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# Effect of regulated prolonged expiration breathing on task related cerebral blood flow velocities: a comparative trial

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## Abstract

**Objectives:** Electrical vagus nerve stimulation increases cerebral blood flow when paired with a cognitive task. Breathing maneuvers, including prolonged expiration breathing are considered to stimulate the vagus nerves behaviorally. Here, effects of prolonged expiration breathing on task related cerebral hemodynamics was studied.

**Material and Methods:** Thirty-four participants (group mean age± SD; 36.24±7.08, m:f = 22:12) were assigned to two groups (prolonged expiration (PE) or non-volitional breathing control (CB). PE group had metronome regulated breathing for 5 minutes at 12 breaths per minute with prolonged expiration, whereas CB group had 5 minutes of non-regulated breathing. Transcranial Doppler sonography evaluated bilateral middle cerebral artery hemodynamics at baseline and during a Trail Making Test-B task before and after either PE or CB.

**Results:** In the PE group alone the peak systolic, diastolic and mean blood flow velocities of left and right MCA increased significantly with decreased pulsatility index during the TMT task, which was completed in less time for the PE group alone ($p<0.05$ in all cases, repeated measures ANOVA).

**Conclusions:** Five minutes of regulated breathing with prolonged expiration increased cerebral blood flow velocities during a cognitive task, while time required to complete the task reduced. Though the findings are preliminary based on the sample size and non-random assignment of participants, the findings support the role of breathing in influencing CBF optimally.

**Keywords:** Middle cerebral artery, blood flow velocities, Trail Making Test B, breathing with longer expiration relative to inspiration, Metronome

## Introduction

Previous published research on the effects of electrical vagus nerve stimulation (VNS) suggest that VNS induces changes in cortical areas and functions [1]. Previously, during a cognitive task, changes in cortical area activity and regional cerebral blood flow were found to be related [2]. This association can explain an increase in task-dependent cerebral blood flow which was recorded when VNS was paired with a verbal fluency task [3].

VNS is usually either delivered by invasive or transcutaneous electrical stimulation[4,5]. Breathing maneuvers are also considered to increase vagus nerve activity [5]. These maneuvers include diaphragmatic breathing, the Valsalva maneuver and extending expiration volitionally [5]. The present study aimed to evaluate cerebral blood flow velocity paired to a cognitive task following a breathing intervention intended to increase vagus nerve activity in participants with normal health. The breathing intervention selected was metronome-regulated prolonged expiration. Prolonged expiration is considered to increase vagus nerve activity as an extension of normal respiratory snus arrhythmia, wherein during inhalation the brainstem cardiovascular center inhibits vagal outflow leading to sympathetic predominance [6, 7], wheareas during exhalation vagal outflow is restored [6, 8].

Hence the present study evaluated transcranial Doppler based cerebral blood flow velocity paired to a trail making task (part B) in healthy participants following either breathing with prolonged expiration or a control period of non-volitional breathing.

## Methods

Participants

Thirty four participants (group average age ± SD = 36.24±7.08 years; male:female 22:12) were recruited with announcements on the notice board of a yoga hospital located in north India. The selected sample size is in agreement with previous studies with similar designs and outcome measures[9, 10] Participants were not given any incentive to participate.

The participants were included if they had (i) normal health as determined by a semi-structured examination, and (ii) right-hand dominance measured by the Edinburgh Handedness Inventory[11] to establish which hand would be used to complete the cognitive task. No participants was excluded based on pre-determined criteria of a history of neurological disorders.
Table 1 outlines the baseline characteristics of the participants. Signed informed consent was obtained from each participant. The study had prior approval from the ethics committee of the institution (approval no. PRF/YRD/023/014).

