing, and through projections to the sensory cortical regions (including the fusiform gyrus), modulates further attentional and perceptual processes (A. K. Anderson & Phelps, 2001; Vuilleumier, Richardson, Armony, Driver, & Dolan, 2004). Although our data strongly support this proposal, given the early timing of the amygdala in our project, these findings will need to be replicated in animals with lesions of amygdala acquired in adulthood.

C) Amygdala and sex differences

The sex differences observed in the scanning patterns of crossmodal social information are interesting because the amygdala has been characterized by sexual differentiation in anatomy and hormonal content. For example, the development of the amygdala in males and females has been noted using structural MRI techniques in both humans (reviewed by Lenroot & Giedd, 2010) and rhesus macaques (Payne, Machado, Bliwise, & Bachevalier, 2010). There is also a growing body of literature from human neuroimaging studies that suggests a mediating role of the amygdala and sex differences in emotion-related activities (reviewed by Hamann, 2005). These differences, as well as the sex differences observed in current study, may be attributed to sexual differentiation of amygdala hormonal content.

Throughout development, the amygdala exhibits higher concentrations of gonadal hormone receptors (specifically androgen receptors) than other neural areas, excluding the hypothalamus (Abdelgadir, Roselli, Choate, & Resko, 1997; Bonsall & Michael, 1992; Michael, Rees, & Bonsall, 1989; Michael, Zumpe, & Bonsall, 1992; Pomerantz & Sholl, 1987; Sholl, Goy, & Kim, 1989; Sholl & Kim, 1990). Additionally, estradiol and testosterone fluctuations are known to affect the maturation of the amygdala (reviewed by Payne & Bachevalier, 2009). The high concentrations of gonadal hormone receptors combined with the expansive connectivity of the amygdala with almost all areas of the brain suggests that the amygdala may serve as a secondary conduit for the effects of androgens on the brain, especially during development. In the absence of the amygdala, modulatory effects of gonadal hormones on behavior may be weaker, resulting in a disruption of the species-typical responses of males and females.

Overall, our characterization of the looking behavior of rhesus macaques with neonatal lesions of the amygdala in response to dynamic audiovisual social signals indicate that the amygdala a) is not a likely locus of audiovisual sensory integration responsible for crossmodal integration of socio-emotional signals, and b) subserves, at least in part, the sex-appropriate, species-typical processing of those cues. The current findings, therefore, strongly support the role of the amygdala in extracting and evaluating the valence of crossmodal socio-emotional signals, proposed by human neuroimaging studies.

5- Does the Orbital Frontal Cortex Modulate Emotional Audiovisual Integration?

As described above, the orbital frontal cortex, like the amygdala, is also poised as a putative convergence zone of affective audiovisual information. And whereas there is some neuroimaging evidence indicating the orbital frontal cortex is involved in the assessment of affect congruence across modalities (Dolan et al., 2001; Loveland et al., 2008), this is the first reported direct assessment of the contributions of the orbital frontal cortex to processing audiovisual affective information.

A) Orbital frontal cortex and integration of crossmodal social cues

The current results indicate that neonatal lesions of the orbital frontal cortex weaken the ability to integrate bimodal social signals. This weakened ability was characterized by inconsistent integration ability across trial types (see Fig D2) and inversely correlated with the extent of damage to the orbital frontal cortex. Importantly, the weaker integration ability was associated with marked differences in scanning patterns, a relationship not detected in the looking behavior of monkeys with neonatal amygdala lesions. Moreover, the differences in the scanning patterns displayed by animals with damage to the orbital frontal cortex (as compared to controls) were distinctive from the differences exhibited by monkeys with neonatal amygdala lesions.

B) Orbital frontal cortex and scanning patterns of faces

Whereas monkeys with neonatal amygdala lesions demonstrated a complete disruption of the species-typical sex differences, monkeys with orbitofrontal damage exhibited more subtle, albeit profound, changes in viewing patterns. As predicted, lesions of the orbital frontal cortex resulted in increased looking to the eye regions. Unlike sham-operated animals that differentiated eye regions of the congruent and incongruent stimulus videos, monkeys with damage to the orbital frontal cortex exhibited a preference for the eyes of both the congruent and incongruent stimulus video. This pattern of exploration resulted in equal looking to the two eye regions and, thus, a weaker preference. This robust preference for the eyes was evident in all trial types (see Fig D3 and Fig D4), suggesting that the animals with orbital frontal cortex damage had difficulty appropriately modulating their looking behavior.

This conclusion is fully supported by a growing number of studies demonstrating that damage to, or dysfunction of, the orbital frontal cortex in humans, monkeys and rodents result in an inability to modify behavioral responses in response to changes in context (Bachevalier, Machado, & Kazama, 2011; Bechara, Damasio, Tranel, & Damasio, 1997; Bissonette et al., 2008; Butter & McDonald, 1970; Butter & Mirsky, 1968; Chudasama & Robbins, 2003; Fellows & Farah, 2003; Hornak et al., 2004; Izquierdo, Suda, & Murray, 2005; B. Jones & Mishkin, 1972; Kazama et al., 2008; Machado & Bachevalier, 2007; McAlonan & Brown, 2003; Meunier, Bachevalier, & Mishkin, 1997; Rolls, Hornak, Wade, & McGrath, 1994; Schoenbaum, Setlow, Nugent, Saddoris, & Gallagher, 2003). Classic demonstrations of this deficit is provided by testing individuals with damage to the orbital frontal cortex in the Iowa Gambling task (Bechara et al., 1997) and by testing monkeys with similar damage in the Reinforcer Devaluation paradigm (reviewed by Murray, 2007). In the Iowa Gambling, subjects select cards from two decks of card. Selection of cards of Deck 1 results in small gains but smaller losses, whereas selection of card of Deck 2 results in large gains but also large losses. Control subjects learned that selecting Deck 1 would result in bigger cumulative gains than selecting Deck 2. Conversely, the patients with ventromedial frontal damage, which included the orbital frontal cortex, did not show signs of learning this contingency and continued to select Deck 2, resulting in very little to no gains. Similarly, in the Reinforcer Devaluation Task, monkeys learn the reward values of different objects (some objects are rewarded with peanuts and other with raisins). After satiation with one of the food (i.e. either peanut or raisin), control animals avoid selecting the objects associated with the devalued food in favor of the other objects, but animals with lesions of orbital frontal areas 11/13 continue to select the objects that have been devalued (Machado & Bachevalier, 2007).

This deficit in flexible modulation of behavioral responses after orbital frontal lesions can also explain the unwavering preference for the eyes displayed by the monkeys with neonatal damage of the orbital frontal cortex. Sham-operated controls exhibit differential looking to the eye and mouth regions across the parameters of Identity and Valence, indicating that they can regulate their behaviors in accord with the differences in 'reward values' of these socio-emotional stimuli. Conversely, the lack of modulation of viewing patterns by monkeys with lesions of the orbital frontal cortex suggests that they did not recognize the differences in reward values and/or could not flexibly alter their looking behavior in response to those differences. This conclusion is strengthened by recent studies on the same monkeys demonstrating that a similar deficit was found when the animals with neonatal orbital frontal lesions were tested in the Reinforcer Devaluation paradigm (Bachevalier et al., 2011; Kazama et al., 2008).

6- Do the Amygdala and Orbital Frontal Cortex Uniquely Contribute to Emotional Crossmodal Integration?

The differences observed between lesion groups, albeit similar, are distinctive. Monkeys with lesions of the orbital frontal cortex show nearly complete inflexibility that effectively weakens their integration ability. By contrast, animals with amygdalar damage exhibit flexibility in their scanning patterns across trial types, but appear to attend to different qualities of the vocalizations than sham-operated controls with no apparent effect on integration ability. Thus, although the abnormal scanning patterns displayed by each lesion group appear to be associated with deficits in the evaluation of the socio-emotional signals, the deficits manifest in slightly different manners.

Interestingly, the amygdala and orbital frontal cortex are highly interconnected, both functionally and neuroanatomically (Bachevalier & Loveland, 2006; Ghashghaei & Barbas, 2002; Ghashghaei et al., 2007; Izquierdo & Murray, 2004, 2010; Kondo et al., 2003). This connectivity may be responsible for both the similarities and distinctions between the lesion effects. As mentioned above, the amygdala has been implicated in modulating the attention to and perception of emotion via feedback projections to association areas as well as primary sensory areas. The amygdala is also reciprocally connected with areas 11 and 13 of the orbital frontal cortex, with the orbital frontal cortex exerting both inhibitory and excitatory influences in the amygdala (Kondo et al., 2003). Accordingly, in the event of dysfunction of one of the structures, it is reasonable to expect at least partial compensation by the other, intact structure.

Given the apparent role of the amygdala in the spontaneous scanning of socially relevant information, especially the eyes, it is plausible that lesions of the orbital frontal cortex could remove modulatory influences from the amygdala resulting in an increased, inflexible attention to the eyes. Conversely, in the absence (or dysfunction) of the amygdala, the orbital frontal cortex may not receive accurate assessments of the socio-emotional information of the stimuli and/or the individual's internal state. This could result in abnormal orbitofrontal modulation of behavior that manifests as disrupted species-specific behavior that shows flexibility (albeit inappropriate flexibility).

Such a relationship explains the apparent redundancies in the functional contributions of the amygdala and orbital frontal cortex to processing socio-emotional information while also highlighting their distinctive roles. It further emphasizes the importance neural networks in normal socio-emotional behavior. More and more, the neuroscientific community is finding that information processing is not a linear assembly line that builds the neural representation of a stimulus in a step-wise manner. This revelation has particular importance to understanding the neural substrates of complex neuropsychological disorders such as autism, schizophrenia, or anxiety disorders. The following section attempts to translate the current findings to the deficits in emotional crossmodal integration.

