Pupillometry as a Glimpse into the Neurochemical Basis of Human Memory Encoding

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Abstract

■ Neurochemical systems are well studied in animal learning; however, ethical issues limit methodologies to explore these systems in humans. Pupillometry provides a glimpse into the brain's neurochemical systems, where pupil dynamics in monkeys have been linked with locus coeruleus (LC) activity, which releases norepinephrine (NE) throughout the brain. Here, we use pupil dynamics as a surrogate measure of neurochemical activity to explore the hypothesis that NE is involved in modulating memory encoding. We examine this using a task-irrelevant learning paradigm in which learning is boosted for stimuli temporally paired with task targets. We show that participants better

recognize images that are paired with task targets than distractors and, in correspondence, that pupil size changes more for target-paired than distractor-paired images. To further investigate the hypothesis that NE nonspecifically guides learning for stimuli that are present with its release, a second procedure was used that employed an unexpected sound to activate the LC–NE system and induce pupil-size changes; results indicated a corresponding increase in memorization of images paired with the unexpected sounds. Together, these results suggest a relationship between the LC–NE system, pupil-size changes, and human memory encoding. ■

INTRODUCTION

Converging evidence from animal research and theoretical models (Seitz & Dinse, 2007; Yu & Dayan, 2005; O'Doherty, Dayan, Friston, Critchley, & Dolan, 2003; Hassani, Cromwell, & Schultz, 2001) suggest a key role of neurochemicals like dopamine (Schultz, 2002; Bao, Chan, & Merzenich, 2001), acetylcholine (Thiel, Friston, & Dolan, 2002; Kilgard & Merzenich, 1998; Bakin & Weinberger, 1996), and norepinephrine (NE; Witte & Marrocco, 1997; Gordon, Allen, & Trombley, 1988; Bear & Singer, 1986) in the plasticity underlying learning; yet, little is known about their role in humans. The study of neurochemical involvement in human learning is difficult because invasive methods are required to directly measure neurochemical release.

However, noninvasive measures such as pupillometry can provide clues into neurochemical activity. For example, pupil size changes (PSCs) have been suggested as surrogate measure of locus coeruleus (LC) activity and its release of NE (Nassar et al., 2012). This relationship is primarily supported by reports of a coupling of activity in the monkey LC and pupil dilation (Aston-Jones, 2005; Rajkowski, Kubiak, & Aston-Jones, 1993). However, recent studies in humans show that PSCs were positively associated with a learning rate (Silvetti, Seurinck, van Bochove, & Verguts, 2013; Nassar et al., 2012) or increased task performance (Murphy, Robertson, Balsters, & O'Connell, 2011). These

data provide evidence that pupil dynamics are related to learning and are consistent with the hypothesized role of NE–LC activity in driving pupil-size dynamics and learning.

Here, we explore the hypothesis that NE causes learning for stimuli present during its release regardless of their relevance to the inducing stimuli (Seitz & Watanabe, 2005, 2009). We explore this hypothesis by examining pupillometry in the context of task-irrelevant learning (TIL), in which learning occurs without attention being directed to the learned stimuli (Seitz & Watanabe, 2003, 2009; Watanabe, Nanez, & Sasaki, 2001). TIL is a robust learning phenomenon with demonstrations ranging from critical flicker fusion thresholds (Seitz, Nanez, Holloway, & Watanabe, 2005, 2006), motion (Watanabe et al., 2002), orientation processing (Nishina, Seitz, Kawato, & Watanabe, 2007), contour integration (Rosenthal & Humphreys, 2010), phonetic processing (Vlahou, Seitz, & Protopapas, 2009), and memory encoding (Leclercq, Le Dantec, & Seitz, 2013). Seitz and Watanabe (2005) suggested a model of perceptual learning where learning results from interactions between spatially diffusive task-driven signals (such as NE) and bottom–up stimulus signals.

Although TIL was discovered in low-level perceptual learning (Seitz & Watanabe, 2003; Watanabe et al., 2001), recent research of fast TIL (Leclercq & Seitz, 2012a, 2012b, 2012c, 2012d; Lin, Pype, Murray, & Boynton, 2010; Swallow & Jiang, 2010) shows increased memorization of images even after a single pairing with a target of a target detection task. We note that "task irrelevant" in the context of fast TIL is used to maintain consistent

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terminology with prior works on the topic and refers to the fact that the memorized images have no predictive relationship to presentation of targets of the target detection task nor are the targets informative of which scene will be tested in the scene-recognition task. Fast TIL-like effects have also been shown in other studies, such as in Krebs, Boehler, De Belder, and Egner (2013) where increased recognition was found for faces paired with incongruent words.

Here, we adopt the fast-TIL paradigm and examine the relationship between pupil dynamics and image memorization. We find that larger PSCs correspond with improved image memorization through TIL. To further test the TIL model, we conduct a second study in which novel sounds (following methods of Nassar et al., 2012, and Seitz & Watanabe, 2009) are paired with images to induce NE release. Results of this study are consistent with the first showing increased PSCs and increased memorization for those images. These results suggest that TIL is a basic mechanism of learning in the brain and is a useful tool by which to understand the involvement of neurochemical signals in learning independent of task-related processing.

METHODS

Participants

Seventy participants were included, 43 in Experiment 1 and 27 in Experiment 2. All participants had normal or corrected-to-normal visual acuity and received course credit for the 1.5-hr session. Participants were excluded because of excessive eye movement (12 in Experiment 1 and 7 in Experiment 2). This left a final sample of 31 participants (19.28 ± 1.4 years old; 20 women, 11 men) in Experiment 1 and 20 participants (19.85 ± 1.5 years old; 9 women, 11 men) in Experiment 2. All participants gave written informed consent, as approved by the University of California-Riverside human research review board.

