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Chelsie L. Cushman Date

Neonatal Amygdalectomy and Visual Scanning Behavior in Monkeys: An Investigation of Scanpath Abnormalities and the Effects of Fixation Definition

By

Chelsie L. Cushman
Master of Arts

Psychology

Hillary R. Rodman, Ph.D.
Advisor

Jocelyne Bachevalier, Ph.D.
Committee Member

Lynne Nygaard, Ph.D.
Committee Member

Kim Wallen, Ph.D.
Committee Member

Accepted:

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

Date

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

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By Chelsie L. Cushman

Previous case studies have shown that a patient with bilateral amygdala damage, S.M., habitually fails to spontaneously fixate the eye region of human faces under free-viewing conditions. Such findings have led researchers to hypothesize that the amygdala is critically involved in directing visual attention to salient, social stimuli in the environment which might contain valuable social information. However, these effects have yet to be demonstrated in others with similar damage. Two rhesus monkeys with extensive bilateral amygdala damage (sustained at one month of age) viewed images of unfamiliar monkey faces as their eye movements were recorded. Contrary to our predictions, subjects showed no obvious disturbances in their visual scanning behavior during the task. Further research is necessary to investigate the possibility that a difference in the timing of the lesions led to the discrepancy in the findings between the current study and previous work with S.M. The current data were also used in order to compare the effects of the application of two different temporal fixation definitions on the outcome(s) of eye tracking data analysis. Because the current subjects represent a special population (i.e., monkeys with brain lesions), the use of a minimum fixation duration lower than the *de facto* standard in the field was warranted. Lowering the duration minimum from the customary 100ms to 55ms increased the number of fixations included in the data set by 41%. Yet, no differences as a function of duration threshold were seen in the percentage of fixations made within and outside of regions of interest (ROIs), the percentage of fixations made within each ROI, the percentage of time spent viewing within and outside of ROIs, or the percentage of time spent viewing each ROI. This suggests that the use of a lower temporal fixation threshold, when justifiable, increases the total amount of data included in analyses, thereby increasing the power of those analyses as well as sensitivity to between-group differences when special populations are being compared to healthy ones. At the same time, use of the lower threshold will not significantly alter the spatiotemporal patterns of fixation on face stimuli.

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For primates, living in a highly social, hierarchical group environment creates the need for certain visual and socio-cognitive abilities. The capacity to extract socially-relevant information from the environment, process that information effectively, and respond in a socially and emotionally appropriate way is of the utmost importance. The face is perhaps the most potent source of visual social signals, as it conveys information regarding the identity, age, gender, emotional disposition, direction of attention, and possibly intentions of another individual or conspecific (Emery, 2000). Though all parts of a face are used to extract socially-relevant information, the eyes are thought to be both the most important and most salient (Emery, 2000). Similarly to humans (e.g., Spezio, Huang, Castelli, & Adolphs, 2007), non-human primates attend primarily to the eye region of the face when presented with a picture of a whole face (Gothard, Brooks, & Peterson, 2009; Keating & Keating, 1982; Nahm, Perrett, Amaral, & Albright, 1997; Sato & Nakamura, 2001), as evidenced by a larger number of fixations made in this region compared to other prominent regions such as the nose or the mouth. This bias has been found regardless of whether the presented face is upright, inverted, or scrambled (Guo, Robertson, Mahmoodi, Tadmor, & Young, 2003), as well as whether the face belongs to a conspecific or a human (Keating & Keating, 1982; Nahm et al., 1997). This tendency to focus attention on the eye region of a neutral face is considered a hallmark of visual scanning patterns with respect to faces.

A Role for the Amygdala in Visual Scanning of Social Stimuli

Though the exact neuroanatomical underpinnings of visual scanning behavior are unknown, there is reason to believe that the amygdala may be central to the production of scanning patterns for face stimuli, specifically. Evidence for this notion comes most directly from an amygdala-damaged patient, S.M., who shows abnormalities in her visual scanning patterns of faces. A well-known patient with very specific, bilateral amygdala damage, S.M. focuses abnormally large amounts of attention (i.e., greater number of fixations) on the mouth region, and abnormally low amounts of attention on the eye region, when viewing a human face (Adolphs, Gosselin, Buchanan, Tranel, Schyns, & Damasio, 2005; Kennedy & Adolphs, 2010; Spezio et al., 2007). S.M. first became famous for her marked impairment in identifying, specifically, fearful facial expressions. Though originally her impairment was thought to reflect a general deficiency in the processing of fear-related stimuli, further investigation linked it to the absence of an automatic shift of attention to the eyes when she viewied a face, as opposed to an aversion to the eyes or an inability to shift gaze to the eye region of a face (Adolphs et al., 2005). Based on these findings, it was postulated that the amygdala directs the visual system to “seek out, fixate, pay attention to, and use” information from the salient eye region of faces.

Individuals with autism also show aberrations in their scanpaths of human faces (Dawson, Meltzoff, Osterling, Rinaldi, & Brown, 1998; Dawson, Osterling, Meltzoff, & Kuhl, 2000; Klin, Jones, Schultz, Volkmar, & Cohen, 2002; Leekam, Hunnisett, & Moore, 1998; Pelphrey, Sasson, Reznick, Paul, Goldman, & Piven, 2002), which are remarkably similar to those of S.M. Eye-tracking studies have demonstrated that individuals with autism exhibit a decrease in looking time at the eye region of face compared to healthy control participants, shifting some attention to the mouth or other, external, regions of the face (Klin et al., 2002; Pelphrey et al 2002; but see Hernandez, Metzger, Magné, Bonnet-Brilhault, Roux, Barthelemy, et al., 2009). Additionally, abnormalities in amygdalar structure and function have been reported in autistic individuals (see Baron-Cohen, Ring, Bullmore, Wheelwright, Ashwin, & Williams, 2000; Baron-Cohen, Ring, Wheelwright, Bullmore, Brammer, Simmons, et al., 1999; Bauman & Kemper, 2005).

Finally, the amygdala has been found to contain cells which are selectively responsive to socially-relevant stimuli such as faces (Brothers & Ring, 1993; Brothers, Ring, & Kling, 1990; Gothard, Battaglia, Erickson, Spitler, & Amaral, 2007; Hoffman, Gothard, Schmid, & Logothetis, 2007; Kawashima, Sugiura, Kato, Nakamura, Hatano, Ito, et al., 1999; Leonard, Rolls, Wilson, & Baylis, 1985). Such selectivity might enable this structure to contribute to the extraction and processing of socially-relevant information, including but not limited to visual scanning of face stimuli.

Case studies, such as those of the visual scanning patterns of amygdala-damaged patient S.M., afford invaluable opportunities to begin the process of discerning brain function. However, once a theory is developed regarding the function of a particular brain region based on a single patient, it becomes necessary to utilize multiple subjects in the context of controlled experiments in order to garner support for that theory. Human subjects with focal, bilateral amygdala damage are extremely rare. Therefore, the central purpose of the current study was to track the eye movements of multiple nonhuman primates with bilateral amygdala lesions as they viewed images of unfamiliar monkey faces, and to provide a detailed, quantitative description of their visual scanning patterns. According to the recently-developed theory regarding amygdala function with respect to visual scanning of faces (mentioned above; Adolphs et al., 2005) based on eye tracking data from S.M., one would predict that nonhuman primates with bilateral amygdala damage would show obvious irregularities in their visual scanning of faces. More specifically, the number of fixations made and time spent on the eye region of a stimulus face would be notably small, while the number of fixations made and time spent on the nose and mouth areas of a stimulus face would be abnormally high.

Defining Fixations: Temporal Criteria

A secondary goal of the study was to investigate the effects that the use of different fixation definitions might have on the outcome(s) of eye tracking data analysis. Though many researchers tend toward customary definitions that have, over time, become common in the fields of human and monkey eye tracking research, some have argued against the use of one-size-fits-all definitions in favor of flexible definitions that depend more on the specific research context or subject population (e.g., Manor and Gordon, 2003). Consequently, there are multiple methods that may be used to decide which pauses or hesitations in the scanpath are treated and analyzed as fixations. However, it remains to be seen whether changing the fixation definition in the context of a special subject population significantly affects the results of data analyses (compared to when standard definitions are used).

Visually scanning an image or object in the environment is characterized by two main events: rapid eye movements called *saccades*, and brief pauses in eye movement called *fixations* (Manor & Gordon, 2003; Rayner, 1998). During a fixation, the fovea is directed toward a particular region of the visual field and focuses on that area for a short period of time. As the eye remains relatively still, visual information is extracted from the visual field, and the visual system begins processing that information. When a fixation ends, the eye moves quickly to focus on another point in the visual field. Input to the visual system is suppressed during this saccadic eye movement, a phenomenon referred to as *saccadic suppression* (Matin, 1974).

With the aid of an eye tracking system such as ISCAN or EyeLink 1000, one is able to track the eye movements of an individual during the presentation of a stimulus. However, in order to quantify those eye movements and describe how the individual looks at a given stimulus, one must first identify fixations in the raw eye movement data. In order to separate saccades and “noise” (such as non-saccadic eye movements and *transient track loss*, when the eye tracker briefly loses track of the eye) from fixations, pre-determined spatial and temporal criteria which serve to define fixations must be applied to the raw data. Most challenging is the task of determining when a saccade ends and a fixation begins, and alternatively, when a fixation ends and a saccade begins. In this respect, a temporal fixation threshold is critically important. If the minimum fixation duration is set too high, true fixations during which visual information is extracted and processed will be lost. On the other hand, if the minimum is too low, noise will inevitably make its way into the data.

