

Memory, emotion, and pupil diameter: Repetition of natural scenes

MARGARET M. BRADLEY AND PETER J. LANG

Center for the Study of Emotion and Attention, University of Florida, Gainesville, Florida, USA

Abstract

Recent studies have suggested that pupil diameter, like the “old-new” ERP, may be a measure of memory. Because the amplitude of the old-new ERP is enhanced for items encoded in the context of repetitions that are distributed (spaced), compared to massed (contiguous), we investigated whether pupil diameter is similarly sensitive to repetition. Emotional and neutral pictures of natural scenes were viewed once or repeated with massed (contiguous) or distributed (spaced) repetition during incidental free viewing and then tested on an explicit recognition test. Although an old-new difference in pupil diameter was found during successful recognition, pupil diameter was not enhanced for distributed, compared to massed, repetitions during either recognition or initial free viewing. Moreover, whereas a significant old-new difference was found for erotic scenes that had been seen only once during encoding, this difference was absent when erotic scenes were repeated. Taken together, the data suggest that pupil diameter is not a straightforward index of prior occurrence for natural scenes.

Descriptors: Memory, Emotion, Pupillometry

Although pupil diameter is primarily controlled by the sensory characteristics of a visual stimulus, including brightness, contrast, distance, spatial frequency, etc. (Barbur, 2004; Loewenfeld, 1999), it is also modulated by psychological variables including task context (e.g., Beatty, 1982; Steinhauer, Siegle, Condray, & Pless, 2004), emotional arousal (e.g., Bradley, Miccoli, Escrig, & Lang, 2008), and memory (e.g., Kafkas & Montaldi, 2011; Naber, Frasle, Rutishauser, & Einhauser, 2013; Vö et al., 2008). Recent recognition studies have reported that pupil diameter is modulated by whether items are old or new when recognizing written words (e.g., Vö et al., 2008), spoken words (Papesh, Goldinger, & Hout, 2012), pictures of objects (Kafkas & Montaldi, 2011), or natural scenes (Naber et al., 2013), prompting speculation that, like the old-new ERP (Rugg & Curran, 2007), pupil diameter may be a (simpler-to-measure) memory index that similarly signals prior occurrence (e.g., Vö et al., 2008; Naber et al., 2013). In the current study, we tested this hypothesis by measuring pupil diameter during recognition of scenes that are repeatedly presented spaced across the encoding phase, a context in which the old-new ERP is reliably enhanced (Ferrari, Bradley, Codispoti, & Lang, in press). If pupil diameter is similarly enhanced by distributed repetition, it would provide further support for its use as a memory index of prior occurrence.

Despite enthusiasm for the use of pupil diameter as a measure of memory (e.g., Goldinger & Papesh, 2012; Naber et al., 2013; Vö et al., 2008), the data relating pupil diameter to recognition is based on studies that vary not only in the stimuli used, but also in the timing and scoring of the pupil data. For instance, Otero, Weekes, and Hutton (2011) assessed pupil diameter for visually presented old and new words, and computed a ratio of the maximum pupil dilation occurring during the final 250 ms of each 2-s presentation and the maximum dilation in the initial 250 ms of each trial. No waveforms were included to further assess temporal aspects of the pupil responses, but quantifying the last 250 ms of a 2-s trial does not suggest an early modulatory difference. Heaver and Hutton (2011), on the other hand, using a 1,750-ms visual presentation of old or new words, also computed a pupil dilation ratio, but used the peak diameter across the 1,750-ms trial and the maximum diameter in a 250-ms prestimulus baseline; again, no waveforms were included to further assess the timing of the peak difference. Papesh et al. (2012) investigated memory for spoken, instead of visually presented, words, and, rather than computing a ratio, calculated peak diameter (deviated from a 200-ms preword baseline) from the onset of the spoken word until 1,000 ms after the old-new button press, reporting greater peak diameter for high-confidence “old” decisions; again, no waveforms were presented to determine the timing of peak modulation.

Studies that include waveforms can assist in establishing the timing of memory effects. For instance, Montefinese, Ambrosini, Fairfield, & Mammarella (2013) presented visual words for 4 s each and expressed each of the resulting pupil samples as a percent change from a 200-ms preword baseline. The accompanying waveforms indicated enhanced change for old, compared to new, items

This work was supported in part by NIMH grants MH098078 and MH094386. Thanks to Petra Houbova, who assisted in initial data acquisition and reduction.

Address correspondence to: Margaret Bradley, Center for the Study of Emotion and Attention, University of Florida, PO Box 112766, Gainesville, FL 32611, USA. E-mail: bradley@ufl.edu

that begins around 500 ms following word onset and was sustained throughout the 4-s presentation. Vö et al. (2008), on the other hand, visually presented words for just 500 ms, followed by a 1,300-ms blank screen, and computed the peak pupil diameter (deviated from a 200-ms preword baseline) across the entire 1,800-ms epoch; accompanying waveforms suggest a difference in pupil diameter between old and new words that begins around 750-800 ms after word onset and is still apparent 1,300 ms following word offset.

When viewing words, the pupillary waveforms show an increase in pupil diameter immediately following stimulus onset (e.g., Vö et al., 2008), whereas, when recognition of natural scenes is assessed, the pupil response often includes an initial constriction (the light reflex), followed by redilation of the pupil. For instance, Naber et al. (2013) presented pictures of old and new scenes (houses, landscapes, vehicles, etc.) for 1 s and quantified the relevant pupil change as the slope of the initial pupil constriction in a window from 300 to 1,000 ms following picture onset, reporting that familiar scenes elicited less constriction in this early time window than novel scenes. Measuring initial constriction, rather than later dilation, suggests that the timing and direction of pupil modulation during recognition of scenes may differ from that obtained when recognizing words. In the current study, we assess both early and late modulation of the pupil during recognition of natural scenes in order to further determine the temporal window in which changes occur.

