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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Sciences with Honors

Department of Neuroscience & Behavioral Biology

2016

Abstract

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Adolescence is a developmental period characterized by behavioral, neurological, and hormonal changes. In addition to substantial social and physical reorientation, this period gives rise to an increased prevalence of psychiatric disorders. To further our understanding of these mental health disorders, it is crucial to first investigate the normal trajectory of social development during adolescence in an animal model. This longitudinal study seeks to document normative socioemotional development in juvenile rhesus macaques by investigating changes in attention to social stimuli prior to adolescence using eye-tracking technology at 4 time points (18, 22, 26, and 36 months) over the pre-pubertal period. Three juvenile male rhesus macaques viewed videos of conspecifics performing stereotyped positive, negative, and neutral facial expressions. Results show that attention to socially salient regions increases with age throughout the pre-pubertal period but has not yet reached adult-like levels at 36 months of age. Additionally, once juveniles reach 36 months, they are able to rapidly modulate their attention to social stimuli based upon emotional content, but not before. These data suggest that social attention and emotion processing abilities continue to develop throughout the pre-pubertal period. This experiment compliments previous research in humans suggesting that adolescence is a developmental window for socioemotional maturation. Future research using this animal model is needed to investigate the role of key brain regions and pubertal hormones in this social reorganization.

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Acknowledgements

I would like to thank my adviser, Jocelyne Bachevalier, PhD, for guiding me with scholarship, support, and expertise throughout this project. Thank you to my committee members, Patricia Bauer, PhD and Daniel Dilks, PhD, for their support and insightful comments on my work. I would also like to thank Lauren Murphy for being a fantastic mentor by pushing me to explore my own ideas, patiently guiding me in learning new techniques and concepts, and encouraging me throughout each stage of this project. Additionally, thank you to the entire Bachevalier lab for all of the stimulating conversations, thoughtful advice, and friendly support throughout my time working in the lab.

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Introduction

Adolescence is a developmental period characterized by substantial changes on both a behavioral and physiological level. More importantly, adolescence is characterized by increased rates of diagnoses of psychiatric disorders including schizophrenia, substance abuse and anxiety disorders (Paus et al., 2008). It is thought that the rise in incidences of these disorders may be due to divergence from normal development patterns in functional, neurobiological, structural, and connectivity changes (Paus et al., 2008). Additionally, there is an association between deviations in normal responses to social emotional stimuli and mental disorders (Paus et al., 2008). Thus, identification, prevention, and treatment of mental health disorders are therefore dependent upon a deeper understanding of the normal course of behavioral and neurobiological development in adolescence.

One approach to gain further knowledge on the behavioral and neurobiological development in adolescence is through the development of an animal model. Although animals do not develop neuropsychiatric disorders, they can provide critical information on the behavioral and physiological changes that occur during adolescence and can contribute to the design of new potential treatments. In contrast to rodents, nonhuman primates are particularly useful because they show similar trajectories of complex social behavior development as well as similar development of brain anatomy and functions seen in humans during adolescence (Machado & Bachevalier 2003). In particular, the long history of the rhesus macaque model in neuropsychological research provides a foundation for testing and evaluating new hypotheses. In both humans and rhesus macaques, adolescence represents a time of behavioral and brain development changes that affect not only the way they interact with their peers but also the way they perceive emotional cues from others. Understanding the behavioral and neural components of social development in rhesus macaques will provide a foundation for future investigations into the contributions of various physical and environmental factors affecting typical social development.

Development of face processing abilities

During social development, the perception of faces and facial expressions in humans undergoes characteristic age-related changes throughout adolescence. When looking at a face, primates obtain crucial information about conspecifics, such as physical characteristics, emotional state, and intentions (Emery, 2000; Ghafanzar et al., 2005; Ghafanzar & Santos, 2004). Additionally, increased fixation durations (viewing) in response to faces in comparison to scenes indicate that there is neural processing specific to extraction of information from face stimuli (Guo et al., 2006). Therefore, the proper functioning of brain regions specialized for facial processing is crucial for successful navigation within complex social environments (Emery 2000). Furthermore, a wide variety of mental health disorders, including Autism Spectrum Disorder, are associated with impaired ability to interpret emotional expressions (Lee et al., 2013; Klin et al., 2002), suggesting that a better knowledge of facial processing development in primates may provide a sensitive tool in investigating deviations from normal development that can lead to mental illness.

Investigation of developing facial processing abilities in humans and nonhuman primates can provide a deeper understanding of the driving forces behind social development. Human studies showed age-related improvements from infancy into adulthood in the perception of emotional face processing, indicating that social development continues throughout adolescence (Scherf 2013). Similarly, juvenile rhesus macaques show continued social development through early adolescence, indicated by changing attention to social stimuli during this period (Murphy;

unpublished data). Additionally, although adult macaques are able to track eye gaze solely based upon eye movements, juvenile macaques rely upon both eye and head movements in order to perform the same function, suggesting that, as in humans, adolescence is a crucial period for development of social skills (Ferrari et al., 2000). Identification of changes in the processing of social stimuli during adolescence in addition to the increased risk for mental health disorders during the same developmental period suggests a potential relationship between factors that drive social development and those that lead to behavioral deficits. Therefore, measuring facial processing abilities over development can be used to investigate variables that may influence social and neurological development during adolescence.

Puberty and social development

Adolescence is marked by social changes as well as an increase in sex hormones; therefore, it is hypothesized that the onset of increases in gonadal hormones initiates the shifts in behavioral changes characteristic of adolescence. Temporal correlations between gonadal hormone release, neurological changes, and behavioral changes suggest that pubertal hormones play a driving role in brain and social development (Blakemore et al., 2010). For example, white matter microstructure development is closely related to pubertal hormone status in boys (Menzies et al., 2015). Additionally, puberty is linked to changes in specific socio-emotional behaviors during this adolescent period, such as increased sensitivity to emotional stimuli as well as decreased cognitive control in response to emotional stimuli (Spear, 2009; Tottenham et al., 2011). Investigation in a rhesus macaque model has found that social behavior development during adolescence is sensitive to gonadectomy. Specifically, gonadal hormones are associated with the acquisition or maintenance of high social standings in rhesus macaques as they enter adulthood (Richards, 2009). Furthermore, the amygdala, an area underlying social behaviors that undergo significant development during adolescence, contains a large number of gonadal hormones receptors (Scherf, 2013), suggesting that puberty-induced hormonal changes may drive alterations in the saliency of various social stimuli. These data further indicate a causal role of pubertal hormones in social maturation throughout adolescence, although this causal role has been difficult to demonstrate in humans.

Face processing maturation coincides with characteristic physiological changes during this developmental period. Age-associated improvements in face processing abilities in conjunction with hormone related reorganization of functional connectivity between face processing systems and emotion processing regions indicate that puberty plays a role in the development of social and behavioral skills during adolescence (Said et al., 2011; Tottenham et al., 2011; Scherf 2012). Face-processing abilities are sensitive to neuroendocrine disorders as well as natural fluctuations of gonadal hormones (Scherf et al., 2012), further supporting the role of puberty-related hormonal changes in adolescent social behavioral development. Furthermore, research shows positive correlations between pubertal progress, as measured by phenotypic pubertal status and hormone levels, and functional connectivity between regions engaged in processing of emotional social stimuli, suggesting that changes in adolescent social behaviors may occur through puberty-driven alterations in neurological connectivity (Klapwijk et al., 2013). Although the period of adolescence shows associations between puberty and social maturation, it is difficult to isolate the effects of specific puberty-related physiological changes and their relation to neurological and behavioral development. Therefore, investigation is needed to determine specific mechanisms of the role of physiological pubertal changes in social maturation.

