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Social and Emotional Development in Pre-Adolescent Macaques

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Social and Emotional Development in Pre-Adolescent Macaques

By

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Advisor: Jocelyne Bachevalier, PhD.

An abstract of
A thesis submitted to the Faculty of the
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Abstract

Social and Emotional Development in Pre-Adolescent Macaques
By Lauren Murphy

Aberrant social and emotional function is a hallmark of many of neuropsychological disorders emerging during adolescence, such as depression and anxiety. To advance a mechanistic understanding of social development during this period, a primate model of adolescence is needed and will provide a vehicle for the application of novel therapies. I propose the rhesus macaque as a model due to the species social complexity and protracted childhood and adolescent period.

To measure social maturation in untrained, nonverbal animals I measured behavioral changes to social and emotional stimuli using infrared eye tracking and an emotional reactivity task in three juvenile and three adult male macaques. Juveniles were tested at 18, 22, and 26 months of age, making this the first longitudinal examination of macaque adolescent social development.

As measured by changes in fixation duration and frequency to the eyes and body of unknown macaques, I report normative adult patterns, as well as developmental changes, in nonhuman social and emotional processing. In adults, the eyes negative social stimuli are looked at more frequently than neutral or positive stimuli. However, this normative pattern is not seen in the late juvenile stage. At 18 months of age, macaques look longer at social stimuli generally, and usually without regard to emotion. By 22 months, this broad social attention significantly decreases within both the body and the eyes. At 26 months, fixation duration once again increases, and is now accompanied by more adult-like patterns of emotional attention. In addition to changes in fixation behavior, measures of emotional reactivity correspond with this pattern, such that 18 month olds are reactive to novel stimuli irrespective of valence, but by 22 and 26 months of age they show an adult-like pattern of increased reactivity only toward novel negative stimuli. Taken together, these data document continuing pre-pubertal changes in social and emotional attention toward an adult-like pattern in a nonhuman primate. These data lay the groundwork for future comparative studies with human subjects that may elucidate the ongoing development of social and emotional processing during the adolescent period.

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INTRODUCTION

In recent years, adolescence has emerged as a key period of social and psychological development. Disruption of this developmental course is associated with exaggerated, impaired, or anomalous development and function, as present in schizophrenia, mood disorders, and substance abuse (Garey, Ong et al. 1998; Thomas, Drevets et al. 2001; White and Swartzwelder 2004). Indeed, the peak age for the onset of mental health disorder in the United States is 14-years-old, square in the midst of pubertal change (Giedd, Keshavan et al. 2008). This dynamic period represents a major social transition during most of the second decade of human life, from parental care to independence, marked by behavioral, neural, and hormonal restructuring (Casey, et al. 2008). Researchers have proposed that these dramatic changes may be linked to differential growth in distinct regions of the social brain (Steinberg, 2005), but hormonal contributions during this period remain understudied (Blakemore, et al. 2010; Casey, et al. 2008). Despite promising new methodology, there has been a dearth of comprehensive, longitudinal research on the ontogeny of social behavior and of what role pubertal development plays. A novel approach to this exciting field is a primate model of adolescence aimed at a deeper understanding of both the behavioral, neural and hormonal changes from late childhood to adolescence in humans. As a first step for developing such an approach, the present study aimed at measuring social development in monkeys, as assessed by face processing abilities and emotional reactivity, together with an assessment of gonadal hormones levels.

Humans and nonhuman primates are tuned to face-like stimuli beginning at birth (Valenza, Simion et al. 1996; Sugita, 2008; Paukner, et al. 2013) leading to experience-dependent development of expertise by adulthood (Leopold, et al. 2010). Emotion recognition comes on board fairly early as well, with human infants demonstrating facial expression discrimination as early as seven months of age (Caron, Caron et al. 1988). Face expertise and emotional perception is spread across the primate order (Leopold, 2010; Burke & Sulikowski, 2013), and adult expertise is likely driven by the salience of facial information throughout development (Tsao, Schweers et al. 2008; Cohen Kadosh, et al. 2013; Tonks, et al. 2007). As such, changes reported in face and emotional processing during development likely support changing social goals, such as mate acquisition, group inclusion, and high social status.

Despite improvement in face processing and emotion processing throughout development (see Herba & Philips, 2004, for a review), some studies suggest a decrement in speed and accuracy of face and emotion processing during late childhood and adolescence (Flin, 1980; Carey, Diamond, Woods, 1983; McGivern, et al. 2002, Taylor, Batty, et al. 2004). However, these data contradict other research demonstrating a continued refinement of social behavior throughout adolescence, culminating in adult expertise (Mondloch, et al. 2003; O'Hearn, et al. 2010). These divergent results may be explained by limitations in the experimental designs used, such as cross-sectional instead of longitudinal studies, lack of non-social control stimuli, and failure to document actual pubertal state at the time of testing. Hence, well-controlled longitudinal studies may provide a better understanding of the development of face processing abilities during adolescence.

The improvements in face processing in late childhood and adolescence represent a refinement of processing speed and strategy rather than a functional reorganization. The speed of identification of familiar faces improves from childhood to adulthood (De sonneville, 2002) and all ages appear to recruit a core face-processing network with minor differences in neural activation during adolescence (Cohen Kadosh, et al. 2013). Additionally, adolescents show improved category specific processing (e.g., house vs. face) compared to children, but lack adult-like processing for individuation (Scherf, Luna, et al. 2011). This result suggests that the functional face processing may become adult-like early, yet refinement of expertise and the development of advanced processing may develop through late adolescence.

As compared to face identification (Johnston, Kauffman, et al. 2011), emotional expertise appears to have a more protracted developmental time course (Herba & Phillips, 2004; Tonks, et al. 2007). Skilled detection of positive and negative emotions may emerge at different times, suggesting later developing cortical regions or connectivity may be essential for mature processing of certain facial expressions. Thus, correct identification of happy faces is present in children (de Sonneville, 2002), whereas accurate identification of anger appears to develop into late adolescence (Thomas, et al. 2007). However, it is unclear if this is a function of the quality of the angry signal itself, the testing paradigms used, or the underlying neural circuitry. Adolescents are faster to process neutral compared to emotional face stimuli, further suggesting that the development of emotional processing is dissociated from that of general face expertise (Taylor, Batty, et al. 2004). The developmental changes underlying these behaviors remain unclear, yet research suggests that the organizational and activational effects of gonadal hormones may play a pivotal role.

During adolescence, the increase in gonadal hormone production leads to dramatic physical changes. Both males and females experience the same facial emotion decrement in adolescence, regardless of pubertal state (McGivern, et al. 2002; Lee, Krabbendem, et al. 2013), although there may be early organizational effects of gender. Preschool girls show a dramatic advantage over boys in emotional processing (Boyatzis, Chazan, Ting, 1993) and females with early, unregulated exposure to testosterone show earlier, and more male-like, processing of negative emotions (Ernst, et al. 2007). Additionally, pubertal state is correlated with increased prefrontal activation to emotional faces, suggesting that hormones and pubertal state are closely linked to emotional development (Moore, et al. 2012). The few studies investigating these relationships suggest an important interaction, but will require further investigation.

Although there have been numerous studies examining the trajectory of face and emotional processing, the nature of human research leaves many gaps in our knowledge. The majority of studies examining changes in face and emotion processing in adolescence do not include measures of pubertal state, despite the high variability in developmental stage at different time points. Other factors affecting face and emotion are often not controlled for, such as early experience (Pollak & Sinha, 2002). Additionally, the lack of longitudinal studies not only fails to account for individual differences, but critically may omit key time points in development. Indeed, multiple studies report a decrement in face and emotion processing in early adolescence (Flin, 1980; Carey, Diamond, Woods, 1983), yet these findings have yet to be replicated or refuted. Increased knowledge on face and emotional processing during this period of development will benefit from novel empirical approach that may take in consideration the gaps highlighted above.

The development of a nonhuman primate model may serve as a proxy for understanding the development of conserved social and emotional processes during adolescence. As earlier human studies call for more control of hormones, pubertal measures, and imaging techniques (Moore, et al. 2012), the monkey presents an ideal model in which to study the hormonal and behavioral implications of social development. The rhesus macaque develops along a similar trajectory to humans and several functions, such as experience-dependent face processing abilities (Sugita, 2008), social reorientation (Ehardt & Berenstein, 1987), emotional development (Parr & Heintz, 2009), and gonadal hormones (Richards, et al. 2009), are adult-like by late adolescence or early adulthood (5 years of age). To this end, we begin a developmental study in monkeys, assessing face processing abilities, emotional regulation, and gonadal hormone levels at different time points from the juvenile period to early adulthood. Here, we report initial results on the development of face processing and emotional reactivity in three male rhesus macaques at the ages of 18, 22 and 26 months.

METHOD

Subjects

Three juvenile male macaques born from mothers living in large social groups at the Yerkes National Primate Research Center (Lawrenceville, GA) were acquired at 10 months of age and brought to the Yerkes National Primate Research Center (Atlanta, GA). They were weaned from their mother at 6 months of age, removed from their social groups and placed in small groups of 3 juveniles each. When moved to the Yerkes Main Center they were kept together in a three-member group on a 12-hour light/dark cycle, with visual access to adults of varied ages and sexes. All juveniles were presumed to be pre-pubertal, as the pubertal rise in gonadal hormones typically emerges around 3 - 4 years of age in male macaques (Bercovitch, 1993; Plant, 2001). They were longitudinally tested at three pre-adolescent time points (aged 18, 22, and 26 months). Actual ages at testing were 17 – 18 months (mean: 17.66 months), 21 – 22 months (mean: 21.33 months), and 25 – 27 months (mean: 26 months).

Three adult male macaques were tested at one time point (aged between 7 and 9 years) and served to establish adult-like behaviors in an identical paradigm. Adult male subjects were also acquired from the Yerkes Field Station where they were mother-reared and lived in large social groups until they were removed from their social groups and re-grouped into smaller bachelor all make groups around 3-4 years of age. Subjects moved to the Yerkes Main Center around 4 years of age, and from that point on were singly housed on a 12-hour light/dark cycle with visual access to other monkeys of varied ages and sexes.

All subjects were middle-ranked and mother-reared in large social groups at the Yerkes Primate Center Field Station (Lawrenceville, GA) until at least six months of age.