Apparatus and Stimuli

An Apple Mac Mini running Matlab (Mathworks, Natick, MA) and Psychophysics Toolbox Version 3.0.8 (Brainard, 1997; Pelli, 1997) were used for stimulus generation and experiment control. Stimuli were presented on a ViewSonic PF817 (Walnut, CA) monitor with resolution of 1600 × 1200 and a refresh rate of 100 Hz. Display items consisted of 2112 scenes depicting natural or manmade environments to ensure that every trial contained a unique set of images. Scenes were obtained from the Massive Memory database (Konkle, Brady, Alvarez, & Oliva, 2010) at 256 × 256 pixels of resolution and then up-sampled and presented at 768 × 768 pixels (18.3° of visual angle). In Experiment 2, environmental sounds were obtained from an online library (Marcell, Borella, Greene, Kerr, & Rogers, 2000). We extracted the first 133 msec of each sound and played them at a sampling rate of 22 kHz through Sennheiser HD 202 headphones (Wedemark, Germany), which have a frequency range of 18 Hz–18 kHz. The background for all displays was gray (luminance of 10 cd/m²). Participants sat with their eyes approximately 57 cm from the screen using the Eyelink 1000 tower mount (Mississauga, Canada), which was used to track eye movement and pupil size fluctuations of the right eye during every 10-msec screen refresh. The eye tracker measured pupil diameter with a resolution of 0.2% of diameter, corresponding to a resolution of 0.01 mm for a 5-mm pupil, and has a spatial resolution of <0.01° root mean squared (SR Research, Ltd., 2005). Pupil dynamics are known to be influenced by luminance levels (Winn, Whitaker, Elliott, & Phillips, 1994); thus, each scene was matched to the average luminance distribution of the 2112 scenes using the histMatch function of the SHINE toolbox to control for luminance fluctuations across the image set (Willenbockel et al., 2010).

Procedure and Design

At the start of the experiment, a randomized target 9-point calibration and validation routine was performed using the EyeLink 1000 software to calibrate gaze and ensure accuracy of pupil dynamic readings.

Each trial consisted of an eight-scene Rapid Serial Visual Presentation (RSVP) stream, each presented for 133 msec followed by an ISI (blank gray screen) of 1000 msec (Figure 1). Targets or distractors were randomly selected each trial and presented within a gray aperture presented in the middle of each scene. Each participant performed 120 trials of the main procedure (described below). Each trial began with the message, "Blink! Whenever you are prepared to not blink press any key to continue," to ensure participants were prepared for each trial. Fifteen blocks of eight trials each were separated at minimum by 10-sec breaks. If a participant blinked or moved their eyes more than 1.5° from fixation during the RSVP stream, they were alerted with the message "Please refrain from blinking or moving your eyes," and the trial was replaced with a new trial containing a novel set of images. Before each trial started, participants were required to maintain fixation on a central red dot (0.1° of visual angle) for a random period of 300–600 msec. To ensure that participants could conduct the task, they performed a practice block of eight trials before initiating the main procedure.

In Experiment 1, participants were instructed to complete two tasks (target detection and then scene recognition) in each trial. The target detection task was on the RSVP stream of alphanumeric characters: targets (numbers 1, 2, or 3) and distractors (A, B, C, D, E, F, G, H, J, K, L, M, N, P, Q, R, S, T, U, or V). For the target detection task, participants had 1500 msec to report the target identity via the number pad keys "0" (if no target presentation), "1," "2," or "3." For the scene recognition task, participants were presented with two side-by-side scenes for 3000 msec and reported which scene had appeared in that trial via the number pad keys "1" or "2."

Figure 1. Cartoon of trial structure. Each scene was presented for 133 msec with an ISI of 1000 msec; eye tracking is recorded every 10 msec. (A) In Experiment 1, each scene could either be distractor paired (DP) or target paired (TP). For the target detection task, after the RSVP stream, participants reported the number if presented or 0 if not. In the scene detection task, participants reported which of the two images was shown in that trial. The test scene could either be a DP or TP scene. (B) In Experiment 2, each scene could be a repeat-sound-paired (RS) scene or a novel-sound-paired (NS) scene. The target detection task was removed, but the scene recognition task remained the same.
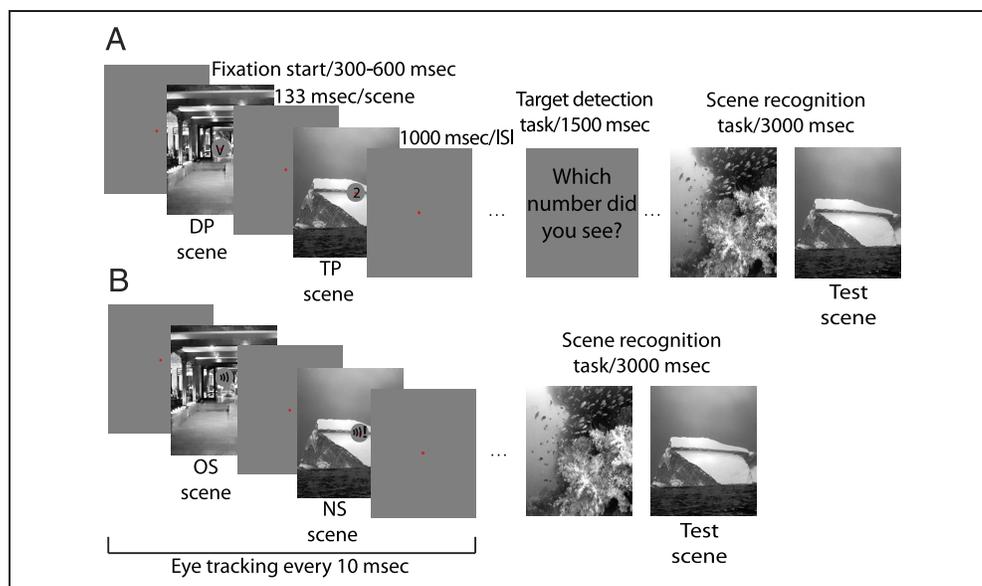
In Experiment 2, alphanumeric characters were replaced with environmental sounds, which were randomly selected from a list of 97 sounds. Participants were told that the sounds were irrelevant to the scene recognition task, which was their single task. In the place of targets, a sound change occurred (novel sound); in place of distractors, one repeating sound (repeat sound) was used. The trial retained the same statistical properties of Experiment 1.

