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# Pupillary response to percutaneous auricular vagus nerve stimulation in alcohol withdrawal syndrome: A pilot trial

M.C. Treiber [a, b, *], J. Grünberger [a, b], B. Vyssoki [a, b], J.C. Szeles [c], E. Kaniusas [d], S. Kampusch [e], H. Stöhr [f], H. Walter [a, b], O.M. Lesch [a, b], D. König [a, b], C. Kraus [a, b]

[a] Department of Psychiatry and Psychotherapy, Medical University of Vienna, Austria
[b] Comprehensive Center for Clinical Neurosciences and Mental Health (C3NMH), Medical University of Vienna, Austria
[c] Division of Vascular Surgery, Department of General Surgery, Medical University of Vienna, Austria
[d] Institute of Biomedical Electronics, Vienna University of Technology, Austria
[e] AURIMOD GmbH, Vienna, Austria
[f] Faculty of Computer Science, University of Vienna, Austria

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ABSTRACT

*Background:* Autonomic symptoms in alcohol withdrawal syndrome (AWS) are associated with a sympathetic-driven imbalance of the autonomic nervous system. To restore autonomic balance in AWS, novel neuromodulatory approaches could be beneficial. We conducted a pilot trial with percutaneous auricular vagus nerve stimulation (pVNS) in AWS and hypothesized that pVNS will enhance the parasympathetic tone represented by a reduction of pupillary dilation in a parasympatholytic pharmacological challenge.

*Methods:* Thirty patients suffering from alcohol use disorder, undergoing AWS, and stable on medication, were recruited in this open-label, single-arm pilot trial with repeated-measure design. Peripheral VNS (monophasic volt impulses of 1 msec, alternating polarity, frequency 1 Hz, amplitude 4 mV) was administered at the left cymba conchae for 72 h, followed by pupillometry under a tropicamide challenge. We assessed craving with a visual analog scale. We used pupillary mean as the dependent variable in a repeated-measures ANOVA (rmANOVA).

*Results:* A repeated-measures ANOVA resulted in a significant difference for pupillary diameter across time and condition ($F_{(2,116)} = 27.97$, $p < .001$, $\eta_p^2 > .14$). Tukey-adjusted *post hoc* analysis revealed a significant reduction of pupillary diameter after pVNS. Alcohol craving was significantly reduced after pVNS ($p < .05$, Cohen's $d = 1.27$).

*Conclusion:* Our study suggests that pVNS activates the parasympathetic nervous system in patients with acute AWS, and that this activation is measurable by pupillometry. To this end, pVNS could be beneficial as a supportive therapy for AWS. Potential confounding effects of anti-craving treatment should be kept in mind.



## Introduction

Alcohol use disorder (AUD) is a recurrent relapsing-related, chronic disease with progressive adverse health consequences and psychological, physical, and behavioral changes (Alarcon et al., 2021). A characteristic of AUD is the desire or compulsion for alcohol, a loss of the controlled amount of drinking and prolonged consumption despite recognizable harmful consequences. Also, there is a development of tolerance toward alcohol and a malaise in the absence of consumption (Jochum et al., 2016; Morse & Flavin, 1992). The chronic consumption of alcohol leads to a pathophysiological adaptation of the system, by an increase in glutamatergic receptors and a decrease in γ-aminobutyric acid (GABA) receptor functions (Roberto, Kirson, & Khom, 2021). Caused by a sympathetic-driven imbalance of the autonomic nervous system (ANS) (Eisenhofer, Whiteside, & Johnson, 1985), autonomic and psychomotor withdrawal symptoms, as well as negative emotional states, occur 6–24 h after cessation of alcohol consumption and

* Corresponding author. Department of Psychiatry and Psychotherapy, Medical University of Vienna, Spitalgasse 23, 1090 Vienna, Austria. Tel.: +43 67762794733.
*E-mail address:* michael.treiber@meduniwien.ac.at (M.C. Treiber).

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increase alcohol craving and risk of relapse (Foy, Kay, & Taylor, 1997; Koob, 2003). Around 90 % of patients undergoing withdrawal management relapse at least once within four years (Carvalho, Heilig, Perez, Probst, & Rehm, 2019), while half of them relapse within the first two weeks after treatment (Manning et al., 2021). Pharmacological treatment of alcohol withdrawal syndrome consists of either benzodiazepines (BDZ) or γ-hydroxybutyrate (GHB). Both drugs are known to have an addiction potential themselves (Lee, Salloum, Lindstrom, & Kathryn McHugh, 2021; van Noorden, Mol, Wisselink, Kuijpers, & Dijkstra, 2017), which underlines the need for better treatments.