In addition to presenting old and new natural scenes during recognition, we manipulate the number and type of repetition for each scene during encoding. Recent data indicate that the centroparietal old-new ERP difference is enhanced when repetitions of natural scenes are distributed (spaced) across an encoding phase, compared to when an item is only seen once previously or is presented repeatedly in a row (massed repetition; Ferrari, Bradley, Codispoli, Karlsson, & Lang, 2013). Moreover, even during incidental free viewing, distributed repetition prompts an enhanced late centroparietal potential that is similar in timing and topography to the old-new ERP (Ferrari et al., in press), as well as activation in regions of posterior parietal cortex, including the precuneus, which have been implicated in episodic retrieval (Bradley et al., 2014). We have interpreted these data as indicating that distributed repetition prompts spontaneous episodic retrieval in both explicit and implicit memory contexts, which may account for the memory advantage for distributed repetitions in the classic “spacing effect” (e.g., Glensberg, 1979; Godbole, Delaney, & Verkoijen, 2014; Greene, 1989). If pupil diameter is similarly modulated by distributed repetitions in the context of explicit recognition and free viewing, it would provide further support for the hypothesis that the pupil is a sensitive measure of episodic retrieval.

In recent studies, we found both attenuation of the initial light reflex and enhanced late pupil diameter when viewing highly arousing pleasant or unpleasant, compared to neutral, pictures during free viewing (Bradley et al., 2008; Henderson, Bradley, & Lang, 2014), and in the current study, we presented both emotionally arousing and neutral scenes. In their previous study assessing pupil diameter and emotional memory, Vö et al. (2008) reported that recognition of old words prompted larger pupil diameter than new words, regardless of whether words were emotional or neutral. On the other hand, whereas emotional words prompted larger dilation than neutral words for new items, similar to our findings with emotional scenes (Bradley et al., 2008), emotional words prompted less dilation than neutral items for old items, resulting in an overall smaller old-new difference for emotional words. The current design allows a reassessment of the effects of emotion on pupil changes

during recognition, using highly arousing natural scenes depicting erotica and violence, as well as a new look at effects of repetition on pupil diameter when viewing emotionally arousing or neutral scenes.

Thus, in the current study, pictures of emotional and neutral natural scenes are presented once or repeated four times using massed (contiguous) or distributed (spaced) repetition in an incidental (free-viewing) context. Then, a recognition test presents old pictures (3 s) that had been presented once or repeated during free viewing, together with new pictures, for a speeded recognition decision. We assess effects of repetition and hedonic content on pupil diameter both during initial constriction in the region of the light reflex, as well as later in the viewing interval. If pupil diameter, like ERP and fMRI data, is a sensitive index of episodic retrieval, we expected to find larger pupil diameter when viewing scenes whose repetitions were spaced, compared to massed, on both the explicit recognition test as well as during initial free viewing.

To quantify the pupil response, we reduce the pupil diameter data in ways similar to ERPs, in which each sample following picture onset is deviated from a 1-s baseline preceding picture onset. The resulting waveforms are used to determine the appropriate time windows for analysis and include both an early (500-1,000-ms postpicture onset) and a late window (1-3-s postpicture onset). Changes in pupil diameter are averaged¹ over the samples in each time window for each participant and condition and submitted to analysis.

Method

Participants

Sixty-five (39 female) participants from University of Florida general psychology courses signed a consent form that was approved by the University of Florida institutional review board, and participated for course credit.

Materials and Design

Stimuli were 72 pictures selected from the International Affective Picture System² (IAPS; Lang, Bradley, & Cuthbert, 2008) to include 36 emotionally arousing pictures (18 erotica/romance, 18 mutilation/threat) and 36 neutral pictures depicting everyday scenes. All pictures portrayed people and were presented in 16-bit grayscale. The 72 pictures were arranged in two sets of 36 such that each pair of pictures in the two sets were matched in brightness and emotional content (e.g., two menacing dogs), and brightness was matched for emotional and neutral pictures.

1. Indices of old-new pupil differences used in previous studies including (a) peak diameter, (b) pupil dilation ratio, and (c) slope of initial constriction were also conducted on the recognition data and are reported for comparison purposes.

2. International Affective Picture System (Lang, Bradley, & Cuthbert, 2008) catalog numbers are: Erotic/Romance. Set 1: 4597, 4611, 4641, 4658, 4659, 4660, 4687, 4690, 4694; Set 2: 4604, 4619, 4653, 4668, 4669, 4693, 4695, 4697, 4800. Everyday Scenes. Set 1: 2102, 2104, 2190, 2221, 2372, 2377, 2383, 2390, 2393, 2410, 2435, 2493, 2506, 2511, 2513, 2579, 2594, 7550; Set 2: 2191, 2210, 2272, 2305, 2312, 2394, 2400, 2411, 2495, 2500, 2510, 2512, 2513, 2515, 2593, 2595, 2630, 2850. Violence. Set 1: 1120, 1300, 3053, 3069, 3140, 3170, 3530, 6212, 6550; Set 2: 1050, 1304, 3030, 3068, 3160, 3191, 3500, 6520, 6560.

During encoding, one set of 36 pictures was presented, half (18) of which were emotionally arousing (9 erotica, 9 violence) and half (18) neutral. Of the 36 pictures, 12 pictures were presented once, 12 were then repeated three more times consecutively (36 trials, massed repetition), and 12 were repeated three more times distributed across the study (36 trials, distributed repetition with a mean lag of 27 pictures, range 24-34), resulting in a total of 108 encoding trials. For analyses of the pupil during free viewing, the first presentation of a picture in the massed or distributed repetition condition was included in the novel condition, resulting in a total of 36 novel trials (18 emotional: 9 erotica/9 violence; 18 neutral), 36 massed repetitions, and 36 distributed repetitions. Different presentation orders varied the input position of specific pictures and counterbalanced conditions such that, across participants, each picture was presented once or repeated with massed or distributed repetition. Each picture was presented for 6 s with a 5-s intertrial interval in a free-viewing context with no mention of a later memory test.

