Does central noradrenergic activity account for attentional analgesia in humans? A pupillometric analysis

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1. Background:

The primary source of noradrenaline to the central nervous system (CNS) is the locus coeruleus (LC), a nucleus located in the dorsal pontine tegmentum. It has vast projections throughout the CNS, driving mechanisms of attention, arousal, emotion and stress (Samuels and Szabadi 2008). Measures of pupil diameter can infer LC activity due to its interactions with the autonomic nervous system. It has influence on both the sympathetic and parasympathetic inputs to the pupil, leading to non-luminescence mediated pupil dilation, tightly correlating with its activity (Liu et al. 2017).

The LC has strong connections with areas such as the anterior cingulate cortex, dorsolateral prefrontal cortex and parietal cortex, providing evidence for its function in selective attention processing (Kihara et al. 2015). This attention-driven LC activity is reflected in a rapid dilation response of the pupils, thus allowing us to measure LC-mediated attentional load (Alnaes et al. 2014).

It has also been shown that the LC modulates noxious stimuli ascending via the dorsal columns (Pertovaara and Almeida 2006). Bidirectional influences produced from optoactivation suggests a function of the LC in descending endogenous analgesia (Hickey et al. 2014, Howorth, Teschemacher and Pickering 2009). This proposes that analgesic properties of the LC are formed by its connections to cortical centres of pain processing and the spinal cord (by descending noradrenergic inhibition). As previously mentioned, attention processes also stimulate LC outputs. Therefore, the LC is thought to be linked to the theory of 'attentional analgesia', whereby there is an analgesic effect of high cognitive load. This theory is supported by previous evidence using functional magnetic resonance imaging (fMRI) (Leung and Stroman 2016, Brooks, Davies and Pickering 2017).
This study aims to assess the pupillary response to attentional demand, noxious thermal stimuli and if an interaction of these factors reflects the 'attentional analgesic' effect.

Hypothesis: There will be a pupil dilation to increased attention demand and to noxious thermal stimulation and an interaction between these two factors.

2. Methods:

2.1: Participants:

Healthy participants will be recruited using posters at the University of Bristol. Anyone with chronic pain, history of neurological or major psychiatric disease, regular use of analgesia or a history of eye disease will be excluded.

2.2: Calibration protocol:

In order to standardise the stimuli/task difficulty across participants, each experimental component will be calibrated to the individual.

2.2.1: Thermal pain calibration:

Brief thermal stimuli will be delivered to the left volar forearm via a contact thermode (30x30 mm) using MEDOC TSA II neurosensory analyser. A 30-second stimulus will be used in which the target temperature will be adjusted pseudorandomly to find a stimulus that produces a consistent 6/10 pain score on a verbal pain rating scale. This temperature will be used as the 'high temperature' condition during the experiment. 'Low temperature' will always be set to 38 °C.

