Eye movements in patients with post-COVID condition

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Abstract: Eye movement control is impaired in some neurological conditions, but the impact of COVID-19 on eye movements remains unknown. This study aims to investigate differences in oculomotor function and pupil response in individuals who suffer post-COVID-19 condition (PCC) with cognitive deficits. Saccades, smooth pursuit, fixation, vergence and pupillary response were recorded using an eye tracker. Eye movements and pupil response parameters were computed. Data from 16 controls, 38 COVID mild (home recovery) and 19 COVID severe (hospital admission) participants were analyzed. Saccadic latencies were shorter in controls (183 ± 54 ms) than in COVID mild (236 ± 83 ms) and COVID severe (227 ± 42 ms) participants ($p = 0.017$). Fixation stability was poorer in COVID mild participants (Bivariate Contour Ellipse Area of $0.80 \pm 1.61°^2$ vs $0.36 \pm 0.65 °^2$ for controls, $p = 0.019$), while percentage of pupil area reduction/enlargement was reduced in COVID severe participants ($39.7 \pm 12.7\%/31.6 \pm 12.7\%$ compared to $51.7 \pm 22.0\%/49.1 \pm 20.7\%$ in controls, $p < 0.015$). The characteristics of oculomotor alterations found in PCC may be useful to understand different pathophysiologic mechanisms.

1. Introduction

The initial concerns about the COVID-19 disease were related to the infection pathways, symptoms, treatment, and fatality rate. Currently, there is also the added concern regarding its long-term impact. In fact, the post-COVID-19 condition (PCC) arises approximately 3 months after the onset of the disease, with symptoms lasting at least 2 months, which cannot be attributed to alternative diagnosis and have a negative impact on daily functioning [1]. These range from fatigue and muscle weakness to discomfort or pain while walking, dyspnea, changed smell and/or taste, anxiety, depression and cognitive impairment [2].

A prevalent symptom of PCC is cognitive dysfunction. Up to 85% of people with PCC have self-reported brain fog [3]. Neuropsychological evaluations have found affected memory, processing speed and executive function in individuals with PCC compared to healthy controls [4]. The impact of neurological deficits on oculomotor control is well known. For instance, patients with Alzheimer's disease (AD) have been shown to have increased latencies and poorer accuracy for saccades, antisaccades and smooth pursuit eye movements [5,6]. A recent meta-analysis has further indicated that there are differences in oculomotor behavior between patients with different levels of AD in response to the gap, step and overlap saccadic paradigms, and therefore these could be of use to make distinctions between AD, mild cognitive impairment and healthy adults

[7]. Similarly, research has shown that Parkinson's disease (PD) also results in eye movements deficits [8,9]. In PD saccades are, in general, less accurate, slower, and have increased latencies [8]. For antisaccades, similar changes in eye movements have been found, in addition to more erratic responses [8]. A number of studies have also indicated abnormalities in smooth pursuit eye movements in PD patients, who exhibited reduced gains and increased presence of saccades [9]. Others have suggested that PD has a significant impact on disparity-driven vergence, resulting in impaired fusion leading to blurred and double vision [10].

Given that eye movement abnormalities have been consistently found in a number of neurological conditions as well as in patients with cognitive impairment, it is reasonable to suggest that the recording of eye movements can also be useful in patients with cognitive affectations resulting from COVID-19, as suggested in Refs. [11–13]. Furthermore, if a relationship between PCC, cognitive impairment, and oculomotor control is found, eye movements metrics could be considered as a biomarker for such condition in the same way that is being explored for other conditions.

The aim of this study was to expand on previous work and investigate differences in oculomotor control and performance of the main types of eye movements (saccades, smooth pursuit, fixation, and vergence) and pupil response between individuals who suffer PCC, and a control group with no reported and/or known previous SARS-CoV-2 infection. In the following section, the methodology of the study is presented by describing the instrumentation and laboratory setup, the participants that took part in the study, the experimental procedure, and how data were analyzed. Then, the differences in eye movements performance between control participants and patients with PCC divided into two sub-groups according to the severity of the acute phase of the disease are reported in the Results section. These findings are discussed in the context of previous literature and pathophysiologic mechanisms, which are still largely unknown. Finally, limitations of the study and the final conclusion are presented.

2. Methods

2.1. Materials

Eye movements and pupil response were measured in binocular viewing using a desktop-mounted EyeLink 1000 Plus (SR-Research Ltd., Ottawa-Ontario, Canada) at 1000 Hz, with the head supported by a chin rest (Fig. 1). Participants were seated 60 cm away from a 17" LCD computer monitor (Dell 1708 FPF, 1280 × 1024 pixels, frame rate 60 Hz) where the visual stimuli for saccades, smooth pursuit, fixation and pupil response tasks were presented. Participants were aligned to the center of the screen and all visual stimuli were fairly central. Given the wide viewing angle of the monitor used (160°) and the improvements in LCD technology, image quality degradation of the peripheral stimuli can be considered to be negligible. In order to evaluate participants' vergence movements, a fixation target consisting of a 2 × 2 mm cross centered on a 7-mm circle printed on a cardboard was mounted on a track with a stepper motor that allowed the movement of the target in depth at a velocity of 2 cm/s [14]. For the eye movement recording, participants wore their habitual near refractive error correction, if any. In line with the country's health regulations at the time of the study, face masks were worn during the data collection; special care was taken so that the participants' masks were well fitted to avoid fogging of lenses during the eye movement recording.