On the recognition test, both sets of 36 pictures were presented, with one set (36) previously seen during encoding (old) and one set (36) not previously presented (new). Of the 36 old pictures, 12 had been seen once during free viewing, 12 were seen in the context of massed repetitions, and 12 were seen in the context of distributed repetitions; of these, half were emotional in content. The 36 new pictures consisted of 18 emotional and 18 neutral scenes that had not been presented before. The specific set of pictures that served as old or new was counterbalanced across participants, and different orders varied the serial position of specific pictures across participants.

On the recognition test, each picture was presented for 3-s with a 5-s intertrial interval. Participants held a button paddle in each hand, and were instructed to press a button as rapidly as possible regarding whether the picture was previously presented (old) or not (new). Response hand was counterbalanced across participants.

Apparatus

Pictures were displayed on a 1,024 × 768 (19") monitor (Samsung SyncMaster 191T), with a distance of approximately 112 cm from the participant's eye to the screen, subtending 19 × 14 degrees of visual angle. Pupil diameter was recorded using an ASL model 504 eye-tracker system (Applied Science Laboratories, Bedford, MA), which allows free movement of the head, and consists of a video camera and an infrared light source pointed at the participant's right eye. A magnetic sensor, attached to a headband, tracked and adjusted for head movement. The recording video camera was located in a box in front of the subject covered by a red translucent screen that obscured it from view. Pupil diameter was recorded for 60 Hz for 2 s prior to picture onset and for the duration of picture presentation with a resolution of 0.1 mm.

Procedure

Upon arrival at the laboratory, each participant signed a consent form and was seated in a small, sound-attenuated, dimly lit room. During the initial free-viewing phase, each participant was instructed that a series of pictures would be displayed and that each picture should be viewed the entire time it was on the screen. There was no mention of a later test of memory. On the subsequent recognition test, each participant was instructed to decide as rapidly as possible whether a picture had been seen before (old) or not (new) and to press the appropriate button held in the right or left hand. Hand of response was counterbalanced across participants.

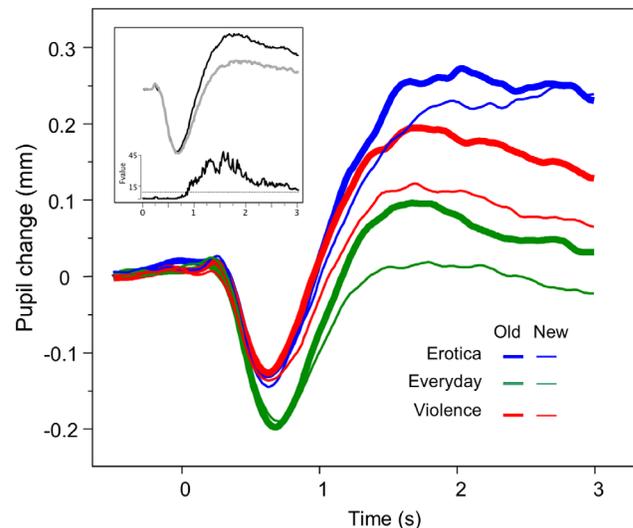


Figure 1. Waveforms showing changes in pupil diameter (deviated from a 1-s pre-picture baseline) during an explicit recognition test of pictures previously presented (old), averaged across repetition condition, and new pictures depicting erotica, everyday scenes, or violence. Inset: Waveforms showing pupil change for old and new pictures averaged across picture content, together with a plot of the value of the *F* statistic testing the difference between old and new pictures at each sampling point. Values above the dotted line are samples in which an old-new difference was significant.

Data Analysis

Samples in which the pupil was obscured due to blinking were identified using ASL Results software, and linear interpolation was used to estimate pupil size. Each sample was deviated from a 1-s prepicture baseline average for each trial and participant. Based on the resulting waveforms during recognition (e.g., see Figure 1), pupil change was averaged in a window from 500 to 1,000 ms following picture onset to estimate differences in initial constriction, and in a window from 1 to 3 s following picture onset to measure late dilation.

For the recognition data, two analyses were conducted. The first repeated measures analysis of variance (ANOVA) included two factors of (1) old-new, in which the data were averaged over old pictures that had been presented once or repeated during free viewing, and (2) hedonic content (erotica, everyday, violence). A second repeated measures ANOVA was confined to old items, and assessed effects of repetition (once, massed, distributed) and hedonic content (pleasant, neutral, unpleasant) on pupil change. For initial viewing, a repeated measures ANOVA included presentation type (novel, massed, distributed) and hedonic content.

For analysis of reaction time (RT) and accuracy on the recognition test, an ANOVA was conducted on responses to old items using type of repetition (once, massed, distributed) and hedonic content (erotica, everyday scenes, violence) as repeated measures.

In all analyses, significant effects (Wilks's lambda) were evaluated at a threshold of $p < .05$.

Results

Explicit Recognition Test

Pupil diameter. Figure 1 illustrates pupil diameter for correctly recognized old pictures, collapsed across repetition condition, and

Table 1. Means (Standard Error) for Dependent Measures During Recognition and Initial Free-Viewing