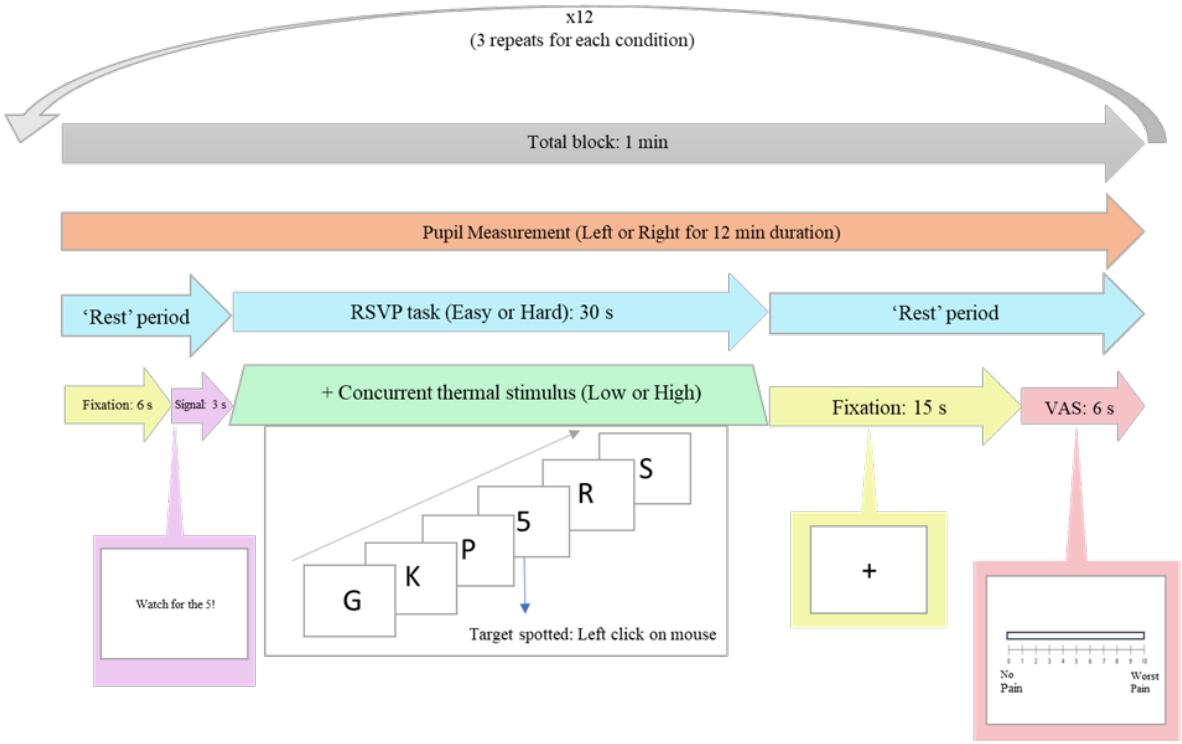
2.2.2: Rapid serial Visual presentation (RSVP) task calibration:

Attentional load will be modulated using 'easy' and 'hard' RSVP tasks. This consists of a stream of numbers and letters presented sequentially in the centre of the screen. The intercharacter interval will be adjusted to alter the speed at which the letters are presented, altering the perceived difficulty of

the task. Each participant will undertake a 12-minute sequence in a darkened room whereby the difficulty will vary (fastest: 32 ms, slowest: 192 ms). They will signal target detection (“5”) using a mouse. I will calculate the ‘hard’ task as the closest intercharacter interval in which the participant managed a 70% target hit accuracy. The easy task will be set at an intercharacter interval of 192 ms.

2.2.3: EyeLink 1000 calibration:

To measure pupil area I will be using EyeLink 1000 (SR Research Ltd. Ontario, Canada) equipment. This measures the area through a video tracker system that uses the centre of a single pupil and the reflection of an infrared illumination on the cornea. Participants will be required to place their head in the tower mount system where the focus of the camera will be adjusted to visualise a single pupil. Standard calibration and validation will then follow. The full experiment will run on one pupil and will then be repeated for the other after a 3-minute rest period. The side of which pupil is tested first will be alternated between participants.



3. Experimental protocol:

After each component has been calibrated, the experiment will run. The participant’s pupils will be measured continuously throughout the whole experiment. There will be 12 1-minute blocks, each consisting of 30-seconds task|thermal stimulus and 30-seconds rest (See image). During the ‘task’ portion of the block, the participants will be asked to refrain from blinking as much as possible, as each blink creates a momentary loss of data. They will however be allowed to blink freely during the ‘rest’ period. The RSVP task will be the same as in the calibration protocol, presented as either ‘easy’ or ‘hard’ in difficulty. A concurrent thermal stimulus (either ‘low’ or ‘high’) will be applied during this 30-seconds. This 2 x 2 factorial design creates four conditions (Easy|Low, Easy|High, Hard|Low, Hard|High) which will each be repeated three times during the experiment. The order by which the conditions are arranged within each 12-minute sequence will be pseudorandomised (no more than 3 low/high temperature conditions together). After each task|temperature portion, the participant will be asked to rate the pain they felt. This will consist of a visual analogue scale from 0-100 whereby 0 means “no pain” to 100 meaning the “worst pain imaginable”. Upon completion measuring one pupil, the participants will be given 3-minutes rest before switching to the other side and repeating the

experiment. The thermode will remain on the left volar forearm. By measuring both pupils without altering the side of thermode, it allows me to collect data from the ipsilateral and contralateral pupils in relation to the thermal stimulus.

