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A new ICT system coupling electromyography and coma recovery scale-revised to support the diagnostic process in disorders of consciousness

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The Coma Recovery Scale-revised (CRS-r) is the gold standard for the behavioral assessment of patients with Disorders of Consciousness (DoCs). However, the misdiagnosis rate is around 40%. For this reason, recent guidelines suggested enhancing the assessment with neurophysiological measures: among these, surface electromyography (sEMG) represents a convenient bedside solution. This work presents the use of the STRIVEfc system, a wearable device that allows CRS-r administration while recording four sEMG signals. STRIVEfc was employed in 40 sessions on 33 DoCs patients and the sEMG was analyzed to look for voluntary and consistent over threshold (OT) muscular activities. Their duration, amplitude, and number were retained and compared between patients in Unresponsive Wakefulness Syndrome (UWS) and Minimally Conscious State (MCS), revealing more numerous and significantly longer OTs in the latter group. Lastly, the EMG information was exploited to enrich the behavioral assessment by building the instrumented CRS-r score (ICRS-r). In 9/16 UWS sessions, the ICRS-r score suggested a higher-level functioning, not translated into a behavioral response, compatible with MCS diagnosis. Overall, the use of STRIVEfc allows to reveal hidden muscular patterns not detectable by the clinician, thus improving the characterization of DoCs patient's functional capabilities and supporting the diagnostic process.

Keywords Electromyography, Disorders of consciousness

The behavioral assessment of consciousness remains the cornerstone in the clinical evaluation of the Disorders of Consciousness (DoCs) following acquired brain injury¹. From a clinical point of view, the Coma Recovery Scale-revised (CRS-r;²) is considered the gold standard for the assessment of the residual level of consciousness in DoCs patients³, since it reduces the misdiagnosis rate between Vegetative State (VS)/Unresponsive Wakefulness Syndrome (UWS) and Minimally Conscious State (MCS) at approximately 40% of the cases⁴, thus achieving a better diagnostic accuracy when compared to clinical consensus⁵. The CRS-r consists of 29 hierarchically ordered items grouped into six subscales evaluating the arousal, auditory, visual, motor, verbal, and communication abilities². Each subscale is linked to a score depending on the highest behavioral responses exhibited by the

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patients after repeated stimuli administration, and the subscale scores correspond to the higher-level behavior manifested by the patients in each specific subscale.

The clinical evaluation of DoCs patients based on the CRS-r administration is challenging due to the presence of many confounders that limit the already impaired behavioral response of these patients. Indeed, to detect a voluntary activity, patients need to be awake, have an almost partially spared motor functioning and retain the voluntary drive to move; furthermore, all these requirements need to be satisfied at the time of examination⁶. Again, behavioral responses sometimes are present, but they can fluctuate and being not replicable; when present, these responses can be inconsistent or incongruent to the examiner, or both. In this framework, even more complicated by the frequent co-existence of language impairments and aphasia in brain injured patients⁷, a proper diagnosis of Unresponsive Wakefulness Syndrome or Minimally Conscious State can be challenging.

Patients in UWS, formerly known as Vegetative State⁸, present only reflexive behaviors and do not show self- and environmental-awareness, despite the presence of periods of arousal and a still preserved, albeit abnormal, sleep-wave cycle. Otherwise, patients with MCS⁹ show low-level voluntary behaviors such as visual fixation/pursuit, oriented movements, and localization to noxious stimulation (MCS-), and even high-level ones such as command following, intelligible verbalization, and/or intentional communication (MCS+)¹⁰. When patients are able to functionally use objects or communicate, and when they show a consistent command following, they are classified as emerging from MCS (eMCS)^{4,11}.

Albeit with some differences, the latest American¹², European¹³, and UK¹⁴ guidelines on the diagnosis of coma and DoCs pave the way for an extended use of the neuroradiological and neurophysiological techniques, potentially capable of revealing the covert residual levels of brain functioning. Such indications are the results of the experience of the last decade when a growing amount of experimental diagnostic paradigms have been implemented in both functional Magnetic Resonance Imaging (fMRI) and electroencephalography (EEG) (see Kondziella et al.¹ for a comprehensive review).

Among the various techniques used to investigate patients' functioning, the use of surface electromyography (sEMG) is a promising research line for the amplification of the CRS-r diagnostic yield^{15–17}, particularly for what regards patient's responsiveness: although consciousness and responsiveness coexist in most cases, recent literature suggests separating the two concepts in the DoCs field¹⁸. This neurophysiological technique has the advantage of being low-expensive and relatively easy to apply at the patient's bedside (i.e., in an ecological context), especially when compared to neuroimaging¹⁶. Indeed, the EMG allows recording sub-threshold movements undetectable or misinterpreted by the examiner during active tasks^{15,17}, thus potentially increasing what could be detected beyond what could be observed by the examiners. The use of EMG could be useful to clarify whether a specific patient's behavior is purposeful and congruent with a response to a specific command, whether it is automatic and/or only coincidentally related to the examiner's command, or both. Indeed, all the previous studies^{15–17} leveraged the EMG coupled to the execution of the same active task (i.e., command following) to better define the patients' level of responsiveness. Furthermore, since command following identifies patients as being in MCS+¹⁹, previous studies showed inconclusive results in detecting low-level behaviors, proving to be ineffective in determining a real diagnostic improvement, especially in the gray zone between UWS and MCS patients; moreover, they showed low sensitivity, as in the study by Habbal et al.¹⁶, where Authors reported that only three out of 20 MCS+ patients showed congruent EMG activity to command, despite all patients showed command following during behavioral assessment. Probably, the lack of agreement between CRS-r and EMG results may be in part explained by the different arousal of MCS patients during the behavioral and EMG assessments, due to the different timing of the two examinations. This issue could be overcome by recording the EMG activity during the CRS-r administration.

