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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 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 non-specifically 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 (Hassani, Cromwell, & Schultz, 2001; O'Doherty, Dayan, Friston, Critchley, & Dolan, 2003; A. R. Seitz & Dinse, 2007; Yu & Dayan, 2005) suggest a key role of neurochemicals like dopamine (Bao, Chan, & Merzenich, 2001; Schultz, 2002), acetylcholine (Bakin & Weinberger, 1996; Kilgard & Merzenich, 1998; Thiel, Friston, & Dolan, 2002) and norepinephrine (NE) (Bear & Singer, 1986; Gordon, Allen, & Trombley, 1988; Witte & Marrocco, 1997) 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.

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Conflict of Interest:

The authors declare no competing financial interests

However, non-invasive measures such as pupillometry can provide clues into neurochemical activity. For example pupil-size changes have been suggested as surrogate measure of locus coeruleus (LC) activity and its release of norepinephrine (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 pupil-size changes were positively associated with a learning rate (Nassar et al., 2012; Silvetti, Seurinck, van Bochove, & Verguts, 2013) 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 (A. Seitz & Watanabe, 2005; A. R. Seitz & Watanabe, 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 (A. R. Seitz & Watanabe, 2003, 2009; Watanabe, Nanez, & Sasaki, 2001). TIL is a robust learning phenomenon with demonstrations ranging from critical flicker fusion thresholds (A. R. 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.

While TIL was discovered in low-level perceptual learning (A. R. Seitz & Watanabe, 2003; Watanabe et al., 2001), recent research of fast task-irrelevant learning (fast-TIL) (Leclercq & Seitz, 2012a, 2012b, 2012c, 2012d; Lin, Pye, 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 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 et al. (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 pupil-size changes 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 et al., 2009) are paired with images to induce NE release. Results of this study are consistent with the first showing increased pupil-size changes 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.

MATERIALS AND METHODS

Participants

A total of 70 participants were run, 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 1.5hr session. Participants were excluded due to excessive eye-movement (12 in Experiment 1 and 7 in Experiment 2). This left a final sample of 31 participants (19.28 ± 1.4 y.o.; 20 females, 11 males) in Experiment 1 and 20 participants (19.85 ± 1.5 y.o.; 9 females, 11 males) 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) was used for stimulus generation and experiment control. Stimuli were presented on a ViewSonic PF817 monitor with resolution of 1600×1200 resolution, and a refresh rate of 100 Hz. Display items consisted of 2112 scenes depicting natural or man-made 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, 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 133ms of each sound and played them at a sampling rate of 22kHz through Sennheiser HD 202 headphones, which have a frequency range of 18Hz–18kHz. The background for all displays was a gray (luminance of 10cd/m^2). Participants sat with their eyes approximately 57cm from the screen using the EyeLink 1000 tower-mount, which was used to track eye movement and pupil-size fluctuations of the right eye during every 10ms 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 $<0.01^\circ$ RMS (Ltd). 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 8-scene Rapid Serial Visual Presentation (RSVP) stream, each presented for 133ms followed by an ISI (blank gray screen) of 1000ms (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. 15 Blocks of 8 trials each were separated at minimum by 10-second 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 300–600ms. To ensure that participants could conduct the task they performed a practice block of 8 trials prior to 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’, ‘V’). For the Target Detection task, participants had 1500ms 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 3000ms and reported which scene had appeared in that trial via the number pad keys ‘1’ or ‘2’.

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 Trials (n=24). 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 couldn’t 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 the total number of responses made for each task, excluding missed trials. In Experiment 1, $7.74 \pm 0.93\%$ (serr) of trials were missed in the Target Detection Task and $9.11 \pm 0.96\%$ in Scene Recognition Task. In Experiment 2, $13.67 \pm 2.54\%$ were missed in the Scene Recognition Task.

For the analysis of pupil dynamics, pupil diameter was normalized by dividing each data point by each subjects’ 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 ($6.77 \pm 0.47\%$) 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 prior to the stimulus of interest, however we note that it is not a stable baseline,

due to 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 pre-trial intervals. Pupil-size change (PSC) was defined as the difference between the trough and peak of pupil-size after stimulus presentation (Figure 2).

RESULTS

Experiment 1 – Task Irrelevant Learning

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 ($73.41\% \pm 1.50\%$) was significantly greater than that for Distractor-paired ($63.94\% \pm 1.16\%$) scenes, $t(30)=3.7534$, $p=0.0004$. Likewise, reaction-times were significantly faster for Target-paired ($1101 \pm 13.2\text{ms}$) compared to Distractor-paired ($1147 \pm 8.2\text{ms}$) scenes, $t(30)=-2.1947$, $p=0.0179$ (Figure 3b). These increased memorization rates and faster reaction times for Target-paired scenes over Distractor paired scenes confirm a TIPL effect.

Pupillometry of TIL

A key question is whether there is a relationship between TIL and pupil-size changes during task performance (Figure 4a). We observed that participants showed a larger pupil-size change (Figure 4b) for Target-paired scenes ($6.01 \pm 0.38\%$) over Distractor paired scenes ($4.33 \pm 0.35\%$), $t(30)=9.5410$, $p<0.0001$, which is consistent with our hypothesis that NE release is associated with Target-processing. On the other hand, we found no baseline pupil-size ($t(30)=-1.1237$, $p=0.8650$) differences (Figure 4c) between Target-paired scenes ($99.91 \pm 0.23\%$) and Distractor paired scenes (100.26 ± 0.18).