The eye tracker was calibrated for each participant using the standard EyeLink 9-point calibration and the stimuli presented later for all tasks were contained within the calibrated area. Visual stimuli were generated with Matlab (MathWorks, Natick-MA, USA) using the Psychophysics Toolbox library [15].
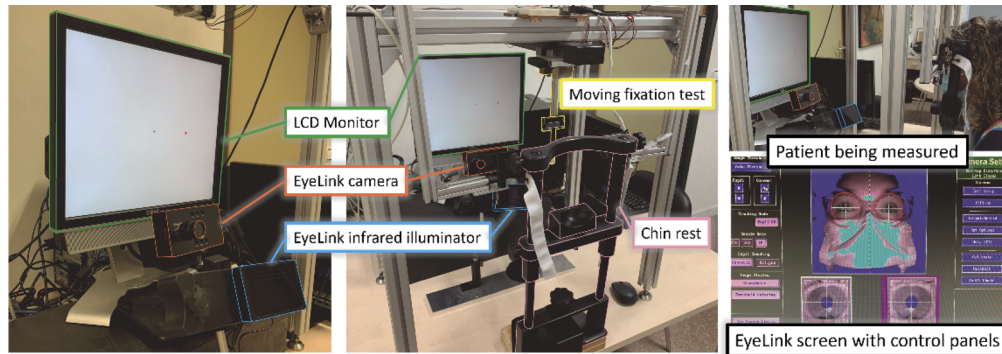
Fig. 1. View of the computer monitor and the eye tracker, which includes a camera and an infrared illuminator (left); general view of the experimental setup, also including the chin rest and the moving fixation target to evaluate participants' vergence movements (middle); and EyeLink screen (right, bottom) during a recording session (right, top).

2.2. Participants

The Nautilus study (ClinicalTrials.gov ID: NCT05307575) is a large multi-center study that aims to evaluate and characterize cognition, mental health, and functional capacity of people who recovered from COVID-19. Participants in the Nautilus study recruited by the Consorci Sanitari de Terrassa (Terrassa, Barcelona, Spain) from November 2021 to November 2022, were invited to participate in this study. This included a total of 94 participants: 70 with PCC and 24 controls. Five PCC (5 females) and four controls (4 females) declined to take part in the study. Thus, the final sample of this study consisted of sixty-five participants with PCC (49 females and 16 males) and 20 controls (14 females and 6 males). Despite the attempts made to balance the gender distribution of the sample, this consisted of a higher proportion of female participants, especially in participants with PCC. This increased proportion of women in our population sample with PCC is in line with the reported higher prevalence of this condition in women than men [3,4,16,17]. At the time of the study a total of 71 participants had received at least one dose of a SARS-CoV-2 vaccine. While all controls received a minimum of two vaccine doses (70% 2 doses and 30% 3 doses), more participants with PCC were unvaccinated (21.5%) or had a single vaccine dose (26%) at the time of the study.

All participants with PCC were diagnosed with a positive PCR test or alternative approved diagnostic method, were symptomatic during the acute phase of the disease and recovered at least 4 weeks before taking part in the study. In addition, participants remained symptomatic and developed PCC, mainly presenting with cognitive deficits. These participants were further divided in: (1) "COVID mild": participants who did not require hospital admission and recovered from the disease at home; and (2) "COVID severe": participants who were admitted to hospital during the acute phase of the disease including some who were admitted to Intensive Care Units (ICU). As part of the Nautilus study, all participants were questioned about their medical history as well as about their COVID-19 experience, including their symptoms, treatment, hospitalization, and time since diagnosis. The most reported symptom was fatigue with a total of 66% of participants in the COVID groups reporting mental and physical fatigue. No vision specific symptoms such as double vision or blur were reported. At a functional level, the most common difficulties reported were related to language, lack of concentration and difficulty in sleeping.

The recruitment of participants for the COVID groups was conducted at local health care centers. Additionally, a group of control participants with self-reported no previous known infection of SARS-COV-2 were recruited via advertisement in social media and local networks.

Participants with symptoms of delirium, an established psychiatric, neurological, developmental disorder, or systemic pathology known to cause cognitive deficits as well as those with motor or sensory alterations were not included in the study. Inclusion criteria were as follows: (1) no diagnosed or suspected diabetes (type I or II), (2) no previous history of interocular surgery or refractive surgery, (3) axial length <26 mm, (4) no diagnosed or suspected glaucoma or other retinal diseases, (5) no diagnosed or suspected strabismus, (6) presence of stereopsis, and (7) corrected near visual acuity of <0.2 logMAR binocularly, the latter ensured that residual uncorrected refractive errors were small and would have minimal impact on eye movements performance.

The protocol was approved by the Drug Research Ethics Committee (CEIm) of Consorci Sanitari de Terrassa (02-20-107-070) and designed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

2.3. Visual tasks and procedure

Data were collected prospectively. Prior to eye movements and pupil response recording, all study participants were screened to confirm good vision and binocularity, and therefore exclude any participant who did not fulfil the inclusion criteria.

