# Oculomotor behaviours and integrative memory functions in the Alzheimer's Clinical Syndrome

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**Running Title:** Pupil size, STMBT, and Alzheimer's Clinical Syndrome

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**Abstract**

**Background.** Biological information drawn from eye-tracking metrics is providing evidence regarding drivers of cognitive decline in Alzheimer disease. In particular, pupil size has proved useful to investigate cognitive performance during online activities.

**Objective.** To investigate the oculomotor correlates of impaired performance of patients with mild Alzheimer's Clinical Syndrome (ACS) on a recently developed memory paradigm, namely the Short-Term Memory Binding Test (STMBT).

**Methods.** We assessed a sample of eighteen healthy controls (HC) and eighteen patients with a diagnosis of mild ACS with the STMBT while we recorded their oculomotor behaviours using pupillometry and eye-tracking. The STMBT assessed the ability to detected changes across two consecutive visual arrays, study and test, presenting 2 bicolored objects. In one condition, changes consisted of new colours in test objects replacing colours presented in the study objects (i.e., Unbound Colours - UC). In the other condition, changes consisted of two studied colours swapping between objects at test (i.e., Bound Colours - BC). We assessed recognition of such changes.

**Results.** As expected, a group (healthy controls vs Alzheimer's Clinical Syndrome) by condition (Unbound Colours vs Bound Colours) interaction was found whereby behavioural group differences were paramount in the Bound Colours condition. Healthy control's pupil dilated significantly more in the Bound Colours than in the Unbound Colours condition, discrepancy not observed in Alzheimer's Clinical Syndrome patients. Furthermore, ROC analysis revealed the abnormal pupil behaviours distinguished Alzheimer's Clinical Syndrome patients from healthy controls with values of sensitivity and specify of 100%, thus outperforming both recognition scores and gaze duration.

**Conclusions**. The biological correlates of Short-Term Memory Binding impairments appear to involve a network much wider than we have thought to date, which expands across cortical and subcortical structures. We discuss these findings focusing on their implications for our understanding of neurocognitive phenotypes in the preclinical stages of Alzheimer Disease and potential development of cognitive biomarkers that can support ongoing initiatives to prevent dementia.

**Keywords:** Eye-tracking; Pupil behaviour; Gaze duration; Short-term Memory Binding Test; Alzheimer disease.

## 1. Introduction

There is consensus about the usefulness of eye-tracking metrics for the study of cognition [1]. Two metrics have proved particularly informative, gaze duration (i.e., sum of consecutive fixation durations within a target) and pupil behaviours. Gaze duration metrics are computed from the sum of fixations, which indicate the time spent looking at a particular location and reflect the amount of attentional resources needed to process the stimulus at that location [2]. Meghanathan, van Leeuwen, & Nikolaev [3] reported that gaze duration appears to be sensitive not only to attention but also to memory and processing load. As a moment-to-moment measure of the focus of attention, gaze path analysis can inform on strategies and approaches people use to solve problems. For example, fixation sequences can be compared across subjects and the probability of shifting from one particular stimulus to another can be calculated, thus informing on potential strategies [4, 1]. As such, gaze duration is prompted to the influence of top-down control mechanisms thus rendering this eye-tracking metric and its underlying brain correlates more vulnerable to response bias (the "gaze duration bias effect" [5]). One other eye-tracking metric which may be able to inform about pathophysiological changes linked to neurodegenerative diseases in a way that is less vulnerable to such a bias is the pupil response. Pupil behaviours appear to be related to fluctuations in autonomic arousal (Bradley et al., 2008), noradrenergic tone (Preuschoff et al., 2011; Einhäuser et al., 2008); Gilzenrat et al., 2010), control states (Ebitz & Platt, 2015; Gilzenrat et al., 2010), and cortical processing (Ebitz & Platt, 2015; Engel et al., 2016). Such mechanisms seemingly underpin pupil behaviours during visual processing and task performance and are independent of mechanisms subserving gaze patterns (McGinley et al., 2015; Ebitz & Moore, 2019). Evidence suggests that neurodegeneration found in the locus coeruleus in the earliest stages of Alzheimer's disease (AD) can explain abnormal pupil responses during cognitively demanding tasks, reflecting early compensatory changes (Granholm et al., 2017). If pupillometric responses accompanying cognitive tests reflect the influence of such subcortical mechanisms, then the proposal that this technique would be less affected by cognitive biases could be tenable. Cognitive load, as indexed by gaze duration, appears to be more influenced by top-down cortical control mechanisms like those coming from the lateral parietal cortex, the medial prefrontal cortex (mPFC) and the anterior superior temporal sulcus (aSTS) (Carlin J, & Calder A, 2013, for a review), while load indexed by pupil behaviours may also reflect the function of upregulation pathways which are mediated through a subcortical pathway. This involves the pretectal nucleus, which controls the pupillary light reflex; the superior colliculus, which mediates orienting responses, including pupil changes to salient stimuli; and the locus coeruleus norepinephrine neuromodulatory system, which mediates relationships between pupil-linked

arousal and cognition (Joshi & Gold, 2020). Hence, these metrics may be differentially sensitive to different stages of the diseases.

The relationship between cognitive load and pupil size has proved useful for measuring cognitive performance during online activities [10]. Recent studies show a strong direct correlation between cognitive load and pupil size, proposing the pupil size as an indicator of how efficiently the processing system operates [11]. The association of pupil size with cognitive processing is thought to result from an inhibitory effect on parasympathetic oculomotor structures through the release of norepinephrine (NE) by the locus coeruleus (LC) [12]. The LC- NE based modulatory mechanisms support attentional shifts to either, external or internal stimuli when a given event becomes more relevant than a coexisting one [11]. NE projections from the LC are sent to virtually all the brain regions with a larger density of projections to areas known to be important for visual processing. Furthermore, the LC sends its strongest innervations to brain areas known to be involved in selective attention processing e.g., parietal cortex and superior colliculus (SC) [13]. Thus, NE plays a crucial role in activating the cortical system and promoting adequate levels of arousal during cognitive performance. Corbetta, Patel, & Shulman [14] proposed that a visual detection system centred on the ventral portions of the temporo-parietal and frontal cortex is responsible for the detection of relevant events, particularly salient stimuli. The ventral detection system is relevant for the type of information that produces pupil change because this network receives strong input from the LC-NE system [13]. Because the LC is a key node of the neural circuitry subserving pupil behaviours, the analysis of externally observable responses which are linked to activity of this specific subcortical loci would provide a robust unbiased measure of cognitive processing demands [15]. There has been an important number of studies linking pupil responses to memory for scenes [16], objects [17], objects in scenes [18] and to memory span [10]. Recently, in a voice recognition study, Papesh *et al.* [19] showed that pupil size reflected part of the recognition memory process. In their study, pupil changes during encoding could accurately track subsequent estimates of memory strength. The researchers reported that it is possible to assess whether pupil behaviour reveals differences in strong versus weak memories across both encoding and retrieval. In Sterpenich *et al.* [20], it was suggested that the LC is engaged during the process of memory retrieval, proposing a role of the LC-NE system in the consolidation of memories. In all the above-mentioned studies, pupil size was used as a metric of the relative amount of effort needed to accomplish a task.