While we failed to find a relationship between baseline pupil-size and TIL, previous studies (e.g. Murphy et al., 2011), have shown that baseline pupil-size is related to performance outcomes. To test for this we examined pupil-size and pupil-size changes as a function of Performance Outcome [Correct, Incorrect] (Figure 4d). Consistent with previous findings, participants showed significantly lower ($t(30)=-2.1386$, $p=0.0407$, two-tail) baseline pupil-size (Figure 4f) for Correct trials ($99.84 \pm 0.20\%$) than Incorrect trials ($100.44 \pm 0.24\%$). On the other hand, we failed to find any significant differences ($t(30)=-0.9283$, $p=0.3607$) between pupil-size changes (Figure 4e) and Correct ($5.09 \pm 0.35\%$) vs. Incorrect scenes ($5.22 \pm 0.39\%$).

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

Experiment 2 - Memory Enhancement due to Alerting Sounds

While 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 were 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 TIPL (A. 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 pupil size changes 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).

Results on the scene memorization task showed significantly increased accuracy for Novel Sound paired scenes ($73.35\% \pm 1.38\%$) compared to Repeat Sound paired scenes ($69.15\% \pm 1.15\%$) ($t(19)=1.84$, $p=0.039$) (Figure 5a) and also significantly faster RT ($t(19)=-2.85$, $p=0.0051$) for Novel Sound paired scenes ($1213 \pm 14.4\text{ms}$), compared to Repeat Sound-paired scenes ($1291 \pm 14.2\text{ms}$) (Figure 5b). This significant increased memorization and faster RT for Novel Sound paired scenes over Repeat Sound paired scenes suggests a TIL effect.

Pupillometry due to Alerting Sounds

Examination of pupil-size dynamics revealed significantly increased pupil-size change for Novel Sound paired scenes ($4.74\% \pm 0.15\%$) compared to Repeat Sound paired scenes ($3.67\% \pm 0.13\%$) ($t(19)=4.2147$, $p=0.0002$) (Figure 6b) and no difference ($t(19)=0.913$, $p=0.19$) between baseline pupil-size for Novel Sound paired scenes ($99.93 \pm 0.21\%$), compared to Repeat Sound-paired scenes ($99.66 \pm 0.21\%$) (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 ($t(19)=1.99$, $p=0.031$) increased pupil-size changes during the to-be-tested scene that was Correct ($4.12\% \pm 0.06\%$) compared to Incorrect ($3.71\% \pm 0.15\%$) (Figure 6e). However, no difference ($t(19)=0.873$, $p=0.20$) between baseline pupil-size before scenes that were Correct ($99.97 \pm 0.12\%$), compared to Incorrect ($99.97 \pm 0.31\%$) (Figure 6f). While these results are different than those of the first experiment, the dynamics of the pupil-size changes were different in this experiment, with a faster initial rise in pupil-size and prolonged elevation of pupil-size after the Novel sounds. Furthermore, even Repeat sounds may have had a different impact on pupil-size changes than the Distractors in Experiment 1. While these differences between experiments are interesting and warrant further study, they don't 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 following that of the Novel Sound. This prolonged effect is consistent with subjective observations of a prolonged "arousal/alerting effect" following the Novel Sounds which was experientially different from a more stayed effect

following the Targets in Experiment 1. We hypothesized that a component of this effect was due to the prolonged release of NE that would in turn lead to improved memorization for scenes presented after the Novel sounds compared to 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 ($F(2,38)=4.78$, $p=0.014$, one-way ANOVA) lower accuracy ($66.12\% \pm 4.04\%$; Figure 7a) and significantly slower ($F(2,38)=58.702$, $p<0.0001$) RTs ($1340 \pm 23.5\text{ms}$; Figure 7b) compared to Novel Sound-paired scenes ($73.35\% \pm 1.38\%$; $1213 \pm 14.4\text{ms}$) and Post-Novel Sound paired scenes ($79.95\% \pm 3.10\%$; $1264 \pm 28.1\text{ms}$). These results show that Novel Sounds not only benefited the paired scene but also had a prolonged effect that lead 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 pupil-size change 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 pupil-size changes 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; Sara, Vankov, & Hervé, 1994) was hypothesized to lead to both task-relevant and task-irrelevant learning. This idea was built upon extant models of arousal and alerting, for which Petersen and Posner (2012) hypothesized to rely upon NE release, and also upon 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 pupil-size changes 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 baseline pupil-size reflects the tonic activation of the LC. In Experiment 1, correct trials exhibited lower average pupil-sizes, while 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 since the tasks (dual vs. single) and stimuli were different. Further, in Experiment 1 the pupil-size change is transiently associated with an increase in TIL while in Experiment 2 there is a prolonged effect of an increase in pupil-size change, which may have contaminated our estimates of baseline pupil size. This transience may also explain why it has no relation to overall accuracy (regardless of condition) while baseline pupil-size is related to overall accuracy. Moreover, given that the Yerkes-Dodson curve is non-monotonic, we are left without the ability to make strong conclusions regarding the relationship between baseline pupil-size 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, 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 TIPL (Learners Group, $n=14$) and those who didn't (Non-Learners Group, $n=17$). Learners performed at least 60% on Target paired scenes and at least 10% greater on tested Target over tested Distractor paired scenes; cut-offs were determined through a binomial cumulative distribution fit. A significant interaction between Group and Trial-type shows that the pupil-size changes differ as a function of condition between groups ($F(1,29)=5.12$, $p=0.031$) and this interaction was driven by a greater difference between Target and Distractor pupil-size changes in the Learners compared to Non-learners ($t(29)=2.26$, $p=0.016$). However, we didn't have sufficient power to conduct this same analysis in Experiment 2, where only 6 (of the 12 participants who showed better performance on novel compared to 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 Pupil Size change (Experiment 1: $r=0.21$, $p=0.26$; Experiment 2: $r=-0.01$, $p=0.96$). Thus, while we are intrigued by larger pupil size changes in Experiment 1 for the Learners, this relationship wasn't highly consistent. Similarly, previous studies (Murphy et al. 2011) have shown negative correlations between pupil size change and baseline pupil size. In the present study we failed to observe a consistent pattern across experiments between Target and Distractor pupil size change and baseline pupil size (Experiment 1, $r=-0.35$, $p=0.26$; Experiment 2, $r=0.11$, $p=0.49$). Altogether, it is difficult to know whether the lack of consistencies of the quantitative relationships between TIL, pupil-size and pupil-size-changes at the individual subject level reflect the noise in the estimates of each of these measures or that we are failing to take into account non-linear interactions between these factors. An issue may be that fast-TIL relies upon 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 line with findings from Murphy et al. (2011) where large pupil-dilations led to an increase in performance followed by a diminishing baseline pupil-size 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 norepinephrine's role in regulating task engagement levels.