At times, both 200ms (Poulton, 1962) and 100ms (Manor & Gordon, 2003) temporal fixation thresholds have been regarded as standards in human eye tracking research. However, a lack of true consensus is apparent as there continues to be considerable variation in the temporal criteria used to define fixations in this field. To date, both 200ms duration thresholds (e.g., Pelphrey et al., 2002; Ryu, Morita, Shoji, Waseda, & Maeda, 2001; Williams, Loughland, Gordon, & Davidson, 1999) and 100ms duration thresholds (e.g., Adolphs et al., 2005; Dahl, Wallraven, Bulthoff, & Logothetis, 2009; Shepherd, Steckenfinger, Hasson, & Ghazanfar, 2010) are commonly used, in addition to 50ms duration thresholds (e.g., Dalton, Nacewicz, Johnstone, Schaefer, Gernsbacher, Goldsmith, et al., 2005; Hernandez et al., 2009). Duration fixation minimums as low as 20ms are occasionally seen (e.g., de Wit, Falck-Ytter, & von Hofsten, 2008) in human eye tracking studies.

Temporal fixation definitions are less variable in the monkey eye tracking literature. The *de facto* standard is 100ms (e.g., Dahl et al., 2009; Guo et al., 2003; Shepherd et al., 2010), a criterion likely borrowed from some current human eye tracking literature. However, there is evidence to suggest that monkey eye movements are characteristically different from those of humans, supporting the notion that standards used for humans may not necessarily be most appropriate for a nonhuman primate subject group. Though both species generally focus their visual attention on similar regions of social stimuli, monkeys tend to make shorter fixations, longer saccades, faster saccades, and to exhibit a more stereotyped fixation pattern (Berg, Boehnke, Marino, Munoz, & Itti, 2009; Shepherd et al., 2010). Given these differences, borrowing a standard temporal fixation threshold established in the human eye tracking literature for use in monkey eye tracking studies might not be appropriate, or further, result in an accurate depiction of monkey visual scanning patterns.

Moreover, as is argued by Manor and Gordon (2003), shying away from standard temporal criteria and considering shorter fixations in the context of clinical populations may be particularly important. Previous work has demonstrated that in conditions such as schizophrenia or autism, eye movements can be affected (e.g., Manor & Gordon, unpublished data, as cited in Manor & Gordon, 2003; Sereno & Holzman, 1995; Ryu et al., 2001; Williams et al., 1999). Including shorter fixations in analyses of eye tracking data may enhance sensitivity when comparing clincial and nonclincial groups, and may affect the outcome of those analyses. For example, in an eye tracking study using abstract and biological stimuli, schizophrenic participants were found to make fewer fixations, fixate for longer, and exhibit a shorter scanpath than control participants when a 100ms temporal fixation threshold was used (Manor & Gordon, unpublished data, as cited in Manor & Gordon, 2003). These differences were not significant when the threshold was increased to 200ms. Lowering the fixation duration minimum may also be best when working with patients with neurological deficits or brain damage, as sensitivity to subtle, between-group differences may be more apparent when shorter fixations are included in analyses. For instance, when shorter fixations are considered, it might be possible to see that one group exhibits increased gaze drifting and a larger number of corrective saccades than the other (Manor & Gordon, 2003).

Given these considerations, it stands to reason that the standard temporal definition of what constitutes a fixation may not be optimal when working with monkeys, let alone monkeys who have sustained some amount of brain damage. Instead, a lower temporal fixation threshold, which would allow for the inclusion and consideration of more of the short registered fixations made by a subject group of this kind, may be more appropriate (personal communication, W. Jones).

Therefore, the focus of the current study was to examine any differences in the results of eye tracking data analyses when a standard temporal fixation threshold and a lower fixation threshold are applied to the data. Identifying statistically significant differences, should they exist, may help us to understand how different temporal definitions of a fixation can affect the outcome of eye tracking data analyses. The data presented herein are relevant to whether or not it is worthwhile to consider moving away from *de facto* standard fixation definitions in favor of lower thresholds when special populations are serving as subjects or participants.

Method

Subjects

Subjects were two male rhesus monkeys (*Macaca mulatta*). Both were neonatally amygdalectomized by MRI-guided neurotoxic injections of ibotenic acid which were intended to affect the entire amygdala in both hemispheres (Table 1). Surgeries were conducted at approximately one month of age (for more details on surgical procedures see Goursaud & Bachevalier, 2007).

Subjects were approximately three years old at the time of testing. Both were raised in large social groups at the Yerkes Field Station until the time of testing, at which point they were removed from their social groups and put into bachelor groups as part of another study. Both animals were chair trained in a modified chair apparatus, using positive reinforcement only, prior to testing. Training proceeded in stages, where subjects were first required to enter the primate chair in order to receive a food (e.g., raisin, fruit loop, or dry noodle) or juice reward. Following relatively consistent performance at this stage of training, they moved to the next stage, were they were required to move their faces to the head hole before they could receive a reward. They were progressively required to bring their own heads partially out of the head hole, bring their own heads fully out of the head hole, hold their own heads out of the head hole, hold their heads out of the head hole as the trainer moved her hand close to the next plate, hold their heads out of the head hole as the neck plate closed, allow head restraints to be placed on the primate chair, and remain relatively calm during exposure to the computer screen used for stimulus presentation. A subject was tested only after completing all training steps during at least three consecutive training sessions. All surgical, training and testing procedures described herein were conducted in accordance with approved Emory University IACUC protocols.

Stimuli

Target stimuli were digital, static, color images of monkey faces obtained from an extended library of digitized monkey faces taken under uniform lighting conditions (obtained from K. Gothard). All external cues – such as cage bars and objects in the background of each picture – were removed using Adobe Photoshop CS3 Version 10.0. Ten face images were used as stimuli, each featuring the face of an unfamiliar monkey with a neutral expression. Five of the pictured monkeys exhibited direct gaze (with respect to the subject), while the other five exhibited averted gaze. Five additional digital, static, color images of objects which would be unfamiliar to the subjects – such as Fabergé eggs, Christmas tree ornaments, and other colorful, complicated or multi-part objects – were also used as stimuli. Such objects were chosen for inclusion in the current set of stimuli so that, in the event that abnormalities were seen in the scanning patterns of face stimuli, it might be determined if those abnormalities were specific to faces. The use of objects as additional stimuli might also aide in determining whether the animals had a general “side” or “area of screen” bias that might have generally influenced their individual scanning patterns.

A priori decisions were made as to what constituted the eye, nose, mouth, and ear regions of each stimulus face (Keating & Keating, 1982). Rectangles were drawn around the eye, mouth, and ear regions of interest, while an ellipse was drawn around the nose region (Adolphs, 2005; Gothard et al., 2009). The eye region was comprised of the iris, pupil, eyebrow and peri-eye areas of each stimulus face (Gothard et al., 2009; Sato & Nakamura, 2001). The nose region included the nostrils and visible bridge of the nose, and the immediately surrounding area (only bare-skinned areas were included). The mouth region was comprised of the entire snout, starting just below the nose and extending to the bottom of the chin (Gothard et al., 2009). The ear regions were comprised of the outer ear as well as the edge of the head where the outer ear was attached. Best estimates were drawn when the outer ears were malformed or covered by hair. Given these criteria for defining the regions of interest (ROIs), and the variability in the sizes of facial features across stimulus faces, the absolute size of ROIs varied between stimuli (Gothard et al., 2009).

In order to draw face-like ROIs on object stimuli, five face stimuli were randomly chosen and their ROIs superimposed on the five object stimuli. This method was employed as opposed to free-drawing ROIs on the object stimuli in order to ensure that the eye, nose, mouth, and ear regions on the object stimuli were realistic and comparable to those drawn on the face stimuli, both in terms of size and placement. Given inconsistencies in the methods used to delineate ROIs in previous research, and a lack of a clear, “best” solution for drawing ROIs, the above criteria were chosen in order to leave room for comparison between the current study and previous studies reporting on face scanpath data in nonhuman primates.

Apparatus and Procedure

Subjects were brought out of their groups or housing areas into a testing room where they were allowed to enter a modified primate chair (28.6 cm wide x 30.5 cm deep x 63.5 cm tall plexiglass enclosure with a head hole at the top), and were further restrained by small plexiglass squares which were attached to the primate chair and situated on either side of the head. Such restraint was necessary in order to keep head movement to a minimum, allowing the eye tracker to hold its position on the pupil of each subject. The chair was positioned approximately 63.5 cm from the monitor used for the presentation of stimuli. Black curtains were hung between the animal and the experimenter during the experiment, and overhead lights were turned off, in an effort to minimize visual distraction. Stimuli were displayed using Presentation software (version 14.5; Neurobehavioral Systems, Albany, CA, USA) on a color monitor (60 Hz, Sony LCD color computer display) with a resolution of 1280 x 1024 pixels and 32 bit color depth. Images were displayed in the center of a black screen at a resolution of 480 x 360 pixels with a 32 bit color depth. An infrared eye tracker (ISCAN, Inc., Woburn, MA, USA) was used to monitor subjects’ eye movements during stimulus presentation.

Each subject completed all trials (i.e., viewed all 15 stimulus images) in one testing session. Stimulus images were presented in random order. Each image appeared only once and was displayed for a total of 2500ms. Between each stimulus presentation a three-dimensional, colorful, rotating object was displayed in order to keep subjects' attention. Small treats such as raisins, dry noodles, and sips of juice were also given between some trials and during the calibration in order to keep subjects motivated to complete the experiment.

