Apomorphine for prolonged disorders of consciousness: a multimodal open-label study

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Summary

Background Apomorphine is a dopaminergic candidate therapy to improve recovery in patients with prolonged disorders of consciousness (PDoC). Behavioural improvements were previously described in non-controlled case series, but its efficacy and neural mechanisms remain largely unknown. This open-label controlled study using multimodal outcome measures investigates the action of apomorphine in severely brain-injured patients.

Methods Thirteen PDoC patients received 30-day subcutaneous apomorphine treatment (n = 6) or standard care (control group, n = 7) in a neurological rehabilitation centre between February 2018 and January 2021. The apomorphine group was monitored 30 days before treatment initiation, during treatment and one year after treatment. Primary outcome measure was defined as changes in behavioural diagnosis using the Coma Recovery Scale—Revised (CRS-R). CRS-R index, recovery of new conscious behaviours, DoC-feeling scores, high-density electroencephalography, and fluorodeoxyglucose positron emission tomography were employed as secondary outcome measures. The control group was monitored with repeated CRS-R only. Registration: EudraCT 2018-003144-23; Clinicaltrials.gov NCT03623828.

Findings Groups (apomorphine vs. control: odds ratio 8.9, 95% CI 3.3–17.8) and study phase (treatment vs. baseline, apomorphine group only: odds ratio 3.9, 95% CI 1.5–10.1) significantly influenced positive changes in behavioural diagnosis. At one-year post-injury, 4/6 patients in the apomorphine group and 1/7 patients in the control group had improved their diagnosis. Similarly, CRS-R index was significantly influenced by study phase (treatment vs. baseline). All items on the DoC-feeling score were rated higher after treatment than before by both family and medical staff. Patients in the apomorphine group recovered more conscious behaviours than control patients. Alpha-band whole-brain connectivity and participation coefficient, as well as alpha-band parieto-temporal connectivity and frontal participation coefficient were higher after treatment than at baseline. Whole-brain metabolism increased by a relative mean of 13.8% after treatment compared to baseline, with a significant effect of timing (pre-vs. post-treatment scans) on regional SUV.

Interpretation Long-lasting consciousness improvements were observed in patients treated with apomorphine, compared to controls and compared to baseline. Changes in brain connectivity and metabolism were observed after treatment, providing insights into possible neurophysiological mechanisms and target areas. This open-label study

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confirmed the feasibility and safety of apomorphine treatment, which may represent a key therapeutic option for PDoC.

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Research in context

Evidence before this study

This study was designed based on the results from two seminal articles reporting behavioural improvements in a series of eight patients with disorders of consciousness treated with subcutaneous apomorphine. A comprehensive search was undertaken in two databases (MEDLINE-Pubmed and Scopus), without time or language filters, for the terms "apomorphine" and "disorders of consciousness". Only these two sources were identified as original patient data reporting the use of apomorphine for patients with disorders of consciousness. A similar search on the clinicaltrials.gov platform allowed to identify a trial protocol posted in 2008 which was suspended before the start of enrolment (NCT00761228). No meta-analysis was possible given the limited evidence available.

Added value of this study

This study is the first report of subcutaneous apomorphine administration in patients with disorders of consciousness that uses up-to-date standardized behavioural outcome measures (CRS-R) and a control group. It is also the first ever account of changes in brain activity (hdEEG, FDG-PET) recorded before and after therapy by apomorphine in brain-injured patients. It confirms the safety, the feasibility and the promising efficacy of this pharmacologic treatment for PDoC patients, using a standardized protocol and reliable multimodal outcome measures.

Implications of all the available evidence

Prior evidence on the efficacy of apomorphine treatment for disorders of consciousness demonstrated promising results given the striking course of recovery described in the two seminal case series, but important limitations in the design precluded any reliable interpretation on the applicability and the reproducibility of the observed outcomes: small sample sizes, heterogeneous treatment duration, lesion aetiology restricted to traumatic brain injury, previous administration of dopamine agonists in some patients and absence of gold-standard outcome measures (CRS-R) or brain activity data. Building on these inaugural foundations, our open-label study provides a next step by providing efficacy data using a control group, recommended outcome measures and a more standardized protocol. These results will have major implications on future research and promote the realization of large-sample placebo-controlled randomized clinical trials that may impact future treatment guidelines for PDoC. Neurophysiological and neuroimaging data could also help understanding the action of pharmacologic agents on the injured brain and facilitate the identification of molecular targets for future drug development, as well as provide biomarkers predicting treatment responsiveness to promote a precision medicine approach.

Introduction

Disorders of consciousness are a group of rare neurological conditions that patients can encounter after coma due to severe brain injury, such as traumatic brain injury, cardiac arrest, ischaemic stroke or brain haemorrhage.[1] Prolonged disorders of consciousness (PDoC—more than four weeks after injury)[2] are traditionally classified in diagnostic categories according to behavioural responsiveness: patients in vegetative state/unresponsive wakefulness syndrome (UWS) display preserved arousal but only reflexive behaviours,[3] patients in minimally conscious state "minus" (MCS–) can demonstrate reproducible non-language-related signs of consciousness[4] while those in minimally conscious state "plus" (MCS+) show language-related behaviours, yet without functional communication.[5] Patients emerge from MCS (EMCS) only when they are able to functionally use objects or communicate.[6]

The diagnostic assessment of PDoC is primarily clinical, and the fluctuating nature of these patients warrants the use of repeated standardized behavioural measures.[7] The Coma Recovery Scale—Revised (CRS-R) is a 23-item tool designed to test all consensual behaviours indicative of MCS and EMCS at the bedside.[6] It is

currently recommended for the behavioural diagnosis of PDoC as it has shown the best content validity and reliability compared to other scales.[8] To complement behavioural assessments, objective measures of brain activity can be used to study regional cerebral function and detect changes induced by consciousness-promoting therapies.[9] High-density electroencephalography (hdEEG) is a practical non-invasive technique to measure brain electrical activity at the bedside, which allows the study of spectral power, functional connectivity and network integration.[10] Fluorodeoxyglucose positron emission tomography (FDG-PET) measures the uptake of radioactive glucose as an indicator of cerebral metabolism, which provides valuable information on synaptic activity in the cortex and deeper structures.[11] In addition to objective measures of consciousness, the DoC-feeling score is a recently proposed, subjective assessment tool which is completed by caregivers to improve diagnostic accuracy in PDoC.[12]