Besides the poor therapeutic outcome and autonomic imbalance, alterations in the brain's neurotransmitter systems, such as GABA, serotonin, and acetylcholine, were found in patients with AUD (Gupta, Khan, Kaur, & Singh, 2021; Nevo & Hamon, 1995). To restore autonomic balance and thereby reduce symptoms in alcohol withdrawal syndrome (AWS), new treatment methods are crucial. The vagus nerve, the largest cranial nerve, mainly responsible for parasympathetic innervation, can be stimulated invasively and non-invasively (Ohemeng & Parham, 2020; Wang et al., 2021). To that end, auricular vagus nerve stimulation (aVNS) seems to be an effective approach to enhance parasympathetic activity (Borovikova et al., 2000). Auricular VNS is clinically used in epilepsy (Lampros, Vlachos, Zigouris, Voulgaris, & Alexiou, 2021), depression (Evensen, Jørgensen, Sabers, & Martiny, 2021), and migraine (Straube, Ellrich, Eren, Blum, & Ruscheweyh, 2015), and is under investigation for cognitive dysfunctions (Cai et al., 2019), anxiety (Burger et al., 2019), and insomnia (Wu et al., 2021) [as reviewed by our group in 2019 (Kaniusas et al., 2019)], conditions often seen in patients with AUD (Schuckit, 2009). It already has been reported that aVNS significantly improves depressive symptoms and sleep quality in patients with AUD after withdrawal (Wang et al., 2021). Non-invasive VNS has further been reported to increase parasympathetic tone in opioid withdrawal (Gazi et al., 2022; Miranda & Taca, 2018).

Pupil size changes, like breathing rate, heart rate variability and sympathetic skin response, serve as psychophysiological markers of the ANS. Moreover, pupil size is determined by balance between the parasympathetic ANS and the sympathetic ANS; measuring the static and dynamic pupillary diameters reflects imbalances of the autonomic regulation (Pomè, Burr, Capuozzo, & Binda, 2020). Like pupil size, it is assumed that pupil oscillation is regulated by a dynamic interaction between parasympathetic (cholinergic) and sympathetic (noradrenergic) activity (Pomè et al., 2020). In the context of reduced cognitive and mnestic performance in AUD patients, the question of an acetylcholine deficiency has been raised (Grünberger et al., 1998). By blocking the cholinergic receptors with the acetylcholine antagonist tropicamide, a larger dilation of the pupil was found in AUD patients (21 days after successful alcohol withdrawal) when compared with healthy controls (Grünberger et al., 1998).

In this pilot study we tested the effect of auricular vagus nerve stimulation on ANS activity by assessing the pupillary reaction to the cholinergic antagonist tropicamide in AWS as a psychophysiological biomarker of the autonomic balance before and after percutaneous auricular vagus nerve stimulation pVNS. Percutaneous VNS is one method of aVNS, using needle electrodes, which are applied through the skin (Samoudi et al., 2017). In a previous analysis of the data set, we explored the maximum pupillary dilation caused by tropicamide before and after pVNS and found that the dilating effect of tropicamide could be roughly halved with pVNS. These results are already published in German (Grünberger et al., 2019). We hypothesized that pVNS will enhance the parasympathetic tone represented by a reduction of pupillary diameter in a parasympatholytic pharmacological challenge with tropicamide after vagal stimulation.

## Materials and methods

### *Patients, inclusion, and exclusion criteria*

Thirty patients, male and female, aged between 20 and 65 years, with diagnosis of AUD according to ICD-10 and DSM-V were recruited between October 2018 and January 2019. Patients were screened and included at an inpatient unit specializing in AUD at the Department for Psychiatry and Psychotherapy at the Medical University of Vienna. All patients had to be able to perform oral and written informed consent to the study protocol, i.e., disoriented or delirious patients were not eligible for this study. In addition, patients were excluded who took medication affecting pupil measurement, such as opioids, acetylcholinesterase inhibitors, or sympathomimetics. Further, patients with a positive pregnancy test, as well as patients with anxiety disorders, phobias, or with current or lifetime history of multiple substance abuse disorder were excluded. Patients suffering from any kind of ocular disease were also excluded. Contraindications to the use of pVNS with the device AuriStim were biochemical implants, hemophilia, and psoriasis vulgaris. All patients were naïve to neurophysiological stimulation, including aVNS. We interviewed and classified all patients according to the “Lesch alcohol typology”, which is a standardized typology for AUD used at our group (Vyssoki et al., 2011). Demographic characteristics were recorded from medical charts at admission to the unit. Patients were instructed not to stop alcohol use prior to admission to avoid AWS-related complications, such as seizures, alcohol hallucinosis, and alcohol withdrawal delirium. For the study protocol, we used two withdrawal therapies for AUD consisting of either oxazepam (BDZ, dose range of 95–350 mg) or GHB (dose range of 3.5–5.3 g) starting at the day of admission. Levetiracetam in the dose range of 1000–1500 mg was prescribed for each patient to prevent potential withdrawal seizures. Patients suffering from sleep disturbances also received trazodone in the dose of 50–150 mg. Patients were on stable medication prior to pVNS and during the stimulation period.