Measure	Picture content		
	Erotica	Everyday	Violence
Recognition			
Initial pupil change ^a (mm)			
New	-.10 (.02)	-.16 (.02)	-.10 (.02)
Old Single	-.09 (.04)	-.15 (.02)	-.09 (.02)
Distributed	-.09 (.03)	-.16 (.02)	-.08 (.03)
Massed	-.11 (.03)	-.15 (.03)	-.06 (.03)
Late pupil change ^b (mm)			
New	.23 (.03)	.00 (.02)	.10 (.03)
Old Single	.37 (.04)	.08 (.03)	.14 (.03)
Distributed	.20 (.03)	.06 (.03)	.17 (.03)
Massed	.23 (.04)	.06 (.03)	.19 (.03)
Reaction time (ms)			
New	1298 (36)	962 (25)	1117 (33)
Old Single	1257 (40)	1046 (26)	1022 (28)
Distributed	977 (32)	842 (20)	855 (19)
Massed	1082 (31)	945 (22)	962 (28)
Accuracy (% correct)			
New	.94 (.01)	.98 (.01)	.97 (.01)
Old Single	.90 (.02)	.89 (.02)	.97 (.01)
Distributed	.99 (.01)	.98 (.01)	.98 (.01)
Massed	.98 (.01)	.97 (.01)	.99 (.01)
Free viewing			
Initial pupil change ^a (mm)			
Novel	-.10 (.03)	-.25 (.02)	-.13 (.02)
Distributed	-.11 (.02)	-.22 (.02)	-.13 (.02)
Massed	-.13 (.02)	-.18 (.02)	-.11 (.02)
Late pupil change ^b (mm)			
Novel	.16 (.03)	-.21 (.03)	.06 (.03)
Distributed	.11 (.02)	-.16 (.02)	.03 (.02)
Massed	.03 (.03)	-.10 (.02)	.04 (.02)

^aAverage change 500-1,000 ms after picture onset.

^bAverage change 1-3 s after picture onset.

new pictures depicting erotica, violence, or everyday scenes. As illustrated in the inset, a significant difference in pupil diameter when recognizing old and new scenes begins around 850 ms after picture onset and continues until picture offset.

Consistent with this, the old-new difference in pupil diameter was not significant in the early time window when averaged over all old pictures, and there was no difference in early constriction as a function of whether a picture had been repeated or only seen once during encoding (see Table 1, top). Rather, in the early window, only a main effect of hedonic content was significant, $F(2,63) = 33.6, p < .0001$, replicating our previous findings that emotional scenes elicit a smaller light reflex than neutral scenes, and this was found for both pleasant, $F(1,64) = 48, p < .0001$, and unpleasant pictures, $F(1,64) = 59, p < .0001$. Similarly, a main effect of hedonic content in the late window, $F(2,63) = 88, p < .0001$, replicated previous findings that pictures of erotica and violence prompt larger late pupil diameter than pictures of everyday scenes, $F_s(1,64) = 172$ and $70, p < .0001$, respectively; pictures of erotica also prompted larger pupil dilation than violent scenes, $F(1,64) = 4, p = .0001$. The interaction of content and old-new was not significant in either the early or late window.³

3. The interaction of content and repetition during explicit recognition was not close to significant ($F < 1$), but if separate tests are nonetheless conducted, the old-new difference when averaged across all old pictures for erotica is not significant. The difference in pupil diameter for old erotic scenes that were presented once and new scenes (see main text and Table 1), however, which is the typical old-new contrast in studies of recognition, is large and significant.

For late pupil change, however, a main effect of old/new,⁴ $F(1,64) = 18.3, p < .0001$, indicated that pupil diameter was significantly larger when correctly recognizing previously presented scenes, compared to new pictures that had never been presented before (see inset, Figure 1). Table 1 lists the mean change in late pupil diameter for correctly recognized old pictures as a function of whether these were originally viewed once or presented repeatedly with either massed or distributed repetition during encoding. A main effect of repetition, $F(2,62) = 3.7, p = .03$, was qualified by a Repetition \times Content interaction, $F(4,60) = 4.2, p = .005$, and followed up by assessing the simple main effects of repetition for each content. There was no evidence that pupil diameter was enhanced for pictures in which multiple repetitions were distributed across the encoding episode: Rather, for pictures of violence and everyday scenes, pupil diameter did not differ as a function of whether an old picture had been presented once or repeated with massed or distributed repetition (see Table 1).

For pictures of erotica, on the other hand, a significant main effect of repetition $F(2,62) = 7.1, p = .002$, indicated that repeated scenes elicited significantly smaller pupil diameter than pictures that were only seen once, whether repetitions were distributed, $F(1,63) = 14.4, p = .0003$, or massed during encoding, $F(1,63) = 8.3, p < .006$. In addition, whereas erotic scenes that had only been viewed once during encoding produced a large and significant old-new difference, $F(1,63) = 12.6, p = .0007$, recognizing erotic scenes that had been repeatedly presented, whether massed or distributed, did not show enhanced pupil diameter compared to viewing new erotic scenes during the recognition test (see Table 1).

Recognition performance. Table 1 lists reaction time and accuracy on the recognition test. Faster responses accompanied correct recognition of pictures that were initially viewed with distributed, compared to massed, repetition, $F(1,64) = 76, p < .0001$, consistent with a significant spacing effect (main effect of presentation, $F(2,62) = 106, p < .0001$); RT was also faster for massed repetitions compared to pictures seen only once during encoding, $F(1,63) = 58, p < .0001$. Pictures of erotica elicited overall slower RT than either pictures of everyday scenes, $F(1,63) = 97, p < .0001$, or violence, $F(1,63) = 78, p < .0001$, which did not differ from each other (content, $F(2,62) = 49, p < .0001$). For new pictures, RT was slower for emotional, compared to neutral scenes (content, $F(2,63) = 57, p < .0001$).

4. Indices of pupil response computed in previous studies to determine old-new pupil differences during recognition were also calculated here, averaging over specific picture content and repetition condition. Following Kafkas and Montaldi (2011) and Vö et al. (2008), peak pupil change was computed across the 3-s trial; the old-new difference was significant, $F(1,64) = 7.2, p = .01$. Following Naber et al. (2013), the slope of the initial constriction (300-1,000 ms) was computed for each trial; the old-new difference was not significant, $F(1,64) = 1.1, p = .30$. The difference in pupil diameter when viewing old and new scenes was also not significant when (a) computing the percent change for each sample across the viewing interval (3 s) by subtracting the baseline from each sample and then dividing by the baseline value and multiplying by 100 (Montefinese et al., 2013); (b) computing a pupil dilation ratio using the maximum pupil diameter in the final 2,750 ms of the trial and the maximum pupil in the initial 250 ms of the trial (Heaver & Hutton, 2011); or (c) computing a pupil dilation ratio using the maximum diameter in the last 250 ms of the trial and the first 250 ms of the trial (Otero et al., 2011). It is likely that some of these measures don't result in significant old-new differences when recognizing natural scenes because the measure was originally defined based on the pupillary response for words, which typically does not involve substantial early constriction.

