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Article

Keywords:

Posted Date: February 22nd, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-3976380/v1>

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Additional Declarations: There is **NO** Competing Interest.

Decreased CO₂ saturation during circular breathwork supports emergence of altered states of consciousness

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Abstract

Altered states of consciousness (ASCs), induced e.g. during psychedelic-assisted therapy, show great potential to treat highly prevalent mental health disorders like depression and posttraumatic stress disorder. However, access to such treatments is restricted by legal, medical, and financial barriers. Here, we explore the potential of circular breathwork to serve as a non-pharmacological and hence more accessible alternative to engage similar therapeutic processes. Scientific studies investigating the clinical effects of breathwork are only just emerging and the underlying physiological and psychological mechanisms are largely unknown. In this study, we address these questions by tracking physiological and experiential dynamics throughout a breathwork session, comparing two popular forms of breathwork: Holotropic Breathwork® and Consciously-Connected breathwork. We show that a reduction in end-tidal CO₂ pressure due to deliberate hyperventilation is instrumental in catalyzing ASCs during breathwork. The ASCs evoked by breathwork were comparable to those produced by psychedelics, and their depth predicted psychological and physiological follow-on effects, including improved well-being and a reduction of depressive symptoms. Further analysis showed that different breathwork traditions produced highly similar physiological, experiential and psychological outcomes. Our findings identify physiological boundary conditions for ASCs to arise in a non-pharmacological context, shedding light on the functional mechanisms of breathwork as well as its potential as a psychotherapeutic tool.

Introduction

In recent years, growing evidence suggests that by evoking altered states of consciousness (ASCs) in a supportive setting, psychedelic-augmented therapy may alleviate some of the most widespread and debilitating forms of mental suffering, including post-traumatic stress disorder, (treatment-resistant) depression and anxiety (Mitchell et al., 2021; Goodwin et al., 2022; Rotz et al., 2023; Raison et al., 2023; Griffiths et al., 2016; Carhart-Harris et al., 2018). While these are hopeful developments, many patients who might benefit from such psychedelic treatments will not have access to them for the foreseeable future, whether due to legal restrictions, medical counterindications or financial limitations. A widely accessible treatment engaging similar therapeutic mechanisms could thus benefit a large population of patients struggling with mental health issues. In the current study, we explore the potential of circular breathwork to be such a complementary therapeutic tool. To this end, we track the acute physiological and experiential dynamics evoked by circular breathwork, as well as their impact on psychological well-being in the wake of a breathwork session.

Circular breathwork is a breathing technique rooted in traditional practices like Tummo and Pranayama Yoga, and subsequently adapted by a multitude of inter-related traditions like, Holotropic Breathwork®, Consciously-Connected, Holorenic and Transformational Breathwork, as well as Rebirthing and others (Fincham, Kartar, et al., 2023). All these traditions have in common that over a prolonged period of time (approx. 15 minutes to several hours), participants sustain a deep, uninterrupted breathing rhythm, typically at a somewhat heightened speed. In addition, breathwork is typically conducted in a communal setting (i.e. in a group and/or in the presence of facilitators) and accompanied by emotionally evocative music. The term ‘circular breathwork’ is thus used here as an umbrella term for a range of techniques in which inhale and exhale are actively connected to each other in a continuous cycle, maintaining an at least somewhat structured form of deliberate hyperventilation, which is embedded in a context of evocative music and social support.

While anecdotal evidence from practitioners of circular breathwork suggests that it can benefit mental health, the first scientific investigations of these claims are only just emerging (e.g. (Bahi et al., 2023; Banushi et al., 2023; Uthaug et al., 2021) for an overview, see (Fincham, Kartar, et al., 2023; Fincham, Strauss, et al., 2023; Lalande et al., 2012)). The studies conducted so far seem to generally demonstrate the benefits of circular breathwork for alleviating stress and anxiety (Balban et al., 2023; Fincham, Kartar, et al., 2023; Fincham, Strauss, et al., 2023; Lalande et al., 2012), reducing depression and PTSD (de Wit & Moraes Cruz, 2021), and enhancing self-

awareness and life satisfaction (Fincham et al., 2023; Uthaug et al., 2021). What's more, these benefits have been hypothesized to be mediated by enhanced psychological openness (Lalande et al., 2012; Rhinewine & Williams, 2007). This constellation of potential mental health benefits appears to closely resemble the one reported for psychedelics (see e.g. (Carhart-Harris et al., 2018; Goodwin et al., 2022; Griffiths et al., 2016; Mitchell et al., 2021; Raison et al., 2023). Consistently with this, the subjective experiences arising acutely during circular breathwork have been tentatively aligned with those produced by psychedelic interventions – both anecdotally and in a small number of explorative studies (Bahi et al., 2023; Metcalf, 1995).

These observations open up several crucial questions regarding the mechanisms and effects of circular breathwork. First, to what extent are the immediate and sustained effects of breathwork comparable to those of psychedelics? And if there are parallels to be drawn, what are the physiological and psychological mechanisms by which a reasonably simple shift in breathing rhythm can give rise to such effects, including altered states of consciousness?

Currently, the only insights into the physiological processes likely triggered by circular breathwork stem to our knowledge (1) from medical studies of hyperventilation (Bednarczyk et al., 1990; Inbar et al., 2022; Krapf et al., 1991; Stäubli et al., 1994; Tercero et al., 2021), which often aim to model the physiological consequences of panic attacks, and (2) from studies examining the physiological consequences of the Wim Hof Method (Kox et al., 2014; Muzik et al., 2018; Zwaag et al., 2022) – a breathing technique that is related to circular breathwork but additionally features intermittent breath-holds and cold exposure. These studies suggest that circular breathwork increases blood oxygenation (Bednarczyk et al., 1990; Zwaag et al., 2022) while reducing CO₂ saturation (Bednarczyk et al., 1990; Inbar et al., 2022; Kox et al., 2014; Krapf et al., 1991; Stäubli et al., 1994; Tercero et al., 2021; Zwaag et al., 2022). This in turn renders blood pH more alkaline (respiratory alkalosis, (Bednarczyk et al., 1990; Kox et al., 2014; Krapf et al., 1991; Nuding et al., 2015; Stäubli et al., 1994; Zwaag et al., 2022)), causing vasoconstriction (Ainslie & Duffin, 2009; Bednarczyk et al., 1990; Bullock et al., 2021; Ito et al., 2003; Kety & Schmidt, 1946; Kontos et al., 1977; Mueller et al., 1977; Stäubli et al., 1994; K. Szabo et al., 2011; Tercero et al., 2021), particularly throughout the neocortex (Posse et al., 1997; Terekhin & Forster, 2006; Wise et al., 2007). However, it is not clear if and how these physiological changes play a causal role in altering participants' conscious experience, and if and how this in turn shapes psychological changes following a breathwork session. Alternatively, the subjective effects of circular breathwork may be largely independent of its physiological impact, relying instead more on psychological context factors like communal emotional expression, group sharing, evocative music, supportive touch by facilitators ('body work') and other contextual elements that breathwork practices are typically embedded in.

The current study aims to for the first time disentangle the contributions of these complementary mechanisms to the subjective experiences evoked by breathwork. To this end, we simultaneously tracked subjects' end-tidal CO₂ pressure (etCO₂) and their subjective experiences throughout a breathwork session, and related them to physiological and psychological changes following the session. With this approach, we show that ASCs evoked by breathwork closely resemble those produced by psychedelics, that the physiological changes associated with decreased etCO₂ are instrumental in triggering such ASCs, and that the depth of the experienced ASCs in turn predicts subacute effects of breathwork on the psychological and physiological level. We believe that these findings not only open up new insights into the functional mechanisms and potential applications of breathwork as a psychotherapeutic tool – more broadly, but they also identify physiological boundary conditions in which ASCs can arise in a non-pharmacological context.

Results

The current study explores the effects of two popular forms of circular breathwork: Holotropic Breathwork® and Consciously-Connected breathwork. To this end, we tracked end-tidal partial CO₂ pressure (etCO₂) and subjective experience depth at six time points throughout a breathwork session in 61 experienced breathwork practitioners (30 participants engaging in Holotropic Breathwork® and 31 participants in Consciously-Connected breathwork). Since Holotropic Breathwork® sessions lasted up to three hours, this equated to a measurement of etCO₂ and subjective experience depth every 25-30 minutes; while Consciously-Connected breathwork sessions lasted approx. 1.5 hours, equating to a measurement every 15-20 minutes. For both breathing techniques, subjects entered breathwork sessions in groups of 7 to 15 participants. To emulate the typical features of breathwork as it is currently practiced, all sessions also featured a group sharing before and after breathwork, as well as evocative music and support by trained facilitators throughout the session (for further details, see Methods). To disentangle the effects of the breathing technique itself from effects of the broader setting of the session, we asked ~70% of all participants (43 of 61) to actively engage in circular breathing (active-breath group), while ~30% of the participants were instructed to adhere to their normal breathing rhythm throughout the session (passive-breath group; see Methods).