Adolescence and brain development

Many systems in the brain are altered during adolescence, but few key areas are thought to play a specific role in social development. The amygdala is identified as a “hub-region,” a highly interconnected structure that interacts with many systems in the brain (Pessoa 2008). The amygdala also undergoes structural changes during adolescence in correlation with the progress of puberty and release of gonadal hormones (Scherf, 2013). Furthermore, this region contains sex hormone receptors further suggesting that puberty-related hormonal changes may drive social behavior maturation dependent upon the amygdala (Scherf, 2013). Adolescence gives rise to an increase in volume and activation of the amygdala that are correlated with pubertal progress (Neufang et al., 2009; Moore et al., 2012). The hyperactivation of this structure during adolescence is seen in response to emotional stimuli (Hare et al., 2008). In addition to showing similar structure and connectivity, amygdalae in rhesus macaques are also implicated in the development of normal social behaviors (Machado & Bachevalier, 2003; Moadab et al., 2015). Thus, the interconnectedness of the amygdala, along with functional changes, suggests that this structure is a fundamental element in the development of social behaviors throughout adolescence (Scherf 2013).

In addition to the amygdala, subregions of the frontal cortex continue to develop throughout adolescence, and changes in these structures are correlated with the development of social behaviors (Machado and Bachevalier 2003). The prefrontal cortex is one of the last neural structures to reach full maturity, with continued development throughout childhood and adolescence (Casey et al., 2000). Although adults rely upon late-maturing prefrontal cortex regions for emotional cue processing, adolescents performing the same task show activity in early-maturing subcortical structures, such as the amygdala and hippocampus (Lau et al., 2011). Additionally, studies have found that adolescents show later development of anger recognition, a prefrontal cortex-dependent ability, in comparison to fear recognition, an amygdala-dependent ability, further implicating this region in social development during adolescence (Thomas et al., 2007). Increased associations between prefrontal cortex regions and processing of socioemotional stimuli during late adolescence further implicate the prefrontal cortex as a target area for functional development during adolescence (Moore et al., 2012). Data indicate that rhesus macaques undergo similar brain development patterns of the prefrontal cortex as the orbitofrontal cortex and the dorsolateral prefrontal cortex may not reach full maturation until 8-12 months and 2 years of age, respectively (Machado & Bachevalier, 2003). The temporal and functional developmental characteristics of the prefrontal cortex suggest that this area may have a causal role in the normal trajectory of social development during adolescence.

In addition to showing characteristic changes in adolescence associated with social development, both the amygdala and the prefrontal cortex have been implicated in mental health disorders that typically present during adolescence. For example, structural and functional abnormalities of the prefrontal cortex are associated with social disorders, including schizophrenia and anxiety disorders (Paus et al., 2008). Similarly, hyperactivation of the amygdala is characteristic of depressive and anxiety disorders (van den Bulk et al., 2014). Additionally, puberty related alterations of the amygdala and prefrontal cortex are thought to modify functional connection between these regions and core face processing regions such as the posterior superior temporal sulcus, leading to changes in social emotion and facial processing abilities (Klapwijk et al., 2013; Scherf et al., 2013). Therefore, deviations from normal adolescent development in these neural structures may cause social deficits that are critical factors in the development of mental health disorders.

Modeling social development

Despite research implicating these brain structures in various psychopathologies, the neurobiological bases for behavioral deficits are often complicated, difficult to identify, and spread across large regions of the brain. Therefore, to advance understanding of the mechanisms of adolescent development, it is crucial to have animal models that allow for manipulation of factors theorized to contribute to mental illness in humans (e.g., gonadal hormone levels, early life stress) (Machado and Bachevalier, 2003). Manipulation of potential contributing factors would make it possible to determine causal forces in the development of social and emotional behaviors. Due to behavioral and neurological similarities, rhesus macaques are frequently used to model interactions between brain and behavior in humans (Machado and Bachevalier, 2003). Building upon previous research on patterns of social attention in adult macaques (Mosher et al., 2011), it is becoming critical to establish a rhesus macaque model for social development by investigating attention towards social stimuli in juvenile monkeys throughout their adolescence. Pilot data indicate that attention to social stimuli develops through adolescence in macaques (Murphy et al. 2014), but the impact of emotional valence is unclear. The acquisition and characterization of normative developmental changes in this animal model will allow for the design of future studies to assess the biological and neural basis of these changes.

Although associations between pubertal progress and behavioral and neurological changes are evident, the underlying mechanisms of these changes remain unclear. Further understanding of these mechanisms is crucial for moving forward with identification, prevention, and treatment of social disorders. Therefore, we seek to create a non-human primate model of social development that will allow for manipulation of variables that may play a causal role in these psychopathologies. This experiment aims to create a rhesus macaque model of adolescence social development by investigating changes in attention to social stimuli over time. Based upon human studies showing increased responsiveness and emotional reactivity to emotional stimuli during adolescence (Tottenham et al., 2011; Spear, 2009), we hypothesize that increased attention to emotional social stimuli and decreased attention to neutral social stimuli will develop over the pre-pubertal period.

Methods

Subjects

This experiment used three male rhesus macaques born into a large social group at Yerkes National Primate Research Center Field Station (Lawrenceville, GA). Subjects were mother reared until 6 months of age, at which time they were removed from the large social group and housed together. At 10 months of age, subjects were moved to the Yerkes National Primate Research Center (Atlanta, GA) where they were group housed under a 12 hour light/dark cycle. Behavioral testing was conducted at 4 time points: 18, 22, 26, and 36 months of age. Puberty-related hormone changes typically occur at 3-4 years in male rhesus macaques (Bercovitch, 1993). Therefore, the time points from 18-26 months were considered pre-pubertal, and the 36 month time point was considered to be the start of puberty.

Research Design

Pre-Training

Before testing began at each time point, the subjects were accustomed to the testing procedures. They were placed in the primate chair, fitted with the custom helmet, and positioned in front of the video monitor. They were rewarded with treats for calm behavior. This acclimation period ended once the subject had maintained calm behavior for at least 15 minutes. Following this acclimation period, each subject underwent formal testing.

Apparatus

The animals were placed 22” from the monitor in a primate chair, which holds the head at a constant height. A thermoplastic helmet was fitted for each subject and used to reduce head movements. At each time point, subject gaze was tracked using a Tobii 120Hz Eye Tracking monitor while subjects viewed a series of social and nonsocial videos. This eye tracking technology uses corneal reflections of infrared light to track eye movements of the subject. Data were recorded at a rate of 60Hz using a Windows laptop running Tobii Studio 3.2.2. At the beginning of each testing session, a 5-point calibration was used to control for variation in distance and head position.