7- Translation to Human Neuropsychopathology

Crossmodal integration of dynamic affective expressions has been identified in seven-month-old infants (Grossmann, Striano, & Friederici, 2005; Soken &

Pick, 1999), emphasizing the importance of this ability in the development of normal social interactions and providing a foundation for the emergence other social cognitive skills. Interestingly, neuropsychological disorders characterized by profound impairments in social skills, such as autism spectrum disorder (Hobson et al., 1988; Loveland, 2005; Loveland, Pearson, & Reddoch, 2005; Loveland et al., 1995), pervasive developmental disorder (Magnee, de Gelder, van Engeland, & Kemner, 2007; Magnee et al., 2008); and schizophrenia (de Gelder et al., 2005; de Jong et al., 2010; de Jong, Hodiamont, Van den Stock, & de Gelder, 2009), also show atypical integration of emotional audiovisual information from faces and voices. In fact, sensory abnormalities were one of the first behavioral characteristics studied in austim (Hermelin & O'Connor, 1970; Hutt, Hutt, Lee, & Ounsted, 1965; Ornitz, 1969), and through the years, many theories have posited that sensory peculiarities are a primary disturbance in autism (reviewed by Iarocci & McDonald, 2006).

Although the abnormalities in emotional crossmodal integration in these disorders have been characterized behaviorally, the neuroanatomical mechanisms of the deficits are poorly understood. One preliminary functional imaging study in normal developing and autistic individuals revealed that the abnormalities in audiovisual emotional associations exhibited by children and adolescents with autism were associated with dysfunctional activity of the orbital frontal cortex (Loveland et al., 2008).

Notably, lesions of the neither the amygdala nor the orbital frontal cortex resulted in a complete replication of the deficits experienced by individuals with any of these neurodevelopmental disorders. Our results suggest that perturbed integration ability is not associated with *isolated* dysfunction of either the orbital frontal cortex or amygdala. Rather, the behavioral deficits are likely the culmination of dysfunction within a network of regions that include the middle and superior temporal areas and the fusiform gyrus, in addition to the amygdala and orbital frontal cortex.

The present findings may also provide insight into another population of patients that, as of yet, do not appear to show deficits in emotional crossmodal integration, but do exhibit abnormalities in assessments of socio-emotional information. Increased aggression is a hallmark characteristic of human psychiatric disorders, such as intermittent explosive disorder, antisocial personality disorder, and behavioral variant frontotemporal dementia, that are thought to involve malfunction of the orbital frontal cortex (recently reviewed by Blair, Peschardt, Budhani, Mitchell, & Pine, 2006; Gansler et al., 2009). Social information processing theories posit that the increased aggression observed in these human disorders is related to a hypersensitivity to cues of threat and hostility that others emit, as well as an overly hostile intent attribution (Crick & Dodge, 1994; Lemerise & Arsenio, 2000).

As detailed in Manuscript III, the scanning patterns of monkeys with neonatal orbitofrontal damage in the current study appear to parallel the way 'high aggressive' human adolescents explore socio-emotional scenes (Horsley, de Castro, & Van der Schoot, 2010). In both studies, participants scanned the same social cues as controls, but fixated on them longer, and appeared to attribute more hostility to the non-hostile cues than normal controls. Thus, our findings suggest that damage to the orbital frontal cortex did not impair the encoding of social information, but rather, the representation of the information. Moreover, the current data indicates that dysfunction or malfunction of the amygdala likely accompanies the purported dysfunction of the orbital frontal cortex in human aggressive disorders.

8- Concluding Remarks

The current data confirm the spontaneous crossmodal integration of socio-emotional signals from conspecifics in surrogate nursery-reared rhesus macaques and demonstrates that integration ability is not dependent on the temporal coincidence of the mouth movements with the rhythm of the acoustic cues. The characterization of scanning patterns suggested divergence in the attentional processes of males and females. Like human women, female rhesus macaques were more sensitive to the relative valence of the stimuli than males. By contrast, male rhesus macaques were more sensitive to factors associated with the identity of the stimulus animals, such as relative dominance status.

Our findings also provide insight into the functional contributions of the amygdala and orbital frontal cortex in processing affective audiovidual information. Both regions are involved in the evaluation of the bimodal socio-emotional stimuli, albeit in slightly different ways. The current results suggest that the amygdala supports the appropriate expression of species-specific sex differences, whereas the orbital frontal cortex supports the flexible modulation of the behavior in response to stimuli assessments. Thus, these regions work in tandem and each region cannot fully compensate for dysfunction in the other region.

Finally, this project emphasizes the importance of characterizing species-typical behavior and using appropriate comparison groups when investigating the functional neuroanatomy of complex socio-emotional behavior.

General Discussion – Tables & Figures.

Table 1: Summary of Crossmodal Integration Abilities and Scanning Patterns.

Group	Crossmodal Integration		Preferred ROI	Sex Differences
	Synchronized	Desynchronized		
Neo-C	+	+	eyes	M: eyes = mouth F: eyes > mouth
Neo-A_{ibo}	+	+	eyes ↑ salience of mouth	M: eyes > mouth F: eyes = mouth
Neo-O_{asp}	-	+	↑ salience of eyes	M: eyes > mouth F: eyes > mouth

Note: (+) signifies normal integration ability; (-) signifies impaired integration ability; (M) = males; (F) = females.

Figure D1: Group Summary of Integration Assessments – All Trials. Percentages of looking time (± sem) to the 'preferred' stimulus video in the Synchronized (S) and Desynchronized (D) conditions across all trials for group Neo-C (solid), group Neo-A_{ibo} (striped) and group Neo-O_{asp} (checkered) in all trials. The dashed line represents chance level of 50%. (*) $p < 0.05$.

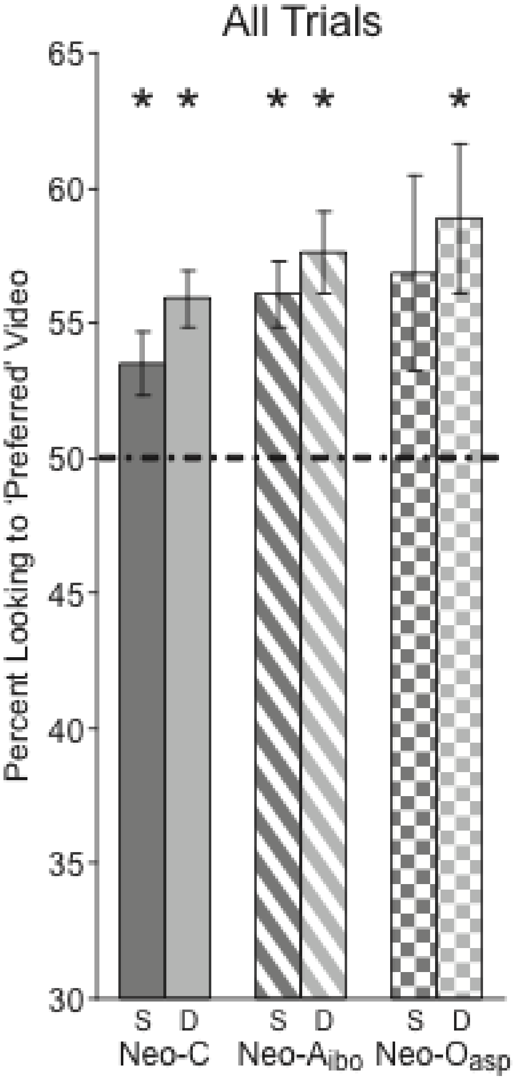


Figure D2: Group Summary of Integration Assessments.

Percentages of looking time (± sem) to the 'preferred' stimulus video in the Synchronized (S) and Desynchronized (D) conditions across all trials for group Neo-C (solid), group Neo-A_{ibo} (striped) and group Neo-O_{asp} (checkered) in (A) Identity Same (S) and Identity Different (D) trials, and (D) Valence Same (S) and Valence Different (D) trials. The dashed line represents chance level of 50%. (*) $p < 0.05$.

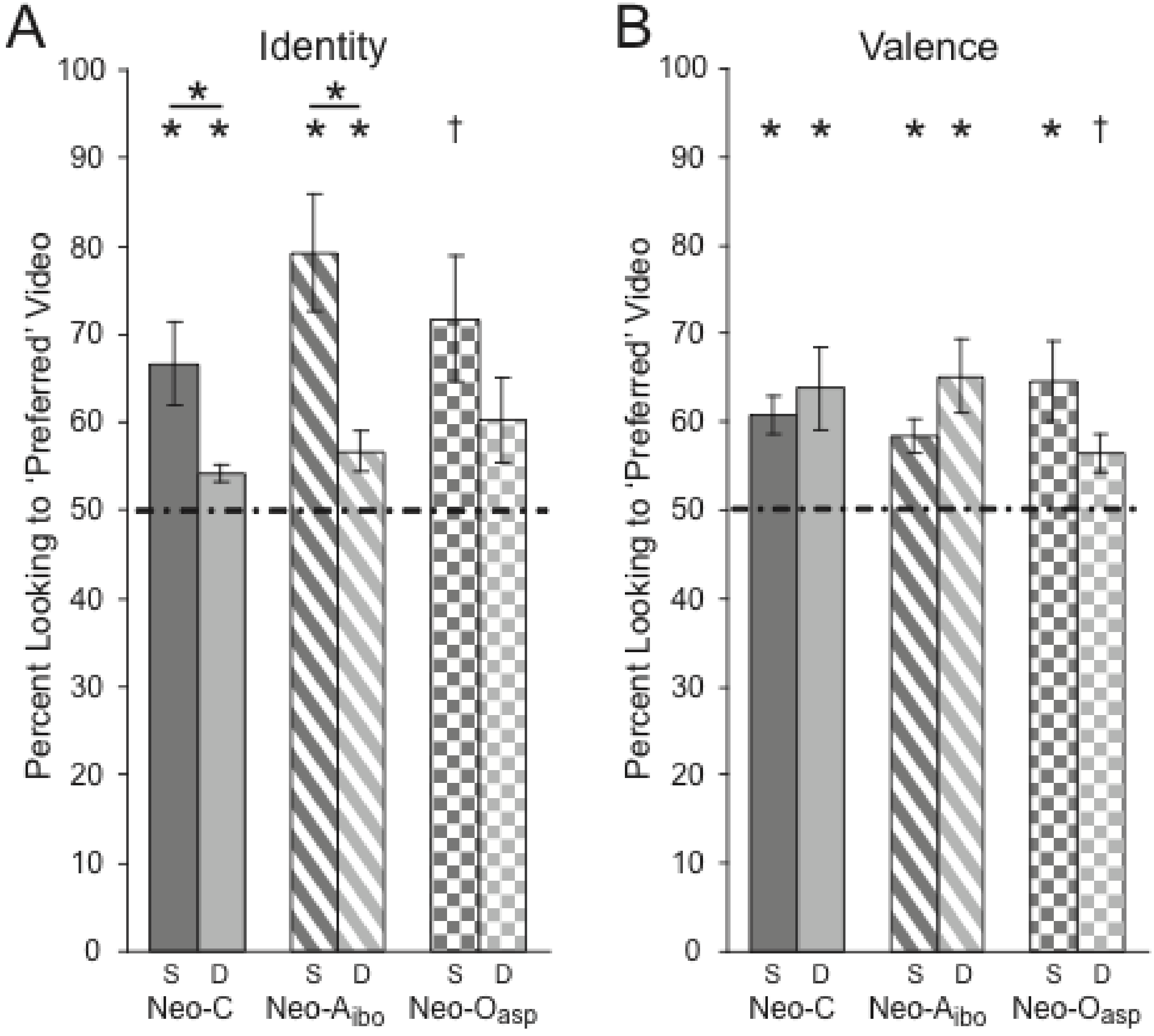


Figure D3: Group Summary of Scanning Patterns – All Trials.

