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SHORT REPORT

Pupillometry as a Potential Objective Measurement of Pain Assessment in Healthy Volunteers

Janika Krafthöfer[1], Sophie-Charlotte Fabig[1], Ralf Baron[1], Janne Gierthmühlen[1,2]

[1]Division of Neurological Pain Research and Therapy, Department of Neurology, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Schleswig-Holstein, Germany; [2]Department for Anesthesiology and Surgical Intensive Care Medicine, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Schleswig-Holstein, Germany

Correspondence: Janika Krafthöfer, Division of Neurological Pain Research and Therapy, Department of Neurology, University Hospital Schleswig-Holstein, Campus Kiel, Arnold-Heller-Str. 3, Haus D, Kiel, 24105, Germany, Tel +49 431 500 23911, Fax +49 431 500 20914, Email janika.krafthoefer@web.de

Background: Pain leads to activation of the autonomic nervous system and thus, among other things, to pupillary reflex dilation (PRD). Previous studies have already confirmed a correlation between the perception of pain and the pupillary reaction, measured using pupillometry. However, the previous study populations were under the influence of medication for analgesia in perioperative setting or suffered from pain. This study examines the relationship between pupillary reaction and pain perception in healthy controls and addresses the question of whether endogenous pain inhibition, clinically tested by conditioned pain modulation (CPM), can be quantified using pupillometry.

Methods: Forty-two healthy volunteers (21 females, 21 males, mean age 27.9 ± 5.8 years, range 20–39 years) were included in this study. The PRD, as a measure of the pupillary reaction (variance from the base diameter in percent), was investigated during baseline, heat application and during CPM testing and results compared to the reported pain intensity on the numerical rating scale (NRS).

Results: The volunteers showed higher variances under painful conditions compared to the measurement at rest corresponding to higher sympathetic activity during pain. Volunteers with a higher variance, ie a stronger pupillary reaction, gave higher pain ratings than subjects with a lower pupil variance. However, there was no correlation between the NRS and PRD. PRD and pain ratings during CPM were significantly lower compared to heat pain application alone. However, there was no correlation between the calculated CPM effect and the PRD.

Conclusion: Pupillometry is capable of objectively reflecting the pain response, eg pain relief through CPM testing. However, the CPM effect calculated from the subjective pain ratings and the objective PRD measurements is not associated suggesting that both measure different aspects of pain perception. It must be discussed whether the CPM effect can be the correct measure for the functionality of the pain system.

Keywords: conditioned pain modulation, descending control, pupillary reaction, pain, sympathetic nervous system

Introduction

The perception of pain is individual and modulated by many factors. Consequently, objectifying pain perception using simple clinical testing would be helpful. Pain leads to autonomic reactions, including pupillary reflex dilation. Higher pain intensities would lead to a greater modulation of pupil dilation via autonomic innervation of the pupil muscles.[1] However, there are contradictory results of associations between pain ratings and pupillary responses.[2–4] Additionally, most studies included patients with perioperative analgesia or chronic pain, but not a healthy cohort. However, analgesics, such as those used perioperatively, influence the pupillary reaction.[5] Therefore, it is unknown whether associations between pain and pupillary reaction also exist in healthy volunteers.

The sensation of pain can be influenced by facilitating and inhibitory mechanisms, which are usually in balance in healthy people.[6] Clinically, part of the endogenous pain inhibition can be tested using Conditioned Pain Modulation (CPM). The CPM effect describes changes in the pain response to a Test Stimulus (TS) triggered by the Conditioned Stimulus (CS).[7] The CPM

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testing is based on subjective pain ratings and is influenced by this subjectivity.[8] Overall, there are anatomical interfaces between endogenous pain inhibition and activation of the autonomic nervous system.[9]

The aims of the study were thus to (a) investigate the relationship between pupillary reaction and pain perception measured with the NRS in healthy controls and (b) addresses the question of whether endogenous pain inhibition, clinically tested by CPM, can be quantified and objectified using pupillometry.

Methods

Forty-two healthy volunteers, classified as healthy according to the EUROPAIN and NEUROPAIN consortia criteria,[10] (21 females, 21 males, mean age 27.9 ± 5.8 years, range 20–39 years) were included. To be classified as healthy, volunteers were not allowed to show abnormalities in the state of health and previous illness questionnaires, as well as in the Hospital Anxiety and Depression Scale[11] and in the quantitative sensory testing.[10,12]

The study adhered to the Declaration of Helsinki and was approved by the Institutional Review Board at Christian-Albrechts-University of Kiel (D454/16). Written informed consent was obtained from all included volunteers.

In all volunteers, pupillometry and CPM testing was performed.