With this premise, in this work we present the first wearable system that integrates the recording of four sEMG signals in the CRS-r administration for the identification of patterns of motor activation. This system, named STRIVEfc, includes an iOS application (STRIVEapp) that guides the clinician in the CRS-r administration and allows communication with two 2-channel bipolar probes for sEMG recording. The STRIVEfc system was employed in a clinical trial recruiting a sample of subacute and chronic DoC patients with the specific aims of: (i) comparing UWS and MCS patients in terms of number of detected over threshold (OT) sEMG activities and related parameters; (ii) determining how the use of the STRIVEfc during the CRS-r administration would possibly impact the patient's evaluation and discrimination between UWS and MCS patients.

Results

Participants

Thirty-three patients with DoCs (mean age $\pm$ SD = 52.9 ± 16.6 years; mean education $\pm$ SD = 11.2 ± 3.58 years; 26 M. See Supplementary Tables S1, S4 and S5 for details) underwent the CRS-r evaluation with STRIVEfc. Table 1 summarizes their clinical characteristics, including time since injury (TSI) expressed in months, CRS-r total score, CRS-r subscales scores, diagnosis (UWS, MCS-, MCS+, or eMCS), and electrodes placement for the four sEMG channels, as STRIVEfc does not impose any constraints on the muscles to be evaluated. For seven patients, the presence of spasticity, fractures, bandages, and/or orthopedic braces forced the use of a single sEMG probe.

Patients 10 and 13 were evaluated twice, while patient 09 underwent six sessions with the STRIVEapp, with an inter-session period of 2 weeks. A total of 40 sessions were available, of which 24 were rated as MCS, MCS+ or eMCS (median CRS-r = 9, 25th percentile = 8, 75th percentile = 13, referred as MCS in the following) and 16 rated as UWS (median CRS-r = 4.5, 25th percentile = 3, 75th percentile = 6). Twenty-one different body segments were evaluated. Out of 21, eight were considered only in a single session and two were considered in two different sessions, being selected in 2.5% and 5% of the total number of sessions, respectively.