Additionally, participants underwent a cognitive examination using the Montreal Cognitive Assessment (MoCA), which is a useful cognitive screening tool for many neurological illnesses assessing several cognitive domains such as visuospatial/executive, naming, memory, attention, language, abstraction, delayed recall and orientation [18]. A MoCA score $\geq$26 is generally considered as normal indicating no cognitive impairment, while scores of <26 are considered reduced and therefore indicate cognitive impairment. The following ranges are often used to grade the severity of the cognitive impairment: 18-25 = mild cognitive impairment, 10-17 = moderate cognitive impairment, and less than 10 = severe cognitive impairment [18]. Considering the study age range and population, published MoCA normative values between 24 and 26 would indicate a mild cognitive impairment [19].

2.3.1. Saccades

Prosaccades/antisaccades were elicited with three different paradigms: Posner overlap, overlap and gap. At the start of each trial, participants were asked to fixate a central fixation target (circular blue target of 0.3°) and look at the peripheral target (circular red target of 0.5°) as fast as possible when it appeared (prosaccades) or to the opposite side relative to the peripheral target trying to match such position at the opposite side (antisaccades). In the case of the overlap paradigm, there was an overlap of 200 ms between the presentation of the central and peripheral stimuli, as the central target was shown for 1500 ms seconds and the peripheral target appeared 1300 ms after the central target onset. The peripheral target disappeared after 1500 ms of presentation. In the Posner overlap paradigm, in addition to the 200 ms overlap between the central and the peripheral targets, a cue to direct participants to the peripheral target location (left or right) ahead of its presentation was introduced [20]. In the gap paradigm, there was no overlap between the central and peripheral stimuli presentation, as the central fixation target was presented for 1500 ms and the peripheral target appeared 200 ms after the central target disappeared. For all conditions, the presented stimuli aimed to elicit saccades of 8.5° amplitude (Fig. 2).

2.3.2. Smooth pursuit

Smooth pursuit eye movements were elicited by asking participants to track a visual stimulus of 0.3° that moved horizontally and vertically following a sinusoidal or linear motion. The visual stimulus appeared at the center of the monitor and immediately after that, it started moving horizontally following a sinusoidal pattern of 24° amplitude. Following this, the same stimulus moved linearly, horizontally then vertically, with the same amplitude (velocity = 10°/s).

Table 1. Metrics used for the characterization of saccades, smooth pursuit, fixation, vergence movements and pupil response.

