MODULATION OF THE INITIAL LIGHT REFLEX DURING AFFECTIVE PICTURE VIEWING

By

ROBERT R. HENDERSON

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To my loving and supportive parents

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Robert R. Henderson Jr.

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Chair: Peter J. Lang
Co-Chair: Margaret M. Bradley
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When pictures of natural scenes are viewed, they first prompt a brief, reflexive, parasympathetic constriction of the pupil, varying in amplitude with the brightness of the image. When the scene is emotionally arousing, reflex constriction is followed by enhanced, sympathetically mediated pupil dilation. The present research was designed to determine whether the initial light reflex itself might also be modulated by emotion. Erotic, violent, and neutral scenes were presented in both natural and scrambled versions, which were identical in brightness. Significantly less initial constriction was found when participants viewed emotional, relative to neutral, natural scenes, with no differences in pupil size as a function or original content when viewing the scrambled versions. Thus, viewing emotionally evocative images modulates both early and late changes in pupil diameter that are not due to differences in brightness, suggesting early central inhibition of the parasympathetically-mediated light reflex.

CHAPTER 1
INTRODUCTION

The Initial Light Reflex

Pupil diameter during visual perception is modulated by several factors, the most important of which is the overall brightness of the visual field. In dark light, pupil diameter increases in order to increase the size of the visual field and lower the threshold for light, and in bright light the pupil constricts in order to decrease glare and improve depth of focus and visual acuity. Constriction of the pupil when viewing bright stimuli serves to protect photoreceptors and prevent damage to the eye (Loewenfeld, 1993).

The pupillary light reflex is an early-occurring constriction that occurs immediately in response to changes in luminance. It is typically elicited by light shining into the right or left pupil; in healthy individuals directing light onto one eye causes similar amplitude constriction in both the stimulated and unstimulated eye (Beatty, 1986). The onset of the light reflex depends on brightness intensity and physical characteristics of the pupillary musculature. With increases in brightness, the rate and amplitude of initial pupillary constriction, as well as rate of subsequent pupillary dilation back to resting pupil diameter, are enhanced (Ellis, 1981). The light reflex does not occur simultaneously with increases in brightness of the visual field, but instead has a built-in delay period due to mechanical limitations of the pupillae sphincter muscle. In humans the light reflex has a minimum built-in delay of 200ms and can be additionally delayed by about 250ms depending on the intensity of the light source (Loewenfeld, 1993).

Neural Mechanisms of Pupillary Control

Pupil diameter is determined by an antagonistic set of muscle groups, the pupillae dilator and pupillae sphincter muscles, which together regulate the amount of light entering the pupil (Beatty & Lucero-Wagner, 2000). Figure 1-1 depicts the general mechanisms controlling the pupillae dilator and pupillae sphincter muscle groups. Contraction of the sphincter muscles causes constriction, whereas contraction of the dilator muscles elicits dilation. These muscle groups work together in accordance with Sherrington's Law, such that contraction of the agonist muscle group is accompanied by relaxation of the antagonistic muscle group, allowing for smoother muscle movements and faster reflex responses (Loewenfeld, 1993).

Activation of the pupillae dilator and sphincter muscles is determined by the relative contributions of the sympathetic and parasympathetic branches of the autonomic nervous system. The parasympathetic branch controls pupillary constriction through its influence on the pupillae sphincter muscles. Pupillary constriction is elicited via projections from the Edinger-Westphal complex of the oculomotor nucleus (the Edinger-Westphal Nucleus) in the midbrain to the pupillae sphincter muscles (Steinhauer, Siegle, Condray, & Pless, 2004), causing them to contract and resulting in a rapid reduction in pupil diameter. This constriction occurs primarily in response to increases in brightness of the visual field. On the other hand, the sympathetic branch of the autonomic nervous system controls pupillary dilation via direct stimulation of pupillae dilator muscles, which is mediated by posterior hypothalamic nuclei (Steinhauer, et al., 2004). Because of their radial orientation (Beatty & Lucero-Wagner, 2000), contraction of the pupillae dilator muscles increases overall pupil diameter, allowing additional light to enter the iris.

Because of the reciprocal nature of the activity of the pupillae dilator and sphincter muscle, contraction of either muscle group is paired with central inhibition of outputs to the opposing muscle group. Thus, the extent of initial pupillary constriction to visual stimuli (i.e. light reflex amplitude) is determined by the degree of parasympathetic activation of the Edinger-Westphal Nucleus and its effects on the sphincter pupillae muscles, as well as to central inhibition of motor outputs to the dilator muscle. Pupil dilation, on the other hand, can be elicited by a combination of central sympathetic inhibition of the Edinger-Wesphal nucleus and direct sympathetic stimulation of the pupillae dilator muscles (Loewenfeld, 1993). Thus, pupil size at any given moment is dependent upon the relative central and peripheral contributions of the sympathetic and parasympathetic branches of the autonomic nervous system.

Factors Affecting Light Reflex Amplitude

The amplitude of the light reflex response may be affected by a variety of physical characteristics, but is most strongly modulated by the intensity of light shining onto the eye. Although brightness is a major contributory factor in determining light reflex amplitude, a number of other features have been proposed to affect the light reflex and overall pupil size. In a large-scale review of her own and other studies, Loewenfeld (1993) found that light reflex amplitude is likely influenced by color and spatial dispersion of the light source, retinal distribution, adaptation of the retina, and speed of onset and duration of the light source. Recent studies are consistent with the notion that many physical features of visual stimuli can impact pupil diameter. In addition to brightness, recent studies have shown that latency and amplitude of pupillary reactions are modulated by spatial frequency (Link, et al., 2006), luminance-contrast, color-contrast (Carle, James, & Maddess, 2013; Rockefeller, & Kennish, 1993), shifting gaze from far to near visual stimuli (Kasthurirangan & Glasser, 2005), and different wavelengths of light (Kimura, & Rockefeller, 1996).

Although the light reflex is typically elicited by physical characteristics of stimuli, when these features are controlled non-physical factors can also impact its modulation. For example, light reflex amplitude is attenuated when subjects anticipate receiving an electric shock, and the size of light reflex attenuation is associated with evaluative ratings of anxiety (Bitsos, Szabadi, & Bradshaw, 1996). This effect is not solely due to anticipating the upcoming stimulus since a low-amplitude burst of noise is insufficient to reduce light reflex amplitude (Bitsios, Szabadi, & Bradshaw, 2004).

Anticipating aversive stimuli is believed to reduce the light reflex response through central inhibition of parasympathetic outputs to the pupillae sphincter muscles rather than sympathetic stimulation of the dilator muscles (Hourdaki, Giakoumaki, Grinakis, Theou, Karataraki, & Bitsos, 2005). Light reflex amplitude is also reduced when subjects engage in tasks that are cognitively demanding (subtract 7), compared to no-task conditions, with light reflex attenuation again reflecting central inhibition of the Edinger-Westphal nucleus (Steinhauer, Condray, & Kasparek, 2000) rather than direct sympathetic stimulation. Overall, attenuation of the light reflex during aversive anticipation and when completing difficult cognitive tasks is likely due to central inhibition of parasympathetic output to the pupillae sphincter muscles, consistent with pupillary mechanisms modulating the amplitude of the light reflex to physical features of visual stimuli.

Pupillary Changes and Emotion

Research on effects of psychological processes on pupil size came into prominence in the United States during the 1960s, following publication of a series of research studies conducted by Eckhard Hess and James Polt, in which the pupil strongly dilated when individuals viewed pleasant pictures and strongly constricted when viewing unpleasant pictures (Hess & Polt, 1960). Although very popular, these studies were afflicted with numerous methodological flaws, including small sample sizes (e.g. n=5), ambiguous stimuli, small picture sets (e.g. n=5), no mention of control for physical characteristics of the stimuli, and no formal statistical analyses.