Besides consensual recommendations for intensive neurological rehabilitation in specialized facilities,[13] different experimental therapies have been tested to actively promote recovery and improve the long-term functional prognosis of patients with PDoC, but there is only limited evidence on their efficacy or safety.[14] Among pharmacologic agents, GABAergic and dopaminergic drugs have been the most extensively studied, essentially in uncontrolled settings, with variable sample sizes and efficacy results.[15] PDoC patients treated with amantadine four to 16 weeks after traumatic brain injury demonstrated a significantly higher rate of recovery during a four-week regimen, which did not persist after treatment withdrawal.[16] Apomorphine is a nonselective dopamine agonist with higher affinity for D2 receptors, which was used in preliminary case series to treat patients with severe traumatic brain injury.[17,18] These studies reported an impressive behavioural progression and functional independence in all patients within one year, but the absence of a control group and the use of suboptimal behavioural outcome measures limit the interpretation of results. Apomorphine, currently marketed for advanced Parkinson's disease, offers a well-documented safety profile with mostly minor adverse reactions that can be avoided with adequate medical monitoring and management.[19] Additionally, its route of administration by subcutaneous infusion allows constant drug delivery and uninterrupted dopaminergic cerebral stimulation during daytime, which may both increase responsiveness and decrease adverse effects.[20]

The use of dopamine agonists to promote recovery of consciousness is driven by strong evidence of imbalances in dopaminergic signalling in animal models[21] and among brain-injured patients.[22] In particular, their action may target critical hubs in dopamine pathways such as the ventral tegmental area,[23,24] ventral striatum[25] and central thalamus.[26] These nodes play a central role in the anterior forebrain mesocircuit model, which postulates that feedback loops between the striatum, globus pallidus interna, central thalamus and cortical areas are essential to support conscious awareness.[27] Compared to amantadine which acts as a non-selective dopamine releasing agent and reuptake inhibitor,[28] apomorphine's direct action on dopamine receptors may be better tailored to treat the presynaptic dopamine depletion observed in PDoC patients.[22] Additionally, the high concentration of D2 receptors in striatal medium spiny neurons[29] may constitute a preferential target for the action of apomorphine and the exogenous restoration of striato-pallidal dysfunctional efferences.[30] It is usually administered in daytime continuous subcutaneous infusions because of its poor oral bioavailability, which offers stable blood levels and a constant stimulation of dopaminergic pathways.

The present study aims to investigate the efficacy and the safety of apomorphine therapy in patients with PDoC. It uses standardized scales as behavioural outcome measures of clinical improvement,[8] as well as objective neuroimaging measures of brain activity. Our main hypothesis is that treatment group (apomorphine vs. control) and phase (treatment vs. baseline, possibly washout vs. baseline) will significantly impact behavioural outcome measures, showing better clinical recovery in the apomorphine treatment conditions. Based on the mesocircuit model, we hypothesize that patients treated with apomorphine will increase striato-pallidal activity, restoring central thalamic activity, and resulting in higher integration of neuronal activity across the cortex. We expect these changes to translate into modifications of brain metabolism, connectivity and network integration, either with a direct effect on mesocircuit hubs (basal ganglia, thalamus, frontal cortex) or with an indirect effect on cortical regions receiving projections from the mesocircuit (primary sensory cortices, default mode network, fronto-parietal network), between pre- and post-treatment assessments. The presence of long-lasting behavioural effects after treatment washout would indicate the possible involvement of durable neurophysiological mechanisms, such as neural plasticity, in critical hubs or pathways of the mesocircuit, whereas a transient recovery pattern returning to baseline after treatment withdrawal would rather suggest a drug-dependent, short-term action of apomorphine.

Methods

Study design and participants

In this open-label non-randomized controlled trial, all new patients admitted to a specialized rehabilitation ward for PDoC in Belgium were screened from February 2018 to January 2021 (Fig. 1). Patients were eligible for this study if they were: 1) aged 18–60 years, 2) clinically stable, independent of mechanical ventilation, 3) diagnosed with UWS or MCS according to the international