In both experiments, three types of trial conditions were utilized: distractor ($n = 48$), target ($n = 48$), and catch ($n = 24$) trials. In distractor (repeat sound) trials, no target was shown, and one random scene was tested in the scene recognition task. In target (novel sound) trials, the target-paired scene was tested. In catch trials, a target was presented, and one of the distractor-paired (repeat sound) scenes was tested. Catch trials were introduced so that participants could not predict when a target would occur or which scene would be tested. Tested scenes and target presentation were counterbalanced across Item Positions 3–6 to avoid primacy and recency effects.

Data Analysis

We calculated accuracy on the target detection task and the scene recognition task by dividing the number of correct trials by total number of responses made for each task, excluding missed trials. In Experiment 1, 7.74 ± 0.93% (serr) of trials were missed in the target detection task and 9.11 ± 0.96% in the scene recognition task. In Experiment 2, 13.67 ± 2.54% were missed in the scene recognition task.

For the analysis of pupil dynamics, pupil diameter was normalized by dividing each data point by each participants' pupil-size session mean. Analysis of pupil size only examined target and distractor trials and excluded trials with eye movements during the RSVP scene stream (6.77 ± 0.47%) resulting in inclusion of 90 trials on average per participant. Baseline pupil size (BPS) was defined as the trough-to-trough mean of pupil size before stimulus presentation. This period was used because it provides an estimate of the pupil size immediately before the stimulus of interest; however, we note that it is not a stable baseline, because of the influence of processing the previous image. Alternative choices of baselines were more problematic because they were less temporally proximal to the stimulus of interest and eye movements and blinks contaminated pretrial intervals. PSC was defined as the difference between the trough and peak of pupil size after stimulus presentation (Figure 2).
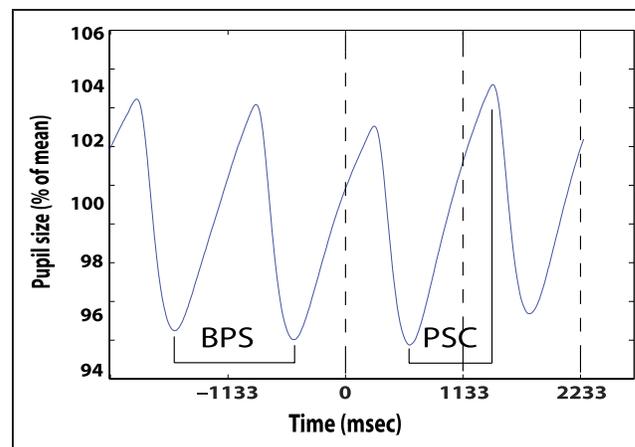

Figure 2. Quantification of pupil dynamics. BPS was defined as the average of the period denoted by BPS. PSC was defined as the difference from the peak minus the trough denoted by PSC.

RESULTS

Experiment 1: TIL

To evaluate whether our procedure led to TIL, we examined memorization rates in the scene recognition task (Figure 3). As hypothesized, accuracy (Figure 3A) for target-paired scenes (73.41 ± 1.50% SEM) was significantly greater than that for distractor-paired scenes (63.94 ± 1.16%), $t(30) = 3.7534, p = .0004$. Likewise, RTs were significantly faster for target-paired (1101 ± 13.2 msec) compared with distractor-paired (1147 ± 8.2 msec) scenes, $t(30) = -2.1947, p = .0179$ (Figure 3B). These increased memorization rates and faster RTs for target-paired scenes over distractor-paired scenes confirm a TIL effect.

Pupillometry of TIL

A key question is whether there is a relationship between TIL and PSCs during task performance (Figure 4A). We observed that participants showed a larger PSC (Figure 4B) for target-paired scenes (6.01 ± 0.38%) over distractor-paired scenes (4.33 ± 0.35%), $t(30) = 9.5410, p < .0001$, which is consistent with our hypothesis that NE release is associated with target processing. On the other hand, we found no BPS ($t(30) = -1.1237, p = .8650$) differences (Figure 4C) between target-paired scenes (99.91 ± 0.23%) and distractor-paired scenes (100.26 ± 0.18%).

Although we failed to find a relationship between BPS and TIL, previous studies (e.g., Murphy et al., 2011) have shown that BPS is related to performance outcomes. To test for this, we examined pupil size and PSCs as a function of performance outcome (correct and incorrect; Figure 4D). Consistent with previous findings, participants showed significantly lower ($t(30) = -2.1386; p = .0407$,

two tailed) BPS (Figure 4F) for correct trials (99.84 ± 0.20%) than incorrect trials (100.44 ± 0.24%). On the other hand, we failed to find any significant differences ($t(30) = -0.9283, p = .3607$) between PSCs (Figure 4E) and correct (5.09 ± 0.35%) versus incorrect (5.22 ± 0.39%) scenes.

These results support a dissociation between PSCs as regulating TIL and baseline pupil in regulating overall performance, with lower BPS leading to more accurate responses and greater target-related PSCs leading to TIL.