Other researchers reported contradicting results in their own investigations of pupillary changes during affective picture viewing. For example, Nunally, Knott, Duchnowski, and Parker (1967) recorded pupillary changes while 30 male college students viewed pictures of a semi-nude female model, as well as male and female models (pleasant), neutral people, and individuals with cancerous growths (unpleasant). Although this study also utilized a small number of images (4 semi-nude, 3 pleasant, 3 neutral, 3 unpleasant), the authors attempted to minimize the likelihood of pupil dilation to physical characteristics by preceding each image with a control image that was adjusted to be darker than the upcoming pictures. In the first part of the experiment, statistical analyses revealed that pupil diameter increased when students viewed the semi-nude female model relative to viewing the same model fully clothed. In the second part of the experiment, pupil dilation occurred when participants viewed pleasant, relative to neutral and unpleasant, pictures, which did not differ from one another. In a more recent study, Aboyoun and Dabbs (1998) found that pupil diameter increased when participants viewed images of unclothed, relative to clothed, individuals. Unpleasant pictures were not presented in this research.

Early investigations of pupillary reactions were limited by the time resolution of pupil recordings, as pupil size was typically measured by using a camera and developing images were hand scored. Head movements were typically limited by locking the participant into a chinrest. Recent technological improvements have allowed for increased temporal resolution and accuracy when recording pupillary changes. Modern eye-tracking software is able to record small changes in pupil size under a variety of ambient conditions by reflecting an infrared light source off of the participant's eye while using facial recognition to track small movements and adjusting the camera to keep the pupil in focus. This allows for a more comfortable and natural experience for the participant than the earlier methodology. More importantly, these advances allow for more frequent monitoring of changes in pupil diameter, with temporal resolutions of 60 to 120 Hz, and much more precise measurement (e.g. +/- 0.5 degree of visual angle).

Using more modern technology, Bradley, Miccoli, Escrig, & Lang, (2008) explored changes in pupil diameter during emotional picture viewing. Unlike the earlier studies, they found that, subsequent to the initial light reflex, pupil diameter was larger when participants viewed either unpleasant or pleasant pictures. That is, pupil diameter was related to emotional arousal rather than the specific hedonic content: Pupil diameter was largest relative to neutral when participants viewed either content, pleasant or unpleasant. In addition, the amplitude of pupil change covaried with the amplitude of skin conductance activity during picture viewing, consistent with an interpretation that these changes in pupil dilation may be sympathetically mediated. Follow-up analyses of these data suggested that highly arousing pictures might also modulate amplitude of the initial light reflex. This conclusion was not clear, however, because of associated variations in stimulus brightness -- prompting the current study.

Research Aims

Although multiple investigations have since replicated the Bradley et al. (2008) findings (e.g. Dietz, Bradley, Okun, & Bowers, 2011; Hermans, Henckens, Roelofs, & Fernandez, 2013), there are as yet no findings confirming that emotional arousal also modulates the amplitude of the initial light reflex during affective picture viewing. In the current study, this issue was examined more closely by presenting highly arousing (erotic and violent) and neutral pictures that were exactly matched in brightness to each other. Moreover, as an additional control for assessing effects of brightness on the initial light reflex, each picture was presented in both an intact and scrambled version. In the scrambled version, pixels were randomly shuffled such that brightness was identical to the intact version but no content (semantic or emotional) remained. If emotional arousal modulates the initial light reflex, we expected that viewing emotional scenes would elicit an attenuated light reflex compared to viewing neutral scenes, but that the light reflex for scrambled images of identical brightness would not vary as a function of original picture content. We also expected to replicate results from previous studies (e.g. Bradley, et al., 2008) showing increased pupillary dilation following the light reflex when participants view emotional, relative to neutral, natural scenes.

To further assess the relationship between brightness, emotional arousal, and pupillary reactions, an additional analysis using each intact picture as the unit of analysis was conducted. We predicted that brightness would account for a substantial amount of variance in light reflex amplitude, replicating previous studies (Bradley, et al., 2008). If the light reflex is modulated when viewing high arousal scenes, we expected that, after removing effects of brightness, ratings of emotional arousal would covary with residual light reflex amplitude. On the other hand, if emotional modulation of pupil diameter does not begin until later in the picture viewing interval, we expected that ratings of emotional arousal would not account for additional variance in the residual light reflex. In summary, this investigation sought to first replicate and then significantly extend prior findings, clarifying the time course of emotional modulation of pupillary changes during affective picture viewing.

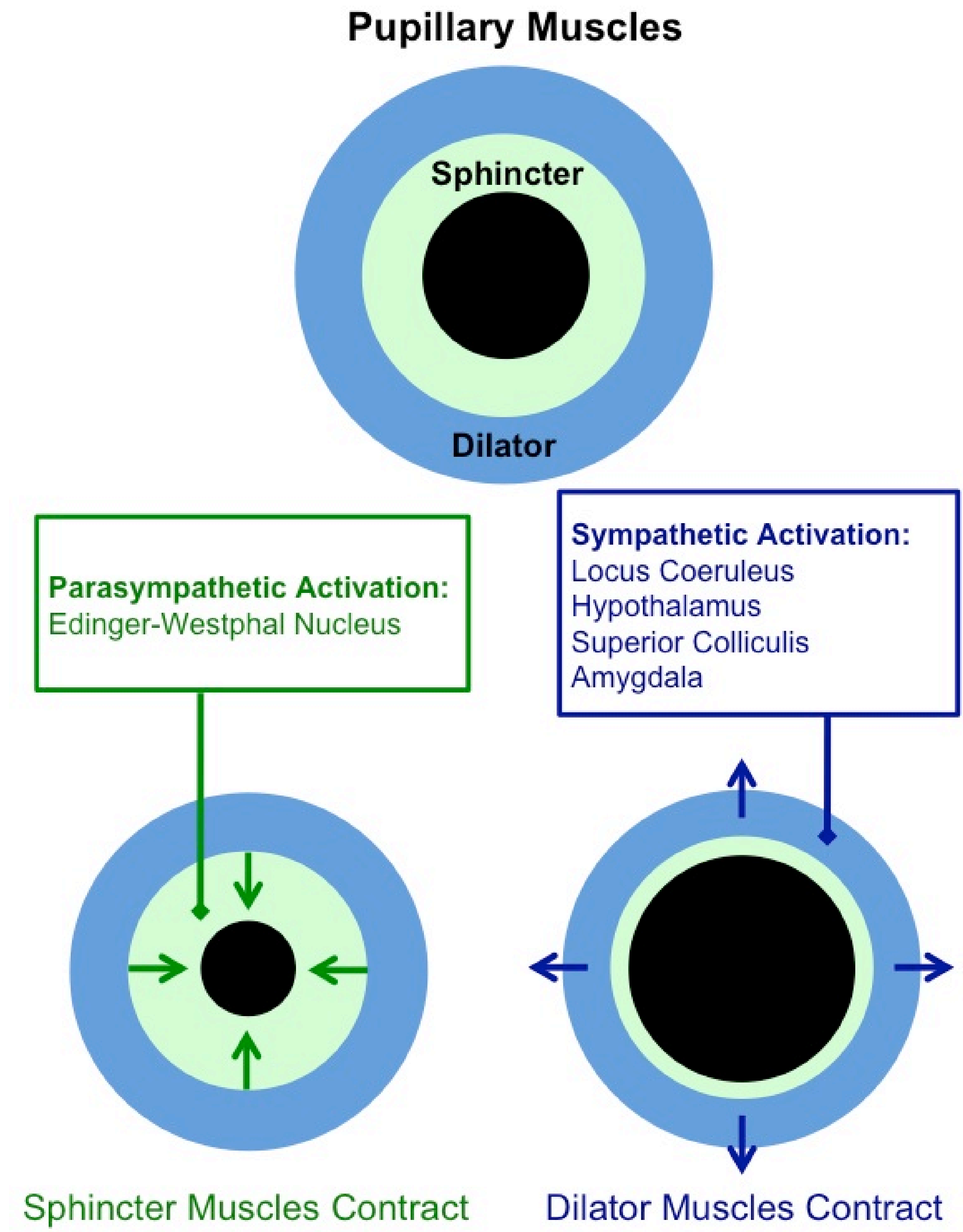

Figure 1-1. Mechanisms of pupillary control. Parasympathetic projections to the sphincter muscles elicit constriction, whereas sympathetic stimulation of the dilator muscles increases pupil diameter.