Pupillometry

The pupillary reaction was measured using a Pupillometry device (AlgiScan, IDMED, France). For all measurements, the right eye was measured for 30 seconds without blinking, and the contralateral eye was left open. The device was held directly to the eye and the Pupil Reflex Dilatation (PRD) were determined, ie, the base diameter of the pupil and its variance from the base diameter was measured in percent (Figure 1). First, the pupillary reaction in bright ambient light was investigated. Afterwards, pupillometry measurements were performed during CPM testing (see below).

CPM Testing

The CPM test was divided into two sections. First, painful heat stimuli were applied for 30 seconds to the right forearm using a peltier thermode (Thermal Sensory Analyzer II, TSA 2001-II, MEDOC, Israel) and the Pain50-temperature, ie, the temperature that resulted in a subjective pain rating of 50 on the Numeric rating scale (NRS, 0 = no pain and 100 = maximum imaginable pain) was determined. This temperature was then used as the individual TS. Afterward, there was a five-minute break. For the CPM test, the TS was first applied for 30 seconds and the volunteers were asked to rate pain on the NRS. At the same time, the pupillary reaction was measured. The left hand was then placed in a cold-water bath with a temperature between 5°C and 10°C (as CS) for 30 seconds. Simultaneously, the TS was applied to the right forearm. During this time, the pupillary reaction was measured and pain ratings were determined. The CPM effect was calculated by: (NRS during heat pain stimulus under CPM) – (NRS during heat pain stimulus).[7]

The correlation between measured parameters was determined by the Spearman correlation. For intergroup comparisons (high/low variance) Mann–Whitney *U*-test was used, for intra individual measurements Wilcoxon-Test was used.

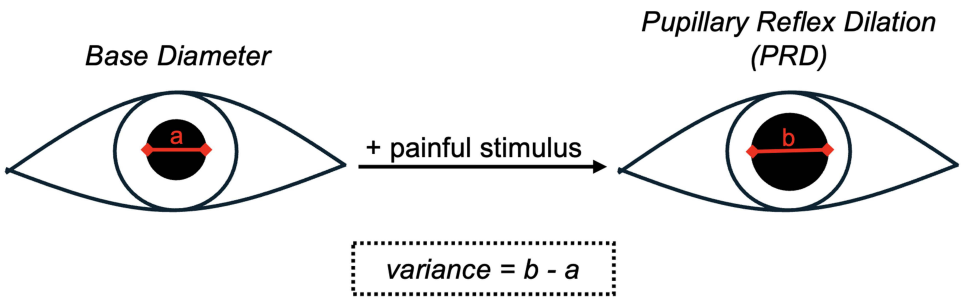

Figure 1 Schematic representation of the measured pupil reaction. Left: First, the base diameter of the pupil (a) is determined. A painful stimulus then leads to Pupillary Reflex Dilation (PRD, b), which is also recorded using pupillometry. The pupil variance results from the PRD (b) – base diameter (a) and is given in percent.

Results

A high variance of the pupillary reaction was observed when only painful heat was applicated, ie, patients showed higher variances under painful condition compared to the measurement in bright ambient light (M_{heat} ± SD: 15.53 ± 11.24% vs $M_{ambient\ light}$ ± SD: 9.21 ± 7.39%, p=0.001) (Figure 2A), corresponding to a higher sympathetic activity during pain.

The variance of the pupillary reaction during CPM was significantly lower compared to the heat pain application alone (M_{heat} ± SD: 15.53 ± 11.24% vs M_{CPM} ± SD: 11.94 ± 7.69%, p=0.043) (Figure 2A). On average, patients showed lower variance in PRD, ie, lower sympathetic activity, during CPM testing. Similarly, the pain ratings on the NRS during CPM testing were significantly lower compared to the pain ratings to the heat application (NRS: M_{heat} ± SD: 59.62 ± 17.36 vs M_{CPM} ± SD: 39.88 ± 21.54, p<0.001), ie, the lower variance and the lower sympathetic activity during CPM testing were in accordance with lower pain ratings.

Based on the median of the variance for the heat pain application (median = 11.7%), patients were grouped into those with high or low PRD. Subjects with higher PRD demonstrated higher pain ratings than those with lower PRD (NRS: $M_{high\ variance}$ ± SD: 65.40 ± 13.96 vs $M_{low\ variance}$ ± SD: 53.83 ± 18.07, p=0.032) (Figure 2B), suggesting that higher pain perception leads to higher variance of pupillary reaction. However, there was no correlation between PRD and reported NRS or calculated CPM.