To first establish if circular breathwork caused CO₂ saturation to drop as previously described for voluntary hyperventilation (Agadzhanyan et al., 2003; Bednarczyk et al., 1990; Krapf et al., 1991; Stäubli et al., 1994), we repeatedly measured etCO₂ throughout the session (see above). To disturb the session as little as possible with these measurements, we asked subjects to exhale into a small, portable CO₂-breathalyser (Masimo, Neuchatel, Switzerland). We then averaged the etCO₂ measured across time points 2-4. We excluded time points 1, 5 and 6 since subjects had either not yet started breathing actively or had returned to normal breathing at these points. As expected, etCO₂ in active breathers dropped significantly throughout the session compared to passive breathers, with an average of 36.7 ± 1.5 versus 22.4 ± 0.8 mmHg (mean \pm SEM; see Fig. 1A). This effect occurred irrespective of Holotropic Breathwork® or Consciously-Connected breathing techniques (Two-way ANOVA; df = 60; Effect of active versus passive breathing: $F = 87.5$; $p = 0$; Effect of breathing technique: $F = 0.0$; $p = 0.97$; Interaction: $F = 0.0$; $p = 0.99$). Effects were even more pronounced for the minimum etCO₂ per subject across time points 2-4, which dropped to 16.6 ± 0.8 mmHg in the active-breathing group but remained at 33.6 ± 1.5 mmHg in the passive-breathing group (mean \pm SEM; Fig. 1B; Two-way ANOVA; df = 60; Active versus passive breathing: $F = 120.0$; $p = 0$; Breathing technique: $F = 0.6$; $p = 0.46$; Interaction: $F = 0.0$; $p = 0.88$). The time course of etCO₂ confirmed that in active-breathing sessions, etCO₂ levels dropped throughout the first half of the session, typically reaching their minimum at measurement time points 2-3, and then gradually rising again towards the end of the session (Fig.

1C). These results indicate that participants adhered to the breathing instructions they were assigned to, with the active-breath group, but not the passive-breath group, reaching decreases in etCO_2 that would typically be expected during deliberate hyperventilation.

Next, we quantified the subjective experiences encountered by participants throughout the session. As a first benchmark, we administered two post-hoc surveys of altered states of consciousness that are classically used to quantify psychedelic experiences: The 11-Dimensional Altered States of Consciousness Scale (11-DASC; (Studerus et al., 2010)), and in a subgroup of 33 participants also the Mystical Experiences Questionnaire 30 (MEQ30, (Barrett et al., 2015; MacLean et al., 2012)). As shown in Figures 2A and B, the subjective experiences reported by the participants were comparable to those triggered by psychedelics. Specifically, the 11-DASC and MEQ30 scores reached by active breathers were significantly higher than scores that would be expected for a placebo treatment (see Methods for composition of reference data set; t-tests for difference from placebo reference scores; 11-DASC: $df = 42$; $t = 6.4$ to 13.9 across 11 sub-scales; all $p < 0.001$; all differences significant at a family-wise error rate of 0.05, based on Dunn-Sidak correction for multiple comparisons; MEQ30: $df = 21$; $t = 10.2$ to 12.9 across 4 sub-scales; all $p < 0.001$; all differences significant at a family-wise error rate of 0.05 based on Dunn-Sidak correction), and frequently approached scores evoked e.g. by a commonly used moderate therapeutic dose of 20-25 mg of psilocybin (see Methods for composition of reference data; t-tests for difference from psilocybin reference scores; 11-DASC: $df = 42$; $t = -10.8$ to 5.6 across 11 sub-scales; all $p = 0$ to 0.12 ; scores significantly lower than psilocybin reference scores in 6 of 11 sub-scales at a family-wise error rate of 0.05, based on Dunn-Sidak correction; MEQ30: $df = 21$; $t = -0.7$ to 0.1 across 4 sub-scales; $p = 0.50$ to 0.94 ; all differences insignificant at a family-wise error rate of 0.05, based on Dunn-Sidak correction). For passive breathers, subjective effects were significantly smaller than for active breathers (Two-way anova; 11-DASC: $df = 670$; passive versus active breath: $F = 94.0$, $p = 0$; 11-DASC sub-scales: $F = 6.4$, $p = 0$; Interaction: $F = 1.4$, $p = 0.19$ MEQ30: $df = 119$; passive versus active breath: $F = 19.0$, $p = 0$; MEQ30 sub-scales: $F = 2.4$, $p = 0.07$; Interaction: $F = 0.7$, $p = 0.53$). However, scores for the passive-breath group still differed noticeably from typical placebo scores (Prugger et al., 2022) (t-tests for difference from placebo reference scores; 11-DASC: $df = 17$; $t = 2.5$ to 7.3 across 11 sub-scales; all $p < 0.05$; differences significant at a family-wise error rate of 0.05 in 10 of 11 sub-scales, based on Dunn-Sidak correction; MEQ30: $df = 7$; $t = 4.1$ to 5.6 across 4 sub-scales; all $p < 0.01$; differences significant at a family-wise error rate of 0.05 in all 4 sub-scales, based on Dunn-Sidak correction). This suggests that context factors like music and communal setting do support somewhat the emergence of ASCs, at least in the case of the experienced breathwork practitioners we tested here. However, such ASCs evoked by the breathwork setting alone were significantly less intense than those triggered by additional deliberate hyperventilation.

To further explore the dynamics of subjective breathwork experiences over the course of the session, we also asked participants to rate the depth of their subjective experience at the same six time points at which etCO_2 was measured throughout the session. To cause minimal disruption to the ongoing session, experience depth was communicated via simple hand signs: One raised finger signified ordinary waking consciousness, while five raised fingers signified deeply altered consciousness, and 2-4 raised fingers correspondingly signified intermediate states (see Methods). When participants were unable to respond to the prompt to rate their subjective experience, this was also recorded as a '5' rating. To test if these hand-sign scores could accurately reflect subjective experience depth, we correlated the average depth ratings across measurement time points 2-4 to the 11-DASC and MEQ30 scores which participants indicated post-hoc (see Figs. 2A-B). All eleven sub-scales of the 11-DASC correlated positively with hand-sign ratings (see Supp. Fig. S1; mean $r \pm \text{SEM} = 0.42 \pm 0.01$; $p < 0.05$ in 10 of 11 sub-scales based on the family-wise error rate given by the Dunn-Sidak correction for multiple comparisons), as did all four sub-scales of the MEQ30 and the total MEQ30 score (see Supp. Fig. S1; mean $r \pm \text{SEM} = 0.37 \pm 0.03$; $p < 0.05$ in 3 of 4 sub-scales based on the family-wise error rate given by the Dunn-Sidak correction). This suggests that hand signs were a valid metric of subjective experience at any given moment.

Consistently with this, the ratings given by hand sign throughout the breathwork session indicated profoundly changed consciousness. The average depth of experience ratings across measurement time points 2-4 were clearly higher in active than passive breathers (mean $\pm \text{SEM}$: 3.46 ± 0.11 versus 2.46 ± 0.20 ; Two-way ANOVA; $df = 60$; Effect of active versus passive breath: $F = 20.4$, $p = 0$), and somewhat higher in Consciously-Connected than Holotropic Breathwork® sessions (Fig 2C; mean $\pm \text{SEM}$: 3.40 ± 0.16 versus 2.92 ± 0.15 ; Two-way ANOVA; $df = 60$; Effect of breathing technique: $F = 9.2$, $p < 0.004$; Interaction : $F = 1.7$, $p = 0.20$). This pattern remained clearly present when considering maximum rather than average experience depths across measurement time points 2-4 (Fig. 1D), both for the difference between active and passive breathing (mean $\pm \text{SEM}$: 4.12 ± 0.12 versus 3.22 ± 0.30 ; Two-way ANOVA; $df = 60$; Active versus passive breath: $F = 10.8$, $p < 0.002$) and between breathwork techniques (mean $\pm \text{SEM}$: 3.88 ± 0.35 versus 2.70 ± 0.40 ; Two-way ANOVA; $df = 60$; Consciously-Connected versus Holotropic Breathwork®: $F = 8.9$, $p < 0.004$; Interaction: $F = 3.0$, $p = 0.09$). The difference in depth ratings between breathwork techniques appeared to be particularly driven by the fact that the passive-breath group rated their experience as deeper in Consciously-Connected than in Holotropic Breathwork® sessions (mean $\pm \text{SEM}$: 2.90 ± 0.30 versus 2.05 ± 0.18 ; post-hoc t-test: $df = 15$; $t = 2.41$; $p = 0.029$).

In terms of temporal dynamics throughout the session, the most intense subjective experiences were likely to occur towards the middle of the session, ramping up during time points 1-2, and

waning again through time points 5 and 6 (Fig. 2E). These dynamics closely follow those of the etCO₂ measurements shown in Figure 1C. Interestingly, they also applied almost identically to Holotropic Breathwork® and Consciously-Connected breathwork (Fig. 2E), despite the fact that the absolute duration of a Holotropic Breathwork® session was almost double that of a Consciously-Connected session (approx. 3 versus 1.5 hours).