CHAPTER 2
METHOD

Participants

Twenty-seven 18-21-year-old (13 male, 82% Caucasian) University of Florida undergraduate students enrolled in General Psychology courses at the University of Florida participated in this study for course credit. Participants were recruited through the university's online research participation scheduling system. The study was approved by the University of Florida Institutional Review Board, and all participants signed an informed consent form prior to participation in the study. Due to limitations of the eye-tracking system, students were ineligible to participate if they required glasses to see the pictures on the screen, although contacts were allowed.

Design and Materials

Stimuli were 36 pictures selected from the International Affective Picture System (IAPS: Lang, Bradley, & Cuthbert, 2008), consisting of 12 erotic (mean pleasure/arousal= 6.6, 6.4), 12 neutral (mean pleasure/arousal=5.2, 3.6), and 12 violent scenes (mean pleasure/arousal= 1.9, 6.4). Table 2-1 shows means and standard deviations of physical features and standardized ratings of emotional arousal, valence, and figure-ground complexity, for the original erotic, violent and neutral pictures. Arousal ratings of erotic and violent pictures were identical, and significantly different from arousal ratings of neutral pictures. Pleasure ratings necessarily differed between each hedonic content, with erotic pictures rated significantly more pleasant than neutral or violent pictures, and violent images rated significantly less pleasant than neutral pictures. All pictures portrayed people, were landscape in orientation and displayed in

256-bit grayscale. Pictures were selected to represent a range of brightness, contrast, and ratings of figure-ground complexity.

Pictures were arranged into twelve sets of three pictures (i.e. triplets of erotic, violent, and neutral images); each set varied in brightness from the other sets. Thus, pictures were selected to include one picture of each hedonic content at twelve different brightness levels. At each brightness level, the triplet included one erotic, one neutral, and one violent picture, all of which were initially very similar in brightness. Pictures within a triplet were then adjusted to be identical in brightness using Adobe Photoshop (version 7.01; Adobe Systems Inc., San Jose, CA). A scrambled version of each picture was then created which randomly rearranged pixels to produce a version that was identical in brightness to the original intact picture. Figure 2-1 shows an example of low and high brightness original triplets and their scrambled versions. The final set of stimuli consisted of 36 intact and 36 scrambled pictures, for a total of 72 pictures.

Each picture was displayed for a 6 s free-viewing period, followed by a varying intertrial interval of 9-12 s that presented a fixation cross on a gray screen. Pictures were arranged such that within a block of 18 trials, participants viewed one intact and one scrambled version of each content at low, medium, and high brightness levels. Picture presentation was counterbalanced such that an intact and scrambled version of each content (i.e. erotic, neutral, violence) was presented within a block of 6 trials; within this block two pictures of low, medium, and high brightness were presented. Across participants, three presentation orders were constructed which counterbalanced whether a specific picture was presented early, middle or late in the series.

Apparatus

Pictures were presented using an IBM-compatible computer running Presentation software (Neurobehavioral Systems, San Francisco, CA). Pictures were displayed on a 19-inch monitor located in the experimental room, at a distance of 30 inches (76.2 cm) from where the participant was seated, subtending 8.9 x 9.1 degrees of visual angle.

Pupil diameter was continuously sampled at 60 Hz from 2-s before picture onset to 250 ms before the end of the inter-trial interval using an ASL model D6 desk mounted remote eye tracker (Applied Science Laboratories, Bedford, MA; see Figure 2-2c). This system consists of a video camera and an infrared light source, which is focused on the participant's right eye. Face recognition is used to track head movements and keep the pupil in focus. The recording video camera was located in front of the participant, situated just below the stimulus presentation monitor. The height of the monitor and eye tracker was adjusted between participants to ensure that each participant's pupil was level with a fixation cross in the center of the screen. Figure 2-2 depicts the setup of the participant room, experimenter view, and eye-tracking camera.

Procedure

After arriving at the laboratory, each participant signed a consent form and was seated in an upright chair in a small, sound-attenuated, dimly lit room. A calibration procedure was conducted in order to ensure accurate measurement of pupil diameter at different gaze locations. The dot distribution and example gaze locations are depicted in Figure 2-3. The participant was instructed to sequentially look at 9 dots that appeared one at a time on the screen while pupil diameter and gaze location were recorded. Several scrambled images of varying brightness were then presented in order to ensure reliable pupil diameter measurement at different levels of picture brightness. This procedure was repeated until pupil diameter was successfully obtained at each gaze location and brightness level. The participant was then instructed to view each picture for the entire time that it was on the screen and to look at the fixation cross at all other times. It was emphasized that participants should sit still throughout the experimental session, as small movements would interfere with data collection. The experimenter monitored gaze location and pupil diameter during each session and adjusted camera focus during the inter-trial interval when necessary (see Figure 2-2b for an example of accurate pupil and gaze location measurement).

Data Reduction

Pupil diameter was converted offline from arbitrary units to millimeters and linear interpolation was used to estimate pupil size for samples in which the pupil was occluded due to blinking. For each trial, pupil diameter during a one-second baseline prior to picture viewing was subtracted from each of the following pupil samples. Five participants were not included in the final analysis due to unsuccessful pupil discrimination on more than 15% of trials.

Based on the average waveform, the initial light reflex was calculated as the mean pupil change in a window from .5 to 1.3 s after picture onset (Figure 3-1a). Trials in which pupil discrimination was less than 50% accurate in this time window were counted as missing (3.2% of total trials). Late pupil diameter was calculated as the mean pupil change in a window from 2 to 6 seconds following picture onset (Figure 3-1b) and included the same trials as in the analysis of the light reflex.

To ensure that any potential differences in pupil size for each picture content were not due to differences in brightness after removing trials, a separate analysis was conducted using only those trials with full-triplets for both intact and scrambled versions of the images (i.e. for each subject, removing all trials which did not have a corresponding same-brightness trial in all six conditions). After removal of these trials 86.4% of original trials remained. The initial light reflex and late pupil diameter were calculated using the same time windows (Light reflex: 0.5-1.3s; late pupil diameter: 2-6s) as in the primary pupil analysis.

Statistical Analyses

Data were analyzed using a Content (3: erotic, neutral, violent) X Mode (2: intact, scrambled) repeated measures analysis of variance for each of the dependent variables (initial light reflex, later pupil diameter), with appropriate follow-up univariate ANOVAs when warranted. Greenhouse-Geisser was used to correct degrees of freedom for sphericity. Tables 3-1 and 3-2 display means and standard errors for all comparisons.