| Metrics [units]	Definition
Pro-saccades and anti-saccades - All metrics are calculated for the three paradigms: **posner overlap, overlap** and **gap**	
Latency [ms]	Time that elapses between the onset of the peripheral target and the onset of the saccade
Duration [ms]	Time difference between the offset and onset of the saccade
Peak velocity [°/s]	Maximum velocity of the eyes during a saccade
Direction [°]	Absolute deviation from horizontal, 0° being perfectly horizontal and 90° perfectly vertical
Amplitude [°]	Distance travelled by the eye during the saccade
N° of correct saccades	Number of saccades with a direction within ± 50° from the peripheral target (Reference angle: 0° for prosaccades; 180° for antisaccades)
Smooth pursuit - All metrics are calculated for the three trajectories: **sinusoidal, horizontal** and **vertical**	
RMS static/dynamic [°]	Root Mean Square error denoting the distance between the target and gaze positions. For each gaze position, in RMS static, the difference is computed to the closest target position regardless of the timestamp, whereas in RMS dynamic, it is computed to the position of the target at the same timestamp
Gain	Ratio of eye velocity to stimulus velocity
Saccades during the smooth pursuit task	
N° of saccades	Number of saccades
Duration sacc [ms]	Mean duration of saccades
Peak velocity sacc [°/s]	Mean peak velocity of saccades
Amplitude sacc [°]	Mean amplitude of saccades
Saccadic component [%]	Percentage of time spent on saccades during the smooth pursuit task
Fixation - All metrics are calculated twice: **before** distractors appear and **with distractors**	
RMS [°]	Root Mean Square error denoting the distance between the fixation target and the measured gaze positions
Bivariate Contour Ellipse Area (BCEA) [°^{2}]	Area of the ellipse drawn around the central 68% of the fitted distribution of gaze positions [24]
AR	Aspect Ratio of the ellipse drawn around the central 68% of the fitted distribution of gaze positions, which indicates the anisotropy of gaze positions [25]
Ellipse direction [°]	Absolute deviation of the fitted ellipse's major axis from horizontal, 0° being perfectly horizontal and 90° perfectly vertical
Saccades during the fixation task	
N° of saccades	Number of saccades with an amplitude < 1° (microsaccades)
Duration sacc [ms]	Mean duration of saccades
Peak velocity sacc [°/s]	Mean peak velocity of saccades
Amplitude sacc [°]	Mean amplitude of saccades
Direction sacc [°]	Mean direction of saccades (0-180°), 0° being perfectly horizontal and 90° perfectly vertical.
Saccadic component [%]	Percentage of time spent on saccades during fixation
Vergence - All metrics are calculated twice: when the target moves away (**Divergence**) and when it returns (**Convergence**)	
RMS(Z) [°]	Root Mean Square error denoting the distance between the target and gaze positions in the z-axis (depth)
Gain	Ratio of eye velocity to stimulus velocity in the z-axis
Saccades during the vergence task	
N° of saccades	Number of saccades
Duration sacc [ms]	Mean duration of saccades
Peak velocity sacc [°/s]	Mean peak velocity of saccades
Amplitude sacc [°]	Mean amplitude of saccades
Saccadic component [%]	Percentage of time spent on saccades during the vergence task
Pupil response	
Miosis time [ms]	Time that elapses between the LED is switched on (t = 30s in Fig. 3) and the maximum constriction is achieved
Area reduction [%]	Area reduction (%) during miosis. The initial value is obtained as the mean pupil area within the last second before switching on the LED (t = 29s and t = 30s in Fig. 3), and the final value corresponds to the maximum constriction measured
Area enlargement [%]	Area growth (%) during mydriasis. The initial value is obtained as that corresponding to maximum constriction, and the final one as the mean pupil area within the last second of the testing condition (t = 74s and t = 75s in Fig. 3)
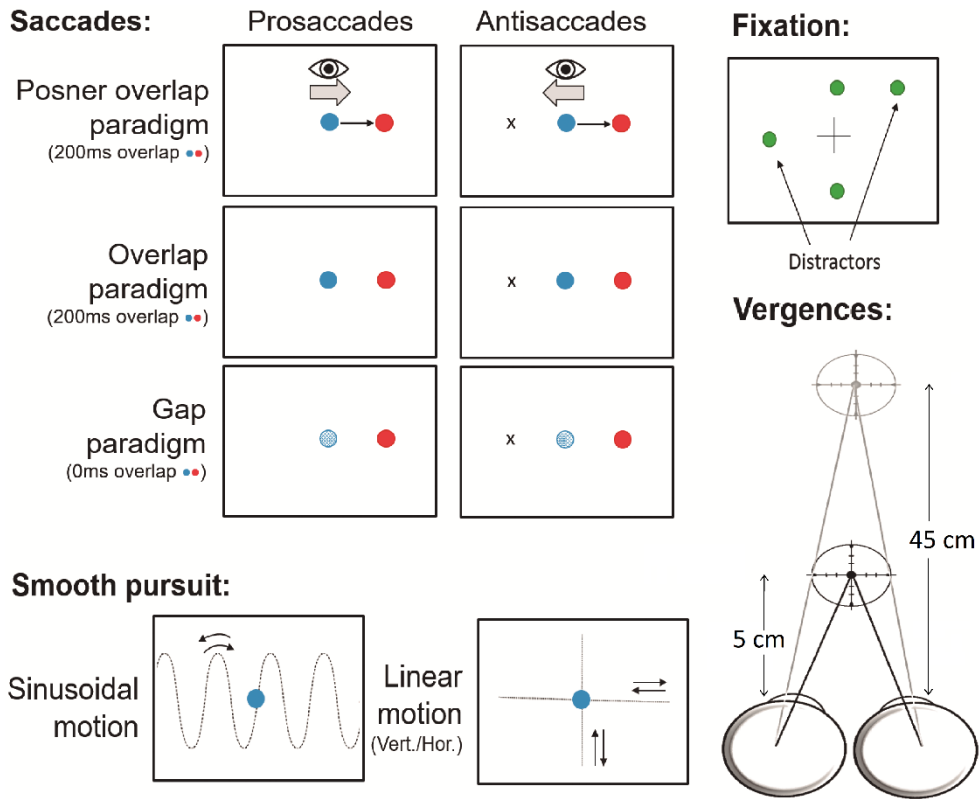
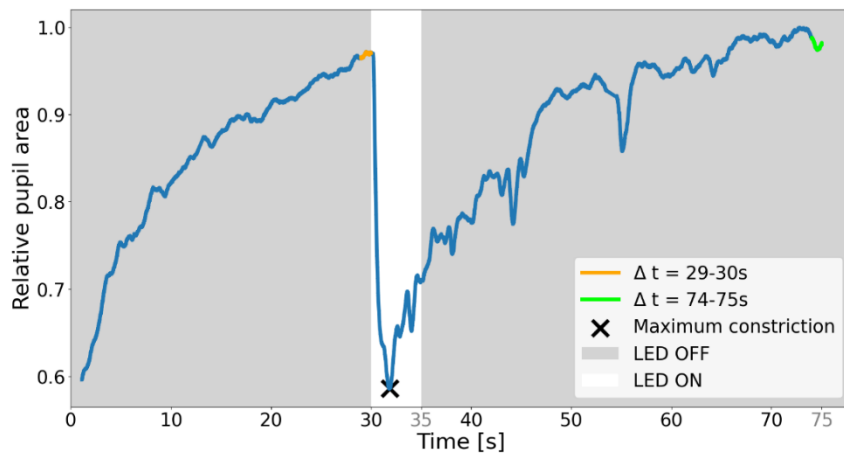**Fig. 2.** Tests used for assessment of saccades, smooth pursuit, fixation and vergences.

Fig. 3. Representative example of a pupil response recording obtained with the EyeLink 1000 Plus at a frame rate of 1000 Hz.

2.3.3. Fixation

During the fixation task, participants' eye movements were recorded while they were fixating a cross of 0.5° situated at the center of the monitor for 40s. After the first 10s, 10 distractors (spots) of 0.3° of diameter appeared every 1.4s at different monitor locations for 200 ms. Participants were asked to maintain their fixation at the central stimulus.

2.3.4. Vergence

Participants were instructed to fixate at the central part of the fixation target at all times while it moved along the motorized track from 5 cm to 45 cm away from them and returned to the initial position.