Stimuli

Social Stimuli: At each time point, the subjects viewed a novel set of 30 social videos depicting unknown adult male and female conspecifics. Each 10-second social video showed one valence of emotional facial expression with positive videos showing lip smacking (n=10), negative videos showing threats (n=10), and neutral videos showing no emotional expressions (n=10). The videos were filmed under similar lighting and background conditions. Social videos were acquired from the lab of Dr. Gothard (Mosher et al., 2011). The valence categorizations of these videos were verified by lab members with extensive experience working with rhesus macaques.

Nonsocial stimuli: Subjects also viewed 10 nonsocial stimuli at each time point. These videos were 10 seconds long and showed moving objects. Videos showed unfamiliar objects with movement across a distinct background. Nonsocial stimuli were visually inspected to exclude any stimuli with face-like configurations.

Design

To examine changes in nonhuman primate social attention over the course of development, the juvenile monkeys were tested at 4 time points with each at least 4 months apart. Testing sessions were limited to 30 minutes to reduce stress on the animals and were repeated until each animal saw the social video set six times and the nonsocial video set 3 times. While the subjects were not experimentally naive, they were naive to the social and nonsocial videos shown at each time point.

Region of Interest (ROI) Analyses

Regions of Interest (ROIs) were drawn on each video stimulus using the Tobii Studio 3.2.2. application. The ROIs for social stimuli included eyes (cheekbones to brow bone), mouth (chin to bridge of nose), face (mouth and eyes), body (entire monkey), and background (entire video) (See Figure 1). The ROIs for nonsocial stimuli included foreground and background (See Figure 1). With the Tobii Studio program, the total viewing duration was calculated for each of ROI.

Each social video shows a rhesus macaque performing a stereotyped facial expression (positive, neutral, or negative). Emotional videos varied in the number of emotional and neutral components. Within a video, the facial expressions displayed by the stimulus monkey were interspersed in between sections of the videos during which the stimulus monkey displayed neutral facial expressions. Therefore, to more specifically analyze viewing duration during the emotional facial expression, we further reduced analyses to viewing duration during the emotional components of the video versus the viewing duration during the neutral components of the videos. Emotional components were defined as portions of the video containing lip smacks for positive stimuli and open-mouthed threats for negative stimuli. Neutral components were defined by a lack of any emotional facial expression. Analyzing the component parts of emotional videos allowed us to directly compare looking patterns during emotional components to looking patterns during neutral components within the social stimuli. Positive and negative videos contained the same duration of emotional components. Thus, within social videos, the positive ones averaged 6.62 seconds (SD = 2.6) of lip smack facial expressions and negative ones averaged 5.77 seconds (SD = 2.4) of threat facial expressions. Further, the number of facial expressions within the positive and negative videos did not differ and averaged 2.7 lip smack facial expressions per positive video and 2.1 threat expressions per negative video.

Finally, within the social videos, the emotional components were isolated by creating ROIs during each portion of the video containing a specific emotional facial expression and during each portion of the video containing neutral facial expressions. Using a custom MATLAB script, fixation durations to each ROI within the emotional and neutral components were summed within a video. The time spent looking at each ROI within the emotional component or neutral component was calculated as a proportion of the time spent looking at either the emotional component or the neutral component.

Statistical Analyses

All statistical analyses were performed using the SPSS statistical package (IBM, 2015).

Aim 1: Whole Video Analyses

To measure changes in social attention due to emotional facial expressions, we ran a repeated-measures ANOVA with valence (positive, negative, neutral) as the within-subject factor for each ROI (body, eyes, mouth). To measure changes in scanning patterns in juveniles over time, we ran a repeated-measures ANOVA with age as the within-subject factor (18, 22, 26, and 36 months) for each ROI.

To compare juveniles' looking towards social and nonsocial videos, we ran a repeated-measures ANOVA with Age (18, 22, 26, and 36) and Stimuli Type (social or nonsocial) as the within-subjects factors. To compare adults' looking towards social and nonsocial videos, we ran a one-way ANOVA with Stimuli Type as the within-subjects factor.

To compare looking patterns towards social stimuli in juveniles with those of adult controls, we ran a one-way ANOVA between the fixations durations of adults and juveniles at each juvenile time point within each ROI.

Aim 2: Emotional Component Analyses

Finally, to analyze the specific effects of emotional components, we separated the neutral and emotional components in the positive and negative social videos. We selected all positive and negative social videos that began with the sequence including neutral-emotional-neutral (Neutral 1–Emotion 1–Neutral 2) components (See Figure 2). Across all videos selected, Neutral 1 averaged 1.21 seconds (SD = 0.92), Emotion 1 averaged 0.60 seconds (SD = 2.38), and Neutral 2 averaged 2.35 seconds (SD = 2.13). For each component at each age, we ran a repeated-measures ANOVA with valence (positive, negative) as the within-subjects factor to assess the impact of emotional content. If the effect of valence reached significance, positive and negative emotional components were analyzed separately. Otherwise, the emotional components for each age and ROI were compared irrespective of valence. To compare the effect of emotional components, we ran a repeated-measures ANOVA with Emotional Component (Neutral 1, Emotion 1, or Neutral 2) as the within-subjects factor at each age and each ROI. Additionally, to directly compare the effect of age on juveniles' attention to social stimuli during the emotional components, we ran a repeated-measures ANOVA on the juveniles' fixation duration specifically during Emotion 1 with Age (18, 22, 26, and 36 months) as the within-subjects factor.

To compare 36 month old juveniles' and adults' attention during the emotional components, we ran a one-way ANOVA between the 36 month juveniles' and adults' fixation durations to Emotion 1 within the eye region.

Results

1. Aim 1: Whole Video Analyses

1.1. Effect of Age on Attention to Social Stimuli

There was no effect of valence on fixation durations to social videos for any ROI or age. Thus, we averaged fixation durations across valence and analyzed the effect of age on juveniles' fixation durations at each ROI. While the analyses did not reach significance, the effect of age reached trend levels for the body and eyes, and had large effect sizes (body: $F(3,6)=3.185$, $p=0.106$, $\eta^2 = 0.614$; See Figure 3; eyes: $F(3,6)=4.252$, $p=0.062$, $\eta^2 = 0.68$; See Figure 4). Thus, the effect of age on the percent fixation duration varied by region of interest of the stimulus. Within the body region, juveniles spent significantly less time fixated on the body of social stimuli at 22 months than at 18 months (18>22, $p=0.002$). Within the eye region, juveniles tended to spend more time looking at the eyes at 36 months of age than they did at 26 months (36>26, $p=0.052$).

1.2. Effect of Age on Attention to Social versus Non-social Stimuli

While there was no effect of Age or Stimuli Type on the juveniles' fixation durations at the social and nonsocial stimuli, there was a trend towards increased looking times at social videos in comparison to nonsocial videos (Age: $F(2,4)=0.525$, $p=0.627$, $\eta^2 = 0.208$; Stimuli type: $F(1,2)=16.819$, $p=0.055$, $\eta^2 = 0.894$). The adults showed a similar trend towards increased looking times at social videos in comparison to nonsocial videos (Stimuli type: $F(1,2)=8.609$, $p=0.099$, $\eta^2 = 0.811$).