### *Study design*

The pilot study applied an open-label, unblinded, repeated-measures design with three time points of pupillometry measurements at two testing days (see Fig. 1). The study protocol and all related procedures were approved by the Ethics Committee of the Medical University of Vienna and registered at clinicaltrials.gov (NCT05131334). For baseline measurements, the static pupil diameter was measured with the head fixed in a position (0′). Then, we administered .05 ml of .01 % tropicamide-solution (.005 μg), an anticholinergic agent leading to pupil dilation, into the left eye. Dynamic pupil measures were conducted 20 min (20′) and 40 min (40′) after tropicamide application. Initially, we scheduled a 60′ measurement, for which data were incomplete, because of patients' restlessness leading to potentially biased recordings. Absolute change (mm) and relative change (%) of the pupil diameter were calculated. All patients were tested at the same time of the day (between 9:00 and 10:00 AM) to exclude diurnal variation in pupillary diameter (Daguet, Bouhassira, & Gronfier, 2019).

Next, neurostimulator AuriStim (AU0115, Multisana GmbH; Vienna, Austria) was placed for pVNS (Samoudi et al., 2017). We stimulated the left ear side, thus ipsilateral to the measured pupillary response, in line with a previous investigation measuring the effect of VNS on the pupil size (Desbeaumes Jodoin, Lespérance,

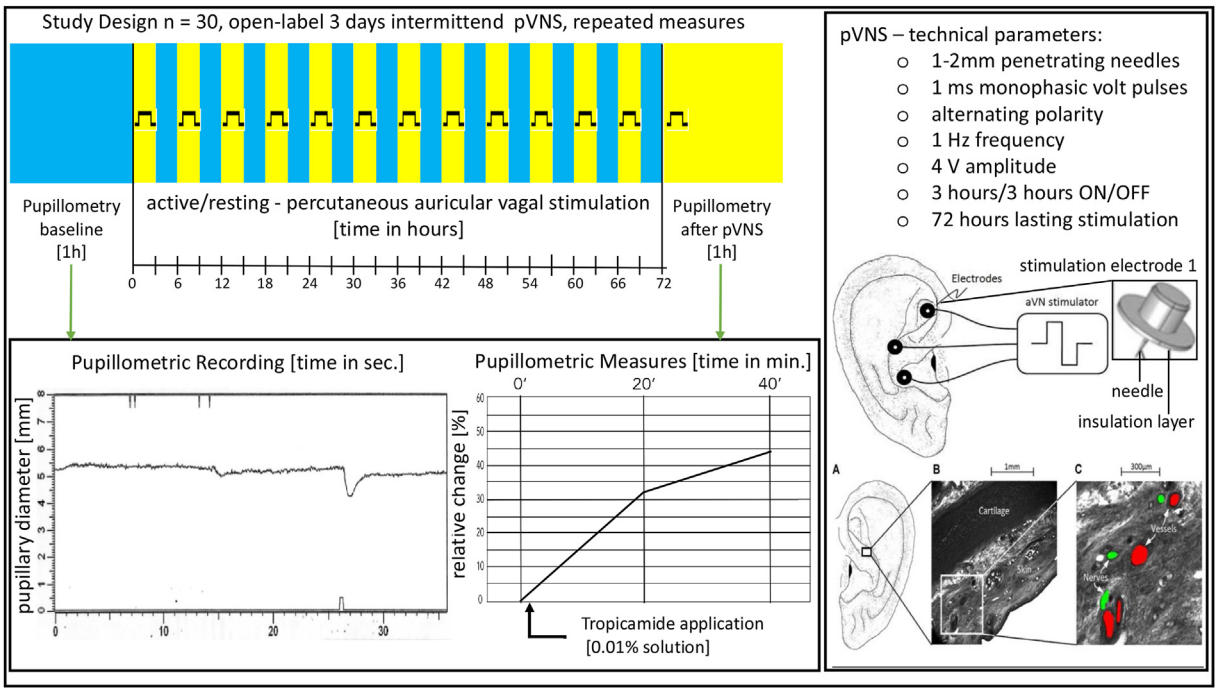


**Fig. 1.** Illustration of the study design. Top left: illustration of the study design. Patients undergoing treatment for alcohol withdrawal underwent two measurements of pupillometry, with baseline on day zero and three days after pVNS. Tropicamide .01 % was used as parasympatholytic challenge during pupillometry. Yellow = active stimulation; blue = resting. Bottom left: sample pupillometric recording as well as measurement time points after tropicamide application. Right: pVNS parameters and schematic placement of the ear electrodes as adapted from Samoudi et al., 2017. pVNS = percutaneous auricular vagus nerve stimulation.

Nguyen, Fournier-Gosselin, & Richer, 2015). After the 72-h stimulation period, patients underwent a second pupil measurement as described above.

We used absolute pupillary diameter as the primary outcome parameter. Since tropicamide is an antimuscarinic drug that produces short duration mydriasis after topical application, the degree of pupillary dilation provoked by tropicamide is a biomarker of the autonomic balance (Grünberger et al., 1999). Secondary outcomes were variables of dynamic pupil change and the measurement of pupillary oscillations calculated by Fourier analysis. We measured alcohol craving with a visual analog scale (Wang, Zhu, Zhou, & Chang, 2017) before and after pVNS.