Research Design

1. Face Processing

1.1 Apparatus

To measure changes in attention to social stimuli, subject's gaze was monitored using a Tobii 120Hz Eye Tracking monitor. Eye tracking technology uses noninvasive infrared light reflections on the subjects' cornea and retina to calculate gaze behavior. These reflections are calibrated using a 5-point calibration paradigm at the start of each testing session to control for subtle changes in distance and head position. Subjects were positioned 22" from the video monitor in a primate chair. The primate chair uses a yoke to keep the subject's head at a constant level, while allowing the animal to comfortably sit in a preferred position. All subjects were trained to sit calmly in the primate chair using positive reinforcement techniques (Fig. 1a). To limit head movements and reduce overall duration of testing sessions, subjects were fitted with custom molded thermoplastic helmets (Fig. 1b). Subjects were trained to calmly wear the helmets using positive reinforcement techniques. Subjects were not trained to look at the monitor or the movies, but were reinforced between sessions or after calibration if they maintained calm and attentive behavior.

1.2 Stimuli

Social: At each time point, juvenile subjects viewed six positive, six neutral, and six negative emotional videos of an unfamiliar monkey (Fig. 2a-c). Each 10-second movie featured similar background and lighting and only one type of emotional expression was displayed on each movie. Adult subjects were shown the same stimuli at one time point. To facilitate comparison with juveniles, the movies shown to juveniles at the 18-month time point are grouped as "Set 1", the 22-month time point as "Set 2", and the 26-month time point as "Set 3". This ensures that any differences between juveniles and adults are not a result of the stimulus characteristics at a given time point.

Nonsocial: Subjects viewed 12 control movies of neutral nonsocial, novel video content. Each 10-second movie included a foreground subject (e.g. train, flower) and background region to facilitate comparison between social and nonsocial movies (Fig. 2d). Neutral nonsocial control movies were excluded if face-like configurations were present. Nonsocial stimuli were only shown to juveniles at 22 and 26-month time points.

1.3 Training

Prior to initiation of data collection, subjects engaged in training days to acclimate to testing conditions. Subjects were placed in the primate chair and helmeted, then brought to the testing room and calibrated while watching nonsocial nature videos. Throughout training, subjects were positively reinforced for calm behavior using small treats, such as raisins, given in between trials. Training continued until subjects could be calibrated and sit calmly for 15 minutes. All subjects received at least three days of training.

1.4 Task

During behavioral recording, the subject was placed in a custom built testing booth containing the Tobii eye tracking monitor and a white noise machine to reduce extraneous visual and auditory stimulation. All eye tracking sessions were limited to 30 minutes to reduce stress to the subjects. Sessions were repeated over successive days until a subject had been exposed to each social movie six times and each nonsocial movie three times. Subjects were not experimentally naïve, but were naïve to social video stimuli.

Juveniles were tested at three time points, approximately four months apart, to assess the development of nonhuman primate social and emotional perception. Adults were tested with the same stimuli and procedures, with stimuli counterbalanced for presentation. Adults did not receive a delay between testing sets, rather experienced all stimuli and procedures consecutively. Testing proceeded in the same order for all juvenile subjects (Fig. 3), though stimuli were counterbalanced within each time point.

1.5 Data Collection

Data was recorded at a rate of 60Hz on a Windows laptop running Tobii Studio 3.2.2. Several regions of interest (ROIs) were drawn on all video stimuli prior to data collection using the Tobii Studio 3.3.2 application. For social videos, regions were defined as eyes (bridge of nose to brow), mouth (nose to chin), face (eyes + mouth), body (outline of monkey body), and background (total viewable movie area, see Figure 1a-c). For nonsocial videos, regions were defined as foreground (outline of object) and background (total viewable movie area; see Figure 1d). Based on these pre-drawn ROIs, several parameters were calculated from the Tobii Studio program: gaze duration, fixation duration, and fixation count. For the fixation duration and fixation count parameters a fixation filter was applied. This filter categorized three gaze points that fell within 40 pixels of diameter within a 200ms time period as a fixation (Holmqvist, et al. 2011). All videos were 720 X 480 pixels, displayed on a 1280 X 1024 resolution monitor.

1.5.1 Fixation Duration

The average fixation duration was calculated to measure the relative salience of each region. Actual fixation duration for each ROI was transformed according to the proportion of time spent in the containing ROI. For example, the average fixation duration in the eye ROI was divided by the average fixation duration of the entire face ROI (which contains the eye ROI). This was repeated for face ROI in relation to the body ROI and for the body ROI in relation to the entire scene ROI. The average proportion of fixation duration at each time point was then averaged across all six presentations of the stimuli and for each emotional valence (positive, negative, and neutral).

1.5.2 Gaze Duration

To ensure that the fixation parameters used above were not too conservative, the average gaze duration within an ROI was calculated. Gaze duration included any gaze point that falls within an ROI, regardless of whether it was part of a fixation or not. Gaze duration was transformed and treated identically to fixation duration, described above.

1.5.3 Fixations per Second

The number of fixations per second at each ROI provides an additional measure of viewing behavior, by indicating whether individuals made few long fixations or more short fixations to each ROI. This measure was then divided by the total fixation duration for that video, obtaining a measure of fixations per second for each ROI. Values were again averaged across all six presentations for each video, and then further averaged for each emotional valence at each time point.

2. Emotional Reactivity

2.1 Apparatus

To measure emotional reactivity, all subjects were tested on the approach/avoid emotional reactivity paradigm (Machado, Kazama, Bachevalier, 2009). Testing was conducted in a Wisconsin General Testing Apparatus (WGTA), which uses a pulley-operated guillotine door to visually separate the subject from the object being presented. The testing tray of the WGTA has three small recessed wells, in which a treat can be placed, as well as open area for the placement of objects. The experimenter is positioned out of sight and operates the pulley door, giving the subject the opportunity to see the objects and retrieve the treat or not.

2.2 Stimuli

In the approach/avoid task, a preferred treat item is presented in front of neutral and fear eliciting objects. Face-like threat stimuli included toys with big eyes and open mouths, such as baby dolls, stuffed animals and children's toys. Non-face threat stimuli included toys known to induce innate fears (spiders, snakes, etc.) or objects animals had learned to fear (handling gloves, capture nets, and pole and collar poles). Neutral items were chosen to match in size and structure the objects, and included items such as plastic jars, garden hoses, and dog toys. A full list and example photos of the approach/avoid items and neutral foils can be found in Figure 4.

2.3 Habituation Pre-training

All subjects received contextual habituation prior to behavioral data collection. All subjects were brought into the WGTA using procedures identical to testing days. Habituation sessions began with the placement of a preferred treat in the center well and an unfamiliar, neutral object in testing position. The experimenter hid out of sight and monitored task completion via video camera. The pulley door was opened, and the subject given 30 seconds to retrieve the treat from in front of an unfamiliar object. If the subject retrieved the treat within 5 seconds, the trial was recorded as successful. If the subject took longer than 5 seconds, the trial was marked a miss. If the subject did not take the treat, the door was closed after 30 seconds and trial marked a miss. Subjects received up to 14 trials per day. Training criterion was defined as 12/14 correct (< 5s) retrievals per session for two consecutive days. Habituation training was repeated prior to each time point for juveniles. Subjects completed initial habituation training in an average of 6 days for juveniles, and 3 days for adults.

2.4 Task

The approach/avoid paradigm measures latency to retrieve a preferred food item (e.g., grape, gummy bear) located in front of a neutral or threatening item. Daily testing was limited to two pairs of threatening and neutral objects, a non-face threat and a face-like one. A daily session began with a one-minute baseline recording during which a treat was presented on the testing tray and subject was able to retrieve it and provided a measure of the latency to retrieve a treat in absence of any stimulus. This was followed by two test trials in which a food treat was placed in a food-well and an object (neutral or threatening) was positioned just beside the treat and presented for a total of one minute. The latency to retrieve the treat was again recorded. The presentation of the neutral and threatening objects in a daily session was counterbalanced across subjects. Each juvenile subject was tested with six objects (three threatening, three neutral) at each time point.

Each adult subject was tested on six objects (three threatening, three neutral) per week for three weeks.

2.5 Data Collection

Testing was recorded using a Sony Handycam with a small LCD screen, allowing sessions to be scored independently, as well as facilitating live observation of the subjects' emotional reactivity. The time elapsed between the opening of the pulley door and the time that the animal retrieved the treat was scored as latency. The latency to retrieve the treat was recorded at the time of testing and was confirmed later by using playbacks of the video recordings and using Noldus Observer to ensure reliability and precision.

3. Statistical Analyses

SPSS© Statistics software was used for all statistical analyses (v.21; IBM Corp.).

3.1 Face processing

To measure the adult visual scanning patterns, we first compared scanning parameters within each ROI for the social video clips with those of nonsocial video clips using repeated-measures ANOVA with social content as the within-subjects measure. Adult scanning patterns for social stimuli were then analyzed for each ROIs considering the valence of social stimuli (positive, neutral, negative) using repeated-measures ANOVAs with emotion as the within-subjects factor. Given that the adult monkeys were tested three times using different sets of stimuli, we also investigated the effects of sets on viewing patterns. At each time point, a repeated measures ANOVA was performed with stimulus set as the within-subjects factor and emotion as the between subjects factor.

For juveniles, attention to social and nonsocial stimuli was compared at 22 and 26 months only, because they were not given the non-social stimuli at 18 months. A repeated-measures ANOVA was used with social content as the within-subjects factor. Social stimuli were then analyzed across the 3 developmental time points (18, 22 and 26 months). For each behavioral parameter, repeated-measures ANOVAs were used with age as the repeated within-subject factor and emotional valence as the between-subjects factor. If a main effect of age was found, an additional ANOVA was performed at each age to measure the effect of emotional valence. To control for potential neurodevelopmental changes in visual processing, attentional patterns to whole scenes were compared using a repeated measures ANOVA with age as the within-subjects factor and emotion as the between subjects factor.

To compare the scanning patterns of the juvenile animals to those of the adults, a two-way ANOVA was performed at each developmental time point for each parameter and each region of interest.

Additionally, when interactions between factors were not significant, planned comparisons were performed between the control group and the experimental group, using one-sided Planned Comparison (Pedhazur, 1982), since this comparison provides more statistical power against Type II error, i.e. not rejecting the null hypothesis when it is false.