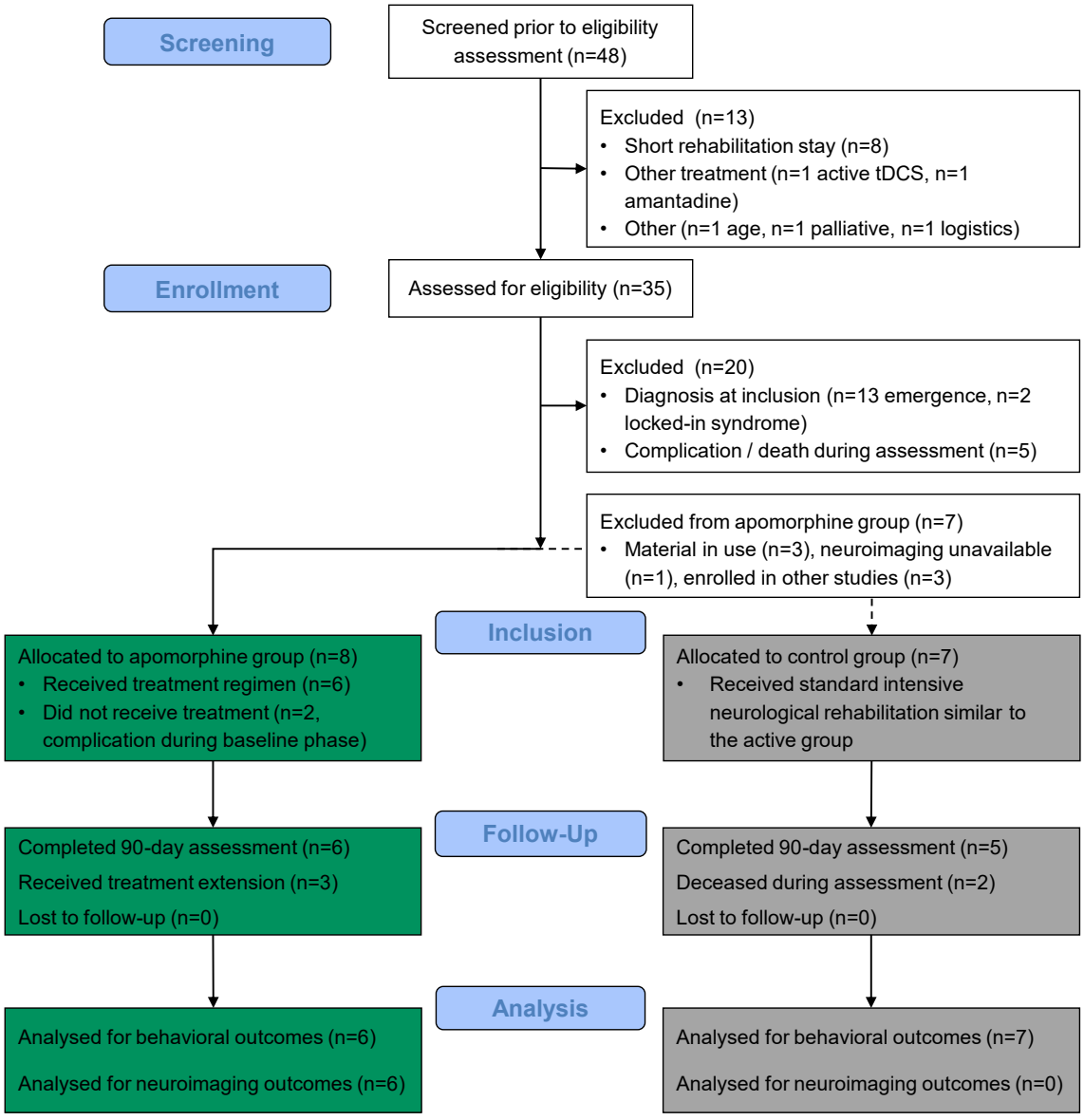

***Fig. 1:* Study flow-chart.** Adapted from the CONSORT 2010 Flow Diagram.[31] tDCS, transcranial direct current stimulation. All 48 patients admitted to the rehabilitation centre within the study period were screened for eligibility. Fifteen patients were deemed eligible, of which eight were included in the apomorphine group and seven in the control group as they could not benefit from active treatment for logistical reasons or enrolment in the control group of other studies. Note that the ineligibility rate (33/48) observed in this study is highly specific to the recruiting rehabilitation centre as it admits patients for short rehabilitation stays, with locked-in syndrome or already in emergence from the minimally conscious state (accounting for 23/33 exclusions).

criteria[7] and based on 2 consistent CRS-R in the last 14 days, and 4) more than 28 days after brain injury. Patients were excluded if they: 1) were more than six months after brain injury, 2) received dopamine (ant) agonists, neurological medications other than anticonvulsant or anti-spasticity, or known QT-interval-prolonging drugs in the last two weeks (or four half-lives), 3) had a corrected QT interval over 480 ms (Bazett's formula) or other risk factors for arrhythmia, 4) had a history of neurological chronic disorder other than the acquired brain injury, or 5) had contraindications to hdEEG or FDG-PET. After inclusion, patients could be withdrawn from the study in the event of severe infections, major surgical procedures, missed treatment days (five unconsecutive days or three consecutive days), severe adverse events, functional deterioration, seizures,

exposure to any above-mentioned prohibited drugs or substantial modification of rehabilitation program.

Sample size was set to six patients completing apomorphine treatment, to gain insight on treatment efficacy, mechanism, safety, and confirm the feasibility of future randomized trials.[32] The control group size resulted from the collection of all available behavioural data for patients fulfilling inclusion criteria in the set time frame, but could not benefit from apomorphine treatment for logistical reasons. Study design and reporting adhered to the CONSORT 2010 Statement, with appropriate changes to account for its non-randomised nature (Appendix 1).[31] The study was approved by the Ethics Committee of the participating centres (neurological rehabilitation centre and neuroimaging facility) and by the Belgian Federal Agency for Medicines and Health Products (EudraCT identifier 2018-003144-23, ClinicalTrials.gov identifier NCT03623828). Written informed consent was obtained from the legal representatives of all participants.

Treatment allocation and administration

Patients were enrolled in the apomorphine treatment group in a prospective way after screening, while eligible patients who could not benefit from treatment (enrolled in control groups of other studies, administration material or imaging unavailable) formed the control group. A part of the control group's data was retrieved retrospectively from health records (evaluations performed as part of clinical routine or other studies). In the apomorphine treatment group, patients received 30 days of subcutaneous infusions of apomorphine, administered daily for 12 h, following a progressive dosage scheme (detailed protocol in Appendix 2). After a 30-day washout, an optional treatment extension was proposed only to patients who showed clinical improvement during the treatment period. Patients in the control group did not receive apomorphine or a placebo treatment, but both groups received standard neurological rehabilitation including physiotherapy, speech therapy, neuropsychological therapy and occupational therapy several times per week.

Assessment methods

After inclusion, all patients in the apomorphine group were assessed weekly with the CRS-R for a 30-day baseline period, during the 30-day treatment phase and then for a 30-day washout period. This OFF-ON-OFF scheme provided an intra-individual control condition, allowing to compare treatment and washout phases to baseline values. Patients in the control group were assessed with repeated CRS-R assessments for at least 90 days. All items of the CRS-R were systematically tested, regardless of successful responses. Adverse events were monitored weekly during treatment and washout phases. In the apomorphine group, patients underwent resting-state hdEEG and FDG-PET once in the last week before treatment initiation, and once again in the first week after treatment completion, using similar acquisition protocols (detailed procedures in Appendix 3). An extended version of the DoC-feeling score[12] was completed by three members of healthcare staff and one close relative immediately before and after treatment (Appendix 4). Follow-up was performed at six months and one year after inclusion with the CRS-R, assessed in person or using remote structured phone interviews with close relatives (Fig. 2). The Disability Rating Scale (DRS)[33] and the Wessex Head Injury Matrix

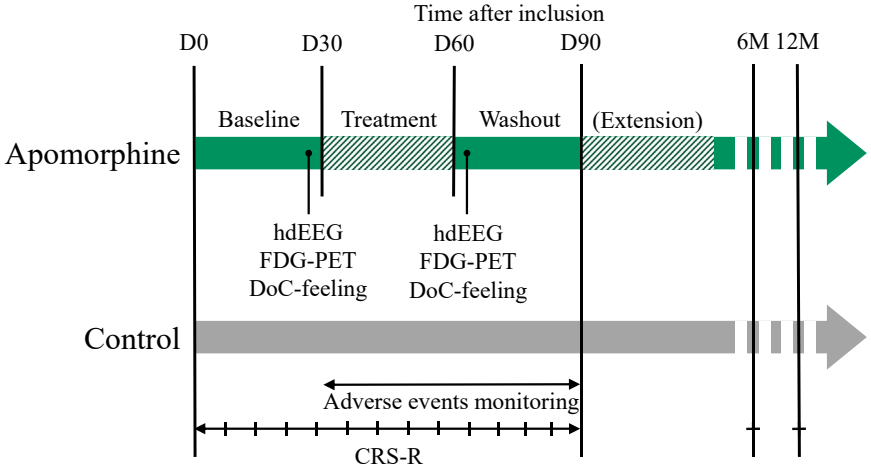

Fig. 2: **Timeline of assessments.** All patients were followed with the Coma Recovery Scale—Revised (CRS-R) and monitored for adverse events, while the apomorphine treatment group also underwent a multimodal assessment before and after the treatment phase. An optional treatment extension was proposed only to patients who showed clinical improvement during the treatment period. D/M, days/months after inclusion; hdEEG, high-density electroencephalography; FDG-PET, fluorodeoxyglucose positron emission tomography; DoC-feeling, extended version of the disorders of consciousness subjective feeling score.[12]

(WHIM)[34] are two neurological rehabilitation scales that allow the monitoring of higher-order behaviours and cognitive functions. They were used to track the progression of patients reaching maximum CRS-R scores.