4. Data analysis:

4.1: Behavioural data:

Results from the RSVP performance were compared using a paired T-test using Easy|Low-Hard|Low as the task reference condition. VAS pain scores were analysed using two-way repeated measures analysis of variance (GraphPad Prism 7). Post-hoc analysis using Bonferroni corrected multiple comparisons explored the interactions between experimental conditions.

4.2: Pupil data:

Each EyeLink data file (left and right for each participant) was transformed using MATLAB (R2018a, MathWorks). The data was smoothed with a Gaussian kernel (SD: 100) and down-sampled to 10 Hz/s. Blinking during the experiment caused a loss of data and so this was corrected with linear interpolation. De-trending was applied using the subtraction of a polynomial function to remove a slow negative drift in pupil area over time. The data was then epoched to isolate the blocks, starting 2 seconds after task onset to 2 seconds after ending (to account for the delay in pupil response). The final transformation was a baseline correction in which the mean pupil area of 2 seconds before the start of each RSVP was subtracted from values within that block. A mean of the three repeats for each condition was then calculated. This produced a 300 decisecond time series for each condition on the left and right (eight time series for each participant). This data was then analysed through two-way repeated measures ANOVA with post-hoc multiple comparisons.

5. Results:

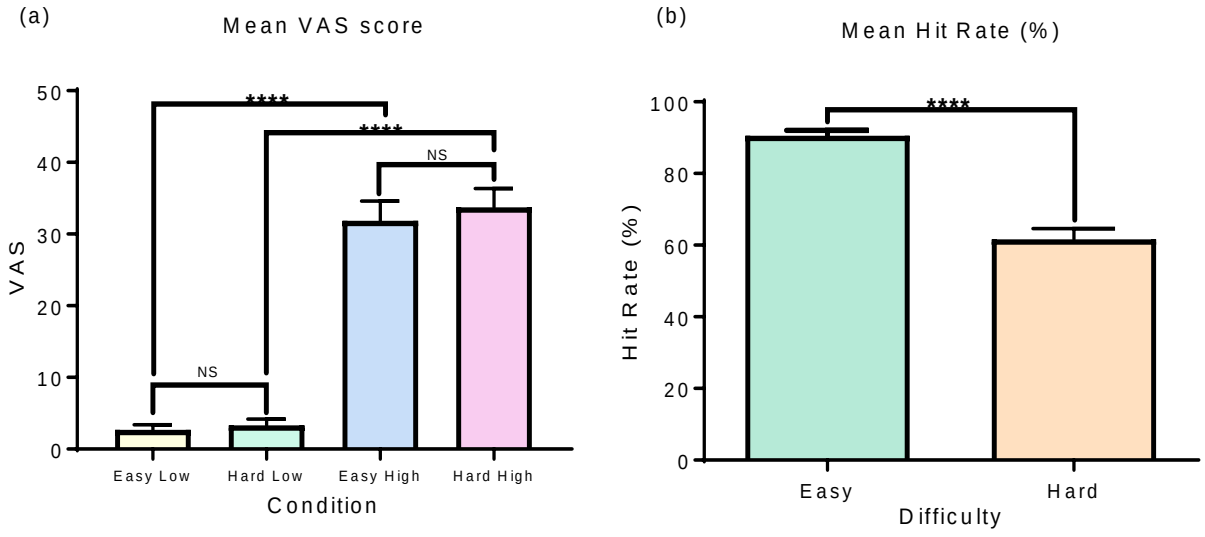


Figure 2: Mean VAS scores and hit rates for all trials (left and right eyes) combined. (a) shows the difference in VAS scores across the conditions for all participants. **(b)** shows the difference in hit rate (%) between the easy and hard task.
Values for **(a)** were produced using a two-way repeated measures ANOVA with post-hoc multiple comparisons (Bonferroni corrected). Values for **(b)** were produced through a paired T-test.
Abbreviations: NS: Not significant ($p>0.05$), ****: $p<0.0001$.