To establish whether physiological and experiential dynamics during the session related to each other in a way that might suggest a causal relationship, we first correlated average etCO₂ values in the active part of the session (time points 2-4) with average experience depth for the same time points. As shown in Figure 3A, there was a considerable correlation (Holotropic Breathwork®: $r = -0.51$; $df = 29$; $p < 0.004$; Consciously-Connected: $r = -0.44$; $df = 30$; $p < 0.02$). This indicated that participants who reached lower etCO₂ levels throughout the session were also more likely to experience deeper ASCs. To explore if this relation held on a moment-by-moment basis, we also quantified the relationship between the raw etCO₂ measurements and experience depth across all six measurement time points in 61 participants (Fig. 3B). As one might expect, the relationship between raw measurements of etCO₂ and experience depth was somewhat more variable than for the same metrics averaged across time (Fig. 3A). Nevertheless, the correlation remained statistically significant (Holotropic Breathwork®: $r = -0.50$, $df = 179$; $p < 0.001$; Consciously-Connected: $r = -0.35$; $df = 185$; $p < 0.001$). What's more, the virtual absence of time points in the upper right and lower left corners of the scatter plot suggests that 1) in the context of breathwork it is difficult to enter intense ASCs without at least some reduction in etCO₂ (e.g. < 50 mmHg) and 2) it is equally difficult to fully maintain ordinary consciousness once etCO₂ drops below a threshold of approximately 20 mmHg.

To further explore how the physiological and experiential effects of breathwork intersected over time, we jointly visualized the dynamics of both parameters throughout the session. As shown in Figures 3C-D, at the beginning of the session, etCO₂ falls and experience depth rises largely in unison. However, in the second half of the session, etCO₂ gradually returns to baseline, while experience depth remains elevated for a longer time. This dynamic suggests a scenario where reduced etCO₂ (and its physiological follow-on effects) serves as a 'trigger condition', initiating deep ASCs, which are then maintained for some time even once etCO₂ has normalized again. This also implies that etCO₂ and experience depth are in fact more closely (and intricately) linked to each other than initially apparent from the scatter plot shown in Figure 3B, which simply pools measurements from early and late portions of the session.

Interestingly, the passive-breath group also showed qualitatively similar dynamics of etCO₂ and experience depth as the active-breath group but confined to a much smaller parameter space. This indicates that there is some effect of context (e.g. evocative music, group setting) and

potentially of ‘social contamination’ by the active-breath group, yet this effect is visibly smaller than that reached with the aid of circular breathing.

Next, we examined if breathwork gave rise to physiological and psychological changes beyond the session itself. Since the physiological and experiential dynamics triggered acutely within the session were so similar across the two breathwork formats studied here, we considered both formats as the same treatment for the purpose of studying follow-on effects. However, statistical comparisons between the sustained effects of Holotropic Breathwork® and Consciously-Connected breathwork are shown in Supplementary Table S1, and confirm that their follow-on effects were largely identical.

To explore if breathwork affected mental well-being beyond the session itself, we tracked follow-on psychological effects in 33 of our 61 participants by administering the self-report version of the 16-item Quick Inventory of Depressive Symptomatology (QIDS-SR16)(Rush et al., 2006) and the Warwick-Edinburgh Mental Wellbeing Scale (WEMWBS)(Tennant et al., 2007) one week before and after the session. Both QIDS-SR16 and WEMWBS showed significant improvements post-session in the active-breath group (Fig. 4A,C; paired t-test; QIDS-SR16: $df = 19$; $t = -4.34$; $p < 0.001$; WEMWBS: $df = 19$; $t = 2.84$; $p < 0.01$). While the passive-breath group was unfortunately underpowered in terms of sample size, the observed data did not suggest a comparable improvement in QIDS-SR16 or WEMWBS scores (Fig. 4B,D; paired t-test; QIDS-SR16: $df = 4$; $t = 0.56$; $p = 0.61$; WEMWBS: $df = 4$; $t = 1.97$; $p = 0.12$).

To test if the follow-up improvements in mental well-being for the active-breath group were a direct result of the physiological and/or experiential processes they encountered throughout the session, we quantified how well follow-on changes in QIDS-SR16 and WEMWBS scores were predicted by 1) reduced $etCO_2$ during the session, and 2) MEQ30 and 11-DASC scores. Figure 4E shows prominent links between acute session parameters and subsequent changes in well-being, as measured by the WEMWBS. Specifically, subacute increases in WEMWBS scores were predicted by lower $etCO_2$ ($r = -0.49$; $df = 23$; $p = 0.014$) during the session, as well as by deeper ASCs, as reflected by higher scores in the MEQ30 ($r = 0.44$; $df = 23$; $p = 0.03$) and in the ‘Oceanic boundlessness’ subscale of the 11-DASC ($r = 0.54$; $df = 23$; $p = 0.006$).

Next, we aimed to establish whether breathwork also caused physiological shifts, particularly in terms of activity in the autonomic nervous system (ANS). To this end, we tracked concentrations of two molecular markers in saliva, extracted directly before and after the breathwork session: The inflammatory marker interleukin-1 beta ($IL-1\beta$), and alpha-amylase (α -amylase), which is a proxy for ANS activity, particularly its sympathetic branch (Ali & Nater, 2020; Nater & Rohleder, 2009).

After the breathwork session, α -amylase levels decreased overall, indicating less engagement of the sympathetic nervous system. Specifically, active breathers showed significantly lower levels of α -amylase post-session (paired t-test; $t=4.09$, $df=38$, $p<0.001$; see Supp. Table S1 for comparison between breathwork formats), while the decrease was smaller and failed to reach significance in the passive-breath group (paired t-test; $t=1.4309$, $df=15$, $p=0.17$) (Fig 5A,B). In contrast, IL-1 β levels increased compared to pre-session levels. In active breathers, levels of IL-1 β showed a significant increase post-session (Fig. 5C; paired t-test: $t=5.6$, $df=38$, $p<0.001$), and we also observed a smaller but statistically significant increase in passive breathers (Fig. 5D; paired t-test: $t=0.6$, $df=15$, $p<0.02$).

These results suggest that the breathwork session evoked qualitatively somewhat similar physiological changes in both groups, which may again reflect the influence of the overall context in which breathwork sessions took place (e.g. Rebecchini, 2021). Interestingly, the lowered α -amylase levels post-session indicate lower activity of the sympathetic nervous system. This may reflect a shift towards parasympathetic activity following emotional and somatic release and relaxation, which would be expected to occur throughout the later stages of a breathwork session (Russo et al., 2017). At the same time, elevated levels of IL-1 β post-session may indicate challenging or stressful experiences that participants might have encountered during breathwork ((Y. Z. Szabo et al., 2020); see Discussion).

We wondered if these physiological changes were simply a result of the physiological processes caused by breathwork, or if they were also shaped by the subjective experiences participants encountered. To answer this question, we first related average etCO₂ during the active phase of the session to subacute physiological changes. etCO₂ did not predict physiological outcomes significantly, suggesting that changes in inflammation and sympathetic recruitment were not driven directly by shifts in O₂-CO₂ balance (Fig. 5E). In contrast, subjective experience did seem to modulate physiological outcomes: All three subscales of the 11-DASC, 'Oceanic boundlessness' ($r=-0.37$; $df=53$; $p=0.006$), 'Ego dissolution' ($r=-0.34$; $df=53$; $p=0.01$) and 'Visual reconstruction' ($r=-0.29$; $df=53$; $p=0.35$), showed negative correlations with IL-1 β change, so that weaker ASCs during the session predicted larger increases in inflammation post-session (Fig. 5E). In other words, the further participants ventured away from every-day consciousness, the less their inflammatory markers increased post-session.

Together, these results indicate a scenario in which decreased etCO₂ is a crucial factor in triggering strong ASCs during breathwork, and the subjective qualities of these ASCs in turn predict subacute outcomes, both in terms of the psychological and physiological effects of breathwork.