Significance level was set at $p < 0.05$ for all analyses and effect sizes were eta squared for ANOVAs. All eta squared values were hand calculated. Finally, given that the parameter “gaze duration’ provided results similar to those for “fixation duration”, indicating no underestimation of looking duration, this parameter is not reported in the result section below.

3.2 Emotional regulation

To measure the effect of stimulus on treat retrieval, latencies were compared for each condition: grape, neutral object, and fearful object. For adults, a repeated measures ANOVA was performed for each condition with Week as the within-subjects measure. For juveniles, a repeated-measures ANOVA was performed for each condition with Age as the within-subjects measure. To compare the effects of fearful stimulus category (face-like, innate, or learned) a repeated measure ANOVA was performed with time as the within subjects factor and stimulus category and group as between subjects factors. To compare latency to retrieve a treat between adults and juveniles, a two-way ANOVA was performed for each condition and each age.

RESULTS

Eye Tracking

1. Adult Scanning Patterns

Tables 1 - 2 and Figure 5 summarize the results of the statistical analyses performed on the adult scanning patterns. There were only few statistical differences that emerged.

1.1 Social vs. nonsocial stimuli

Adults fixated longer on social stimuli than nonsocial stimuli, as reflected by longer fixation duration to social stimuli (Condition: $F(1,2)=9.31$, $p=0.09$, $\eta^2=0.80$, Table 1).

1.2 Looking at social stimuli

Adults showed different patterns of looking across all ROIs depending on emotional valence of the stimuli. The main effect of emotion on both fixation duration and fixation frequency to eyes of social stimuli (Fixation Duration: $F(2,4)=1.31$, $p=0.37$, $\eta^2=0.40$; Fixation Frequency: $F(2,4)=5.03$, $p=0.08$, $\eta^2=0.72$) did not reach significance but the effect sizes were large. Specifically, adults had a tendency to fixate more frequently on the eyes of negative as compared to positive stimuli (Negative > Positive, $p < 0.01$; see Figure 5b), and conversely, made fewer fixations per second to the body region of negative than neutral stimuli (Emotion: $F(2,4)=9.15$, $p=0.03$, $\eta^2=0.82$; Negative < Positive, $p < 0.023$; Negative < Neutral, $p < 0.06$; see Figure 5a).

1.3 Effect of Stimulus Set

In addition, looking patterns in the adults varied according to the stimulus sets (see Figure 5c). Although difference in the length of fixation across the 3 sets did not reach significance for the Body (Set: $F(2,12)=3.04$, $p=0.09$, $\eta^2=0.33$), it did for the Eye region (Set: $F(2,12)=2.42$, $p=0.013$, $\eta^2=0.29$). . However, the effect sizes were large and post-hoc comparisons revealed that adults fixated longer on the body region of stimulus Set 3 than stimulus Set 2 (Set 3 > Set 2, $p < 0.01$; see Figure 5c), more frequently on the body of Set 1 than Set 2 (Set 1 > Set 2, $p = 0.07$), and fixated longer on the eye region of Set 1 than Set 2 (Set 1 > Set 2, $p = 0.05$).

2. Juvenile Scanning Patterns

Tables 3 - 4 and Figures 6 – 9 summarize the results of the statistical analyses performed on the juvenile scanning patterns.

2.1 Social versus nonsocial stimuli

Scanning patterns to social versus nonsocial stimuli were investigated only at 22 and 26 months. At 22 months, juvenile monkeys did not differentiate between social and nonsocial stimuli, as reflected by the same amount of time fixating ($F(1,2) = 0.17$, $p = 0.73$; Figure 6a) at both types of stimuli, although they displayed more frequent looks at the nonsocial stimuli (Condition: $F(1,2)=34.56$, $p=0.03$, $\eta^2=0.95$; see Figure 6b).

Interestingly, by 26 months of age, they showed a more adult-like pattern, given that they spent more time scanning the social than the nonsocial stimuli. Thus, they displayed longer fixation duration (Condition: $F(1,2)=40.52$, $p=0.02$, $\eta^2=0.96$; see Figure 6a) and more frequent fixations (Condition: $F(1,2)=9.59$, $p=0.09$, $\eta^2=0.83$; see Figure 6b) towards social stimuli than nonsocial stimuli.

This age difference in looking patterns across social and nonsocial stimuli were reflected by a significant Age X Condition interaction for fixation duration ($F(1,4)=8.85$, $p=0.04$, $\eta^2=0.68$) and fixation frequency ($F(1,4)=39.29$, $p<0.01$, $\eta^2=0.91$).

2.2 Looking at social stimuli

2.2.1 Body

At 18 months of age, juveniles had longer fixation to the body than at 22 or 26 months (Age: $F(2,6)=13.36$, $p<0.01$, $\eta^2=0.70$; $18 > 22$: $p<0.01$; $18 > 26$: $p=0.07$; $22 < 26$: $p=0.07$; see Figure 7a), and as a result had shorter fixation duration towards the eyes than at 26 months ($p < 0.03$; Figure 8a). By 22 months of age, fixation duration to the body decreased as compared to 18 and 26 months ($p < 0.01$ and $p < 0.08$, respectively; Figure 7a).

Looking patterns to the body varied according to the valence of the stimuli only at 18 and 26 months of age (Emotion: $F(2, 4)=3.69$, $p<0.01$, $\eta^2=0.37$), such that increased looking was mostly directed towards the body of negative stimuli at 18 months (Negative > Neutral, $p < 0.09$; see Figure 7b). A strong effect size at 22 months of age indicated an effect of emotion (Emotion: $F(2,4)=1.56$, $p=0.32$, $\eta^2=0.50$), though the post-hoc comparisons did not reach significance. By contrast, when reaching 26 months of age, they made significantly less fixations per second on the body of negative stimuli compared to neutral stimuli (Emotion: $F(2,4)=5.08$, $p=0.08$, $\eta^2=0.72$; Negative < Neutral; $p=0.02$; see Figure 7c).

Looking patterns across the body of social stimuli varied according to Age and Emotion (figure 7b, c). The Age X Emotion interaction for fixation frequency failed to reach significance ($F(4,12)=2.51$, $p=0.10$, $\eta^2=0.40$), but the effect size was large.

2.2.2 Face

Average fixation duration and fixation frequency to the face did not change with age (Age: $F(2,12)=0.19$, $p=0.83$, $\eta^2=0.03$ and $F(2,12)=2.51$, $p=0.12$, $\eta^2=0.29$, respectively; Tables 3 and 4), though there was a large effect size for the fixation frequency. Post-hoc comparisons suggest a trend toward greater fixation frequency to the face at 26 months compared to 22 months, but this fails to reach significance (22 < 26: $p=0.07$). The overall interaction between Age and Emotion did not reach significance, indicating that viewing patterns to the face did not change according to the valence of the stimuli at any age.

2.2.3 Eye

Fixation duration and fixation frequency towards the eyes was significantly shorter at 22 months of age than at 18 and 26 months (Age: $F(2,6)=19.02$, $p<0.01$, $\eta^2=0.76$; 22 < 18, $p < 0.01$; 22 < 26, $p < 0.01$; 26 > 18, $p = 0.03$ and $F(2,6)=6.23$, $p=0.01$,

η^2=0.51; 18 > 22, $p < 0.06$; 26 > 22, $p < 0.02$, respectively; Figures 8a and b). For fixation duration and fixation frequency, the Age X Emotion interaction reached significance and had a large effect size ($F(4,12)$=7.50, p<0.01, η^2=0.37). Yet differences in looking patterns across emotions failed to reach significance at each age (18 months: $F(2,4)$=1.16, p<0.40, η^2=0.37; 22 months: $F(2,4)$=0.83, p<0.50, η^2=0.30; 26 months: $F(2,4)$=5.35, p<0.07, η^2=0.73). Overall, this interaction indicated that, at 18 and 22 months, animals had a tendency to fixate longer at neutral faces than at faces with emotional valence, whereas the opposite pattern was observed at 26 months, when animals fixated more at faces with emotional valence than at neutral faces.

For fixation frequency, the Age X Emotion interaction approached significance with a large effect size ($F(4,12)$=3.18, p=0.05, η^2=0.51).Thus, at 26 months juveniles made more fixations per second to the eye region of negative stimuli than neutral stimuli (Emotion: $F(2,4)$=7.67, p=0.04, η^2=0.79; Negative > Neutral, $p < 0.09$; see Figure 8c).

2.2.4 Social Scenes

Juvenile attentional patterns to social scenes did not differ at 18, 22, or 26 months of age, as measured by fixation duration (Age: $F(2,12)$=0.66, p=0.54, η^2=0.09) and fixation frequency (Age: $F(2,12)$=0.88, p=0.92, η^2=0.01). Additional, there was no interaction of Age X Emotion for either fixation duration (Age X Emotion: $F(4,12)$=0.30, p=0.87, η^2=0.08) or frequency (Age X Emotion: $F(4,12)$=0.61, p=0.66, η^2=0.17).

3. Comparison between Juveniles and Adults

For these analyses, we compared visual scanning patterns of 18 month olds to those of Adult Set 1, those of 22 month olds to Adult Set 2, and those of 26 months olds to Adult Set 3. Statistical analyses are summarized in Tables 5-6 and significant comparisons are illustrated in Figure 9.

Across emotional valence, 18 month olds averaged significantly longer fixation duration to the body region of all stimuli compare to adults (Group: $F(1,5)=13.70$, $p=0.02$, $\eta^2=0.75$). Additionally, juveniles fixated longer to the body of positive, neutral, and negative stimuli than adults, as indicated by large effect size for each emotion (Positive: $F(1,5)=3.55$, $p=0.13$, $\eta^2=0.40$; Neutral: $F(1,5)=6.39$, $p=0.07$, $\eta^2=0.61$; Negative: $F(1,5)=13.35$, $p=0.02$, $\eta^2=0.78$; see Figure 9a). Adults, however, fixated more frequently than juveniles on the face of negative stimuli, as indicated by large effect size (Face: $F(1,5)=1.75$, $p=0.24$, $\eta^2=0.26$).