It is possible that eye movements made within the 1.5-degree 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 degrees (Gagl et al., 2011) and are unlikely an influence in our data. To address whether there were movement differences we conducted a 2x2 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 subject and found no interaction in either Experiment 1 ($F(30,90)=1.875$ $p=0.1811$) or Experiment 2 ($F(19,57)=2.177$ $p=0.1565$). Another concern is that even though 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). While 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. Out of the 2112 scenes that could possibly be presented (including the Blink set) and tested participants were tested on 1,315 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) ($t(1314)=0.3388$, $p=0.7348$) 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. While 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, while 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.

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References

- Aston-Jones G. Brain structures and receptors involved in alertness. *Sleep Med.* 2005; 6(Suppl 1):S3–7. [PubMed: 16140243]
- Bakin JS, Weinberger NM. Induction of a physiological memory in the cerebral cortex by stimulation of the nucleus basalis. *Proceedings of the National Academy of Sciences.* 1996; 93(20):11219–11224.
- Bao S, Chan VT, Merzenich MM. Cortical remodelling induced by activity of ventral tegmental dopamine neurons. *Nature.* 2001; 412(6842):79–83. [PubMed: 11452310]
- Bear MF, Singer W. Modulation of visual cortical plasticity by acetylcholine and noradrenaline. *Nature.* 1986; 320(6058):172–176. [PubMed: 3005879]
- Bouret S, Sara SJ. Network reset: a simplified overarching theory of locus coeruleus noradrenaline function. *Trends in neurosciences.* 2005; 28(11):574–582. [PubMed: 16165227]
- Brainard DH. The Psychophysics Toolbox. *Spat Vis.* 1997; 10(4):433–436. [PubMed: 9176952]
- Gagl B, Hawelka S, Hutzler F. Systematic influence of gaze position on pupil size measurement: analysis and correction. *Behavior research methods.* 2011; 43(4):1171–1181. [PubMed: 21637943]
- Gordon B, Allen EE, Trombley PQ. The role of norepinephrine in plasticity of visual cortex. *Prog Neurobiol.* 1988; 30(2–3):171–191. [PubMed: 3125564]
- Harley CW. A role for norepinephrine in arousal, emotion and learning?: limbic modulation by norepinephrine and the Kety hypothesis. *Progress in Neuro-psychopharmacology and Biological Psychiatry.* 1987; 11(4):419–458. [PubMed: 3321150]
- Harley CW. Norepinephrine and dopamine as learning signals. *Neural plasticity.* 2004; 11(3–4):191–204. [PubMed: 15656268]
- Hassani OK, Cromwell HC, Schultz W. Influence of expectation of different rewards on behavior-related neuronal activity in the striatum. *J Neurophysiol.* 2001; 85(6):2477–2489. [PubMed: 11387394]
- Kilgard MP, Merzenich MM. Cortical map reorganization enabled by nucleus basalis activity. *Science.* 1998; 279(5357):1714–1718. [PubMed: 9497289]
- Konkle T, Brady TF, Alvarez GA, Oliva A. Conceptual distinctiveness supports detailed visual long-term memory for real-world objects. *J Exp Psychol Gen.* 2010; 139(3):558–578. [PubMed: 20677899]
- Krebs RM, Boehler CN, De Belder M, Egner T. Neural Conflict–Control Mechanisms Improve Memory for Target Stimuli. *Cerebral Cortex.* 2013
- Leclercq V, Hoffing RC, Seitz AR. Uncertainty in fast-task-irrelevant perceptual learning boosts learning of images in women but not men. *Journal of Vision.* 2014 in press.
- Leclercq V, Le Dantec CC, Seitz AR. Encoding of episodic information through fast task-irrelevant perceptual learning. *Vision Res.* 2013
- Leclercq V, Seitz AR. Enhancement from targets and suppression from cues in fast task-irrelevant perceptual learning. *Acta Psychol (Amst).* 2012a; 141(1):31–38. [PubMed: 22842471]
- Leclercq V, Seitz AR. Fast task-irrelevant perceptual learning is disrupted by sudden onset of central task elements. *Vision Res.* 2012b; 61:70–76. [PubMed: 21810439]
- Leclercq V, Seitz AR. Fast-TIPL occurs for salient images without a memorization requirement in men but not in women. *PLoS ONE.* 2012c; 7(4):e36228. [PubMed: 22558395]
- Leclercq V, Seitz AR. The impact of orienting attention in fast task-irrelevant perceptual learning. *Atten Percept Psychophys.* 2012d; 74(4):648–660. [PubMed: 22287208]
- Lin JY, Pype AD, Murray SO, Boynton GM. Enhanced memory for scenes presented at behaviorally relevant points in time. *PLoS Biol.* 2010; 8(3):e1000337. [PubMed: 20305721]