Subject	TSI (months)	CRS-r total score	Subscales scores Au, Vi, M, Ve, C, Ar	Diagnosis	Electrodes placement Ch1, Ch2, Ch3, Ch4
01	6	8	0, 3, 2, 1, 0, 2	MCS	R TA, R GM, R BB, R EDC
02	2	5	1, 1, 0, 1, 0, 2	UWS	R FDS, R BB, R CS, R ZM
03	3	14	4, 5, 2, 1, 0, 2	MCS+	R LH, R ECU, L LH, L FCU
04	4	7	2, 3, 0, 0, 0, 2	MCS	L BB, L FCU, R BB, R FCU
05	3.9	18	3, 4, 5, 2, 1, 3	MCS+	L FCU, L TRB, R FCU, R TRB
06	1.5	7	1, 3, 0, 1, 0, 2	MCS	L BB, L ECU, R BB, R ECU
07	0.8	17	3, 4, 6, 1, 1, 2	MCS+	L FCU, L BB, R FCU, R BB
08	2	8	1, 3, 2, 1, 0, 1	MCS	R FCU, R BB, L FCU, L ECU
09 S1	2	7	2, 0, 2, 1, 0, 2	UWS	L FCU, L BB, R TA, R GM
09 S2	3	6	2, 0, 2, 1, 0, 1	UWS	L BB, L ECU, R TA, R GM
09 S3	4	9	1, 3, 2, 1, 0, 2	MCS	L APB, L FCU, L TA, L GM
09 S4	4.5	8	1, 3, 1, 1, 0, 2	MCS	L FDS, L FCU, L GM, L TA
09 S5	5	8	1, 3, 1, 1, 0, 2	MCS	L FCU, L FDS, L TA, L GM
09 S6	5.5	10	2, 3, 2, 1, 0, 2	MCS	L FCU, L APB, L GM, L TA
10 S1	8	6	1, 0, 2, 1, 0, 2	UWS	R BB, R FCU, L FCU, L BB
10 S2	9	5	1, 0, 1, 1, 0, 2	UWS	L FCU, L BB, R BB, R FCU
11	3.9	7	1, 1, 2, 1, 0, 2	UWS	R EDC, R FCR, L EDC, L FCR
12	103.2	7	1, 0, 2, 2, 0, 2	UWS	R FDS, R BB, L OEC, L BB
13 S1	56.7	2	0, 0, 1, 1, 0, 0	UWS	R FDS, R BB, L TA, L PL
13 S2	58.2	3	0, 0, 0, 1, 0, 2	UWS	R EDL, R AH, R ECU, R FCU
14	106.8	4	1, 0, 0, 1, 0, 2	UWS	L EDL, L AH, L EDC, L FDS
15	15.3	15	2, 4, 4, 3, 0, 2	MCS	R FCU, R ECU, R TA, R GM
16	74.4	0	0, 0, 0, 0, 0, 0	UWS	L FCU, L ECU, R TA, R SO
17	128.1	4	1, 0, 0, 1, 0, 2	UWS	R ECU, R FCU, L OEC, R CS
18	67.8	3	1, 0, 0, 1, 0, 1	UWS	R M, R CS, R ECU, R APB
19	3.4	8	2, 3, 1, 1, 0, 1	MCS	R EDL, R GM, N.A., N.A.
20	5.8	9	2, 3, 1, 1, 0, 2	MCS	L GM, L TA, L APB, L ECU
21	8.4	9	1, 3, 2, 1, 0, 2	MCS	R GM, R TA, L ECU, L APB
22	5.5	4	1, 0, 1, 0, 0, 2	UWS	N.A., N.A., R CS, L CS
23	4.7	12	4, 0, 5, 1, 0, 2	MCS	R FDS, R ECU, N.A., N.A.
24	5.4	9	2, 3, 1, 1, 0, 2	MCS	R CS, R FCU, L APB, L ECU
25	8.6	9	3, 1, 1, 2, 0, 2	MCS	L TA, L EDL, R CS, L OEC
26	3.1	7	2, 3, 0, 1, 0, 1	MCS	R EHL, R GM, R APB, R FCU
27	1.1	7	1, 2, 2, 1, 0, 1	MCS	L GM, L TA, R FCU, R FCR
28	1.5	7	0, 3, 1, 1, 0, 2	MCS	L FCU, L APB, R FCU, R APB
29	8.3	5	1, 1, 2, 1, 0, 0	UWS	L OEC, R OEC, N.A., N.A.
30	2	22	4, 4, 6, 3, 2, 3	eMCS	R FDS, L FDS, R EDL, L EDL
31	4.4	11	4, 3, 2, 1, 0, 1	MCS+	N.A., N.A., R FCU, R BB
32	2	3	0, 0, 2, 0, 0, 0	UWS	R APL, R ECU, N.A., N.A.
33	3	16	4, 3, 5, 2, 0, 2	MCS+	N.A., N.A., R FDS, R ECU

Table 1. Clinical characteristics and electrodes placement for all patients. *TSI* time since injury, *Au* auditory subscale, *Vi* visual subscale, *M* motor subscale, *Ve* verbal subscale, *C* communication subscale, *Ar* arousal subscale, *CS* corrugator supercilii, *OEC* outer eye canthus, *ZM* zygomaticus major, *M* masseter, *FCU* flexor carpi ulnaris, *FCR* flexor carpi radialis, *ECU* extensor carpi ulnaris, *BB* biceps brachii, *TRB* triceps brachii, *FDS* flexor digitorum superficialis, *EDC* extensor digitorum communis, *APB* abductor pollicis brevis, *APL* abductor pollicis longus, *LH* lumbricals of the hand, *TA* tibialis anterior, *PL* peroneus longus, *GM* gastrocnemius medialis, *SO* soleus, *EDL* extensor digitorum longus, *AH* adductor hallucis, *EHL* extensor hallucis longus, *R* right, *L* left, *N.A.* not applied.

Comparison between UWS and MCS sessions

Sessions rated as UWS and MCS were firstly compared in terms of CRS-r total score and subscales scores to assess their clinical differences. Table 2 reports the results of the statistical analysis. Coherently with the diagnostic procedure, UWS sessions were rated significantly lower in the total CRS-r score. The auditory and visual subscales differentiated the two groups the most.

It is worth to notice that the two groups of patients did not differ statistically for age (UWS: median = 59.00, interquartile range = 35.00; MCS: median = 57.00, interquartile range = 24.30; $U_{(31)} = 125.5$; $p = 0.883$), sex

Scale	UWS	MCS	p-value
auditory	1 (0.5–1)	2 (1–3)	0.0018
visual	0 (0–0)	3 (3–3)	1.31e-07
motor	1 (0–2)	2 (1–3)	0.061
verbal	1 (1–1)	1 (1–1)	0.072
communication	0 (0–0)	0 (0–0)	0.155
arousal	2 (0.5–2)	2 (2–2)	0.086
CRS-r	4.5 (3–6)	9 (8–13)	3.18e-07

Table 2. Median (25th–75th percentiles) CRS-r subscales and CRS-r total scores in UWS (*n* = 16) and MCS (*n* = 24) sessions. The last column reports the p-value of the Mann Whitney test between the two groups. Significant values are given in bold.

distribution ($\chi^2 = 1.17$; $p = 0.279$), years of education (UWS: median = 13.00, interquartile range = 5.00; MCS: median = 13.00, interquartile range = 5.00; $U_{(31)} = 125.5$; $p = 0.871$), and TSI (UWS: median = 8.00, interquartile range = 72.40; MCS: median = 3.65, interquartile range = 3.50, $U_{(31)} = 80.5$; $p = 0.070$). More details can be found in Supplementary Table S2 for the descriptive statistics of the two groups, Supplementary Table S4 for the sex distribution, and S3 and S5 for the statistical analysis.