Experiment 2: Memory Enhancement because of Alerting Sounds

Although the results of Experiment 1 are consistent with our hypothesis for the role of NE in TIL, the dual task in Experiment 1 may recruit processes that may impact pupil dilation and thus confound the results. Thus, to find independent evidence of the role of NE in TIL, we adopted an approach suggested by Nassar et al. (2012), where alerting sounds played as task-irrelevant stimuli drove learning on a numerical inference task. This is consistent with Seitz and Watanabe's model of TIL, which predicts that rewarding or alerting signals are sufficient to induce TIL (Seitz & Watanabe, 2005); this is also consistent with research that unexpected stimuli have been shown to phasically drive LC neurons (Sara & Bouret, 2012) and pupil dynamics (Murphy et al., 2011). We thus hypothesized that unexpected (alerting) sounds would lead to larger PSCs than expected sounds and that these alerting sounds would also lead to greater memorization of the paired scenes. To test this hypothesis, we replaced the target detection task with a stream of task-irrelevant sounds, where one sound (repeat sound) replaced distractors and a set of novel sounds replaced targets (Figure 1B).
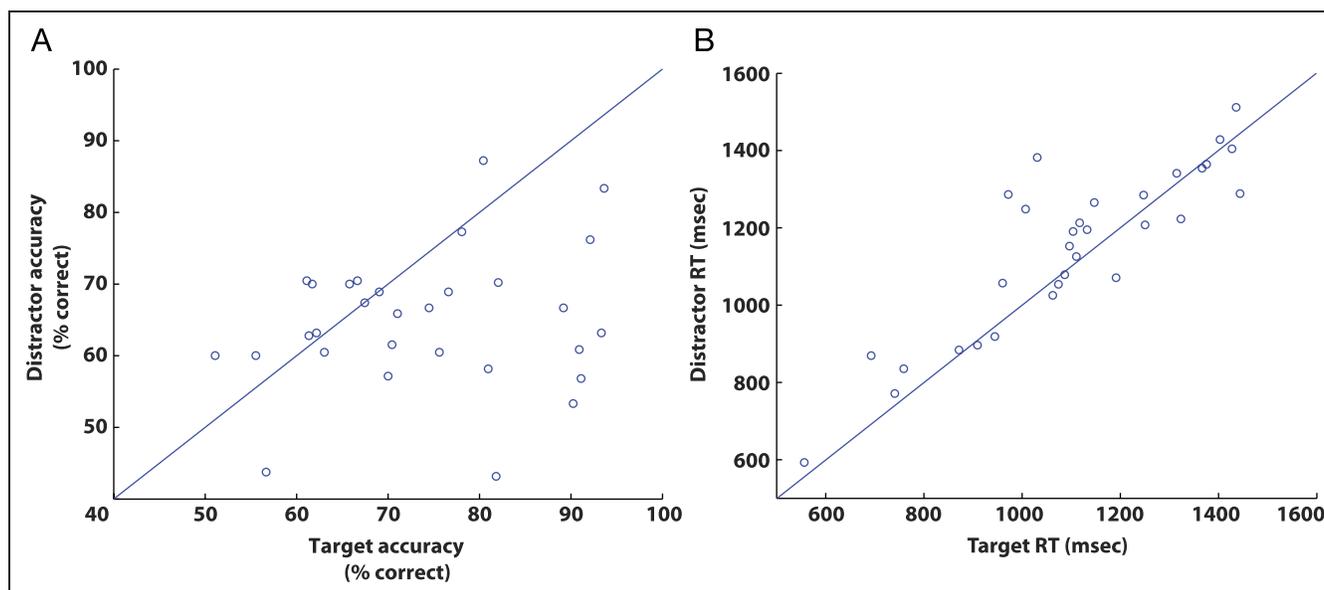