By 22 months, juveniles fixated less at the face (Group: $F(1,5)=6.77$, $p=0.06$, $\eta^2=0.67$; see Figure 9b) and body (Group: $F(1,5)=2.51$, $p=0.19$, $\eta^2=0.39$) of neutral stimuli than adults, as indicated by large effect size where p-values did not reach significance. Twenty-two month olds also fixated less frequently than adults to the face and eyes of all stimuli (Face: $F(1,5)=2.22$, $p=0.21$, $\eta^2=0.39$; Eyes: $F(1,5)=3.90$, $p=0.22$, $\eta^2=0.50$). Within each emotion, 22 month olds fixated less frequently at the face and eyes of positive (Face: $F(1,5)=2.18$, $p=0.21$, $\eta^2=0.35$; Eyes: $F(1,5)=3.25$, $p=0.15$, $\eta^2=0.45$) and negative (Face: $F(1,5)=2.51$, $p=0.17$, $\eta^2=0.33$; Eyes: $F(1,5)=4.38$, $p=0.10$, $\eta^2=0.47$) neutral (Face: $F(1,5)=0.86$, $p=0.41$, $\eta^2=0.18$; Eyes: $F(1,5)=1.16$, $p=0.34$, $\eta^2=0.24$), as indicated by large effect size.

Finally, 26 month olds fixated longer than adults at the eyes of social stimuli regardless of emotional valence (Group: $F(1,5)=2.04$, $p=0.23$, $\eta^2=0.34$), as indicated by large effect size. Twenty-six month olds fixated longer at the eyes of positive (Group:

$F(1,5)=1.89$, $p=0.24$, $\eta^2=0.32$), neutral (Group: $F(1,5)=0.08$, $p=0.08$, $\eta^2=0.02$; See Figure 9c) and negative (Group: $F(1,5)=5.20$, $p=0.09$, $\eta^2=0.57$; see Figure 9d) stimuli, as indicated by large effect size or significant p-values. Twenty-six month olds also fixated more frequently to the eyes of negative stimuli than adults, as indicated by large effect size (Group: $F(1,5)=2.10$, $p=0.21$, $\eta^2=0.29$).

Emotional Reactivity

Table 7 and Figure 10 summarize the results.

Latency to retrieve the treat in the grape alone condition or when in front of neutral objects remained constant across the three testing weeks for the adults. However, adults were significantly slower to retrieve the treat in the first week than in the second and third weeks when faced with a fearful object (Negative, Week: $F(2,46)=3.92$, $p=0.03$, $\eta^2=0.15$; 1st week > 2nd week, $p < 0.06$; 1st week > 3rd week, $p < 0.02$; see Figure 10a). At all three weeks, adults were significantly slower to retrieve a treat from in front of a negative stimulus, compared to a neutral or the grape only condition (Week 1: $F(2,34)=11.98$, $p<0.01$, $\eta^2=0.41$; Week 2: $F(2,34)=6.17$, $p<0.01$, $\eta^2=0.27$; Week 3: $F(2,34)=5.85$, $p<0.01$, $\eta^2=0.26$).

By contrast, juveniles retrieved the grape more slowly at 18 months of age than at 22 or 26 months in all conditions, as demonstrated by a significant effect of Age for Grape only (Age: $F(2,30)=3.45$, $p=0.05$, $\eta^2=0.19$; see Figure 10b), neutral stimuli (Age: $F(2,30)=8.82$, $p<0.01$, $\eta^2=0.37$; Figure 10c), as well as for negative stimuli (Age: $F(2,30)=5.94$, $p<0.01$, $\eta^2=0.28$; Figure 10d). The effect of age was not significantly modulated by the type of fearful stimulus presented (Age X Stimulus Category: $F(4,54)=1.23$, $p=0.31$, $\eta^2=0.09$).

Only at 18 months, did juveniles differ from adults, displaying slower overall latency to retrieve the reward. This group difference was significant for neutral stimuli (Group: $F(1,40)=5.84$, $p=0.03$, $\eta^2=0.12$) and during the grape alone conditions (Group: $F(1,41)=4.98$, $p=0.03$, $\eta^2=0.11$).

DISCUSSION

The data document the maturation of perception of and reactivity towards social and emotional stimuli prior to the onset of puberty in macaques. They also contributed to the literature on normative adult visual scanning patterns. Despite the small sample size in these studies, important results emerged. Thus, adult macaques treated negative social stimuli differently than neutral or positive ones with an increased attention to the eye regions of negative stimuli compared to other valences. These adult patterns, however, were not observed in juvenile monkeys from 18 to 26 months of age. First, it was only at the oldest age of 26 months that young monkeys, like adults, spent more time investigating social compared to nonsocial stimuli. Second, at the youngest age of 18 months, monkeys showed an overall heightened attention to the body region of conspecific stimuli with less frequent fixation towards the eyes, and increased attention to stimuli irrespective of their valence. This overall heightened attention decreased by 22 months of age, and salient regions within the social stimuli (importantly, the face and eyes) were less explored than in the adults. Third, only by 26 months of age, did the juvenile scan patterns begin to consistently differ depending on the valence of the social stimuli and approached the adult patterns, such that at this age, they looked more frequently at the eyes of negative stimuli, as did the adults. These data represent the first longitudinal investigation of the development of social gaze behavior in preadolescent male macaques, and the main results will be discussed in turn.

Adult Visual Scanning Pattern

Adults fixate differently on negative conspecific stimuli, compared to stimuli of neutral or positive valence. This was evidenced by a significant increase in the number of fixations per second towards the eyes of conspecifics displaying threat gestures. These scanning patterns differed from those previously reported in adult macaques. Gothard and colleagues (2004) showed an inverse scanning pattern with greater attention to the eyes for stimuli displaying affiliative signals (lip-smacks), and equal attention to the eyes and mouth for stimuli displaying threat gestures. The discrepancy between the two sets of results may have resulted from different procedures and parameters used. Thus, whereas we looked at spontaneous scanning patterns, the previous study employed a paired-comparison task to assess the effect of familiarity and novelty on viewing patterns, such that the successive repetition of the same conspecific within a trial may have differentially influenced the viewing patterns of the monkeys. Second, whereas we used short videos of conspecifics displaying neutral, positive or negative emotions, the previous study used static images probably carrying less emotional information. Finally, the longer gaze at positive faces in the previous study and the more frequent looks at negative faces in the present study, are in fact congruent with monkeys' reactivity to emotional signals emitted by conspecifics. Thus, when viewing threatening gestures from conspecifics, monkeys tend to display short, but more frequent, looks at threatening faces than at affiliative faces.

Unfortunately there is little data on the normative viewing patterns of adult primates for social and nonsocial stimuli. Where available, our results are consistent with findings that adult male rhesus spend a greater proportion of their fixations on the eyes as opposed to the mouth of conspecific stimuli (Ghazanfar, et al. 2006), and tend to fixate longer on faces than non-face stimuli (Guo, et al. 2006). In humans, typically developing adults spend a larger proportion of their time fixating on the eyes compared to the mouth of social stimuli (Mazzola, et al. 2006; Dahl, et al. 2009). Additionally, Eisenbarth and Alpers (2011) reported that happy emotional faces elicit fewer fixations on the eyes compared to negative (fearful and sad) faces, and similarly, Schurgin and colleagues (2014) demonstrated greater fixation time to negative compared to positive faces. However, these findings contradict those of Becker and Detweiler-Bedell (2009), suggesting that adults actively avoided fixating on negative faces. This study measured fixation patterns toward still images so incongruous results may be an artifact of the differences in testing procedures.

An unexpected result was the tendency that adults monkeys to fixate longer to the bodies of stimuli in Set 3 and the eyes of Set 1 than Set 2, as well as more frequently on the body of Set 1 than Set 2. Visual stimuli were separated into homogenous sets, each portraying monkeys of various sexes and ages in the same setting. After examination of the video clips across the three sets, we have not identified major changes in the qualities or characteristics of the conspecifics displayed. Additionally, the inconsistent nature of these effects makes their interpretation difficult. However, this difference could have impacted a potential age difference reported in viewing patterns of the juveniles at 18 month (Set 1), 22 months (Set 2) and 26 months (Set 3). Indeed, juveniles did fixate longer on the eye of social stimuli at 18 months (Set 1) than at 22 months (Set 2), and this effect was highly significant ($p<0.01$). However, there was no effect of age (or Set) on the frequency of fixations to the body of social stimuli in juveniles. Additionally, at 26 months (Set 3), juveniles did not display greater fixation to the body of the stimuli than at younger ages.

Visual Scanning Pattern During Development

At the youngest age of 18 months, young monkeys displayed an overall heightened attention to all stimuli irrespective of the emotional gestures emitted by the conspecifics. This increased attention was reflected by longer fixations at the body of the stimulus monkeys as compared to those at older ages, and longer fixations to the face of neutral, compared to emotional, stimuli. At this age, they also looked and fixated significantly longer than adults viewing the same stimuli. This result suggests that social stimuli are particularly salient and attractive at 18 months of age, consistent with the heightened exploration found in late childhood in male macaques (Suomi, 1997).

By 22 months of age, juveniles did not spend more attention to social movies than nonsocial movies, as adults did, and they looked significantly less than adults towards salient areas of social stimuli. In addition to significantly shorter fixation to the body region, 22 month olds also looked for shorter durations and significantly less at the eye region than at 18 and 26 months. In contrast to the adult pattern, 22 month olds looked less at the face and eyes in positive, neutral, and negative stimuli. These viewing patterns suggest that there is a decrease in attention to social stimuli more broadly, as well as to salient regions of the eyes and the face at 22 months of age. This decrement may correspond to the observed face processing decrement in late childhood/early adolescence in humans. Numerous studies have documented a decrement in face processing accuracy, emotion matching, or neural activity patterns to social stimuli in the early adolescent stage, primarily around ages 10 – 13 (Flin, 1980; Carey, Diamond, Woods, 1983; McGivern, et al. 2002, Taylor, Batty, et al. 2004). However, although these human studies have limitations due to their cross-sectional design, the findings from the current longitudinal studies in monkeys lent support to the proposal that face processing becomes weaker in late childhood/early adolescence in primates. Additional information on the levels of testosterone of the monkeys at each time points should indicate whether or not the changes in face processing abilities may be linked to the onset of puberty.