Then, EMG-related parameters were investigated. The sEMG signals, acquired during each sensory stimulation of a given CRS-r item (named “item trial” in the following), were pre-processed (filtering, rectification, and envelope extraction) and inspected to identify representative resting activities (RRA). The RRAs were exploited to compute a threshold for the sEMG of each item trial, allowing to recognize OT sEMG activities. Two parameters were computed on each OT and grouped within item trials: the duration in seconds (*Duration_Trial*) and the normalized root mean square difference with respect to the corresponding RRA in μV (*nRMS_Diff_Trial*). The third parameter was the number of detected OT activities. More details are available in the Methods section, both for data processing and parameters computation (see Fig. 4 for the analysis workflow). For this analysis, all muscles were grouped together to align with the real CRS-r administration. Results for the verbal and communication subscales are not reported in Table 3 as the electrodes positioning did not allow to evaluate a sufficient number of item trials to run the statistical tests.

The OT duration was always higher for the MCS than for the UWS sessions, with significant differences in total CRS-r, auditory, and visual subscales. The OT normalized RMS difference (*nRMS_Diff_Trial*) resulted to be significantly greater for the MCS group in the auditory subscale, while the opposite trend was revealed in the motor one. The total number of items with an identified OT resulted greater in MCS than in UWS (MCS = 26.5 (34); UWS = 21.5 (13.5); $p = 0.147$), but this expected trend was not supported by a statistically significant difference.

Impact of the use of STRIVEfc on the diagnosis

The impact of the adoption of the system was quantified by the agreement between the behavioral responses detected by the clinician and the presence of sEMG OT activities identified by the proposed sEMG analysis. Considering the former as the true reference, a contingency table was built at the item trial level (see Methods section). Then, CRS-r scoring rules were applied to obtain the same table (Table 4) at the item level, which was further differentiated between UWS and MCS sessions and across CRS-r subscales (the communication scale was excluded due to the lack of items that could be featured in the analysis). The distribution of TP, TN, FP, and FN is also reported in percentage with respect to the total number of items considered. Considering single muscles in the whole CRS-r, FCU, TA, ECU, and BB accounted for 21.1%, 14.8% 12.7% and 12% of total FPs, respectively. The trend was consistent in the different CRS-r subscales. Interestingly, the CS muscle was responsible for 22.2% of the FPs in the visual subscale. The same muscles were the most involved in sessions characterized by the presence of FNs: FCU 18.89%, TA 7.78%, ECU 14.44% and BB 7.78%. Additionally, APB and GM activity was measured in 7.78% and 10% of FP cases.

The results in Table 4 were the basis for the computation of the instrumented CRS-r score (ICRS-r). The ICRS-r score was constructed by adding FP (i.e., items for which the EMG revealed muscular activity in the absence of recognizable behavioral response) to the available CRS-r total score. Figure 1 depicts the results of the statistical comparison between the scores obtained by the CRS-r scale and the corresponding ICRS-r scale for the whole sample, and separately for UWS and MCS patients. The detailed results are here reported as median (25th–75th percentiles) for CRS-r and ICRS-r, respectively: 7 (5–9) and 10 (8–12) for the whole sample ($p = 5.91e-08$); 4.5 (3–6) and 7.5 (5–9) for UWS patients ($p = 3.72e-04$); 9 (8–13) and 12 (9.5–15.5) for MCS patients ($p = 3.39e-05$).

In Fig. 2, the scatterplot of the ICRS-r score (y-axis) with respect to the original CRS-r score (x-axis) is presented. Each point represents a session, and it is light blue or black if the patient was diagnosed as MCS or UWS, respectively. The dimension of the point is bigger if more sessions were characterized by the same score both in the CRS-r and ICRS-r. The panel of interest is highlighted in green and includes nine different sessions in which the patient was behaviorally diagnosed as UWS (black points) but obtained an ICRS-r greater than 7, thus compatible with a MCS diagnosis. The session characterized by CRS-r = 5 and ICRS-r = 7 would also be associated with the MCS group, as the ICRS-r revealed the presence of consistent sEMG responses to the “Consistent Movement to Command” item in the auditory subscale². For these sessions, the ICRS-r would

suggest a higher-level functioning. It is noteworthy that five out of the eight patients who had a CRS-r score of 7 obtained the MCS diagnosis because they exhibited high-level behaviors on a single subscale of the CRS-r².

Considering the separation in the two groups of diagnosis generated by the ICRS-r, the comparison between the obtained median (25th–75th percentiles) *OT_Number* in the whole CRS-r was 16 (7–19) and 27 (21–48.5) for UWS and MCS patients, respectively. These values resulted to be significantly different (p -value = 0.0026).