Outcome measures

The primary outcome was predefined in the study protocol[32] as a change of behavioural diagnosis assessed with the CRS-R, considering the six following ordered categories: dead, coma, UWS, MCS–, MCS+ and EMCS. Secondary behavioural outcomes included: CRS-R index, a metric ranging from 0 to 100 calculated from CRS-R subscores to reliably express and compare levels of consciousness[35]; emergence of new conscious behaviours assessed by the CRS-R (i.e., items denoting MCS/EMCS never reported during baseline assessments–see Appendix 5); DoC-feeling scores; and the occurrence of adverse events. Secondary neuroimaging outcomes included: hdEEG spectral power, connectivity, participation coefficient and degree; FDG-PET whole-brain and regional standardized uptake values (SUV).

Statistical analysis

Demographic parameters were compared between groups using Wilcoxon rank-sum tests for continuous variables and Fisher's exact tests for categorical variables. Primary outcome (change in behavioural diagnosis) was analysed using a mixed-effects ordinal logistic regression models fitted with the Laplace approximation (R version 4.2.3 using RStudio version 2023.12.1 + 402) with random intercepts at the patient-level, in two separate conditions: a) considering both groups with treatment allocation (apomorphine, control), time since injury, age, baseline diagnosis and aetiology (traumatic vs. non-traumatic) as independent variables, and b) considering only the apomorphine group with study phase (baseline, treatment, washout), time since injury, age, baseline diagnosis and aetiology (traumatic vs. non-traumatic) as independent variables (Appendix 6). Intraclass correlation coefficients and model fit characteristics (Akaike's information criterion, Bayesian information criterion, log likelihood) were employed to compare models. Effect size of independent variables are reported as estimates of odds ratio (OR) and 95% confidence intervals (95% CI). CRS-R index was analysed using mixed-effects linear regression models using the same random and fixed effects as for the primary outcome, and results are presented as coefficient estimates (CE) of independent variables with 95% CIs. Presence of new behaviours in the CRS-R and DoC-feeling scores were compared descriptively.

HdEEG data were pre-processed and analysed separately for delta, theta, alpha and beta frequency bands at scalp-level (see detailed analysis procedures in Appendix 3). Whole-brain and regional analyses were conducted at the group-level considering pre-defined regions of interest (ROIs). Differences between pre- and post-treatment spectral power values and functional connectivity were calculated, and the network topological reorganization was assessed from connectivity matrices using graph theory measures of number of connections (degree) and network integration (participation coefficient).[10] The participation coefficient gives an estimate of the inter-module connectivity strength, which reflects the level of functional integration within the network. Connectivity, degree and participation coefficient values were compared with 26 healthy controls at rest.

FDG-PET images were pre-processed and regional voxel-wise analysis of patients' metabolism was performed at the single-subject level to derive: 1) Global changes in brain glucose metabolism, by computing whole-brain SUV for each normalized FDG-PET image, which was then compared to the average global SUV of 33 healthy controls. Pre-to-post-treatment percentage change in global SUV was computed for each patient, and then averaged across all patients, to derive a mean percentage change in global SUV, presented descriptively; 2) Regional SUV, by computing SUV in 119 brain regions for each patient from normalized FDG-PET images. Similar to behavioural data, a mixed-effect linear regression model was used to evaluate the effect of timing (pre vs. post-treatment) on SUV values (see detailed analysis procedures in Appendix 3).

Significance level was set at 0.05 for all statistical analyses and FDR-corrected for multiples comparisons when indicated. Analyses were performed blindly using anonymized datasets, without information on the patient's diagnosis, age, sex, time since injury or timing (pre- or post-treatment) of the exams.

Role of the funding source

The funding sources of this study were not involved in decisions related to study design; collection, analysis, and interpretation of data; writing of the report; and the decision to submit the paper for publication.

Results

Patient characteristics

Forty-eight patients admitted consecutively were screened for inclusion in the 35-month study period. Thirteen patients were excluded based on their medical record and 20 additional patients were further excluded after eligibility assessment. Eight patients were enrolled in the apomorphine group, of which six received apomorphine and successfully completed the study protocol and remote follow-up. Four of these patients were selected for a treatment extension, of which three completed the extension phase (one patient died of unrelated complications during extension—see Appendix 7 for a breakdown of individual apomorphine doses received). The two patients excluded after enrolment had to be transferred to acute care units due to infections before treatment initiation, and were

therefore not included in the analyses as they were never exposed to apomorphine therapy. Data from seven patients were collected (four prospectively, three retrospectively) to constitute the control group (Fig. 1; individual patient data in Appendix 8).

In the control group, the behavioural assessment of three patients (C4, C6, C7) had to be temporarily paused (duration range 43–85 days) because of sanitary restrictions related to COVID-19. All three patients had a stable behavioural diagnosis with no change after the pause compared to all previous assessments.

There was no significant difference between the two groups in terms of sex, age, time since injury, baseline diagnosis or aetiology (all p-values >0.15–Table 1, Appendix 8A).

Behavioural results

Three of six patients in the apomorphine group improved their behavioural diagnosis between the baseline period and the end of the washout follow-up, and four out of six patients showed an improvement at 6-month and 12-month time points compared to baseline. One patient emerged from the MCS during baseline and reached maximum CRS-R scores, but continued to improve in higher-order rehabilitation scales (DRS –10 points and WHIM +51 points after washout compared to baseline). In comparison, two of seven patients in the control group had improved their behavioural diagnosis between the initial 30 days and the following 60 days after inclusion. Only one patient out of seven had maintained this improvement at 6-month and 12-month time points (Fig. 3A, Appendices 8B, C, and 9).

The mixed-effects ordered logistic regression model considering all patients showed a significant effect of group (apomorphine vs. control, OR 8.9, 95% CI 3.3–17.8) on behavioural diagnosis (primary outcome). The analysis in the apomorphine group revealed a significant effect of study phase on behavioural diagnosis, both for treatment phase (OR 3.9, 95% CI 1.5–10.1) and washout phase (OR 4.4, 95% CI 1.3–14.8) compared to baseline (Fig. 4, Appendix 10).

The evolution of the CRS-R index followed that of the behavioural diagnosis, with all six (100%) patients in the apomorphine group increasing their median score between the baseline phase and the end of the washout (median improvement +19.3 points). All (100%) had maintained this improvement at the 6-month follow-up assessment (median improvement +25.9 points), and five out of six (83%) at the 12-month follow-up (one patient died of unrelated causes; median improvement +34.6 points). In comparison, three patients out of seven (43%) in the control group showed an improvement 90 days after inclusion, only two (29%) could maintain this improvement at the 6-month follow-up and one (14%) at the 12-month assessment (median CRS-R index changes –0.7, –1.1 and –3.8 points, respectively) (Fig. 3B, Appendix 8B and C). Using the CRS-R index as outcome variable, mixed-effects linear models on all patients did not demonstrate a significant effect of group (apomorphine vs. control, CE 12.4, 95% CI –14.5 to 39.2). In the apomorphine-only condition, study phase (treatment vs. baseline: CE 7.9, 95% CI 1.1–14.9; washout vs. baseline: CE 17.3, 95% CI 10.7–24.9) showed significant effects on the CRS-R index (Appendix 10).