1.3. Comparison of Adult and Juvenile Social Attention

When we compared percent fixation durations of the adults with those of the juveniles for each time point within each ROI, there were specific differences within each time point. While they didn't differ at 18 or 26 months, they looked less at all ROIs at 22 months (Eyes: $F(1,17)=5.422$, $p=0.033$, $\eta^2 = 0.012$; Mouth: $F(1,17)=4.907$, $p=0.042$, $\eta^2 = 0.235$; Body: $F(1,17)=8.691$, $p=0.009$, $\eta^2 = 0.352$; See Figure 3) and differed in multiple ROIs at 36 months. Specifically, at 36 months, juveniles spent more time fixated at the eyes ($F(1,17)=12.208$, $p=0.003$, $\eta^2 = 0.433$; See Figure 4), spent less time fixated at the mouth ($F(1,17)=8.071$, $p=0.012$, $\eta^2 = 0.335$), and spent the same time fixated at the body ($F(1,17)=0.065$, $p=0.801$, $\eta^2 = 0.004$; See Figure 5).

2. Aim 2: Emotional Component Analyses

2.1. Effect of Emotional Components on Juvenile Attention to Social Stimuli

When considering the entire length of the social videos, the analyses did not show a strong effect of valence (see Aim 1). To further investigate whether juvenile monkeys looked differently at positive versus negative social videos, we analyzed their visual exploration within the emotional and neutral components of each video. We compared the first neutral components, first emotional components, and second neutral components of social videos at each age, and found that attention to these three components varied with age.

At 18 months, there was no significant effect of valence on juveniles' attention to emotional components. Juveniles' did not modulate attention based on emotional components within any of the ROIs (body: $F(2,34)=0.750$, $p=0.480$, $\eta^2=0.042$; eyes: $F(2,34)=0.878$, $p=0.425$, $\eta^2=0.049$; see Figure 6a; mouth: $F(2,34)=0.483$, $p=0.621$, $\eta^2=0.028$).

At 22 months, there was no effect of valence on the mouth or body. However, within the eye region there was a significant effect of valence such that juveniles looked more at Neutral 1 during positive videos than during negative videos ($F(1,2)=58.043$, $p=0.017$, $\eta^2=0.967$; positive > negative, $p=0.017$). Thus, emotional components were examined separately for positive and negative valence. There was an effect of Emotional Component at the eyes only for positive social videos such that juveniles looked at Neutral 1 more than Neutral 2 (Positive: $F(2,10)=6.338$, $p=0.017$, $\eta^2=0.559$; Neutral 1 > Neutral 2, $p=0.018$; Negative: $F(2,28)=0.705$, $p=0.502$, $\eta^2=0.048$). Further, we analyzed the two videos included in the emotional component analyses for 22 months separately, and found that there was a trend toward an effect of emotional components at the eyes for one video ($F(2,4)=5.719$, $p=0.067$, $\eta^2=0.741$), but not the other ($F(2,4)=1.403$, $p=0.345$, $\eta^2=0.412$). There was no significant effect of emotional components on juveniles' fixation durations at the mouth or body (Mouth: $F(2,40)=0.569$, $p=0.571$, $\eta^2=0.028$; Body: $F(2,40)=0.369$, $p=0.694$, $\eta^2=0.028$).

There was no significant effect of valence on juveniles' attention to emotional components. At 26 months, there was no effect of emotional components on juveniles' fixation durations at the body ($F(2,46)=0.148$, $p=0.810$, $\eta^2=0.006$), eyes ($F(2,46)=0.205$, $p=0.815$, $\eta^2=0.009$; see Figure 6c), or mouth ($F(2,46)=0.365$, $p=0.696$, $\eta^2=0.016$).

At 36 months, there was no significant effect of valence on juveniles' attention to emotional components. There was a significant effect of emotional components on juveniles' fixation durations to the eyes ($F(2,50)=4.450$, $p=0.017$, $\eta^2=0.151$), and mouth ($F(2,50)=4.362$, $p=0.018$, $\eta^2=0.149$), but not to the body ($F(2,50)=2.773$, $p=0.072$, $\eta^2=0.100$). Specifically, attention to the eyes increased from Neutral 1 to Emotion 1 (Eyes: Neutral 1 < Emotion 1, $p=0.016$). Additionally, attention to the mouth increased from Neutral 1 to Emotion 1 before decreasing during Neutral 2 (Mouth: Neutral 1 < Emotion 1, $p=0.013$; Emotion 1 > Neutral 2, $p=0.038$). However, there was no difference between the two neutral components (Neutral 1 < Neutral 2, $p=0.484$).

To investigate the effect of age on attention to emotional content, we analyzed the effect of age on the juveniles' looking times during Emotion 1 of the social videos. There was no effect of Valence on juveniles' attention to the eyes, mouth or body (Eyes: $F(1,2)=04.237$, $p=0.176$, $\eta^2=0.679$; Mouth: $F(1,2)=0.123$, $p=0.756$, $\eta^2=0.058$; Body: $F(1,2)=0.182$, $p=0.182$, $\eta^2=0.669$). There was no effect of Age on juveniles' attention to the eyes, mouth, or body during the emotional component (Eyes: $F(3,6)=0.856$, $p=0.513$, $\eta^2=0.300$; Mouth: $F(3,6)=0.220$, $p=0.879$, $\eta^2=0.099$; Body: $F(3,6)=0.763$, $p=0.555$, $\eta^2=0.276$).

Finally, to compare the juveniles' and adults' attention to emotional content, we analyzed the difference between juveniles' and adults' attention during the emotional component. There was no significant difference between 36 month old juveniles' and adults' looking times during emotional components.

2.2. Effect of Emotional Components on Adult Attention to Social Stimuli

When emotional and neutral components were analyzed, the effect of the emotional expression on adults' looking times varied by region of interest but not by valence. So that for both positive and negative videos, there was a significant effect of emotional component for the eyes ($F(2,152)=3.476$, $p=0.033$, $\eta^2=0.044$; see Figure 6e), but not for body and mouth ($F(2,152)=2.178$, $p=0.117$, $\eta^2=0.028$; $F(2,152)=0.911$, $p=0.404$, $\eta^2=0.012$). Thus, for the eyes, Emotion 1 was significantly greater than both Neutral 1 and Neutral 2, but there was no difference between the two neutral components (Neutral 1 < Emotion 1, $p=0.041$; Emotion 1 > Neutral 2, $p=0.017$; Neutral 1 > Neutral 2, $p=0.591$).

Discussion

This study resulted in two main findings: (1) juvenile rhesus macaques show continued development of social attention between the ages of 18 and 36 months but have not yet reached an adult like pattern, and (2) only close to the onset of puberty can juvenile rhesus macaques rapidly modulate their attention to social stimuli based upon presence of emotional facial expressions. These findings will be discussed in turn.