Recent evidence suggests that oculomotor behaviours linked to cognitive performance can reliably inform on the presence of the Alzheimer's Clinical Syndrome[1] (ACS) [21]. The approach comprises the analysis of gaze duration responses during performance on the short-term memory binding test (STMBT). The STMB function declines in patients with dementia due to AD [22] and in those who will inevitably develop dementia due to familial AD but are still asymptomatic as demonstrated by traditional neuropsychological tasks [22, 23, 24]. STMB is an integrative memory function known to support the conjunction of features necessary to create objects' identity [25, 26]. Such a function relies on regions along the visual ventral stream but is independent of the hippocampus [27, 28, 29]. Parra and collaborators recently showed that STMB deficits in patients in the prodromal stages of familial AD are associated to altered patterns of brain connectivity which seem to involve not only parietal-occipital regions but also the frontal lobes [30, 31, 32]. This network seems to share nodes with the network controlling attention mechanisms. For example, Mayer et al. [33] carried out an fMRI study and found that encoding information into visual working memory and visual selective attention require access to common neural resources. The interplay between attention and visual working memory can be investigated through the analysis of oculomotor behaviours during task performance [7, 8, 34]. Oculomotor behaviours can inform not only on the acquisition of visual information at fixation, but also the accumulation of information in memory [3, 8]. Fernández *et al*. [21] recently showed that changes in gaze durations significantly predict the presence of AD when such an oculomotor behaviour is linked to performance on the STMBT. From a practical perspective, these results suggest that such a combined analysis can differentiate patients with AD from healthy controls. The STMBT has been considered a cognitive marker of AD [35]. Hence, the associated information drawn from eye movement behaviours holds value as a potential biomarker for the disease. From a theoretical perspective these findings suggest that the brain network responsible for attention control may be reactive to the cognitive load posed by a task condition that requires feature integration. Earlier studies provide support to this notion confirming that attention may be necessary to form bindings in perception and hold them in memory [36, 37]. Mayer et al. [33] suggest that competition for resources shared by visual attention and WM encoding can limit processing capabilities in distributed brain networks. Taken together this evidence indicates that meeting the additional attentional

[1] Alzheimer's Clinical Syndrome is the recommended terminology for clinically ascertained multi- (or single-) domain amnestic syndrome or a classic syndromal variant (i.e., what has historically been labeled "possible or probable AD"). It applies to both mildly impaired and demented individuals. The term "Alzheimer's disease" is reserved for situations where neuropathologic or biomarker evidence of the disease (i.e., Aβ plaques and pathologic tau deposits) is present (Jack et al., 2018).

demands of encoding feature bindings during the STMBT may be the aspect of the test that patients with AD find particularly challenging [31].

The evidence reviewed above warrants investigation of pupil behaviours during STMB performance. Further support to the feasibility of this investigation comes from a recent study which found that pupillary responses during a simple digit recall task could hold biomarker properties for AD [10]. Evidence has now accrued suggesting that the circuitry that regulates pupillary responses might be targeted by AD in its earliest stages [38]. If the hypotheses about the unbiased nature of pupil behaviours holds true, such an eye-tracking metric might prove more sensitive to AD than gaze duration. The present study addressed this hypothesis by analysing pupil size during the STMBT [22]. To examine whether pupil behaviours linked to STMB impairments are informative of AD pathology, we compared ACS patients' and healthy controls' pupil responses during performance on a STMBT developed by Parra, Abrahams, Logie, and Della Sala [39] which has been used to assess AD samples [22]. A key feature of this STMBT is that it controls for memory load across baseline (Unbound Colours) and the core condition (Bound Colours) with the only difference being greater processing load for the latter (i.e., processing colours and their combination) relative to the former (processing colours). We therefore hypothesized that if pupil size reflect processing demands, controls' pupil size will increase significantly more than that of ACS patients when they bind visual features in STMB. Moreover, we were also interested in investigating if abnormal patterns of pupil behaviour-memory binding performance would be informative of the disease presence at the individual level. Although the current studied focused on pupil behaviours, we were interested in exploring how well this oculomotor responses would discriminate patients from controls relative to eye-tracking and behavioural measures we have previously investigated [21]. Based on this earlier study, and considering recent observations by Granholm et al. [10], we hypothesized that gaze duration and behavioural responses will differ significantly when comparing controls and ACS patients, whereby controls will produce longer gaze durations and more correct target recognitions while binding visual features in short-term memory. However, pupil behaviours would outperform these outcome measures.

## 2. Methods

### *2.1. Participants*

Data were acquired from 18 healthy older adults (controls) with mean age of 69 years (SD=3.6) and mean education of 17.1 (in years) and from 18 patients with mean age 68 (SD=2.2 years) and mean education 13.6 (in years) ($F(1, 34)= 1.49$, $p =0.23$ and $F(1, 34)= 22.23$, $p =0.001$, age

