Pupil diameter does not covary with learning during single-session Neurofeedback Training

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***Abstract*—One of the major challenges in neurofeedback (NFB) research is that a large proportion of users have difficulty or cannot learn to control their neural activity when provided feedback. Studying learning from a fundamental perspective, using independent measures, during NFB training is crucial to address this challenge. Our aim in this study was to investigate whether the pupil diameter can be used as a biomarker for learning neural self-regulation. Twenty healthy subjects performed four sessions of NFB training to control different electroencephalogram (EEG) power features. We did not observe any differences in pupil diameter across NFB sessions between learners and non-learners. In addition, no correlation was found between the pupil diameter and the targeted EEG power features during NFB training for all subjects. Our results indicate that the pupil diameter does not covary with learning neural self-regulation, possibly due to confounding factors such as mental fatigue. Further studies are needed to explore whether other eye-related parameters can be linked to learning during NFB training.**

I. Introduction

In neurofeedback (NFB) training, a subject is provided real-time feedback reflecting the activity of a specific brain feature. While the subject is testing different strategies to control the feedback, a process of learning to self-regulate the brain feature (i.e. neural self-regulation) is taking place [1]. An extensive number of observations show that not everyone can learn neural self-regulation given the experimental conditions and are thus referred to as non-learners [2]. Determining whether a subject has learnt neural self-regulation is often done by inspecting the brain feature that was targeted by the NFB across time and comparing it to a baseline or threshold. However, as this procedure is subject to ambiguous interpretations, and thus is not optimal for all subjects, an independent biomarker for learning neural self-regulation could better differentiate between learners and non-learners.

Eye-related parameters, such as pupillometry, could potentially be used for monitoring learning in NFB training tasks as it has been shown to identify different neural dynamics [3]. In particular, pupil dilation has been shown to not only respond to variations in ambient light levels [4], but early observations show that the pupil diameter varies systematically in relation to cognitive demands, attention and effort [5], [6]. In the early 60's, the effects of repeating the same memory task on the pupil diameter were studied [7] and they concluded that the pupil sensitivity decreased throughout the task. This implied that as subjects adapted to the task's difficulty, and the task became less demanding, the pupil response and diameter tended to decrease. Investigations into the link between pupil dilation and learning is scarce. However, existing work suggests that the pupil diameter decreases when learning has taken place in a cognitive task [8]. Therefore, in a NFB setting, pupil diameter for learners could serve as an indicator of task proficiency, while in non-learners, could reflect the level of effort invested.

This study investigated the relation between pupil diameter and learning during NFB training. By specifically comparing learners and non-learners of neural self-regulation, the interaction between across-session changes in pupil diameter and EEG power features were analysed.

II. Methodology

A. Participants

This study reports the findings from a cohort of twenty healthy adults (15 males and 5 females), aged between 20 and 39 years (M = 27.60, SD = 5.57). Participant recruitment primarily targeted students and employees at Mälardalen University, supplemented by a small number of individuals outside of the University. The recruitment process and study procedures followed ethical guidelines, and the research protocol received approval from the Swedish ethical review authority under reference number 2021–03121.

B. Recordings

Eye-tracking and Electroencephalogram (EEG) activity were simultaneously collected during the NFB training. The real-time neurofeedback system was developed in LabVIEW (National Instruments) using MATLAB (MathWorks) for all signal processing. Eye-tracking activity was recorded exclusively for offline data analysis.

1) Eye-Tracking: Pupillometry was recorded with the Smart Eye Aurora XO eye-tracking system. Smart Eye Pro 9.2 Software was used for the first 5 participants and Smart Eye Pro 10 for the remaining. Validated with 0.3 gaze accuracy (degrees), this system currently operates at a sampling rate of 250 Hz [9]. An expansion box from Smart Eye was integrated to ensure time synchronization with the EEG device.

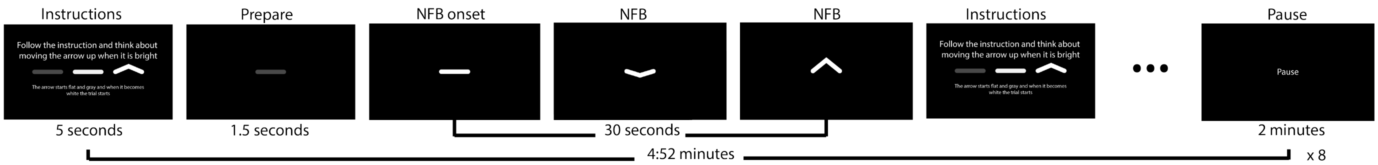


Fig. 1: Example of the NFB training protocol [11].