(1) Age-Related Development of Social Attention

Juvenile rhesus macaques show age-related changes in attention to salient regions of social stimuli. With age, attention to the body region decreased and was replaced by a greater attention to the eyes region. Allocating more time to the eyes of social stimuli and less to the body suggests that juvenile rhesus macaques undergo continued refinement of social attention throughout the pre-adolescent period. However, attention to the mouth region did not change with age, further suggesting that this effect is limited to emotionally salient regions, such as the eyes. When compared to viewing patterns in adults, juveniles fixated significantly less at 22 months but comparably at 26 months for all ROIs. However, at 36 months, juveniles fixated significantly more at the eyes, less at the mouth, and comparably at the body. At all ages, juveniles showed similar looking patterns towards social versus nonsocial videos as adults, with a trend towards looking more at social videos. Although neither group showed a significant effect of stimuli type on fixation duration, this may be due to the novelty of objects shown in nonsocial videos in comparison to social videos. Overall, the findings suggest that, in this pre-adolescent period, the juveniles have not yet reached adult-like levels of social attention and are still undergoing development at this time.

These findings are consistent with previous social development research in rhesus macaques showing that juvenile macaques use qualitatively different strategies to track social movements than the adults do (Ferrari et al., 2000). While there is no research investigating looking patterns of juvenile macaques in response to social stimuli, previous work shows that adults spend more time viewing the eye region of social videos (Gothard et al., 2004). Our findings contribute to fill the gap in our knowledge regarding adolescent social maturation by showing that juvenile macaques develop the ability to modulate attention to socially salient regions over the pre-pubertal period. Evidence for differences between adult and juvenile social strategies, coupled with our findings, support the idea of continued social development throughout adolescence. Additionally, our findings are consistent with human research demonstrating the importance of the eyes as a socially salient region necessary to navigate social interactions (Baron-Cohen et al., 1997). The social importance of the eyes, with the shifts in social relationships during adolescence (Brown, 2004), matches our findings showing juveniles' age-related modifications in attention to the eye region of social stimuli. The present findings together with previous data indicate that socioemotional behavioral development does not culminate in childhood but rather continues well throughout adolescence.

Adolescence-specific physiological changes may underlie the distinct social behavior changes seen in juvenile rhesus macaques. Our results show that changes in social attention occur only at the final time point (36 months), which is closest to the start of puberty in male rhesus monkeys. This finding together with data from human studies showing puberty-linked social development suggests that pubertal hormone changes drive social development in adolescence (Richards, 2009; Spear, 2009; Tottenham et al., 2011; Said et al., 2011).

Although the neural substrate for such developmental changes has yet to be determined, the amygdala and the prefrontal cortex are two brain regions known to be critical for social development and are not fully mature by adolescence. Previous human studies have shown activational differences between adolescents and adults in these regions in response to facial processing (Hare et al., 2008; Monk et al., 2003; Thomas et al., 2007; Moore et al., 2012). These data in conjunction with evidence for structural changes in these regions during adolescence (Scherf, 2013; Neufang et al., 2009; Machado & Bachevalier, 2003) suggest that puberty-driven changes in these key brain regions may be responsible for the social behavioral reorganization seen during this developmental window.

(2) Age-Related Development of Emotion Processing

Our second finding indicated that although there was no effect of valence on social attention in whole video analyses, separation of emotional and neutral components of social videos revealed that there was an effect of emotional content, but not valence, on attention to social stimuli. Analyses comparing neutral and emotional components within the same video show that, close to the onset of puberty, juveniles are rapidly modulating their attention to social stimuli based upon the emotional content, similar to adults. Specifically, at 18 and 26 months, juveniles spend the same amount of time fixating on the emotional and neutral components of social stimuli at all ROIs. However, at 22 months, juveniles looked significantly more at the first neutral component in comparison to the second neutral component. Further analyses showed that this decrease was driven by increased looking times at the first neutral component of the positive videos only. Inspection of the individual videos revealed that only one of the two positive videos at 22 months had the pattern of increased looking times during Neutral 1. This effect may be seen in this one video because of a shorter Neutral 1 component. A short first neutral component may result in higher looking times due to the effect of novelty on visual attention. Finally, at 36 months, juveniles looked more at the eyes of emotional components than at the first neutral component.

Juveniles' looking patterns to the eyes were similar to the adults such that they rapidly modulated their attention between the first neutral and the first emotional components. While the juveniles' showed similar attention levels as adults during the emotional components, their looking patterns are not yet at adult-like levels, as their attention to the eyes did not decrease during the second neutral component as it did in the adults. Furthermore, at the mouth, 36 month old juveniles looked more during the emotional component than during either of the neutral components, while adults did not modulate attention to the mouth based upon emotional content.

The juveniles' patterns of emotional attention approaches that of the adults at the eyes but not the mouth suggesting that emotion processing maturation is underway, but not yet complete, during adolescence.

Though there is a lack of research on adolescent socioemotional development in non-human primates, our findings are consistent with research on socioemotional behaviors in adult rhesus macaques. Data show that adults show increased attention towards emotional social videos in comparison to neutral social videos (Gothard et al., 2004). As is seen in adult rhesus macaques, by 36 months of age juveniles are also able to modulate their attention to social videos based upon the presence of emotional facial expressions. However, as these behaviors are not present at earlier time points, the development of this ability may not emerge until close to the onset of puberty. Furthermore, while juveniles do show the ability to modulate social attention based upon emotional content, their looking patterns are still distinct from those of adults. The lack of difference between attention to Emotion 1 and Neutral 2 within the eye region, suggests that juveniles are in the process of developing the ability to modulate attention based upon emotional content, however they have not reached full maturation. Differences in attention to the mouth region during neutral and emotional components of social stimuli in juveniles but not adults suggests that juveniles may still rely upon the mouth as a socially salient region providing social information while the adults, with more refined emotion processing abilities, no longer depend upon social information from this region.

Our results are also consistent with human adolescent studies. Adolescents exhibit age-related improvements in emotional facial recognition and emotion discrimination abilities, although these abilities are not yet at adult levels (Thomas et al., 2007; Tottenham et al., 2011). Additionally, previous research shows associations between increased emotional reactivity to social stimuli and pubertal maturation (Spear, 2009). Our results capture the emergence emotion-specific modulation of attention in the 36-month-old juveniles, and support the continued development of emotion processing abilities during adolescence. Although our juveniles showed patterns approaching adult-like emotional attention at the eyes at 36 months, they still showed differences in emotional attention at both the eyes and mouth suggesting that emotional processing maturation improves during adolescence but continues into adulthood, similar to the differences between adolescents and adults seen in humans. While the fundamentals of facial processing may be in place in childhood, these data suggest that adolescence is a developmental window for the refinement of emotional discrimination abilities to reach adult levels of emotion processing.

In addition to distinct behavioral characteristics of emotion processing during adolescence, previous research showed associations between emotion processing abilities and pubertal progress. An fMRI study on human adolescents demonstrated a hyperactivation of the amygdala in response to emotional stimuli in comparison to adults (Hare et al., 2008). Similarly, the prefrontal cortex is less active in adolescents than adults in response to emotion processing (Lau et al., 2011). Furthermore, the functional changes in response to emotional stimuli during adolescence and puberty-related structural changes of the amygdala and prefrontal cortex suggest that these brain regions may be driving the behavioral changes observed at these ages (Neufang et al., 2009; Moore et al., 2012; Menzies et al., 2015). Thus, all together the data suggest that emotion-specific modulation of social attention seen only at the start of puberty is consistent with the idea that gonadal hormone fluctuations associated with the onset of puberty initiate changes of these brain regions leading to refinement of emotion processing.