Five of six patients (83%) in the apomorphine group exhibited new conscious behaviours during the treatment and washout phases, compared to three out of seven patients (43%) in the control group during the same period (Fig. 5). When considering only language-related items, three patients (50%) in the apomorphine group and one patient (14%) in the control group regained new language-related behaviours. Apomorphine patients recovered an average of 2.2 conscious behaviours (1.3 language-related) per patient in the same time frame, while control patients recovered an average of 0.6 conscious behaviours per patient (0.14 language-related) (Fig. 5).

In the apomorphine group, DoC-feeling questionnaires were completed before and after treatment by three members of healthcare staff for all patients, and by one member of close family for four of the six patients (no visits allowed during the COVID-19 lockdown for the two remaining). Results demonstrated that all items

	Control (n = 7)	Apomorphine (n = 6)	p-value
Age[a]			
Years	44 (36–52)	40 (26–49)	0.67
Sex[b]			
Female	4 (57%)	2 (33%)	0.38
Male	3 (43%)	4 (67%)	
Time since injury[a]			
Days	93 (70–96)	100 (95–104)	0.15
Diagnosis[b]			
UWS	4 (57%)	1 (17%)	0.48[c]
MCS–	2 (29%)	3 (50%)	
MCS+	1 (14%)	2 (33%)	
Aetiology[b]			
TBI	3 (43%)	4 (67%)	0.38
Non-TBI	4 (57%)	2 (33%)	
CRS-R index[a]			
Score 0–100	4.8 (4.0–22.4)	14.7 (4.3–60.9)	0.52

p-values are calculated using Wilcoxon rank-sum tests for continuous variables (age, time since injury, CRS-R index) and Fisher's exact tests for categorical variables (sex, diagnosis, aetiology). UWS, unresponsive wakefulness syndrome; MCS–, minimally conscious state minus; MCS+, minimally conscious state plus; TBI, traumatic brain injury; CRS-R, Coma Recovery Scale–Revised. [a]Median (interquartile range). [b]Number (percentage). [c]p = 0.18 when considering only UWS vs. MCS.

Table 1: **Demographic and clinical characteristics of patients at inclusion.**

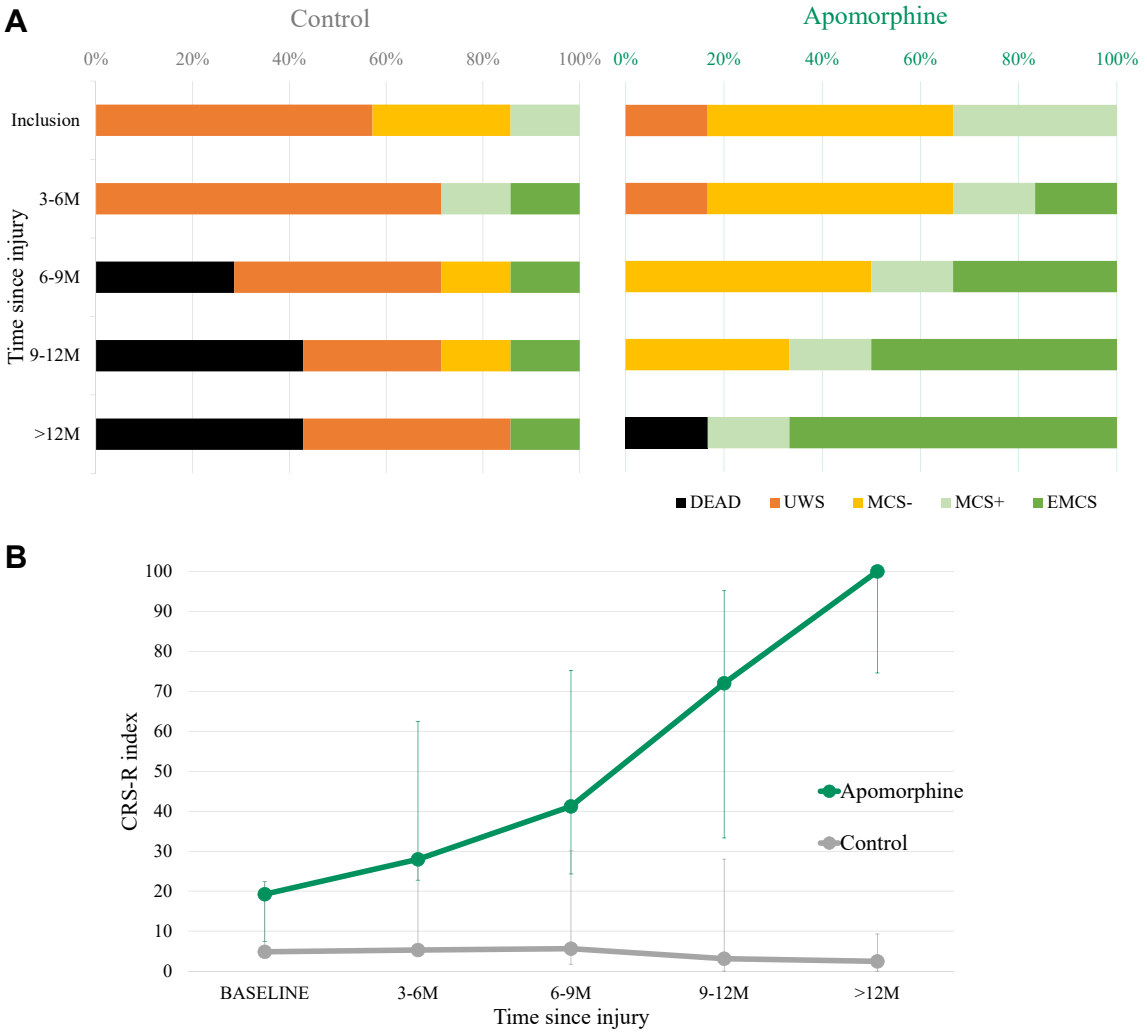

***Fig. 3:* Longitudinal evolution of behavioural outcome measures. A.** Evolution of behavioural diagnosis in the two groups across four time windows. For each patient, the most frequent diagnosis for all observations made within the given time window are displayed. **B.** Evolution of the CRS-R index (median—interquartile range) for the apomorphine (green) and the control (black) groups. For each patient, the median CRS-R index value was computed for all observations within each time window, and the median and quartile values were then calculated for each group. M, months; UWS, unresponsive wakefulness syndrome; MCS–, minimally conscious state minus; MCS+, minimally conscious state plus; EMCS, emergence from the MCS; CRS-R, Coma Recovery Scale–Revised.

were scored higher after treatment than before treatment when considering the mean difference for all patients. Family rated all items higher than healthcare professionals in both pre- and post-treatment conditions, with mean pre-post relative increases of 32.5% (staff) and 16.9% (family). Highest improvements were reported in communication by family (45% higher) and in spontaneous motor function by healthcare staff (25% higher) (Fig. 6; Appendix 8D).