Discussion

The assessment of the level of consciousness in DoCs patients relies on the presence of behavioral responses according to the CRS-r, which demonstrated strong feasibility, thus becoming the gold standard for the evaluation of this clinical population². However, some limits must be accounted for in a diagnostic evaluation based only on the CRS-r. Indeed, the CRS-r requires stringent criteria to define a certain level of consciousness, based primarily on the ‘voluntary’ or ‘goal-directed’ patients’ motor output clinically detected through visual inspection by the examiner²⁰. The main issue is thus represented by the difficulty in distinguishing goal-directed from non-goal-directed motor outputs at a behavioral level. As a consequence, an ever-increasing body of evidence^{1,3,13,21,22} suggested the adoption of instrumental tools to add valuable information to the actual level of responsiveness of DoCs patients.

To support the complex diagnostic process in DoCs patients, this work presented the STRIVEfc system, a tool that enables the recording of four sEMG channels during the administration of the CRS-r, thus overcoming the limitations of the previous studies adopting the same technique^{15–17}. Indeed, STRIVEfc was employed in the framework of the standard DoCs assessment and guaranteed flexibility during CRS-r administration, in terms of proposed commands (i.e., what is asked in the CRS-r items) and examined muscles: both could be tailored to the patient’s conditions. The STRIVEfc system was used during the CRS-r administration in 33 DoCs patients, for a total of 40 sessions. The sEMG signal was then analyzed to explore differences between patients diagnosed with UWS and MCS (either MCS–, MCS+, or eMCS; aim i) and assess the impact of the system on patients’ diagnosis (aim ii).

In the proposed protocol, the analysis of the EMG signals was particularly complex because it could not follow the EMG processing traditionally adopted to identify volitional activation during movements. Indeed, DoCs patients might not be able to generate the maximal voluntary contraction (MVC) and/or completely relax specific muscles, actions which are required to normalize the acquisitions of interest for the successive analysis. Thus, a calibration phase to identify the baseline was necessary for each channel before starting the CRS-r administration. It consisted of the recording of the sEMG signals without providing external stimuli. However, the strategy was less reliable than anticipated, even though the step of electrodes testing (“sEMG Test” in Fig. 3) was implemented to ensure that the quality of the signal was sufficient. Indeed, the muscles conditions and/or the patient’s posture changed over time in several sessions, introducing slow fluctuations on the recorded baseline, possibly due to the long-lasting CRS-r assessment. This made the automatization process very challenging: to avoid recognizing such fluctuations as a tentative of producing behavioral responses to external stimuli, the automatic approach based on the calibration baseline was replaced by the identification of representing resting activities, given the impossibility to repeat the acquisitions with a different strategy for baseline recording. A second issue was produced by the intrinsic flexibility of the system, allowing the clinician to personalize the choice of the muscles to record on different patients. While maximizing the probability of identifying positive sEMG responses, it reduced the sample size of the activities obtained for each muscle. In our study, this issue was mitigated by performing an analysis merging all the muscles. The approach also aligns with the CRS-r, as it looks for behavioral outcomes in the most responsive body segments, regardless of which they are. This inter-muscular analysis was directly possible on parameters such as the EMG OT activities duration (*Duration_Trial*), but it required further processing for parameters dependent on the EMG signal amplitude (*nRMS_Diff_Trial*). Such a problem was not addressed in the literature. In Bekinshtein and Habbal^{15,16}, only intra-subject and intra-muscle analyses were conducted to assess the degree of difference in the EMG response between target and control commands. On the other hand, the group analysis performed by Lesenfants et al.¹⁷ regarded a parameter, named *EMG score*, depending on the number of detected OT activities only. As a result, a novel methodology was proposed to normalize the sEMG envelopes consisting of two steps. Firstly, the sEMG envelope was normalized by its maximum value, so that the amplitude of all EMG recordings was in the same range ([0,1]). However, a second step was necessary, as the first normalization did not consider any envelope property other than its maximum, thus altering eventual differences among waveforms (e.g., a value equal to 1 in an almost flat envelope or in an envelope characterized by an evident peak would capture different aspects of the muscular activity). The maximum outlier level²³ (a dimensionless measure of OT deviation from its resting threshold) among all OT activities within a session was considered for the second normalization step, to also include information regarding the envelope waveform.