Table 2-1. Physical features and standardized ratings of intact pictures.

		Mean (SD)		
		Erotic	Neutral	Violent
Physical Features	Brightness	0.45 (0.13)	0.45 (0.13)	0.45 (0.13)
	Contrast	0.05 (0.02)	0.06 (0.02)	0.05 (0.03)
Standardized Ratings	Hedonic Valence	6.61 (0.34)	5.17 (0.42)	1.88 (0.24)
	Arousal	6.37 (0.36)	3.57 (0.39)	6.45 (0.32)
	Complexity	3.90 (0.36)	3.70 (2.06)	3.99 (2.06)

Note: Pictures depicting erotica, neutral events, and violence were selected to be identical in brightness, and highly similar in contrast and ratings of figure-ground complexity. Erotic and violent pictures were identical in ratings of emotional arousal and significantly different from neutral scenes. Erotic, neutral, and violent pictures necessarily differed in ratings of hedonic valence. Scrambled versions were identical in brightness to intact images.

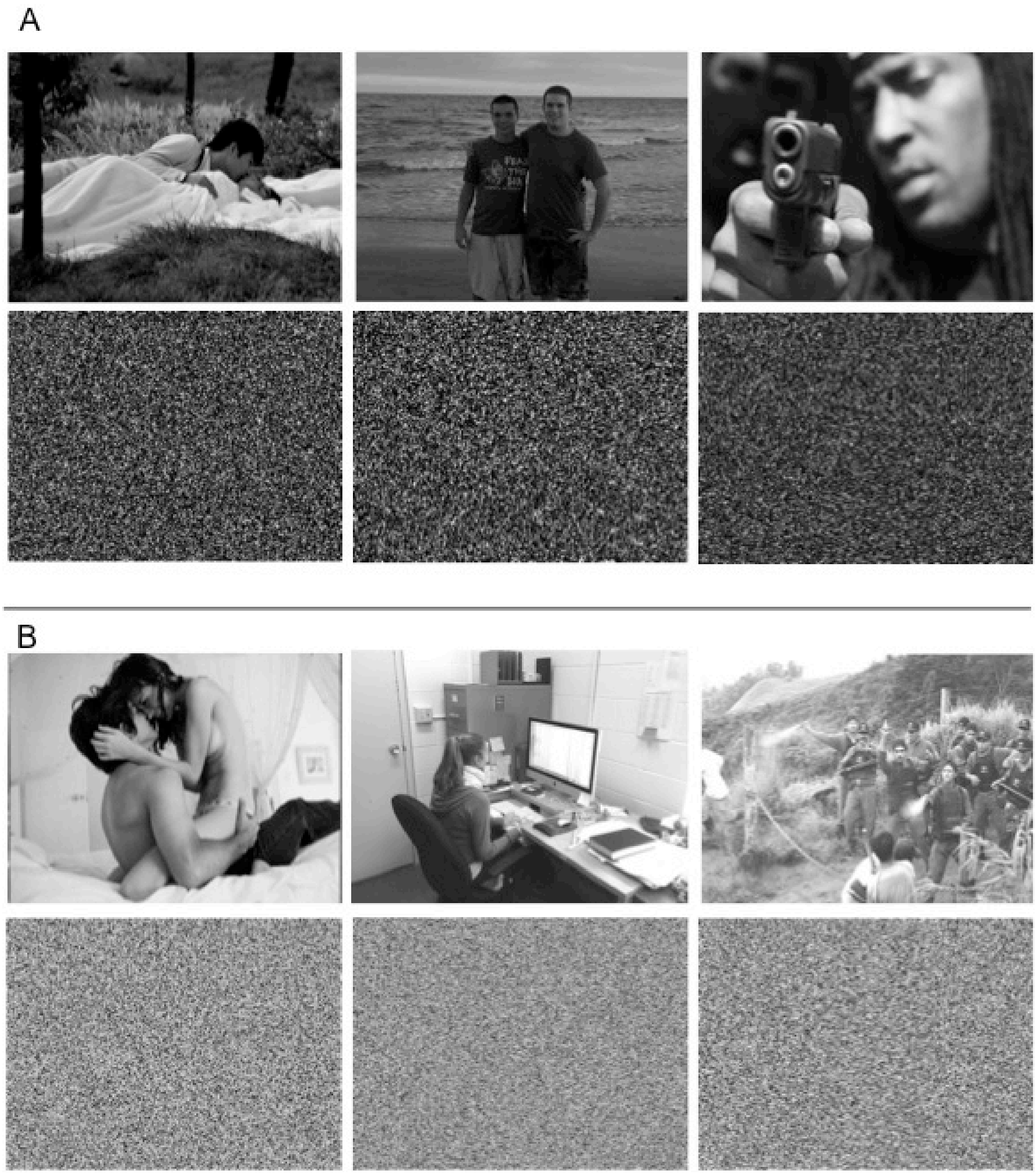

Figure 2-1. Example of identical brightness triplets. A. Example of a low brightness triplet. B. Example of a high brightness triplet. Brightness was varied across triplets but identical between erotic, violent, and neutral images within each triplet.

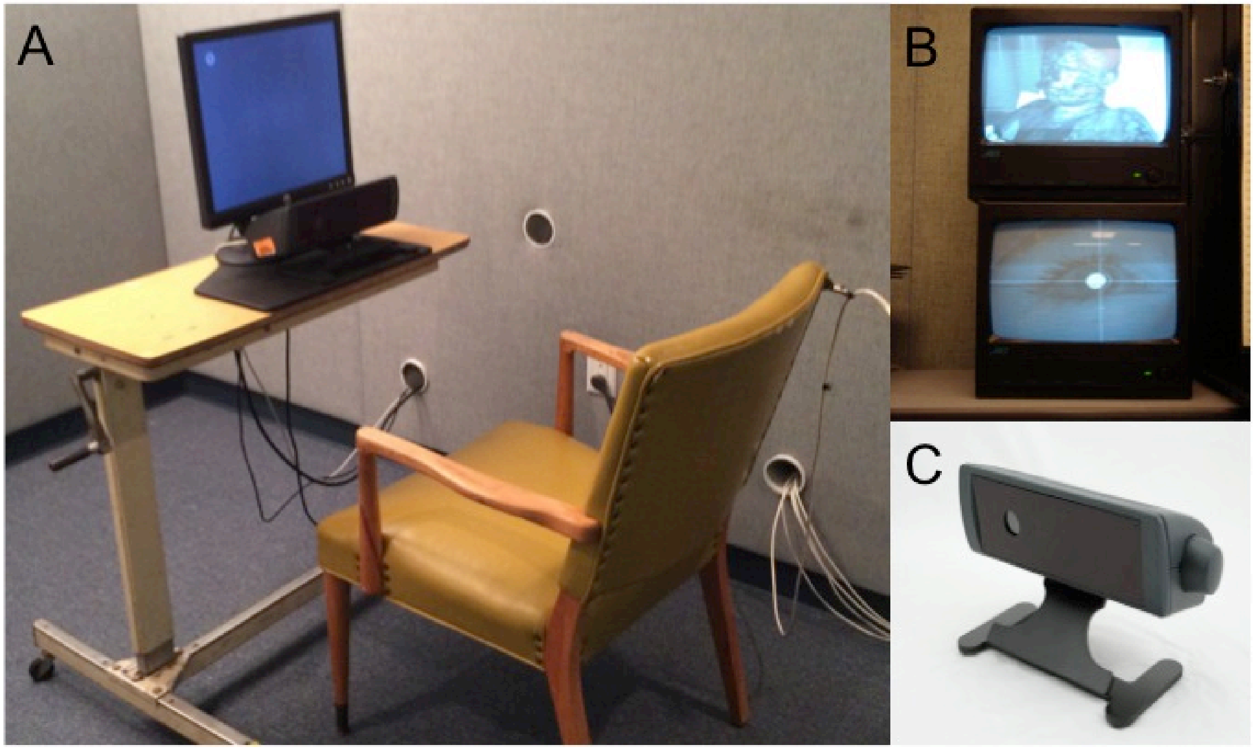

Figure 2-2. Experiment set-up. A. Participants were seated 30 inches away from the monitor. The height of the monitor and eye-tracker were adjusted so that each participant's eye was level with the fixation cross in the center of the screen. B. Experimenter view of monitors showing participant's point of gaze and accuracy of pupil size measurement. C. The eye-tracking camera, which uses infrared light to maintain pupil discrimination and face recognition to adjust for head movements.