2.3.5. Pupil response

Participants were asked to fixate on a white cross (0.5°) on a black background placed at the center of the monitor in scotopic room illumination conditions. A white LED (865 lx at the pupil plane; 120° viewing angle) was placed 5 cm away from the participants' eyes. The LED was initially switched off for 30s allowing pupil dilation (mydriasis). Then it was switched on causing pupil constriction (miosis), and it was switched off 5s later (t = 35s), allowing mydriasis again. Pupil size was recorded during 75s. Figure 3 illustrates the pupil response recording obtained from a study participant; pupil oscillations can be observed, the small ones are related to the noise of the eye tracker (small amplitude, high frequency oscillations) and the large ones correspond to physiologically normal oscillations (lower frequency) known as Hippus [21].

2.4. Data analysis

Eye tracking data were analyzed using custom algorithms in Python. Periods of 200 ms before and after each blink detected by the EyeLink software were removed, data were smoothed using a second order Savitzky Golay filter of 21 samples length (21 ms). Saccades were detected using an adaptive velocity threshold that varies according to the signal noise [22]. Saccades during the smooth pursuit task were detected with an adaptative acceleration threshold as in Ref. [23]. Table 1 presents all eye movements and pupil response parameters.

2.5. Statistical analysis

The distribution of the data was evaluated using Shapiro-Wilk normality test. To investigate differences between groups, parametric and non-parametric ANOVA tests were conducted accordingly. Bonferroni (homogeneous variances), Games-Howell (heterogeneous variances) post-hoc, and Mann-Whitney U tests were used to analyze normally and non-normally distributed parameters. A p-value <0.05 was considered significant. The IBM SPSS software package version 18.0 (IBM SPSS Inc., Chicago-IL, USA) was used.

3. Results

Eye movement data from twelve participants were discarded from further analysis: eleven of these participants (3 controls, 7 COVID mild and 1 COVID severe) did not meet the inclusion criteria and one control participant had poor quality eye tracking calibration. Therefore, eye movement recordings from 16 controls (10 females and 6 males), 38 COVID mild (34 females and 4 males) 19 COVID severe (10 female and 9 male) participants were included in the analysis. Mean calibration accuracy was 0.74 ± 0.30°, 0.78 ± 0.70° and 0.86 ± 0.72°, for controls, COVID mild and COVID severe groups, respectively.

Table 2 and Fig. 4 show the most relevant demographic and clinical (visual and cognitive) characteristics of participants.

Statistically significant differences were found between control and COVID mild participants for stereopsis (p = 0.029) and MoCA values (p = 0.004) as well as between control and COVID severe participants (stereopsis p = 0.008, MoCA p = 0.015), indicating worse levels of stereopsis and cognitive abilities in participants with PCC. There were no cognitive differences between the two groups of COVID participants (MoCA p = 0.952). For contrast sensitivity at 1.5cpd, differences were only found between control and COVID mild participants (p = 0.037).
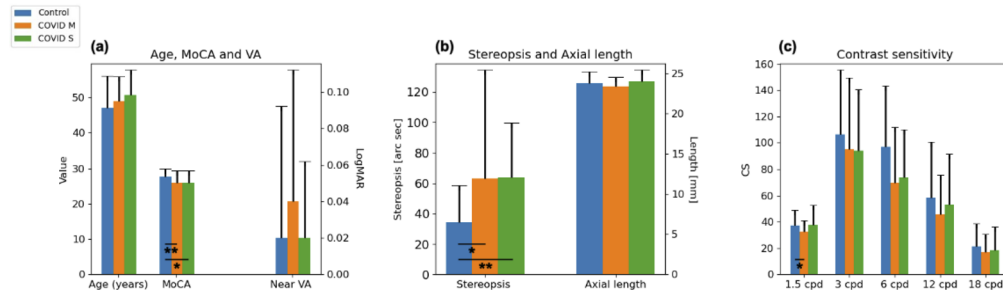
Fig. 4. Demographic and clinical (visual and cognitive) characteristics of Controls, COVID Mild (M) and COVID Severe (S) groups: Age, MoCA and visual acuity (VA) (a), stereopsis and axial length (b), and contrast sensitivity (CS) (c). Parameters that showed statistically significant differences between groups are indicated as follows: *, p ≤ 0.05; **, p ≤ 0.01 on the pairwise post-hoc tests.

Table 2. Demographic and clinical (visual and cognitive) characteristics of healthy controls and PCC participants divided into the COVID mild (M) and COVID severe (S) groups. VA, visual acuity; CS, contrast sensitivity; cpd, cycles per degree; MoCA, Montreal Cognitive Assessment. Significant p values (<0.05) are in bold.

Parameter	Control	COVID M	COVID S	P value
Age (years)	47.01 ± 8.17	49.02 ± 5.99	50.75 ± 6.06	0.248
Near VA (logMAR)	0.02 ± 0.07	0.04 ± 0.07	0.02 ± 0.04	0.741
Stereopsis (arc sec)	34.33 ± 21.74	63.02 ± 68.97	63.79 ± 33.27	**0.020**
Axial length (mm)	23.78 ± 1.02	23.42 ± 0.70	23.98 ± 1.01	0.127
CS 1.5 cpd	37.19 ± 8.75	32.63 ± 5.54	37.89 ± 11.82	**0.046**
CS 3 cpd	106.44 ± 46.28	95.05 ± 51.86	94.26 ± 43.45	0.539
CS 6 cpd	96.88 ± 43.96	69.84 ± 39.45	73.95 ± 33.19	0.125
CS 12 cpd	58.31 ± 39.65	45.68 ± 27.40	53.42 ± 35.43	0.590
CS 18 cpd	21.19 ± 14.54	16.82 ± 11.07	18.37 ± 14.99	0.706
MoCA	27.69 ± 1.25	25.84 ± 2.55	25.89 ± 2.51	**0.010**

Some saccadic parameters were found to be different between groups (Table 3 and Fig. 5). While differences in the prosaccade gap latency were found between the control and both COVID groups (p = 0.025 and p = 0.035) (Fig. 5(a)), post-hoc tests revealed differences in the antisaccade latency with the overlap paradigm only between the control and COVID mild groups (p = 0.018) (Fig. 5(b)). These results suggest longer latencies for some saccadic paradigms in participants with PCC compared to controls.