Figures

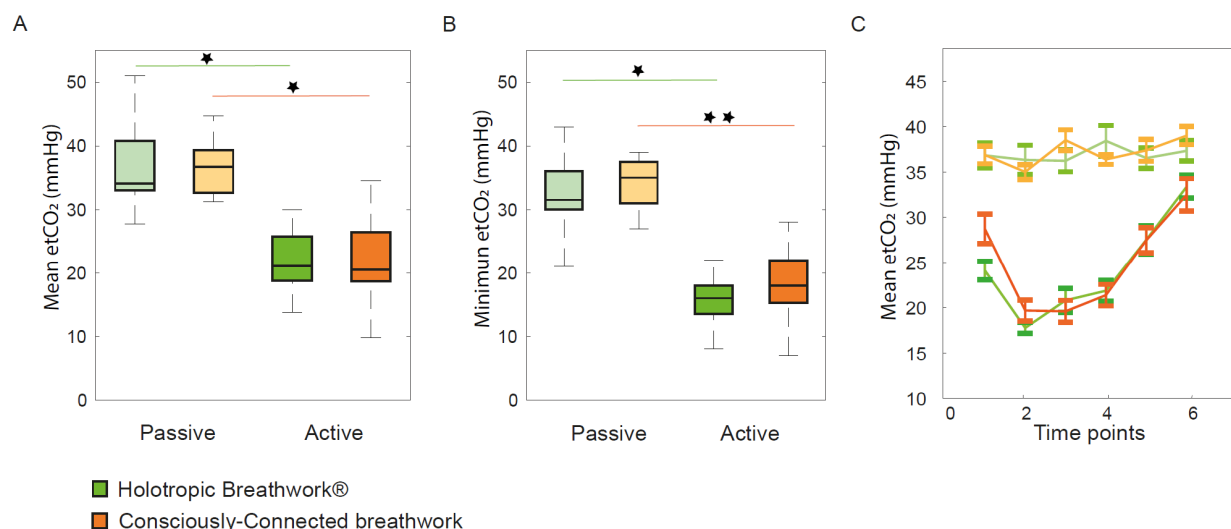


Figure 1. End-tidal CO₂ saturation during breathwork. (A) Boxplot of mean partial end-tidal CO₂ pressure (etCO₂) per subject, pooled across the active portion of the breathwork session (time points 2-4 out of 6). Central line: Median. Box outline: 25th and 75th percentile. Whiskers: 10th and 90th percentile. Green: Holotropic Breathwork®. Orange: Consciously-Connected breathwork. Desaturated colours (on the left): Passive-breath control groups. Saturated colours (on the right): Active-breath groups. Both active-breath groups showed significantly lower etCO₂ than their passive-breath counterparts. (B) Same as A for minimum etCO₂ per subject across measurement time points 2-4. Differences between active and passive breath groups are even more pronounced, with active-breath participants reaching etCO₂ as low as 10-20 mmHg throughout the session. (C) Time course of etCO₂ across all six measurement time points. etCO₂ decreased rapidly at the start of the session, reaching their minimum around measurement time points 2-3, and then increasing gradually towards the end of the session.

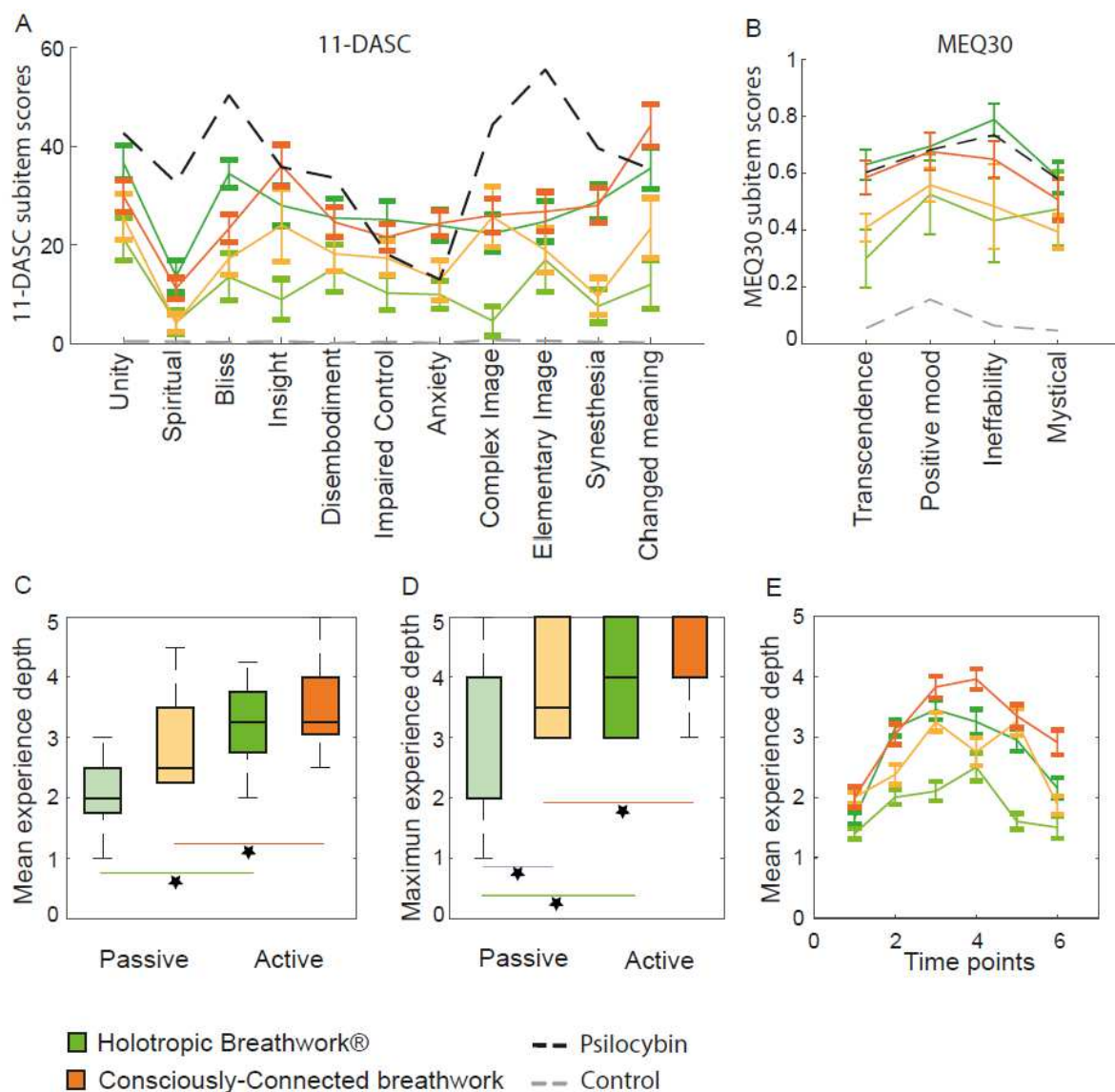


Figure 2. Experience depth during breathwork. (A) Subjective experience triggered by breathwork, as assessed by the 11-Dimensional Altered States of Consciousness Scale (11-DASC). Error bars: SEM. Colour scheme is the same as in Figure 1, with saturated colours denoting active-breath groups and desaturated colours denoting the passive-breath control groups. Black dashed line: Typical 11-DASC scores for a dose of 20-25mg Psilocybin, pooled across five relevant clinical studies of psilocybin-augmented therapy extracted from the Altered States of Consciousness Database (see Methods). Grey dashed lines: Typical 11-DASC scores for placebo treatments in three clinical studies extracted from the same data base. (B) Same as A for the four subscales of the Mystical Experiences Questionnaire 30 (MEQ30). Black dashed lines: Typical MEQ30 scores for a dose of 20-25mg Psilocybin, pooled across four relevant clinical studies from the Altered States of Consciousness Database (see Methods). Grey dashed lines: Same for placebo

treatments applied in two studies. Both 11-DASC and MEQ30 revealed profound changes of consciousness in active breathers, and on a smaller scale also in passive breathers. (C) Boxplot of mean experience depth per subject, determined by hand signs given on a scale of 1-5 (1= everyday consciousness, 5 = deeply altered consciousness; see Methods), which were then pooled across measurement time points 2-4. Central line: Median. Box outline: 25th and 75th percentile. Whiskers: 10th and 90th percentile. Colour scheme as in A and B. Active-breath groups on average indicated deeper experiences than their passive-breath counter parts. (D) Same as C for the maximum experience depth. (E) Same time course as shown in Figure 1C, but for experience depth. In both active-breath groups, consciousness is increasingly altered at the start of the session, reaching its maximum around measurement time points 3-4, and then gradually reverts to baseline. A qualitatively similar but quantitatively shallower dynamic can be seen in both passive-breath groups.