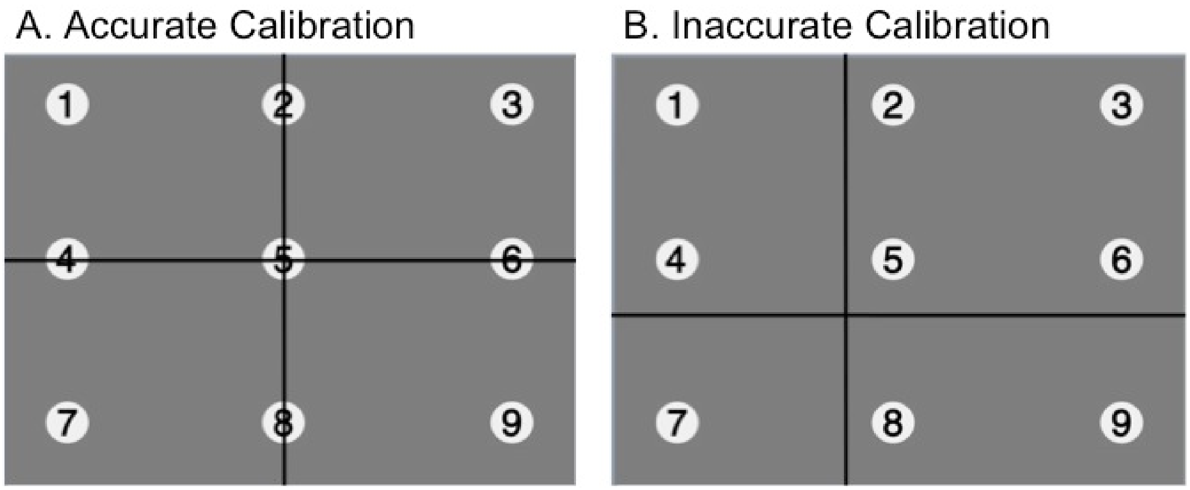

Figure 2-3. An example of the calibration procedure. A. Example of an accurate calibration to the center dot. B. An example of unsuccessful calibration to the center dot. Calibration was repeated until accurate pupil discrimination and point of gaze coordinates were obtained for all dots on the screen and at varying levels of brightness.

CHAPTER 3
RESULTS

Primary Pupil Analysis

Light Reflex

Figure 3-1a illustrates the change in pupil diameter during free-viewing of natural scenes, showing a clearly attenuated light reflex response when viewing intact erotic and violent, compared to neutral, scenes. Significant main effects of picture content, $F(2,20) = 23.5$, $p < .001$, and mode, $F(1,21) = 179.9$, $p < .001$, were accompanied by a significant interaction between content and mode, $F(2,20) = 13.4$, $p < .001$. Follow-up analyses revealed significant effects of picture content when viewing intact $F(2,20) = 35.1$, $p<.001$, but not scrambled, pictures. For intact pictures, viewing either erotic or violent scenes prompted significantly less early pupil constriction, compared to neutral pictures, $F(1,21) = 107.7$, $p< .001$ and $F(1,21)= 14.5$, $p < .005$, respectively. Viewing erotic scenes prompted less constriction than violent scenes, $F(1, 21) = 15.4$, $p < .001$.

Early modulation of the initial light reflex was not due to differences in brightness, as size of the initial light reflex did not differ by original hedonic content when participants viewed scrambled versions of these pictures that were identical in brightness, $F(2,20) = 0.48$, $p> .6$ (see figure 3-1, inset). Overall, pupil constriction was larger when viewing scrambled, compared to intact, scenes (all F's $1(1, 21) > 58$, $p < .001$).

Late Pupil Diameter

Pupil diameter later in the viewing interval was also modulated, with a larger response for emotional than neutral scenes (Figure 3-1b). Significant main effects were obtained for picture content, $F(2,20) = 55.3$, $p< .001$, mode, $F(1,21) = 169.1$, $p < .001$, and their interaction, $F(2,20) = 28.7$, $p < .001$. Follow-up analyses revealed significant effects of picture content for intact pictures only, $F(2,20) = 72.0$, $p<.001$. Viewing erotic and violent pictures was associated with significantly larger increases in late pupil diameter, compared to neutral scenes, $F(1,21)= 200.6$, $p < .001$ and $F(1, 21) = 19.1$, $p < .001$, respectively, with viewing erotic scenes again eliciting larger changes than violent scenes, $F(1,21) = 49.3$, $p < .001$. Overall, pupil diameter during the later viewing interval was reduced when participants viewed scrambled, compared to intact, images (all F's $(1,21) > 70$, $p < .001$). Again, pupil diameter did not vary among scrambled pictures.

Picture Analysis

Figure 3-2 depicts average pupillary changes when participants viewed intact erotic, violent, and neutral pictures at low and high brightness levels. As can be seen, light reflex amplitude was strongly associated with picture brightness. To further determine whether the initial light reflex is reliably modulated by emotional content, a hierarchical regression was conducted using each intact picture as the unit of analysis, assessing first effects of brightness and then emotional arousal. As expected, light reflex amplitude was highly correlated with picture brightness, $F(1, 34) = 106.6$, $p < .001$, $R^2 =.75$, with pictures higher in brightness prompting significantly larger light reflexes than lower brightness pictures. After removing effects due to brightness, however, rated emotional arousal of each picture (Lang et al., 2008) continued to account for significant variance in the amplitude of the initial light reflex, $F(2,33) = 74.9$, $p < .001$, R^2 increase = .06.

Full Triplet Pupil Analysis

Pictures of erotica, violence and neutral content were originally designed as triplets in which brightness was exactly matched. Because some trials were lost due to excessive pupil loss or poor pupil discrimination, it is possible that, in the preceding analyses, brightness may have still varied among data retained in the analysis. To confirm that these effects were not do to subtle differences in brightness resulting from missing trials, a second analysis was conducted using only those trials in which data was available for both the intact triplet and its scrambled version. In this analysis, approximately 86% of the previous data was retained.

Light Reflex.

Pupillary reactions during free-viewing of natural scenes again resulted in attenuated light reflex responses when viewing intact erotic and violent, compared to neutral, natural scenes (Figure 3-3a). Results were identical to those in the full analysis. Significant main effects of picture content, $F(2,20) = 12.31$, $p < .001$, and mode, $F(1,21) =, 228.5$, $p < .001$, were found, accompanied by a significant interaction between content and mode, $F(2,20) = 13.9$, $p < .001$. Significant effects of picture content were found when participants viewed intact, $F(2,20) = 26.0$, $p<.001$, but not scrambled, pictures, with viewing intact erotic and violent images prompting attenuated light reflex amplitudes relative to viewing intact neutral scenes, $F(1,21) = 51.8$, $p< .001$ and $F(1,21)= 15.9$, $p < .001$, respectively. The light reflex was further attenuated when participants viewed erotic, compared to violent, scenes, $F(1, 21) = 10.04$, $p < .005$.