Percentages of looking time (± sem) to the eyes (e), mouth (m), and other (o) for both the congruent and incongruent stimuli combined for group Neo-C (solid), group Neo-A_{ibo} (striped) and group Neo-O_{asp} (checkered). (*) $p \leq 0.05$; (†) $p \leq 0.08$.

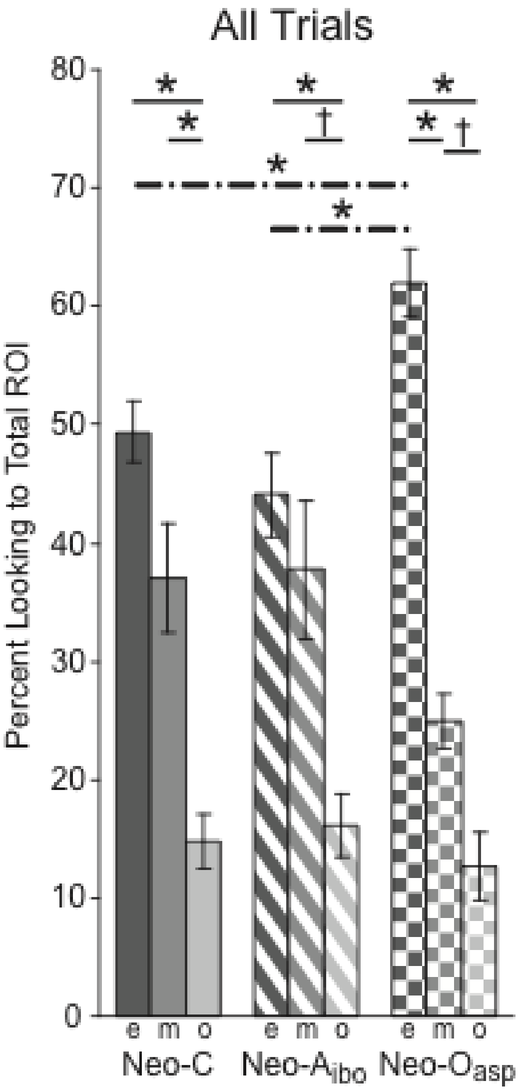


Figure D4: Group Summary of Scanning Patterns – Identity and Valence.

Percentages of looking time (± sem) to the eyes (e), mouth (m), and other (o) for both the congruent and incongruent stimuli combined for group Neo-C (solid), group Neo-A_{ibo} (striped) and group Neo-O_{asp} (checkered) in (A) Identity Same trials, (B) Identity Different trials, (C) Valence Same trials, and (D) Valence Different trials. (*) $p \le 0.05$; (†) $p \le 0.08$.

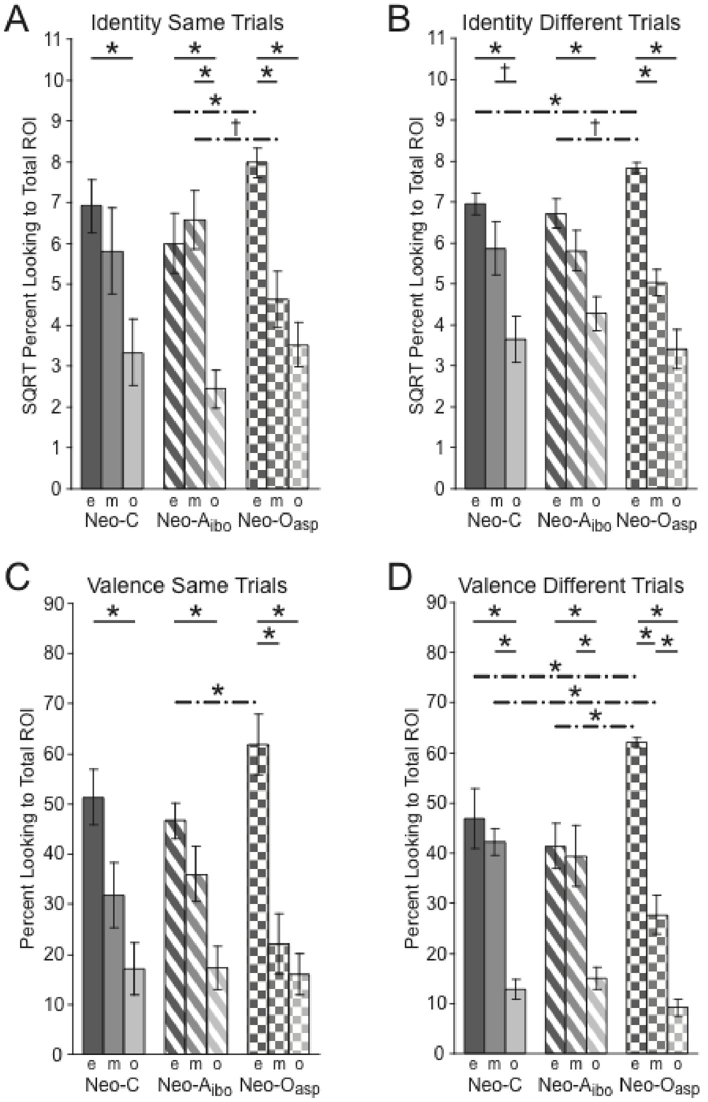


Figure D5: Group Summary of Sex Differences – All Trials.

Percentages of looking time (± sem) to the combined eyes (diamonds) and combined mouth (circles) males and females of Neo-C (grey lines, filled symbols), group Neo-A_{ibo} (black lines, filled symbols) and group Neo-O_{asp} (dashed lines, open symbols). (*) $p \leq 0.05$; (†) $p \leq 0.08$.

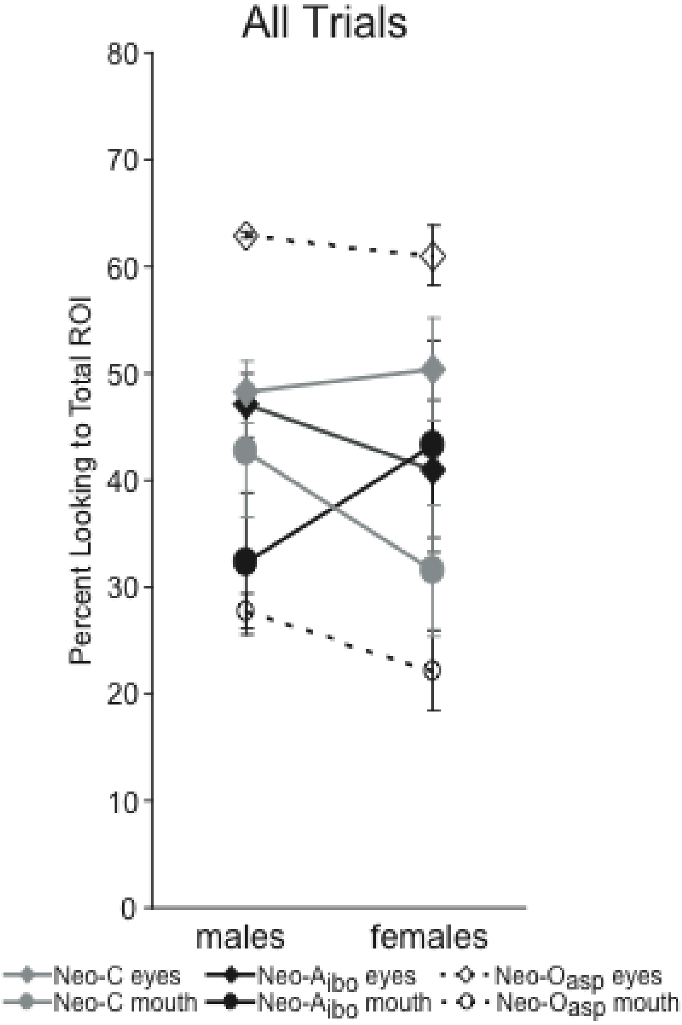


Figure D6: Group Summary of Sex Differences – Identity and Valence.

Percentages of looking time (± sem) to the combined eyes (diamonds) and combined mouth (circles) males and females of Neo-C (grey lines, filled symbols), group Neo-A$_{ibo}$ (black lines, filled symbols) and group Neo-O$_{asp}$ (dashed lines, open symbols) in (A) Identity Same trials, (B) Identity Different trials, (C) Valence Same trials, and (D) Valence Different trials. (*) $p \leq 0.05$; (†) $p \leq 0.08$.