and education respectively). It is worth noting that the STMBT has proved insensitive to the level of education of the assessed individual ([22] see also [40]), a feature that has led to the suggestion that this task is a cross-cultural cognitive marker for AD [41]. Our sample consisted of 13 controls and 13 patients who were assessed by Fernandez et al. [21] plus 5 new patients and 5 new controls who were recruited for the present study. Controls underwent the same neurological and cognitive assessments completed by patients. A trained neurologist was responsible for confirming the healthy status . Patients were diagnosed based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria which were applied by a psychiatrist. They were recruited at the Hospital Municipal and at Clinica Privada Bahiense, both of Bahía Blanca, Buenos Aires, Argentina. All patients underwent a detailed clinical interview asking about medical history, physical/neurological examination and thyroid function test. All the patients underwent biochemical analysis (haemoglobin, full blood count, erythrocyte sedimentation rate, urea and electrolytes, blood glucose), to rule out other common pathologies such as psychiatric diseases, Traumatic Brain Injury, Cardio Vascular Disease, Brain Tumours or Infectious diseases of the CNS. Patients were excluded if: (1) they suffered from any medical conditions other than dementia that could account for, or interfere with, their cognitive functioning; (2) had evidence of vascular lesions in CT or MRI scans; (3) had evidence for an Axis I diagnosis (e.g. major depression or drug abuse) as defined by the DSM-IV. To be eligible for the study, patients had to have at least one caregiver providing regular care and support. Patients taking cholinesterase inhibitors (ChE-I) were not included. None of the subjects was taking hypnotics, sedative drugs or major tranquillizers. Those participants with a diagnosis of Ophthalmologic diseases such as glaucoma, visually significant cataract or macular degeneration were not eligible to participate in the study. Subjects' visual acuity was 20/20 or corrected to 20/20 as confirmed by an ophthalmological assessment. The Ishihara's test was used to rule out colour blindness. The investigation adhered to the principles of the Declaration of Helsinki. All patients and all control subjects signed an informed consent prior to their inclusion in the study which was obtained according to the Declaration of Helsinki. The study received approval by the Ethics Committee of the Hospital Municipal de Agudos (Bahía Blanca, Buenos Aires, Argentina). As our sample of patients was identified based on clinical criteria and not on biomarker evidence, we decided to adhere to recent recommendations regarding the terminology that should be used in such cases [42], and refer to our patient sample as Alzheimer's Clinical Syndrome (ACS).

The mean score of ACS patients on the Mini-Mental State Examination (MMSE) [43] was 23.1 (SD = 2.2) and 29.7 (SD = 03) in Controls ($F(1, 34)= 596.4$, $p =0.001$). The mean score of ACS

patients on the Adenbrook's Cognitive Examination - Revised (ACE-R) [44] was 66.4 (SD = 15.7) and in Controls was 98.5 (SD = 1.5) ($F(1, 34)= 366.5$, $p =0.001$). The mean score of ACS patients in the INECO Frontal Screen (IFC) [45] was 19.3 (SD = 3.4) and of 29.3 (SD = 0.7) in Controls ($F(1, 34)= 366.5$, $p =0.001$). The mean score of ACS in the Trial Making Test A (TMT-A) [46] was 66.9 (SD = 31) and of 35.8 (SD = 12) in Controls ($F(1, 34)= 6998$, $p =0.001$). These outcomes from the neuropsychological assessment suggest that our patients group presented with mild dementia.

### *2.2. The STMBT and eye movement assessment*

Stimuli were presented on the centre line of a 20" LCD Monitor (1024 x 768 pixels resolution). Participants sat at a distance of 60 cm from the monitor. Head movements were minimized using a chin rest. Eye movements were recorded with an EyeLink 1000 Desktop Mount (SR Research) eyetracker, with a sampling rate of 1000 Hz and an eye position resolution of 20-s arc. All recordings and calibration were binocular. Dim lighting conditions were kept constant throughout testing sessions and although the stimuli were not equiluminant, the same visual arrays were presented to all the participants recruited into the study. A pupil size normalization procedure was applied on each individual trial, dividing pupil size data by the mean baseline value drawn from the fixation period. Pupil size during blinks and saccades was replaced by a linear interpolation from the last valid sample before the beginning of blink or saccade to the first valid sample after the end of each event. Stimulus presentation and data collection programs were developed using C++.Participant's gaze was calibrated with a standard 13-point grid for both eyes. After validation of calibration, the STMBT began. During the task, participants were presented with arrays of object shapes in random positions of a 3x3 virtual grid which sustained $10^{o}$ of visual angle. The stimulus was constructed following the layouts developed by Parra et al. [39], which was defined by a shape and a frame area. The shape or frame area of the stimulus (each representing 50% of the surface) was filled with a colour. The procedures used to select the colours and the psychophysical features of the colours selected were reported in Parra et al. [39]. During the task, participants were presented with arrays of two object shapes. Based on the study by Parra et al. [39] this seems an optimal array size to identify STMB impairments in AD patients (see also [22] and [47] for recent evidence).

Trials began with a fixation screen (i.e., a cross) shown for 250 ms. This was followed by a study display presented for 2000 ms (Figure 1). After an unfilled interval of 900 ms, the test

display was presented until the participant responded. There was then an inter-trial interval of 1000 ms. In half of the trials objects on both displays were the same. In the other half, the objects in the test display showed different colours from those in which they were presented during the study display. Object locations in the test display were always randomly changed to render location an uninformative feature. Participants were requested to detect whether the study and test displays consisted of the "same" or "different" items and to respond verbally accordingly. Responses were manually typed by a trained instructor.

Two experimental conditions were used (Figure 1). In the Unbound Colour (UC) condition both the shape and frame areas of each object were shown in different colours. In the "different" trials, the colour from either the shape (50%) or the frame (50%) area of the two objects was replaced by a new colour that had not appeared in the study display. Participants were told to focus on colours and not on their associations as the change would consist of new colours. In the condition assessing memory for Bound Colours (BC) both the shape and frame area were also of different colours. However, in the "different" trials the two objects swapped either the colour from the shape area (50%) or from the frame area (50%). Participants were told that colours and their associations were both relevant as sometimes colours would be rearranged in different combinations during the test display. For each condition participants performed 15 practice trials followed by 32 test trials. The "same" and "different" trials were fully randomized and conditions were blocked and counterbalanced across participants. The task takes approximately 5 minutes per condition (i.e., BC and UC) and participants can take breaks after each condition for as long as they need it.

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Insert Figure 1 about here

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### *2.3. Statistical analysis*

Statistical analyses were performed in R version 3.1.1 (RDevelopment Core Team). Group differences in the STMBT were tested with ANOVA and linear regression. We used a between-subjects factor Group (Controls vs ACS patients) and as within-subjects factors Condition (UC

vs BC) and Memory Stage (Encoding vs. Retrieval). The dependent variable of the linear model was the normalized pupil size as function of % of correct recognition. Additionally, we carried out ROC analysis to determine the sensitivity and specificity of the pupil size and behavioural responses (i.e., score) when performing the STMBT.