2) Electroencephalogram (EEG): EEG was recorded at 1 kHz using 64 active electrodes arranged as per the extended 10-20 electrode placement method (Brain Products ActiCHamp). To record horizontal eye movements and measure electrooculogram (EOG), two additional passive electrodes were incorporated and placed 1 cm lateral to the left and right outer canthi of the eye. To detect vertical eye movements, one of the active electrodes was placed below the right eye and the reference electrode was placed on the tip of the left nostril. Impedances were maintained below 40 kOhms throughout all sessions.

C. Data analyses

All signal processing and statistic analyses were performed using MATLAB (versions R2022b and R2023a, MathWorks Inc., USA). The following sections distinguish and describe the steps involved in online (1) and offline (2) data analyses.

1) online EEG signal processing: During the real-time recordings, streamed EEG data was windowed in 250 ms windows (overlapped by 150 ms) and pre-processed with the following steps: 1) DC offset removal, 2) adaptive filtering to remove eye and muscle artifacts, 3) Laplacian filter and 4) Discrete Fourier Transform to compute the Power Spectral Density (PSD) [10].

2) offline EEG and pupillometry processing: Raw EEG data saved by the PyCorder software was used and bandpass-filtered from 1 Hz to 40 Hz, using *pop_eegfiltnew*, a default linear (zero-phase) non-causal FIR filter from EEGLAB. Larger artifacts and noisy channels were removed with Artifact Subspace Reconstruction (ASR) (settings: 40, -1, 0.85, 4, 20, 0.25). Independent component analysis (ICA) was applied and eye-related artifacts were removed from raw data (not processed) using the ICLabel function in EEGLAB. Then ASR was applied again to remove smaller artifacts on ICA decomposed data. Similarly to real-time processing, the Laplacian filter was applied and finally, power was extracted using complex Morlet wavelets and converted to dB and downsampled to 100 Hz. Frequencies in the range of 1 Hz to 40 Hz were retained for analysis.

Raw pupillometry data was first upsampled from 250 Hz to 1000 Hz using a zero-order hold filter. Only data from the NFB trials was extracted and analyzed. The data was then corrected in several steps: blinks and outlier noise were identified and removed by using a representative portion of the data where the mean and standard deviation were affected by minimal noise. This portion of data required a manual selection for each session, pupil diameter values below 2mm were considered abnormal and excluded. Two participants had to be excluded because eye-tracking was not recorded. Additionally, six subjects had to be partially omitted from the analysis due to excessively noisy pupil data. For analyzing trends in pupil diameter modulation across sessions, pupil data was normalized for each session and a smoothing average filter was applied using a window size of 1 minute.

D. Neurofeedback Training

Previous findings from this dataset were reported in [11], without overlap with the results in the current paper. Participants performed four NFB training sessions on separate days. Each session displayed feedback that represented a different EEG power feature. The order of the regulated power features in the NFB sessions was randomized for each subject to avoid learning effects or task difficulty influence. The participants sat in front of a computer screen and were instructed to make an arrowhead point as much upward as possible (see fig.1). Importantly, no specific strategies on how to control the arrowhead were provided other than to control the arrowhead using only their mind, without any muscle involvement. Each session comprised 2 runs, each with 32 trials of 30 seconds, with a 5-second pause between trials. These trials were divided into 8 blocks of 8 trials with 2-minute breaks between each block. A longer break occurred between the two runs (after about 16 minutes) for rest and snacks.

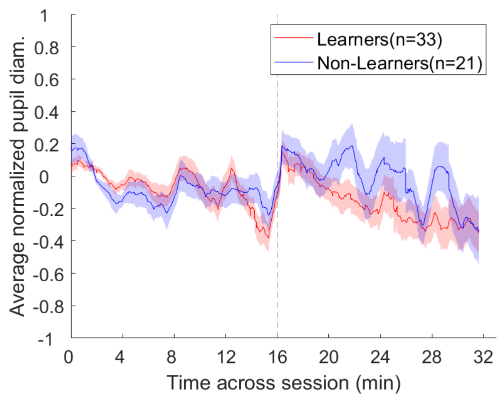
Before each trial, participants received visual instructions on the screen, that were then replaced by a grey horizontal line that turned white at the trial's onset. As the trial started, each subject actively tried to control the arrowhead, of which movements represented the averaged power of an EEG frequency band over a set of electrodes. Each session targeted a different frequency band and a set of electrode locations (i.e. power features): i) frontal midline Theta, ii) occipital Alpha, iii) centrotemporal SMR and iv) Central Beta, commonly used in EEG NFB studies [1], [2].