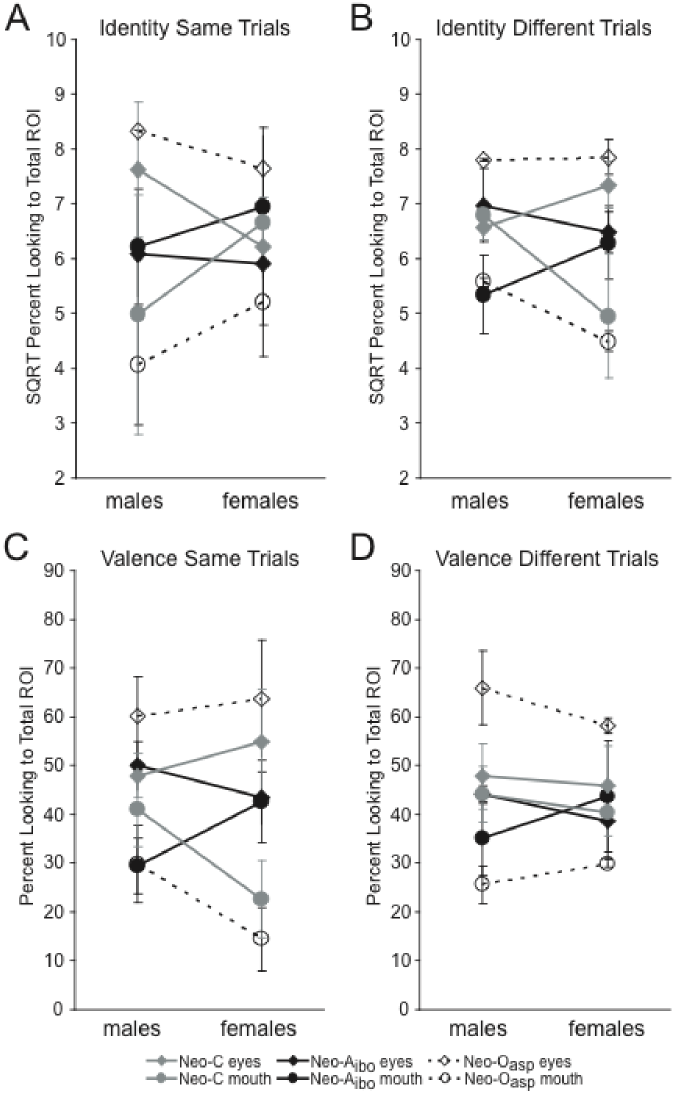
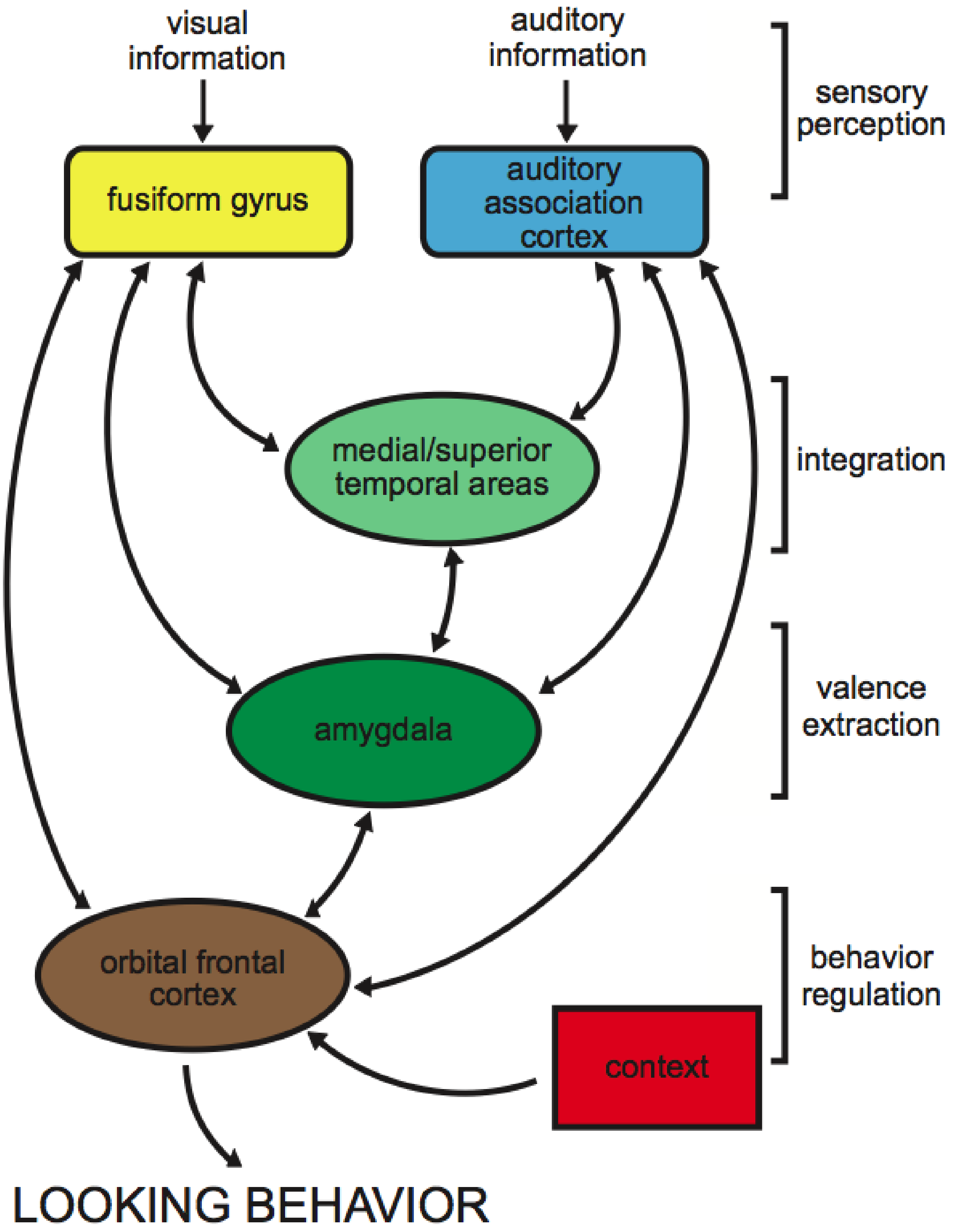


Figure D7: Representation of Neural Network Subserving Emotional Crossmodal Integration.



GENERAL REFERENCES

Abdelgadir, S. E., Roselli, C. E., Choate, J. V., & Resko, J. A. (1997). Distribution of aromatase cytochrome P450 messenger ribonucleic acid in adult rhesus monkey brains. *Biol.Reprod., 57*(4), 772-777.

Adolphs, R. (1999). Social cognition and the human brain. *Trends Cogn Sci., 3*(12), 469-479.

Adolphs, R. (2002). Recognizing emotion from facial expressions: Psychological and neurological mechanisms. *Beh.Cog.Neurosci.Rev., 1*(1), 21-62.

Adolphs, R. (2003). Is the human amygdala specialized for processing social information? *Ann.N.Y.Acad.Sci., 985*, 326-340.

Adolphs, R. (2010). What does the amygdala contribute to social cognition? *Ann N Y Acad Sci, 1191*, 42-61.

Adolphs, R., Gosselin, F., Buchanan, T. W., Tranel, D., Schyns, P., & Damasio, A. R. (2005). A mechanism for impaired fear recognition after amygdala damage. *Nature, 433*, 68-72.

Adolphs, R., Sears, L., & Piven, J. (2001). Abnormal processing of social information from faces in autism. *J.Cogn Neurosci., 13*, 232-240.

Adolphs, R., Tranel, D., & Damasio, A. R. (2003). Dissociable neural systems for recognizing emotions. *Brain Cogn, 52*(1), 61-69.

Amaral, D. G., Behniea, H., & Kelly, J. L. (2003). Topographic organization of projections from the amygdala to the visual cortex in the macaque monkey. *Neuroscience, 118*(4), 1099-1120.

Amaral, D. G., Price, J. L., Pitkanen, A., Carmichael, S. T., & Aggleton, J. P. (1992). Anatomical organization of the primate amygdaloid complex. In

The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction (pp. 1-66). New York: Wiley-Liss, Inc.

Ambadar, Z., Schooler, J. W., & Cohn, J. F. (2005). Deciphering the enigmatic face: the importance of facial dynamics in interpreting subtle facial expressions. *Psychol Sci, 16*(5), 403-410.

Anderson, A. K., & Phelps, E. A. (2001). Lesions of the human amygdala impair enhanced perception of emotionally salient events. *Nature, 411*(6835), 305-309.

Anderson, S. W., Bechara, A., Damasio, H., Tranel, D., & Damasio, A. R. (1999). Impairment of social and moral behavior related to early damage in human prefrontal cortex. *Nat Neurosci, 2*(11), 1032-1037.

Bachevalier, J., & Loveland, K. A. (2006). The orbitofrontal-amygdala circuit and self-regulation of social-emotional behavior in autism. *Neurosci.Biobehav.Rev., 30*(1), 97-117.

Bachevalier, J., Machado, C. J., & Kazama, A. M. (2011). *Behavioral outcomes of late-onset or early-onset orbital frontal cortex (areas 11/13) lesions in rhesus monkeys*. Paper presented at the New York Acadamy of Sciences.

Bachevalier, J., & Meunier, M. (2005). The neurobiology of social-emotional cognition in nonhuman primates. In A. Easton & N. J. Emery (Eds.), *The Cognitive Neuroscience of Social Behavior* (pp. 19-57). East Sussex: Psychology Press.

Barbas, H. (1988). Anatomic organization of basoventral and mediodorsal visual recipient prefrontal regions in the rhesus monkey. *J Comp Neurol, 276*(3), 313-342.

Barbas, H. (1993). Organization of cortical afferent input to orbitofrontal areas in the rhesus monkey. *Neuroscience, 56*(4), 841-864.

Barbas, H., & Blatt, G. J. (1995). Topographically specific hippocampal projections target functionally distinct prefrontal areas in the rhesus monkey. *Hippocampus, 5*(6), 511-533.

Baron-Cohen, S., Ring, H. A., Wheelwright, S., Bullmore, E. T., Brammer, M. J., Simmons, A., et al. (1999). Social intelligence in the normal and autistic brain: an fMRI study. *Eur.J Neurosci., 11*(6), 1891-1898.

Baxter, M., & Murray, E. (2000). Reinterpreting the behavioural effects of the amygdala lesions in nonhuman primates. In J. Aggleton (Ed.), *The amygdala: A functional analysis* (pp. 545-568). New York, NY: Oxford University Press.