We used the *lmer* program of the *lme4* package (version 0.999999-2) (Bates & Maechler, & Bolcher, 2013) for estimating fixed and random coefficients. This package is supplied in the *R* system for statistical computing (version 3.0.1; R Development Core Team, 2013) under the GNU General Public License (Version 2, June 1991).

The dependent variable of the Linear Mixed Model (LMM) was pupil size. Fixed effects in LMM terminology correspond to regression coefficients in standard linear regression models. They can also estimate slopes or differences between conditions. In addition, we estimated how strongly the mean pupil size varied with participants and conditions by fitting crossed random intercepts for participants. Instead of estimating a slope or the difference between conditions, random effects estimate the variance that is associated with the levels of a certain factor.

The LMM applied for modeling the pupil size is given by the following formula, PUPIL SIZE_MODEL <- lmer(data, formula = (Pupil size) ~ ((BC vs. UC ) * (ACS vs. Controls) + ((1|id)),

In addition, we applied a LMM for modeling the gaze duration using the following formula, Log Gaze Duration <- lmer(data, formula = (log Gaze Duration) ~ ((BC vs. UC ) * (ACS vs. Controls) + ((1|id)), (See appendix for the supplementary Table).

Regression coefficients (*bs*) standard errors (*SEs*) and t-values (*t=b/SE*) are reported for the LMMs. In general, given the large number of observations, subjects entering our analysis and the comparatively small number of fixed and random effects estimated, the *t*-distribution is equivalent to the normal distribution for all practical purposes (i.e., the contribution of the degrees of freedom to the test statistics is negligible). Our criterion for referring to an effect as

significant is $t = b/SE > \pm 1.95$. Finally, we calculated and reported p-values for each variable included in the model.

## 3. Results

### *3.1 Correct recognition during the STM binding task*

Mean behavioural data during the STMBT is shown in Figure 2. Relative to Controls, patients with ACS showed a large drop in performance on the Bound Colours (BC) condition (68% and 91% of correct responses for ACS patients and controls, respectively) a discrepancy not found during the Unbound Colour (UC) condition (86% and 92% of correct responses for ACS patients and controls, respectively). To analyse performance, a two-way mixed ANOVA was used. The between-subject factor was Group (controls vs. ACS patients) and the within-subjects factor was Condition (BC vs. UC). The ANOVA resulted in a main effect of Condition: ($F$=96.03, p<0.001) but not of Group: ($F$=4.03, ns). The Condition by Group interaction was significant ($F$=12.13, p<0.001). Four post-hoc comparisons, two across groups (i.e., UC: ACS vs. controls and BC: ACS vs. controls) and two across conditions (ACS: UC vs. BC and controls: UC vs. BC) were carried out to further investigate the interaction (corrected p-value = 0.005). They revealed that performance of ACS patients on the BC was significantly poorer than that of controls (t=5.33, p=0.001), and their own performance on the UC condition (t=4.21, p=0.001). No other contrast resulted in significant differences.

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Insert Figure 2 about here

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### *3.2. STMBT and pupil size*

Table 1 presents (a) pupil size data across Memory Stages (Encoding vs. Retrieval) and Task conditions (BC and UC) as function of % of correct recognition. It also presents outcomes from the statistical comparisons including (b) interactions of Condition x Group, and (c) interactions of Condition x Group x Memory Stages.

*Averaging across predictors.* When we analysed the effect of Memory Stage on pupil size we noted a significant effect reflecting increased pupil size during Retrieval relative to Encoding (See Table 1 and Figure 3). The analysis of the interaction between Memory Stage (Encoding

vs. Retrieval) and Task Condition (BC vs. UC) revealed that overall, pupil size was significantly larger during the Retrieval stage of memory than during the Encoding stage and that was true regardless of Task Condition (See Table 1 and Figure 3).

*Interaction of Group x Condition*. We then evaluated whether pupil size was differentially affected during the BC condition in ACS patients relative to controls. As shown in Table 1 and in Figure 3, the mean pupil size significantly increased in controls as compared to ACS patients only in BC condition.

*Interaction between Group x Memory Stages across Task Condition*. As Table 1 shows, the two-way interaction between *Group and Memory Stage* was significant for the BC condition but not for the UC condition. Further analysis to reveal the source of this interaction included contrasts between memory stages for each group separately and between groups for each memory stage separately for the BC condition only. These contrasts revealed that ACS patients' pupil size during encoding was significantly smaller than during retrieval ($t=-9.83$; $p=0.0001$). Such a discrepancy was less pronounced in controls ($t=2.35$; $p=0.01$). Moreover, differences between ACS patients and controls during encoding were much larger ($t=11.12$; $p=0.0001$) than those observed during retrieval ($t=7.62$; $p=0.0001$) (See Figure 3).

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Insert Table1 about here

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Insert Figure 3 about here

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When analyzing Gaze duration, controls' encoding responses differ from those seen in ACS patients (for a complete description See Supplementary Material, Table 2 and Figure 5). Controls seem to meet the encoding demands of the BC task more effectively than ACS patients. Mirroring Fernandez et al., 2018 [21] ACS patients however, showed shorter gaze duration particularly during the encoding stage of the BC condition.

The analyses carried out so far suggest that the discrepancy between UC and BC during the encodings stage provides the best distinction between AD patients and controls. We subjected this hypothesis to further analyses using ROC curves. We decided to also enter behavioural data and gaze duration data which, as reported previously [21], help distinguish between ACS patients and controls. We were interested in investigating whether pupil behaviours would outperform gaze duration and memory scores in the distinction of ACS patients and controls.

ROC analyses revealed that pupil size linked to STMBT performance achieved 100% sensitivity and 100% specificity to distinguish between controls and ACS patients. This is in strike contrast with the classification power shown by behavioural responses (i.e., percentage of correct recognition) which achieved acceptable but lower levels of accuracy (83% sensitivity and 81% specificity) (Figure 4). Finally, gaze duration, achieved 73% of sensitivity and 74% specificity to distinguish between controls and ACS patients. We compared the AUC across classifiers. We found that the AUC for Pupil size was significantly higher than that of memory scores ($p=0.03$) and gaze duration ($p=0.002$). The AUC for memory scores and gaze duration did not significantly differ ($p=0.32$).