*Percutaneous auricular vagus nerve stimulation (pVNS)*

For auricular vagal stimulation we used the device AuriStim (AU0115, Multisana GmbH; Vienna, Austria). Using a green light-emitting diode (LEDs) flashlight, blood vessels in the cymba conchae of the left ear side were visualized for the unaided eye. Minimal invasive needles (penetrating 1–2 mm) were placed close to the vessel bifurcations to provide efficient stimulation of the auricular branch of the vagus nerve. The built-in microchip controlled monophasic volt pulses of 1 msec, with alternating polarity, a frequency of 1 Hz, and an amplitude of 4 V (Samoudi et al., 2017). Stimulation lasted for 72 h in total with alternating on/off periods of 3 h. The stimulation intensity was imperceptible enough that patients were not awakened by stimulation. Thereafter, the device was removed, and patients continued their therapy consisting of anti-withdrawal medication.

*Quantitative pupillometry*

Before recording, patients were acquainted to the test environment (160 lux) in a noise-protected room (3 × 4 m) for 3 min. Patients had their heads held steady in a chin and forehead rest, the patients positioned their left eyes in front of the camera and fixed a black dot at 1.6 m distance to prevent accommodation (Grünberger et al., 1999). We performed pupillometry using a custom-made non-invasive personal computer-based infrared digital video pupillometer system, as was used in prior studies (Grünberger, Linzmayer, Majda, Reitner, & Walter, 1996, 1998, 1999). It comprises 28 incorporated 5-mW, infrared LEDs to provide continuous illumination of the eye, with a wavelength of 850 nm to achieve high contrast filming condition, and to which the pupil does not respond (Herbst, Sander, Milea, Lund-Andersen, & Kawasaki, 2011). The system integrates a calibrated light stimulation of fixed intensity (145 lux) and duration (300 msec) to induce a light-evoked pupillary reaction. The system allows a precise (.01-mm limit) and rapid (50 Hz) measure of the vertical diameter, and automatically determines static pupil size (averaged over the first 25.6 s of recording) and a series of dynamic pupillary parameters after light stimulation. Dynamic parameters include resting pupil diameter, the latency for constriction, the amplitude of constriction, the constrictive ratio, the duration of constriction, and peak constriction velocity. Based on the measurement of static pupillary diameter (first 25.6 s), the pupillometer calculates pupillary oscillations by Fourier analysis (FA), reflecting the activation of the central nervous system (Pomè et al., 2020). For each recording we analyzed pupillary oscillations with an FA as a biomarker of the central nervous activation and as described earlier by members of our group (Grünberger et al., 1999). Prior to FA a linear interpolation was applied to artefacts such as blinking and missing data by a built-in noise removal program. For FA we used five individual frequency bands: 0–.2 Hz, .21–.4 Hz, .41–.6 Hz, .61–.8 Hz, .81–1 Hz, and whole power spectrum .0–1 Hz (Grünberger et al., 1998).

*Craving measures*

We used a visual analogue scale (VAS) for measuring craving levels for alcohol. The VAS has been utilized frequently to assess cravings (Shahbabaie et al., 2014; Wang et al., 2017). Each patient used a pen to mark a location along a 10-cm line. The lower (i.e., 0) and upper (i.e., 10) ends of the VAS represent "no craving at all" and "extremely intense craving", respectively (Wang et al., 2017). The VAS was measured at baseline and following 72 h of pVNS.

*Statistical analyses*

All statistics were performed using the statistical software 'R4.0.5' (cran.r-project.org). For each patient and for each condition, static and dynamic diameter of the pupil were recorded (baseline 0′, 20′, and 40′ recording and pVNS 0 ′, 20′, and 40′ recording). Data were analyzed for outliers and checked for normality by visual inspection of data spread.

For pupillary differences of the main outcome parameter after pVNS, we used pupillary mean as the dependent variable under stable environmental and pharmacological circumstances in a repeated-measures ANOVA (rmANOVA) with R's built-in 'aov-function'. *Post hoc t* tests between baseline and after pVNS for each measurement time point were conducted with estimated marginal means in the 'emmeans' package and corrected for multiple comparisons with Tukey's method. All significance thresholds were set at $p < .05$. This analysis was corrected for sex and age. To investigate differences in secondary pupillary outcomes, an rmANOVA was conducted. Additionally in the present analysis, we checked potential differential effects of pVNS in two anti-craving regimens (BZD, GHB) in an explorative sub-analysis. Referring to the previous publication (Grünberger et al., 2019), we assessed differences in the alcohol craving visual analogue scale using a *t* test for dependent samples. To interpret effect sizes, we included Partial Eta Square ($\eta_p^2$) values for significant rmANOVA effects (small: $\eta_p^2 = .01$, moderate: $\eta_p^2 = .06$, large: $\eta_p^2 = .14$) (Sink & Mvududu, 2017) and Cohen's *d* for each significant *t* test (small: $d = .20$, moderate: $d = .50$, large: $d = .80$) (Cohen, 2013).