- Loftus GR, Masson MEJ. Using Confidence-Intervals in within-Subject Designs. *Psychonomic Bulletin & Review*. 1994; 1(4):476–490. [PubMed: 24203555]
- Ltd SR. EyeLink User Manual. 2005
- Marcell MM, Borella D, Greene M, Kerr E, Rogers S. Confrontation naming of environmental sounds. *Journal of Clinical and Experimental Neuropsychology*. 2000; 22(6):830–864. [PubMed: 11320440]
- Murphy PR, Robertson IH, Balsters JH, O'connell RG. Pupillometry and P3 index the locus coeruleus–noradrenergic arousal function in humans. *Psychophysiology*. 2011; 48(11):1532–1543. [PubMed: 21762458]
- Naber M, Nakayama K. Pupil responses to high-level image content. *Journal of vision*. 2013; 13(6):7. [PubMed: 23685390]
- Nassar MR, Rumsey KM, Wilson RC, Parikh K, Heasley B, Gold JI. Rational regulation of learning dynamics by pupil-linked arousal systems. *Nat Neurosci*. 2012; 15(7):1040–1046. [PubMed: 22660479]
- Nishina S, Seitz AR, Kawato M, Watanabe T. Effect of spatial distance to the task stimulus on task-irrelevant perceptual learning of static Gabors. *Journal of Vision*. 2007; 7(13):1–10. [PubMed: 17997630]
- O'Doherty JP, Dayan P, Friston K, Critchley H, Dolan RJ. Temporal difference models and reward-related learning in the human brain. *Neuron*. 2003; 38(2):329–337. [PubMed: 12718865]
- Pelli DG. The VideoToolbox software for visual psychophysics: transforming numbers into movies. *Spat Vis*. 1997; 10(4):437–442. [PubMed: 9176953]
- Petersen SE, Posner MI. The attention system of the human brain: 20 years after. *Annual review of neuroscience*. 2012; 35:73.
- Rajkowski J, Kubiak P, Aston-Jones G. Correlations between locus coeruleus (LC) neural activity, pupil diameter and behavior in monkey support a role of LC in attention. *Society for Neuroscience Abstract*. 1993; 19:974.
- Rosenthal O, Humphreys GW. Perceptual organization without perception: the subliminal learning of global contour. *Psychol Sci*. 2010; 21(12):1751–1758. [PubMed: 21098739]
- Sara SJ, Bouret S. Orienting and Reorienting: The Locus Coeruleus Mediates Cognition through Arousal. *Neuron*. 2012; 76(1):130–141. [PubMed: 23040811]
- Sara SJ, Vankov A, Hervé A. Locus coeruleus-evoked responses in behaving rats: a clue to the role of noradrenaline in memory. *Brain research bulletin*. 1994; 35(5):457–465. [PubMed: 7859103]
- Schultz W. Getting formal with dopamine and reward. *Neuron*. 2002; 36(2):241–263. [PubMed: 12383780]
- Seitz A, Watanabe T. A unified model for perceptual learning. *Trends Cogn Sci*. 2005; 9(7):329–334. [PubMed: 15955722]
- Seitz AR, Dinse HR. A common framework for perceptual learning. *Curr Opin Neurobiol*. 2007; 17(2):148–153. [PubMed: 17317151]
- Seitz AR, Nanez JE, Holloway SR, Watanabe T. Visual experience can substantially alter critical flicker fusion thresholds. *Hum Psychopharmacol*. 2005; 20(1):55–60. [PubMed: 15568203]
- Seitz AR, Nanez JE, Holloway SR, Watanabe T. Perceptual learning of motion leads to faster flicker perception. *PLoS ONE*. 2006; 1:e28. [PubMed: 17183655]
- Seitz AR, Watanabe T. Psychophysics: Is subliminal learning really passive? *Nature*. 2003; 422(6927):36. [PubMed: 12621425]
- Seitz AR, Watanabe T. The phenomenon of task-irrelevant perceptual learning. *Vision Res*. 2009; 49(21):2604–2610. [PubMed: 19665471]
- Silvetti M, Seurinck R, van Bochove M, Verguts T. The influence of the noradrenergic system on optimal control of neural plasticity. *Frontiers in Behavioral Neuroscience*. 2013; 7
- Swallow KM, Jiang YV. The Attentional Boost Effect: Transient increases in attention to one task enhance performance in a second task. *Cognition*. 2010; 115(1):118–132. [PubMed: 20080232]
- Thiel CM, Friston KJ, Dolan RJ. Cholinergic modulation of experience-dependent plasticity in human auditory cortex. *Neuron*. 2002; 35(3):567–574. [PubMed: 12165477]