The arrowhead was updated every 100 ms. The arrowhead's direction depended on whether the power magnitude was above or below a threshold calculated during the first 8 trials (4 minutes) of NFB. Positive feedback for successful neural self-regulation was always represented by the arrowhead pointing upwards. That is, when neural activity was above the threshold for up-regulated features and when the neural activity was below the threshold for down-regulated features. A learner was defined as successfully having the power magnitude of the targeted power feature above (Theta and Alpha) or below (Beta and SMR) the threshold for at least 50% of the NFB session (after the initial 4-minute threshold calculation). The angle of the arrowhead was determined by the ratio of the power magnitude to the threshold. The threshold was initiated at a constant value of 0.2. During the first eight trials, the threshold was dynamically recomputed every second based on the median of the 95th percentile of power values. Subsequently, for all other trials, it remained fixed at the median of the 95th percentile of the initial eight trials.

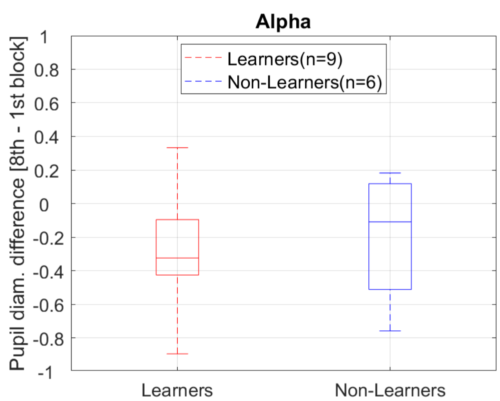
The delay between EEG activity (last sample in the online data window) and visual feedback display was determined using a photodiode attached to the screen and ranged between 70 ms and 80 ms, similarly to [12].

III. Results

First, we analyzed the pupil diameter across NFB sessions for learners and non-learners, following the methods outlined in the previous section and described in a parallel study by the same authors [11]. The illustrated results (fig.2 and fig.3) were based on the saved offline EEG and pupillometry data.



(a)

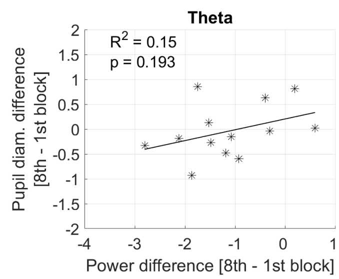


(b)

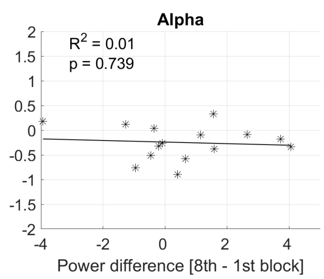
Fig. 2: Pupil diameter for learners and non-learners. (a) Average pupil diameter (normalized with standard error) across all sessions for learners (red) and non-learners (blue). The dotted vertical line represents the approximate time when a longer break occurred. (b) Difference in pupil diameter (normalized) between the last and first blocks for the Alpha feature. Lines within the boxes show the median and edges correspond to the 25th and 75th percentiles.

Our analysis show that independent of whether the subject learned to self-regulate the EEG power feature, a general decreasing pupil diameter trend can be observed across each run when all sessions are concatenated (fig.2A). At the start of the second run, the pupil diameter appears to be reset to the same size as at the start of the session. No consistent difference in pupil diameter can be visually observed between learners and non-learners (fig.2A). Comparing the pupil diameter of the first and last block for alpha regulation, no significant difference is observed between learners and non-learners (fig.2B; Wilcoxon rank-sum test; p=0.5959). The other power features were omitted in this comparison due to an insufficient amount of learners/non-learners.