Figure 3. Scene recognition task performance. Participants exhibited (A) increased accuracy for target-paired scenes over distractor-paired scenes and (B) decreased RTs for target-paired scenes compared with distractor-paired scenes.
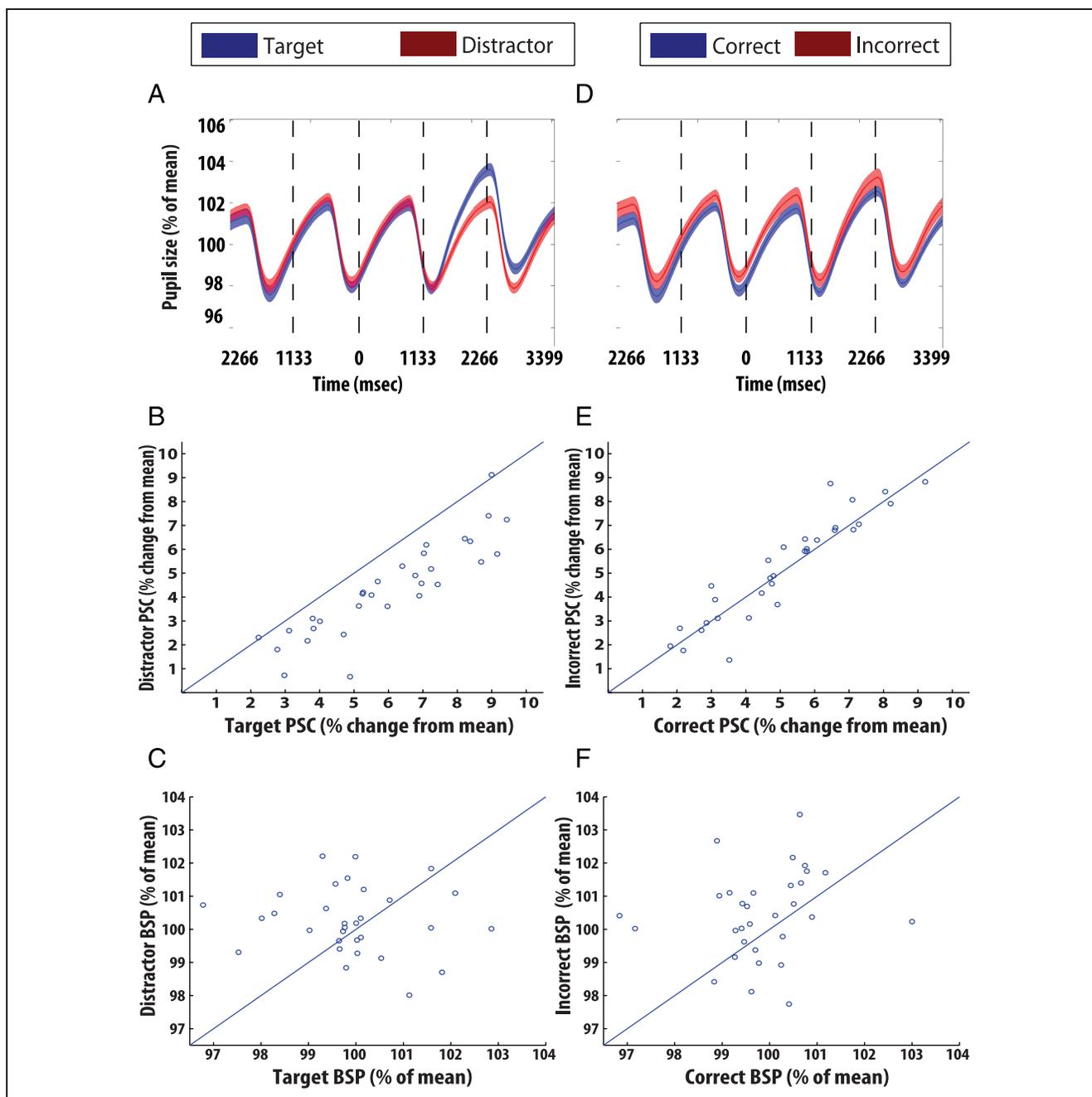
Figure 4. Pupil dynamics in Experiment 1. (A, D) Pupil size fluctuates with each stimulus presentation with 0 representing the onset of the to-be-tested target- or distractor-paired scene. Dashed lines represent times of stimulus onset. (B) Significant *t* tests indicated that targets compared with distractors induced larger PSCs, whereas (C) no differences in BPS were observed. (E) No difference was found in stimulus-induced PSC between correct and incorrect trials. (F) Correct trials show a lower BPS than incorrect trials. Shading (A, D) represents within-subject standard error (Loftus & Masson, 1994).

Results on the scene memorization task showed significantly increased accuracy for novel-sound-paired scenes (73.35 ± 1.38%) compared with repeat-sound-paired scenes (69.15 ± 1.15%; *t*(19) = 1.84, *p* = .039; Figure 5A) and also significantly faster RT (*t*(19) = −2.85, *p* = .0051) for novel-sound-paired scenes (1213 ± 14.4 msec) compared with repeat-sound-paired scenes (1291 ± 14.2 msec; Figure 5B). This significantly increased

memorization and faster RT for novel-sound-paired scenes over repeat-sound-paired scenes suggest a TIL effect.

Pupillometry because of Alerting Sounds

Examination of pupil-size dynamics revealed significantly increased PSC for novel-sound-paired scenes (4.74 ± 0.15%) compared with repeat-sound-paired scenes (3.67 ± 0.13%;

$t(19) = 4.2147, p = .0002$; Figure 6B) and no difference ($t(19) = 0.913, p = .19$) between BPS for novel-sound-paired scenes (99.93 ± 0.21%) compared with repeat-sound-paired scenes (99.66 ± 0.21%; Figure 6C). These replicate the pattern of results seen for TIL.

Interestingly, we also found a relationship between performance outcome (Figure 6D) and pupil changes, with significantly ($t(19) = 1.99, p = .031$) increased PSCs during the to-be-tested scene that was correct (4.12 ± 0.06%) compared with incorrect (3.71 ± 0.15%; Figure 6E). However, no difference ($t(19) = 0.873, p = .20$) between BPS before scenes that were correct (99.97 ± 0.12%), compared with incorrect (99.97 ± 0.31%; Figure 6F). Although these results are different than those of the first experiment, the dynamics of the PSCs were different in this experiment, with a faster initial rise in pupil size and prolonged elevation of pupil size after the novel sounds. Furthermore, repeat sounds may have had a different impact on PSCs than the distractors in Experiment 1. Although these differences between experiments are interesting and warrant further study, they do not strongly bear on our central observation that novel sounds led to a TIL-like effect.

Prolonged Effects of Alerting

As stated above, the novel sounds produced a prolonged change in pupil size that persisted into the stimulus presentation periods after that of the novel sound. This prolonged effect is consistent with subjective observations of a prolonged "arousal/alerting effect" after the novel sounds, which was experientially different from a more stayed effect after the targets in Experiment 1. We hypothesized that a component of this effect was because of the prolonged release of NE that would in turn lead to improved memorization for scenes presented after the novel sounds compared with scenes presented before the novel sounds. To test this hypothesis, we examined performance in trials in which the tested scene was presented earlier or later in the image sequence relative to the novel sound.

Results of the scene recognition task revealed a significant main effect of Accuracy as a function of Trial Type [pre-novel sound, novel sound, post-novel sound] with pre-novel-sound-paired scenes having significantly ($F(2, 38) = 4.78, p = .014$, one-way ANOVA) lower accuracy (66.12 ± 4.04%; Figure 7A) and significantly slower ($F(2, 38) = 58.702, p < .0001$) RTs (1340 ± 23.5 msec; Figure 7B) compared with novel-sound-paired scenes (73.35 ± 1.38%; 1213 ± 14.4 msec) and post-novel-sound-paired scenes (79.95 ± 3.10%; 1264 ± 28.1 msec). These results show that novel sounds not only benefited the paired scene but also had a prolonged effect that led to increased accuracy and decreased RTs for scenes presented after the novel sound.