By 26 months of age, the scanning pattern of juveniles begins to reflect that of adults. That is, for the first time, young monkeys spent more time looking at social stimuli than nonsocial stimuli as adult monkeys did. In addition, they dedicated more of their attention to negatively valenced stimuli, but had not yet reached adult levels. Indeed, the normal emotional regulation seen in the adults appears to emerge at this age, as 26 month olds spend longer looking at the eyes of positive stimuli, and make more frequent, and shorter fixations, to the eye region of negative stimuli. However, at this age, they still attended longer to the eye regions of positive, neutral, and negative stimuli, and more frequently to the eyes of negative stimuli, compared to adults, suggesting that they have yet to reach an adult pattern of visual attention.

Taken together, these findings are consistent with research in humans suggesting that adolescents show consistent adult-like social attention, but have not fully matured to adult levels. In a study by O’Hearn and colleagues, children, adolescents and adults were compared for face, eye, and mouth recognition accuracy (O’Hearn, et al. 2010). Their study demonstrated that adolescents (around 14-years of age) showed marked improvements compared to children, but have yet to reach adult-like levels of expertise. However, the cross-sectional design of this study, as well as the face-matching paradigm, make direct comparison with the present results difficult. Finally, the ongoing development of face processing skills into adolescence is also supported by electrophysiological findings demonstrating slower adult-like neural activity response to faces, compared to other visual stimuli in adolescence (Taylor, et al. 1999).

Emotional Reactivity During Development

The findings of the emotional reactivity task are consistent with the eye tracking results. At 18 months of age, juveniles were more reactive in all conditions, indicated by heightened latency to retrieve the treat. When compared with adults, 18 month olds were more reactive in the nonthreatening conditions, but behaved adult-like to the fearful condition. By 22 months of age, the juvenile pattern matched that of adults. This is consistent with reports of increasing emotional reactivity in late childhood in rhesus macaques (Raper, et al. 2013). Our findings suggest that emotional regulation and attentional patterns to conspecific and emotional stimuli mature through late childhood and adolescence.

Effects of Social Experience

As reported above, subjects in each group differed in their levels of social experience prior to testing. The adult group was mother reared until natural weaning around one year of age (Bowman & Lee, 1995), and lived for at least 5 years in large, naturalistic social groups before being removed and brought to the Yerkes Main Center, where subjects were pair or singly housed. Juvenile subjects were mother reared until at least 6 months of age, roughly 6 months prior to natural weaning (Bowman & Lee, 1995). Additionally, when removed from maternal care subjects were also removed from the large natal social group, limiting complex social experience to only six months. Finally, juvenile subjects were peer-reared in a three-member group, again differing from adult subjects. Limited social experience may account for significant differences in the perception of social stimuli, as well as negatively impact typical behavioral and emotional function (Stevens, et al. 2009; Ljungberg & Westlund, 2000). To control for variability in rearing condition, juvenile subjects will be tested at later time points and again compared to control adults. A lack of significant difference in looking patterns as late adolescents or adults would indicate that the differences seen in adolescence are a product of typical development, rather than atypical rearing conditions.

Effects of Habituation

All subjects were received familiarization training prior to engaging in behavioral data collection, as outlined above. However, due to the longitudinal nature of this study, subjects necessarily had more experience with testing and procedures by the end of the study than at the start. The significant changes in attention to social stimuli and emotional reactivity documented in this study may result from changes in familiarization to the testing procedures or habituation to the testing stimuli. Methods employed to minimize the effects of habituation included the use of novel stimuli at every testing point, as well as measures of baseline social attention and emotional reactivity. Indeed, juveniles maintained consistent whole scene attention to social stimuli at all ages, indicating that changes seen between 18 and 26 months are limited to socially salient information, not a result of habituation or familiarization. Additionally, in emotional reactivity tasks, juveniles were significantly more reactive to nonthreatening (neutral) stimuli than adults at the first time point. This heightened emotional reactivity during test may be associated with recent exposure to a fearful stimulus during testing procedures, as all subjects completed identical habituation procedures. This failure to regulate emotion in the presence of a nonthreatening stimulus at 18 months of age, but not at 22 or 26, suggests late maturation of adult-like emotional reactivity in male macaques.

Future Directions

As noted above, this is the first longitudinal examination of behavioral changes in patterns of attention to social and emotional stimuli. Although these data suggest early development toward an adult-like pattern prior to pubertal onset, the measure of testosterone levels at the same time points will clearly inform whether the behavioral changes correlate with changes in gonadal hormone levels. In addition, it is still unclear what impact the rising of gonadal hormones will have later in adolescence (4-5 years). This will be tested by continuing the study of these same subjects at later ages, with collection of visual scanning behavior and serum testosterone.

Finally, as highlighted above, existing research on the developmental and hormonal contributions to social processing abilities lacks direct translational comparisons across species. However, eye tracking and emotional reactivity measures in nonhuman primates, as those described in the present study, may illuminate interesting developmental changes in response to social and emotional stimuli during adolescence and provide critical knowledge for a better understanding of the developmental changes observed in humans. From this nonhuman primate model, it appears that changes in social development begin prior to pubertal onset, though the mechanisms driving these changes are still poorly understood. Future studies should include replication of the results with a larger, heterosexual, sample size as well as complementary studies of human adolescents using the same paradigms.

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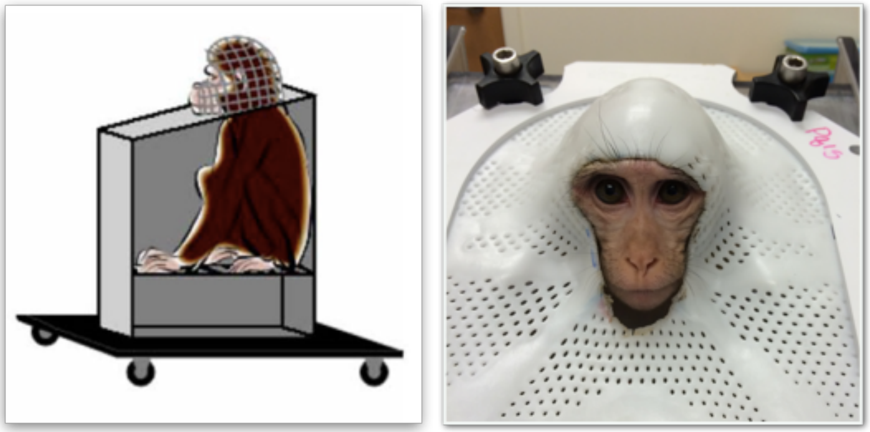


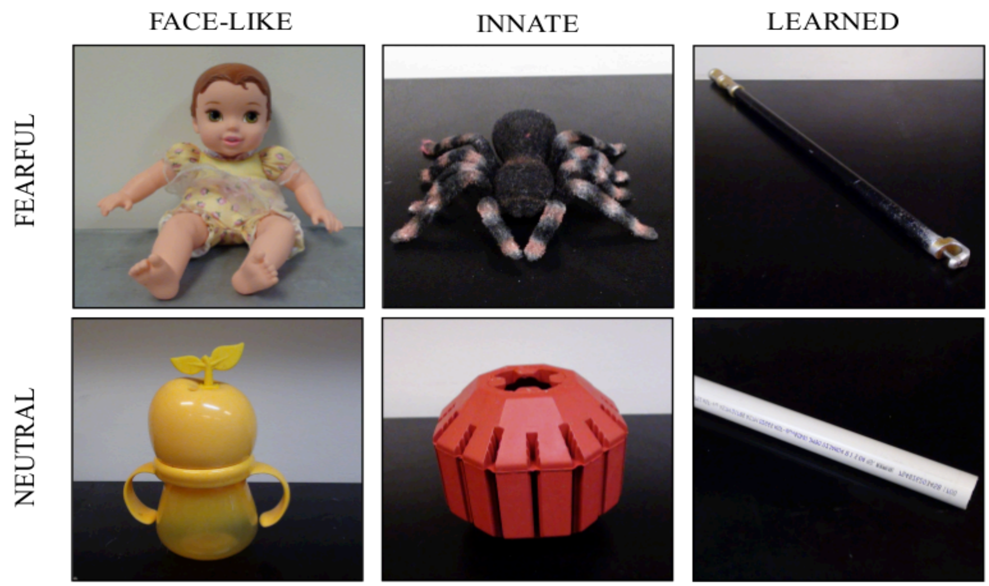
Figure 1

Figure 2

Blood Draw - Baseline
Eye Tracking Social: 4 positive, 4 neutral, 4 negative (X 6 presentations) Control: 12 nonsocial (X 3 presentation)
Emotional Reactivity Task
Blood Draw - 22 months
Eye Tracking Social: 4 positive, 4 neutral, 4 negative (X 6 presentations) Control: 12 nonsocial (X 3 presentation)
Emotional Reactivity Task
Blood Draw - 18months
Eye Tracking Social: 4 positive, 4 neutral, 4 negative (X 6 presentations) Control: 12 nonsocial (X 3 presentation)
Emotional Reactivity Task
Blood Draw - 26 months

Figure 3

Emotional Reactivity Objects



Face-Like	Neutral	Innate	Neutral	Learned	Neutral
Elmo	Bucket	Snake 1	Jump Rope	Capture Net	Mop
Doll 1	Cup	Spider 1	Red Kong	Pole	PVC Pipe
Monster Toy	Pink Stuffed Toy	Snake 2	Tigger Tail	Handling Glove	Latex Glove
Doll 2	Pink Box	Spider 2	Blue Toy		
Spongebob	Tan Ball	Snake 3	Tan Hose		
Doll 3	Water Bottle	Spider 3	Blue Kong		
Red Devil	Stuffed Dice				