5.1: Behavioural results: The VAS scores showed a large amount of variability between participants. High temperatures (Mean: 45.75°C, Range: 43°C-48°C) produced a mean VAS score of 32.2 (Range: 3-69), much lower than the mean VAS score of 58.1 (Range 35-75) that was produced during MEDOC calibration. There was a significant difference in mean VAS scores between the four conditions ($p<0.0001$, $F(3,228)= 265.3$), as seen in **Figure 2(a)**. Post-hoc analysis showed a significant increase in VAS score at high temperatures (Easy|Low to Easy|High, $p<0.0001$, $F=37,76=4.46$), leading to the conclusion that the high temperature conditions did produce pain. There was no main effect of task on VAS score at either low or high temperatures (Low: $p=0.9766$, High: $p=0.5959$), signifying that attentional load did not alter temperature perception and thus no effect of attentional analgesia was seen.

Figure 2 (b) presents the mean hit rates for the easy and hard task without the influence of temperature (Easy|Low, Hard|Low). There was a significant difference ($p<0.0001$, $t(37)=10.2$) in hit rate between the tasks with the average easy and hard hit-rates being 90.51% (CI: 87.47-93.55) and 61.6% (CI:55.56-67.65) respectively. Performance in the task was not degraded by the

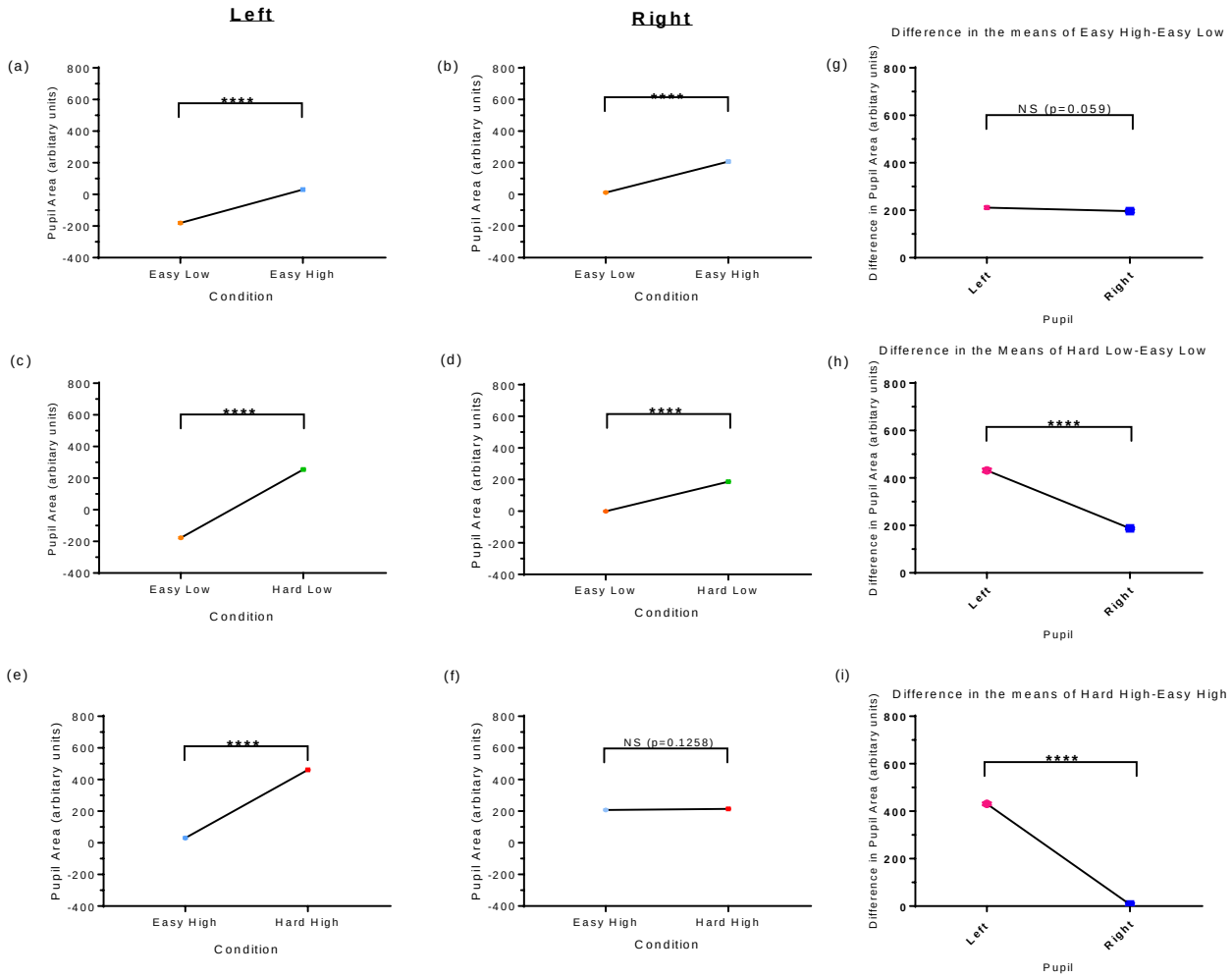


Figure 3: (a)-(f) Mean pupil area for each condition showing the effect of temperature, task and attention at high temperatures in either pupil. Error bars represent ±95% CI (Minimal variation seen). **(g)-(i) show the difference between the condition means in the left and right pupils.** The graphs present a main effect of temperature **(a) (b)**, attention **(c) (d)** and an attention|temperature interaction **(e)**. There is a lateralised effect of task at both low and high temperatures **(h) (i)**.
NS= Not significant ($p>0.05$), ****: $p<0.0001$