- Vlahou, E.; Seitz, AR.; Protopapas, A. Implicit learning of non-native speech stimuli. Paper presented at the Acoustical Society of America; Portland, OR.. 2009.
- Watanabe T, Nanez JE, Sasaki Y. Perceptual learning without perception. *Nature*. 2001; 413(6858): 844–848. [PubMed: 11677607]
- Watanabe T, Nanez JE Sr, Koyama S, Mukai I, Liederman J, Sasaki Y. Greater plasticity in lower-level than higher-level visual motion processing in a passive perceptual learning task. *Nat Neurosci*. 2002; 5(10):1003–1009. [PubMed: 12219093]
- Willenbockel V, Sadr J, Fiset D, Horne GO, Gosselin F, Tanaka JW. Controlling low-level image properties: the SHINE toolbox. *Behavior research methods*. 2010; 42(3):671–684. [PubMed: 20805589]
- Winn B, Whitaker D, Elliott DB, Phillips NJ. Factors affecting light-adapted pupil size in normal human subjects. *Investigative Ophthalmology & Visual Science*. 1994; 35(3):1132–1137. [PubMed: 8125724]
- Witte EA, Marrocco RT. Alteration of brain noradrenergic activity in rhesus monkeys affects the alerting component of covert orienting. *Psychopharmacology (Berl)*. 1997; 132(4):315–323. [PubMed: 9298508]
- Yerkes RM, Dodson JD. The relation of strength of stimulus to rapidity of habit-formation. *Journal of Comparative Neurology and Psychology*. 1908; 18(5):459–482.
- Yu AJ, Dayan P. Uncertainty, neuromodulation, and attention. *Neuron*. 2005; 46(4):681–692. [PubMed: 15944135]

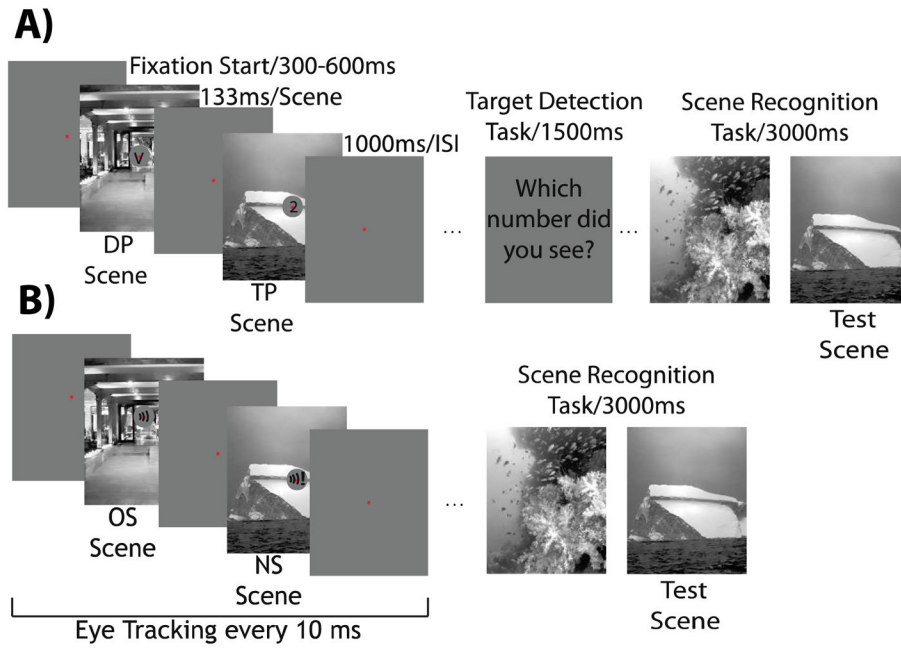


Figure 1. Cartoon of trial structure

Each scene was presented for 133ms with an ISI of 1000ms, eye tracking is recorded every 10ms. **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.