Figure 4

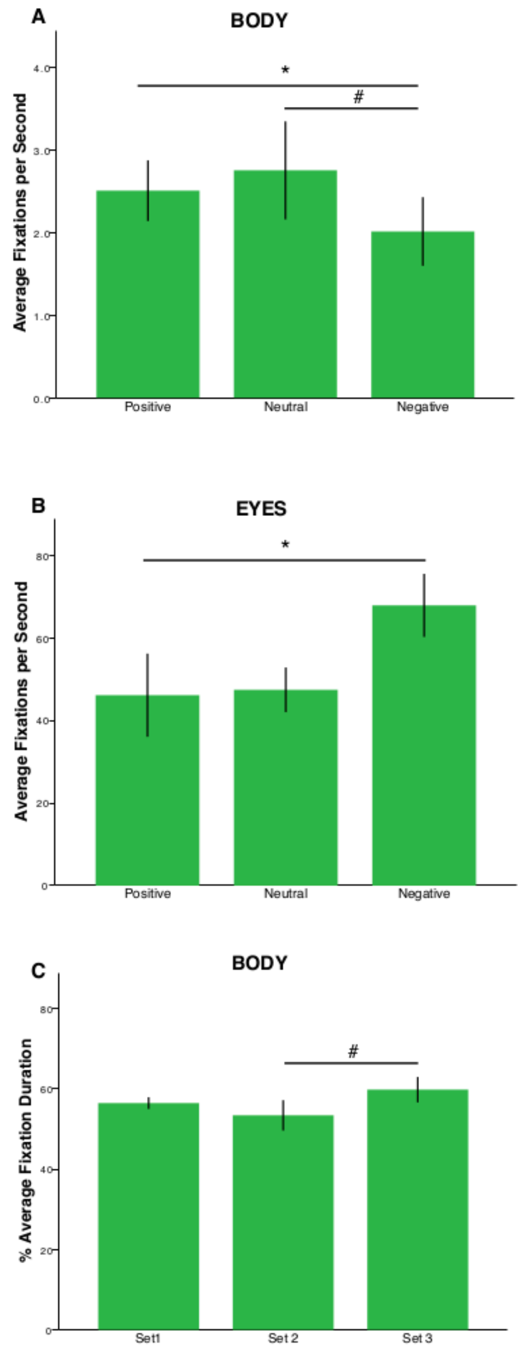

A
BODY
Average Fixations per Second
4.0
3.0
2.0
1.0
0.0
*
#
Positive
Neutral
Negative
B
EYES
Average Fixations per Second
80
60
40
20
0
*
Positive
Neutral
Negative
C
BODY
% Average Fixation Duration
80
60
40
20
0
#
Set1
Set 2
Set 3

Figure 5

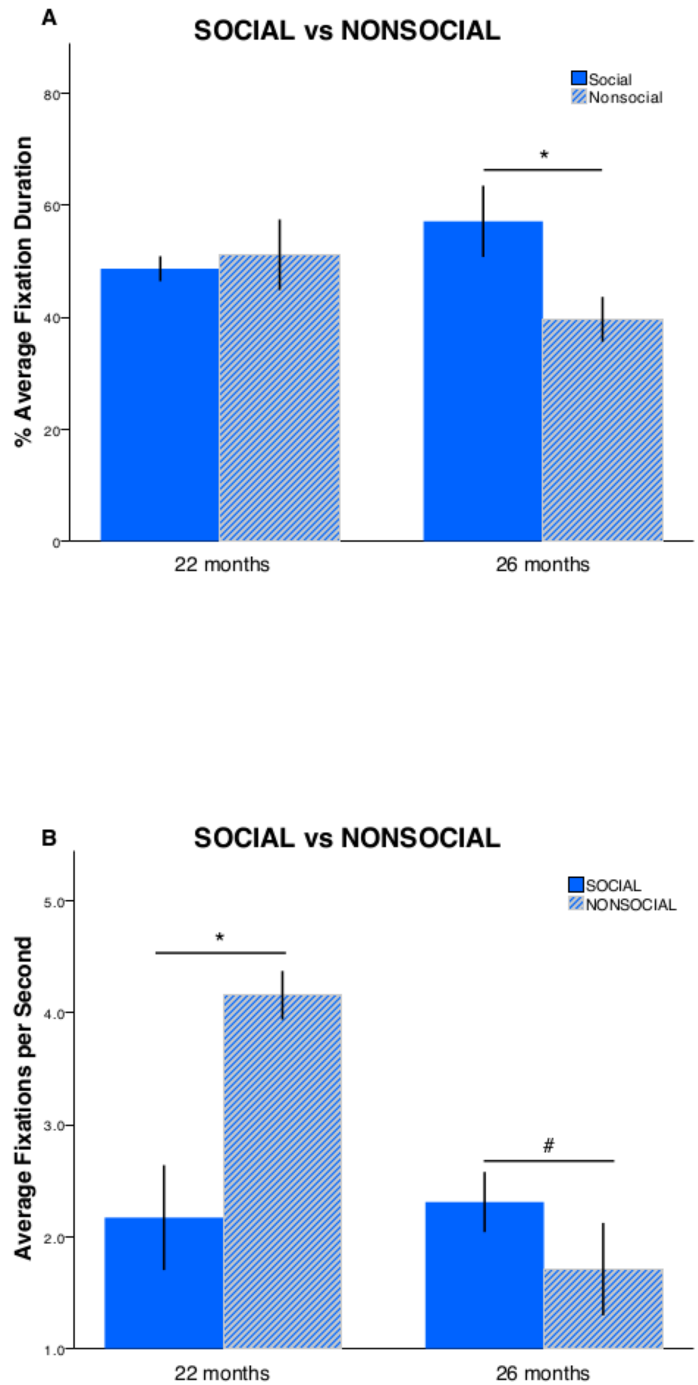

A
SOCIAL vs NONSOCIAL
Social
Nonsocial
% Average Fixation Duration
80
60
40
20
0
*
22 months
26 months
B
SOCIAL vs NONSOCIAL
SOCIAL
NONSOCIAL
Average Fixations per Second
5.0
4.0
3.0
2.0
1.0
*
#
22 months
26 months

Figure 6

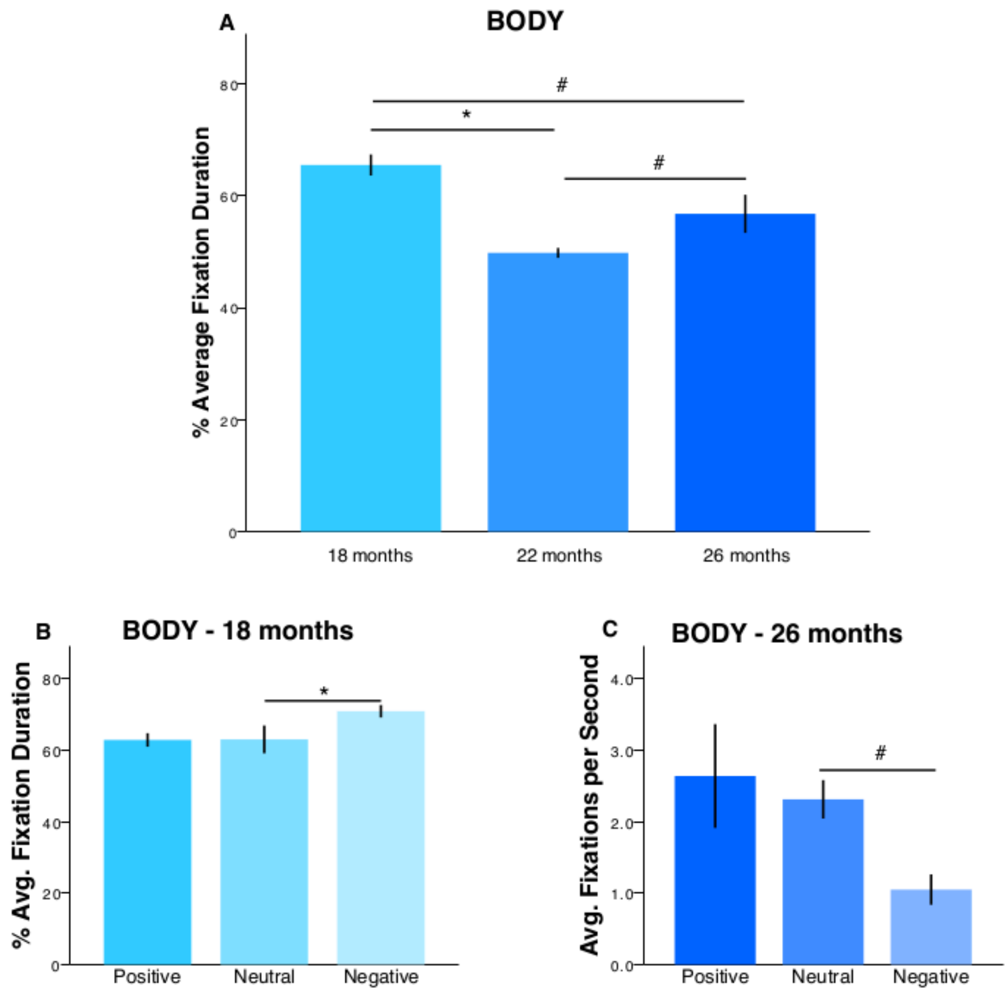

A
BODY
% Average Fixation Duration
80
60
40
20
0
#
*
#
18 months
22 months
26 months
B
BODY - 18 months
% Avg. Fixation Duration
80
60
40
20
0
*
Positive
Neutral
Negative
C
BODY - 26 months
Avg. Fixations per Second
4.0
3.0
2.0
1.0
0.0
#
Positive
Neutral
Negative