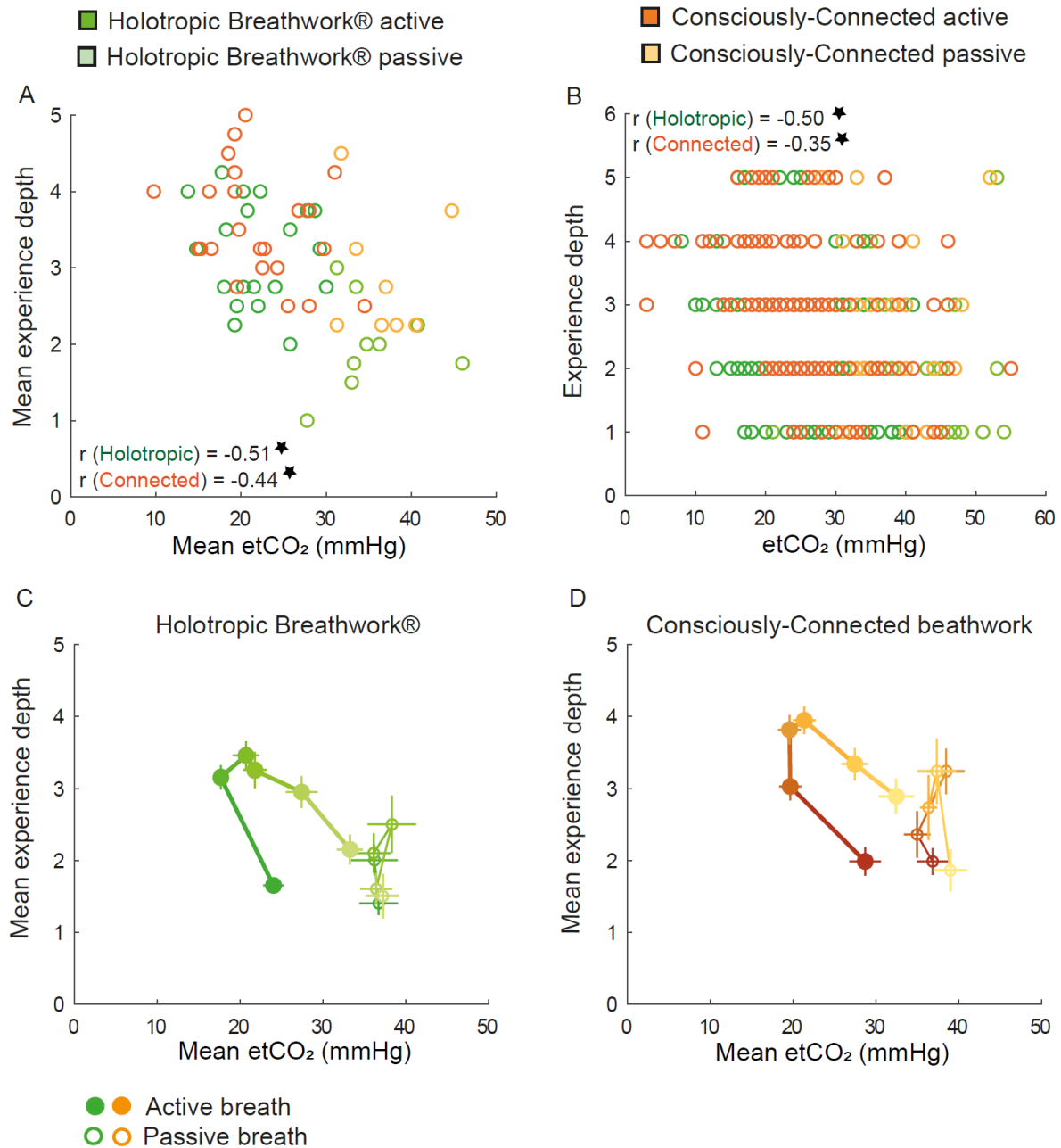


Figure 3. Relationship between end-tidal CO₂ saturation and experience depth. (A) Scatter plot showing mean experience depth per subject, averaged across measurement time points 2-4, as a function of mean end-tidal CO₂ pressure (etCO₂) averaged across the same interval. Colour scheme as in Figures 1 and 2. Mean etCO₂ and experience depth were negatively correlated for both Holotropic Breathwork® and Consciously-Connected breathwork, indicating that lower etCO₂ levels throughout the session were associated with deeper altered states of consciousness. (B) Same as A, but for six raw, i.e. non-averaged, measurements of etCO₂ and experience depth per subject. etCO₂ and experience depth were again negatively correlated. (C) Average trajectory

of etCO_2 and experience depth throughout a session of Holotropic Breathwork®. Filled circles: Average etCO_2 and experience depth at each time point, averaged across all participants of the active-breath group. Thin horizontal and vertical lines: SEM for etCO_2 and experience depth, respectively, for each time point. Darker colours signify measurement time points at the beginning of the session, light colours denote time points towards the end of the session. Open circles: Same for the passive-breath control group. (D) Same as C for Consciously-Connected breathwork. In both cases, experience depth initially increases when etCO_2 decreases, but then persists for some time even once etCO_2 begins to return to normal. Similar trajectories, however on a much smaller scale, were observed also for passive breathers.

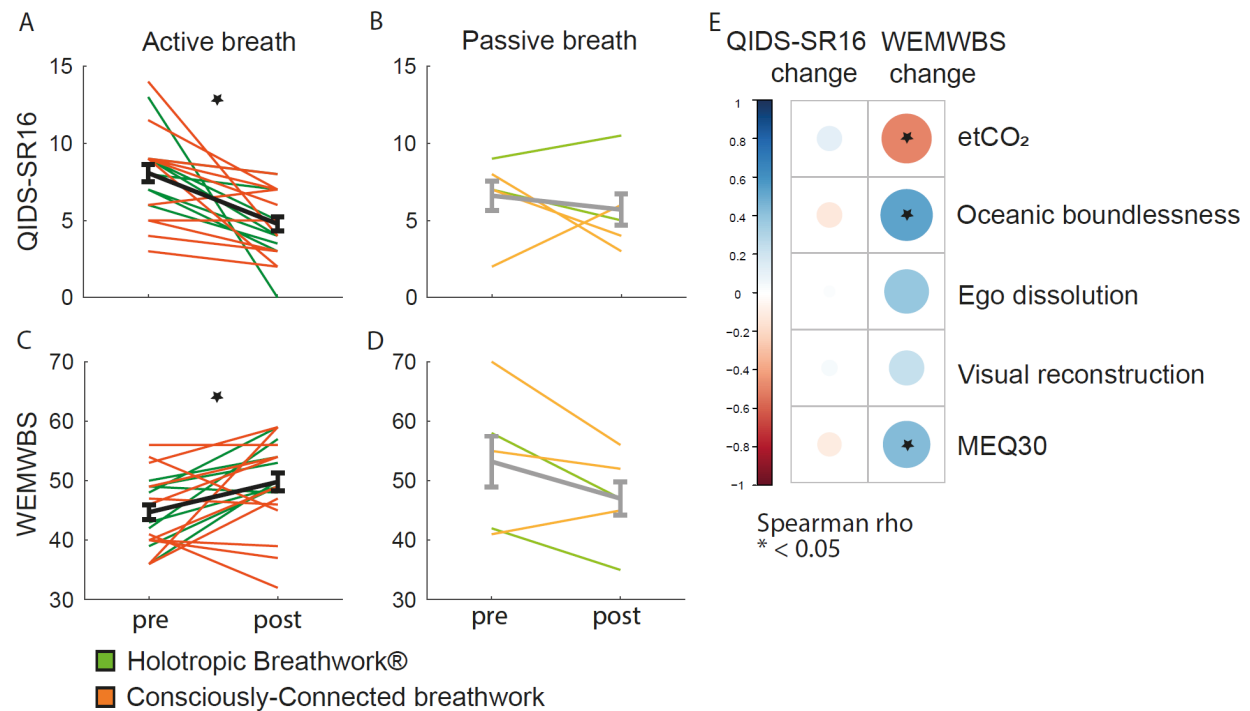


Figure 4. Psychological effects of breathwork. (A) Scores of the Self-Report Quick Inventory of Depressive Symptomatology (QIDS-SR16) one week before and after the breathwork session, completed by a total of 27 participants in the active-breath group. Orange lines: Participants in Consciously-Connected breathwork. Green lines: Participants in Holotropic Breathwork®. Black line and error bars: Mean \pm SEM across participants. Star indicates statistically significant decrease in QIDS-SR16 scores at an Alpha level of 0.05. (B) Same as A for the passive-breath group that completed follow-on questionnaires (n=5). Light orange: Consciously-Connected breathwork. Light green: Holotropic Breathwork®. Grey line and error bars: Mean \pm SEM. (C) Same as A for scores in the Warwick-Edinburgh Mental Wellbeing Scale (WEMWBS). Star indicates statistically significant increase in WEMWBS scores at an Alpha level of 0.05. (D) Same as B for WEMWBS scores. (E) Correlations between post-breathwork changes in QIDS-SR16 and WEMWBS scores on the one hand, and acute aspects of the breathwork session on the other hand, specifically average etCO₂ during the active part of the session, as well as scores in the Mystical Experiences Questionnaire 30 (MEQ30) and the three subscales of the 11-Dimensional Altered States of Consciousness scale (11-DASC), which are termed 'Oceanic boundlessness', 'Ego dissolution' and 'Visual reconstruction'. Correlation strength is colour-coded (see in-figure legend). Stars indicate statistically significant correlations. Correlations were computed based on 32 participants who completed questionnaires at the 1-week follow-up of the breathwork session. Post-breathwork changes in QIDS-SR16 scores were not significantly predicted by any of the acute parameters of the breathwork session. In contrast, improvements in WEMWBS scores were predicted by lower etCO₂ and by deeper ASCs during the session, indicated by both MEQ30 and 11-DASC scores.