Measures and Analyses

The number and duration of fixations made on each stimulus image with respect to ROIs were measured with the aid of ISCAN eye tracking software. The proportion of fixations falling onto and outside of ROIs, as well as the proportion of viewing time dedicated to ROIs, was then calculated (e.g., Guo et al., 2003; Keating and Keating, 1982; Pelphrey et al., 2002).

Fixations were defined using two different sets of criteria, as described in detail below. However, before either criterion could be applied, it was necessary to remove as much "noise" (e.g., recording noise, non-saccadic eye movements, transient track loss) as possible from the raw eye movement data. In the current study, the eye tracker was set to record eye movements at a rate of 60hz. Thus, the location of the subject's eye was sampled once every 16.7ms. In order to conclude with any degree of confidence that a subject has indeed made a fixation, his eye must register in approximately the same location for at least two consecutive samples. This means that the shortest pause in the scanpath that can possibly be considered as a fixation within the limitations of the eye tracking software is one that lasts for at least 33ms. Thus, all recorded fixations shorter than 33ms were discarded outright, and were not included in any analyses.

Due to limitations of the ISCAN software itself, the durations of all recorded fixations were automatically rounded to the nearest hundredth of a second. For example, any pause in the scanpath that was between 15 and 24ms in duration registered as a fixation that was 0.02s in length. Similarly, pauses in the scanpath that were between 25 and 34ms in duration registered as fixations that were 0.03s long. Consequently, it was impossible to differentiate between pauses in the scanpath that fell into the same "duration category". All fixations that registered as 0.02 or 0.03s in duration were discarded, because they most likely did not meet the 33ms duration criterion for being considered as true fixations, described above (note that 34ms fixations may have been included in this group).

Visual scanning patterns of amygdalectomized subjects. Given the small sample size of the experimental group, and that the planned control group was not able to be successfully tested, no attempt was made to formally compare the data gathered from the amygdalectomized animals to data from sham-lesion or intact animals. Instead, the current study, which should be viewed as exploratory in nature, used descriptive statistics in order to provide a quantitative account of the visual scanning patterns of two amygdalectomized macaques with respect to unfamiliar monkey faces. Any trends seen in the current data should be investigated in a follow-up study which tests a larger sample, including both amygdalectomized and sham-lesion macaques, using the same procedure.

Defining a fixation: Standard duration threshold versus lower threshold for special populations. For the purposes of comparing the outcomes of eye tracking data analyses when a standard or a lower minimum fixation duration are utilized, two different temporal criteria were used in the current study.

To begin, a temporal method for determining what constitutes a fixation which does not take a given data set into consideration was employed. As a 100ms temporal fixation threshold has become the standard in the monkey eye tracking literature (e.g., Dahl et al., 2009; Guo et al., 2003; Shepherd et al., 2010) this criterion was chosen for application to the raw eye movement data. That is, only those recorded fixations which lasted for at least 100ms were included as true fixations and subsequently analyzed as described above. The data were then reanalyzed in the same manner after a lower threshold was applied to the eye movement record. In order to determine exactly where this lower threshold would be placed, a "data-driven" method for temporally defining fixations was used. This method (W. Jones, personal communication, September 2, 2011) requires that the shortest 5% of recorded fixations be discarded as eye movement and recording noise. The remaining 95% of recorded fixations are regarded as true fixations and included in subsequent analyses.

In order to employ the "5% cutoff" method, all fixations made by both animals during testing sessions, regardless of the stimulus on which they were made, were compiled into a cumulative distribution graph where the duration of each of those fixations was the variable of interest (Figure 1). A frequency distribution table was also made. The task of determining and discarding the shortest 5% of recorded fixations was also made somewhat difficult by the aforementioned eye tracking software limitations. Following 0.02 and 0.03s, the next longest fixation made by either subject registered as 0.05s in duration. Again, this included any fixations that were between 45 and 54ms long. Though fixations of this duration constituted 7.4% of all recorded fixations, all fixations falling into this 0.05s duration category were discarded in order to ensure that the shortest 5% of fixations had been removed. Thus, the temporal fixation threshold, as determined by the data themselves, was set at 55ms.

Comparative analysis. In order to determine whether the utilization of different thresholds for defining fixations affects the results of eye tracking data analyses appreciably, the results of data analyses following the application of a 100ms minimum duration were compared statistically to the results of data analyses following the application of the “5% cutoff” rule (or, the 55ms minimum duration).

Repeated Measures ANOVAs were used to determine whether the distribution of fixations falling within or outside of ROIs, of fixations falling within each ROI, the amount of time spent viewing ROIs, and the amount of time spent viewing each ROI, differed significantly depending on the temporal fixation threshold.

Results

Description of Visual Scanning Patterns of Amygdalectomized Subjects

The proportion of fixations falling onto and outside of ROIs, as well as the proportion of viewing time dedicated to ROIs, was calculated for each animal separately. For the purposes of simplicity, the data set consisting of fixations no less than 100ms in duration were used to calculate the descriptive statistics given in this section. Figure 2 contains example scanpaths produced by both subjects while free-viewing both averted and direct gaze stimulus faces.

Fq12. The average number of fixations per stimulus image was 4.8 (*SD* = 1.92) for averted gaze faces, 6.6 (*SD* = 1.52) for direct gaze faces, 4.6 (*SD* = 1.52) for objects, and 5.7 (*SD* = 1.89) for all faces, regardless of gaze direction. Average fixation duration for fixations made on averted gaze faces was 0.21 seconds (*SD* = 0.03); direct gaze was 0.20 seconds (*SD* = 0.65); objects was 0.16 seconds (*SD* = 0.26); all faces was 0.21 seconds (*SD* = 0.05).

The average proportion of fixations made on the stimulus image (i.e., the face or object in the center of the computer screen as opposed to the black areas of the screen that were not taken up by the stimulus) was 92% (*SD* = 17.89) for averted gaze faces, 100% (*SD* = 0) for direct gaze faces, 76.0% (*SD* = 1.30) for objects, and 96.0% (*SD* = 12.65) for all faces.

The average proportions of fixations falling within specific ROIs as a function of stimulus type can be seen in Table 2. The average proportions of time spent viewing specific ROIs as a function of stimulus type are shown in Table 3.

Based on the area of the stimulus image subsumed by the eye region, the average probability of fixating in this region by chance alone was 12.3%. (The area of the eye regions of object stimuli were not included in this average because all ROIs drawn on object stimuli were taken directly from ROIs drawn on face stimuli.) Fq12 averaged 44.6% (± 15.9) fixations in the eye region in the case of face stimuli, and 14.0% (± 21.9) in the case of object stimuli. In addition, he spent 53.2% (±22.8) of his image viewing time, on average, on the eye region of face stimuli, compared to 15.7% (±25.9) for object stimuli. For face stimuli, both the percentage of fixations made in the eye region (One sample t-test; $t = 6.44$, $p < 0.001$) and the percentage of time spent viewing the eye region ($t = 5.69$, $p < 0.001$) were significantly greater than chance level. These same measures were not significantly different than chance in the case of object stimuli ($t = 0.17$, $p > 0.05$ for fixations made in eye region; $t = 0.29$, $p > 0.05$ for time spent viewing eye region).

The probability of the first fixation falling within the eye region was 60% for face stimuli and 40% for object stimuli. The probability of any fixation falling within the eye region was 43.9% for face stimuli, and 13.0% for object stimuli.

Ht12. The average number of fixations per stimulus image was 6.0 (*SD* = 1.22) for averted gaze faces, 4.0 (*SD* = .82) for direct gaze faces, 5.4 (*SD* = 1.34) for objects, and 5.1 (*SD* = 1.45) for all faces. Average fixation duration for fixations made on averted gaze faces was 0.17 seconds (*SD* = 0.03); direct gaze was 0.25 seconds (*SD* = 0.08); objects was 0.22 seconds (*SD* = 0.03); all faces was 0.20 seconds (*SD* = 0.07).

The average proportion of fixations made on the stimulus image was 100% ($SD = 0.00$) for averted gaze faces, 91.67% ($SD = 16.65$) for direct gaze faces, 93.80% ($SD = 8.52$) for objects, and 96.30% ($SD = 11.11$) for all faces.

The average proportions of fixations falling within specific regions of interest as a function of stimulus type can be seen in Table 2. The average proportions of time spent viewing specific regions of interest as a function of stimulus type are shown in Table 3.

As previously stated, the probability of any given fixation falling in the eye region of a stimulus image by chance was 12.3%, given the area of the image subsumed by that region. Ht12 fixated the eye region 73.1% (±19.0) of the time in the case of face stimuli, and 53.7% (±17.4) of the time in the case of object stimuli. In addition, he spent 77.7% (±19.0) of his image viewing time, on average, on the eye region of face stimuli, compared to 57.2% (±24.8) for object stimuli. For face stimuli, both the percentage of fixations made in the eye region (One sample t-test; $t = 9.58$, $p < 0.001$) and the percentage of time spent viewing the eye region ($t = 10.31$, $p < 0.001$) were significantly greater than chance level. These same measures were also significantly different than chance in the case of object stimuli ($t = 5.31$, $p < 0.01$ for fixations made in eye region; $t = 4.05$, $p < 0.05$ for time spent viewing eye region).

The probability of the first fixation falling within the eye region was 55.6% for face stimuli and 20.0% for object stimuli. The probability of any fixation falling within the eye region was 67.4% for face stimuli, and 51.9% for object stimuli.