Discussion

The study showed that objectifying pain responses using pupillometry is possible. Volunteers with higher pain ratings showed significantly higher PRD than volunteers with lower ratings. Furthermore, the pain relief and thus lower pain ratings achieved by the CPM were significantly reflected in PRD, ie, PRD was lower under CPM testing compared to heat application alone. Overall, we could objectify the CPM response using pupillometry. However, there was no correlation between the calculated CPM effect or the NRS and PRD. This suggests that both measure different aspects of pain perception: NRS is a subjective rating, which depends on many factors (eg biopsychosocial aspects).[13] The CPM effect is calculated from subjective pain ratings. As an objective tool, pupillometry shows the respective autonomic pain response independent from subjective rating; consequently, there can be no correlation between the subjective and influenceable NRS or CPM effect, which is based on NRS, and the objective pupillometry. Therefore, it needs to be discussed whether a CPM effect based on subjective reports can be used as a parameter to assess the status of the

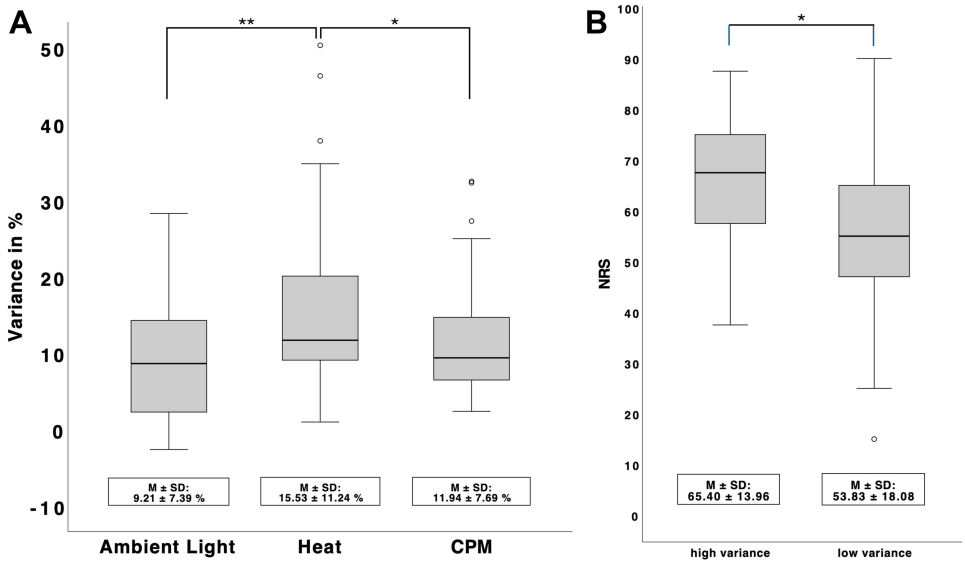

Figure 2 (**A**) Measured variances of the pupil in percent under different several conditions. *Ambient Light:* Variance during 30 seconds in ambient light without any stimulus. *Heat*: Variance during the individual Pain50-temperature was applied on the right forearm for 30 seconds. *CPM*: Variance during the individual Pain50-temperature was applied on the right forearm while the left hand was in the cold-water bath for 30 seconds. In addition to the box plot, the mean (M) with standard deviation (SD) is given for each condition. *p<0.05; **p<0.01. (**B**) Comparison of reported pain ratings on the NRS between the groups with a high variance (left), ie greater than the median, and the group with a lower variance than the median (right). *p<0.05.

nociceptive system. Previous studies have already shown inter- and intra-individual differences in CPM effects, most of which cannot be explained.[8] Additionally, healthy subjects can have low CPM values without suffering from pain.[14]

While former studies have examined a possible correlation between pupillometry and pain ratings in perioperative and emergency settings, our study seems to be the first to answer this question for a larger group of healthy volunteers. Furthermore, we aimed to find out if the pupillary reaction can be modulated by the activation of the endogenous pain inhibitory system during CPM testing, following the recommendations of Yarnitsky et al.[7]

In a 2023 study,[4] 313 patients in the emergency room were asked to rate their pain on the NRS during triage and the pupillary response, in detail Pupil Light Reflex (PLR) and Pupil Rest Under Ambient Light (PUAL) was measured. In this study, no significant associations between pain ratings and pupillometry were found. The patients with moderate pain did not differ in pupillometry compared to patients with severe pain (NRS $\geq$ 4),[4] while our Results showed significant differences between the groups.

The different results compared to the study by Gregoire et al[4] could be due to the different modes of pupillometry used. In our study, we utilized PRD and not PLR/PUAL. Furthermore, the patients of Gregoire et al[4] were recruited on site in the emergency room, while the participants in our study were healthy and explicitly selected and prepared for participation in advance, ie, the participants could prepare themselves cognitively and emotionally for this examination, which might not apply to the patients from the ER. This could also lead to a changed pupillary reaction.