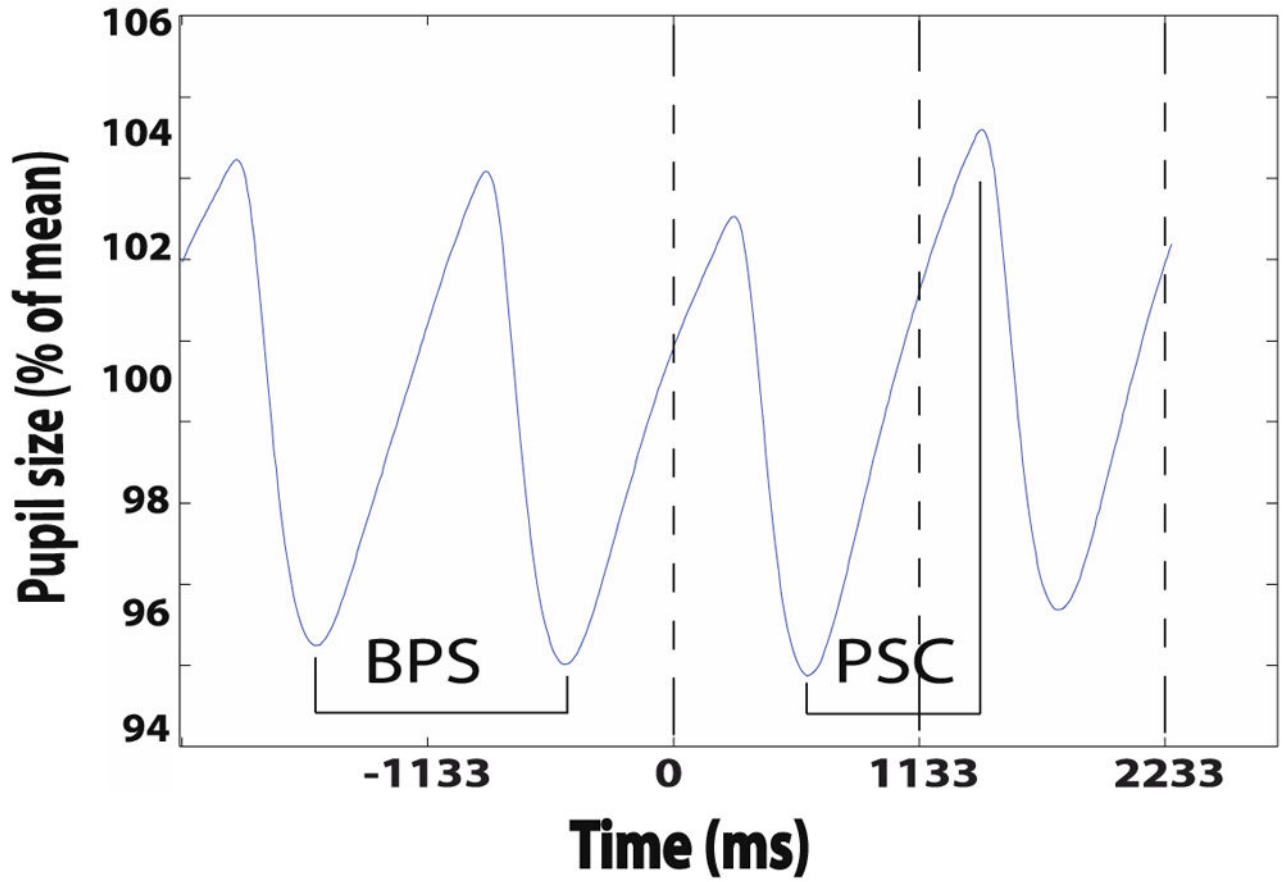


Figure 2. Quantification of Pupil dynamics

Baseline pupil-size (BPS) defined as the average of the period denoted by BPS. Pupil-size change (PSC) defined as the difference from the peak minus the trough denoted by PSC.

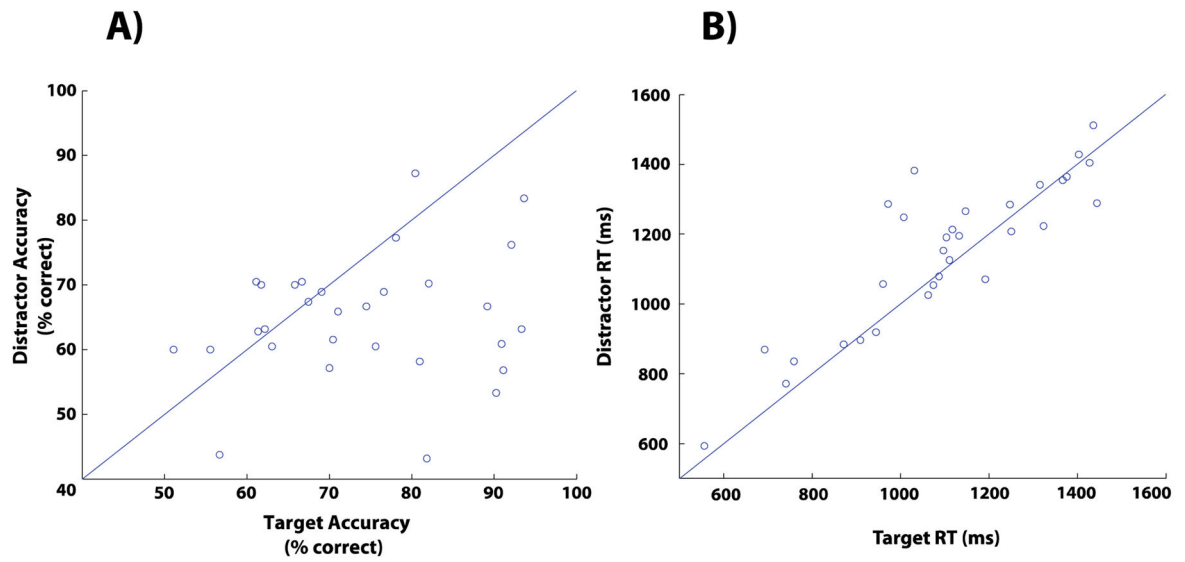


Figure 3. Scene Recognition Task Performance

Participants exhibited **A)** increased accuracy for Target-paired scenes over Distractor-paired scenes and **B)** decreased RTs for Target-paired scenes compared to Distractor-paired scenes.