**Study design**

The present study was a comparative controlled trial. A researcher who had no other part in the trial assigned the thirty-four participants into two groups, i.e., an intervention (PE) and a control group (CB) based on age and gender. The PE group received a metronome guided breath intervention with prolonged expiration (inspiration:expiration, 28:72) at a breath frequency of 12 breaths per minute while the CB group was asked to look at the metronome without consciously modifying their breath. The hemodynamics of bilateral middle cerebral arteries (MCA) of each group were monitored at (i) baseline for five minutes, (ii) during Trail Making Test B prior to PE or CB, (iii) during PE or CB (7 minutes; this included 2 minute training), and (iv) during Trail Making Test B following PE or CB. In the training period, the participants experienced modifying their breath using the metronome visual cues. The session structure is provided in Figure 1. Respiration was recorded throughout each session.

**Assessments**

Cerebral blood flow was assessed at baseline, during TMT B (recorded twice) and during PE or CB.

Transcranial Doppler sonography (Digi-LiteTM-Rimed Ltd., Israel) was used to monitor the bilateral middle cerebral arteries (MCA) of the participants. The insonation of both MCA was recorded simultaneously by placing distinct 2.0 Mega Hz ultrasound probes (RIMED, SN 17-3681) at the transtemporal acoustic window. The probes were connected to a headframe to minimize motion and maintain a constant angle of the MCA insonation depth at 40–65 mm from the skull surface. Adjustments were made in the sample volume to receive visually stable and an acoustically optimal ultrasound signal.

The following settings were used for TCD recordings: (i) spatial-peak temporal-average intensity: 420 mW/cm$^2$; (ii) sample volume of 15 mm in length and (iii) thermal index of 1.6.

The average peak systolic velocity (PSV; measured in cm/s), the end-diastolic velocity (EDV in cm/s), mean flow velocity (MFV in cm/s), and pulsatility index (PI) were recorded at rest and during the assessment of TMT B test.

Peak systolic velocity is defined as the initial peak observed on a TCD waveform during each cardiac cycle. In contrast, end-diastolic flow velocity (EDV) is assessed within the range of 20 to 50% of the peak systolic velocity (PSV) values.

The mean flow velocity is determined by adding one-third of the difference between peak systolic velocity (PSV) and end diastolic velocity (EDV) to the EDV value. In contrast, the Pulse-Velocity index (PI) is specified by dividing the difference between EDV and PSV by MFV. The TMT B was conducted as a paper and pencil assessment in which participants were asked to connect numbers (1–13) and letters (A–L) in an alternating, ascending sequence (i.e., 1, A, 2, B, 3, C,..., 13, L) with both speed and accuracy. Two separate sheets of TMT B were used prior to and following PE or CB to prevent the learning effect on the TMT B performance.

**Breath characteristics**.

A strain gauge transducer (MP 45 Biopac Student Lab, U.S.A.) was used to record the respiration. The transducer measures the changes in abdominal circumference that occur as the participant breathes. This method was selected as the respiratory metrics using this method are highly correlated to measurements from a medical grade continuous spirometer taken on healthy participants at rest.

The respiration recording was represented as a wave with peaks representing the expansion of the abdominal cavity during inspiration and troughs indicating the contraction of the abdominal cavity during expiration. The duration of inspiration in each respiratory cycle was measured by determining the time in seconds from the trough of a preceding wave to the peak of that wave. In contrast, the expiration duration was calculated by measuring the time from the peak of one wave to the trough of the following wave in the next respiratory cycle. The inspiration to expiration ratio (i:e) was also calculated. The average breaths per minutes in each state was determined by counting the number of peaks and are expressed as the average number of breaths

The respiration rate was determined by manually counting the number of peaks and is presented as the average per minute in each state. The difference in volume between inspiration and expiration was measured (in mV) as the amplitude of the waveform generated by changes in abdominal volume during respiration.

**Nijmegen questionnaire.**

The Nijmegen questionnaire was used to identify any signs of hyperventilation immediately after the two breathing sessions. The questionnaire has been widely used to detect the symptoms of hyperventilation and has been proven to have good reliability and validity [12]

**Interventions**

In the entire duration of a session, participants in both groups were seated in chairs with their feet flat on the ground, their spines straight, and their necks and spines in alignment. Participants in the two groups were given the intervention or control session using a metronome which was designed by the Medical Intelligence and Language Engineering Laboratory, Department of Electrical Engineering, Indian Institute of Science, Bangalore, India to regulate breathing [13]. The breath intervention was administered in the trial using a visual cue that represented an expanding/contracting circle in response to inspiration/expiration.