A 2017 study,[15] examining the relationship between PRD and pain ratings in 28 healthy volunteers, showed that reported pain intensity from a single heat pain stimulus and PRD was positively correlated. This applies to short-lasting and intense pain stimuli under tightly controlled conditions. We also detected the same trend in our results, but only when groups were previously divided into high/low PRD. However, we measured the PRD only using the temperature which the subjects rated previously as NRS 50, and its influence by CPM. In the study by Eisenach et al,[15] the subjects were given prescribed temperatures, first in a training run with increasing intensity, then in a random order that the subjects were asked to rate on the NRS.

Eisenach et al described that pupillometry was distracted by CPM testing, ie, there was no correlation between PRD and NRS anymore.[15] In our study, we observed that pupillometry reflects the current pain response. During CPM testing, pain ratings were significantly lower due to pain relief. At the same time, the measured pupil variance was significantly lower, so all in all, pupillary reaction can objectify pain in different situations and reflects the pain response independently of individual pain ratings. As described above, the calculated CPM effect is the variable that can be distracted because it is based on subjective pain ratings, which are influenced by many factors.[8]

The activity of the sympathetic nervous system and thus the pupillary reaction are influenced by many factors that we excluded in advance. We therefore only included young, healthy volunteers who had not previously taken any medications affecting the autonomic nervous system and who previously avoided, eg, caffeine or heavy meals. However, not all disruptive factors, eg reduced sleep, could be ruled out. Although pathological anxiety was ruled out by several questionnaires, the anticipation of the cold-water bath as an unpleasant stimulus can inhibit the pupillary reaction.[16] It is possible that the fear of the cold-water bath differs between the volunteers. Furthermore, it should be noted that our study is quite small with 42 participants which limits concluding remarks. The question arises as to whether the individual confounding variables can be minimized in a larger study population.

Further studies explaining the anatomical pathways could be interesting. The LC is activated by nociceptive stimuli and can profoundly modulate pain neurotransmission and experience,[17] however, the exact mechanisms behind this activity in humans are largely unexplored. Recent evidence confirms the utility of pupil diameter as a measure of LC activity. There is a tight correlation between pupil diameter and BOLD activity in a dorsal pontine cluster overlapping with an established LC atlas[18] in individuals at rest and during a two-stimulus oddball task[19] or during systematically manipulated cognitive load.[20] Thus, the locus coeruleus (LC) could be a possible interface between pupillary response and pain perception.[15]

In this study, we used a device shielding the measured eye from ambient light by an opaque screen, but the contralateral eye was open compared to other studies. This could have influenced the measured PRD via the indirect pupillary reaction. However, as intra individual measurements were evaluated in this study, we consider this limitation of lower importance.

Conclusion

Our results showed objectifying pain response based on pupillary reaction is possible. It turns out that, compared to previous studies, pupillometry can also be used in healthy volunteers as an objective measurement of pain reaction. Overall, there were no correlations between PRD and the subjective NRS or the calculated CPM effect, which is based on subjective NRS. However, it should be noted that pupillometry is capable of objectively reflecting pain response, eg pain relief through CPM testing. Consequently, a correlation between the subjective measures and pupillometry is not possible, as both measure different aspects of pain perception. It must be discussed whether the CPM effect can be the correct measure for the functionality of the pain system. Pupillometry as an objective tool might be more suitable for this purpose.

Abbreviations

CPM, Conditioned Pain Modulation; CS, Conditioned Stimulus; LC, Locus coeruleus; NRS, Numeric Rating Scale; PRD, Pupil Reflex Dilation; TS, Test Stimulus.

Data Sharing Statement

Individual-level data cannot be shared due to data protection reasons, but derived data can be shared on request to the corresponding author (J.K.).