Figure 7

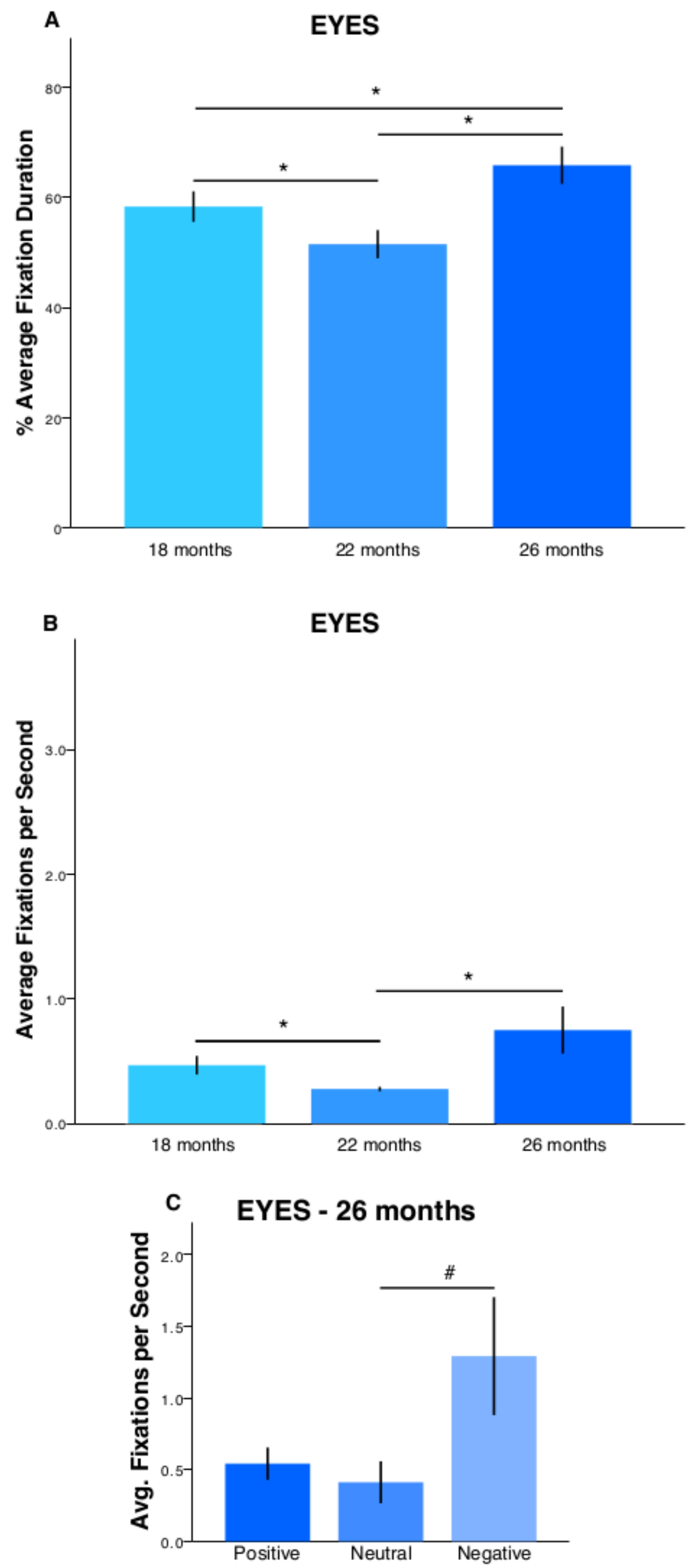

A
EYES
% Average Fixation Duration
0
20
40
60
80
*
*
*
18 months
22 months
26 months
B
EYES
Average Fixations per Second
0.0
1.0
2.0
3.0
*
*
18 months
22 months
26 months
C
EYES - 26 months
Avg. Fixations per Second
0.0
0.5
1.0
1.5
2.0
#
Positive
Neutral
Negative

Figure 8

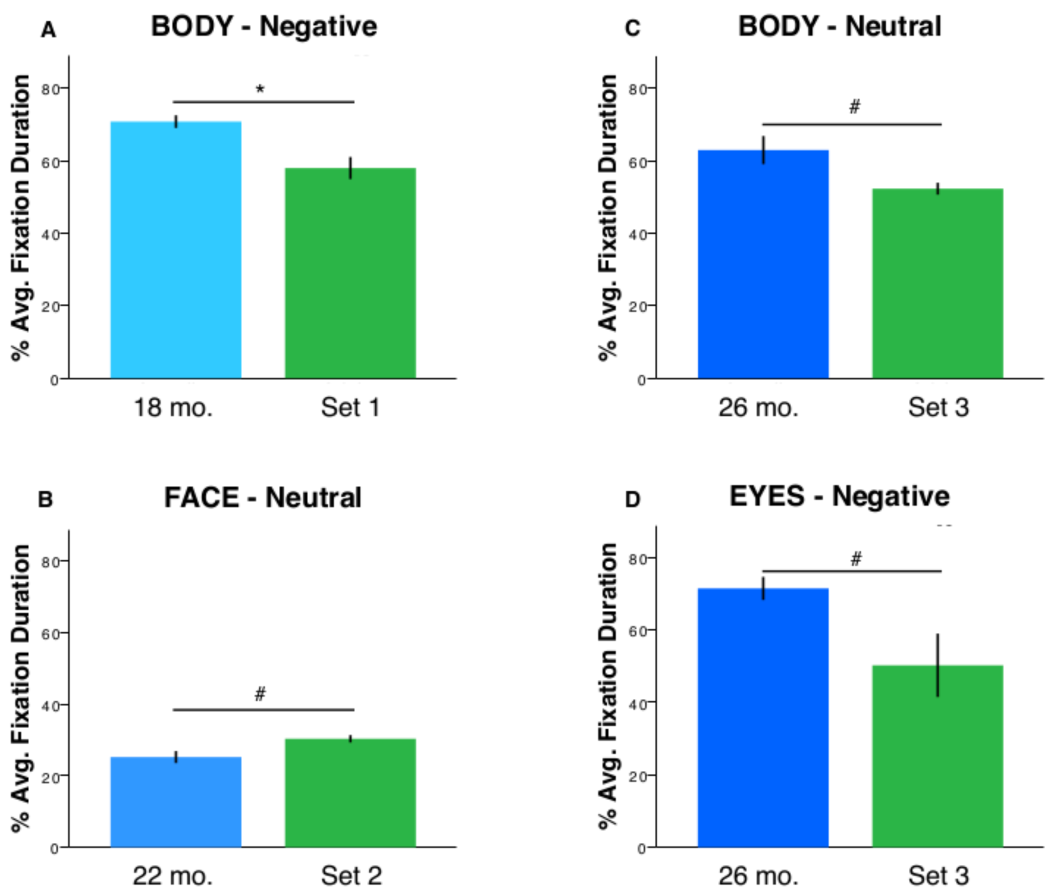

A
BODY - Negative
% Avg. Fixation Duration
0
20
40
60
80
*
18 mo.
Set 1
C
BODY - Neutral
% Avg. Fixation Duration
0
20
40
60
80
#
26 mo.
Set 3
B
FACE - Neutral
% Avg. Fixation Duration
0
20
40
60
80
#
22 mo.
Set 2
D
EYES - Negative
% Avg. Fixation Duration
0
20
40
60
80
#
26 mo.
Set 3

Figure 9

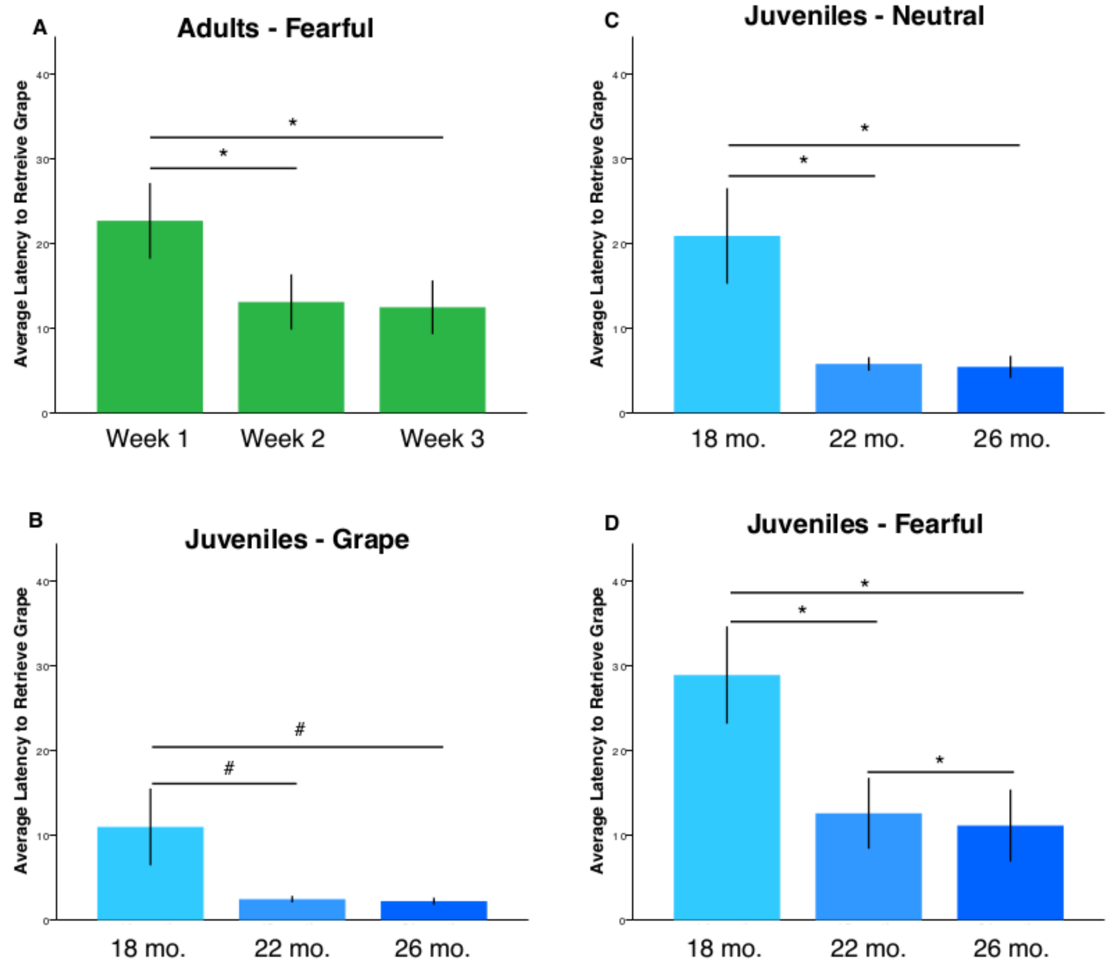

A
Adults - Fearful
Average Latency to Retreive Grape
Week 1
Week 2
Week 3
C
Juveniles - Neutral
Average Latency to Retrieve Grape
18 mo.
22 mo.
26 mo.
B
Juveniles - Grape
Average Latency to Retrieve Grape
18 mo.
22 mo.
26 mo.
D
Juveniles - Fearful
Average Latency to Retrieve Grape
18 mo.
22 mo.
26 mo.