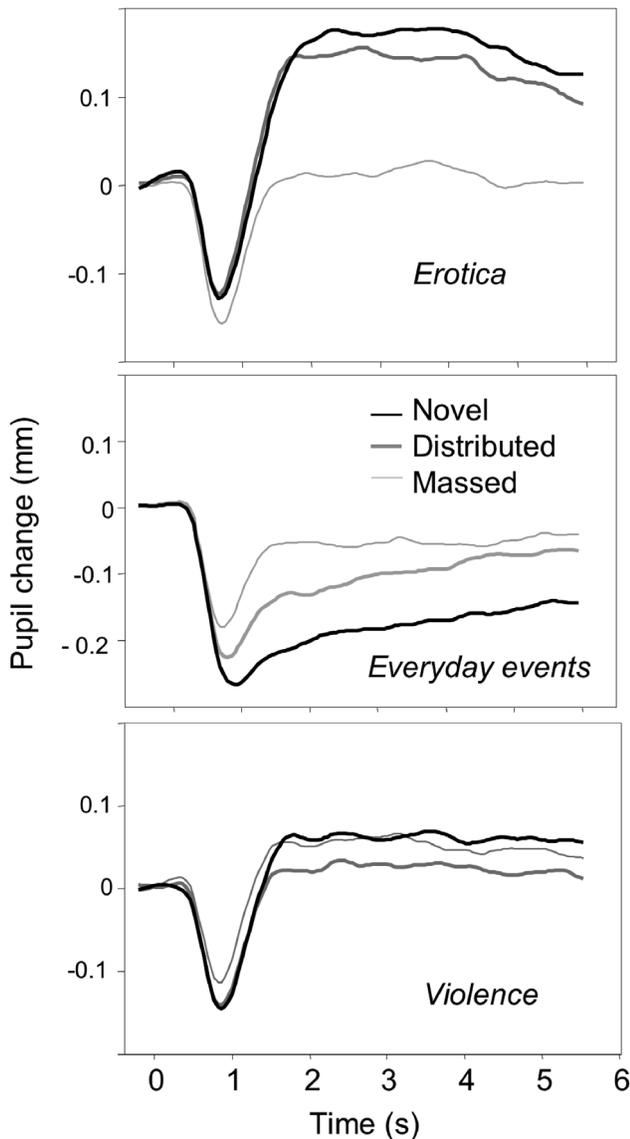


Figure 2. Waveforms showing changes in pupil diameter (deviated from a 1-s prepicture baseline) during initial free viewing of pictures that were novel or presented four times in a row (massed repetition) or spaced across the encoding phase (i.e., distributed repetition), separately for scenes depicting erotica (top), everyday scenes (middle), and violence (bottom).

Accuracy on the immediate recognition test was generally high (see Table 1). Compared to pictures presented once, accuracy was enhanced for scenes viewed with distributed repetition, $F(1,64) = 42$, $p < .0001$, as well as massed repetitions $F(1,64) = 30$, $p < .0001$,⁵ main effect $F(2,63) = 20.8$, $p < .0001$. Recognizing new pictures of erotica was slightly less accurate than recognizing novel neutral scenes, $F(1,64) = 20$, $p < .0001$, or scenes of violence, $F(1,64) = 4.98$, $p = .03$, which did not differ (content, $F(2,63) = 9.89$, $p = .0002$).

5. A marginal interaction of Content \times Repetition, $F(4,61) = 2.52$, $p = .06$, in accuracy suggested that differences due to repetition were not found when recognizing scenes of violence, which were recognized uniformly well.

Free Viewing

Figure 2 illustrates pupil diameter during initial free viewing for scenes that were novel and for scenes whose repetitions were massed or distributed; Table 1(bottom) lists early and late pupil changes during free viewing. In general, none of the early or late pupillary changes during initial free viewing are consistent with a hypothesis of enhanced diameter when viewing scenes whose repetitions were spaced (distributed) across the encoding phase, compared to when viewing massed repetitions or to novel scenes.

Thus, analysis of early constriction during free viewing resulted in a main effect of hedonic content, $F(2,53) = 59$, $p < .001$, replicating the differences found on the recognition test, as well as previously, in which, compared to neutral scenes, emotionally arousing scenes attenuate the initial light reflex (erotic: $F(1,64) = 69$, $p < .0001$; violent: $F(1,64) = 52$, $p < .0001$). In addition, as clearly illustrated in Figure 2, repetition did not significantly modulate the amplitude of the early light reflex for either pleasant or unpleasant scenes, whereas the amplitude of initial constriction varied significantly for neutral scenes, $F(2,63) = 5.8$, with novel pictures of everyday events prompting more initial constriction than repeated pictures, $F(1,64) = 8.1$, $p = .006$, and distributed repetitions prompting more constriction than massed repetitions, $F(1,64) = 6.8$, $p = .01$.