100ms versus 55ms Fixation Duration Threshold

Effects on data set. Of 407 total registered potential fixations (collapsed across subjects and stimulus category), 153 met the standard 100ms temporal fixation threshold and were included in subsequent analyses.

Almost 48% of those fixations that met the 100ms criterion registered in the eye region of the stimulus image, 11.8% in the nose region, 2% in the mouth region, 3.9% in the ear region, 27.5% outside of regions of interest, and 7.2% outside of the stimulus image.

When the duration threshold was lowered to 55ms in accordance with the “5% cutoff” method for temporally defining fixations, an additional 63 fixations were included in subsequent analyses. The overall distribution of fixations with respect to the stimulus images did not change appreciably when these shorter fixations were added back into the data set. Forty-four percent of them registered in the eye region, 10.6% in the mouth region, 2.3% in the mouth region, 3.7% in the ear region, 32.5% outside of ROIs, and 6.9% outside of the stimulus image.

Effects on descriptive statistics. The primary goal of the following comparison was to determine whether the use of different temporal fixation thresholds can have a significant impact on the results of eye tracking data analyses. As such, statistical analyses of differences between descriptive statistics for the 100 and 55 ms fixation duration thresholds were collapsed across Ht12 and Fq12, and across all 10 face stimuli. Graphical representation of all means and standard deviations mentioned below can be seen in Figures 3 – 6.

Repeated Measures ANOVAs were used to determine whether the distribution of fixations falling on and off ROIs, or the distribution of fixations falling within each ROI, differed significantly depending on the temporal fixation threshold being used. A Bonferroni correction was used to calculate the alpha level which takes into account multiple comparisons: 0.025.

For percentage of fixations made on versus off of ROIs, there was a significant main effect of ROI position ($F_{1, 36}$ = 67.376, $p > 0.001$). On average, a larger percentage of the total number of fixations that were made on the stimulus fell within ROIs (M = 73.29, SD = 21.70) than outside of ROIs (M = 23.90, SD = 18.69). There was no significant main effect of duration threshold ($F_{1, 36} = 0.642$, $p > 0.025$), and no significant interaction between duration threshold and ROI position ($F_{1, 36} = 2.261$, $p > 0.025$).

For percentage of fixations made on each ROI out of the total number of fixations made on the stimulus, the assumption of sphericity was violated and a Greenhouse-Geisser correction was used. There was a significant main effect of ROI ($F_{1.947, 70.078} = 81.311$, $p < 0.001$). Collapsed across threshold value, a larger percentage of fixations were made in the eye region of the stimulus face ($M = 55.186$, $SD = 22.447$) than on the nose ($M = 11.846$, $SD = 15.661$), mouth ($M = 2.896$, $SD = 8.367$), or ears ($M = 4.939$, $SD = 10.041$). There was no main effect of threshold value ($F_{1.947, 70.078} = 1.674$, $p > 0.025$), and no interaction between ROI and threshold ($F_{1.947, 10.078} = 0.233$, $p > 0.025$).

For the average proportion of time spent viewing ROIs versus areas of the stimulus image not included in any ROI (as a percentage of total trial time), there was a significant main effect of ROI position ($F_{1, 36} = 97.813$, $p < 0.001$). On average, regardless of duration threshold, a larger percentage of trial time was spent viewing ROIs ($M = 35.842$, $SD = 11.966$) than non-ROIs ($M = 9.421$, $SD = 8.829$). There was no significant main effect of threshold ($F_{1, 36} = 2.389$, $p > 0.025$), nor was there a significant interaction between ROI position and duration threshold ($F_{1, 36} = 0.052$, $p > 0.025$).

Finally, in terms of the proportion of time spent viewing each ROI out of total trial time, the assumption of sphericity was violated and a Greenhouse-Geisser correction was used. There was a significant main effect of ROI ($F_{1.665, 59.957} = 85.202$, $p < 0.001$). On average, collapsed across duration threshold, a larger proportion of time was spent viewing the eye region of a stimulus face ($M = 27.958$, $SD = 13.432$), than the nose ($M = 4.873$, $SD = 6.160$), mouth ($M = 0.895$, $SD = 2.730$), or ears ($M = 2.116$, $SD = 4.859$). There was no main effect of threshold ($F_{1,}$

$_{36} = 1.007$, $p > 0.025$), and no interaction between threshold and ROI ($F_{1.665,\ 59.957} = 0.203$, $p > 0.025$).

These results were not affected when object stimuli were included in analyses.

Discussion

Visual Scanning Behavior of Amygdalectomized Nonhuman Primates

Two rhesus monkeys with extensive, bilateral amygdala damage were found to devote more attention to the eyes than to the nose or mouth of unfamiliar monkey faces during a free-viewing task. This was evidenced by a larger proportion of fixations made, and a larger proportion of total trial time spent, in the eye region of the stimulus images compared to the nose and mouth regions. Additionally, the scanpaths produced by both subjects while viewing images of monkey faces did not reveal any conspicuous disruptions in the visual scanning behavior of either subject.

Visual scanning of face versus object stimuli. Though the data presented herein were not normalized for ROI size, the probability of any given fixation falling in the large eye ROI was greater for face stimuli than for object stimuli. In addition, Fq12 fixated and gazed at the eye region of face stimuli significantly more than would be expected by chance given the area of the stimulus images that was subsumed by the eye ROI. In the case of object stimuli, the proportion of fixations falling in this region, as well as time spent viewing this region, was close to chance level. Similarly, Ht12 fixated and gazed at the eye region of face stimuli significantly more than would be expected by chance. While he did fixate and gaze at this region more than would be expected by chance in object stimuli as well, he appeared to pay less attention to the eye region of object stimuli than he did to the eye region of face stimuli. He was also considerably more likely to make his first fixation in the eye region for face stimuli than for object stimuli. These data suggest that the disproportionate amount of visual attention directed at the eye region of the face stimuli in the current study is not a result of chance due to the size of the ROI, but rather, because this region contained a facial feature of interest and meaning to the subjects. (i.e., the eyes of another monkey).

A comparison of the general scanning patterns of both animals on the two categories of images further shows that face stimuli were scanned differently than object stimuli. Both subjects devoted more visual attention (i.e., fixations and viewing time) to the eye region than to the nose and mouth regions for both types of images. However, the ratio of attention directed at the eyes to attention directed at the nose and mouth was always higher for face stimuli, indicating that the distribution of both subjects' attention was more heavily skewed towards the eyes over the nose and mouth when they were viewing faces. Similarly, the ratio of visual attention directed at designated ROIs to attention directed at regions of the stimulus image not falling within an ROI was higher for face stimuli than for object stimuli in both subjects. This suggests that both animals distributed their attention to ROIs, such as the nose, mouth, eyes, and ears, more heavily when a face was presented than when an unfamiliar object was presented.

Individual differences in scanning patterns. Both animal subjects showed evidence of scanning faces differently than objects, and both dedicated a considerable amount of visual attention to the eye region of face stimuli, contrary to our predictions given their lesions. However, there were some notable differences in the visual scanning behavior of the two animals. Most obviously, Ht12 visually scanned objects and faces very similarly, while Fq12 exhibited a much more dramatic difference in his visual scanning of these two types of stimuli in the direction that would be expected (i.e., much less attention directed at the "eye region" of an object). Ht12 also tended to devote more visual attention to ROIs in general, and to the eye region specifically, regardless of stimulus category, compared to Fq12.

There are several possible explanations for these individual differences. It is, of course, plausible that the differences in face scanning behavior result from differences in the extent of the lesion in each animal. However, Ht12 sustained a larger amount of total amygdala tissue damage than Fq12; so, it is somewhat puzzling that he would direct more attention to the eye region of face stimuli than Fq12. Based on previous literature (Adolphs et al., 2005; Kennedy & Adolphs, 2010), one would predict that, because he sustained more total amygdala damage, Ht12 would show greater abnormalities in his face scanning behavior (i.e., less visual attention paid to the eyes).

It is possible that Ht12 may simply have an upper visual field bias of some sort, perhaps as a result of his specific pattern of brain damage, which may differ from Fq12's damage on a fine scale (i.e., Ht12 may have sustained damage to a particular subdivision of the amygdala that remains intact in Fq12). This might explain the high percentage of fixations he made on, and time he spent viewing, the eye region of both face *and* object stimuli. On the other hand, Ht12's propensity to view the eye region of stimulus images, specifically face images, may have been due to his high rank in his social group at the time of testing. The rank of a conspecific has previously been shown to influence the amount of reward associated with viewing and gathering information from the face of that conspecific on the part of the viewer (Deaner, Khera, & Platt, 2005). Because of their respective ranks, Ht12 and Fq12 may have perceived the rank or dominance of the monkeys featured in the current stimuli differently, which may have led one to look at the eyes of face images more or less than the other. Though this hypothesis does not offer a direct explanation for Ht12’s object scanning behavior, it may explain why he spent more time viewing the eyes of face stimuli than did Fq12.

Finally, variations in the serotonin transporter gene have been shown to generally influence anxiety levels in both humans and monkeys, and have specifically been shown to influence the amount of time rhesus monkeys spend viewing the eye region of conspecific faces (Watson, Ghodasra, & Platt, 2009). Again, this theory does not offer an explanation for why Ht12 devoted a considerable amount of visual attention to the “eye region” of object images. However, differences in serotonin transporter gene alleles may explain why Fq12 generally committed less visual attention to the eye region of faces than Ht12, despite the fact that Ht12 sustained more amygdala damage. The specific serotonin transporter gene alleles possessed by these subjects is not known at this time. Future studies should seek to test additional animals with similar damage under the procedure used currently so that it might be possible to correlate eye tracking measures with damage extent, location of damage, social rank, and serotonin transporter gene alleles.