Figure 10

Adult Average Fixation Duration

Parameter	*F statistic*	*df*	*P value*	η^2
Social / Non-social	9.31	1, 2	0.09*	0.80
Emotion: Body	0.17	2, 4	0.85	0.08
Emotion: Face	1.12	2, 4	0.41	0.36
Emotion: Eyes	1.31	2, 4	0.37	0.40
Set: Body	3.04	2, 12	0.09*	0.33
Set: Face	0.08	2, 12	0.92	0.01
Set: Eyes	2.42	2, 12	0.13	0.29

Table 1

Adult Average Fixations per Second

Parameter	*F statistic*	*df*	*P value*	η^2
Social / Non-social	8.47	1, 2	0.10*	0.81
Emotion: Body	9.15	2, 4	0.03*	0.82
Emotion: Face	7.50	2, 4	0.04*	0.79
Emotion: Eyes	5.03	2, 4	0.08*	0.72
Set: Body	1.45	2, 12	0.27	0.20
Set: Face	0.57	2, 12	0.58	0.09
Set: Eyes	0.05	2, 12	0.95	0.01

Table 2

Juvenile Average Fixation Duration

Parameter	*F statistic*	*df*	*P value*	η^2
Age X Social Condition	8.85	1, 4	0.04**	0.67
Social Condition: 22 mo.	0.17	1, 2	0.72	0.08
Social Condition: 26 mo.	40.52	1, 2	0.02**	0.96
Age: Body	13.36	2, 12	<0.01**	0.66
Age: Face	0.19	2, 12	0.83	0.03
Age: Eyes	19.02	2, 12	<0.01**	0.48
Age X Emotion: Body	0.47	4, 12	0.75	0.05
Age X Emotion: Face	0.06	4, 12	0.99	0.02
Age X Emotion: Eyes	7.50	4, 12	<0.01**	0.37
Emotion: 18 mo. - Body	3.69	2, 4	0.12	0.65
Emotion: 22 mo. - Body	1.56	2, 4	0.32	0.50
Emotion: 26 mo. - Body	0.06	2, 4	0.95	0.03
Emotion: 18 mo. - Face	20.90	2, 4	<0.01**	0.99
Emotion: 22 mo. - Face	2.91	2, 4	0.17	0.57
Emotion: 26 mo. - Face	0.03	2, 4	0.97	0.02
Emotion: 18 mo. - Eyes	1.16	2, 4	0.40	0.37
Emotion: 22 mo. - Eyes	0.83	2, 4	0.50	0.30
Emotion: 26 mo. - Eyes	5.35	2, 4	0.07*	0.73

Table 3

Juvenile Average Fixations per Second

Parameter	*F statistic*	*df*	*P value*	η^2
Age X Social Condition	39.29	1, 4	<0.01**	0.53
Social Condition: 22 mo.	34.56	1, 2	0.03**	0.95
Social Condition: 26 mo.	9.59	1, 2	0.09*	0.83
Age: Body	1.41	2, 12	0.28	0.11
Age: Face	2.51	2, 12	0.12	0.27
Age: Eyes	6.23	2, 12	0.01**	0.34
Age X Emotion: Body	2.51	4, 12	0.10*	0.40
Age X Emotion: Face	0.45	4, 12	0.77	0.10
Age X Emotion: Eyes	3.18	4, 12	0.05*	0.34
Emotion: 18 mo. - Body	1.29	2, 4	0.37	0.39
Emotion: 22 mo. - Body	0.45	2, 4	0.67	0.18
Emotion: 26 mo. - Body	5.08	2, 4	0.08*	0.72
Emotion: 18 mo. - Face	0.05	2, 4	0.95	0.03
Emotion: 22 mo. - Face	0.40	2, 4	0.69	0.17
Emotion: 26 mo. - Face	1.95	2, 4	0.26	0.73
Emotion: 18 mo. - Eyes	0.47	2, 4	0.66	0.19
Emotion: 22 mo. - Eyes	3.06	2, 4	0.16	0.60
Emotion: 26 mo. - Eyes	7.67	2, 4	0.04**	0.79

Table 4

Adult - Juvenile Comparison - Average Fixation Duration

Parameter	*F statistic*	*df*	*P value*	η^2
18 mo./Set 1 - BODY	13.70	1, 5	0.02**	0.75
18 mo./Set 1 - Positive	3.55	1, 5	0.13	0.40
18 mo./Set 1 - Neutral	6.39	1, 5	0.07*	0.61
18 mo./Set 1 - Negative	13.35	1, 5	0.02**	0.78
18 mo./Set 1 - FACE	0.01	1, 5	0.91	0.00
18 mo./Set 1 - Positive	0.02	1, 5	0.89	0.00
18 mo./Set 1 - Neutral	0.05	1, 5	0.83	0.00
18 mo./Set 1 - Negative	0.51	1, 5	0.52	0.11
18 mo./Set 1 - EYES	0.10	1, 5	0.77	0.19
18 mo./Set 1 - Positive	0.52	1, 5	0.51	0.12
18 mo./Set 1 - Neutral	0.54	1, 5	0.50	0.10
18 mo./Set 1 - Negative	0.01	1, 5	0.91	0.00
22 mo./Set 2 - BODY	0.46	1, 5	0.54	0.11
22 mo./Set 2 - Positive	0.18	1, 5	0.70	0.07
22 mo./Set 2 - Neutral	2.51	1, 5	0.19	0.39
22 mo./Set 2 - Negative	0.32	1, 5	0.60	0.07
22 mo./Set 2 - FACE	1.38	1, 5	0.31	0.29
22 mo./Set 2 - Positive	0.46	1, 5	0.54	0.11
22 mo./Set 2 - Neutral	6.77	1, 5	0.06*	0.67
22 mo./Set 2 - Negative	0.75	1, 5	0.43	0.15
22 mo./Set 2 - EYES	0.06	1, 5	0.82	0.02
22 mo./Set 2 - Positive	0.02	1, 5	0.90	0.00
22 mo./Set 2 - Neutral	0.53	1, 5	0.51	0.11
22 mo./Set 2 - Negative	0.02	1, 5	0.91	0.00
26 mo./Set 3 - BODY	0.12	1, 5	0.75	0.02
26 mo./Set 3 - Positive	0.00	1, 5	0.99	0.00
26 mo./Set 3 - Neutral	0.06	1, 5	0.82	0.02
26 mo./Set 3 - Negative	0.47	1, 5	0.53	0.10
26 mo./Set 3 - FACE	0.12	1, 5	0.75	0.03
26 mo./Set 3 - Positive	0.33	1, 5	0.60	0.09
26 mo./Set 3 - Neutral	0.00	1, 5	0.99	0.00
26 mo./Set 3 - Negative	0.21	1, 5	0.67	0.04
26 mo./Set 3 - EYES	2.04	1, 5	0.23	0.34
26 mo./Set 3 - Positive	1.89	1, 5	0.24	0.32
26 mo./Set 3 - Neutral	0.08	1, 5	0.08*	0.02
26 mo./Set 3 - Negative	5.20	1, 5	0.09*	0.57

Table 5

Adult - Juvenile Comparison - Average Fixations per Second

Parameter	*F statistic*	*df*	*P value*	η^2
18 mo./Set 1 - BODY	0.35	1, 5	0.59	0.08
18 mo./Set 1 - Positive	0.89	1, 5	0.40	0.18
18 mo./Set 1 - Neutral	0.08	1, 5	0.79	0.03
18 mo./Set 1 - Negative	0.51	1, 5	0.51	0.09
18 mo./Set 1 - FACE	1.09	1, 5	0.36	0.21
18 mo./Set 1 - Positive	0.04	1, 5	0.86	0.01
18 mo./Set 1 - Neutral	0.84	1, 5	0.41	0.17
18 mo./Set 1 - Negative	1.75	1, 5	0.24	0.26
18 mo./Set 1 - EYES	0.23	1, 5	0.66	0.05
18 mo./Set 1 - Positive	0.27	1, 5	0.63	0.07
18 mo./Set 1 - Neutral	0.08	1, 5	0.80	0.02
18 mo./Set 1 - Negative	1.57	1, 5	0.27	0.24
22 mo./Set 2 - BODY	0.41	1, 5	0.56	0.09
22 mo./Set 2 - Positive	1.36	1, 5	0.31	0.25
22 mo./Set 2 - Neutral	0.00	1, 5	0.92	0.00
22 mo./Set 2 - Negative	1.25	1, 5	0.31	0.20
22 mo./Set 2 - FACE	2.22	1, 5	0.21	0.39
22 mo./Set 2 - Positive	2.18	1, 5	0.21	0.35
22 mo./Set 2 - Neutral	0.86	1, 5	0.41	0.18
22 mo./Set 2 - Negative	2.51	1, 5	0.17	0.33
22 mo./Set 2 - EYES	3.90	1, 5	0.12	0.50
22 mo./Set 2 - Positive	3.25	1, 5	0.15	0.45
22 mo./Set 2 - Neutral	1.16	1, 5	0.34	0.24
22 mo./Set 2 - Negative	4.38	1, 5	0.10*	0.47
26 mo./Set 3 - BODY	0.11	1, 5	0.76	0.03
26 mo./Set 3 - Positive	0.02	1, 5	0.91	0.00
26 mo./Set 3 - Neutral	1.20	1, 5	0.34	0.23
26 mo./Set 3 - Negative	0.41	1, 5	0.55	0.08
26 mo./Set 3 - FACE	0.53	1, 5	0.51	0.12
26 mo./Set 3 - Positive	0.67	1, 5	0.46	0.14
26 mo./Set 3 - Neutral	0.55	1, 5	0.50	0.12
26 mo./Set 3 - Negative	0.06	1, 5	0.82	0.01
26 mo./Set 3 - EYES	0.59	1, 5	0.49	0.13
26 mo./Set 3 - Positive	0.61	1, 5	0.48	0.13
26 mo./Set 3 - Neutral	0.01	1, 5	0.94	0.00
26 mo./Set 3 - Negative	2.10	1, 5	0.21	0.29

Table 6

Emotional Reactivity

Parameter	*F statistic*	*df*	*P value*	η^2
Juvenile				
Age: Grape	3.45	2, 30	0.05*	0.19
Age: Fearful	5.94	2, 30	0.01**	0.28
Age: Neutral	8.82	2, 30	0.01**	0.37
Adult				
Age: Grape	1.90	2, 46	0.16	0.08
Age: Fearful	3.92	2, 46	0.03**	0.15
Age: Neutral	0.72	2, 46	0.49	0.03
Juvenile vs Adult				
Grape - 18 months	5.39	1,40	0.03	0.12
Fearful - 18 months	0.77	1,41	0.39	0.02
Neutral - 18 months	4.99	1,41	0.03	0.11
Group X Fearful Stimulus	1.17	4,48	0.34	0.40

Table 7