## Results

For the final analyses, we included 30 patients (12 females) suffering from AUD and acute alcohol withdrawal syndrome; for psychosocial variables please see Table 1. For a precise and efficient vagal stimulation all patients received the same needle placements at the left ear (shown in Fig. 1).

*Percutaneous vagus stimulation inhibits pupil dilation*

The change of pupillary diameter across time was assessed. For the primary outcome parameter of this study, pupillary diameter at time points 0′, 20′, and 40′ before and after pVNS, a 3 × 2 rmANOVA resulted in a significant difference for pupillary diameter across time and condition ($F_{(2,116)} = 27.97$, $p < .001$, $\eta_p^2 > .14$; see Fig. 2 and Table 1).

Tukey-adjusted *post hoc* analysis revealed a significant increase of pupillary diameter after pVNS for 0′ ($p = .013$, Cohen's $d = -.33$), 20′ ($p = .015$, Cohen's $d = .31$), and 40′ ($p = .002$, Cohen's $d = -.39$). The decrease of pupil dilation (diameter 40′ minus diameter 0′) induced by tropicamide following pVNS remained robust across 28 individuals (see Fig. 2B). For two patients we found no differences in pupillary dilation between conditions.

We assessed alcohol craving with a vertical visual analogue scale. After pVNS, a *t* test for dependent samples showed a significant reduction of subjective alcohol craving [$t(29) = 3.03$, $p = .005$, Cohen's $d = 1.27$].

In addition, significant differences were obtained in resting diameter ($F_{(2,116)} = 13.4$, $p < .001$, $\eta_p^2 > .14$) and minimum diameter ($F_{(2,113)} = 7.12$, $p < .001$, $\eta_p^2 > .06$). No significant difference in the rmANOVA was observed for amplitude of constriction ($p = .282$) and constrictive ratio ($p = .956$). Due to recording failures, time-dependent variables such as latency and time to constriction, had to be excluded from data analysis. Recording failure was caused by blinking at the time of light stimulation.

Due to missing data, 13 patients had to be excluded from the FA. By analyzing data from the remaining 17 patients at baseline and after pVNS with an rmANOVA, a trend-wise increased ($F_{(2,66)} = 3.07$, $p = .054$) amplitude in low-frequency bands was demonstrated (sum of the frequency bands: .01–1.0 Hz). Tukey's *post hoc* analysis revealed a significant contrast between conditions (baseline vs. pVNS) at 0′ measurement ($p = .008$, Cohen's $d = -.48$), while no significance was observed at the 20′ and 40′ time points.

To check for potential differential effects of pVNS in two anti-craving regimens (BZD, GHB), an explorative sub-analysis was performed. An rmANOVA yielded no significant differences ($F_{(2,112)} = 1.52$, $p = .224$) between the different psychopharmacological anti-craving therapies. The averages of pupillary diameters are summarized in Table 1.

## Discussion

In this study, we measured the pupillary reaction to pVNS in a pharmacological challenge to the anticholinergic agent tropicamide in patients with acute alcohol withdrawal syndrome. As predicted, pupil dilation was reduced after pVNS compared to baseline. Our results, although without sham control, justify further controlled studies of pVNS during alcohol withdrawal, whereby pupillometry might serve as a marker of ANS activation.

In patients suffering from AWS, an autonomic shift toward sympathetic activity (Ingjaldsson, Laberg, & Thayer, 2003) with reduced vagal modulation (Herbsleb et al., 2013) and a cholinergic deficit (Nevo & Hamon, 1995) results in autonomic symptoms, a negative affective state, and a decline of cognitive functions, symptoms that often cause relapse to alcohol consumption (Manning et al., 2021). Previous studies have shown that aVNS modulates neurotransmission systems such as acetylcholine (Rosas-Ballina et al., 2011), GABA (Keute, Ruhnau, Heinze, & Zaehle, 2018), serotonin (Li et al., 2018), and norepinephrine (Berger et al., 2021; Sharon, Fahoum, & Nir, 2021). In addition, VNS improves depressive symptoms and sleep quality in patients with AUD after withdrawal (<link id=bib_wang_et_al_2021b>Wang, Xu, et al., 2021; </link>). Vagus nerve stimulation is currently approved for epilepsy (Lampros et al., 2021) and depression (Fang et al., 2016), conditions that have frequent comorbidities with AUD (Miller, DiBello, Merrill, Neighbors, & Carey, 2020; Woo, Kim, Ko, Kim, & Kim, 2022). Accordingly, with increases in acetylcholine concentrations, our results demonstrate a reduction of pupillary dilation provoked by tropicamide after pVNS. Consistent with enhanced norepinephrine concentrations we found larger pupillary diameter after pVNS at time point 0, thus while not affected by tropicamide.