As for the first aim, which focused on the comparison between UWS and MCS sessions, the obtained results confirm the findings by Lesenfants et al.¹⁷ in terms of the number of OT activities, which was higher in the MCS group, although statistical significance was not reached. This trend is also consistent with the clinical definition of MCS, which implies the presence of a wide range of behavioral responses, such as the localization of noxious stimuli and command following¹⁰, and underlying muscular activation. As it was in Habbal et al.¹⁶, a high number of FP (around 40% in the whole sample) was revealed in the current study. A discussion on this topic can be found in the following paragraph. Despite the implementation of a control on the minimum duration of OTs to exclude spasms and the visual inspection of the envelopes to find the most suitable RRA, it is unlikely that all identified OT activities correspond to a volitional, consistent tentative of response to a CRS-r item. It must be noted that the analysis was solely based on the available sEMG signals: after excluding non-meaningful electrodes for specific stimuli, the body segment the behavioral response was expected from was not a variable available for more precise investigations. The evaluation carried out in the literature^{15–17}, where the same body

Parameter	Scale or subscale	N UWS, N MCS	UWS	MCS	p-value
Duration_Trial [s]	CRS-r	292, 579	1.80 (0.89–3.22)	2.29 (1.15–4.50)	3.04e-04
Duration_Trial [s]	Auditory	128, 209	1.76 (0.90–2.75)	2.01 (1.07–3.88)	0.037
Duration_Trial [s]	Visual	49, 127	1.01 (0.65–1.94)	1.91 (0.92–3.31)	0.003
Duration_Trial [s]	Motor	112, 243	2.61 (1.12–4.55)	2.98 (1.35–5.48)	0.102
nRMS_Diff_Trial [μV]	CRS-r	292, 579	0.17 (0.11–0.26)	0.19 (0.11–0.28)	0.573
nRMS_Diff_Trial [μV]	Auditory	128, 209	0.15 (0.09–0.22)	0.20 (0.10–0.28)	0.001
nRMS_Diff_Trial [μV]	Visual	49, 127	0.19 ± 0.11	0.19 ± 0.10	0.825
nRMS_Diff_Trial [μV]	Motor	112, 243	0.23 (0.15–0.31)	0.17 (0.11–0.28)	0.006
OT_Number [#]	CRS-r	16, 24	21.50 (16.00–29.50)	26.50 (18.00–52.00)	0.147
OT_Number [#]	Auditory	16, 24	10.00 (4.00–11.50)	8.00 (4.00–17.00)	0.638
OT_Number [#]	Visual	16, 24	3.50 (0.00–5.50)	6.00 (2.50–13.50)	0.070
OT_Number [#]	Motor	16, 24	8.00 (3.50–10.00)	12.00 (5.00–19.00)	0.103

Table 3. Results of the comparison between the UWS and MCS sessions in terms of the parameters of interest obtained from the sEMG analysis. The numerosity (N UWS, N MCS) and mean ± standard deviation or median (25th–75th) is reported for the CRS-r and for the auditory, visual, and motor subscales. Significant values are given in bold.

	TP	TN	FP	FN	tot	TP/tot	TN/tot	FP/tot	FN/tot
CRS-r items	48	63	90	25	226	21.2%	27.9%	39.8%	11.1%
CRS-r items UWS	12	27	41	3	83	14.5%	32.5%	49.4%	3.6%
CRS-r items MCS	36	36	49	22	143	25.2%	25.2%	34.3%	15.4%
Auditory subscale items	22	15	30	13	80	27.5%	18.8%	37.5%	16.2%
Auditory subscale items UWS	10	9	13	2	34	29.4%	26.5%	38.2%	5.9%
Auditory subscale items MCS	12	6	17	11	46	26.1%	13%	37%	23.9%
Visual subscale items	16	22	19	9	66	24.2%	33.3%	28.8%	13.6%
Visual subscale items UWS	1	7	12	1	21	4.8%	33.3%	57.1%	4.8%
Visual subscale items MCS	15	15	7	8	45	33.3%	33.3%	15.6%	17.8%
Motor subscale items	9	22	41	3	75	12%	29.3%	54.7%	4%
Motor subscale items UWS	0	7	16	0	23	0%	30.4%	69.6%	0%
Motor subscale items MCS	9	15	25	3	52	17.3%	28.9%	48%	5.8%
Verbal subscale items	1	4	0	0	5	20%	80%	0%	0%
Verbal subscale items UWS	1	4	0	0	5	20%	80%	0%	0%
Verbal subscale items MCS	0	0	0	0	0	N.A.	N.A.	N.A.	N.A.

Table 4. The comparison of the behavioral responses (true values) and the identified OT EMG activity (predicted values) in terms of true positive (TP), true negative (TN), false positive (FP), false negative (FN) and their percentage for items separately for the UWS and MCS sessions and across subscales.

segment was evaluated repeatedly for a limited number of target commands, was not possible in the current protocol. The trend of the *nRMS_Diff_Trial* parameter was not univocal. While significantly higher for the MCS group in the auditory subscale, the opposite was found in the motor subscale. This result could partially be explained by the stronger differentiation between the two diagnostic groups in the auditory behavioral responses rather than in the motor ones. In the UWS sessions, flexion withdrawal was recognized in seven sessions out of 16: such reflexes could have been characterized by a marked electromyographical activation, even higher than the MCS patients’ ones. The existing literature does not offer a direct comparison either, as in the already cited studies amplitude parameters were compared intra-subject between target command and control command or resting states. Here, the comparison was with the corresponding RRA, which was visually identified in the signal: it cannot be excluded that fluctuations in the RRA altered the parameter computation. Surely, signal normalization had an impact on this parameter and is a topic that warrants further study in the future: as already mentioned, the comparison of amplitude-based sEMG parameters between subjects posed a great challenge in the current work, given the absence of MVC and a “real” relaxation phase. Lastly, *Duration_Trial* revealed a neat trend, with OT durations significantly greater in the MCS group. This result is robust, as the parameter depended exclusively on the logic of OTs detection. Although this parameter is not investigated in the literature, it could suggest the increased capability of MCS patients to perform sustained contractions – not always translated into a behavioral response – during CRS-r administration.