No severe adverse effects were reported during the study. One patient died of respiratory complications during the treatment extension (P1), which were found to be unrelated to the experimental treatment after formal investigation. Mild effects including vomiting (6/6 patients), skin erythema (4/6) and local skin nodules (1/6) were observed and were always adequately managed by antiemetics, adaptation of apomorphine dosage, infusion site rotation and local skin care, without the need for pausing therapy. No unintended effect was reported in the control group.

Neuroimaging results

HdEEG connectivity was significantly higher after apomorphine treatment, at the whole-brain level ($t = -2.12$, $p = 0.044$) and for parieto-temporal interactions ($t = -3.57$, $p = 0.048$ FDR-corrected) in the

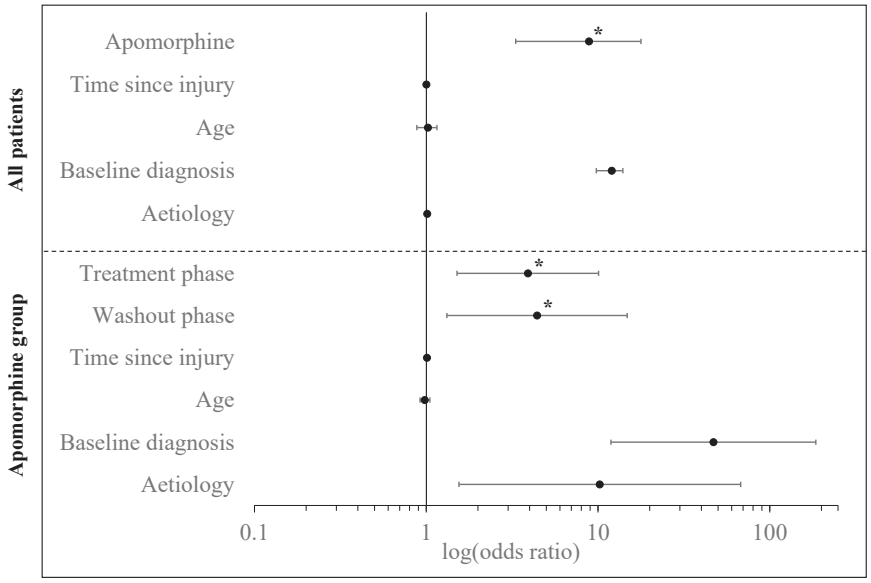

***Fig. 4:* Effect sizes of the mixed-effects model on primary outcome (behavioural diagnosis).** Forest plot displaying the decimal logarithm of the odds ratio to predict a change in behavioural diagnosis for the fixed effects included in the mixed-effects ordered logistic regression models, in the two analysis conditions (all patients and apomorphine group only). Apomorphine: odds ratio of the apomorphine group compared to the control group, Treatment and Washout phase: odds ratio compared to the baseline phase. Asterisks denote significant effects and whiskers indicate 95% confidence intervals. Interpretation of confounder coefficients is subject to special caution and may lead to misleading conclusions.[36]

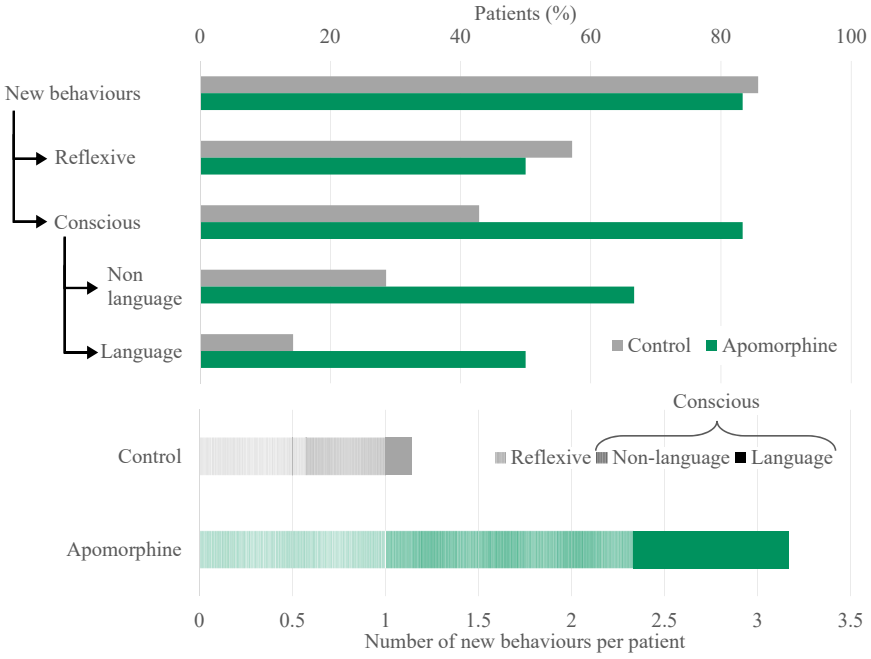

***Fig. 5:* Presence of new behaviours.** The upper panel displays the percentage of patients in each group who demonstrated new behaviours during treatment and washout phases. The lower panel shows the number of new behaviours developed per patient in each group for the same period. A new behaviour is defined as a Coma Recovery Scale–Revised (CRS-R) item which was never previously reported during baseline assessments. Treatment and washout phases for the control group are defined as day 31–60 and 61–90 after inclusion, respectively (similar to the apomorphine group). CRS-R items (without arousal) were grouped into reflexive or conscious according to the scale guidelines, and conscious behaviours were further divided into language- and non-language-related.