Comparison with previous literature. Human patients with amygdala damage have been reported to show moderate to severe impairments in identifying fearful facial expressions (as well as sad, angry, and/or surprised facial expressions, although to a lesser extent; e.g., Broks, Young, Maratos, Coffey, Calder, Isaac, et al., 1998). This impairment was believed to be one component of a general fear-processing deficit. In 2005, a seminal paper was published in which a specific mechanism for impaired fear recognition was offered (Adolphs et al.). A patient with bilateral amygdala damage, S.M., participated in several eye tracking experiments, which demonstrated her inability to make use of information from the eye region of human faces in order to distinguish emotional expressions. This inability was linked to her lack of spontaneous fixation on the eye region of faces, regardless of facial expression. The scanpaths produced by S.M. while viewing stimulus faces during both free-viewing and emotion judgment tasks revealed marked irregularities in her visual scanning behavior. The scanpaths of healthy, adult humans are often characterized by an “inverted triangle pattern”, produced when fixations are concentrated on the internal features of the face (a majority of fixations fall in the eye region and the rest fall in the nose and mouth; see Adolphs et al., 2005, or Pelphrey et al., 2002, for examples). In contrast, S.M.’s scanpaths often began and remained entirely within the nose and mouth regions of a stimulus face, or were somewhat erratic (i.e., fixations falling on both internal and external facial features and lacking a clear pattern). Though some fixations were voluntarily made on the eye region of stimulus faces, her lack of attention to this region was readily evident in her scanpaths.

Interestingly, when specifically instructed to do so, S.M. was capable of fixating on the eye region of stimulus faces. During these trials, her ability to identify fearful facial expressions improved such that her scores were comparable to those of control participants. Based on this evidence, it was concluded that S.M., on account of her amygdala damage, lacks the ability to spontaneously fixate on the eye region of faces and make use of that information for the purposes of expression identification. Based on these findings, it was purported that the amygdala is responsible for modulating visual attention, directing the visual system to seek and fixate on salient social stimuli in the environment that might contain valuable social information.

In accordance with this theory of critical amygdala involvement in the visual scanning of social stimuli such as faces, one would predict abnormal visual scanning behavior from nonhuman primates with extensive, bilateral amygdala damage. Like S.M., these animals might devote some viewing time to the eye region of a stimulus face; however, their scanpaths would reveal a dramatic lack of visual attention paid to this salient, information-rich area. In contrast, the two amygdalectomized monkeys used as subjects in the current study showed no observable, gross irregularities in their scanpaths of unfamiliar monkey faces.

Because the current subjects were likely amygdalectomized before the amygdala was fully developed, it is possible that by three years of age (i.e., the time of testing), neural compensation significant enough to mask gross scanpath abnormalities had already occurred. The amygdala is thought to mature early in life compared to, for example, visual cortical areas (Johnson, 2005). Neurogenesis in this area is completed before birth (post-gestational day 50 in rhesus monkeys according to Kardower, Piecinski, & Rakic, 1992); and, though little data are available on the exact age at which the amygdala is fully developed, it is often estimated at approximately three months in monkeys (Sabatini, Ebret, Lewis, Levitt, Cameron, & Mirnics, 2007). Amygdalectomies were performed on the current subjects at approximately one month of age. In contrast, S.M. sustained amygdala damage in late childhood as a side effect of Urbach-Wiethe disease. This important difference may explain the discrepancy in the results of the current and previous studies. Future studies should seek to test the effects of amygdala damage sustained during adulthood on performance in the visual scanning task used currently. If the same kinds of marked abnormalities that are seen in S.M.'s scanpaths are also observed in the scanpaths of these subjects, it would suggest that only amygdala damage sustained later in life leads to gross disturbances in visual scanning behavior, at least when stimuli are socially relevant.

Though neural compensation after early amygdala damage may conceivably explain the current findings, there does exist another possible explanation: that the amygdala does not play as large of a role in the production of visual scanning patterns of social stimuli as was originally proposed. Data from patients with amygdala damage, individuals with autism, and neuroanatomical research on amygdala structure and function, together, have led researchers to posit that the amygdala forms part of a neural complex involved in the socio-emotional development of a social primate (Brothers, 1990; Emery, 2000), along with the orbital frontal cortex, cingulate cortex, and the superior temporal cortex which also contains face and eye gaze-sensitive cells (Perrett, Smith, Potter, Mistlin, Head, Milner, et al., 1985). However, there is yet little empirical evidence demonstrating a critical role for the amygdala in the processing of visual social signals, aside from case studies featuring a single patient (Adolphs et al., 2005; Kennedy & Adolphs, 2010; Spezio et al., 2007). It is more likely that the amygdala functions as part of a "social brain network" or "eye direction detector network" that is responsible for the extraction and processing of social information, including visual scanning of socially-significant environmental stimuli. In other words, it is unlikely that a single brain structure, even one as complex as the amygdala, "micromanages" saccades in the way that Adolphs and colleagues' theory (2005) seems to imply.

This is not to say that the amygdala plays no role in visual scanning of social stimuli. It is quite possible that there are subtle abnormalities in the visual scanning behaviors of these neonatally amygdalectomized subjects which would not be readily apparent in the scanpaths they produce while viewing a face. For example, they may indeed make less use of the eye region of a stimulus face (as evidenced by less time spent viewing and fewer fixations made on the eye region) compared to control subjects. The group differences may simply not be as dramatic as those seen previously (Adolphs et al., 2005). Also, they make more corrective saccades than neurologically intact animals. In fact, the current subjects showed a very large number of very short registered fixations (much more than other sham-lesion rhesus monkeys, albeit of a different age, having more experience with these types of experimental tasks, and raised under different conditions; C. Cushman, unpublished data). While some of these registered fixations are likely noise, it is possible that others represent very short fixations preceding corrective saccades. In order to determine whether this is the case, the scanpaths should be measured before and after these very short registered fixations are included. If the scanpath length does not increase appreciably with the addition of the short registered fixations, then many of them are likely fixations preceding corrective saccades.

Temporal Fixation Definition: Standard versus Study-Specific Thresholds

No significant differences were found between typical eye tracking study measures when a standard temporal fixation minimum (100ms) and a lower, study-specific minimum (55ms) were used. The average percentage of fixations made on and off of ROIs, and the average percentage of total trial time spent viewing within and outside of ROIs, did not differ significantly as a function of the minimum fixation duration criterion that was applied to the raw eye movement data. Likewise, the average percentage of fixations made on the eye, nose, mouth and ear regions of a stimulus face, and the average percentage of total trial time spent viewing these ROIs, did not differ significantly as a function of the fixation duration threshold.

Following these findings, one might conclude that, at least in the context of an eye tracking experiment investigating visual scanning of faces and using rhesus monkeys as subjects, it does not matter whether the standard 100ms threshold or a study-specific threshold is used; averages for all dependent measures of interest will be, essentially, the same. It is possible that with a larger number of stimuli, which would render a larger total number of fixations made across both animals, some mean differences may have reached significance; main effects of threshold criterion might have been easier to detect as well. However, in most cases the mean differences were so close, and the *p* values so high, that it is unlikely that additional data would have changed the results.

If lowering the threshold in a systematic way (i.e., using the 5% cutoff method) generally does not significantly affect the outcome of eye tracking data analysis compared to the standard threshold, then it is worthwhile to consider using a lower, study-specific threshold in certain cases for the following reason. Incorporating shorter fixations into data analyses may have the benefit of contributing power to a sparse data set. A particular subject or group of subjects may not make many fixations, or the duration of stimulus presentation may be so short that subjects simply do not have time to make many fixations. In these situations, the application of a standard fixation threshold (e.g., 100ms) may result in the exclusion of some visuocognitively relevant fixations and leave the researcher with very few fixations to analyze. Indeed, this was a concern in the current study. With a relatively small number of stimuli as well as subjects, there was a small number of fixations to include in data analyses to begin with. Because a considerable number of registered fixations were very short, the application of the standard 100ms duration threshold resulted in the elimination of a large percentage of registered fixations (62.4%). The notion of lowering the threshold a bit in order to regain some potentially valuable data was additionally justified by the fact that, though a 100ms duration threshold has been argued to be appropriate for experimental tasks involving visual scanning of faces or objects that use humans as participants (Manor & Gordon, 2003), monkeys typically make shorter fixations and longer saccades than do humans (Berg et al., 2009; Shepherd et al., 2010).

Furthermore, lower fixation duration minimums may be valuable when working with special populations – particularly when comparing data obtained from special populations to data obtained from healthy ones. The incorporation of shorter fixations in data analyses, and the associated increase in statistical power, may allow for the detection of between-group differences that would not reach significance if longer fixations, exclusively, were considered in analyses (Manor & Gordon, unpublished data, cited in Manor & Gordon, 2003). Additionally, the inclusion of short fixations may be valuable when working with individuals with neurological deficits or brain damage, as those shorter fixations may give an indication of general, subtle eye movement abnormalities (e.g., corrective saccading; see Manor & Gordon, 2003).