Analysis of the late pupil changes during free viewing resulted in a main effect of content, $F(2,63) = 154$, $p < .0001$, replicating previous studies showing enhanced late pupil diameter when viewing emotionally arousing, compared to everyday, scenes (erotica: $F(2,64) = 248$, $p < .0001$; violence: $F(1,64) = 137$, $p < .0001$). A significant interaction of Presentation \times Content, $F(4,61) = 9.5$, $p < .0001$, was not due to different effects of emotion when viewing novel or repeated scenes. Rather, as illustrated in Figure 3, emotionally arousing pictures of erotica and violence prompted significantly larger late pupil diameter than pictures of everyday scenes, regardless of whether pictures were novel or repeated (content, $F(2,63) = 154$, $p < .0001$; $F_s(2,63) = 72, 62, 31$, $ps < .0001$, for novel, distributed, and massed repetition, respectively; quadratic $F(1,64) = 129, 125, 65$, $ps < .0001$, for novel, massed, and distributed, respectively), and pictures of erotica elicited larger changes than pictures of violence, $F(1,64) = 8.1$, $p = .006$.

The significant interaction of Presentation \times Content for late pupil change was instead due to unique effects of repetition for each picture content, as illustrated in Figure 2. For erotica, $F(2,63) = 8.4$, $p < .001$, late pupil diameter was significantly attenuated for massed repetitions, compared to either novel picture viewing, $F(1,64) = 15.3$, $p < .0001$, or to distributed repetitions, $F(1,64) = 8.2$, $p = .005$, which did not differ from one another. For pictures of violence (Figure 2, bottom), on the other hand, pupil diameter did not differ whether pictures were novel or repeated, $F < 1$. For neutral pictures (Figure 2, middle), the pattern of modulation followed the pattern found during initial constriction, with the least late pupil dilation when viewing novel, compared to repeated, pictures (presentation: $F(2,62) = 10.3$, $p < .0001$; massed, novel: $F(1,62) = 17$, $p < .0001$; distributed, novel: $F(1,62) = 19$, $p < .0001$) and for distributed, compared to massed, repetitions, $F(1,62) = 5$, $p = .03$.

Discussion

During explicit recognition, late pupil diameter was larger for correctly recognized old pictures, compared to pictures that had never been seen before, regardless of hedonic content. This pupil old-new

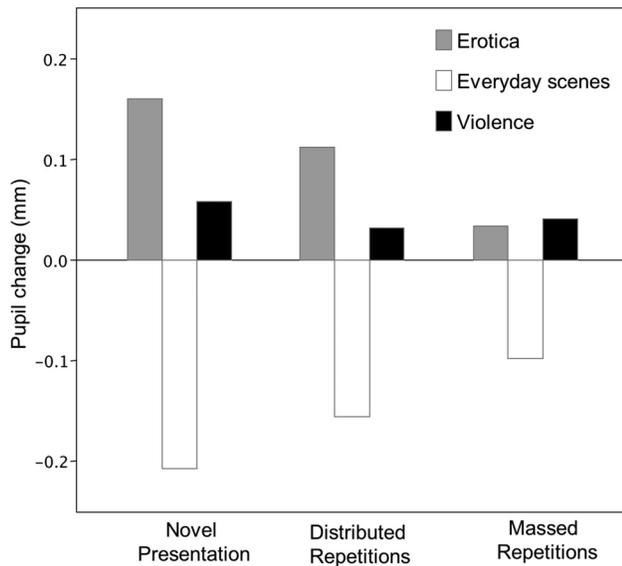


Figure 3. Mean late pupil change during initial free viewing shows enhanced pupil diameter when viewing scenes of erotica or violence, compared to scenes of everyday events, whether pictures were novel or repeated with massed or distributed repetition.

effect replicates previous studies reporting enhanced pupil diameter when recognizing old, compared to new, stimuli (e.g., Kafkas & Montaldi, 2001; Montefinese et al., 2013; Otero et al., 2011; Papesh et al., 2012; Vö et al., 2008). On the other hand, pupil diameter was not reliably enhanced when recognizing or viewing scenes whose repetitions were spaced across the encoding phase, a condition that improves memory and underlies the classic spacing effect (Ebbinghaus, 1885; see Godbole et al., 2014, for an overview) and which reliably prompts enhanced positivity in the ERP (Ferrari et al., 2013, in press). Rather, modulation of pupil diameter was not similar to differences found as a function of stimulus repetition in either explicit or implicit memory contexts when measuring neural activity.

Temporal Window of Old-New Differences

We assessed pupil change both early in the viewing interval, in the region of the initial light reflex (constriction), as well as later pupil diameter. During recognition, we did not find a significant old-new difference in either the amplitude or slope of initial constriction, whereas Naber et al. (2013) did report an old-new difference in the slope of initial constriction (300–1,000 ms after picture onset) when recognizing natural scenes. Inspection of Naber et al.'s (2013, Figure 4) waveforms, however, show that the difference in pupil diameter between old and new scenes begins very near to completion of the light reflex, and is then accompanied by larger pupil diameter for familiar, compared to novel, pictures that is sustained for up to 3 s following picture onset. This pattern is almost identical to the modulation found here, and taken together, the data suggest that old-new differences in pupil diameter when viewing natural scenes are maximal later, rather than early, in the viewing interval. This temporal window also agrees well with differences in pupil size found when recognizing words in which old-new differences tend to begin around 750 ms after stimulus and continue for a sustained duration (e.g., Vö et al., 2008). Because the late changes in pupil diameter are quite sustained, we found statistically similar old-new differences regardless of whether pupil change was averaged over

samples or peak change calculated, although an average is typically preferred as it does not rely on a single sampling point.

Repetition and Hedonic Content

None of the effects of repetition on pupil diameter during either explicit recognition or free viewing were consistent with the hypothesis of enhanced pupil diameter for scenes encoded in the context of distributed repetitions. Rather, repetition had different effects on pupil diameter, depending upon specific picture content. On the one hand, replicating our previous studies, not only did novel scenes that were emotionally arousing prompt smaller light reflexes (Henderson et al., 2014) and larger late dilation than neutral scenes (Bradley et al., 2008), but these differences continued to be found when scenes were repeated (either massed or distributed) during both recognition and free viewing, demonstrating remarkable stability of emotional modulation across a variety of experimental contexts. We have suggested that emotionally arousing scenes prompt a type of “natural selective attention” that occurs in the absence of instructions or task-relevance and reflects motivational activation (e.g., Bradley, 2009; Lang, Bradley, & Cuthbert, 1997).