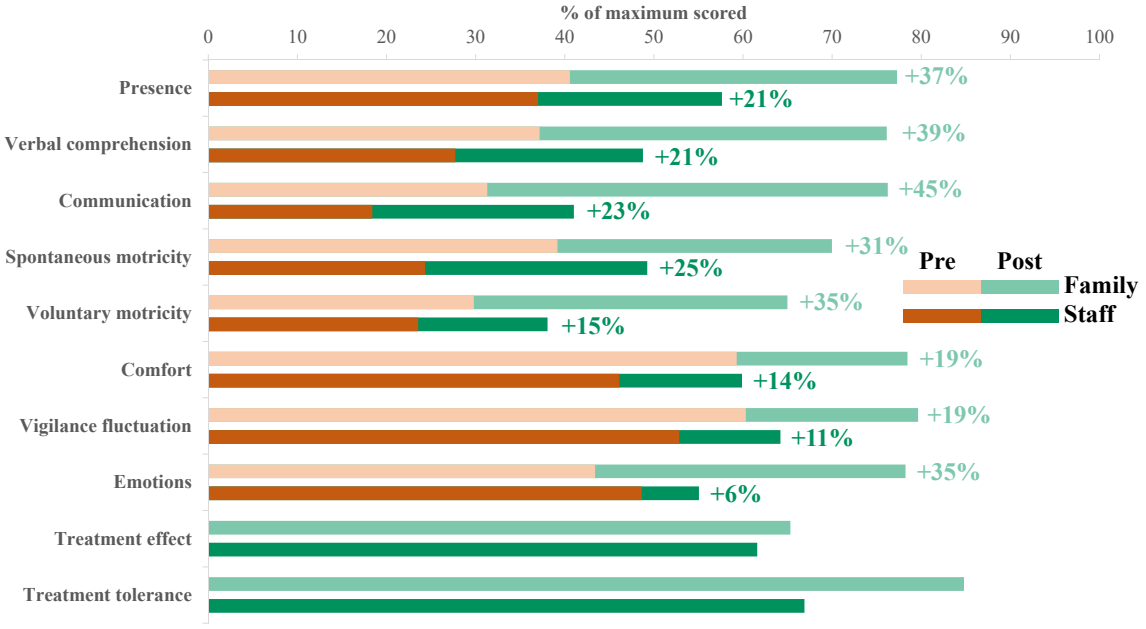

Fig. 6: **DoC-feeling questionnaire results.** Ratings by family (one rater) and staff (average of three raters) pre- and post-treatment on an eight-item clinical questionnaire using visual analogue scale reports of perceived patient status over the last seven days. The post-treatment questionnaire featured two additional items on treatment efficacy and tolerance.

alpha frequency band (Fig. 7). Alpha network participation coefficient was significantly higher after apomorphine treatment at the whole-brain level (t = −3.23, p = 0.012) and in the frontal region (t = −3.12, p = 0.039 FDR-corrected). No significant difference was found for spectral power, number of connections (degree) and for other frequency bands.

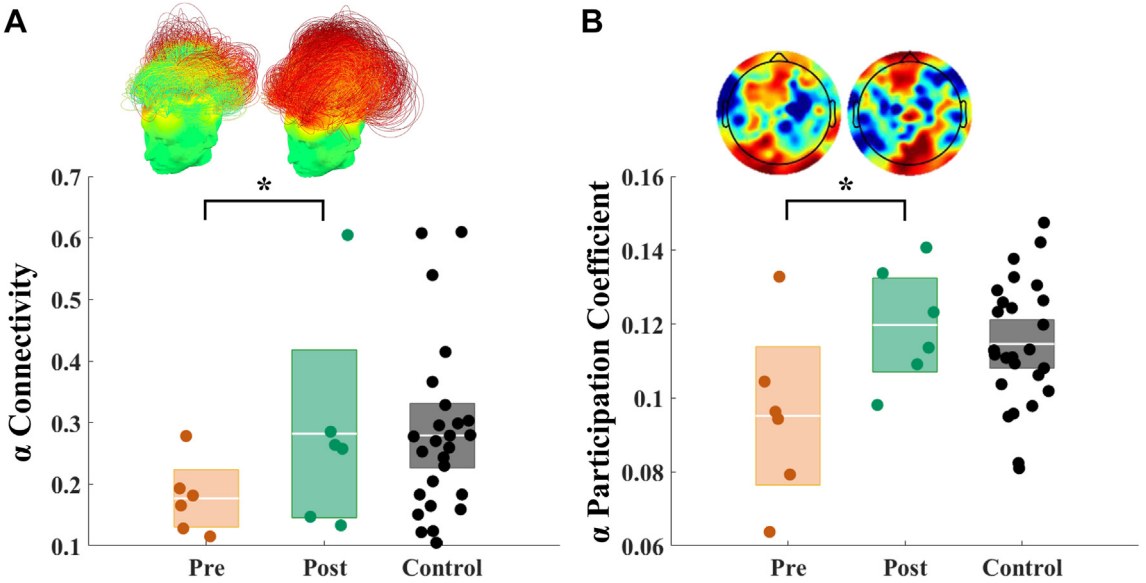

Fig. 7: **High-density electroencephalography.** Whole-brain connectivity (**A**) and participation coefficient (**B**) values in the alpha band are displayed for patients in the apomorphine group before and after treatment, as well as for 26 healthy controls. 3D topographs of alpha mean brain network connectivity and 2D topographs of alpha participation coefficient averaged over the six patients pre- and post-treatment, colour-coded for connection/participation strength (from blue to red), are displayed over the corresponding boxplots. Whole-brain connectivity and participation coefficient are increased in the post-treatment condition compared to before treatment for the alpha frequency band, returning to near-normal mean values.

Whole-brain metabolism measured by FDG-PET SUV indicated a mean decrease of 52.7% (range 33–69%) compared to healthy controls before treatment initiation, and a mean decrease of 46.5% (range 28–66%) after treatment completion, corresponding to a mean relative +13.8% (range –5.2%–+37.3%) improvement of global SUV (Fig. 8A, Appendix 11). The mixed-effect model fitted with regional SUV values confirmed these findings and demonstrated a significant effect of timing on SUV (before vs. after treatment, CE 0.38, 95% CI 0.30–0.45—Fig. 8B, Appendix 12).

Discussion

The results of this controlled open-label study demonstrated a significant effect of treatment allocation on the primary outcome. Patients treated with 30-day apomorphine subcutaneous infusions improved their behavioural diagnosis after treatment initiation compared to the control group, including individual patients with non-traumatic brain injuries for the first time. The apomorphine-group analysis allowed to further study the temporal dynamics of recovery in the treatment group throughout the OFF-ON-OFF treatment design. The model revealed significant effects of both treatment and washout phases compared to baseline. Patients treated with apomorphine displayed a higher rate of conscious behaviours than controls, which was even more pronounced for language-related behaviours. These findings confirm preliminary behavioural reports of efficacy[17,18] with additional robustness and replicability owing to the use of recommended behavioural measures and standardized periods for baseline, treatment and washout.

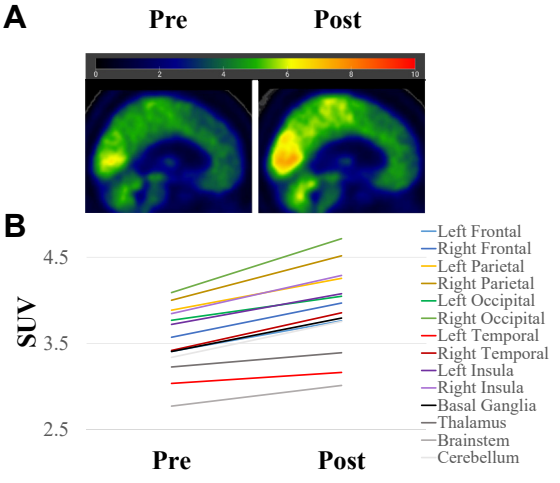

Fig. 8: **FDG-PET standardized uptake values (SUV) before and after apomorphine treatment. A.** Sagittal view of whole-brain SUV averaged over all patients in the apomorphine group (n = 6), demonstrating a predominant increase in posterior areas after treatment compared to before. **B.** Regional SUV averaged over the 14 brain macrostructures used to design the mixed model, before and after treatment, averaged over all patients in the apomorphine group.