Pupillometry has widely been used as a diagnostic tool to evaluate activity of the autonomic nervous system (Venkata Sivakumar, Kalburgi-Narayana, Kuppusamy, & Bachali, 2020) and to assess autonomic balance in AWS (Herbsleb et al., 2013). Still, pupil-related measures as a biomarker for aVNS are discussed controversially. While several studies reported dilation of static pupillary diameter after short term aVNS (Capone et al., 2021; Mridha et al., 2021; Sharon et al., 2021), others could not replicate these findings and reported no changes of static pupillary size

**Table 1**
Demographic and clinical characteristics.

| Measure | Total (N = 30) | | GHB Group (N = 13) | | BDZ Group (N = 17) | | *p* |
|---|---|---|---|---|---|---|---|
| | N | % | N | % | N | % | |
| Female/male | 12/18 | 40/60 | 6/7 | 46/54 | 6/11 | 35/65 | |
| Lesch 1/2/3/4 | 3/2/13/12 | 10/7/43/40 | 0/0/6/7 | 0/0/46/54 | 3/2/7/5 | 20/10/41/29 | |
| History of seizures | 7 | 23 | 3 | 23 | 4 | 24 | |
| Sleep disturbances | 21 | 70 | 10 | 77 | 11 | 65 | |
| Suicidality (lifetime history) | 5 | 17 | 2 | 15 | 3 | 18 | |
| Previous hospitalization | **N** | **mean** | **N** | **mean** | **N** | **mean** | |
| | 24 | 4.0 | 9 | 4.7 | 14 | 5.7 | |
| | **mean** | | **mean** | | **mean** | | |
| Height (cm) | 174 ± 8.2 | | 175 ± 9.1 | | 174 ± 7.9 | | .746 |
| Weight (kg) | 76.3 ± 13.9 | | 77.3 ± 13.1 | | 75.3 ± 15.1 | | .708 |
| Age at baseline | 50 ± 9.2 | | 52 ± 9.6 | | 48 ± 8.5 | | .285 |
| Age IAM | 19 ± 5.4 | | 18 ± 3.8 | | 20 ± 6.7 | | .409 |
| Daily drinks | 18.83 ± 7.36 | | 20.75 ± 8.18 | | 17.65 ± 6.89 | | .279 |
| Pupillometry condition | | | | | | | |
| pupillary diameter | baseline | pVNS | baseline | pVNS | baseline | pVNS | |
| 0 min. | 3.73 ± .80 | 4.03 ± 1.0* | 3.49 ± .64 | 3.83 ± .83* | 3.91 ± .87 | 4.18 ± 1.13 | |
| 20 min. | 4.72 ± .86 | 4.42 ± 1.05* | 4.38 ± .77 | 4.29 ± .99 | 4.98 ± .90 | 4.52 ± 1.11* | |
| 40 min. | 5.16 ± .82 | 4.79 ± 1.05* | 4.87 ± .70 | 4.71 ± .96 | 5.38 ± .86 | 4.85 ± 1.14* | |
| Analog scale | | | | | | | |
| VCS (0–10) | 3.59 ± 2.41 | 1.05 ± 1.47* | 2.13 ± .94 | .33 ± .22* | 4.43 ± 2.65 | 1.47 ± 1.74* | |

Note: IAM – initial alcohol misuse; OAA – onset of alcohol addiction; VCS – visual craving scale (0–10); * = *t* test statistically significant difference between baseline and pVNS condition $p < .05$; GHB – γ-hydroxybutyrate.

(Burger, van der Does, Brosschot, & Verkuil, 2020; D'Agostini et al., 2021; Keute, Demirezen, Graf, Mueller, & Zaehle, 2019). To our advantage, we used an intermittent but continuous stimulation mode over 72 h to enable the balancing of the ANS, which outlasts pulse durations of negative studies. It has been reported that there is no habituation effect to 1 % tropicamide solution after one week (Arad et al., 2021), thus a habituation to .01 % tropicamide solution after 72 h can be excluded. Further, we used a VAS for the assessment of alcohol craving, which is an easy and effective method to assess subjective alcohol craving (Alarcon et al., 2021).

The pupil is under central and autonomic control and is therefore an interesting non-invasive biomarker of autonomic activity in clinical patients with alcoholism. It has been proven that pupil size is a versatile marker for cholinergic and noradrenergic neuromodulation (Radetz & Siegel, 2022). Neuroanatomically, parasympathetic motoneuron projections from the Edinger-Westphal nucleus, which is part of the oculomotor complex, to the ciliary ganglion cause constriction through activation of the pupillary sphincter. Sympathetic projections from the hypothalamus course through the spinal cord and the superior cervical ganglion and