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References

1. Chapman CR, Oka S, Bradshaw DH, Jacobson RC, Donaldson GW. Phasic pupil dilation response to noxious stimulation in normal volunteers: relationship to brain evoked potentials and pain report. *Psychophysiology*. 1999;36(1):44–52. doi:10.1017/S0048577299970373
2. Höfle M, Kenntner-Mabiala R, Pauli P, Alpers GW. You can see pain in the eye: pupillometry as an index of pain intensity under different luminance conditions. *Int J Psychophysiol*. 2008;70(3):171–175. doi:10.1016/j.ijpsycho.2008.06.008
3. Connelly MA, Brown JT, Kearns GL, Anderson RA, St Peter SD, Neville KA. Pupillometry: a non-invasive technique for pain assessment in paediatric patients. *Arch Dis Child*. 2014;99(12):1125–1131. doi:10.1136/archdischild-2014-306286
4. Gregoire C, Charier D, de Bergeyck R, et al. Comparison between pupillometry and numeric pain rating scale for pain assessments in communicating adult patients in the emergency department. *Eur J Pain*. 2023;27(8):952–960. doi:10.1002/ejp.2137
5. Larson MD, Kurz A, Sessler DI, Dechert M, Bjorksten AR, Tayefeh F. Alfentanil blocks reflex pupillary dilation in response to noxious stimulation but does not diminish the light reflex. *Anesthesiology*. 1997;87(4):849–855. doi:10.1097/00000542-199710000-00019
6. Yarnitsky D. Role of endogenous pain modulation in chronic pain mechanisms and treatment. *Pain*. 2015;156(Suppl 1):S24–S31. doi:10.1097/01.j.pain.0000460343.46847.58
7. Yarnitsky D, Bouhassira D, Drewes AM, et al. Recommendations on practice of conditioned pain modulation (CPM) testing. *Eur J Pain*. 2015;19(6):805–806. doi:10.1002/ejp.605
8. Graeff P, Itter A, Wach K, Ruscheweyh R. Inter-individual differences explain more variance in conditioned pain modulation than age, sex and conditioning stimulus intensity combined. *Brain Sci*. 2021;11(9):1186. doi:10.3390/brainsci11091186
9. Bruehl S, Chung OY. Interactions between the cardiovascular and pain regulatory systems: an updated review of mechanisms and possible alterations in chronic pain. *Neurosci Biobehav Rev*. 2004;28(4):395–414. doi:10.1016/j.neubiorev.2004.06.004
10. Gierthmühlen J, Enax-Krumova EK, Attal N, et al. Who is healthy? Aspects to consider when including healthy volunteers in QST--based studies-a consensus statement by the EUROPAIN and NEUROPAIN consortia. *Pain*. 2015;156(11):2203–2211. doi:10.1097/j.pain.0000000000000227
11. Zigmond AS, Snaith RP. The Hospital Anxiety and Depression Scale. *Acta Psychiatr Scand*. 1983;67(6):361–370. doi:10.1111/j.1600-0447.1983.tb09716.x
12. Rolke R, Baron R, Maier C, et al. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): standardized protocol and reference values. *Pain*. 2006;123(3):231–243. doi:10.1016/j.pain.2006.01.041
13. Gatchel RJ, Peng YB, Peters ML, Fuchs PN, Turk DC. The biopsychosocial approach to chronic pain: scientific advances and future directions. *Psychol Bull*. 2007;133(4):581–624. doi:10.1037/0033-2909.133.4.581
14. Nuwailati R, Bobos P, Drangsholt M, Curatolo M. Reliability of conditioned pain modulation in healthy individuals and chronic pain patients: a systematic review and meta-analysis. *Scand J Pain*. 2022;22(2):262–278. doi:10.1515/sjpain-2021-0149
15. Eisenach JC, Curry R, Aschenbrenner CA, Coghill RC, Houle TT. Pupil responses and pain ratings to heat stimuli: reliability and effects of expectations and a conditioning pain stimulus. *J Neurosci Methods*. 2017;279:52–59. doi:10.1016/j.jneumeth.2017.01.005
16. Bitsios P, Szabadi E, Bradshaw CM. The fear-inhibited light reflex: importance of the anticipation of an aversive event. *Int J Psychophysiol*. 2004;52(1):87–95. doi:10.1016/j.ijpsycho.2003.12.006
17. Pertovaara A. The noradrenergic pain regulation system: a potential target for pain therapy. *Eur J Pharmacol*. 2013;716(1–3):2–7. doi:10.1016/j.ejphar.2013.01.067
18. Keren NI, Lozar CT, Harris KC, Morgan PS, Eckert MA. In vivo mapping of the human locus coeruleus. *Neuroimage*. 2009;47(4):1261–1267.
19. Murphy PR, O'Connell RG, O'Sullivan M, Robertson IH, Balsters JH. Pupil diameter covaries with BOLD activity in human locus coeruleus. *Hum Brain Mapp*. 2014;35(8):4140–4154. doi:10.1002/hbm.22466
20. Alnæs D, Sneve MH, Espeseth T, Endestad T, van de Pavert SH, Laeng B. Pupil size signals mental effort deployed during multiple object tracking and predicts brain activity in the dorsal attention network and the locus coeruleus. *J Vis*. 2014;14(4):1. doi:10.1167/14.4.1