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Insert Figure 4 about here

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## 4. Discussion

The current study was aimed at investigating whether abnormal pupil behaviours accompany impaired performance on a task known to be a cognitive marker for AD [22]. Such biological evidence would grant this cognitive marker property of a biomarker for this type of dementia. The key findings of this study are: (1) altered pupil size do accompany poor STMB abilities in ACS patients, (2) such impaired pupil responses in ACS patients seem to be paramount during memory encoding, (3) pupil size outperforms behavioural (i.e., percentage of correct recognition) and other oculomotor responses, i.e. gaze duration [21] as a classification tool to distinguish between normal ageing and dementia in the ACS. These findings have both theoretical and practical implications which we discuss in turn.

### *4.1. Pupillometry and STMB in the ACS*

Pupil size increases during cognitive tasks in response to increased demands [10]. The results here presented provide evidence supporting the notion of biologically costly memory binding functions operating within the same feature dimension (i.e., colour). Parra et al.[39] had previously demonstrated that behaviourally, processing BC in STM is more resource demanding than processing the same number and type of UC (mirroring Parra et al. [22] and Fernandez et al. [21] results) and that such a cost is independent of age. Parra et al. [22] then revealed that this age-insensitive STMB function is very sensitive to AD regardless of its clinical variant (i.e., sporadic or familial AD). These findings from sporadic AD were recently replicated by Fernandez et al. [21]. Moreover, by adding eye-tracking to such an assessment, biological underpinnings of STMB abilities are revealed. ACS patients and controls showed similar patterns of pupil dilation during the UC condition. This suggests that the impairment found in these patients is unlikely due to general STM functions or attentional deficits. However, ACS patients and controls produced well-differentiated pupil dilation when performing the BC condition. As we hypothesized, relative to ACS patients, controls showed a significant increase in their pupil size when binding colour-colour targets (see Figure 3), seemingly due to the increased cognitive effort required to process the binding between colours in STMB. Meghanathan et al. [3] proposed that whereas gaze duration is sensitive to both memory and processing load, pupil size is only sensitive to processing load. Our data seem to support this view. In the context of the current STMB paradigm, memory load, as defined by the number of to-be-remembered items, is held constant across the UC and BC condition. The only difference between these conditions is the need to bind features together in the latter. Feature binding, as a function subserving object-based representations in STM, seems to be linked to processing demands [48] rather than to capacity demands (see [25, 26]).

Our current results not only confirm that altered pupil behaviours are linked to STMB impairments in ACS but they also unveil the potential source of such memory impairments. ACS patients' pupil dilated significantly less during the encoding of BC than during their retrieval. This suggests that inefficient encoding mechanisms could be the source of impairments found in these patients during the STMBT, in line with our previous work [21]. Similar conclusions were presented by Parra et al., Pietto et al. [32, 32] using EEG brain connectivity and ERP analysis respectively. These results are both encouraging and intriguing. They raise questions as to the potential relationship between visual STMB as a function supporting object identity formation, the neuroanatomical correlates of pupil behaviours during such a test, and ACS pathology. We will address this relationship next.

Reduced pupil responses during the encoding of BC into visual STM may be the output of inefficient subcortical-cortical integration mechanisms responsible for activating the attentional pointers that keeps features integrated within object representations [49, 37]. Pupil dilation correlates with activity in the LC, superior colliculus, right thalamus, and the anterior attention network which manages competing demands for working memory resources [14]. It has been suggested that the LC-NE circuit is engaged during the process of memory consolidation [20]. In Alnaes et al. [12], pupil size increased when tasks required more cognitive effort. Recently, Kelly et al. [50] proposed that LC cell loss appears to happen early in the clinical progression of AD, concurrent with cell loss in the nucleus basalis and entorhinal cortex (see also [38]). The researchers proposed that the central NE projection system is essential for cognitive function and in turn, LC neural degeneration contributes to cognitive dysfunctions. Previously, Ross et al. [51] reported that the duration of AD progression correlated significantly with neuronal loss in the LC. Analyses of post-mortem AD brain tissues of LC indicate that cell loss reaches as high as 50% in the rostral region of the nucleus, and further, this is associated to a 31% reduction of cortical NE levels [52]. Arendt et al. [53] demonstrated a significant loss (~13%) of neuromelanin positive LC neurons in subjects classified as MCI/prodromal AD compared to those classified as controls. Subjects classified as mild/moderate AD exhibited ~30–45% LC cell loss compared to controls. The magnitude of LC degeneration and associated cortical NE depletion correlate with severity of dementia and cognitive impairment [10]. Therefore, given the links between pupil size and the functions of the LC neuromodulatory system, as well as between the LC and AD, pupil responses during the STMBT seem to provide biomarker evidence of the functional integrity of brain circuits that are affected in the earliest stages of ACS.

Recently, Castellotti et al., [54] suggested that the pupil diameter is also sensitive to top-down modulation, and consequently the pupil diameter could be modulated by cortical pathways in addition to the subcortical proposed system. It seems that pupillary constriction results from the activation of the subcortical Edinger-Westphal nucleus (EW) [55] (Gamlin & Clarke, 1995), and there are some known modulatory inputs from cortical areas to this circuit. First, EW activity is enhanced by inputs from the visual cortex [56, 57] (Becket Ebitz & Moore, 2017; Binda & Gamlin, 2017) and the superior colliculus [58, 59, 60, 61, 62] (Gamlin, 2006; Joshi & Gold, 2019; Joshi, Li, Kalwani, & Gold, 2016; Wang & Munoz, 2015; Wang & Munoz, 2012). Other possible inputs could come directly from the prefrontal cortex, in particular from

the frontal eye field (FEF), or indirectly through the striate cortex, the oculomotor regions in the parietal cortex and the superior colliculus that are modulated by FEF [56] (Becket Ebitz & Moore, 2017). EW nucleus also receives inhibitory input from the sympathetic system through projections from locus coeruleus [60, 63] (Joshi et al., 2016; Peinkhofer et al., 2019). A reduction of this inhibitory inputs could result in a pupillary constriction [59, 64] (Joshi & Gold, 2019; Wilhelm, 2002).