**Interventional group** (PE group).
Participants performed breathing with a metronome, which had visual cues to regulate the breathing frequency at 12 breaths per minute. Each breath cycle consisted of inspiration lasting 1.4 seconds and expiration lasting 3.6 seconds, resulting in a ratio of inspiration to expiration of 0.38.

**Control group** (CB)
The non-volitional breathing control group observed the metronome without consciously modifying their breathing patterns.

**Data analyses**
Separate repeated measures ANOVA were carried out to determine the differences in (i) peak systolic velocity (PSV), (ii) end-diastolic velocity (EDV), (iii) mean flow velocity (MFV) and (iv) pulsatility index (PI) (1) during the breath intervention sessions compared to baseline and (ii) during TMT before and after the three breath intervention sessions using SPSS Version 24.0. Each ANOVA had one within-subjects factor, i.e., States; four levels included (i.e., baseline, during TMT B prior to the intervention or control sessions, during intervention or control sessions and during TMT B after intervention or control sessions) and one between subjects factors with two levels (i.e., PE/ intervention group and CB/control group).

**Results**
Thirty-four participants completed the study. Table 1 provides the baseline characterstics of the participants. The two groups did not differ in age and, gender distribution and the scores of the Nijmegan questionnaires (p>0.05; unpaired t-test).
Repeated measures analysis of variance (RM-ANOVA)

**Table 1: Baseline characteristics of the participants**

| Characteristics | Intervention group | Control group | P-value |
|---|---|---|---|
| Number of participants (n) | 17 | 17 | |
| Number of male participants (n) | 11 | 11 | |
| Number of female participants (n) | 6 | 6 | |
| Age in years (mean±SD) | 36.24±7.08 | 36.06±6.77 | 0.94$ |
| Nijmegen questionnaire (scores) | 13.00±10.67 | 14.08±10.18 | 0.77$ |

$p>0.05, based on unpaired t-test.

The ANOVA results for the interaction between Sessions and States for variables related to (a) respiratory characteristics, (b) cerebral blood flow velocities, and the time taken to complete the TMT B are presented in Table 2. A significant interaction of the Sessions and States for any variable indicates their interdependence.

| Factors | Variables | Left Middle Cerebral Artery | | | Right Middle Cerebral Artery | | |
|---|---|---|---|---|---|---|---|
| | | F | df | p-value | F | df | p-value |
| Groups x States | Peak Systolic Velocity | 22.143 | 2.527, 80.877 | <0.001 | 30.247 | 2.578, 82.490 | <0.001 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Mean Flow Velocity | 35.954 | 2.165, 69.291 | <0.001 | 38.508 | 1.960, 62.708 | <0.001 |
| Pulsatility Index | 16.447 | 1.938, 62.028 | <0.001 | 15.606 | 1.748, 55.929 | <0.001 |
| End-Diastolic Velocity | 38.333 | 2.238, 71.626 | <0.001 | 35.726 | 1.819, 58.198 | <0.001 |
| Trail Making Test | 1.9 | 1.00, 32.00 | 0.178 | | | |
| Respiration rate (breaths per minute) | 45.46 | 1.00, 32.00 | <0.001 | | | |
| Ratio of duration of inspiration to expiration | 8.92 | 1.00, 32.00 | 0.005 | | | |

**Table 2. Details of the repeated measures ANOVA for (i) cerebral blood flow velocities, (ii) time taken to complete Trail making test B, and (iii) respiration rate and the ratio of the duration of inspiration to expiration.**

*Post hoc* analyses

(a) Respiratory characteristics for validation of the breath intervention

The PE group attained an inspiration:expiration ratio of 0.60 (SD 0.20)] which was a statistically significant reduction from baseline ($p<0.05$).