For the smooth pursuit task, the mean amplitude of saccades exhibited during horizontal motion was significantly larger in both COVID groups compared to the control group (p = 0.021 and p = 0.036) (Fig. 5(c)). Differences between groups in some parameters related to the accuracy of fixation and vergence movements were also found. For instance, the BCEA before the presentation of distractors was significantly different between control and COVID groups (p = 0.019 and p = 0.045), and the RMS during the presentation of distractors was also different between control and COVID mild participants (p = 0.022) (Fig. 5(d)). Similarly, the mean direction of saccades conducted during the presentation of distractors also showed significant differences between the control and COVID mild groups (p = 0.016) (Fig. 5(d)). In general, these results suggest poorer fixation stability in the COVID mild group compared to the other groups.
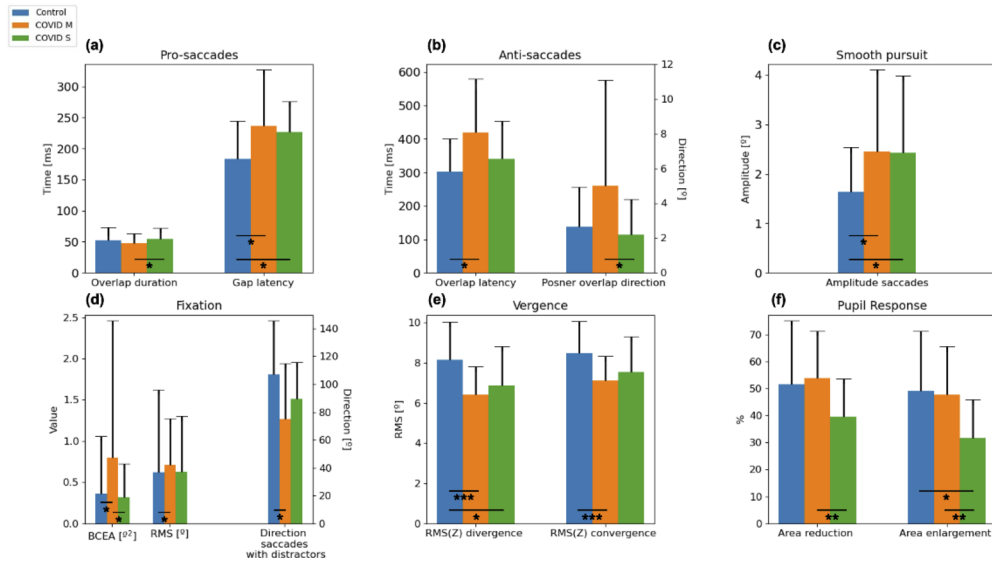
Fig. 5. Parameters that showed statistically significant differences between Controls, COVID Mild (M) and COVID Severe (S) groups for the following tasks: pro-saccades (a), anti-saccades (b), smooth pursuit (c), fixation (d), vergence (e), and pupil response (f). *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$ on the pairwise post-hoc tests. BCEA, Bivariate Contour Ellipse Area; RMS, Root Mean Square error.

Table 3. Eye movements and pupil response parameters that showed statistically significant differences between Controls, COVID Mild (CM) and COVID severe (CS) groups. Significant p values (<0.05) are in bold. BCEA, Bivariate Contour Ellipse Area; RMS, Root Mean Square error.

Task	Parameter	Control	COVID M	COVID S	P value			
					ANOVA	C/CM	C/CS	CM/CS
Pro-saccades	Overlap duration (ms)	52 ± 14	47 ± 80	55 ± 10	**0.035**	0.399[a]	1.00[a]	**0.040**[a]
	Gap latency (ms)	183 ± 54	236 ± 83	227 ± 42	**0.017**	**0.025**[b]	**0.035**[b]	0.859[b]
Anti-saccades	Overlap latency (ms)	302 ± 85	418 ± 174	341 ± 100	**0.029**	**0.018**[c]	0.243[c]	0.093[c]
	Posner overlap direction (°)	2.63 ± 2.07	5.01 ± 5.82	2.19 ± 18	**0.034**	0.133[c]	0.676[c]	**0.013**[c]
Horizontal smooth pursuit	Amplitude saccades (°)	1.63 ± 0.82	2.45 ± 1.57	2.43 ± 1.47	**0.048**	**0.021**[c]	**0.036**[c]	0.912[c]
Fixation	Direction saccades with distractors (°)	106.89 ± 35.79	75.01 ± 36.99	89.63 ± 23.01	**0.017**	**0.016**[a]	0.480[a]	0.431[a]
	BCEA before distractors (°²)	0.36 ± 0.65	0.80 ± 1.61	0.32 ± 0.35	**0.025**	**0.019**[c]	0.683[c]	**0.045**[c]
	RMS with distractors (°)	0.62 ± 0.95	0.71 ± 0.51	0.63 ± 0.62	**0.046**	**0.022**[c]	0.573[c]	0.102[c]
Vergence	RMS(Z) divergence (°)	8.15 ± 1.68	6.43 ± 1.18	6.88 ± 1.73	**0.024**	**0.001**[c]	**0.017**[c]	0.851[c]
	RMS(Z) convergence (°)	8.49 ± 1.41	7.13 ± 0.99	7.56 ± 1.53	**0.004**	**<0.001**[c]	0.053[c]	0.948[c]
Pupil response	Area reduction (%)	51.66 ± 21.98	53.97 ± 15.81	39.68 ± 12.47	**0.015**	0.945[a]	0.275[a]	**0.009**[a]
	Area enlargement (%)	49.09 ± 20.65	47.66 ± 16.47	31.59 ± 12.68	**0.011**	0.978[c]	**0.046**[c]	**0.003**[c]