### *4.2. Eye-tracking metrics during STMB can yield AD biomarkers*

Our results show that both gaze duration and pupil size during the encoding of feature bindings in visual STM are oculomotor behaviours severely affected by ACS (see Figure 3). However, when comparing gaze duration and pupil behaviours using ROC analysis (see Figure 4), pupil size was able to distinguish ACS patients from controls with 100% sensitivity and specificity. This classification power was significantly better than that achieved by both memory scores and gaze duration. We predicted that pupil behaviours would be more informative of AD pathology than gaze duration because the latter would be impacted by top-down biased mechanisms whereas the former would reflect the function of more biologically driven bottom-up functions which are known to be affected in the very early stages of AD [13, 65, 38]. Although the results here presented support this hypothesis, they also open new questions. The interplay between cortically driven feature binding mechanisms and subcortically driven attention mechanisms seemingly needed to process (e.g., encode) such bindings in visual STM, and the role of such a network in the impaired abilities observed in people at risk of AD dementia and in those already affected, will need to be investigated.

Future studies will be needed to investigate if such specificity holds for oculomotor behaviours. Based on our current data it would be difficult to disentangle the precise contribution of poor encoding or retrieval functions, as informed by pupil size, to memory performance. However, our data suggest that pupil size discrepancies in the BC vs UC condition during encoding was

significantly larger than that during the retrieval stage (t=8.93 and t=3.95 respectively). Such a large impact of the ACS on binding functions supporting working memory encoding will carry forward to the retrieval stage, as less information will need to be searched for and retrieved by patients compared to healthy controls. Although under such circumstances it would be difficult to identify the precise contribution of poor retrieval functions to memory binding impairments, such contributions cannot be ruled out and will need further research.

In addition, future studies may explore whether maximal pupil sizes, which could be reached by prolonging the encoding period, would modify the outcomes here presented. Moreover, we classified patients based on clinical outcomes (ACS) so we cannot ascertain that AD was always the underlying pathology. Future studies will require AD biomarkers to enhance the diagnosis.

In sum, the analysis of pupil size during the STMBT provides biomarker evidence of the ACS thus granting this novel test properties of a cognitive biomarker for such a disease. These findings create new opportunities to explore the potential of such a low-cost and easy to use methodology (i.e., peripheral biomarkers) as a screening tool to support dementia prevention initiatives globally.

## 5. Acknowledges

We acknowledge the support from ViewMind inc. We acknowledge the support from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) provided to Gerardo Fernández. We also acknowledge the support from The Carnegie Trust via the Carnegie Research Incentive Grant awarded to Mario A Parra in collaboration with Gerardo Fernández. Finally, we acknowledge commentaries and technical support from Marcela Schumacher.

## 6. Funding

This project was supported by The Carnegie Trust, Carnegie Research Incentive Grant (Trust reference: RIG007838) awarded to Mario A Parra in collaboration with Dr. Gerardo Fernández.