presence of pain (Hard|Low-Hard|High T-test: p=0.1389, t(37)=1.102). This indicates the task was calibrated sufficiently to meet the >90% (Easy), <70% (Hard) criteria.

5.2: Pupil results:

5.2.1: Main effect of attention, temperature and an attention x temperature interaction:

The difference in mean pupil areas between conditions is shown by **Figure 3**. There is a main effect of temperature (Figure **3a** (p, Mean ± SEM): p<0.0001, 211.1 ± 5.005, Figure **3b**: p<0.0001, 196.5 ± 5.984) and attention (Figure **3c**: p<0.0001, 431.7 ± 7.309, Figure **3d:** p<0.0001, 187.5 ± 4.512) in both pupils. There is an interaction of temperature and attention present in the left pupil only (Figure **3e**: p<0.0001, 431 ± 10.4). A lateralised effect of attention is seen at low and high temperatures (Figure **3h & 3i**: p<0.0001), but there is no lateralisation present between the ipsilateral (left) or contralateral pupils in response to pain (Figure **3g**: p=0.059). As this is close to the value of significance, the biological size of this difference was investigated (15 arbitrary units with no overlap of the SEMs). It suggests that there may have been a small lateralisation, but due to experimental underpowering a significant difference in pupils was not produced.

5.2.2: Lateralisation of pupil response:

Analysis between the raw data of the left and right pupils is seen in **Figure 4.** There was a significant difference in response to the hard task at high temperatures (p=0.014, F(1,19)=7.332). This shows an ipsilateral effect of pupillary response to during low temperatures (p=0.2751, F(1,19)= 1.263). This contrasts the mean results (**Figure 3h)** suggesting the importance of time as a factor in the analysis. There was no significant difference between the left and right pupils in response to low or high temperatures (p=0.9419, F(1,19)=0.0054). As this is close to significance it could be considered as a lateralised effect of temperature.