[a]Parametric ANOVA – Bonferroni post-hoc

[b]Parametric ANOVA – Games-Howell post-hoc (non-normal distribution of variances)

[c]Non-parametric ANOVA – Independent t-tests Mann-Whitney U-tests for post-hoc comparisons

The RMS along the z-axis during convergence was significantly different between control and COVID mild participants (p < 0.001) and during divergence between control and both COVID groups (p = 0.001 and p = 0.017) (Fig. 5(e)). These differences suggest that during vergence, the eyes positions of COVID participants were closer to the stimulus than in controls.

Finally, pupillary response was found to be impaired in COVID severe participants. The percentage of miosis was different between the two COVID groups (p = 0.009), and the percentage of mydriasis was found to be reduced in COVID severe participants compared to participants in the control (p = 0.046) and COVID mild (p = 0.003) groups (Fig. 5(f)). These results suggest a reduction in pupil contraction and dilation in participants who suffered COVID and required hospitalization during the acute phase.

4. Discussion

In this study, the performance of individuals with PCC in saccadic, smooth pursuit, fixation and vergence eye movements tasks as well as their pupil response was compared to that of age-matched healthy controls. Participants with PCC were further classified into two groups based on whether they required hospital admission during the acute phase of the infection (COVID mild or COVID severe).

The mechanisms to explain the origin of cognitive dysfunction in patients with PCC are still unknown. In addition, pathophysiologic mechanisms might differ as a function of the severity of the disease, as suggested by the presence of different symptoms and signs during the acute phase [26]. While silent hypoxemia is a common feature in severe SARS-CoV-2 infections [27] and it has been associated to long-term slower mental speed processing [28], the pathophysiologic mechanisms of PCC in cases with mild or absent symptoms during the acute phase might be related to direct brain infection through the olfactory channel [29,30].

In general, we found that participants with PCC tended to be slower than controls in some saccadic paradigms in agreement with Garcia-Cena et al. [13]. They exhibited increased prosaccadic latencies (gap paradigm), and those in the COVID severe group also showed slightly increased saccadic duration compared to the COVID mild group. Carbone et al. (2022) reported no significant differences in saccadic latency with the overlap paradigm, whereas they found statistically significant differences in the latency of antisaccades [12]. This finding also agrees with the results reported by Garcia-Cena et al. in a sample of 9 individuals with PCC and Kelly et al. in a larger cohort of younger participants [11–13]. Antisaccades represent a challenging task, as it involves an inhibitory process to suppress a saccade towards the visual stimulus before the initiation of a voluntary saccade in the opposite direction [31]. In this study, the antisaccadic latency of only the COVID mild group was significantly longer compared to that of controls, and they also tended to show more variability in the direction of antisaccades. Accordingly, mild COVIDs are associated with a stronger participation of structures involved in inhibitory processes than severe COVIDs. The mechanisms by which this phenomenon operates are unknown, but it suggests that COVID mild affectation is distinct from the severe one.

The fact that the amplitude of saccades that occurred during horizontal smooth pursuit was significantly larger in participants in the COVID groups compared to control participants might be an indirect indication of disrupted smooth pursuit movements in PCC patients [32]. Kelly et al. found that a significant number of participants exhibited an increased number of saccades during smooth pursuit compared to normative values [11]. Although the number of saccades was not significantly different among groups in our study, their greater amplitude might imply the presence of larger errors of the smooth pursuit movements to correct. Prediction plays a major role in the performance of smooth pursuit movements [33]. It is feasible that presenting unpredictable stimulus' trajectories would have resulted in more obvious disruption of smooth pursuit movements if the top-down systems involved in prediction are damaged in PCC patients.

To our knowledge, this is the first study to measure the fixation stability of patients with PCC, and the results suggest that those in the COVID mild group exhibited an increased fixation instability compared to participants in the control and COVID severe groups. Although the BCEA was significantly different between groups only before the presentation of distractors, the BCEA values with distractors followed a similar trend (controls: $0.40 \pm 0.85^{\circ 2}$, COVID mild: $0.87 \pm 1.70^{\circ 2}$, COVID severe $0.56 \pm 1.28^{\circ 2}$). Similarly, the mean RMS before the presentation of distractors (controls: $0.54 \pm 0.53^{\circ}$, COVID mild: $0.75 \pm 0.55^{\circ}$, COVID severe $0.58 \pm 0.37^{\circ}$) were qualitatively similar to those computed after the presentation of distractors despite not reaching statistical significance. In this case, the fact that fixation stability was poorer in the COVID mild than the COVID severe group might not be explained solely by altered inhibition processes, as similar trends were found before and during the presentation of distractors.