Light reflex amplitude did not differ when participants viewed scrambled versions of these pictures that were identical in brightness, $F(2,20) = 0.07$, $p>.9$ (see Figure 3-3, inset). As in the previous analysis, light reflex amplitude was attenuated when participants viewed scrambled, compared to intact, pictures (all F's (1, 21) > 48, p < .001).

Late Pupil Diameter

As in the original analysis, later pupil diameter was also modulated by picture content, F(2,20)= 44.8, p<.001, mode, F(1,21)= 196.1, p<.001, and their interaction, F (2,20) = 25.9, p<.001. Significant effects of picture content were elicited when participants viewed intact pictures, F(2,20) = 52.3, p<.001, with no differences when viewing scrambled images, F(2,20) = 0.51, p>.6. Significantly larger changes in late pupil diameter were found when participants viewed erotic or violent, compared to neutral, scenes, F(1,21)= 105.6, p < .001 and F(1, 21) = 17.8, p < .001, respectively. Pupil diameter was again greater when participants viewed erotic, relative to violent, pictures, F(1,21) = 42.7, p < .001. Late Pupil diameter was similarly reduced when participants viewed scrambled, compared to intact, images (all F's (1,21) > 60, p < .001).

Picture Analysis

Assessing effects of brightness and emotional arousal via a hierarchical regression analysis using intact picture as the unit of analysis revealed similar results to the primary pupil analysis. Light reflex amplitude remained strongly associated with picture brightness, F(1,34) = 196.3, p<.001, R^2= .76, with ratings of emotional arousal accounting for significant variance in light reflex amplitude once effects of brightness were removed, F (2,33) = 16.3 , p<.001, R^2 increase= .07.

Table 3-1. Mean pupil diameter change (mm) during free-viewing of intact erotic, neutral, and violent scenes.

		Mean (SD)		
	Analysis	Erotic	Violent	Neutral
Light Reflex	Primary	-0.07 (0.04)	-0.20 (0.06)	-0.32 (0.04)
	Full Triplet	-0.04 (0.21)	-0.15 (0.30)	-0.30 (0.26)
Late Pupil Diameter	Primary	0.52 (0.07)	0.15 (0.08)	-0.12 (0.06)
	Full Triplet	0.53 (0.36)	0.20 (0.43)	-0.10 (0.35)

Note: Means (standard errors) of pupil diameter change (mm) during free-viewing of erotic, neutral, and violent scenes during the initial light reflex (.5 - 1.3 seconds) and late pupil diameter (2 – 6 seconds). Results of the primary and full triplet analyses revealed identical patterns of modulation.

Table 3-2. Mean pupil diameter change (mm) during free-viewing of scrambled images.

		Mean (SD)		
	Analysis	Erotic	Violent	Neutral
Light Reflex	Primary	-0.61 (0.04)	-0.63 (0.05)	-0.64 (0.05)
	Full Triplet	-0.60 (0.23)	-0.61 (0.23)	-0.60 (0.33)
Late Pupil Diameter	Primary	-0.57 (0.07)	-0.60 (0.05)	-0.67 (0.06)
	Full Triplet	-0.58 (0.35)	-0.60 (0.25)	-0.58 (0.35)

Note: Means (standard errors) of pupil diameter change (mm) during free-viewing of scrambled versions of each original hedonic content (erotic, neutral, and violent) during the initial light reflex (.5 - 1.3 seconds) and late pupil diameter (2 – 6 seconds). Pupil diameter was not modulated during viewing of scrambled images of original hedonic content in either time period. Results of the primary and full triplet analyses revealed identical patterns of modulation.

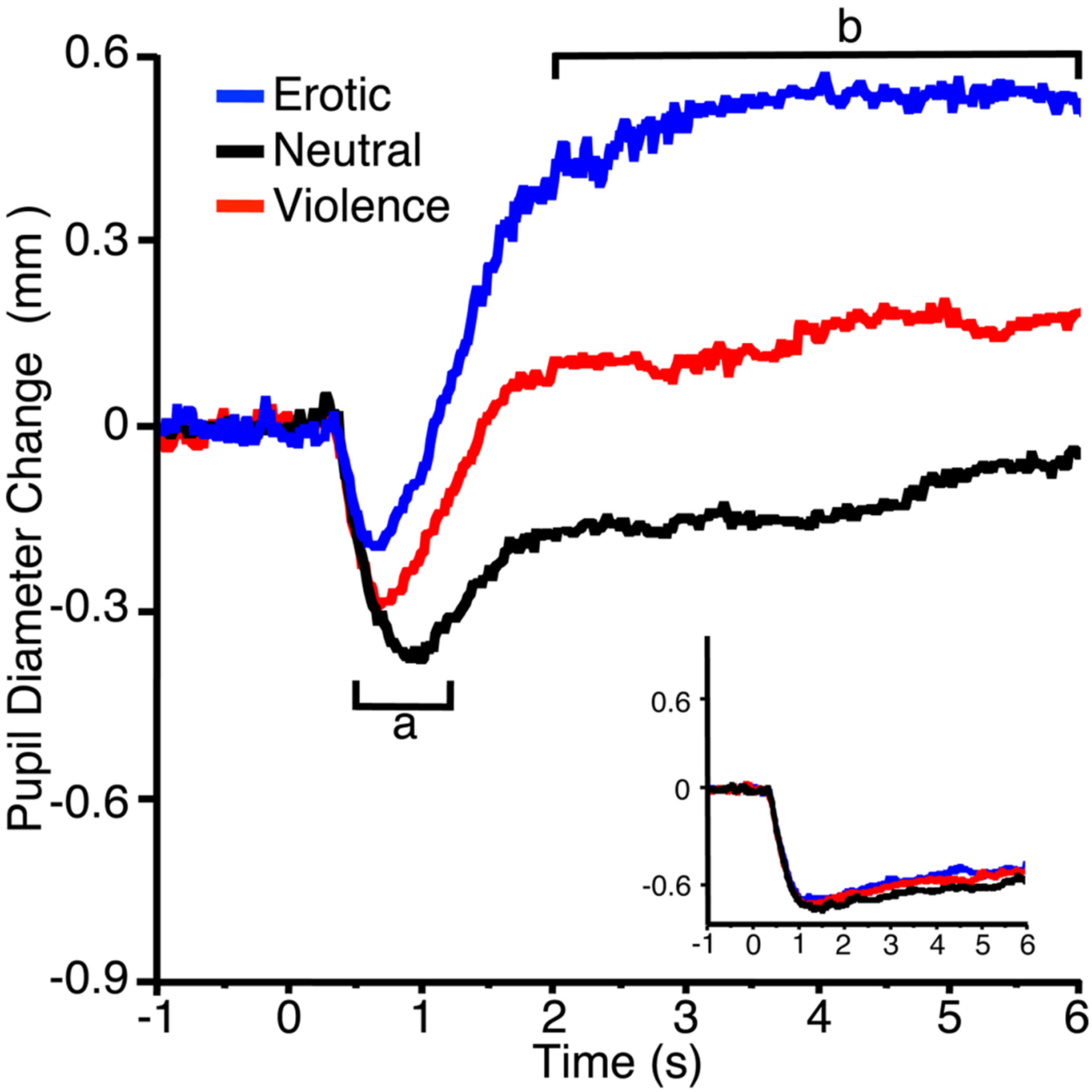

Figure 3-1. Change (mm) in pupil diameter from a 1-s baseline preceding picture onset when viewing original erotic, neutral, and violent scenes. a) The mean light reflex was averaged in a window from .5 to 1.3 s following picture onset, and b) later pupil diameter was averaged in a window from 2 to 6 s post picture onset. Inset: Pupil size when viewing scrambled versions did not differ as a function of original picture content in either time period.