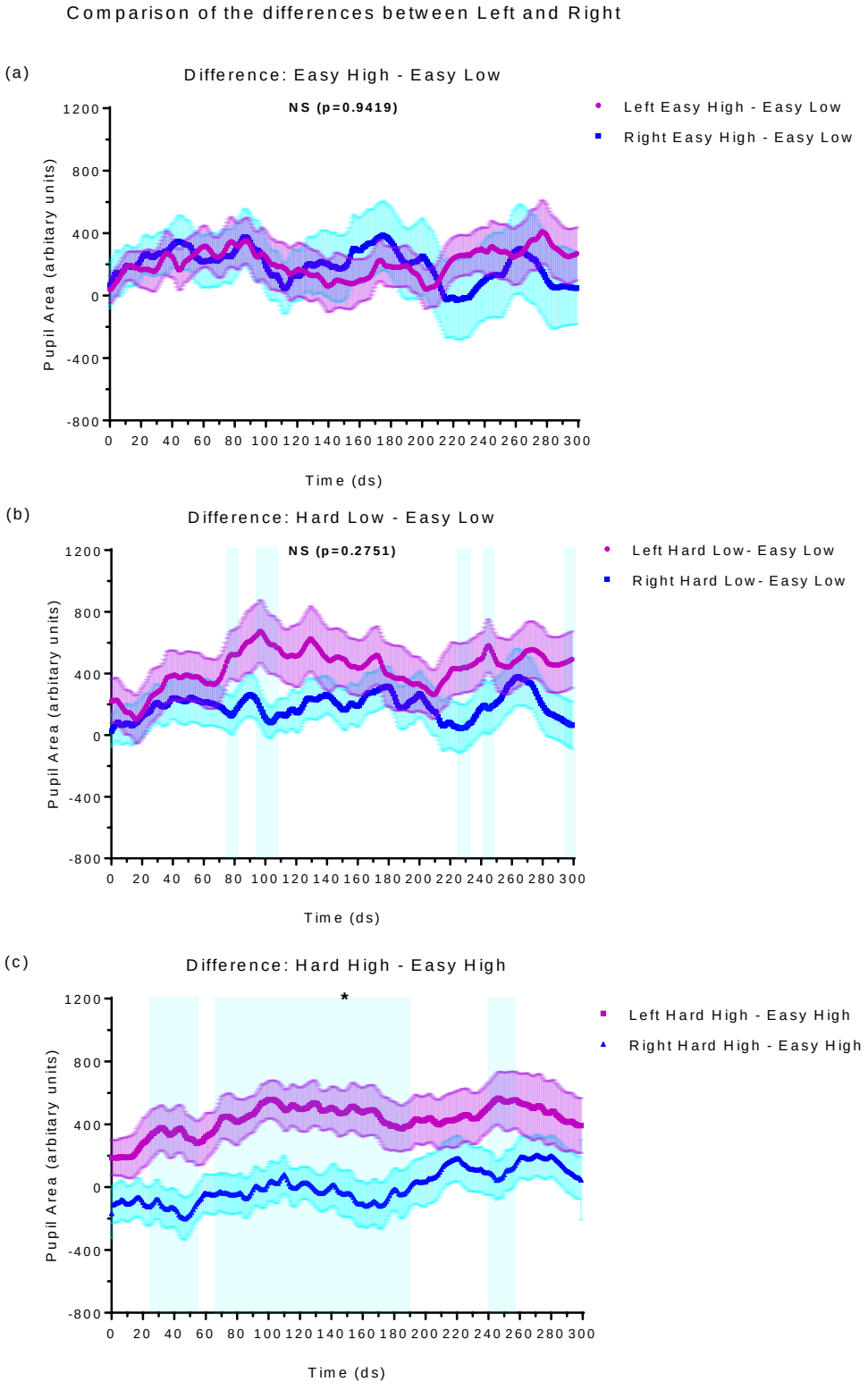


Figure 4: Comparisons between the left and right pupil to determine if effects were lateralised. The graphs show the mean difference ± SEM of two conditions across the time series in the left and right pupils. The shaded columns signify time points at which the difference between the left and right pupils is statistically significant ($p<0.05$). Significance values were determined from Bonferroni corrected post-hoc multiple comparisons (undertaken on **(b)** and **(c)** only) after two-way repeated measures ANOVA. The significance of side is indicated at the top of each graph.
NS: Not significant $p>0.05$, *: $p<0.05$