Limitations

This study is the continuation of a pilot study and consists of three, male juvenile rhesus macaques. Male and female juvenile rhesus macaques engage in different social behaviors on different timelines throughout development (Machado & Bachevalier, 2003). Additionally, puberty in male and female macaques involves different gonadal hormones and emerges at different ages (i.e. 3 years in the females and 4-5 years in the males). These gender differences may result in distinct trajectories of adolescent socioemotional development for males and females. Thus, caution is required to apply the current results to juvenile macaques in general.

Furthermore, this study was limited to only three individuals that were housed together and raised in a laboratory setting. With three macaques from the same social group, there is individual variability in dominance status. Research shows that individual differences and rank in the social hierarchy may affect social behaviors and therefore looking patterns at videos of conspecifics (Mosher et al., 2011). Our results show that the highest ranking juvenile typically had longer looking times at social videos than the other two juveniles. This difference may be due to higher levels of testosterone in the more dominant male. Blood samples collected at each time point will allow us to investigate associations between social attention development and testosterone levels. These juveniles were also raised in an atypical social setting for macaques, as they were raised in a laboratory from 10 months on with altered social experiences. Although the juveniles of the present study had social interactions, they were not challenged with the more complex social interactions normally encountered in large social groups containing not only young animals but also adult males and females. Despite these limitations, important, novel findings result from this experiment.

Future Directions

To address the limitations of this study, future studies should include larger sample sizes including male and female individuals from a variety of social groups. While these data suggest that there is an effect of pre-pubertal maturation on social development, investigation of fluctuations in testosterone (or estrogen for the females) would help to elucidate the role of gonadal hormones in these behavioral changes. Finally, to investigate the hypothesis that gonadal hormones play a causal role in social development during adolescence, researchers could use our behavioral paradigm to investigate the effects of the absence of gonadal hormones on social attention development during adolescence.

In addition, the present study focused on behavioral social changes over the pre-pubertal period, however previous studies indicate that functional and connectivity alterations in the brain are associated with the behavioral changes. Future studies using an animal model are needed to investigate how the neurological changes characteristic of adolescence drive this behavioral maturation. Although the present data have advanced our understanding of the complex time-course of socioemotional development during primate adolescence, research into the connection between neurological, hormonal, and behavioral changes could further our understanding of normal and abnormal socioemotional development during adolescence.

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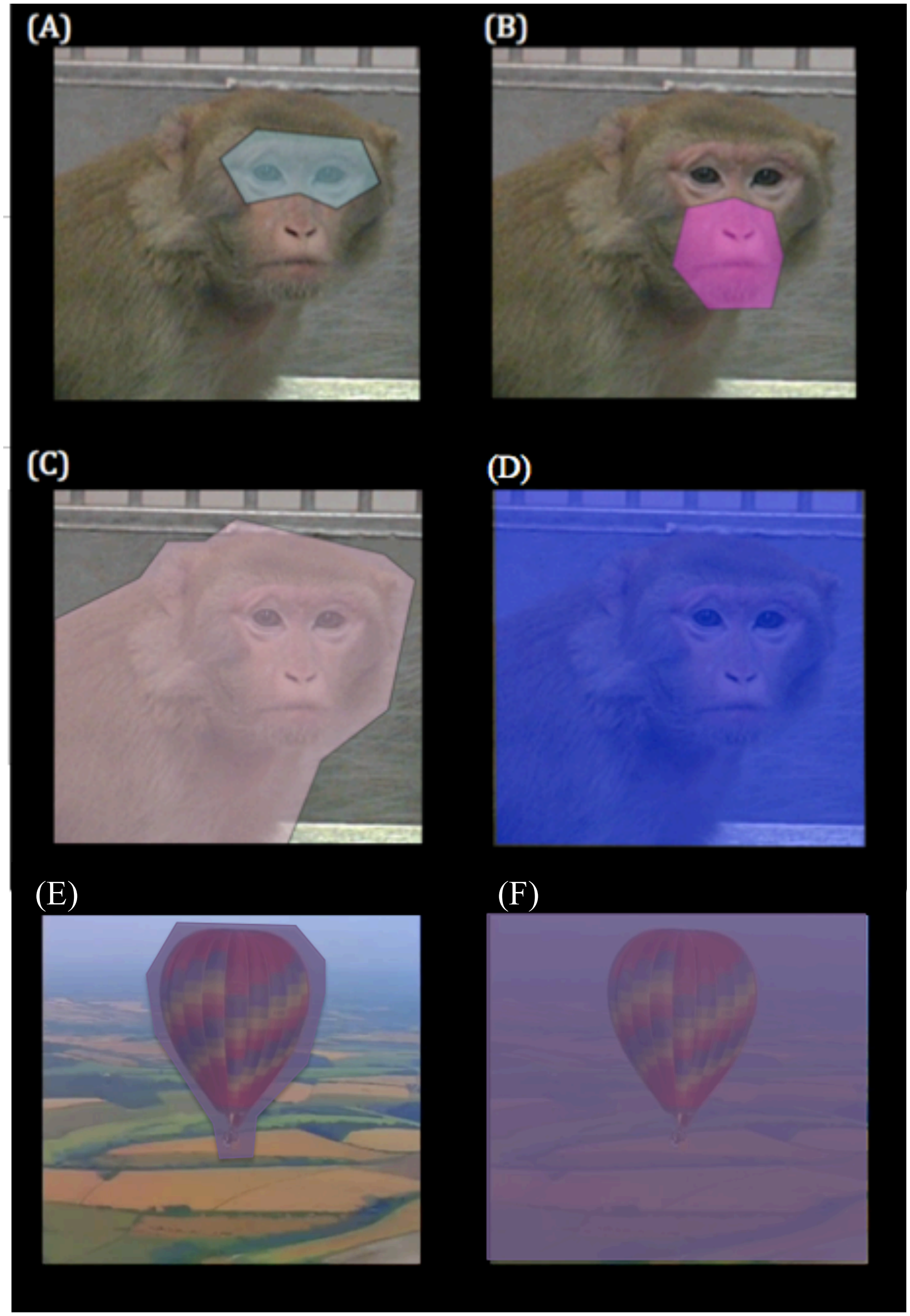

Figure 1. Social and Nonsocial Stimuli Regions of Interest. Social stimuli regions of interest included (A) Eyes: from brow bone to cheekbones, (B) Mouth: from bridge of the nose to bottom of the chin, (C) Body: all parts of the monkey, (D) Scene: entire video. Nonsocial stimuli regions of interest included (E) Object, (F) Scene: entire video.