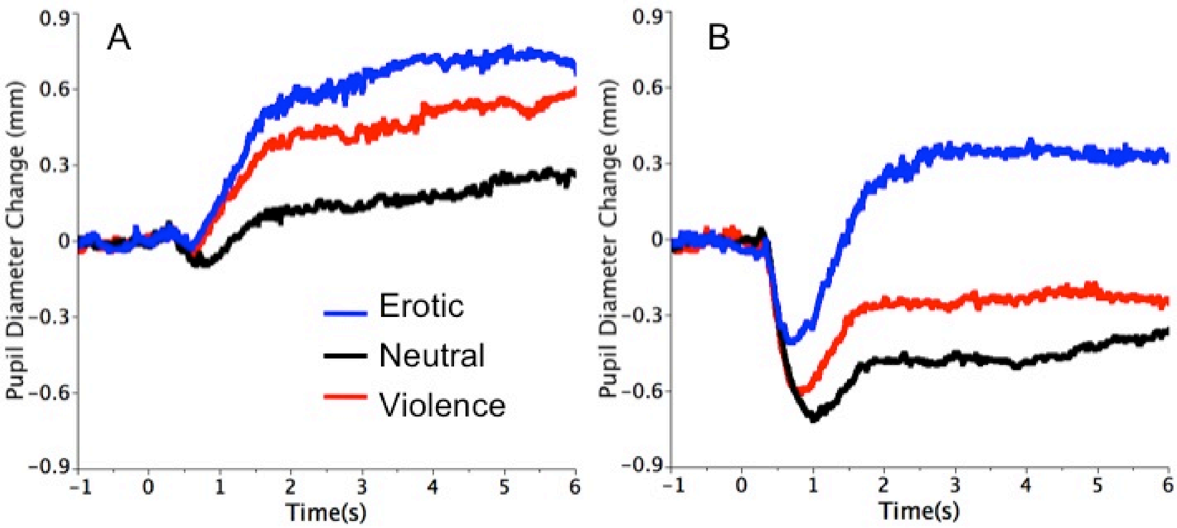

Figure 3-2. Pupil diameter change (mm) when viewing low and high brightness natural scenes. A. Mean pupil change during viewing of low brightness erotic, neutral, and violent scenes. B. Mean change in pupil diameter when viewing high brightness natural scenes. The light reflex was enhanced when participants viewed high brightness, relative to low brightness, pictures. The light reflex and later pupil diameter were consistently modulated by emotional content during viewing of both darker and lighter natural scenes.

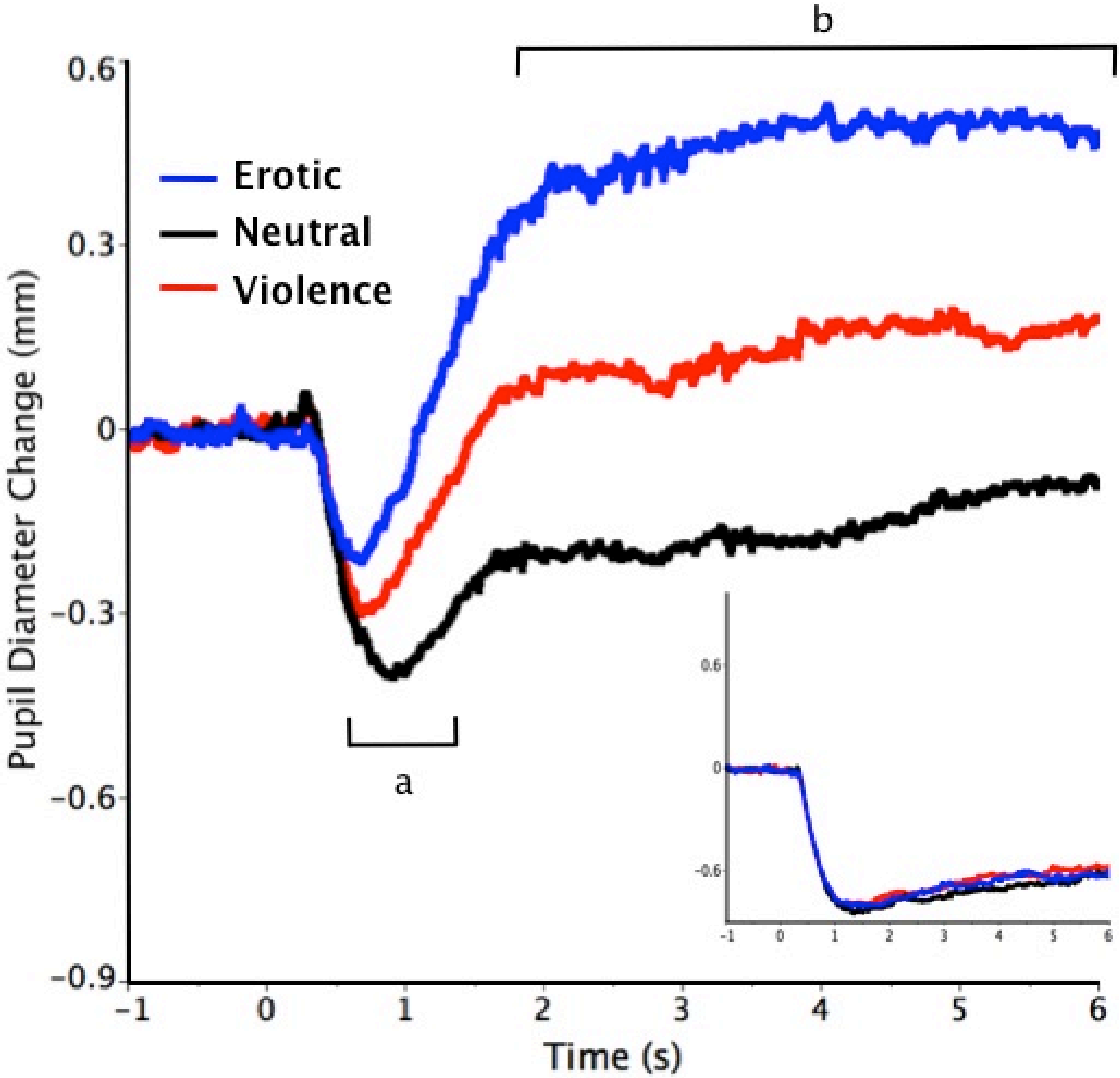

Figure 3-3. Pupil Diameter change (mm) using only full triplet trials. Change (mm) in pupil diameter from a 1 s baseline preceding picture onset when viewing the original erotic, neutral or violent scenes using only full triplet trials. a) Initial light reflex; b) Late pupil diameter. Inset: Pupil size when viewing scrambled versions. Results were identical to the primary analysis.

CHAPTER 4
DISCUSSION

Interpretation and Significance

When viewing emotionally engaging pictures, modulatory effects on pupil diameter are apparent as early as the initial light reflex. Although the light reflex is much more strongly modulated by perceptual factors such as brightness, this early constriction was nonetheless significantly attenuated when participants viewed highly arousing pictures of erotica and violence, compared to neutral images (see Figure 3-1). When participants viewed scrambled versions of each intact scene, which were identical in brightness but without semantic content, neither the initial light reflex or later pupil dilation varied as a function of original picture content, confirming that the attenuation in light reflex amplitude found when viewing intact emotional, compared to neutral, pictures did not reflect subtle differences in brightness. Furthermore, ratings of emotional arousal substantially predicted differences in light reflex amplitude once effects of brightness were removed, again demonstrating early modulation of pupil diameter when viewing emotionally engaging natural scenes.