Baxter, M. G., Parker, A., Lindner, C. C., Izquierdo, A. D., & Murray, E. A. (2000). Control of response selection by reinforcer value requires interaction of amygdala and orbital prefrontal cortex. *Journal of Neuroscience, 20*, 4311-4319.

Beauchamp, M. S. (2005). See me, hear me, touch me: multisensory integration in lateral occipital-temporal cortex. *Curr.Opin.Neurobiol., 15*(2), 145-153.

Beauchamp, M. S., Lee, K. E., Argall, B. D., & Martin, A. (2004). Integration of auditory and visual information about objects in superior temporal sulcus. *Neuron, 41*(5), 809-823.

Bechara, A., Damasio, H., & Damasio, A. R. (2003). Role of the amygdala in decision-making. *Ann N Y Acad Sci, 985*, 356-369.

Bechara, A., Damasio, H., Tranel, D., & Damasio, A. R. (1997). Deciding advantageously before knowing the advantageous strategy. *Science, 275*(5304), 1293-1295.

Biele, C., & Grabowska, A. (2006). Sex differences in perception of emotion intensity in dynamic and static facial expressions. *Exp Brain Res, 171*(1), 1-6.

Bissonette, G. B., Martins, G. J., Franz, T. M., Harper, E. S., Schoenbaum, G., & Powell, E. M. (2008). Double dissociation of the effects of medial and orbital prefrontal cortical lesions on attentional and affective shifts in mice. *J Neurosci, 28*(44), 11124-11130.

Blair, R. J., Morris, J. S., Frith, C. D., Perrett, D. I., & Dolan, R. J. (1999). Dissociable neural responses to facial expressions of sadness and anger. *Brain, 122 (Pt 5)*, 883-893.

Blair, R. J., Peschardt, K. S., Budhani, S., Mitchell, D. G., & Pine, D. S. (2006). The development of psychopathy. *J Child Psychol Psychiatry, 47*(3-4), 262-276.

Blood, A. J., Zatorre, R. J., Bermudez, P., & Evans, A. C. (1999). Emotional responses to pleasant and unpleasant music correlate with activity in paralimbic brain regions. *Nat Neurosci, 2*(4), 382-387.

Blundell, P., Hall, G., & Killcross, S. (2001). Lesions of the basolateral amygdala disrupt selective aspects of reinforcer representation in rats. *J Neurosci, 21*(22), 9018-9026.

Bonsall, R. W., & Michael, R. P. (1992). Developmental changes in the uptake of testosterone by the primate brain. *Neuroendocrinology, 55*(1), 84-91.

Breiter, H. C., Etcoff, N. L., Whalen, P. J., Kennedy, W. A., Rauch, S. L., Buckner, R. L., et al. (1996). Response and habituation of the human amygdala during visual processing of facial expression. *Neuron, 17*(5), 875-887.

Broks, P., Young, A. W., Maratos, E. J., Coffey, P. J., Calder, A. J., Isaac, C. L., et al. (1998). Face processing impairments after encephalitis: amygdala damage and recognition of fear. *Neuropsychologia, 36*(1), 59-70.

Buchan, J. N., Pare, M., & Munhall, K. G. (2008). The effect of varying talker identity and listening conditions on gaze behavior during audiovisual speech perception. *Brain Res, 1242*, 162-171.

Butter, C. M., & McDonald, J. A. (1970). Effects of orbital frontal lesions on aversive and aggressive behaviors in rhesus monkeys Journal Article: 1970-13903-001 English Journal Article. *Journal of Comparative & Physiological Psychology, 72*(1).

Butter, C. M., & Mirsky, A. F. (1968). Emotional responses toward humans in monkeys with selective frontal lesions. *Physiology & Behavior, 3*(2).

Calvert, G. A. (2001). Crossmodal processing in the human brain: insights from functional neuroimaging studies. *Cereb.Cortex, 11*(12), 1110-1123.

Calvert, G. A., Bullmore, E. T., Brammer, M. J., Campbell, R., Williams, S. C., McGuire, P. K., et al. (1997). Activation of auditory cortex during silent lipreading. *Science, 276*(5312), 593-596.

Calvert, G. A., & Campbell, R. (2003). Reading speech from still and moving faces: the neural substrates of visible speech. *J Cogn Neurosci, 15*(1), 57-70.

Campanella, S., & Belin, P. (2007). Integrating face and voice in person perception. *Trends Cogn Sci, 11*(12), 535-543.

Chudasama, Y., & Robbins, T. W. (2003). Dissociable contributions of the orbitofrontal and infralimbic cortex to pavlovian autoshaping and discrimination reversal learning: further evidence for the functional heterogeneity of the rodent frontal cortex. *Journal of Neuroscience, 23,* 8771-8780.

Clark, L., Cools, R., & Robbins, T. W. (2004). The neuropsychology of ventral prefrontal cortex: decision-making and reversal learning. *Brain Cogn, 55*(1), 41-53.

Collignon, O., Girard, S., Gosselin, F., Roy, S., Saint-Amour, D., Lassonde, M., et al. (2008). Audio-visual integration of emotion expression. *Brain Res, 1242*, 126-135.

Collignon, O., Girard, S., Gosselin, F., Saint-Amour, D., Lepore, F., & Lassonde, M. (2010). Women process multisensory emotion expressions more efficiently than men. *Neuropsychologia, 48*(1), 220-225.

Crick, N., & Dodge, K. (1994). A review and reformulation of social information-processing mechanisms in children's social adjustment. *Psychol Bull, 115*(1), 74-101.

Critchley, H. D., Daly, E. M., Bullmore, E. T., Williams, S. C., Van Amelsvoort, T., Robertson, D. M., et al. (2000). The functional neuroanatomy of social behaviour: changes in cerebral blood flow when people with autistic disorder process facial expressions. *Brain, 123 (Pt 11)*, 2203-2212.

Dalton, K. M., Nacewicz, B. M., Johnstone, T., Schaefer, H. S., Gernsbacher, M. A., Goldsmith, H. H., et al. (2005). Gaze fixation and the neural circuitry of face processing in autism. *Nat.Neurosci., 8*, 519-526.

Darwin, C. (1872). *The expression of the emotions in man and animals* (2nd ed.). New York, NY: D Appleton & Co.

de Gelder, B., & Vroomen, J. (2000). The perception of emotions by ear and by eye. *Cogn & Emot, 14*(3), 289-311.

de Gelder, B., Vroomen, J., Annen, L., Masthof, E., & Hodiamont, P. (2003). Audio-visual integration in schizophrenia. *Schizophr Res, 59*(2-3), 211-218.

de Gelder, B., Vroomen, J., de Jong, S. J., Masthoff, E. D., Trompenaars, F. J., & Hodiamont, P. (2005). Multisensory integration of emotional faces and voices in schizophrenics. *Schizophr Res, 72*(2-3), 195-203.

de Jong, J. J., Hodiamont, P. P., & de Gelder, B. (2010). Modality-specific attention and multisensory integration of emotions in schizophrenia: reduced regulatory effects. *Schizophr Res, 122*(1-3), 136-143.

de Jong, J. J., Hodiamont, P. P., Van den Stock, J., & de Gelder, B. (2009). Audiovisual emotion recognition in schizophrenia: reduced integration of facial and vocal affect. *Schizophr Res, 107*(2-3), 286-293.

de Waal, F. B. (1996). Macaque social culture: development and perpetuation of affiliative networks. *J Comp Psychol, 110*(2), 147-154.

Dixon, N. F., & Spitz, L. (1980). The detection of auditory visual desynchrony. *Perception, 9*(6), 719-721.

Dolan, R. J., Morris, J. S., & de Gelder, B. (2001). Crossmodal binding of fear in voice and face. *Proc.Natl.Acad.Sci.U.S.A, 98*(17), 10006-10010.

Ekman, P., & Friesen, W. (1976). Pictures of Facial Affect. Palo Alto, CA: Consulting Psychologist Press.

Ethofer, T., Anders, S., Erb, M., Droll, C., Royen, L., Saur, R., et al. (2006). Impact of voice on emotional judgment of faces: an event-related fMRI study. *Hum Brain Mapp, 27*(9), 707-714.

Ethofer, T., Anders, S., Wiethoff, S., Erb, M., Herbert, C., Saur, R., et al. (2006). Effects of prosodic emotional intensity on activation of associative auditory cortex. *Neuroreport, 17*(3), 249-253.

Ethofer, T., Pourtois, G., & Wildgruber, D. (2006). Investigating audiovisual integration of emotional signals in the human brain. *Prog Brain Res, 156*, 345-361.

Falck-Ytter, T., & von Hofsten, C. (2011). How special is social looking in ASD: a review. *Prog Brain Res, 189*, 209-222.

Fellows, L. K., & Farah, M. J. (2003). Ventromedial frontal cortex mediates affective shifting in humans: evidence from a reversal learning paradigm. *Brain, 126*(Pt 8), 1830-1837.

Frey, S., Kostopoulos, P., & Petrides, M. (2000). Orbitofrontal involvement in the processing of unpleasant auditory information. *Eur J Neurosci, 12*(10), 3709-3712.

Fugate, J. M., Gouzoules, H., & Nygaard, L. C. (2008). Recognition of rhesus macaque (Macaca mulatta) noisy screams: evidence from conspecifics and human listeners. *Am J Primatol, 70*(6), 594-604.

Gansler, D. A., McLaughlin, N. C., Iguchi, L., Jerram, M., Moore, D. W., Bhadelia, R., et al. (2009). A multivariate approach to aggression and the orbital frontal cortex in psychiatric patients. *Psychiatry Res, 171*(3), 145-154.

Ghashghaei, H. T., & Barbas, H. (2002). Pathways for emotion: Interactions of prefrontal and anterior temporal pathways in the amygdala of the rhesus monkey. *Neuroscience, 115*(4), 1261-1279.

Ghashghaei, H. T., Hilgetag, C. C., & Barbas, H. (2007). Sequence of information processing for emotions based on the anatomic dialogue between prefrontal cortex and amygdala. *Neuroimage, 34*(3), 905-923.

Ghazanfar, A. A., Chandrasekaran, C., & Logothetis, N. K. (2008). Interactions between the superior temporal sulcus and auditory cortex mediate dynamic face/voice integration in rhesus monkeys. *J Neurosci, 28*(17), 4457-4469.