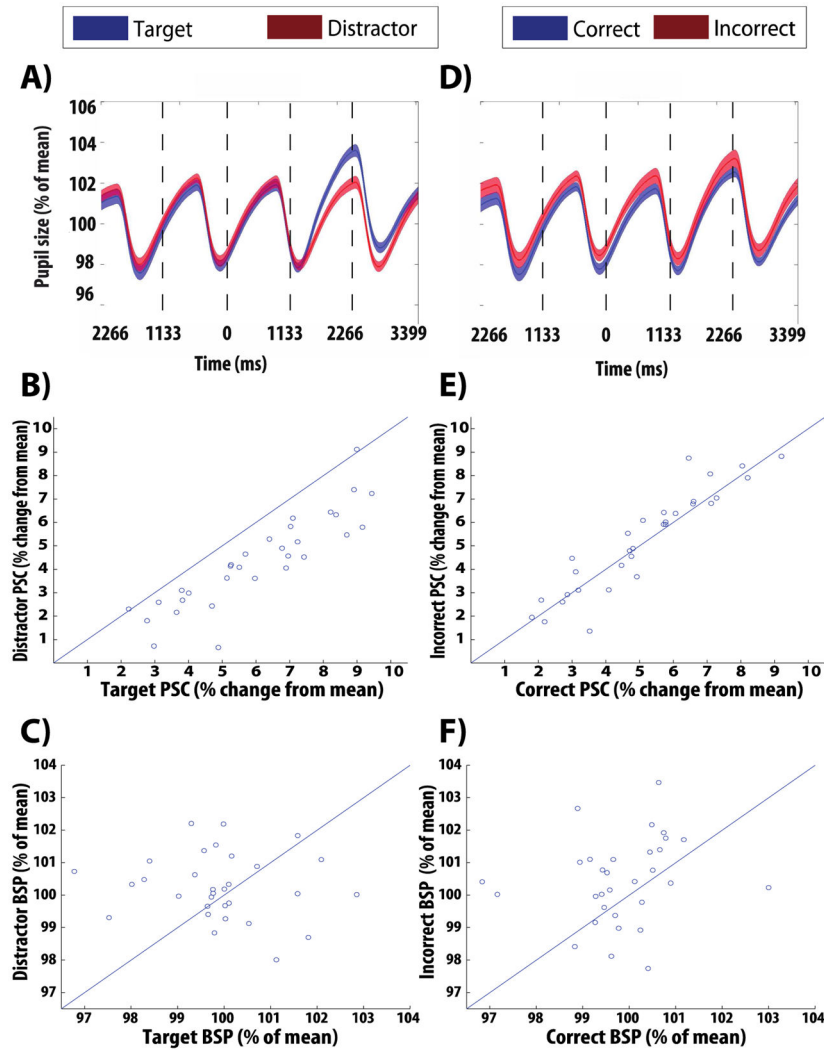


Figure 4. Pupil Dynamics in Experiment 1

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 to Distractors induced larger pupil-size changes while **C)** no differences in baseline pupil-size were observed. Shading **(A)** represents within-subject standard error (Loftus & Masson, 1994). **E)** No difference was found in stimulus induced pupil-size change between Correct and Incorrect trials. **F)** Correct trials show a lower baseline pupil-size than Incorrect trials. Shading **(D)** represents within-subject standard error.

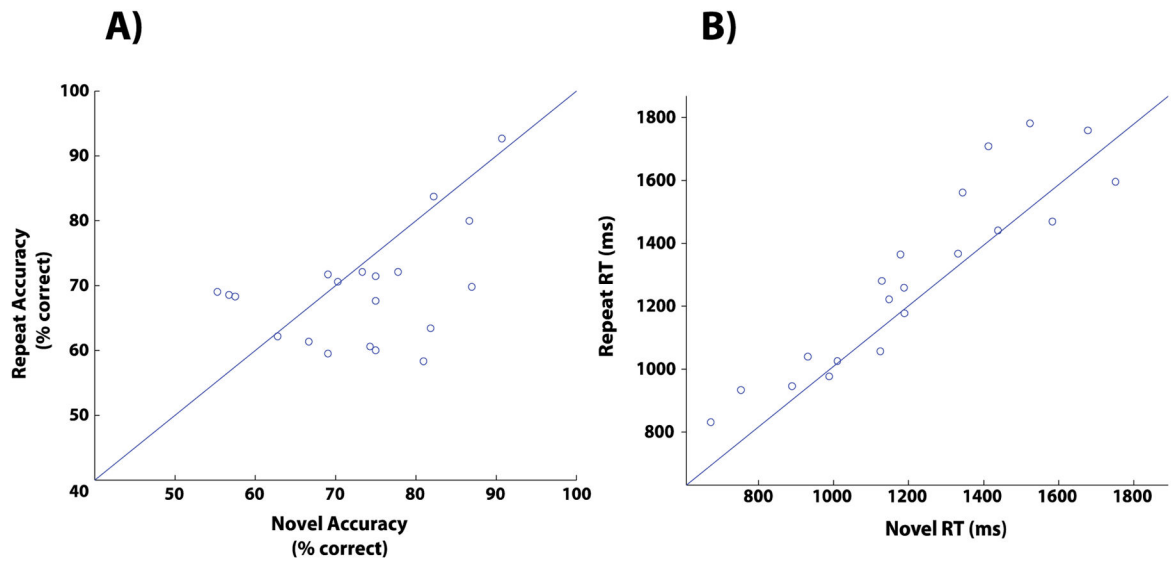


Figure 5. Scene Recognition Task Performance

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 to Repeat Sound-paired scenes.