Despite its apparent subjectivity, the use of the DoC-feeling tool[12] provided a valuable proxy of patients' evolution perceived by family and healthcare staff. There is increasing evidence that the implementation of patient-centred outcome measures is beneficial to the success of clinical trials, and subjective evaluations by caregivers is an important parameter to take into account alongside research-driven variables.[37] We observed higher subjective ratings in all items after apomorphine treatment and specific differences in perceived changes between staff and family. Indeed, higher baseline values and higher overall improvements were reported by the family, with a focus on communication and emotions, contrasting with the healthcare staff who ranked emotions as the least improved feature, and spontaneous motor function as the largest improvement. These may reflect diverging points of view and priorities in the perception of recovery by caregivers, suggesting the need for new clinically meaningful outcomes in research on brain-injured patients. Both staff and family also reported good tolerance to treatment. The absence of severe adverse event observed during the study and the successful management of mild adverse reactions confirmed the feasibility and the safety of subcutaneous apomorphine therapy in our patient sample, paralleling previous literature on Parkinson's disease.[19] Future research protocols featuring larger samples, multicentre designs and longer follow-up durations will be necessary to formally confirm the safety of apomorphine in this vulnerable population and generalize our findings.

The use of neuroimaging and neurophysiological secondary outcome measures in the apomorphine treatment group allowed investigating global and regional changes in brain activity between pre- and post-treatment conditions, across independent and complementary techniques. It opened the perspective for future identification of biomarkers predicting therapy responsiveness, an important milestone towards precision medicine for PDoC patients.[38] In particular, the increase in whole-brain EEG alpha functional connectivity and participation coefficient mirrors previous findings on the key role of alpha dynamic connectivity in the modulation of consciousness, in brain injury[10,39,40] but also in anesthesia,[41] meditation[42] or hypnosis.[43] Increases observed in alpha frontal participation coefficient and alpha parieto-temporal connectivity may highlight the central role of these regions in the action of apomorphine on consciousness. Indeed, increased participation coefficient has been previously observed in frontal, midline and temporal areas as the level of consciousness improved.[44] Electroencephalographic changes were associated with significant increases in brain metabolism measured by SUV using a mixed-effects model fitted to FDG-PET regional data.

These findings indicate the presence of multimodal changes in the treatment group both in behavioural responsiveness and brain activity occurring between the baseline phase and the end of treatment phase, which would not be expected to occur spontaneously in the course of 30 days among patients with PDoC. The significant effect of the group on the evolution of behavioural indices at different time points corroborate these findings and seem to indicate an effect of apomorphine on recovery of consciousness.

This study has limitations which require special caution in the interpretation of results. First, the limited sample size may have provided insufficient statistical power to demonstrate the contribution of specific brain activity outcome measures, and decreased the accuracy of the mixed-effects model estimates, as indicated by the wide confidence intervals on the group variable. Small populations increase the sensitivity to individual variations, and the diagnostic change of a single patient may impact the group estimates significantly. The high serial correlation of our repeated observations over time (as indicated by intraclass correlation coefficients) demonstrate within-patient interdependency in our dataset, which reflects the consistency and standardization of CRS-R evaluations, but decreases the amount of independent information at hand. Statistical comparisons of baseline group characteristics were also limited in power by sample sizes, and the absence of significant baseline differences do not warrant the absence of unbalanced characteristics between patient groups (notably in terms of diagnosis or aetiology). The potential impact of such imbalances is however mitigated by the inclusion of baseline characteristics in the mixed effects model, and the much better evolution of the apomorphine group than published datasets on the natural evolution of MCS patients only.[45] The limited statistical power may also be responsible for the non-significant effect of apomorphine group on the evolution of CRS-R index, despite its significant effect on clinical diagnosis (as both effects go in the same direction). The availability of neuroimaging and neurophysiological exams was limited to the apomorphine group, which allowed us to compare data between pre- and post-treatment time points but not between groups, therefore leaving the possibility that observed changes could have partly resulted from spontaneous recovery. In the absence of formal dose-finding studies, administration schemes were based on previous evidence derived from Parkinson's disease and few brain-injured patients. Further specific research is needed to determine the optimal therapeutic windows in this population. Notably, our study did not include therapeutic drug monitoring, which could have helped to confirm that apomorphine blood levels remained constant and to identify possible individual variations in absorption. Last, the open-label design may have introduced some degree of bias in the course of recovery process (the therapists and the family being aware of the treatment allocation), especially for subjective assessments such as the DoC-feeling score. The large infrastructure required to implement a double-blind placebo condition was beyond the scope of this study, however the chance of assessment bias was reduced by the use of objective (e.g., neuroimaging data analysed blindly by predefined pipelines) or standardized (e.g., CRS-R administered by accredited examiners following a protocol) outcome measures. The efficacy of apomorphine treatment will need to be formally confirmed in larger-sample, randomized placebo-controlled multicentre clinical trials.

While the need for a global framework to bolster the development of consciousness-promoting therapies has been recently advocated,[14] this study provides new evidence for the use of dopaminergic agents in PDoC. For the first time, we provide data on safety and long-term efficacy both at the behavioural and neural level, using standardized, repeated and recommended measures, on patients with several types of brain lesions, that confirm the promising character of subcutaneous apomorphine therapy to promote long-lasting recovery of consciousness. On a broader level, our results demonstrate the complementarity of independent, patient-centred, multimodal outcome measures, which could help promote the success of future research on new therapies for PDoC and close the gap towards better coma care.

Contributors

LRDS, OG and SL realized the initial literature search on previous available evidence. LRDS, NL, NF, JS, RDZ, NDS, SL and OG designed the protocol of the study. LRDS, NL, ES, EACB, AT, and DD collected the data. OG accessed and verified the data. NL, DD, RH and DL ensured the medical management of patients during the study. SVG was responsible of correct management and delivery of pharmaceutical products. LRDS, RP, AS and ND analysed the data. LRDS, ES, RP, AS, AT, ND, SL and OG were responsible of data interpretation. LRDS and OG drafted the manuscript. All authors provided critical review of the manuscript. LRDS was responsible of submitting the manuscript for publication.

Data sharing statement

A table summarizing individual patient demographic and behavioural data is available in Appendix 8.

Complete behavioural and neuroimaging datasets from this study are available upon reasonable request to OG at ogosseries@uliege.be.

Declaration of interests

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2024.102925.

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