This is also the first study to analyze vergence movements in patients with PCC. The task chosen in our experimental protocol evaluated convergence and divergence tracking responses to a stimulus moving along the midline from a typical viewing distance (45 cm) up to a short distance close to the maximum convergence capability (5 cm). Eye movements recordings were visually inspected to ensure that all participants included in this analysis could fuse the stimulus throughout the range of tested distances. Initially, it was hypothesized that RMS error during convergence and divergence would be higher in the COVID groups if they exhibited longer vergence latencies or less accurate responses than controls. However, the opposite trend was found. Further research is needed to examine the origin of these differences. Other tasks targeting vergence responses related to cognitive processing of visual information [34–37] may be more appropriate to detect possible anomalies in vergence attributable to PCC.

Previous studies have described autonomic nervous system dysfunctions associated to SARS-CoV-2 infection, which may persist in cases of PCC [38,39]. The pupillary light reflex is controlled by the autonomic nervous system. Thus, quantitative evaluation of the pupil response to light could be used to assess sympathetic and parasympathetic systems' damage associated with COVID-19 [40,41]. Our results suggested decreased miosis and mydriasis amplitudes in COVID severe participants. This implies a greater damage in both sympathetic and parasympathetic systems probably related to hypoxia during the acute phase of the infection.

Altered visual acuity and contrast sensitivity in individuals with COVID-19 who were hospitalized has been previously found [42]. Our results seem to differ from these as similar visual acuities were found between groups and contrast sensitivity was only found to be reduced for lower spatial frequencies in COVID mild participants compared to controls. However, it should be noted that the visual function assessment conducted in our study was a screening assessment with the objective to confirm good vision and binocularity ensuring that participants met the inclusion criteria, and that they were able to clearly see the visual stimuli displayed on the screen. A more detailed visual function assessment should be conducted to further understand the impact of PCC on visual acuity and contrast sensitivity.

In relation to the eye movement and pupil response results obtained in this study, it should be noted that large standard deviations were found for a number of parameters. The large standard deviations are likely to indicate heterogeneity within groups, and therefore future research should consider the investigation of eye movements in individuals with PCC with a similar neuropsychological profile, similar symptoms or similar symptom severity.

There are further limitations related to the COVID participants sample studied. First, eye movement data from participants prior to SARS-CoV-2 infection were not available. The comparison of eye movement control and performance in individuals before and after recovering from COVID-19 would have provided further insight into the impact of SARS-CoV-2 infection on eye movements. However, eye movement recordings are not a standard ophthalmic/ophthalmological procedure, and therefore previous eye movement data from the study participants are not available. In addition, considering the COVID-19 pandemic and its course, it was not feasible to obtain

eye movement recordings prior to SARS-CoV-2 infection in our participants. In line with other studies investigating eye movements abnormalities after COVID-19 disease [11–13], we have studied eye movements in participants after recovering from the acute phase of the condition, but that remain symptomatic after this. Another limitation is that the control group consisted of participants with self-reported no previous known infection of SARS-CoV-2. It could be argued that this group may include individuals with undiagnosed and asymptomatic COVID-19, and that this may consequently impact on the eye movement findings. However, this study was interested in evaluating eye movement control in participants with diagnosed PCC, who frequently report neurological symptoms and appear to have reduced scores in neuropsychological evaluations. Our control participants were healthy with no signs, symptoms, or history of psychiatric and/or neurological disorders. Hence, if some control participants had an asymptomatic COVID-19 infection, the fact that they were asymptomatic during the acute phase of the disease and had no reported history of neurological signs or symptoms indicate that cognitive abnormalities resulting from possible asymptomatic COVID-19 are unlikely in this population. This is further supported by the fact that all control participants had a MoCA score of ≥ 26 and the mean MoCA score in the control group was higher than that obtained in the COVID-19 groups. In contrast, there were participants in the COVID groups with MoCA scores <26 (34% of participants in the COVID mild group and 42% of participants in the COVID severe group). For these reasons, the possible inclusion of some participants with asymptomatic COVID-19 with no neurological concerns, signs or symptoms, and demonstrating good cognitive abilities (MoCA scores ≥ 26) in the control group, would have limited impact on the study results. Finally, the collection of blood samples to determine the presence of SARS-CoV-2 antibodies would not have been useful to identify individuals with undiagnosed and asymptomatic COVID-19 as all control participants had received two doses of the vaccine at the time of the study.

5. Conclusion

This study showed the possibility to identify abnormal eye movements and pupil response behavior in individuals with PCC using a relatively short and simple battery of oculomotor tasks. This quick and easy-to-apply task complements conventional neuropsychological assessments of cognitive function and could be sensitive in detecting cognitive impairment at an early stage. Further research is needed to determine the correlation between eye movement performance and the results of conventional neuropsychologic tests. This would provide new insight into the use of eye movements metrics as biomarkers to detect PCC. Hence, ongoing work aims to focus on assessing the relationship between eye movement metrics and neuropsychological tests scores including further analysis of the MoCA domains as well as other neuropsychological tests specific for the study of different cognitive function. In addition, the nature of each altered oculomotor parameter and the characteristics of patients exhibiting the disruption might be useful to discern different pathophysiologic mechanisms of PCC.

Disclosures. The authors declare no conflicts of interest.

Data availability. Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

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