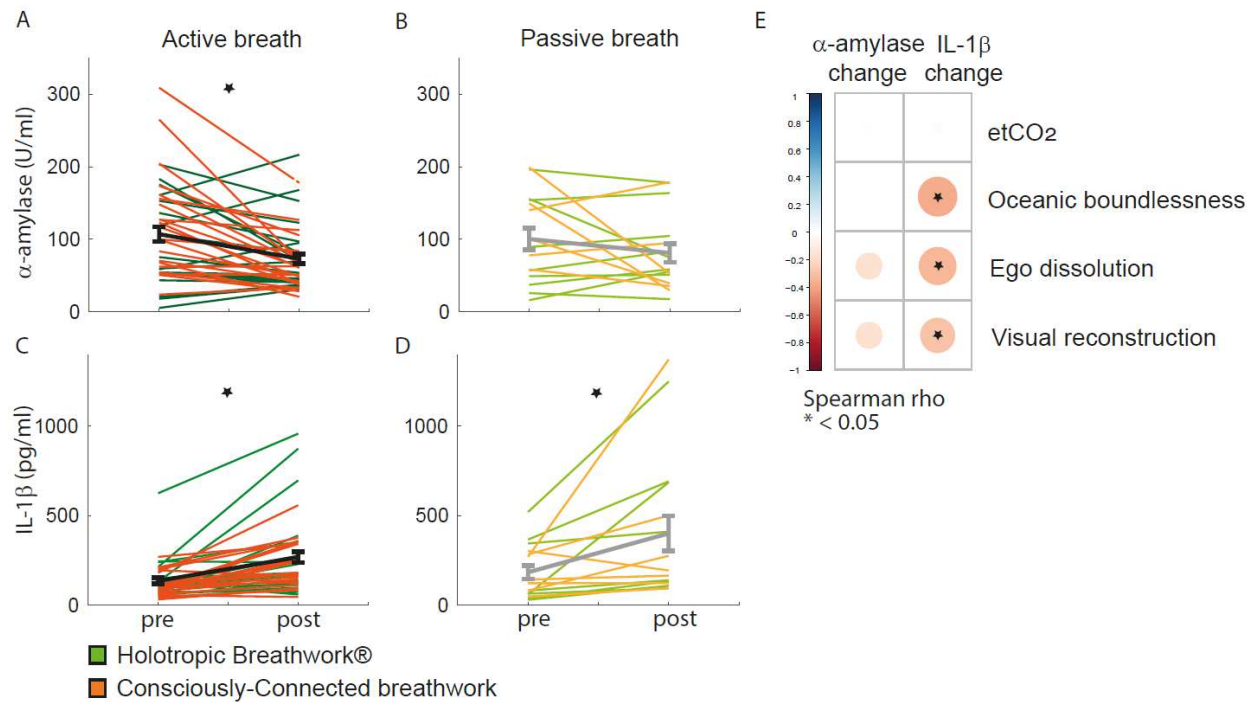


Figure 5. Subacute physiological effects of breathwork. (A) Concentration of the autonomic nervous system activity marker alpha-amylase (α -amylase) directly before and after the breathwork session, completed by a total of 39 participants in the active-breath group. Orange lines: Participants in Consciously-Connected breathwork. Green lines: Participants in Holotropic Breathwork®. Black line and error bars: Mean \pm SEM across participants. Star indicates statistically significant decrease in α -amylase at an Alpha level of 0.05. (B) Same as A for passive-breath participants (n=15). Light orange: Consciously-Connected breathwork. Light green: Holotropic Breathwork®. Grey line and error bars: Mean \pm SEM. (C) Same as A for concentrations of the inflammatory marker interleukin-1 beta (IL-1 β). Star indicates statistically significant increase in IL-1 β at an Alpha level of 0.05. (D) Same as B for concentrations of IL-1 β . Star indicates statistically significant increase in IL-1 β at an Alpha level of 0.05. (E) Same as Figure 4E for correlations between the acute aspects of the breathwork session and the post-breathwork changes in α -amylase and IL-1 β levels. IL-1 β increases were linked to subjective experiences, but not etCO₂, during breathwork, such that deeper ASCs predicted smaller increases in IL-1 β post-session. Change in α -amylase was not significantly predicted by any of the acute parameters of the breathwork session.

Discussion

In the present study, we have demonstrated that the experiential effects of circular breathwork are comparable to those evoked by psychedelics. By tracking physiological and experiential dynamics throughout the time course of a breathwork session, we were able to show that while part of this effect appears to be supported by general context (including music and social support), deep ASCs appear to be facilitated by the physiological processes triggered by circular breathwork, represented here by the reduction in etCO_2 . By comparing these effects in two popular and complementary formats of breathwork practice – Holotropic Breathwork® and Consciously-Connected breathwork – we could demonstrate that these different breathwork traditions engage similar physiological processes and also produce comparable experiential and psychological outcomes.

The subjective effects of breathwork in this sample were measured with two classical self-report questionnaires used in psychedelic research: MEQ30 and 11-DASC. Scores for both questionnaires were similar to those expected in the context of psychedelic-augmented therapy, with 5 of 11 sub-scales of the 11-DASC and all 4 sub-scales of the MEQ indicating no significant difference from experiences typically evoked by e.g. a dose of 20-25 mg of psilocybin (Hirschfeld, Prugger et al., 2023; Liechti et al., 2017; see Fig. 2A-B and Database Analysis section of Methods). These results are in line with the recently published work by Bahi et al (2023), but stronger than the effects reported by Uthaug et al (2021). This suggests that similarly to psychedelic experiences, different breathwork settings may deliver different experiential qualities (e.g. Golden et al., 2022). What's more, self-reports one week post-session indicated improvements in general well-being and a reduction of depressive symptoms; and the depth of the acute breathwork experience predicted such positive subacute outcomes. This dynamic resembles results that have been reported for psychedelic therapy, where the intensity of mystical experiences is also associated with subsequent improvements in mental health outcomes (Griffiths et al., 2008). This positions circular breathwork as a viable alternative in cases where psychedelic therapy may be indicated but not accessible.

How does breathwork evoke these stark subjective effects? Our study indicates that while the general setting of a breathwork session certainly plays a role in enhancing subjective experiences, the physiological changes driven by deliberate hyperventilation, quantified here in terms of decreased etCO_2 , are a key factor in evoking ASCs during breathwork. Decreases in etCO_2 were strongly linked to deeper ASCs, and intense ASCs were virtually never reached unless etCO_2 fell below standard levels of approx. 35 mmHg (see Figs. 3A-B). Conversely, when etCO_2 fell below approx. 20 mmHg, it was virtually guaranteed to trigger at least some (and often a strong) departure from ordinary waking consciousness. This effect is particularly intriguing because in

non-breathwork-related circumstances, an etCO_2 of 20 mmHg or less would be considered a sign of severe physiological malfunctions, e.g. of the heart or lungs (Arena & Sietsema, 2011; Inbar et al., 2022). This observation tallies with the concept of Pivotal Mental States introduced in 2020 by Brouwer & Carhart-Harris (Brouwer & Carhart-Harris, 2020). In this conceptual framework, unusual and overwhelming physiological challenges such as sensory overload or pain, especially when delivered in ritual contexts like e.g. sweat lodges, ritual tattooing, or religious fasting, can cause neuronal processing to transition into a state of heightened perception and learning. This switch is hypothesized to be mediated by a drastic upregulation of serotonin signalling (Brouwer & Carhart-Harris, 2020). In this scenario, classical psychedelics would be a pharmacological avenue to 'hijack' this innate system. By perturbing the normal equilibrium of blood gas concentrations, the physiological processes set in motion by breathwork fall squarely into the category of potential non-pharmacological triggers for such Pivotal Mental States. This notion is also consistent with our observation that once triggered, ASCs can self-sustain for an extended period of time (see Fig. 3). This dynamic could be explained by the fact that once neuronal processing has transitioned into a Pivotal Mental State, the initial physiological trigger for this switch is not required to remain present.

Another interesting and perhaps unexpected aspect of our findings is just how much the effects of Holotropic Breathwork® and Consciously-Connected breathwork resemble each other, both in terms of within-session physiological and experiential dynamics (Figs. 1-3), and of psychological and physiological follow-on effects (Figs 4-5 and Supp. Table S1). This similarity is particularly remarkable given that, while both breathwork practices share the same core breathing technique, their overall format is not identical, especially with regards to duration: At a typical duration of three hours, Holotropic Breathwork® sessions last approximately twice as long as Consciously-Connected breathwork sessions. The fact that in-session dynamics are nevertheless so similar (see Fig. 3C,D) might be due to the participants' pre-existing expectations and experience of a specific timeline, as well as the guidance from facilitators and the corresponding musical cues, which follow a similar overall dynamic (increasingly active breathing – emotional release – relaxation), but adjusted to the total session duration of each breathwork style.

Note that while in both breathwork formats, the passive-breath group reported less intense subjective experiences than the active-breath group, participants in the passive-breath group still did seem to experience some alterations of their conscious state (see Figs. 2 and 3). To some extent this effect is likely to be a true reflection of the role that context plays in a breathwork session. In fact, prominent context factors of breathwork (e.g. music, group sharing, social support by group and facilitators) are specifically designed to evoke and support emotional processing. As such, in our view the fact that the breathwork setting alone can alter consciousness to some extent does not put the effectiveness of the circular breathing technique

itself in question. Rather, it reflects the fact that breathwork traditions have successfully developed contextual elements to enhance the processes the breathing technique itself is meant to evoke. In addition, the setting effects measured in this study are likely heightened due to the fact that it was conducted in experienced breathwork practitioners. It stands to argue that simply due to their prior experience, subjects were better able to shift their processing away from everyday consciousness when experiencing the (to them) familiar setting of a breathwork session. It is therefore likely that if the same experiential metrics were recorded in naive subjects, the passive-breath group would report experience depths closer to ordinary consciousness than in this study – a hypothesis that should be tested in future.

Our self-report metrics also indicated improvements in overall well-being and depressive symptoms one week after the session (see Fig. 4). It is however important to keep in mind that this effect may have been enhanced by prior convictions of the participants: Since we worked with experienced practitioners, our participants are likely to perceive breathwork as a practice that generally enhances their quality of life. Therefore, they might be expecting to see improved well-being post-session. Nevertheless, the subacute increases in well-being reported here were in line with previous reports (Bahi et al., 2023; Fincham, Strauss, et al., 2023; Uthaug et al., 2021), and improvements depended on ASC depth throughout the session – a relationship that has also been reported for psychedelic experiences (Hirschfeld et al., 2023). Together, these results suggests that improvements in well-being were substantial beyond the contribution e.g. of placebo or expectation effects.