Given the complex control of the pupil by both sympathetic and parasympathetic influences on the sphincter and dilator muscles, however, a continuing question is whether both the attenuated light reflex as well as the enhanced late dilation found for emotionally arousing scenes reflect the same underlying process. The differential effects of repetition on the early and late components of the pupillary response during free viewing could be taken as evidence for separate mechanisms. Thus, for instance, repetition of erotic scenes, whether massed or distributed, had no effect on initial constriction, whereas massed repetition resulted in greatly attenuated late pupil diameter. If late dilation reflects sympathetic nervous system activity (e.g., Bradley et al., 2008), such attenuation could indicate habituation of sympathetic arousal due to repetition. Relatedly, later recognition of repeated erotic scenes—whether massed or distributed—was also associated with attenuated late dilation, compared to recognizing pictures that had been viewed only once during encoding, which prompted a large old-new difference in pupil diameter. Accuracy, however, was also lowest for erotic scenes presented once, suggesting that task difficulty might play a role in enhancing late pupil dilation during recognition. The absence of any of these repetition effects on the initial light reflex for erotic scenes, however, suggests that the early modulation is resistant to both habituation and task effects.

Whereas repetition of erotic scenes had a number of modulatory effects on pupil diameter, repetition of scenes of violence did not affect pupil diameter during either recognition or free viewing. For these pictures, both early and late pupil changes were completely unaffected by repetition, consistently resulting in both an attenuated initial constriction and enhanced late diameter, compared to viewing neutral scenes. Although these data suggest slower or no habituation of sympathetic arousal for scenes of violence, novel erotic scenes also prompted significantly greater late pupil dilation (but not early constriction) than novel scenes of violence, and it is possible that the attenuation of late dilation for erotic scenes with repetition primarily reflects greater initial arousal for novel erotic pictures. Thus, for instance, for massed repetitions, pupillary changes when viewing erotic and violent scenes did not differ during either recognition or free viewing in either time window.

Repetition also had no effect on early or late pupil diameter when recognizing scenes of everyday events that had been

presented previously. Rather, during free viewing only, a large initial constriction was found for novel pictures of everyday events that was most attenuated for massed repetitions, which is a difficult pattern to interpret. These differences in initial constriction, however, are not consistent with a hypothesis of larger diameter when viewing distributed repetitions, as massed, rather than distributed, repetitions showed the greatest difference from novel picture processing. Regardless of the ultimate explanation for this pattern of modulation during free viewing of neutral scenes, it is important to note that differential early constriction occurs in a context in which the identical sensory/perceptual stimulus is viewed at each repetition, making explanations based on differences in brightness, contrast, or other sensory features of the picture itself less probable.

Is Pupil Diameter An Index of Memory?

Taken together, the lack of pupil enhancement for items presented with distributed repetition during both explicit recognition and incidental free viewing are very different from repetition effects found when measuring old-new ERPs, suggesting that, at the least, these autonomic and neurophysiological measures are not identically sensitive to factors that affect memory. This, of course, raises the question of whether pupil diameter during recognition is specifically indexing prior occurrence, or is perhaps related to some other task variable. Previous studies investigating memory using pupillary measures have produced mixed results with respect to the issue of whether the old-new difference in pupil diameter is an accurate measure of memory strength. Supporting its interpretation as a marker of memory, Heaver and Hutton (2011) found enhanced pupil diameter for old, compared to new, words regardless of whether participants were instructed to respond old-new (standard instructions) or to respond to all items as new, prompting them to conclude that the pupil is an accurate measure of memory, and Papesh et al. (2012) found greater pupil enhancement for words spoken in the same, compared to different, voice at test, consistent with the better memory typically found for items that match at study and test (e.g., encoding specificity principle, Tulving & Thomson, 1973).

On the other hand, both Montefinese et al. (2013) and Otero et al. (2011) found enhanced pupil diameter for false alarms—new words mistakenly identified as old—leading Montefinese et al. to propose that pupil diameter does not index memory strength, but, rather, is related to subjective judgments regarding prior occur-

rence. When pupil diameter at encoding is used to predict later memory performance (“subsequent memory”), reports include the expected finding that increased dilation at encoding is associated with better memory for words (Papesh et al., 2012), but also that there is no relationship between pupil dilation at encoding and later memory for words (Võ et al., 2008). When pupil diameter was measured during the encoding of pictures of natural or man-made objects (Kafkas & Montaldi, 2011), larger pupil constriction, rather than dilation, at encoding predicted better memory, inconsistent with the findings of both Papesh et al. (2012) and Võ et al. (2008) using words. The lack of repetition enhancement in pupil diameter found here for scenes whose presentations were spaced across the encoding phase provides additional data that are inconsistent with a hypothesis that pupil diameter measures memory.

Summary

On the one hand, like the old-new ERP, late pupil dilation was significantly enhanced when correctly recognizing old, compared to new, pictures on an explicit recognition test. On the other hand, pupil diameter was not enhanced for scenes presented with distributed, compared to massed, repetition during either explicit recognition or free viewing—contexts in which both ERPs and fMRI show unique modulation for distributed repetitions that are consistent with a hypothesis of episodic retrieval. Although it is not surprising that an autonomic measure, such as pupil dilation, differs from brain-based measures of memory, initial reports of an old-new difference in pupil diameter led some to propose that the pupil may be a simple, easier to implement and to analyze measure that could serve as a proxy for more difficult and time-consuming brain-based measures (e.g., Goldinger & Papesh, 2012; Naber et al., 2013; Võ et al., 2008). The current data do not strongly encourage such a proposal. Rather, it is possible that pupil dilation during recognition may reflect additional task-related factors such as difficulty, similarity, subjective confidence, or other aspects of an explicit recognition task. Most generally, during both recognition and free viewing, the effects of repetition and emotion showed distinct modulatory patterns representing the coordinated control of pupil diameter by parasympathetic and sympathetic activity. In this complex servo-motor system, pupil diameter during recognition reflects the net effect of multiple influences on autonomic nervous system activity, and is not a straightforward index of memory strength or prior occurrence.