DISCUSSION

Our results show that pupil-size dynamics are associated with increased memorization of images. In Experiment 1, PSC was related to the performance advantage that arises from target processing in a standard TIL paradigm. Experiment 2 further tested this model by testing whether novel sounds, which also stimulate the LC, would lead to TIL. Consistent with the model, novel sounds led to increased PSCs and learning. Together, these data support the hypothesis that NE release guides human memory.

These results are consistent with a model of TIL put forth by Seitz and Watanabe (2005) where "phasic" activation of the LC, which results in NE release throughout the nervous system (Bouret & Sara, 2005; Harley, 1987, 2004;
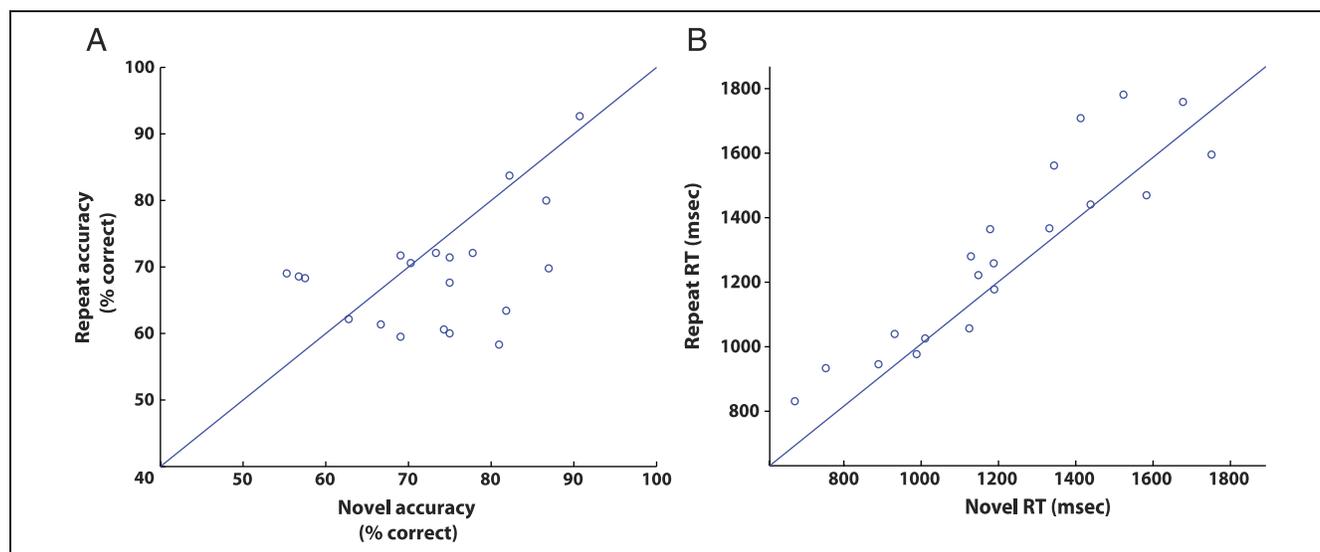

Figure 5. Scene recognition task performance. Participants exhibited (A) increased accuracy for novel-sound-paired scenes over repeat-sound-paired scenes and (B) decreased RT for novel-sound-paired scenes compared with repeat-sound-paired scenes.
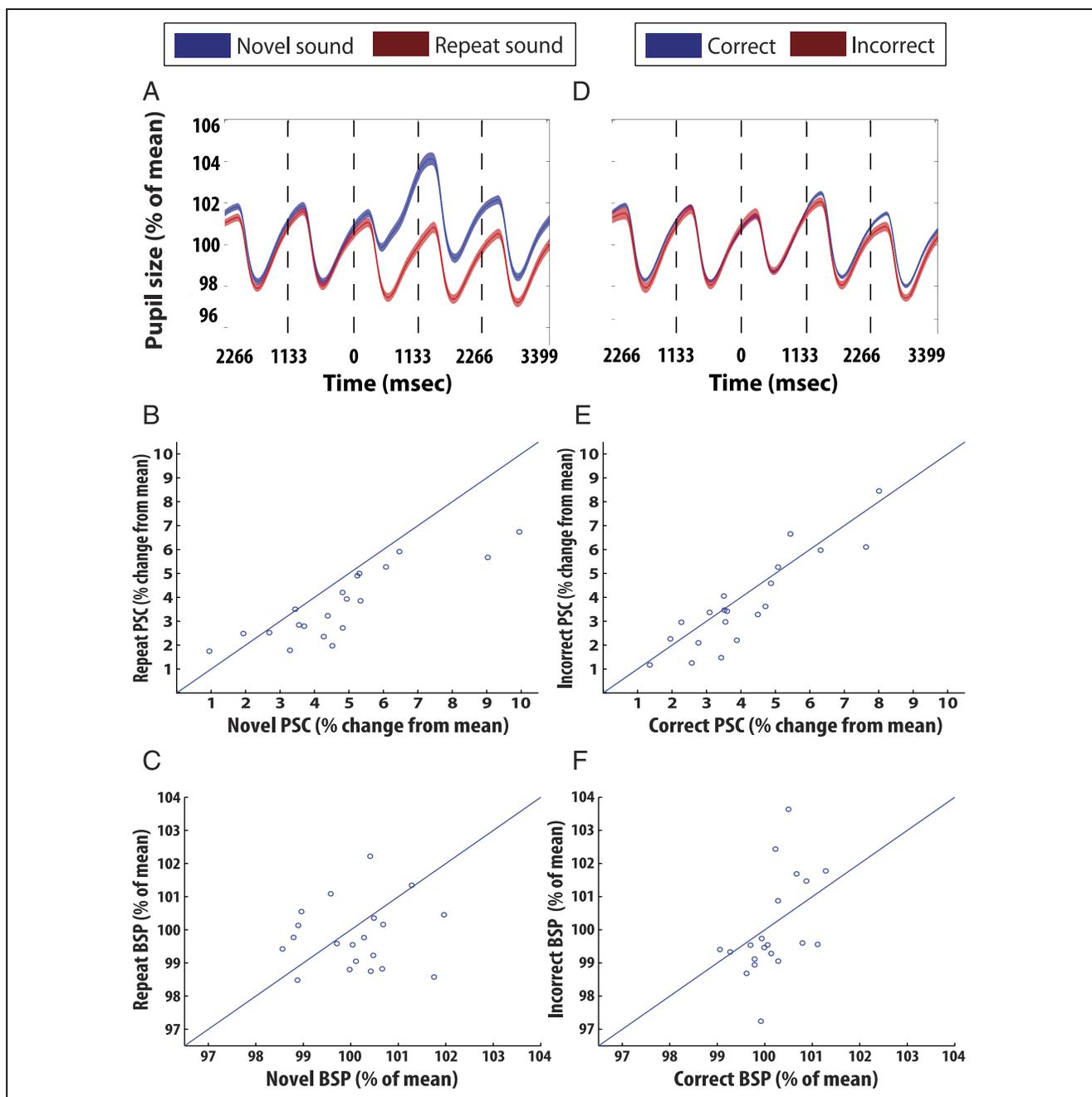
Figure 6. Pupil dynamics in sound experiment. (A, D) Pupil size fluctuates with each stimulus presentation with 0 representing the onset of the to-be-tested novel- or repeat-sound-paired scene. Dashed lines represent times of stimulus onset. (B) Significant *t* tests indicated that novel compared with repeat sounds induced larger PSCs, whereas (C) no difference between BPS was observed. (D) Pupil dynamics during correct and incorrect trials showed (E) increased PSC during correct trials compared with incorrect trials. (F) No significant difference in BPS between correct and incorrect trials was observed. Shading (A, D) represents within-subject standard error.