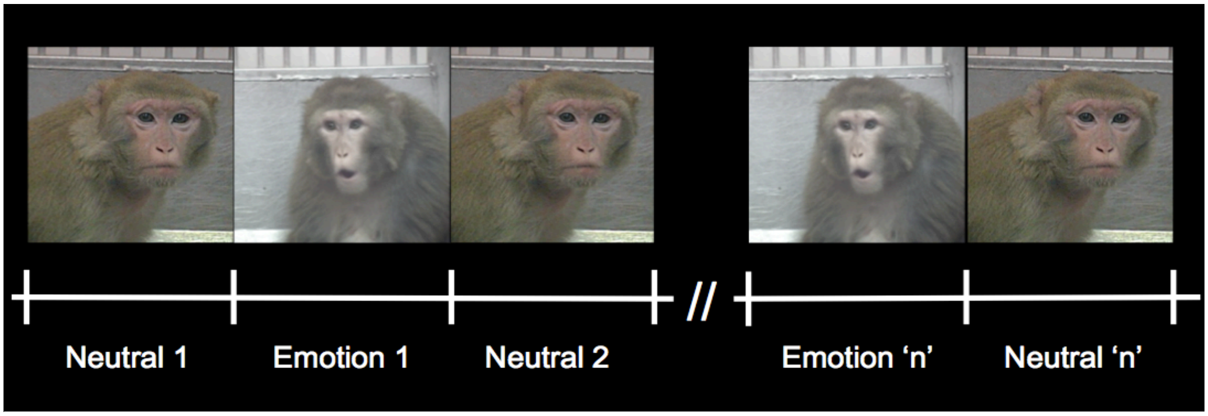

Figure 2. Separation of Emotional and Neutral Components of Social Stimuli. We limited emotional component analyses to the sequence Neutral 1 – Emotion 1 – Neutral 2 in positive and negative videos. Subsequent emotional and neutral components in these videos were not included in analyses.

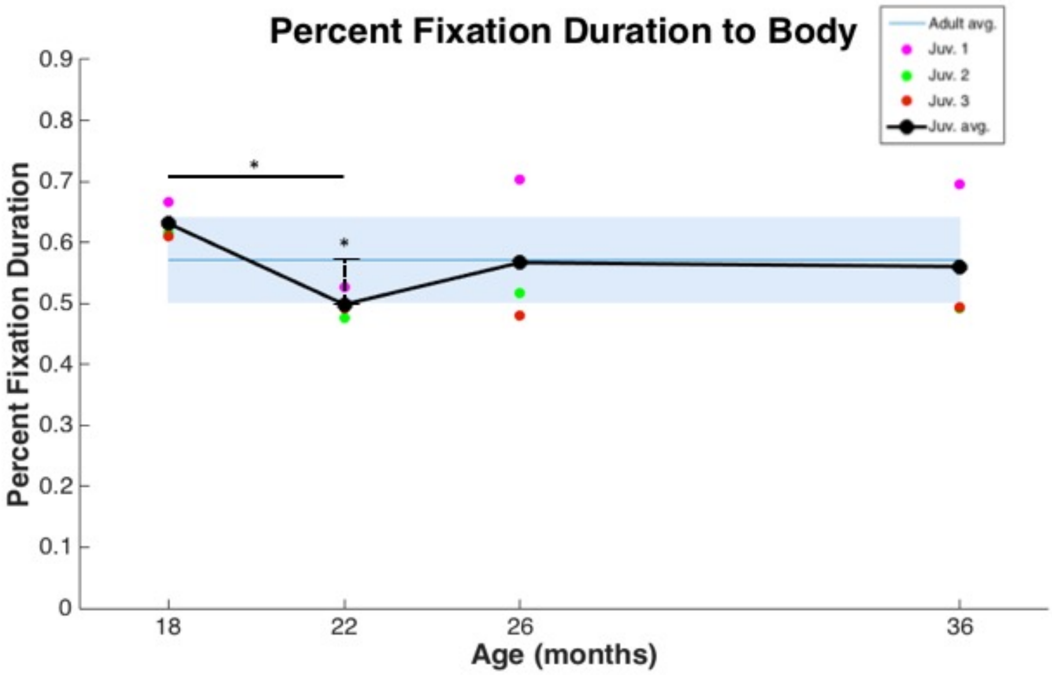

Figure 3. Effect of Age on Social Attention to the Body. Juveniles' attention to the body changed with age, with juveniles looking less at the body at 22 months than at 18 months. In comparison to adults, juveniles looked significantly less at the body at 22 months. (* signifies $p < 0.05$)

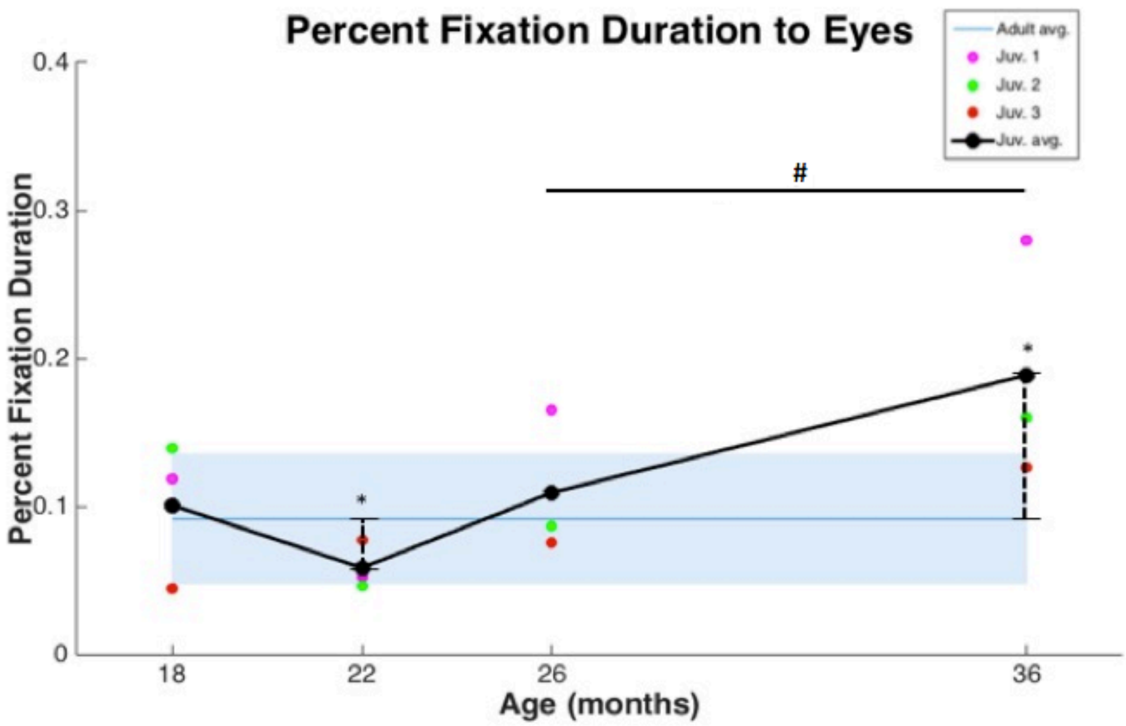

Figure 4. Effect of Age on Social Attention to the Eyes. Juveniles' attention to the eyes changed with age, with trends towards longer looking times at 36 months than at 26 months. In comparison to adults, juveniles looked significantly less at the eyes at 22 months and significantly more at 36 months. (* signifies p < 0.05, # signifies p=0.052)