6. Discussion

The experiment showed a significant effect of attention in the left, but not right, pupil at both low and high temperatures. The difference between the two pupils was significant during attentionally demanding tasks at high temperatures. Although attention was shown to affect the pupil, it was not shown to affect the VAS scores, signifying no attentional analgesic response. Temperature did increase VAS scores but did not significantly affect either pupil when the factor of time is included. However, there is an indication of lateralisation between the pupil responses to temperature

6.1: Why was there a lateralised effect of task?

The significant lateralisation in the pupillometry results, with relation to attentional load, is consistent with previous studies. A physiological difference in two pupils in the absence of any pathological significance is called benign anisoconia (BA). This was produced in the study by Poynter (2017) who analysed numerous factors that may have a causal link to BA. One of these factors was attentional functioning. He discovered significant BA (left pupil had a greater dilation response) was produced during demanding attention tasks. This is consistent with previous reports of right-sided hemispheric asymmetry in visuospatial attention networks (Burtis et al. 2014, Muri et al. 2002). This suggests that if pupil diameter is controlled by the autonomic nervous system, then there must be an autonomic asymmetry present for lateralisation to occur. Studies analysing this autonomic asymmetry found an associated reduction in sympathetic input to the smaller pupil in patients with previously diagnosed BA (Rosenberg 2008). This leads us to conclude that the left pupil received a larger sympathetic stimulus relative to the right pupil during the harder RSVP task. A study in animals (Liu et al. 2017) supported this theory as they showed that sympathetic nervous system drives LC-mediated lateralised dilation.

Together, this evidence suggests why a significant lateralisation was produced with high attentional demand during noxious stimulation. The construct of attention requires a complex neural network to generate concentration and focus. A number of brain loci have been shown to be involved, including the DL-PFC; ACC; posterior parietal cortex and the lateral pulvinar nucleus (Mesulam 1983). There is direct evidence for LC stimulation by such areas, specifically a strong connection between the DL-PFC to bilateral LC nuclei (Jodoj, Chiang and Aston-Jones 1998, Jodo and Aston-Jones 1997). We hypothesise that during the attentionally demanding tasks the afferent output from the DL-PFC to the LC was greater, as attentional load drives LC excitation (Arnsten and Goldman-Rakic 1984). Considering the previous evidence of right-sided hemispheric asymmetry, it is thought that the left LC received a higher output from the DL-PFC compared to the right. Therefore, if the left LC experienced a larger top-down effect of the attention task, then the subsequent noradrenergic output to the left IML (via α1-adrenoreceptors) was higher than on the right side. This produced the lateralised pupil dilation. As the DL-PFC bilaterally stimulates the LC, it explains why the right pupil did dilate, but to a lesser extent (Jodoj et al. 1998).

There are many factors that could have contributed to a lateralised response to task. As the evidence in human subjects is limited, we can only postulate the pathways that may have led to this result.

6.2: Why was there no difference in VAS between attentional tasks?

Although the pupillometry results show a difference between task difficulties during high temperatures, the corresponding pain scores were not significant (Easy|High-Hard|High: p=0.5959). This is contrary to expectations as the concept of attentional analgesia is well established (Brooks et al. 2017, Tracey et al. 2002). If LC activity is increased due to high cognitive load, it is thought to subsequently heighten noradrenergic outputs of descending control by means of pontospinal excitation (Hwang et al. 2001, Howorth et al. 2009). The Easy|High-Hard|High comparison was designed to test this theory.