The control group had an average i/e ratio of 0.92 (SD 0.15)], not lower than pre.

(b) Bilateral cerebral blood flow during PE or CB compared to respective baseline state

During PE (i) PSV of the left and right MCA, (ii) mean flow velocity of the left and right MCA, and (iii) diastolic velocity of the left and right MCA decreased significantly compared to baseline state ($p<0.05$; in all cases). Also, the pulsatility index of the left and right MCA increased significantly during PE in comparison to baseline state ($p<0.05$; in all cases).

Bilateral cerebral blood flow during the TMT-B.

The PE group showed a significant increase in (i) PSV of the left and right MCA, (ii) mean flow velocity of the left and right MCA, and (iii) diastolic velocity of the left and right MCA during TMT B ($p<0.05$; in all cases). Also, the pulsatility index of the left and right MCA decreased significantly during the TMT B in the PE group ($p<0.05$; in all cases).

Performance in TMT B.

The time taken to complete the TMT B (in seconds) showed a significant difference between states ($F=7.37$, df=1.00, 32.00, $p=0.011$) with a significant reduction in the time taken to complete TMT B in the PE group alone ($p=0.011$).

For the PE group the group mean ±SD values of time taken to compete the TMT B (in seconds) before PE was 89.12±32.92 and after PE was 72.18±39.17. For the CB group mean ±SD values of time taken to compete the TMT B (in seconds) before CB was 87.65±28.30 and after CB was 82.12±29.31.

The group mean ±SD values of (i) cerebral blood flow velocities, and (ii) Trail making test B and respiratory characteristics are provided in Table 3 A and 3 B.

**Table 3 A. Changes in cerebral blood flow velocities. Values are group mean±SD**

**Intervention group (n = 17)**

| **Session** | **Left MCA** | | | | **Right MCA** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Peak velocity** | **Mean velocity** | **Pulsatility index** | **Diastolic velocity** | **Peak velocity** | **Mean velocity** | **Pulsatility index** | **Diastolic velocity** |
| Baseline | 75.24±16.63 | 46.00±10.63 | 0.98±0.22 | 31.38±8.98 | 74.87±17.11 | 46.13±11.70 | 0.97±0.23 | 31.76±10.02 |
| Before intervention during Trail making test B | 78.28±16.16 | 49.23±10.09 | 0.91±0.20 | 34.70±8.61 | 77.07±17.26 | 48.80±11.77 | 0.89±0.21 | 34.67±10.15 |
| During intervention | 60.30±15.49*** | 33.58±10.01*** | 1.29±0.42*** | 20.17±8.65*** | 61.00±16.53*** | 34.14±10.88*** | 1.25±0.30*** | 20.71±8.97*** |
| After intervention during Trail making test B | 80.72±16.49 | 53.47±12.01@@, | 0.79±0.15@@, | 39.85±10.49@@@, | 79.36±18.22 | 52.17±12.85@, | 0.80±0.16 | 38.57±10.92@@, |

**Control group (n = 17)**

| **Session** | **Left MCA** | | | | **Right MCA** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Peak velocity** | **Mean velocity** | **Pulsatility index** | **Diastolic velocity** | **Peak velocity** | **Mean velocity** | **Pulsatility index** | **Diastolic velocity** |
| Baseline | 65.94±15.92 | 40.86±9.97 | 0.93±0.22 | 28.33±8.08 | 60.81±14.94 | 37.35±10.10 | 0.97±0.23 | 25.62±8.49 |
| Before interventio | 67.22±18.40 | 43.34±11.82 | 0.86±0.21 | 31.37±9.42 | 60.83±15.89 | 38.58±10.33 | 0.89±0.16 | 27.41±8.29 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| n during Trail making test B control | | | | | | | | |
| During control session | 63.45±16.60 | 39.95±10.36 | 0.89±0.22 | 28.20±8.34 | 58.29±15.45 | 36.35±10.15 | 0.93±0.22 | 25.37±8.44 |
| After intervention during Trail making test B control | 66.73±17.51 | 43.65±11.51 | 0.81±0.19 | 32.11±9.42 | 58.37±16.93 | 37.11±11.85 | 0.91±0.24 | 26.47±9.72 |