By using the 5% cutoff method to lower the fixation duration threshold to 55ms in the current study, it was possible to add a number of fixations back into the data set. These fixations contributed to the averages calculated on typical eye tracking study measures, such as the number of fixations falling within particular ROIs or the amount of time spent viewing ROIs, without significantly affecting those averages (compared to the averages obtained under the 100ms duration threshold criterion). Furthermore, data obtained from the current subjects to which the lower, study-specific threshold was applied may, in the future, be compared to data from rhesus monkeys with sham-lesions tested in the same experiment under the same procedure. The inclusion of the shorter fixations may allow for the detection of between-group differences that would otherwise be lost, and may also contribute to the power of the analyses.

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Table 1

Summary of Amygdala Damage

	Damage to L Amyg.	Damage to R Amyg.	Average Damage Across Hemispheres
Fq12	**84.9%**	**50.5%**	**67.7%**
Ht12	**92.3%**	**77.3%**	**84.8%**

Note. Percentages represent the proportion of the total estimated volume of the structure that was damaged by ibotenic lesion (J. Raper & J. Bachevalier, unpublished data). Ht12 sustained additional damage (4.4% to right hippocampus, 0.3% to left entorhinal cortex, and 0.7% to left perirhinal cortex). Fq12 sustained no additional brain damage.

Table 2

Proportion of Fixations Falling Within Specific Regions of Interest

	Region					
	% on Eyes	*% on Nose*	*% on Mouth*	*% on Ears*	*% on ROI*	*% off ROI*
Averted Gaze Face						
Fq 12	48.09*(8.81)*	13.33*(0.89)*	0.00*(0.00)*	6.66*(14.91)*	68.09*(22.50)*	28.57*(20.27)*
Ht 12	64.50*(15.04)*	6.50*(9.29)*	4.00*(8.94)*	10.00*(14.91)*	85.00*(14.91)*	15.00*(14.91)*
Direct Gaze Face						
Fq12	41.05*(21.36)*	19.20*(23.41)*	6.66*(14.91)*	3.33*(7.46)*	70.25*(21.53)*	29.75*(21.53)*
Ht12	83.75*(19.73)*	11.25*(13.15)*	0.00*(0.00)*	0.00*(0.00)*	95.00*(10.00)*	5.00*(10.00)*
Object						
Fq12	14.00*(21.90)*	6.60*(14.91)*	0.00*(0.00)*	0.00*(0.00)*	20.66*(21.65)*	79.33*(21.65)*
Ht12	53.67*(17.41)*	17.30*(20.47)*	0.00*(0.00)*	5.00*(11.18)*	76.00*(18.77)*	24.00*(18.77)*
All Faces						
Fq12	44.57*(15.85)*	16.26*(20.03*	3.33*(10.54)*	5.00*(11.25)*	69.17*(20.79)*	29.16*(19.73)*
Ht12	73.06*(19.03)*	8.61*(10.69)*	2.22*(6.67)*	5.56*(11.78)*	89.44*(13.28)*	10.56*(13.28)*

Note. Means are given as percentages. Standard deviations are included in parentheses.

Table 3

Proportion of Time Spent Viewing Specific Regions of Interest

	Region					
	% on Eyes	*% on Nose*	*% on Mouth*	*% on Ears*	*% on ROI*	*% off ROI*
Averted Gaze Face						
Fq12	22.08*(10.57)*	3.76*(6.41)*	0.00*(0.00)*	2.24*(5.01)*	28.08*(10.51)*	9.44*(10.54)*
Ht12	26.64*(7.00)*	3.20*(4.60)*	0.80*(1.79)*	4.72*(8.14)*	35.36*(5.74)*	4.64*(4.82)*
Direct Gaze Face						
Fq12	24.88*(17.21)*	7.20*(8.09)*	2.00*(4.47)*	0.80*(1.79)*	34.88*(13.73)*	15.60*(11.00)*
Ht12	34.1*(17.14)*	4.00*(5.66)*	0.00*(0.00)*	0.00*(0.00)*	38.10*(18.37)*	1.30*(2.60)*
Object						
Fq12	4.24*(6.95)*	1.6*(3.57)*	0.00*(0.00)*	0.00 *(0.00)*	5.84*(6.64)*	16.00*(4.54)*
Ht12	28.32*(16.64)*	6.72*(7.71)*	0.00*(0.00)*	1.20*(2.68)*	36.24*(11.80)*	9.44*(5.47)*
All Faces						
Fq12	23.48*(13.54)*	5.48*(7.12)*	1.00*(3.16)*	1.52*(3.63)*	31.48*(12.07)*	26.88*(20.53)*
Ht12	29.95*(12.25)*	3.56*(4.77)*	0.44*(1.33)*	2.62*(6.27)*	36.58*(12.05)*	3.16*(4.15)*

Note. Means are given as percentages of total trial time (2.5s). Standard deviations are included in parentheses.

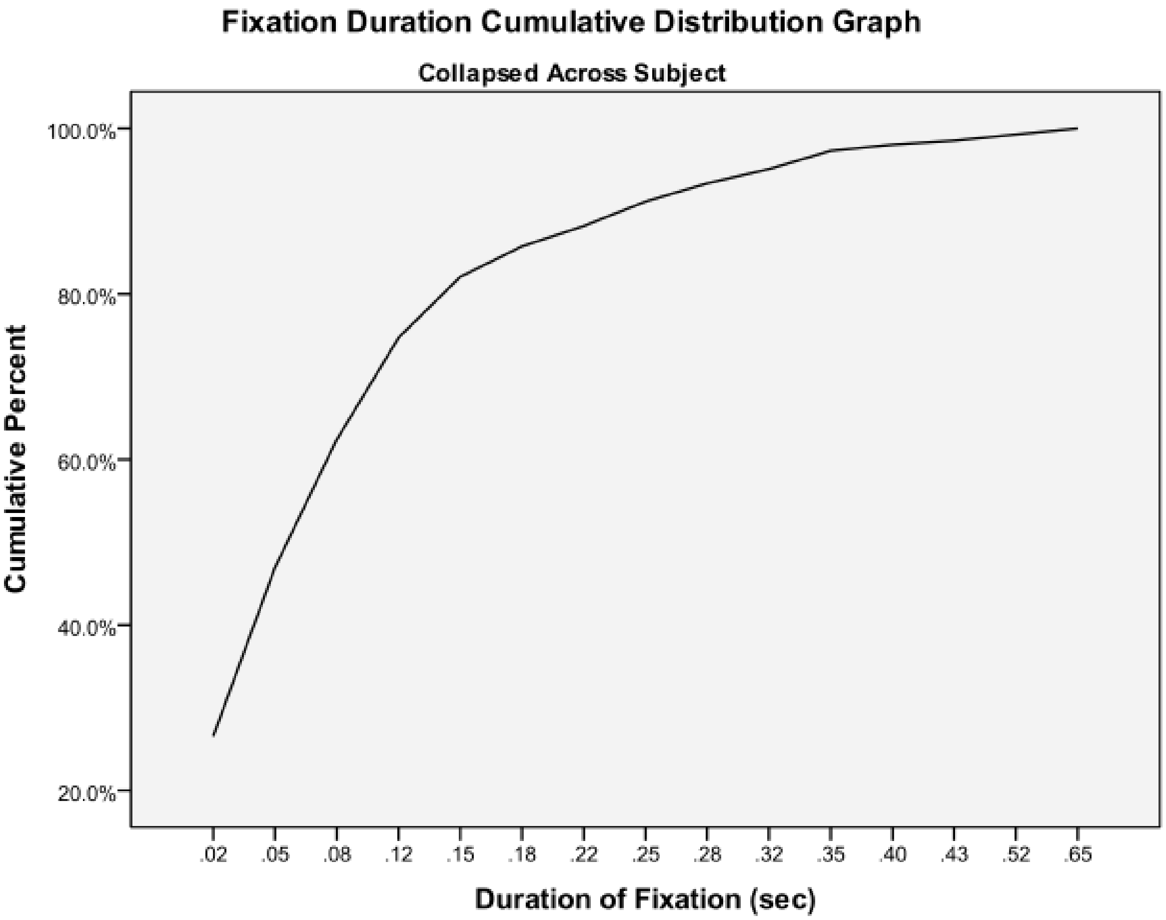
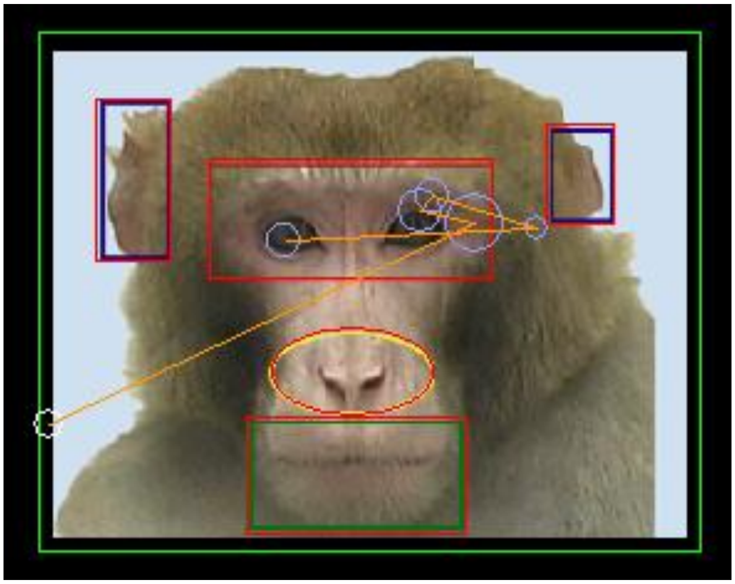
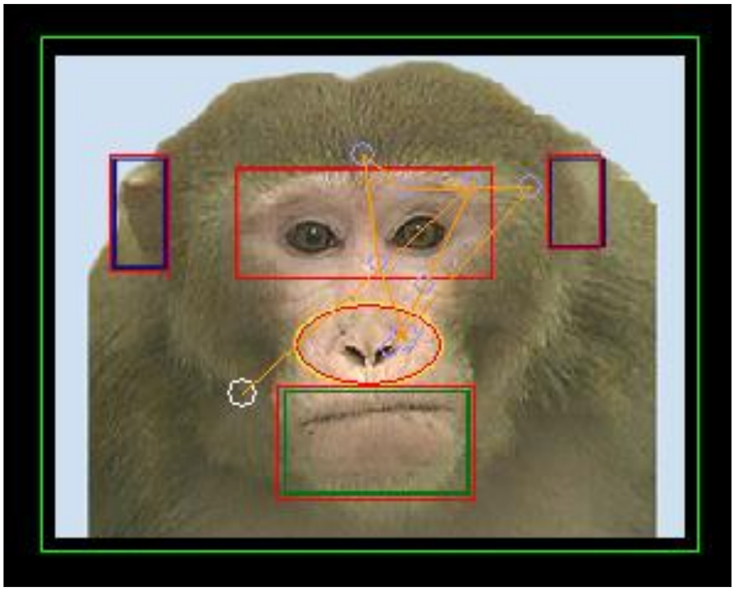