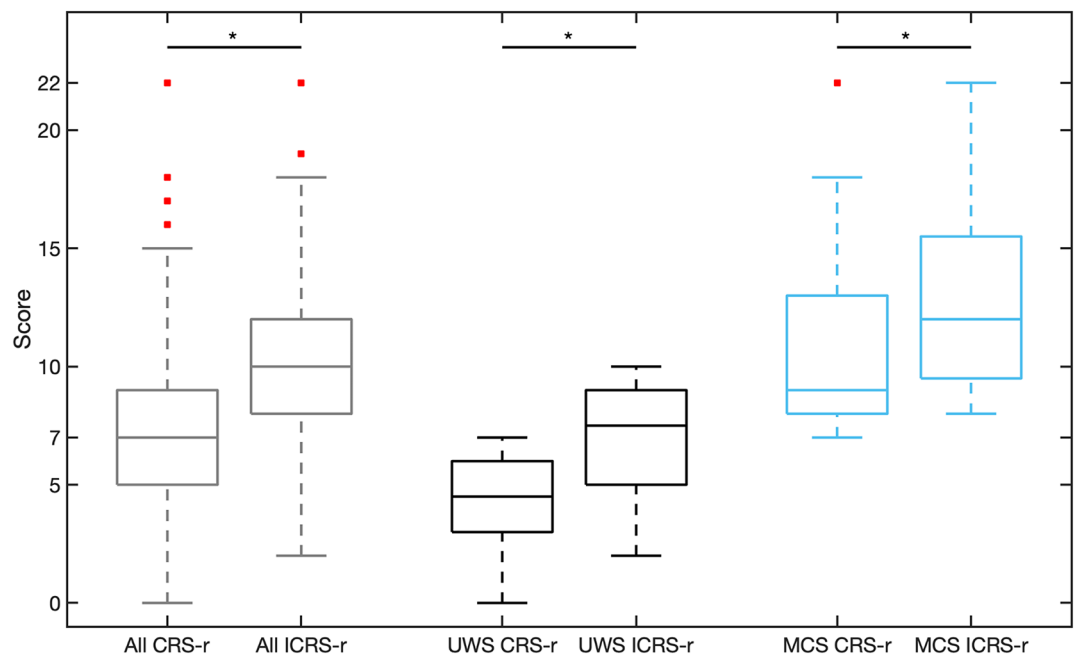


Fig. 1. CRS-r (first box in each pair) vs. ICRS-r (second box in each pair) scores boxplots. The boxplots are represented for all sessions (grey boxes on the left), UWS sessions (black boxes in the center) and MCS sessions (light blue boxes on the right). The horizontal black lines with the ‘*’ symbol indicate a statistically significant difference between the distributions.

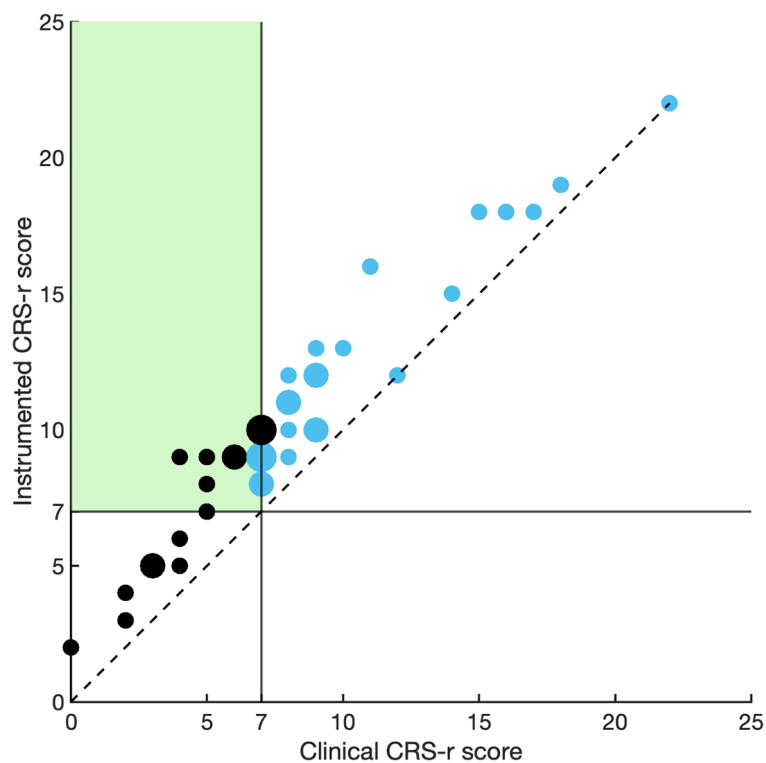


Fig. 2. CRS-r vs. ICRS-r scores scatterplot. The black and blue dots represent sessions in which the patients were diagnosed as UWS and MCS respectively. The size of the dot is larger if there are multiple sessions with the same values (x, y). The green area represents the patients for whom the ICRS-r would suggest a higher level of functioning.