Because the pupil is sensitive to slight variations in brightness, pictures were originally selected to form same-brightness triplets of erotic, violent, and neutral content. However, since some trials were lost due to poor pupil discrimination, it was possible that brightness may have varied when using all remaining trials in the primary analysis. Therefore, a follow-up analysis using only those trials in which complete data was available for the original triplet and its scrambled version (see Figure 3-3) was conducted, confirming findings of attenuated light reflex amplitudes when participants viewed erotic or violent, compared to neutral, pictures, and of a significant association between emotional arousal and light reflex amplitude after removing effects of brightness.

Modulation of the initial light reflex was larger when participants viewed erotic, compared to violent, scenes. This is consistent with previous studies finding greater physiological reactivity when young adults view pictures of erotic couples, compared to when viewing other pictures rated similarly in emotional arousal. For example, Schupp, Cuthbert, Bradley, Hillman, Hamm, and Lang (2004) recorded startle reflexes and event-related potentials during free-viewing of emotional pictures varying in terms of their motivational significance. Pictures depicting erotica elicited significantly greater late positive potentials and increased inhibition of the P3 component to startle probes, compared to viewing other pictures which were rated similarly in terms of emotional arousal (e.g. human threat, animal threat, mutilation) suggesting greater allocation of attentional resources when viewing erotica. Erotic stimuli also prompt greater electrodermal reactions than similarly-rated arousing unpleasant pictures in young adults (Lang, Bradley, & Cuthbert, 1997), suggesting that erotic pictures are more emotionally arousing in this population and that self-report may not identify these differences as well as physiological measures. Despite this heightened response when viewing erotica, pupil diameter was nonetheless reliably and significantly modulated when participants viewed arousing pictures of erotica and violence, compared to neutral images.

Pupil diameter at any given moment is determined by the amount of co-activation of sympathetic and parasympathetic influence on pupillary musculature. Although the specific mechanism behind emotional modulation of the light reflex has not been determined, one view is that pupil diameter reflects changes in locus coeruleus activity during processing of motivationally significant stimuli (Gilzenrat, Nieuwenhuis, Jepma, & Cohen, 2010), based on studies indicating that locus coeruleus activity is strongly associated with changes in pupil diameter. The locus coeruleus has inhibitory influences on the Edinger-Westphal nucleus as well as excitatory projections to preganglionic sympathetic neurons in the spinal cord (Samuels & Szbadi, 2008), both of which can elicit changes in pupil diameter. However, in animal studies utilizing electrical stimulation of parasympathetic fibers in the oculomotor nerve, it has been demonstrated that light reflex amplitude is primarily determined by contractions of the pupillae sphincter muscles (Clarke, R. J., 2007) rather than direct sympathetic stimulation of the dilator muscle. Thus, one possibility is that viewing scenes high in emotional arousal alters the firing rate of neurons in the locus coeruleus, inhibiting projections from the Edinger-Westphal nucleus to the pupillae sphincter muscles and attenuating the light reflex.

On the other hand, increased late pupil diameter is likely due to continued inhibition of the Edinger-Westphal Nucleus as well as direct sympathetic stimulation of the pupillae dilator muscles by posterior hypothalamic nuclei (Steinhauer, et al., 2004). Pupil dilation may also be elicited by increased activity of the superior colliculus, as microstimulation of this area in monkeys elicits reliable increases in pupil diameter (Wang, Boehnke, White, & Munoz, 2012). Future studies utilizing pharmacological blockades of appropriate receptors in the sympathetically-mediated dilator and parasympathetically-mediated sphincter muscles (e.g. Steinhauer, et al., 2004) will be useful in determining the specific mechanisms underlying emotional modulation of the light reflex (and later pupil dilation). However, previous research suggests that light reflex modulation is due to central inhibition of the Edinger-Westphal nucleus, which may be mediated by changes in locus coeruleus activity, with subsequent dilation elicited by continued central inhibition of the Edinger-Westphal nucleus as well as direct sympathetic stimulation of the dilator muscle.

Limitations and Future Research Directions

This study measured pupil diameter in a population of college-age students at the University of Florida. Older adults have been shown to have smaller resting pupil and reduced light reflex amplitudes to flashes of light relative to young adults (Bitsios, Prettyman, & Szabadi, 1996). Previous studies have demonstrated similar emotional modulation of late pupil diameter in healthy older adults (e.g. Dietz, et al., 2011) but it is unclear whether emotional modulation of the light reflex will replicate in differently aged populations.

The scrambled and intact pictures used in this study necessarily differed in terms of spatial frequency and contrast. Although pupil size did not differ among scrambled versions of erotic, neutral, and violent scenes, these images did elicit a significantly larger initial constriction than the intact version of the picture. Larger light reflexes are found for stimuli higher in spatial frequency (Link et al., 2006), however, which is consistent with the enhanced constriction when viewing scrambled, compared to intact, images found here. In addition to physical differences, specific contents of erotica, and violence were used in this study. Future studies utilizing a wider range of affective pictures that are controlled for spatial frequency may be useful in better understanding the relationship between emotional arousal and light reflex amplitude.

Summary

The initial light reflex is attenuated when participants view emotionally arousing, compared to neutral, natural scenes, suggesting early and rapid effects of emotional arousal on pupillary changes during free-viewing of affective pictures. These findings cannot be explained by subtle variance in picture brightness, as scrambled versions of these same scenes, identical in brightness to the intact images, did not elicit reliable differences in pupil diameter. One interpretation is that early modulation of pupil diameter reflects changes in the firing rate of neurons in the locus coeruleus, but additional studies are needed to determine the precise mechanisms. Most broadly, these data show that differences in emotional arousal prompt changes in the initial light reflex beyond the effects of brightness alone, suggesting that emotional effects are immediate and then continue to modulate pupillary responses over periods of picture exposure.

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BIOGRAPHICAL SKETCH

Robert R. Henderson was born on July 18, 1987 in Woodbury, New Jersey. One of two children, he grew up in Upper Township and West Deptford, New Jersey, graduating from West Deptford High School in 2006. He earned his B.S. in Psychology from the University of Delaware (UD) in 2010. While at UD, Robert completed one year of research in an infant cognition lab and one year of research in a clinical psychophysiology laboratory, where he completed an honors senior thesis investigating inhibitory control in overweight children and healthy control participants.

Following his graduation from UD, Robert was employed as a post-baccalaureate research assistant at the Center for the Study of Emotion and Attention (CSEA) in Gainesville, Florida. While at the CSEA, Robert ran studies investigating psychophysiological processes during affective picture viewing and memory tasks, collected standardized ratings of affective pictures and texts, and helped to measure and record physiological data from patients with Anxiety Disorders. He also assisted with administrative duties and data analysis for several other research projects and was able to gain valuable research exposure at annual Society for Psychophysiological Research conferences, where he presented multiple first and second author posters.

Robert is currently pursuing a Ph.D. in clinical psychology as a graduate student in the Clinical & Health Psychology program at the University of Florida. He continues to work in the CSEA, studying emotional and cognitive processes in undergraduates and patients diagnosed with anxiety disorders.

After completing his Ph.D. program, Robert hopes to complete an internship in an academic clinic or VA prior to beginning a post-doctoral research career utilizing psychophysiological methods in an academic environment.