References

- Barbur, J. L. (2004). Learning from the pupil: Studies of basic mechanisms and clinical applications. In L. M. Chalupa and J. S. Werner (Eds.), *The visual neurosciences* (pp. 641–656). Cambridge, MA: MIT Press.
- Beatty, J. (1982). Task-evoked pupillary responses, processing load, and the structure of processing resources. *Psychological Bulletin*, *91*, 276–292.
- Bradley, M. M. (2009). Natural selective attention: Orienting and emotion. *Psychophysiology*, *46*, 1–11.
- Bradley, M. M., Costa, V. D., Ferrari, V., Codispoti, M., Fitzsimmons, J. R., & Lang, P. J. (2014). Imaging distributed and massed repetitions of natural scenes: Spontaneous retrieval and maintenance. *Human Brain Mapping*. Advance online publication. doi: 10.1002/hbm.22708
- Bradley, M. M., Miccoli, L., Escrig, M. A., & Lang, P. J. (2008). The pupil as a measure of emotional arousal and autonomic activation. *Psychophysiology*, *45*, 602–607. doi: 10.1111/j.1469-8986.2008.00654.x
- Ebbinghaus, H. (1885). *Memory: A contribution to experimental psychology*. New York, NY: Columbia University Press.
- Ferrari, V., Bradley, M. M., Codispoti, M., Karlsson, M., & Lang, P. J. (2013). Repetition and brain potentials when recognizing natural scenes: Task and emotion differences. *Social, Cognitive, Affective Neuroscience*, *8*, 847–854.
- Ferrari, V., Bradley, M. M., Codispoti, M., & Lang, P. J. (2015). Massed and distributed repetition of natural scenes: Brain potentials and oscillatory activity. *Psychophysiology*. Advance online publication. doi: 10.1111/psyp.12424
- Glenberg, A. M. (1979). Component-levels theory of the effects of spacing of repetitions on recall and recognition. *Memory & Cognition*, *7*, 95–112.
- Godbole, N. R., Delaney, P. F., & Verkoijen, P. P. J. L. (2014). The spacing effect in immediate and delayed free recall. *Memory*, *22*, 462–469. doi: 10.1080/09658211.2013.798416
- Goldinger, S. D., & Papesh, M. H. (2012). Pupil dilation reflects the creation and retrieval of memories. *Current Directions in Psychological Science*, *21*, 90–95. doi: 10.1177/0963721412436811
- Greene, R. L. (1989). Spacing effects in memory: Evidence for a two-process account. *Journal of Experimental Psychology: Learning, Memory, and Cognition*, *15*, 371–377.

- Heaver, B., & Hutton, S. B. (2011). Keeping an eye on the truth? Pupil size changes associated with recognition memory. *Memory, 19*, 398–405. doi: 10.1080/09658211.2011.575788
- Henderson, R. Bradley, M. M., & Lang, P. J. (2014). Modulation of the initial light reflex during affective picture viewing. *Psychophysiology, 51*, 815–818.
- Kafkas, A., & Montaldi, D. (2011). Recognition memory strength is predicted by pupillary responses at encoding while fixation patterns distinguish recollection from familiarity. *Quarterly Journal of Experimental Psychology, 64*, 1971–1989. doi: 10.1080/17470218.2011.588335
- Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1997). Motivated attention: Affect, activation, and action. In P. J. Lang, R. F. Simons, & M. Balaban (Eds.), *Attention and orienting* (pp. 97–135). Mahwah, NJ: Erlbaum.
- Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (2008). *International affective picture system (IAPS): Affective ratings of pictures and instruction manual. Technical Report A-8*. University of Florida, Gainesville, FL.
- Loewenfeld, I.E. (1999). *The pupil: Anatomy, physiology, and clinical applications*. Boston, MA: Butterworth and Heinemann.
- Montefinese, M., Ambrosini, E., Fairfield, B., & Mammarella, N. (2013). The “subjective” pupil old/new effect: Is the truth plain to see? *International Journal of Psychophysiology, 89*, 48–56.
- Naber, M., Frassle, S., Rutishauser, U., & Einhauser, W. (2013). Pupil size signals novelty and predicts later retrieval success for declarative memories of natural scenes. *Journal of Vision, 13*, 1–20. doi: 10.1167/13.2.11.
- Otero, S. C., Weekes, B. S., & Hutton, S. B. (2011). Pupil size changes during recognition memory. *Psychophysiology, 48*, 1346–1353. doi: 10.1111/j.1469-8986.2011.01217.x
- Papesh, M. H., Goldinger, S. D., & Hout, M. C. (2012). Memory strength and specificity revealed by pupillometry. *International Journal of Psychophysiology, 83*, 56–64. doi: 10.1016/j.ijpsycho.2011.10.022
- Rugg, M. D., & Curran, T. (2007). Event-related potentials and recognition memory. *Trends in Cognitive Science, 11*, 251–257.
- Steinhauer, S., Siegle, G., Condray, R., & Pless, M. (2004). Sympathetic and parasympathetic innervation of pupillary dilation during sustained processing. *International Journal of Psychophysiology, 52*, 77–86.
- Tulving, E., & Thomson, D. (1973). Encoding specificity and retrieval processes in episodic memory. *Psychological Review, 80*, 352–373.
- Vö, M. L., Jacobs, A. M., Kuchinke, L., Hofmann, M., Conrad, M., Schacht, A., & Hutzler, F. (2008). The coupling of emotion and cognition in the eye: Introducing the pupil old/new effect. *Psychophysiology, 45*, 130–140.

(RECEIVED October 13, 2014; ACCEPTED March 12, 2015)