Figure 1. Cumulative frequency distribution of fixation durations which includes all fixations made by Ht12 and Fq12 while viewing each of the 15 stimulus images (neutral, averted gaze, unfamiliar monkey faces; neutral, direct gaze, unfamiliar monkey faces; and unfamiliar objects).

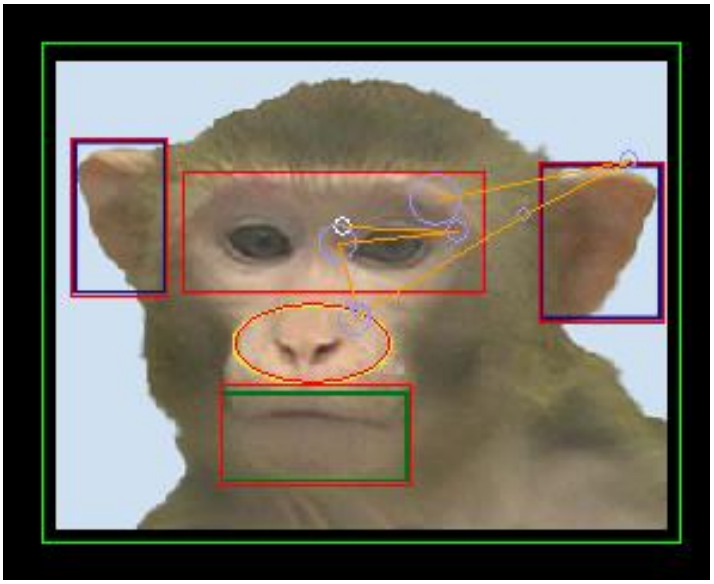
a



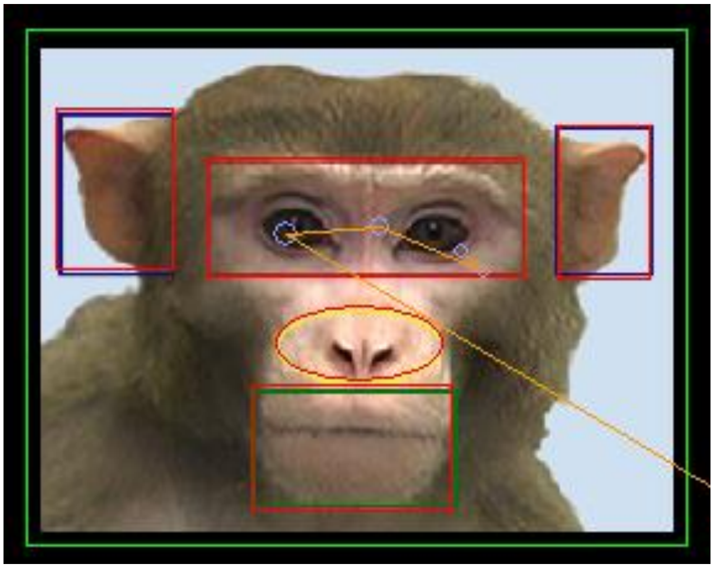
b



c



d



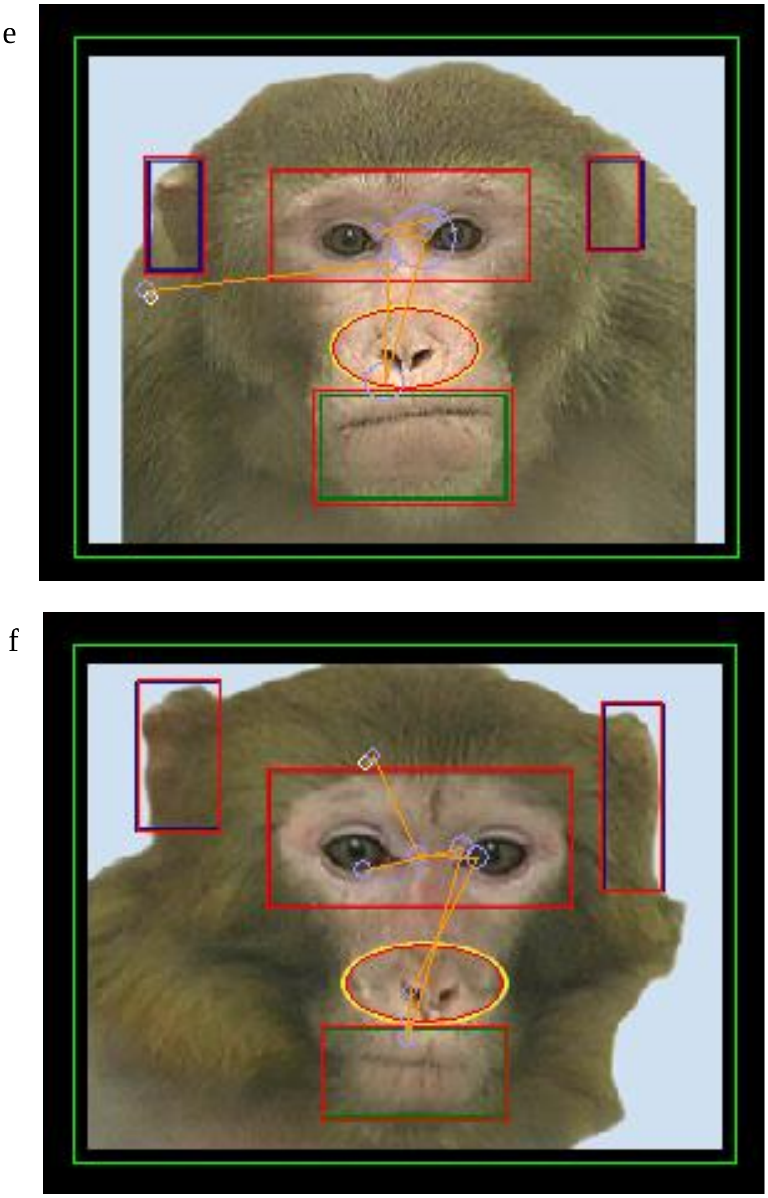


Figure 2. Examples of scanpaths produced by subjects during free face viewing. Total trial time for each stimulus image was 2.5s. Saccades are indicated by orange lines; fixations are indicated by purple circles (where circle size corresponds to fixation duration). **a-c**, Scanpaths from Fq12. **d-f**, Scanpaths from Ht12. **a, b, d, e**, direct gaze face stimuli; **c, f**, averted gaze stimuli. Gross irregularities (e.g., all fixations concentrated in nose region of the stimulus face) were not seen in two macaques with bilateral amygdala lesions.

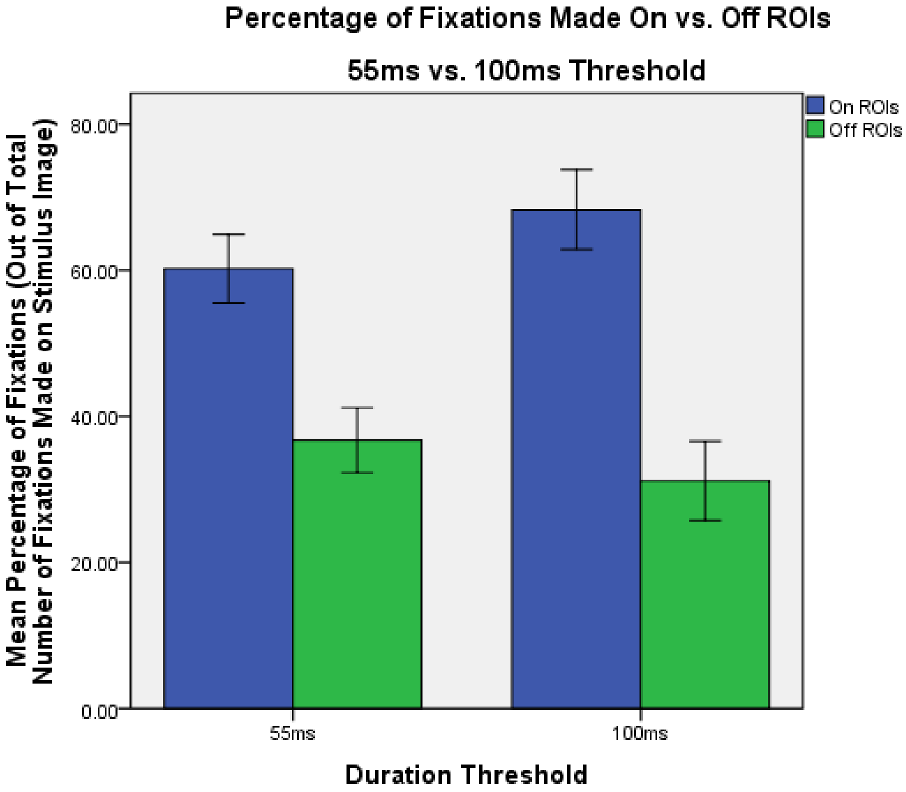


Figure 3. Average percentage of fixations falling on versus off of ROIs out of the total number of fixations made on the stimulus image. Averages for both the 55ms and 100ms duration thresholds are shown. The distribution of fixations falling within versus outside of ROIs did not differ significantly as a function of duration threshold.