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FIGURES

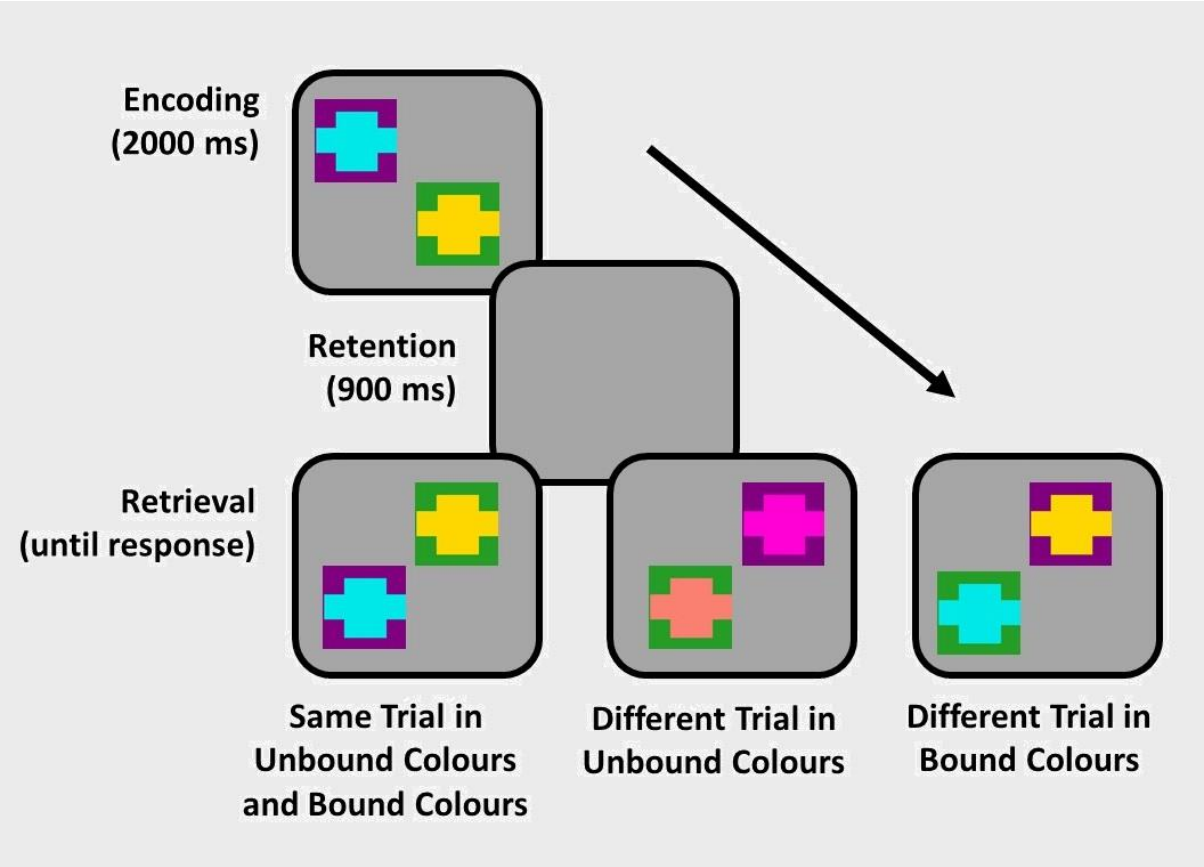


**Figure 1**. **Binding Task**. Trial design for the two conditions of the STM task.

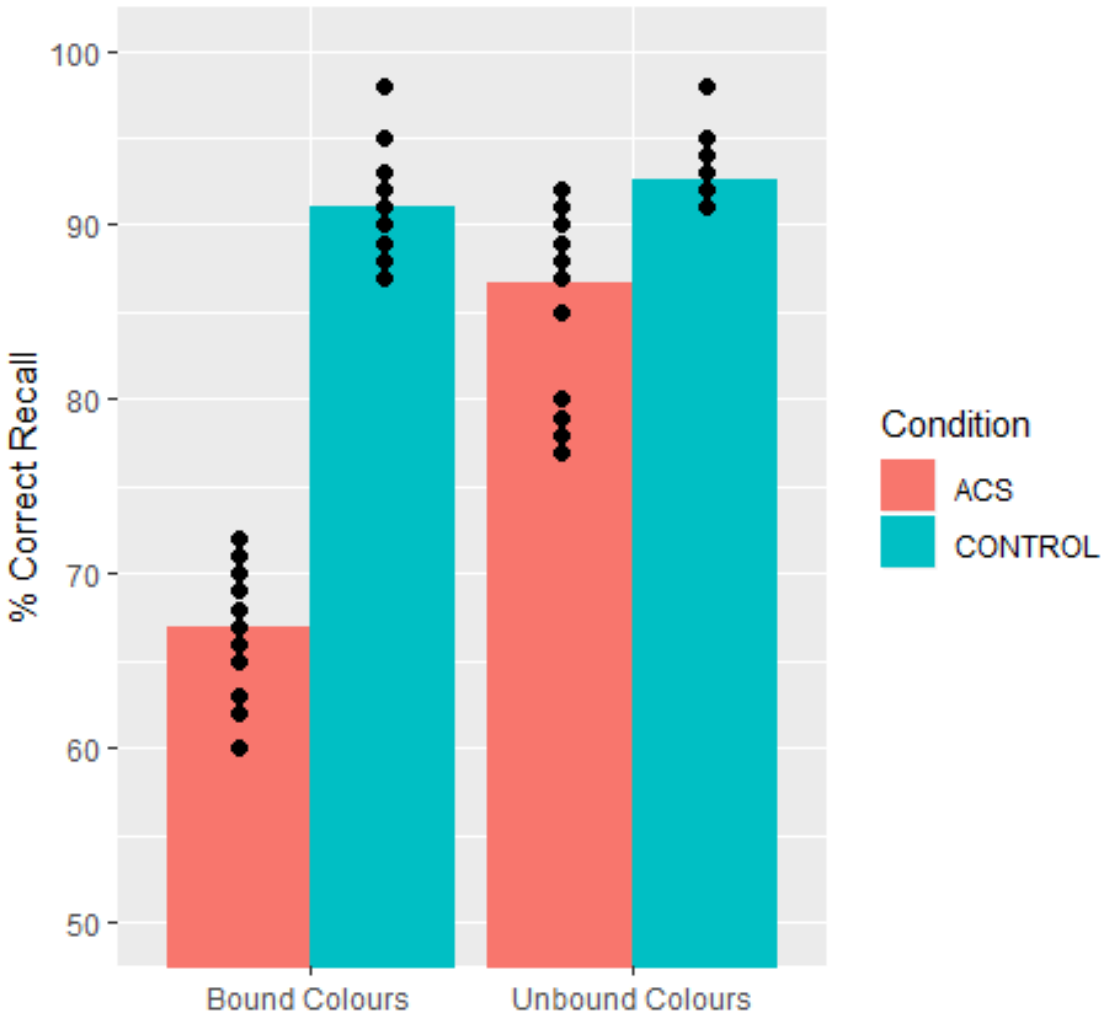


**Figure 2. Behavioural Response.** Corrected recognition during the two experimental conditions in both controls and ACS patients (error bars = standard errors of the mean).