However, the determination of pain is conditional upon many factors. Pain is a construct of consciousness and is therefore highly subjective to individual perceptions, emotions and memory (Coghill 2010). This subjectivity increases the variability of VAS scores as numerous factors can alter a participant's perception of pain from one moment to the next. For example, calibration of the MEDOC was undertaken soon after consenting. Although calibration itself familiarises the participant with the equipment, it is likely that their stress and anxiety levels were higher than normal. Many studies show stress-induced hyperalgesia (SIH), in which participants experience greater levels of pain due to the interaction between emotional processes, such as valence (whether a sensation is pleasant or unpleasant) and arousal (state of excitement or calm) (Crettaz et al. 2013). If the balance of these factors is shifted towards negative emotion with moderate arousal then pain perception is enhanced (Rhudy and Williams 2005)**.** This too is altered by other factors such as personality traits, gender and expectation. If we conclude that during the calibration phase SIH was induced, then the pain ratings to the thermal stimuli were heightened. Therefore, as stress levels reduced over the time course of the experiment, the noxious stimuli would have had a lower impact than previously. This was reflected in the mean VAS score of 32.2 compared to 58.1 in the calibration.

The protocol used by Brooks, Davies and Pickering (2017) elicited a significant attentional analgesic response to thermal stimuli. However, as this study used fMRI and not pupillometry to monitor neural activity, it did not have the added confound of attentional strain caused by the instruction of not to blink during the RSVP task. This could have had a significant effect on the task perceptions, even if the task itself was deemed easy/difficult by hit rate analysis. An analgesic effect, if produced, could have been masked by the confounding SIH response of this strain.

6.3: Why was there no significant pupil response to pain?

Pain can reliably cause bilateral pupil dilation as a marker of an autonomic response to noxious stimuli, with or without a conscious awareness of the pain itself (Yang, Niemann and Larson 2003).The comparison of Easy|Low-Easy|High produced no significant effect of temperature in either pupil. However, the left pupil was close to meeting the value of significance (p=0.0562) compared to the right pupil (p=0.3166) and temperature was significant when time was removed as a factor (**Figure 4a & b)**. This provides a promising suggestion that there was a lateralised effect to pain, an idea supported by research modulating LC activity in rats (Liu et al. 2017).

A possible explanation for the lack of significance lies with the temperature range used. Eisenach et al (2017) were able to show a correlation between bilateral pupil diameter and a temperature dependent increase in pain report to thermal stimuli. They however used higher temperatures (49℃ & 50℃) for the experiment, which as the maximum temperature used in this experiment was 48℃ may suggest a reason for the difference in results.

6.4: Limitations of the experiment:

Although the RSVP task and thermal stimuli did correctly induce high attention demand and pain sensation, there were still limitations of the experimental protocol. A large limitation is the subjectivity produced from verbal or scalar ratings. Although this variability was accounted for by calibrating the MEDOC to correspond to individual pain ratings, there was still a great amount of inconsistency within an individual.

Another limitation of the experiment was using a visual attention task. A visual task was chosen because it allows for more accurate titratability. However, the task itself introduced several confounding factors. The letter presentation, changes in luminosity and variability of pupil position all caused fluctuations in pupil area. The pupil time series was baseline corrected to account for some of this variability. However, if the experiment were to be repeated an auditory task, such as an auditory 'oddball' paradigm, may be more appropriate (Swick et al. 1994).

6.5: Future perspectives:

As our results showed a significant lateralisation of cognitive load at high temperatures, more research could explore this effect further. There is currently very little evidence on lateralised effects of pain or task on pupil response in humans. Therefore, more robust experiments, such as bilateral pupillometry considering the effect of eye dominance (Chaumillon et al. 2017), could be developed. It could explore whether the response is reflected using auditory tasks or greater variations in noxious stimuli.

6.6: Conclusion:

In conclusion, attention demanding tasks during noxious thermal stimuli produced a dilation response lateralised to the left pupil. There was also a significant dilation to attentional load at low temperatures in the left pupil. This lateralisation is a novel finding that has not previously been found in humans.

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Appendices:

Ethics:

All procedures were carried out with the informed written consent of the subjects and were in accordance with the ethical procedures approved by the University of Bristol Faculty of Science Human Research Ethics committee (Reference: 59828). The experiment followed appropriate risk assessments and COSHH regulations.