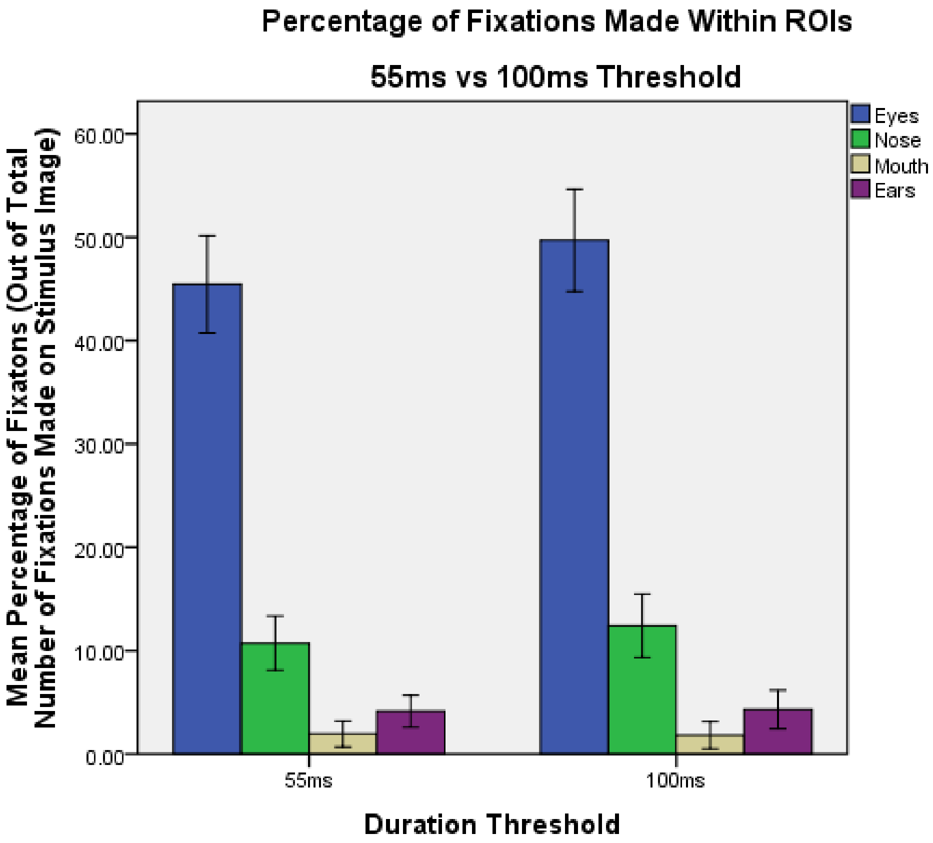


Figure 4. Average percentage of fixations made on the eye, nose, mouth and ear regions of stimulus images. Averages for both the 55ms and 100ms duration thresholds are shown. The distribution of fixations made on ROIs did not differ significantly as a function of duration threshold.

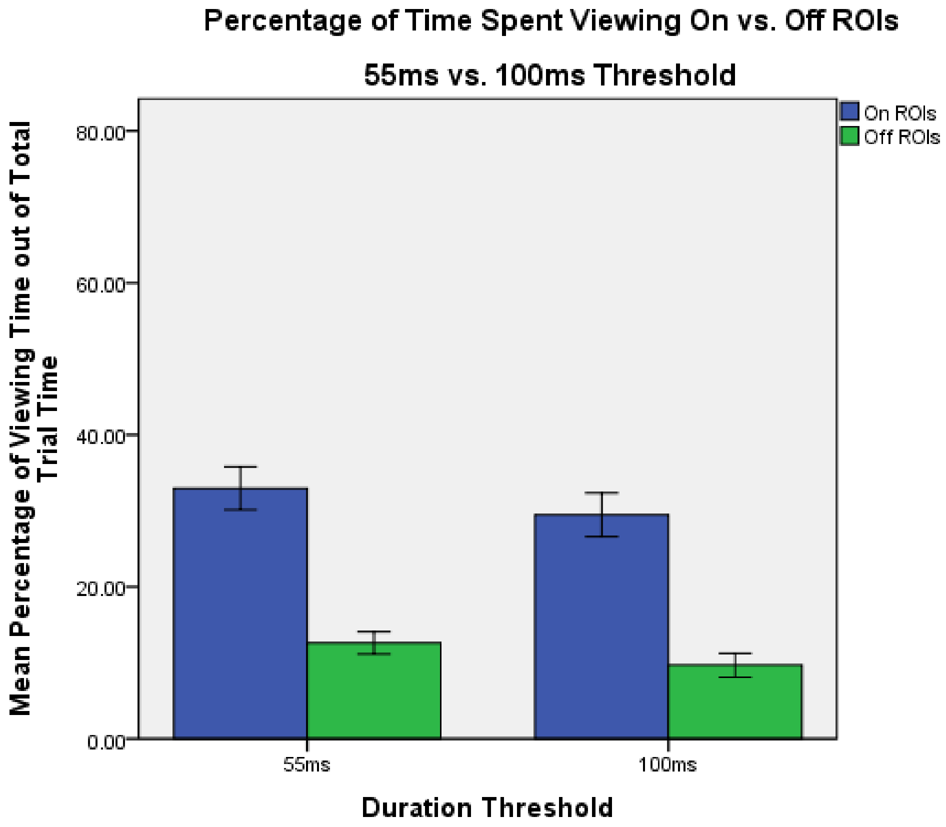


Figure 5. Average percentage of time spent viewing on versus off of ROIs out of the total trial time (2.5s). Averages for both the 55ms and 100ms duration thresholds are shown. The distribution of time spent viewing ROIs versus other areas of the stimulus image did not differ significantly as a function of duration threshold.

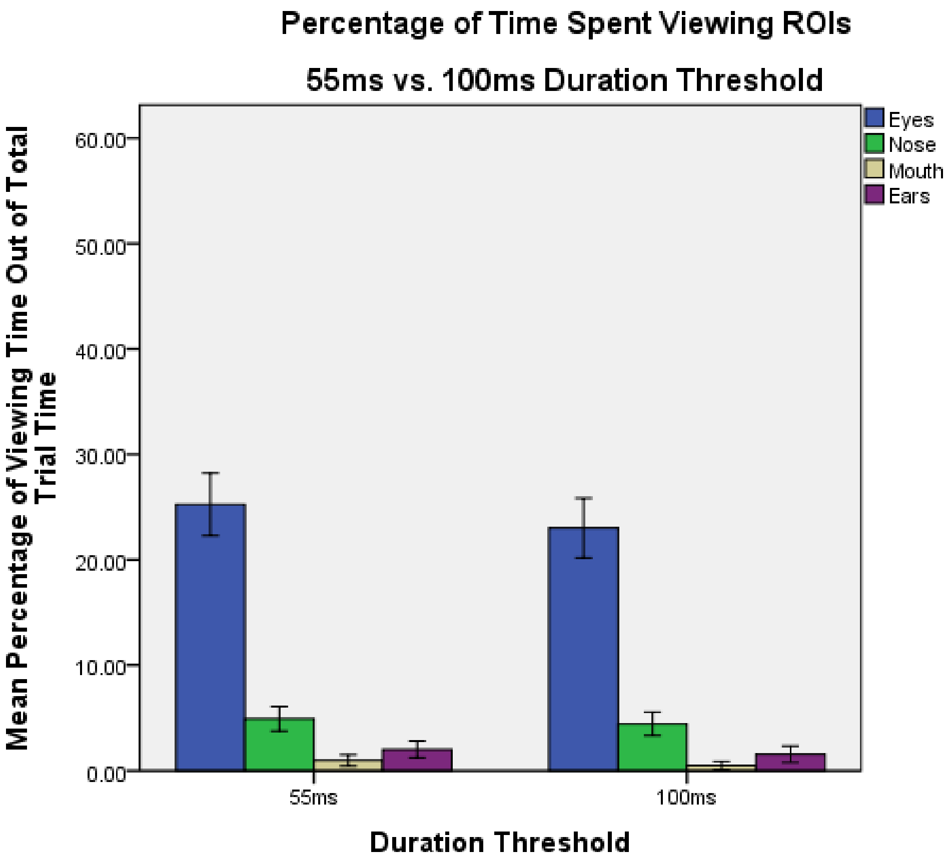


Figure 6. Average percentage of time spent viewing the eye, nose, mouth and ear regions of stimulus images out of total trial time (2.5s). Averages for both the 55ms and 100ms duration thresholds are shown. The distribution of time spent viewing ROIs did not differ significantly as a function of duration threshold.