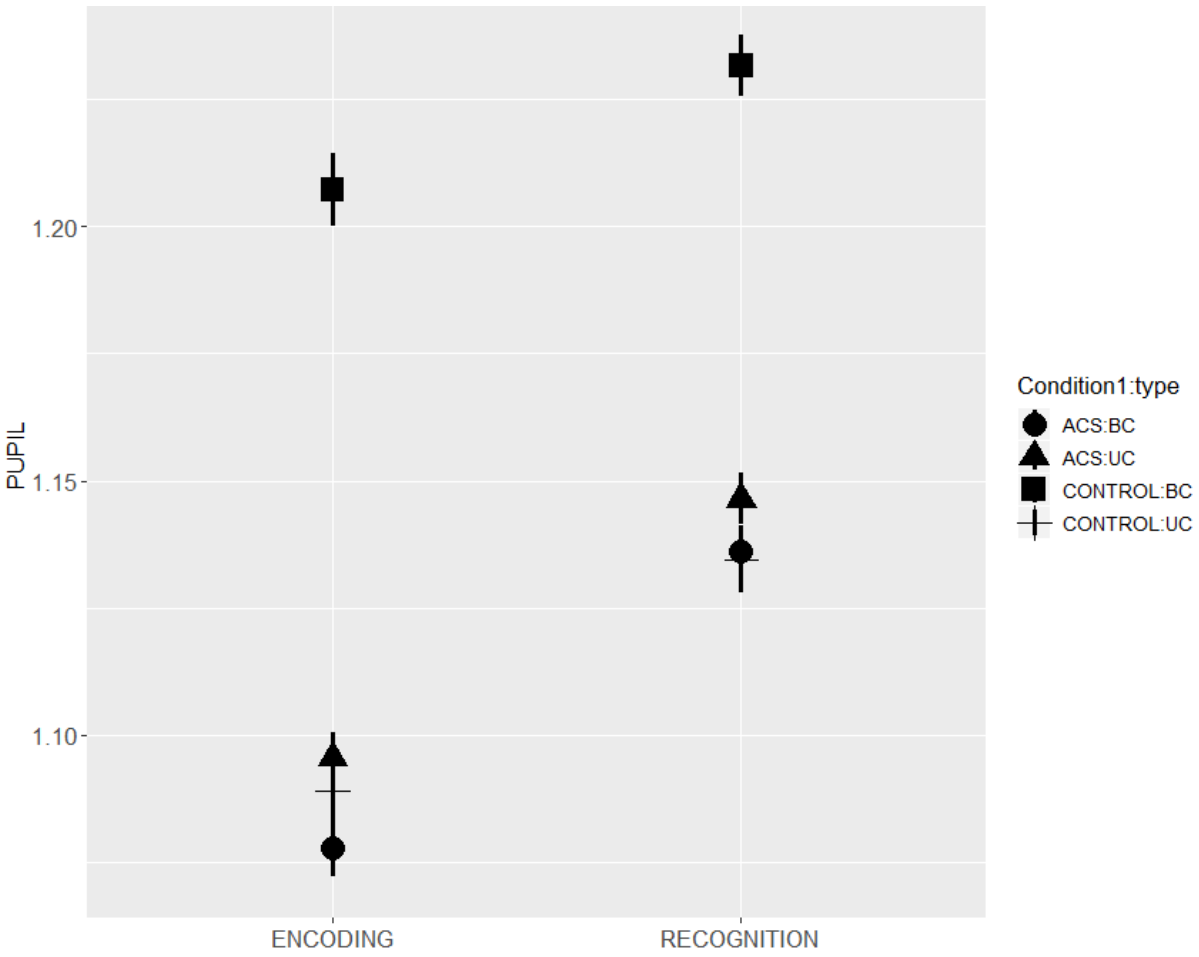


**Figure 3. Regression with pupil size, memory binding and group.** Effect of binding task on pupil size in control and in ACS patients during Encoding and Recognition moments. Panel show effects of Lineal Model. Bar errors denote 95%confidence intervals. Pupil size is plotted on a transformed scale for correspondence with the LM.

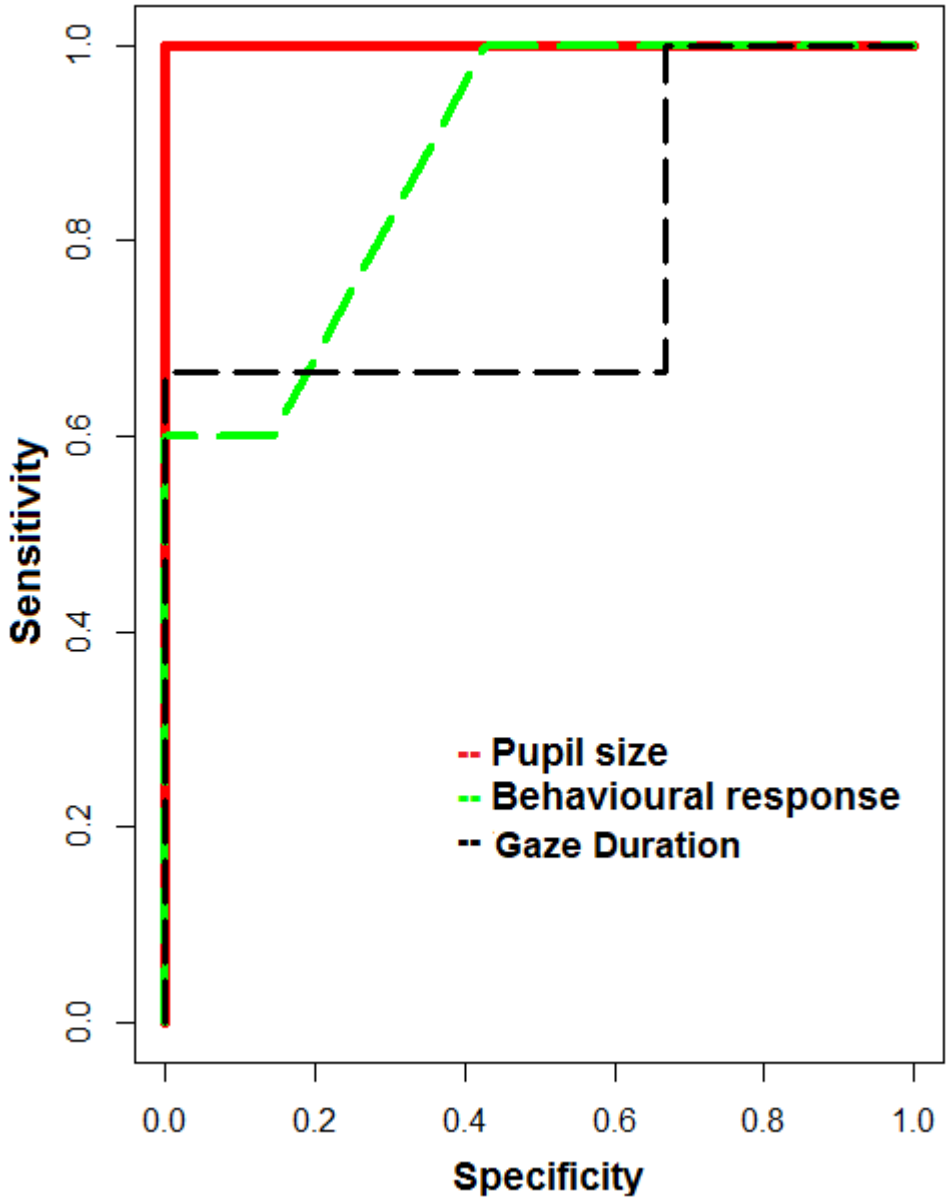


**Figure 4. ROC Analysis.** ROC analysis with performance on pupil size, on behavioural response and Gaze duration during the visual short-term memory (VSTM) binding task for ACS patients and controls.

**TABLE**

| | Pupil size | | | |
|---|---|---|---|---|
| | Mean | SE | t-value | p-value |
| Normalized Pupil diameter | 1.104 | 0.004 | **265.46** | 0.000 |
| Encoding vs. Retrieval | 0.014 | 0.005 | **2.63** | 0.008 |
| UC vs. BC X Encoding vs. Retrieval | 0.075 | 0.016 | **4.54** | 0.000 |
| **Group x Task Condition** | | | | |
| Control vs. ACS X BC | 0.107 | 0.012 | **8.93** | 0.000 |
| Control vs. ACS X UC | -0.017 | 0.011 | -1.54 | 0.121 |
| **Group x Task Condition x Memory Stage (Encoding vs. Retrieval)** | | | | |
| Control vs. ACS X BC X Enc. vs. Ret. | 0.063 | 0.015 | **3.95** | 0.000 |
| Control vs. ACS X UC X Enc. vs. Ret. | 0.003 | 0.003 | 1.04 | 0.294 |

**Table 1.** Pupil size data across Memory Stages (Encoding vs. Retrieval) and Task conditions (BC and UC) as function of % of correct recognition and outcomes from LM models.