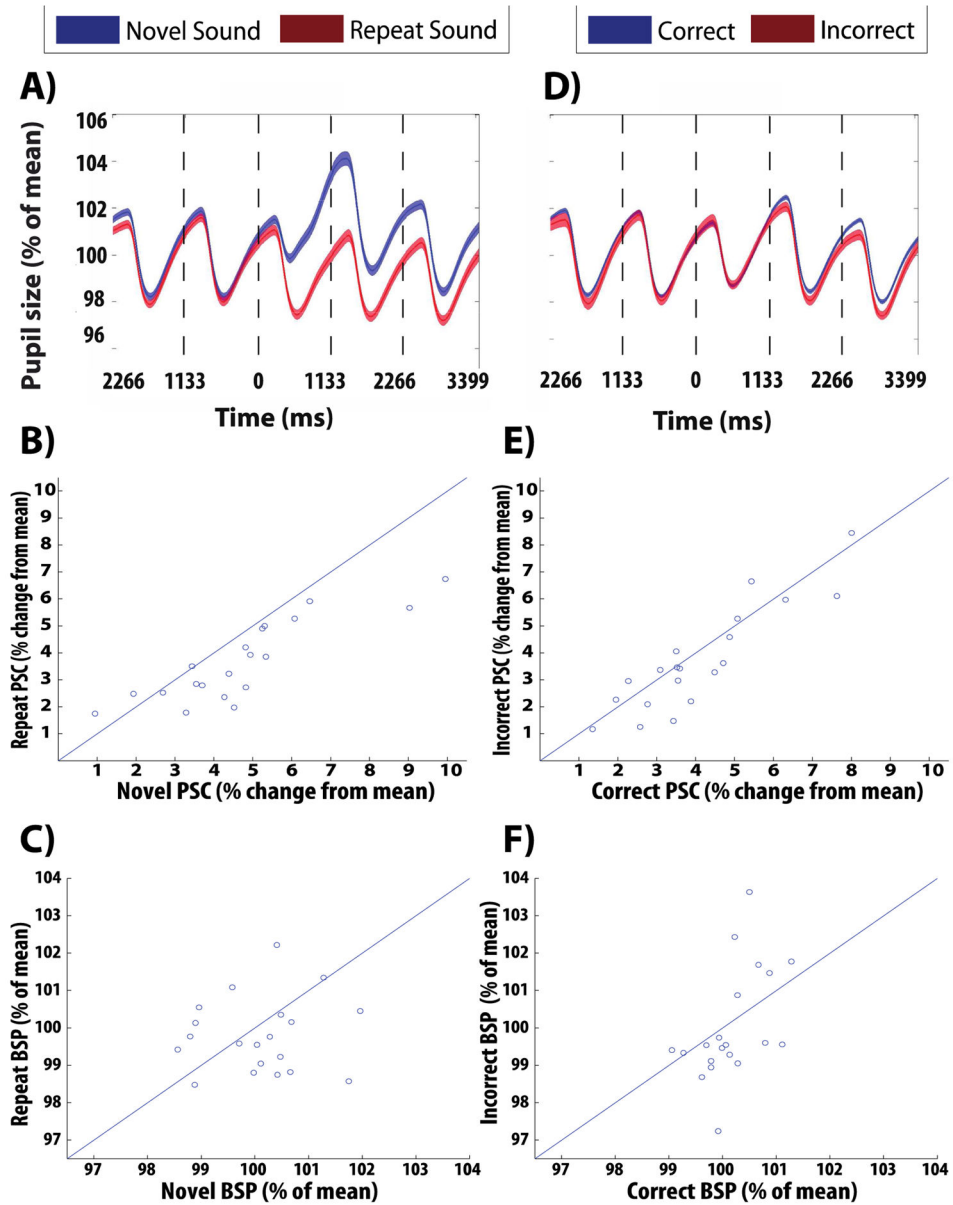


Figure 6. Pupil Dynamics in Sound Experiment

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 to Repeat Sounds induced larger pupil-size changes while **C)** no difference between baseline pupil-size was observed. **D)** Pupil dynamics during Correct and Incorrect trials showed **E)** increased pupil-size change during Correct trials compared to Incorrect trials. **F)** No significant difference in baseline pupil-size between Correct and Incorrect trials was observed. Shading (**A, D**) represents within-subject standard error.

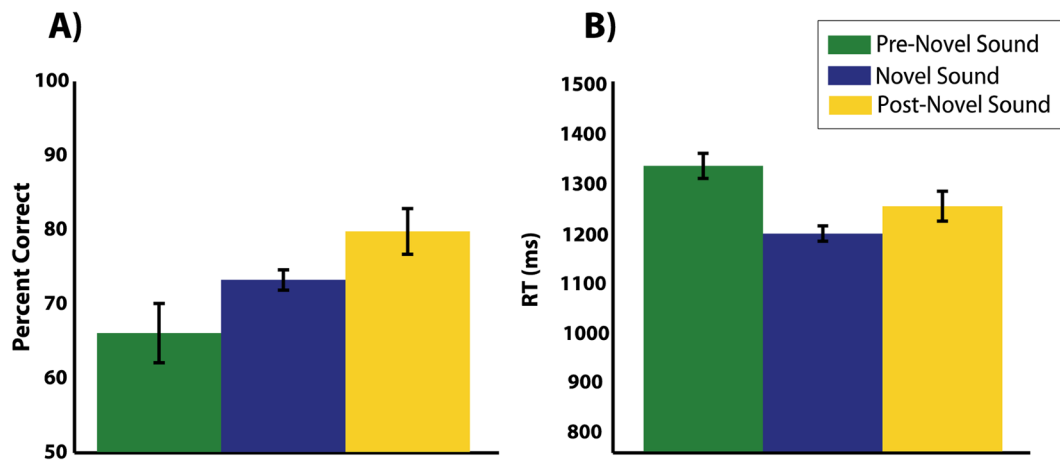


Figure 7. Catch Trial Accuracy and RT

Performance by condition before and after Novel Sound presentation during Catch trials indicates **A)** increased accuracy for Post-Novel Sound-paired scenes compared to Pre-Novel Sound-paired scenes and **B)** decreased RT for Post-Novel Sound-paired scenes, compared to Pre-Novel Sound-paired scenes. Error bars are within-subject standard error.