Ghazanfar, A. A., & Lemus, L. (2010). Multisensory integration: vision boosts information through suppression in auditory cortex. *Curr Biol, 20*(1), R22-23.

Ghazanfar, A. A., & Logothetis, N. K. (2003). Neuroperception: facial expressions linked to monkey calls. *Nature, 423*(6943), 937-938.

Ghazanfar, A. A., Maier, J. X., Hoffman, K. L., & Logothetis, N. K. (2005). Multisensory integration of dynamic faces and voices in rhesus monkey auditory cortex. *Journal of Neuroscience, 25*(20), 5004-5012.

Gil-da-Costa, R., Braun, A., Lopes, M., Hauser, M. D., Carson, R. E., Herscovitch, P., et al. (2004). Toward an evolutionary perspective on conceptual representation: species-specific calls activate visual and affective processing systems in the macaque. *Proc.Natl.Acad.Sci.U.S.A, 101*(50), 17516-17521.

Gorno-Tempini, M. L., Pradelli, S., Serafini, M., Pagnoni, G., Baraldi, P., Porro, C., et al. (2001). Explicit and incidental facial expression processing: an fMRI study. *Neuroimage, 14*(2), 465-473.

Gothard, K. M., Brooks, K. N., & Peterson, M. A. (2009). Multiple perceptual strategies used by macaque monkeys for face recognition. *Anim Cogn, 12*(1), 155-167.

Gothard, K. M., Erickson, C. A., & Amaral, D. G. (2004). How do rhesus monkeys (Macaca mulatta) scan faces in a visual paired comparison task? *Anim Cogn, 7*(1), 25-36.

Goulet, S., & Murray, E. A. (2001). Neural substrates of crossmodal association memory in monkeys: the amygdala versus the anterior rhinal cortex. *Behav Neurosci, 115*(2), 271-284.

Goursaud, A. P., & Bachevalier, J. (2007). Social attachment in juvenile monkeys with neonatal lesion of the hippocampus, amygdala and orbital frontal cortex. *Behav Brain Res, 176*(1), 75-93.

Goursaud, A. P., & Bachevalier, J. (in preparation). Face processing and emotion regulation are altered by orbitofrontal cortex lesion in adult monkeys (Macaca mulatta).

Goursaud, A. P., Bachevalier, J., Jones, W., & Klin, A. (2003). *The use of eye-tracking technology to study social visual pursuit in monkeys (Macaca mulatta).* Paper presented at the University of Texas Neuroscience Symposium.

Gouzoules, H., Gouzoules, S., & Marler, P. (1986). Vocal communication: A vehicle for the study of social relationships. In R. Rawlins & M. Kessler (Eds.), *The Cayo Santiago Macaques: history, behavior and biology* (pp. 111-129). Albany, NY: State University of New York Press.

Gouzoules, S., Gouzoules, H., & Marler, P. (1984). Rhesus monkey (Macaca mulatta) screams: representational signaling in the recruitment of agonistic aid. *Anim Behav, 32*, 182-193.

Grossmann, T., Striano, T., & Friederici, A. D. (2005). Infants' electric brain responses to emotional prosody. *Neuroreport, 16*(16), 1825-1828.

Guo, K., Robertson, R. G., Mahmoodi, S., Tadmor, Y., & Young, M. P. (2003). How do monkeys view faces?--A study of eye movements. *Experimental Brain Research, 150*(3), 363-374.

Hackett, T. A., Stepniewska, I., & Kaas, J. H. (1999). Prefrontal connections of the parabelt auditory cortex in macaque monkeys. *Brain Res, 817*(1-2), 45-58.

Hamann, S. (2005). Sex differences in the responses of the human amygdala. *Neuroscientist, 11*(4), 288-293.

Hauser, M., Evans, C., & Marler, P. (1993). The role of articulation in the production of rhesus monkey, Macaca mulatta, vocalizations. *Anim Behav, 45*, 423-433.

Haxby, J. V., Hoffman, E. A., & Gobbini, M. I. (2000). The distributed human neural system for face perception. *Trends Cogn Sci, 4*(6), 223-233.

Haxby, J. V., Hoffman, E. A., & Gobbini, M. I. (2002). Human neural systems for face recognition and social communication. *Biol Psychiatry, 51*(1), 59-67.

Hermelin, B., & O'Connor, N. (1970). *Psychological experiments with autistic children.* Oxford: Pergamon.

Hobson, R. P., Ouston, J., & Lee, A. (1988). Emotion recognition in autism: coordinating faces and voices. *Psychol.Med., 18*(4), 911-923.

Holland, P. C., & Gallagher, M. (2004). Amygdala-frontal interactions and reward expectancy. *Curr Opin Neurobiol, 14*(2), 148-155.

Hornak, J., Bramham, J., Rolls, E. T., Morris, R. G., O'Doherty, J., Bullock, P. R., et al. (2003). Changes in emotion after circumscribed surgical lesions of the orbitofrontal and cingulate cortices. *Brain, 126*(Pt 7), 1691-1712.

Hornak, J., O'Doherty, J., Bramham, J., Rolls, E. T., Morris, R. G., Bullock, P. R., et al. (2004). Reward-related reversal learning after surgical excisions in orbito-frontal or dorsolateral prefrontal cortex in humans. *J Cogn Neurosci, 16*(3), 463-478.

Hornak, J., Rolls, E. T., & Wade, D. (1996). Face and voice expression identification in patients with emotional and behavioural changes following ventral frontal lobe damage. *Neuropsychologia, 34*(4), 247-261.

Horsley, T. A., de Castro, B. O., & Van der Schoot, M. (2010). In the eye of the beholder: eye-tracking assessment of social information processing in aggressive behavior. *J Abnorm Child Psychol, 38*(5), 587-599.

Humphreys, G. W., Donnelly, N., & Riddoch, M. J. (1993). Expression is computed separately from facial identity, and it is computed separately for moving and static faces: neuropsychological evidence. *Neuropsychologia, 31*(2), 173-181.

Hutt, S. J., Hutt, C., Lee, D., & Ounsted, C. (1965). A behavioural and electroencephalographic study of autistic children. *J Psychiatr Res, 3*(3), 181-197.

Iarocci, G., & McDonald, J. (2006). Sensory integration and the perceptual experience of persons with autism. *J.Autism Dev.Disord., 36*(1), 77-90.

Izquierdo, A., & Murray, E. A. (2004). Combined unilateral lesions of the amygdala and orbital prefrontal cortex impair affective processing in rhesus monkeys. *J Neurophysiol, 91*(5), 2023-2039.

Izquierdo, A., & Murray, E. A. (2010). Functional interaction of medial mediodorsal thalamic nucleus but not nucleus accumbens with amygdala and orbital prefrontal cortex is essential for adaptive response selection after reinforcer devaluation. *J Neurosci, 30*(2), 661-669.

Izquierdo, A., Suda, R. K., & Murray, E. A. (2005). Comparison of the effects of bilateral orbital prefrontal cortex lesions and amygdala lesions on emotional responses in rhesus monkeys. *J Neurosci, 25*(37), 8534-8542.

Jones, B., & Mishkin, M. (1972). Limbic lesions and the problem of stimulus--reinforcement associations. *Experimental Neurology, 36*(2), 362-377.

Jones, E. G., & Powell, T. P. (1970). An anatomical study of converging sensory pathways within the cerebral cortex of the monkey. *Brain, 93*(4), 793-820.

Jones, J. A., & Callan, D. E. (2003). Brain activity during audiovisual speech perception: an fMRI study of the McGurk effect. *Neuroreport, 14*(8), 1129-1133.

Kadunce, D. C., Vaughan, J. W., Wallace, M. T., Benedek, G., & Stein, B. E. (1997). Mechanisms of within- and cross-modality suppression in the superior colliculus. *J Neurophysiol, 78*(6), 2834-2847.

Kanwisher, N., & Yovel, G. (2006). The fusiform face area: a cortical region specialized for the perception of faces. *Philos Trans R Soc Lond B Biol Sci, 361*(1476), 2109-2128.

Kayser, C., & Logothetis, N. K. (2007). Do early sensory cortices integrate cross-modal information? *Brain Struct Funct, 212*(2), 121-132.

Kazama, A. M., Glavis-Bloom, C., & Bachevalier, J. (2008). *Neonatal amygdala and orbital frontal cortex lesions disrupt flexible decision-making in adult macaques*. Paper presented at the Society for Neuroscience.

Klopp, J., Marinkovic, K., Chauvel, P., Nenov, V., & Halgren, E. (2000). Early widespread cortical distribution of coherent fusiform face selective activity. *Hum Brain Mapp, 11*(4), 286-293.

Kondo, H., Saleem, K. S., & Price, J. L. (2003). Differential connections of the temporal pole with the orbital and medial prefrontal networks in macaque monkeys. *Journal of Comparative Neurology, 465*(4), 499-523.

Kreifelts, B., Ethofer, T., Grodd, W., Erb, M., & Wildgruber, D. (2007). Audiovisual integration of emotional signals in voice and face: an event-related fMRI study. *Neuroimage, 37*(4), 1445-1456.

Kuraoka, K., & Nakamura, K. (2007). Responses of single neurons in monkey amygdala to facial and vocal emotions. *J Neurophysiol, 97*(2), 1379-1387.

LaBar, K. S., Crupain, M. J., Voyvodic, J. T., & McCarthy, G. (2003). Dynamic perception of facial affect and identity in the human brain. *Cereb Cortex, 13*(10), 1023-1033.

Lansing, C. R., & McConkie, G. W. (1999). Attention to facial regions in segmental and prosodic visual speech perception tasks. *J Speech Lang Hear Res, 42*(3), 526-539.

Laurienti, P. J., Perrault, T. J., Stanford, T. R., Wallace, M. T., & Stein, B. E. (2005). On the use of superadditivity as a metric for characterizing multisensory integration in functional neuroimaging studies. *Exp Brain Res, 166*(3-4), 289-297.

Lee, G. P., Meador, K. J., Smith, J. R., Loring, D. W., & Flanigin, H. F. (1988). Preserved crossmodal association following bilateral amygdalotomy in man. *Int J Neurosci, 40*(1-2), 47-55.