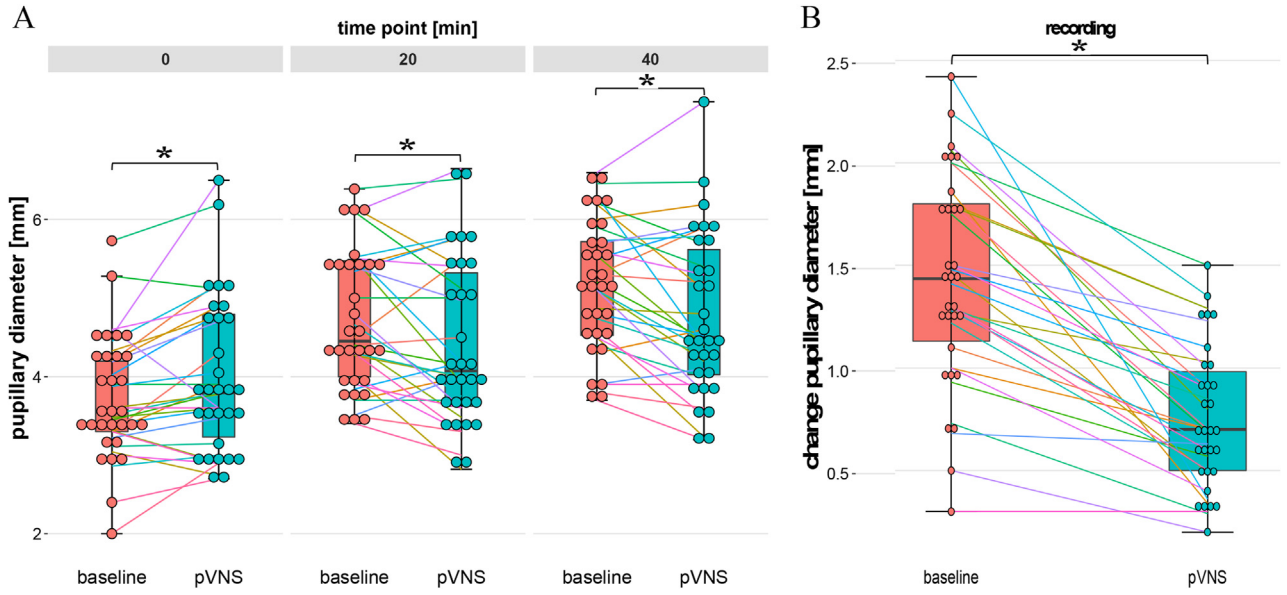


**Fig. 2.** **A**) Mean pupillary diameters, averaged over all patients for each measure and condition. Repeated-measures analysis of variance (rmANOVA) comparisons between baseline and after three consecutive days of pVNS. Patients were treated with oxazepam or γ-hydroxybutyric acid and received additive percutaneous vagus nerve stimulation (pVNS) for three days consecutively (3 h on/3 h off). Lines depict individual changes (baseline vs. after pVNS) of the mean pupillary diameter at each time. An rmANOVA revealed reduced pupil dilation provoked by tropicamide after three days of pVNS compared to baseline values. *Post hoc t* tests revealed significant differences between conditions for each time point. **B**) Absolute change of pupillary diameter (40′ minus 0′) after receiving tropicamide, with robust diminution of pupillary dilation after pVNS for 28 out of 30 patients. Time point 0′ is prior to administration of tropicamide.

activate the pupillary dilator (Diamond, 2001). Previous studies demonstrated that VNS leads to activation of the nucleus of the solitary tract (Fang et al., 2016), which further projects to the dorsal raphe nucleus, parabrachial nucleus, and the locus coeruleus; the last two project to the cholinergic, preganglionic part of the EW supplying the ciliary ganglion (Kozicz et al., 2011). It has been reported that aVNS can enhance vagal activity (Borovikova et al., 2000) that results in higher concentrations of acetylcholine (Rosas-Ballina et al., 2011). Consistently, we found reduced pupillary dilation after administration of the anticholinergic agent tropicamide for pVNS condition, most likely reflecting a parasympathetic activation with higher concentrations of acetylcholine. Further, the LC is a key link between pupillary diameter and the ANS. Activity in the LC, resulting in noradrenergic transmission, has been correlated with dilation of the pupil (Desbeaumes Jodoin et al., 2015), which is consistent with our finding of a larger pupillary diameter for pVNS condition at 0′ recording compared to baseline condition, reflecting an activation of the LC-norepinephrine system (Sharon et al., 2021). Regarding the neuromodulatory effects of VNS on cholinergic (Rosas-Ballina et al., 2011) as well as noradrenergic (Berger et al., 2021; Sharon et al., 2021) transmission, a fundamental question remains unclear. Since the cortical correlates of pupillary dynamics remain largely unknown (Radetz & Siegel, 2022), it is hard to disentangle the observed effects of pVNS on the pupil size, namely, dilation of static pupillary diameter (prior to tropicamide administration at 0′) and the opposite effect, thus resulting in reduced dilation following tropicamide administration. However, our results might contribute to the ongoing research regarding pupillary size as a biomarker of central cholinergic and noradrenergic activation due to VNS (Lloyd, Wurm, de Kleijn, & Nieuwenhuis, 2023).