Sara, Vankov, & Hervé, 1994), was hypothesized to lead to both task-relevant learning and TIL. This idea was built on extant models of arousal and alerting, for which Petersen and Posner (2012) hypothesized to rely on NE release, and also on models of unexpected uncertainty in learning (Yu & Dayan, 2005) where NE release occurs for unpredictable stimuli, such as the unpredictable onset of a target in the stimulus stream. However, until now, there was no experimental evidence testing this hypothesis.

Here, building upon findings of a connection between NE release and pupil dilation (Aston-Jones, 2005; Rajkowski et al., 1993), we find that PSCs are transiently related to the difference in accuracy for the target- and distractor-paired scenes.

Apart from phasic changes in the LC, longer-term "tonic" changes in the LC have been shown to relate to performance reflective of the Yerkes–Dodson curve (Yerkes & Dodson, 1908). The Yerkes–Dodson curve has provided

evidence of LC activation in which the low and high ends of the curve are associated with low performance and low task engagement, whereas activation in the middle of the curve is associated with optimal performance and increased task engagement. Previous studies have suggested that BPS reflects the tonic activation of the LC. In Experiment 1, correct trials exhibited lower average pupil sizes, whereas incorrect trials had higher average pupil sizes. However, this pattern was not replicated in Experiment 2. Still, it is difficult to directly compare Experiments 1 and 2 because the tasks (dual vs. single) and stimuli were different. Furthermore, in Experiment 1, the PSC is transiently associated with an increase in TIL, whereas in Experiment 2, there is a prolonged effect of an increase in PSC, which may have contaminated our estimates of BPS. This transience may also explain why it has no relation to overall accuracy (regardless of condition), whereas BPS is related to overall accuracy. Moreover, given that the Yerkes–Dodson curve is nonmonotonic, we are left without the ability to make strong conclusions regarding the relationship between BPS and performance as the full range of baselines is unknown and was not manipulated in this experiment.

An important factor in learning research is one concerning the impact of individual differences. Previous studies have found that significant individual differences can occur in the fast-TIL paradigm (Leclercq, Cohen Hoffing, & Seitz, 2014; Leclercq & Seitz, 2012c). As such, one question to address is whether these individual differences in TIL also contribute to differences in pupil dynamics. We first examined whether there were significant group-wise differences when participants in Experiment 1 were separated into those who showed TIL (learners group, $n = 14$) and those who did not (nonlearners group, $n = 17$). Learners performed at least 60% on target-paired scenes and at least 10% greater on tested target-paired scenes over tested distractor-paired scenes; cutoffs were determined through a binomial cumulative distribution fit. A significant inter-action between group and trial type shows that the PSCs differ as a function of condition between groups ($F(1, 29) = 5.12, p = .031$), and this interaction was driven by a greater difference between target and distractor PSCs in the learners compared with nonlearners ($t(29) = 2.26, p = .016$). However, we did not have sufficient power to conduct this same analysis in Experiment 2, where only six (of the 12 participants who showed better performance on novel compared with repeated paired sounds) met our strict criteria of "learners." Furthermore, there were no correlations between the difference scores of target–distractor accuracy and target–distractor PSC (Experiment 1: $r = .21, p = .26$; Experiment 2: $r = -.01, p = .96$). Thus, although we are intrigued by larger pupil sizes changes in Experiment 1 for the learners, this relationship was not highly consistent. Similarly, previous studies (Murphy et al., 2011) have shown negative correlations between PSC and BPS. In this study, we failed to observe a consistent pattern across experiments between target and distractor PSC and BPS (Experiment 1: $r = -.35, p = .26$; Experiment 2: $r = .11, p = .49$). Altogether, it is difficult to know whether the lack of consistencies of the quantitative relationships between TIL, pupil size, and PSCs at the individual subject level reflects the noise in the estimates of each of these measures or that we are failing to take into account nonlinear interactions between these factors. An issue may be that fast TIL relies on a single pairing between an image and a target and that this may not produce as reliable of an induction of learning as found in slow TIL experiments, which involve many thousands of stimulus–reinforcement pairings.