Lemerise, E. A., & Arsenio, W. F. (2000). An integrated model of emotion processes and cognition in social information processing. *Child Dev, 71*(1), 107-118.

Lenroot, R. K., & Giedd, J. N. (2010). Sex differences in the adolescent brain. *Brain Cogn, 72*(1), 46-55.

Loveland, K. (2005). Social-emotional impairment and self-regulation in Autism spectrum disorders. In J. Nadel & D. Muir (Eds.), *Typical and impaired emotional development* (pp. 365-382). Oxford: Oxford University Press.

Loveland, K., Pearson, D., & Reddoch, S. (2005). *Anxiety symptoms in childrem and adolescents with autism.* Paper presented at the International Meeting for Autism Research.

Loveland, K., Steinberg, J. L., Pearson, D. A., Mansour, R., & Reddoch, S. (2008). Judgments of auditory-visual affective congruence in adolescents with and without autism: a pilot study of a new task using fMRI. *Percept Mot Skills, 107*(2), 557-575.

Loveland, K., Tunali-Kotoski, B., Chen, R., Brelsford, K., Ortegon, J., & Pearson, D. (1995). Intermodal perception of affect in persons with autism or Down syndrome. *Develop Psychopath, 7.*

Machado, C. J., & Bachevalier, J. (2007). The effects of selective amygdala, orbital frontal cortex or hippocampal formation lesions on reward assessment in nonhuman primates. *Eur J Neurosci, 25*(9), 2885-2904.

Machado, C. J., Kazama, A. M., & Bachevalier, J. (2009). Impact of amygdala, orbital frontal, or hippocampal lesions on threat avoidance and emotional reactivity in nonhuman primates. *Emotion, 9*(2), 147-163.

Magnee, M. J., de Gelder, B., van Engeland, H., & Kemner, C. (2007). Facial electromyographic responses to emotional information from faces and voices in individuals with pervasive developmental disorder. *J Child Psychol Psychiatry, 48*(11), 1122-1130.

Magnee, M. J., de Gelder, B., van Engeland, H., & Kemner, C. (2008). Audiovisual speech integration in pervasive developmental disorder: evidence from event-related potentials. *J Child Psychol Psychiatry, 49*(9), 995-1000.

Marler, P. (1965). Communication in monkeys and apes. In I. DeVore (Ed.), *Primate behavior: Field studies of monkeys and apes* (pp. 544-584). New York: Hold, Rinehardt and Winston.

Massaro, D., & Egan, P. (1996). Perceiving affect from the voice and face. *Psychon Bull Rev, 3*, 215-221.

McAlonan, K., & Brown, V. J. (2003). Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. *Behav Brain Res, 146*(1-2), 97-103.

McDonald, A. J. (1998). Cortical pathways to the mammalian amygdala. *Prog Neurobiol, 55*(3), 257-332.

McGaugh, J. L., Ferry, B., Vazdarjanova, A., & Roozendaal, B. (2000). Amygdala: role in modulation of memory storage. In J. Aggleton (Ed.), *The amygdala: A functional analysis* (2nd ed., pp. 391-424). Oxford: Oxford University Press.

McGurk, H., & MacDonald, J. (1976). Hearing lips and seeing voices. *Nature, 264*(5588), 746-748.

Meunier, M., Bachevalier, J., & Mishkin, M. (1997). Effects of orbital frontal and anterior cingulate lesions on object and spatial memory in rhesus monkeys. *Neuropsychologia, 35*(7), 999-1015.

Michael, R. P., Rees, H. D., & Bonsall, R. W. (1989). Sites in the male primate brain at which testosterone acts as an androgen. *Brain Res, 502*(1), 11-20.

Michael, R. P., Zumpe, D., & Bonsall, R. W. (1992). The interaction of testosterone with the brain of the orchidectomized primate fetus. *Brain Res, 570*(1-2), 68-74.

Miller, J. (1982). Divided attention: evidence for coactivation with redundant signals. *Cogn Psychol, 14*(2), 247-279.

Mitchell, R. L., Elliott, R., Barry, M., Cruttenden, A., & Woodruff, P. W. (2003). The neural response to emotional prosody, as revealed by functional magnetic resonance imaging. *Neuropsychologia, 41*(10), 1410-1421.

Morris, J. S., Friston, K. J., Buchel, C., Frith, C. D., Young, A. W., Calder, A. J., et al. (1998). A neuromodulatory role for the human amygdala in processing emotional facial expressions. *Brain, 121 (Pt 1)*, 47-57.

Morris, J. S., Friston, K. J., & Dolan, R. J. (1998). Experience-dependent modulation of tonotopic neural responses in human auditory cortex. *Proc Biol Sci, 265*(1397), 649-657.

Morris, J. S., Frith, C. D., Perrett, D. I., Rowland, D., Young, A. W., Calder, A. J., et al. (1996). A differential neural response in the human amygdala to fearful and happy facial expressions. *Nature, 383*(6603), 812-815.

Morrison, S. E., & Salzman, C. D. (2010). Re-valuing the amygdala. *Curr Opin Neurobiol, 20*(2), 221-230.

Muller, V. I., Habel, U., Derntl, B., Schneider, F., Zilles, K., Turetsky, B. I., et al. (2011). Incongruence effects in crossmodal emotional integration. *Neuroimage, 54*(3), 2257-2266.

Murray, E. A. (2007). The amygdala, reward and emotion. *Trends Cogn Sci, 11*(11), 489-497.

Nahm, F. K., Tranel, D., Damasio, H., & Damasio, A. R. (1993). Cross-modal associations and the human amygdala. *Neuropsychologia, 31*(8), 727-744.

Nakamura, K., Kawashima, R., Ito, K., Sugiura, M., Kato, T., Nakamura, A., et al. (1999). Activation of the right inferior frontal cortex during assessment of facial emotion. *J Neurophysiol, 82*(3), 1610-1614.

O'Doherty, J., Rolls, E. T., Kringelbach, M. L., Calvert, G. A., Spence, C., & Stein, B. E. (2004). Neuroimaging studies of cross-modal integration for emotion. In *The handbook of multisensory processes* (pp. 563-579). New York: Bradford Books.

O'Scalaidhe, S. P., Wilson, F. A., & Goldman-Rakic, P. S. (1997). Areal segregation of face-processing neurons in prefrontal cortex. *Science, 278*(5340), 1135-1138.

O'Scalaidhe, S. P., Wilson, F. A., & Goldman-Rakic, P. S. (1999). Face-selective neurons during passive viewing and working memory performance of rhesus monkeys: evidence for intrinsic specialization of neuronal coding. *Cereb Cortex, 9*(5), 459-475.

Olson, I. R., Gatenby, J. C., & Gore, J. C. (2002). A comparison of bound and unbound audio-visual information processing in the human cerebral cortex. *Brain Res Cogn Brain Res, 14*(1), 129-138.

Ornitz, E. M. (1969). Disorders of perception common to early infantile autism and schizophrenia. *Compr Psychiatry, 10*(4), 259-274.

Pandya, D. N., & Kuypers, H. G. (1969). Cortico-cortical connections in the rhesus monkey. *Brain Research, 13*(1), 13-36.

Parr, L. A. (2011). The evolution of face processing in primates. *Philos Trans R Soc Lond B Biol Sci, 366*(1571), 1764-1777.

Partan, S. R. (2002). Single and multichannel signal composition: Facial expressions and vocalizations of rhesus macaques (Macaca mulatta). *Behaviour, 139*, 993-1027.

Partan, S. R. (2004). Multisensoty animal communication. In G. Calvert, C. Spence & B. Stein (Eds.), *The Handbook of Multisensory Processes* (pp. 225-242). Cambridge, MA: The MIT Press.

Partan, S. R., & Marler, P. (1999). Communication goes multimodal. *Science, 283*(5406), 1272-1273.

Payne, C., & Bachevalier, J. (2009). Neuroanatomy of the developing social brain. In M. de Haan & M. Gunnary (Eds.), *Handbook of developmental social neuroscience* (pp. 38-59). New York: Guilford Publications.

Payne, C., Machado, C. J., Bliwise, N. G., & Bachevalier, J. (2010). Maturation of the hippocampal formation and amygdala in Macaca mulatta: a volumetric magnetic resonance imaging study. *Hippocampus, 20*(8), 922-935.

Perrault, T. J., Jr., Vaughan, J. W., Stein, B. E., & Wallace, M. T. (2003). Neuron-specific response characteristics predict the magnitude of multisensory integration. *J Neurophysiol, 90*(6), 4022-4026.

Phelps, E. A. (2006). Emotion and cognition: Insights from studies of the human amygdala. *Annu.Rev.Psychol., 57*, 27-53.

Phillips, M. L., Young, A. W., Scott, S. K., Calder, A. J., Andrew, C., Giampietro, V., et al. (1998). Neural responses to facial and vocal expressions of fear and disgust. *Proc Biol Sci, 265*(1408), 1809-1817.

Pierce, K., Haist, F., Sedaghat, F., & Courchesne, E. (2004). The brain response to personally familiar faces in autism: findings of fusiform activity and beyond. *Brain, 127*(Pt 12), 2703-2716.

Pomerantz, S. M., & Sholl, S. A. (1987). Analysis of sex and regional differences in androgen receptors in fetal rhesus monkey brain. *Brain Res, 433*(1), 151-154.

Poremba, A., Saunders, R. C., Crane, A. M., Cook, M., Sokoloff, L., & Mishkin, M. (2003). Functional mapping of the primate auditory system. *Science, 299*(5606), 568-572.

Pourtois, G., de Gelder, B., Bol, A., & Crommelinck, M. (2005). Perception of facial expressions and voices and of their combination in the human brain. *Cortex, 41*(1), 49-59.

Radeau, M. (1994). Auditory-visual spatial interaction and modularity. *Curr Psychol Cogn, 13*(1), 3-51.

Raper, J., Kazama, A., & Bachevalier, J. (2009). *Blunted fear reactivity after neonatal amygdala and orbital frontal lesions in rhesus monkeys*. Paper presented at the Society for Neuroscience.

Raper, J., Wilson, M., Sanchez, M., & Bachevalier, J. (2011). *Neonatal amygdala lesions alter emotional and neuroendocrine responses to an acute stressor in adult rhesus monkeys*. Paper presented at the Gordon Research Conference: Amygdala in Health and Disease.