One interesting feature of our self-report metrics was that according to the scoring criteria of the QIDS-SR16, a portion of participants reported symptoms of mild, and in three cases moderate, depression one week before the breathwork session (see Fig 4A-B). These somewhat elevated baseline levels of depressive symptoms might be explained in several ways. First, given the high prevalence of diagnosed depression (Otte et al., 2016), it is reasonable to assume that mild levels of undiagnosed depression may be even more prevalent across the population. Beyond that, practices involving ASCs, including psychedelics and breathwork, have been reported to increase emotional openness (Erritzoe et al., 2019; Lebedev et al., 2016; MacLean et al., 2011; Wagner et al., 2017). As such, participants might simply be more aware of e.g. feelings of sadness or discomfort, and more willing to report them. Another intriguing possibility arises from the observation that one week post-session, most participants in the active-breath group reported decreased depressive symptoms (see Fig. 4A). As such, one could hypothesize that generally well-functioning persons with mild mental health disorders (such as mild depression) might take up breathwork as a form of self-medication, which might help them to alleviate symptoms for days, potentially weeks, post-session. Further experiments will be needed to disentangle these possibilities.

In terms of physiological changes triggered by breathwork, the coexistence of decreased markers of sympathetic nervous system activity (α -amylase) but increased inflammatory immune responses (IL-1 β) may seem contradictory at first glance, particularly since enhanced immune system activity would typically be thought to reflect increased rather than decreased stress. One potential explanation for this pattern is that by disturbing the normal equilibrium of blood gases, a breathwork session initially represents a temporary stressor to the body. This is likely to trigger activity across several stress response systems, particularly the sympathetic nervous system and hypothalamic-pituitary axis, which may in turn upregulate the immune system (Ali & Nater, 2020; Engeland et al., 2019). Thus, we hypothesize that breathwork initially triggers the sympathetic nervous system and thereby activates the immune system, leading to an increase in IL-1 β . As the session progresses, and participants return to relaxation, the ANS may then transition from sympathetic to parasympathetic activity, resulting in lower levels of α -amylase post-session. If true, this dynamic would be consistent with the notion of breathwork as a cathartic process, which involves experiencing and expressing challenging emotions and sensations in order to arrive at feeling of release and relaxation (Lalande et al., 2012; Rhinewine & Williams, 2007; Weiss et al., 2023). To test this theory, future studies should monitor biomarker profiles more comprehensively by tracking pro- and anti-inflammatory cytokines over a longer time frame, as well as throughout the session. It is also important to note that our analysis was based on saliva samples, where the status of mucosal immunity and overall mouth health may have contributed to the observed IL-1 β levels. For future studies, it would be useful to measure plasma levels of cytokines as well as immune cell phenotypes to get a more comprehensive picture of how circular breathwork affects the immune system.

Finally, work on related breathing techniques, especially the Wim Hof Method, suggests that in the long run, engaging in circular breathwork may lead to a sustained decrease of sympathetic reactivity to mental stress (Fonkoue et al., 2020) and may ultimately act as an anti-inflammatory (Kox et al., 2014). In line with previous results (Kox et al., 2014; Zwaag et al., 2022), our findings suggest that while breathwork may have anti-inflammatory effects in the long term, during the session it appears to act as a temporary challenge to the system, increasing inflammation. Such seemingly paradoxical effects can be interpreted as processes of hormesis, where physical challenges such as fasting or physical workouts provide short-term stressors to the body that increase physical fitness long-term. Further longitudinal studies of breathwork and its psychological and physiological long-term effects will be needed to test these hypotheses.

In summary, our results indicate that breathwork can be an effective tool to enhance psychological well-being. The psychological processes by which it does so seem to resemble those engaged by psychedelic-augmented therapy, including highly similar ASCs whose depth predicts

subsequent improvements in well-being, as well as the modulating function of setting (music, social support and other context factors). Challenging physiological processes, including decreased etCO_2 , seem to be central in triggering deep breathwork experiences. This observation is consistent with the concept of pivotal mental states, which hypothesizes that unusual physiological challenges such as heat/cold exposure, fasting, sensory overload, but also psychedelic substances, can trigger intrinsically driven states of altered, heightened, and less filtered perception, as well as heightened learning and plasticity (Brouwer & Carhart-Harris, 2020). The physiological and experiential dynamics evoked by breathwork seem to tick all the boxes to qualify as a potential trigger for such pivotal mental states. On a more conceptual level, the co-existence of feelings of challenge and relaxation, of sympathetic and parasympathetic activity has been interpreted as a hallmark of experiences of awe (Monroy & Keltner, 2023). Recent work has highlighted feelings of awe as a vital ingredient of human well-being (Monroy & Keltner, 2023). Breathwork, like psychedelic states, may provide a physiological and experiential access point to this emotional space.

Methods

Subjects and Ethics

The study enrolled 61 experienced breathwork practitioners (33 female, 28 male) via online advertisements in various breathwork communities. The average age of participants was 33.1 ± 7.1 years (Range: 20 to 55 years). Three participants reported being unemployed, eight reported being in education, the rest reported being self-employed ($n=17$) or employed ($n = 27$). None of these attributes differed significantly across the experimental groups that participants were assigned to.

The study was conducted at the MIND Foundation office in Berlin and approved by the ethics committee of the Ärztekammer Berlin (File number Eth-55/21; Project Code PPEIA). Written informed consent was given by all participants prior to the participation in the study after having an individual short interview with the study coordinator. Participants were included if they were between 18 and 60 years old, provided written informed consent and had previously experienced at least five immersive breathwork sessions. Exclusion criteria were: Presence of a mental health disorder in the forms of schizophrenia, psychoses, personality disorders (e.g. borderline or antisocial personality disorder) or a history of substance use disorder during the last 6 months (according to DSM-5). Cardiovascular diseases (e.g. inadequately treated arterial hypertension, heart arrhythmia), chronic lung disease (e.g. bronchial asthma) and chronic obstructive pulmonary disease, pregnancy, and the presence of a chronic disease of the central nervous system (especially epilepsy) were also excluded. At least one week before the session, potential participants filled out a questionnaire investigating all exclusion criteria. If they met all criteria for inclusion according to this questionnaire, participants next conducted a 'check-in interview' per phone call with the study coordinator, where they were informed about the structure of the study (including session length, explanation of CO₂ measurements, and online questionnaires pre- and post-session where applicable). During this phone interview, participants were also asked once more about potential exclusion criteria to make sure e.g. relevant diagnoses had not been overlooked. Finally, participants received their personal pseudonymized code, which they used both in the session itself but also for the online questionnaires pre- and post-session.

Participants were assigned to an experimental group in a two-step procedure. First participants volunteered either for a one-time Holotropic Breathwork® (N=30) or Consciously-Connected (N=31) breathwork session. On the day of the session, every participant then checked in with the study coordinator, completed a COVID-test, and underwent a short medical examination to check for physiological exclusion criteria (resting heart rate > 100 beats per minute, resting blood pressure > 160, obvious issues in lung function that could be detected by a medical doctor using a stethoscope). In the second step, participants got handed a randomly matched envelope

assigning them either to the passive or active breath group. In this way, participants were randomly assigned to the active- or passive-breath groups at a ratio of approximately 70:30, so that ultimately 43 out of 61 participants (20/30 in Holotropic Breathwork® sessions, and 23/32 in Consciously-Connected breathwork) were in the active-breath groups where they followed the instructions for the respective breathwork format they had chosen, while 18 participants (10 for Holotropic®, and 8 for Consciously-Connected breathwork) were in the passive-breath groups, adhering to their normal breathing rhythm.

Intervention: Holotropic Breathwork® and Consciously-Connected breathwork

The Holotropic Breathwork® session lasted approximately 3h while the Consciously-Connected session lasted 1.5h. Each session comprised of 7-15 participants. All sessions were supported by experienced breathwork facilitators trained in the respective breathing technique. Facilitators were not informed of the experimental group of the participants (but may have been able to infer experimental groups by observing breath patterns). In both formats, a group sharing was conducted before and after the breathwork session, where participants were invited to express thoughts and feelings about their current experiences. Also in both formats, facilitators could offer physical support (e.g. a hand on the shoulder) to participants that were encountering intense emotional experiences, in accordance with the physical support ('body work') typically offered in both breathwork formats. Throughout the session, 2-3 study helpers assisted with measurements of etCO₂ and experience depth, while interfering as little as possible in the participants' experiences.

For the Holotropic Breathwork® sessions, the day consisted of 2 consecutive breathing sessions, which participants experienced together in pairs, such that half of the group (irrespective whether they had been assigned to the active- or passive-breath condition) would breathe first, and the other half would play the role of sitters who were there to provide emotional and practical support (e.g. by providing water or a blanket). Sitters were not counted as part of the passive-breath group – in fact, data from sitter sessions were not analysed at all. In the second breathing session, each pair of participants reversed roles, such that sitters from the first session would be breathers (assigned either to the passive or active breath group) in the second session, and vice versa. Each session was supported by 2-4 facilitators per group. Right after the session participants were invited to engage in mandala drawing (a form of graphic expression of the experience where participants get a white paper with a round shape drawn on it and fill that with colours). This type of drawing is traditionally used as a form of non-verbal integration of the breathwork experience in Holotropic Breathwork® settings.