***p<0.001, *post-hoc* analyses with Bonferroni adjustment when blood flow velocities during intervention or during control states were compared with respective baseline states

$^{@}$p<0.05, $^{@@}$p<0.01, $^{@@@}$p<0.001, *post-hoc* analyses with Bonferroni adjustment when blood flow velocities recorded during trail making test B after intervention were compared with blood flow velocities recorded during trail making test B before intervention

**Discussion**

Following PE there was a 8.86% increase in bilateral middle cerebral artery (MCA) flow velocities during completion of a cognitive task whereas CB showed no task related change in MCA velocities.

These results are comparable with those obtained with electrical VNS, where VNS paired with a verbal fluency task increased CBF related to the task, believed to be due to VNS promoting cerebral cortical activation[3]. In the present study prolonged expiration is believed to enhance respiratory sinus arrhythmia (i.e., R–R intervals are shortened during inspiration and lengthened during expiration associated) [14], with increased cardiac vagus nerve activity related to complex synergistic central, peripheral and mechanical factors [15-17].

With the background of flow-activation coupling of cerebral blood flow-neural activity seen in normal health [18] and the knowledge that transcranial Doppler sonography measures offer high temporal resolution to examine change in areas supplied by the MCA [19], certain inferences may be made. The TMT-B task-related increase in MCA cerebral blood flow velocities seen here suggests increased activation in most of the lateral surface of the cerebral cortex supplied by the MCA.

The Trail Making Test Part B (TMT-B) is used to assess set-shifting and inhibition [20]. The area of supply of the MCA is compatible with areas involved in completion of TMT-B. A study on areas involved in the TMT-B completion reported lesions within the prefrontal cortex were found to predict longer raw TMT-B completion times[21]. Hence in the present study breathing-induced stimulation of the vagus nerve may have increased activity in the prefrontal cortex [22, 23]

Following the PE breathing intervention participants required less time to complete TMT-B. This may be explained by activation of areas involved in performing the task (as described above), as well as efferent vagal effects associated with better relaxation conducive to performing well. Improved time in TMT-B is consistent with a previous report of better scores in a cancellation task following breathing with prolonged expiration [24].

Apart from task related effects, in the non-task related periods cerebral blood flow velocities following PE breathing were significantly lower than the non-task related flow velocities recorded before PE breathing. These results are comparable to earlier reports of decreased MCA flow velocities following regulated yoga breathing with prolonged expiration and low frequency breathing [9, 10]. Based on flow-activation coupling[18], periods of low neural activity such as slow wave sleep lead to reduced MCA flow velocities [18]. The decrease in non-task flow velocities was unlikely to be due to changes in carbondioxide levels due to comparable scores in the Nijmegen test for hyperventilation in PE and CB groups.

The increase in PI when velocities decrease in during low i/e breathing compared to baseline and decrease in PI when task related velocities increase after low i/e breathing supports low neural activity during low i/e as PI measures resistance to flow. In contrast when task related velocities increase, PI decreases facilitating blood flow to the required region.

The study was limited by (i) a small sample size, (ii) non randomized distribution of the participants to the two groups and (iii) absence of measures of efferent vagal nerve activity.

In summary, after PE breathing during completion of a cognitive MCA flow velocities increased with less time required for TMT-B. In comparison there were no changes in task related MCA hemodynamics after CB. The findings suggest that PE breathing may facilitate increase in CBF as required for a task, while reducing time required to complete the task.

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**Author contributions**
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**Competing interests**
Authors state no conflict of interest.

**Informed consent**
Informed consent was obtained from all individuals included in this study.

**Ethical approval**
Institutional ethical committee approval (PRF/YRD/023/014) was obtained.

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