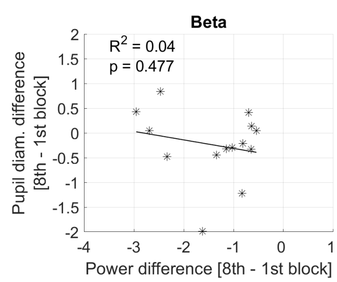
As no evidence for a relationship between pupil diameter modulation and learning neural self-regulation was observed, we next wanted to investigate whether across-session modulations in pupil diameter correlated with EEG power for each feature (fig.2). To investigate the linear correlation between these two parameters, we calculated the Pearson correlation between the pupil diameter difference between the first and last block and the EEG power difference between the first and last block of each NFB session. Results indicate that none of the frequency bands show a significant correlation with the pupil diameter (Pearson correlation; Theta, R^2=0.15, p=0.193; Alpha, R^2=0.01, p=0.739; Beta, R^2=0.04, p=0.477; SMR, R^2=0.01, p=0.741).



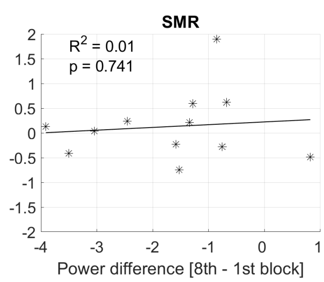
(a)



(b)



(c)



(d)

Fig. 3: Pearson correlation between pupil diameter and EEG power during NFB training. Plotted are the differences between the last and first blocks of pupil diameter and EEG power for (a) Theta, (b) Alpha, (c) Beta, and (d) SMR. The black lines represent linear regression fits for each feature.

IV. Discussion

In this study, participants trained on learning to modulate different EEG frequency bands across a set of electrodes without being provided an explicit strategy. The learning process can therefore be expected to contain an initial phase of higher cognitive load [13] during which the participants tested different strategies while trying to control the feedback. This initial phase could go on for the entire session if the participant was unsuccessful in finding an efficient strategy, thus being categorized as a non-learner. On the contrary, if the participant succeeded in finding a strategy that allowed them to efficiently modulate the EEG feature and hence would be categorized as a learner, cognitive load would reasonably decrease as the participant learned to control the feedback. Our results showed no significant differences in pupil diameter between learners and non-learners across the NFB session. However, we observed a general decrease in pupil diameter throughout each run. As pupil dilation has also been described to covary with mental fatigue, specifically decreasing with time-on-task and increased mental fatigue [14], our results may be an indication of this process. The increase of the pupil diameter after the long break at the beginning of the second run further suggests this as the longer break with snacks (and turning on the lights) would likely make the participants alert again. Previous work shows that pupil diameter increases with the amount of cognitive effort and workload [7], [15], [16]. However, moments of higher cognitive load during the NFB task (i.e. assumably when the subjects were actively trying to find an efficient strategy for controlling the feedback) are unknown and are likely to fluctuate during the task. Further confounding factors may arise due to individual cognitive strategies, attention fluctuations, or arousal levels [17]. While ensuring an alert state and employing engaging tasks might reduce these confounds, our goal was to evaluate pupil diameter as a biomarker for learning neural self-regulation in a common NFB setting. When further exploring the relationship between the pupil diameter and EEG power in different frequency bands, no significant correlation was found with any of the EEG features that were controlled in the NFB sessions (fig.3 A-D). In contrast to other work showing that parieto-occipital EEG alpha activity correlates to pupil diameter [18], [19], our study consisted of actively modulating occipital alpha power in the absence of an explicit cognitive task. Both Ceh et al. (2020) and Montefusco-Siegmun et al. (2022) describe a positive correlation between alpha activity and pupil diameter during inactive rest and fixation, respectively. The learning nature of the NFB task and the active modulation of alpha activity in our study may explain the absence of correlation with the pupil diameter, however further studies are needed to confirm this.

V. Conclusion

This work explored the relationship between pupil diameter and learning neural self-regulation with NFB training. The results revealed that pupil diameter does not serve as a reliable marker for assessing learning dynamics in NFB training. A decreasing pupil diameter across each session run was observed, possibly due to mental fatigue. Despite other work showing a correlation between alpha and pupil diameter during inactive tasks, our findings indicate no correlation with any of the NFB power features, including alpha. Our results also demonstrate the complex interpretation of pupil dilation due its multi-faceted nature. Furthermore, adjusting our study design could enhance control over involuntary and task-irrelevant pupil dilation, leading to a more detailed understanding of the relationship between pupil dynamics and learning during NFB.

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