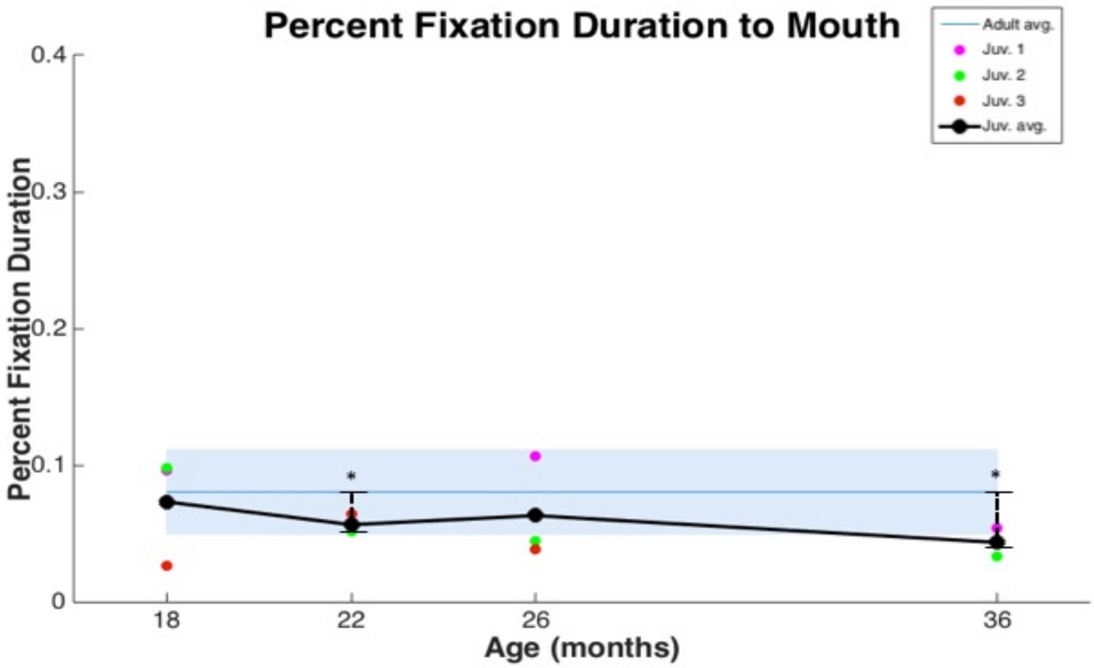

Figure 5. Effect of Age on Social Attention to the Mouth. There was no effect of age on juveniles' attention to the mouth. In comparison to adults, juveniles looked significantly less at the mouth at 22 and 36 months. (* signifies $p < 0.05$)

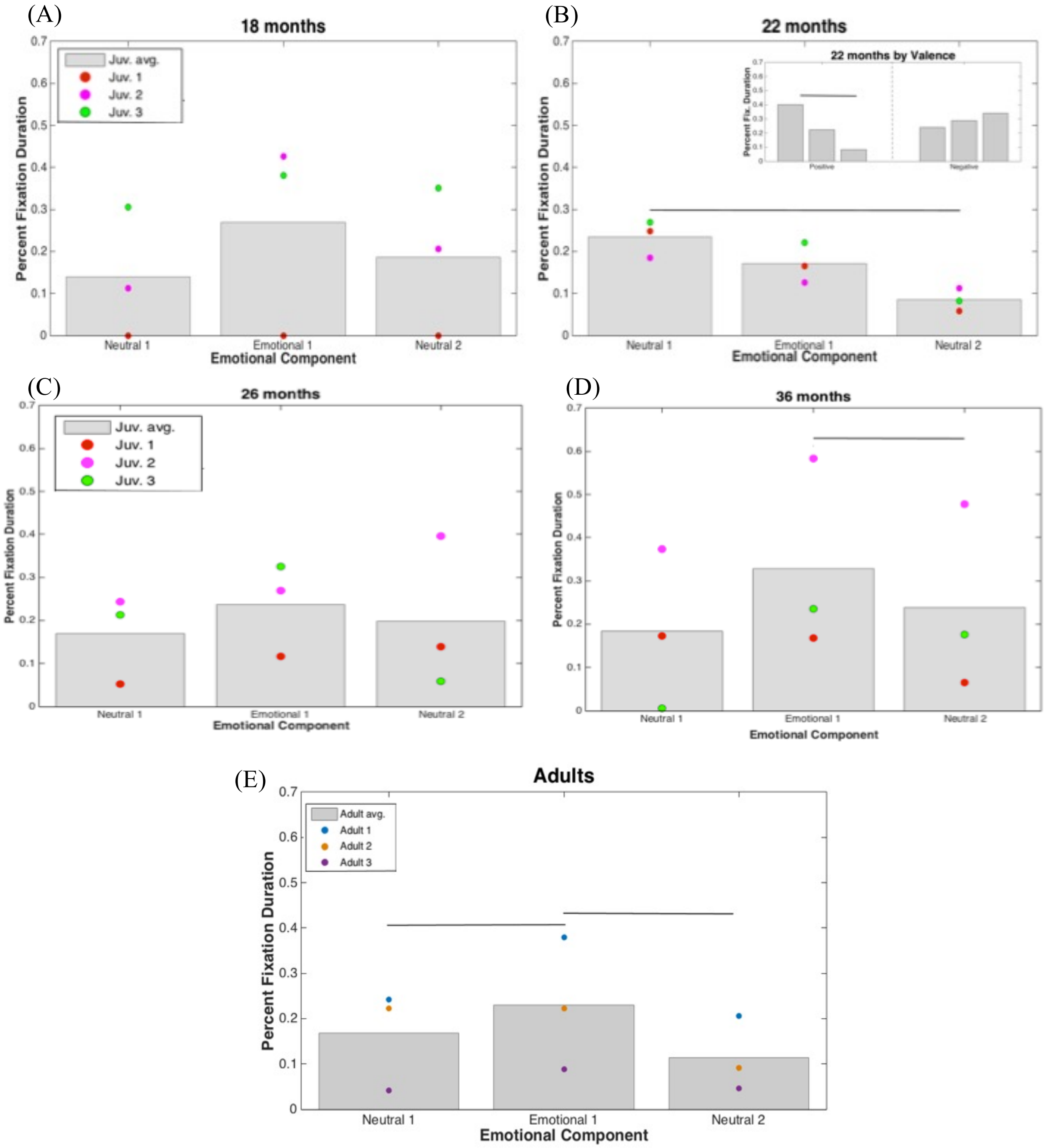

Figure 6. Effect of Emotional Components on Social Attention to the Eyes. (A) 18 months: Juveniles' attention did not differ by emotional component. (B) 22 months: Juveniles' attention was significantly greater at Neutral 1 than at Neutral 2. This effect was driven by increased looking times at Neutral 1 during positive social videos. (C) 26 months: Juveniles' attention did not differ by emotional component. (D) 36 months: Juveniles' attention was significantly greater at Emotion 1 than at both Neutral 1 and Neutral 2. (E) Adults: Adults' attention was significantly greater at Emotion 1 than at both Neutral 1 and Neutral 2. (solid line signifies $p < 0.05$)