Pupillary oscillations, frequency, and amplitude are modulated by cortico–diencephalic activity. Correlations between pupillary oscillations and vigilance have been stated (Yüzgeç, Prsa, Zimmermann, & Huber, 2018). While lower amplitude reflects a central deactivation, as found in severe cognitive deficits (Grünberger et al., 1999) and AUD (Grünberger et al., 1998), higher amplitudes were reported in mindful meditation, which was linked with cortical excitability (Pomè et al., 2020), thus reflecting vigilance. In FA we found a trend-wise increased amplitude in low frequency bands (sum of the frequency bands: .00–1.0 Hz) with a significant contrast between conditions (baseline and pVNS) at 0′ measurement. This is consistent with the finding of decreased amplitude in AUD patients when compared with healthy controls (Grünberger et al., 1998).

Alcohol craving following detoxification causes a crucial problem in clinical treatment of AUD. Reintroduced as a criterion for the definition of alcohol use disorders, alcohol craving is an important factor regarding treatment dropout and relapse. It has been reported that craving is highly associated with negative affectivity, depression, and anxiety and that these negative affects expand during withdrawal (Petit et al., 2017). Although still preliminary at this stage, since this effect could also be mediated by anti-craving treatment, we found hints for reduced alcohol craving between baseline and following pVNS treatment in our study.

In our study, patients received either BDZ or GHB as psychopharmacological AWS treatment. Low-dose GHB leads to an increase in dopaminergic activity in the mesolimbic system (4–6 g daily dose, based on a standard dose of 50 mg/kg/day) (van den Brink et al., 2018), while in higher doses (7–9.5 g daily dose) GHB is an agonist on GABA-B receptors, thus having a sedative effect resulting in reduced concentrations of extracellular acetylcholine in the hippocampus (Nava, Carta, Bortolato, & Gessa, 2001). Benzodiazepines are known to act via agonism on GABA-A receptors, thus having a sedative and anxiolytic effect, without any effect on the cholinergic system (Huron, Giersch, & Danion, 2002). Here, we couldn't observe significant different effects of pVNS in two anti-craving regimens.

## Limitations

For this open-label trial, some limitations should be stated. First, our pilot study was not controlled, nor did we compare it with a reference group. Thus, a potential effect of anti-craving treatments or placebo effects cannot be excluded at this time. However, since previous evidence demonstrated that benzodiazepines, along with the sedative effect, have no impact on the static (Bitsios, Szabadi, & Bradshaw, 1998) and the dynamic pupillary diameter after topical administration of tropicamide (Hou, Samuels, Langley, Szabadi, & Bradshaw, 2007), we argue that it is more likely that the changes measured on the pupil in this study are attributed to pVNS. This is further supported by findings that sham stimulation at the auricle did not cause changes in pupil size (Capone et al., 2021). The possibility of a placebo effect should be kept in mind. To this end, a pVNS control would have been desirable, which is, however, difficult to implement with the pVNS system applied here. Aside from that, regarding 0′ measurements, alternate factors affecting pupillary size, such as mood, medication, and treatment duration cannot be excluded since we did not include a sham control. Further, a limiting factor concerning 0′ measurements is that we cannot exclude participation itself in the study as a potential stressor affecting pupillary diameter. Taken together, to address these limitations, sham-controlled studies are necessary and are under preparation by our team. Also, regarding test-retest of the method, a minimal habituation effect was found for repeated measures on two consecutive days using 1 % tropicamide solution (McCormack, 1990), but no habituation was found when retested after seven days (Arad et al., 2021). Thus, in our study, reduced mydriasis after three days due to repeated doses of .01 % tropicamide solution seems very unlikely. For pupillometric recordings, we used a custom-built system, to the disadvantage of post-processing possibilities. Also, we lack raw pupillary data that are not processed by the built-in noise removal program. Missing statistics on proportions of noise extraction automatically removed by the program is a limitation of this dataset. Finally, the follow-up was limited to three days; thus, long-term therapeutic effects of pVNS in alcohol withdrawal remain to be elucidated.

In conclusion, our results provide evidence that pVNS mediates changes of autonomic activity. Pupillometry could serve as a simple, reliable, non-invasive, and low-cost approach sensitive to autonomic modulation techniques such as VNS. On the long term, therapeutic interventions in the ANS might reduce alcohol craving and risk of relapse. To this end, pVNS could be beneficial as a supportive therapy for AWS. Further evaluation and controlled trials of the therapeutic effects of auricular VNS as an additive treatment in alcohol withdrawal syndrome are warranted.

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## Author contributions

MCT and JG were responsible for the study concept and design. MCT collected data and DK recruited patients in collaboration with HW, BV, and OML. JG, HS, JCS, EK, and SK conceived the method and MCT processed and analyzed the data together with CK. MCT and CK wrote the manuscript. All authors contributed to the interpretation of findings, provided critical revision of the manuscript for

important intellectual content, and approved the final version for publication.

## Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available due to privacy or ethical restrictions.

## Declaration of competing interest

SK holds, shares, and is employed by AURIMOD GmbH. JCS holds shares of the Multisana GmbH. All other authors declare no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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