Rasia-Filho, A. A., Londero, R. G., & Achaval, M. (2000). Functional activities of the amygdala: an overview. *J.Psychiatry Neurosci., 25*, 14-23.

Rendall, D., Rodman, P., & Emond, R. (1996). Voal recognition of individuals and kin in free-ranging rhesus monkeys. *Anim Behav, 51*, 1007-1015.

Robins, D. L., Hunyadi, E., & Schultz, R. T. (2009). Superior temporal activation in response to dynamic audio-visual emotional cues. *Brain Cogn, 69*(2), 269-278.

Rolls, E. T. (1992). Neurophysiology and functions of the primate amygdala. In J. Aggleton (Ed.), *The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction* (pp. 143-165). New York, NY: Wiley-Liss.

Rolls, E. T. (1999). *The brain and emotion*. Oxford, England: Oxford University Press.

Rolls, E. T. (2000). The orbitofrontal cortex and reward. *Cereb.Cortex, 10*, 284-294.

Rolls, E. T., Critchley, H. D., Browning, A. S., & Inoue, K. (2006). Face-selective and auditory neurons in the primate orbitofrontal cortex. *Exp Brain Res, 170*(1), 74-87.

Rolls, E. T., & Grabenhorst, F. (2008). The orbitofrontal cortex and beyond: from affect to decision-making. *Prog Neurobiol, 86*(3), 216-244.

Rolls, E. T., Hornak, J., Wade, D., & McGrath, J. (1994). Emotion-related learning in patients with social and emotional changes associated with frontal lobe damage. *J Neurol Neurosurg Psychiatry, 57*(12), 1518-1524.

Romanski, L. M., & Goldman-Rakic, P. S. (2002). An auditory domain in primate prefrontal cortex. *Nat Neurosci, 5*(1), 15-16.

Romanski, L. M., Tian, B., Fritz, J., Mishkin, M., Goldman-Rakic, P. S., & Rauschecker, J. P. (1999). Dual streams of auditory afferents target multiple domains in the primate prefrontal cortex. *Nat.Neurosci., 2*(12), 1131-1136.

Rowe, C. (1999). Receiver psychology and the evolution of multicomponent signals. *Anim Behav, 58*(5), 921-931.

Rowell, T., & Hinde, R. (1962). Vocal communication by the rhesus monkey (Macaca mulatta). *Proc Zool Soc Lond, 138*, 279-294.

Saito, D. N., Yoshimura, K., Kochiyama, T., Okada, T., Honda, M., & Sadato, N. (2005). Cross-modal binding and activated attentional networks during audio-visual speech integration: a functional MRI study. *Cereb Cortex, 15*(11), 1750-1760.

Salzman, C. D., & Fusi, S. (2010). Emotion, cognition, and mental state representation in amygdala and prefrontal cortex. *Annu Rev Neurosci, 33*, 173-202.

Saunders, R. C., & Rosene, D. L. (1988). A comparison of the efferents of the amygdala and the hippocampal formation in the rhesus monkey: I. Convergence in the entorhinal, prorhinal, and perirhinal cortices. *J Comp Neurol, 271*(2), 153-184.

Saunders, R. C., Rosene, D. L., & Van Hoesen, G. W. (1988). Comparison of the efferents of the amygdala and the hippocampal formation in the rhesus monkey: II. Reciprocal and non-reciprocal connections. *J Comp Neurol, 271*(2), 185-207.

Schoenbaum, G., & Esber, G. R. (2010). How do you (estimate you will) like them apples? Integration as a defining trait of orbitofrontal function. *Curr Opin Neurobiol, 20*(2), 205-211.

Schoenbaum, G., Roesch, M. R., Stalnaker, T. A., & Takahashi, Y. K. (2009). A new perspective on the role of the orbitofrontal cortex in adaptive behaviour. *Nat Rev Neurosci, 10*(12), 885-892.

Schoenbaum, G., Setlow, B., Nugent, S. L., Saddoris, M. P., & Gallagher, M. (2003). Lesions of orbitofrontal cortex and basolateral amygdala complex disrupt acquisition of odor-guided discriminations and reversals. *Learn Mem, 10*(2), 129-140.

Schultz, R. T., Gauthier, I., Klin, A., Fulbright, R. K., Anderson, A. W., Volkmar, F., et al. (2000). Abnormal ventral temporal cortical activity during face discrimination among individuals with autism and Asperger syndrome. *Archives of General Psychiatry, 57*(4), 331-340.

Schultz, R. T., Grelotti, D. J., Klin, A., Kleinman, J., Van der, G. C., Marois, R., et al. (2003). The role of the fusiform face area in social cognition: implications for the pathobiology of autism. *Philos.Trans.R.Soc.Lond B Biol.Sci., 358*, 415-427.

Schultz, R. T., Romanski, L., & Tsatsanis, K. (2000). Neurofunctional models of autistic disorder and asperger syndrome: Clues from neuroimaging. In A. Klin, F. Volkmar & S. Sparrow (Eds.), *Asperger Syndrome* (pp. 172-209). New York: Guilford Press.

Schultz, W., Tremblay, L., & Hollerman, J. R. (2000). Reward processing in primate orbitofrontal cortex and basal ganglia, *Cerebral Cortex* (Vol. 10, pp. 272-283).

Scott, S. K., Young, A. W., Calder, A. J., Hellawell, D. J., Aggleton, J. P., & Johnson, M. (1997). Impaired auditory recognition of fear and anger following bilateral amygdala lesions. *Nature, 385*(6613), 254-257.

Seltzer, B., & Pandya, D. N. (1978). Afferent cortical connections and architectonics of the superior temporal sulcus and surrounding cortex in the rhesus monkey. *Brain Research, 149*(1), 1-24.

Sholl, S. A., Goy, R. W., & Kim, K. L. (1989). 5 alpha-reductase, aromatase, and androgen receptor levels in the monkey brain during fetal development. *Endocrinology, 124*(2), 627-634.

Sholl, S. A., & Kim, K. L. (1990). Androgen receptors are differentially distributed between right and left cerebral hemispheres of the fetal male rhesus monkey. *Brain Research, 516*(1), 122-126.

Sliwa, J., Duhamel, J. R., Pascalis, O., & Wirth, S. (2011). Spontaneous voice-face identity matching by rhesus monkeys for familiar conspecifics and humans. *Proc Natl Acad Sci U S A, 108*(4), 1735-1740.

Soken, N. H., & Pick, A. D. (1999). Infants' perception of dynamic affective expressions: do infants distinguish specific expressions? *Child Dev, 70*(6), 1275-1282.

Sprengelmeyer, R., Rausch, M., Eysel, U. T., & Przuntek, H. (1998). Neural structures associated with recognition of facial expressions of basic emotions. *Proc Biol Sci, 265*(1409), 1927-1931.

Stanford, T. R., Quessy, S., & Stein, B. E. (2005). Evaluating the operations underlying multisensory integration in the cat superior colliculus. *J Neurosci, 25*(28), 6499-6508.

Stanford, T. R., & Stein, B. E. (2007). Superadditivity in multisensory integration: putting the computation in context. *Neuroreport, 18*(8), 787-792.

Stefanacci, L., Clark, R. E., & Zola, S. M. (2003). Selective neurotoxic amygdala lesions in monkeys disrupt reactivity to food and object stimuli and have limited effects on memory. *Behav Neurosci, 117*(5), 1029-1043.

Stein, B. E. (1998). Neural mechanisms for synthesizing sensory information and producing adaptive behaviors. *Exp Brain Res, 123*(1-2), 124-135.

Stein, B. E., & Meredith, M. (1993). *Merging of the senses*. Cambridge: MIT Press.

Stein, B. E., & Wallace, M. T. (1996). Comparisons of cross-modality integration in midbrain and cortex. *Prog Brain Res, 112*, 289-299.

Sugihara, T., Diltz, M. D., Averbeck, B. B., & Romanski, L. M. (2006). Integration of auditory and visual communication information in the primate ventrolateral prefrontal cortex. *J Neurosci, 26*(43), 11138-11147.

Treisman, A. (1996). The binding problem. *Curr Opin Neurobiol, 6*(2), 171-178.

van Atteveldt, N., Formisano, E., Goebel, R., & Blomert, L. (2004). Integration of letters and speech sounds in the human brain. *Neuron, 43*(2), 271-282.

Vuilleumier, P., & Pourtois, G. (2007). Distributed and interactive brain mechanisms during emotion face perception: evidence from functional neuroimaging. *Neuropsychologia, 45*(1), 174-194.

Vuilleumier, P., Richardson, M. P., Armony, J. L., Driver, J., & Dolan, R. J. (2004). Distant influences of amygdala lesion on visual cortical activation during emotional face processing. *Nat Neurosci, 7*(11), 1271-1278.

Webster, M. J., Bachevalier, J., & Ungerleider, L. G. (1994). Connections of inferior temporal areas TEO and TE with parietal and frontal cortex in macaque monkeys. *Cereb.Cortex, 4*(5), 470-483.

Wildgruber, D., Hertrich, I., Riecker, A., Erb, M., Anders, S., Grodd, W., et al. (2004). Distinct frontal regions subserve evaluation of linguistic and emotional aspects of speech intonation. *Cereb Cortex, 14*(12), 1384-1389.

Wildgruber, D., Pihan, H., Ackermann, H., Erb, M., & Grodd, W. (2002). Dynamic brain activation during processing of emotional intonation: influence of acoustic parameters, emotional valence, and sex. *Neuroimage, 15*(4), 856-869.

Wright, T. M., Pelphrey, K. A., Allison, T., McKeown, M. J., & McCarthy, G. (2003). Polysensory interactions along lateral temporal regions evoked by audiovisual speech. *Cereb.Cortex, 13*(10), 1034-1043.

Young, A. W., Hellawell, D. J., Van De Wal, C., & Johnson, M. (1996). Facial expression processing after amygdalotomy. *Neuropsychologia, 34*(1), 31-39.

Yukie, M. (2002). Connections between the amygdala and auditory cortical areas in the macaque monkey. *Neurosci Res, 42*(3), 219-229.