An interesting finding in Experiment 2 was that scenes tested after the novel sound also showed enhanced memorization. These results are consistent with previous findings of fast TIL where prolonged benefits for scenes were found after the presentation of a target arrow, which was similarly thought to alert participants to the RSVP stream of scenes (Leclercq & Seitz, 2012d). This result is also in
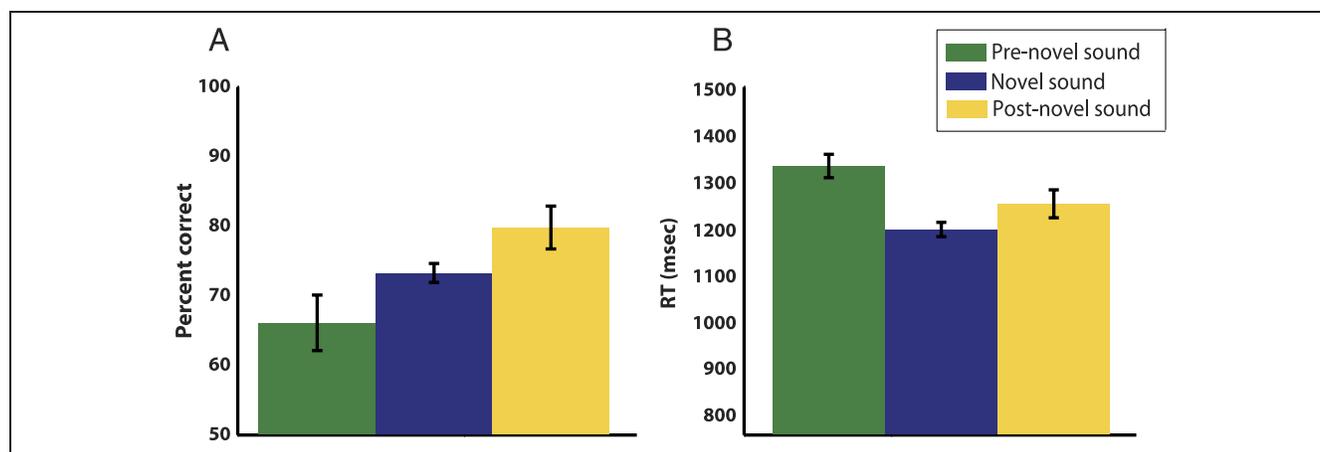
Figure 7. Catch trial accuracy and RT. Performance by condition before and after novel sound presentation during catch trials indicates (A) increased accuracy for post-novel-sound-paired scenes compared with pre-novel-sound-paired scenes and (B) decreased RT for post-novel-sound-paired scenes compared with pre-novel-sound-paired scenes. Error bars indicate within-subject standard error.

line with findings from Murphy et al. (2011), where large pupil dilations led to an increase in performance followed by a diminishing BPS and decreased performance in the context of an oddball task where a key was pressed when an unpredictable sound occurred. They proposed that this pattern of task reengagement and disengagement was reflecting NE's role in regulating task engagement levels.

It is possible that eye movements made within the 1.5° fixation window may have influenced target- or novel-sound-mediated pupil measurements through direct influence on the pupil or through measurement error. Recent research has detailed that pupil size can be confounded by eye position with standard eye trackers, such as the Eyelink that we used here (Gagl, Hawelka, & Hutzler, 2011). To avoid this potential confound, all experiments were conducted using a gaze-contingent display where fixation was required during all points of task performance. Errors induced by gaze position are minimal within the central 2° (Gagl et al., 2011) and are unlikely an influence in our data. To address whether there were movement differences, we conducted a 2×2 repeated-measures ANOVA on Condition (target, distractor) by Coordinate (x, y) using the mean x and y position during the target and subsequent ISI presentation across trials for each participant and found no interaction in either Experiment 1 $(F(30, 90) = 1.875, p = .1811)$ or Experiment 2 $(F(19, 57) = 2.177, p = .1565)$. Another concern is that, although luminance was equated across all images, targets and distractors may have exhibited local scene content and luminance differences, both of which have been demonstrated to influence pupil dynamics (Naber & Nakayama, 2013). Although unlikely, as target and distractor scenes were randomly selected from a set of 960 scenes and randomized across participants, we confirmed that targets were equally likely to be distractors by computing the conditional probability of an image being a target given it was tested. Of the 2112 scenes that could possibly be presented (including the blink set) and tested, participants were tested on 1315 of these scenes. A one-sample t test on the tested target conditional probability from .5 (whether it was equally likely to be tested as a distractor; $t(1314) = 0.3388, p = .7348$) fails to find any difference in probability that a given image was tested as a target or as a distractor. As such, we are reasonably confident that our findings of pupil size are not simple confounds of eye movements or image content.

Our results suggest that pupillometry combined with TIL, in which task factors can be manipulated independently from the stimuli that are being learned, provides a useful approach to study the mechanisms of learning and memory. Although these results are consistent with the effects of arousal or alerting on learning, we suggest that a mechanistic model that relates arousal and learning to neurochemical systems like the LC–NE system provides a more parsimonious model of the processes involved. However, although our results demonstrate a relationship between pupil dynamics and TIL, there are substantial individual subject differences, and further work will be required to clarify the quantitative relationship between pupil dynamics and TIL. Furthermore, further research such as psychopharmacological and fMRI studies will be required to confirm the neurochemical basis of TIL.

Acknowledgments

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