In the case of the Consciously-Connected breathwork session, one breathwork session was conducted jointly for all participants (active- and passive-breath group) at the same time (there were no sitters). Sessions were accompanied by 2 facilitators per group.

End-tidal CO₂ pressure

End-tidal partial CO₂ pressure (etCO₂; i.e. partial pressure of CO₂ at the end of exhalation) was measured at 6 time points throughout the breathwork session using a portable CO₂-breathalyser (capnography) device (EMMA, produced by Masimo, Neuchatel, Switzerland). Before the session participants were instructed how to use these capnography devices. At each measurement time point, the study helpers would alert participants with a touch on the shoulder, then put the device in the participant's hand, and together lead their hand to their mouth, where the participants would inhale freely and then exhale as fully as possible through a tube attached to the device. The breathalyser then displayed both the wave-form of CO₂ pressure throughout the exhalation (capnographic display), and the etCO₂, which was noted down by the study helpers.

Subjective experience

Experience depth was assessed at the same six time points as etCO₂ throughout the session, using hand signs to give a depth rating from 1 to 5. One finger raised indicated normal waking consciousness, while five fingers or inability to raise hand were signalling deep altered states of consciousness. Two to four raised fingers indicated intermediate states (see below). The hand signs were demonstrated and explained to all participants before the beginning of the session. More detailed examples of criteria for the subjective depth ratings, which were also verbally shared with the participants before the start of the session were as follows:

- 1) Normal ability to speak, normal analytical thinking, clear awareness of time, space and current context, normal experience of self, including awareness of biographical data, social contacts, and conscious control over actions.
- 2) Mostly normal thinking and speaking ability, mild changes e.g. in perception, emotional experience of spontaneous body movements.
- 3) Reduced analytical thinking, partial loss of awareness over time and space, reduced awareness of self, potentially some non-immersive visual or auditory hallucinations, emotional experiences of involuntary body movements.
- 4) Loss of analytical thinking, strongly reduced awareness of self, time and space, reduced reaction to external stimuli, potentially visual or auditory hallucinations, uncontrolled emotional expression, or body movements.
- 5) Loss of awareness of self, time, and space, strongly reduced perception of external stimuli, strongly reduced ability to speak or control physical movements, potentially intense and

immersive visual or auditory hallucinations, uncontrolled emotional expression, or body movements.

Sub-acute self-reports on subjective experience

Subjective experience of study participants was assessed immediately after the breathwork session using widely established scales for measuring altered states of consciousness: the 11-Dimensional Altered States of Consciousness Scale (11-DASC, (Studerus et al., 2010)) and Mystical Experiences Questionnaire (MEQ30, (Barrett et al., 2015; MacLean et al., 2012)). 11-DASC subscales were computed as: “Oceanic boundlessness” (experience of unity, spiritual experience, blissful state, insightfulness), “Ego dissolution” (disembodiment, impaired control and cognition, anxiety) and “Visual reconstruction” (complex imagery, elementary imagery, audio-visual synaesthesia, changed meaning of perception). The questionnaires were handed out on paper and took around 20-25 min to complete.

Self-reports on mental health and well-being

To explore follow-on effects of breathwork on mental health and wellbeing, the first half of the participants (33 out of 61) were asked to fill out self-report questionnaires one week before and one week after the breathwork session (these metrics could not be recorded for the second half of participants for logistical reasons). The self-report version of the 16-item Quick Inventory of Depressive Symptomatology (QIDS-SR16) (Rush et al., 2006) was used to assess depressive symptoms and the Warwick-Edinburgh Mental Wellbeing Scale (WEMBWS) (Tennant et al., 2007) was used to measure overall wellbeing. These questionnaires were administered as online surveys using a secure platform (implemented by Gravity Forms), hosted on the MIND Foundation’s website. Entries were pseudonymized, i.e. participants identified with their pseudonymized study code only. The questionnaires took around 10min in total to fill out.

Measurement of α -amylase and IL-1 β in saliva

Levels of salivary α -amylase and IL-1 β were assessed before and after the breathwork session. Prior to session participants were instructed how to use saliva collection tubes (Salivette, Sarstedt Germany). After sample collection, tubes were frozen and kept at -20C until analysis using enzyme-linked immunosorbent assay (ELISA). On the day of assays, saliva samples were thawed completely, vortexed and centrifuged at 1500 x g for 15 min to remove mucins that precipitate during freezing. Samples and assay kits (salivary α -amylase assay kit cat no. 1-1902; salivary IL-1 β ELISA kit cat no. 1-3902) were brought to room temperature before use and assays were performed following manufacturer’s instruction (Salimetrics). All samples were run in duplicates. Plates were read using Clario Star plate reader (BMG Labtech) and final α -amylase and IL-1 β concentrations calculated according to protocol’s instructions using MARS data analysis software.

Data Analysis

Questionnaires that had been administered on paper were subsequently transcribed by hand into Excel tables, and all transcriptions were checked independently by at least one other person. Data were then analysed using custom-written code in MATLAB and R (Version 4.2.3).

Statistics

Statistical tests were conducted using standard functions (e.g. anova, ttest and ttest2) in MATLAB. Significant results were reported at $p < 0.05$. Throughout the study, we employed the following statistical tests.

1) Group differences between active and passive breath groups as well as across breathwork techniques in terms of mean/min etCO₂ and mean/max experience depth ratings:

Two-way ANOVA with interaction term, with active/passive breath and breathing technique as predictors.

2) Differences in 11-DASC and MEQ scores from reference scores, either for psilocybin or placebo treatment (see data base analysis below):

Individual one-group t-tests for differences from reference scores per questionnaire sub-scale; with Dunn-Sidak correction for multiple comparisons across sub-scales. These t-tests were conducted independently for active and passive breath groups.

3) Differences in 11-DASC and MEQ scores between active and passive breath groups:

Two-way ANOVA with interaction term, with active/passive breath and questionnaire sub-scale as predictors

4) Pre-post measurements of QIDS-SR16 scores, WEMBWS scores and α -amylase and IL-1 β levels: Paired t-tests of pre- versus post-values. T-tests were conducted separately for passive and active breath groups.

5) All correlations were computed as Spearman correlations. Where multiple correlations were computed, statistical significance was corrected for multiple comparisons using the Dunn-Sidak correction.

Data base analysis of MEQ30 and 11-DASC reference scores

To estimate reference scores of typical MEQ30 and 11-DASC outcomes for psychedelic versus placebo treatments, we relied on data reported in the Altered States Database (ASDB; <https://alteredstatesdb.org/>; see (Prugger et al., 2022)). To estimate reference scores for ASCs triggered by psilocybin, we searched the database for studies in which participants had received

approx. 20-25 mg of psilocybin (at an adult body weight of 75-80kg) and completed the MEQ30 and/or 11-DASC. To gather reference scores for MEQ30 and 11-DASC after a placebo treatment, we searched studies that had administered psychopharmacologically neutral liquids as a placebo treatment (water, saline, saline with glucose, lactose, and in one case a very low concentration of alcohol solution). The studies included as a result of this search strategy are listed in the tables below. For each questionnaire sub-scale, reference scores were then computed as a weighted average of mean questionnaire score per study, weighted by the number of participants in each study, as can be seen here:

$$S = \frac{\sum_1^n s_n \times p_n}{\sum_1^n p_n}$$

, where S is the overall score in a questionnaire sub-scale, n is the number of included studies, s_n is the average score in each study, and p_n is the number of participants in each study.

Table 1: Studies included in the database analysis of 11-DASC scores.

	Study	Participants	Treatment
Placebo	(Carhart-Harris et al., 2016)	20	Saline
	(Schmidt et al., 2012)	19	Saline + Glucose
	(Murray et al., 2022)	18	Water
Psilocybin	(Carhart-Harris et al., 2018)	20	25 mg
	(Smigielski et al., 2019)	20	0.315mg/kg
	(Madsen et al., 2019)	2	24mg
	(Carbonaro et al., 2018)	20	20mg/70kg
	(Smigielski et al., 2020)	17	0.230mg/kg

Table 2: Studies included in the database analysis of MEQ30 scores.

	Study	Participants	Treatment
Placebo	(Wießner et al., 2023)	24	Alcohol solution
	(Carbonaro et al., 2018)	20	Lactose
Psilocybin	(Barsuglia et al., 2018)	18	20 mg / 70kg
	(Griffiths et al., 2016)	50	0.22-0.3mg/kg
	(Nicholas et al., 2018)	12	0.3mg/kg
	(Carbonaro et al., 2018)	20	20mg/70kg

Acknowledgements

We thank all breathwork practitioners and facilitators who took part in the study, particularly Anja Haberlandner, Paul Auls and Sonia Yolanda Paez. We thank Marieke Schölvink, Abdellatif Nemri and Isabel Dziobek for comments on earlier versions of the manuscript. This work was generously supported by the Tiny Blue Dot Foundation, the MIND Foundation, and the MatKat Foundation.

Conflicts of Interest

All co-authors declare no conflict of interest.

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