The STRIVEfc system allowed to compare the gold standard evaluation performed by the clinician during the administration of the CRS-r with the evaluation produced after the sEMG data analysis and the identification of OT sEMG activities (ICRS-r, aim ii). Firstly, the degree of agreement was measured at the item trial and item levels. Starting with the results on the CRS-r, the high rate of FPs at the item level should not lead to the conclusion that the proposed system is oversensitive, given the peculiar scenario where it is applied. Indeed, the DoCs diagnostic

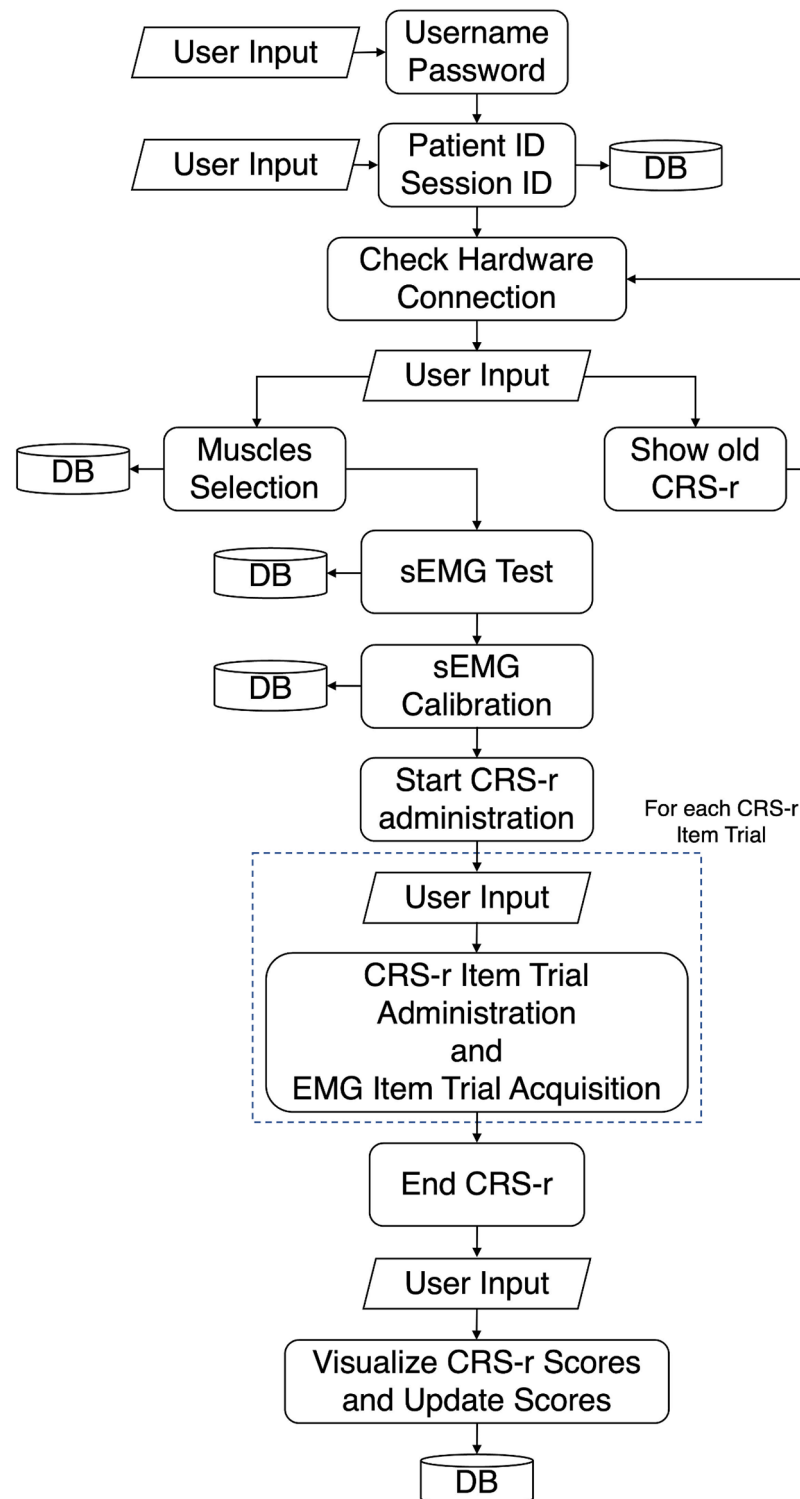


Fig. 3. Workflow of the STRIVEapp for CRS-r administration and sEMG recording. The “User Input” blocks refer to inputs required from the user, which can be either the insertion of a field (e.g., password or selected muscles) or the clicking of a button (e.g., visualization of old CRS-r or selection of CRS-r item). The “DB” blocks refer to all the information stored in the database at the end of the administration.

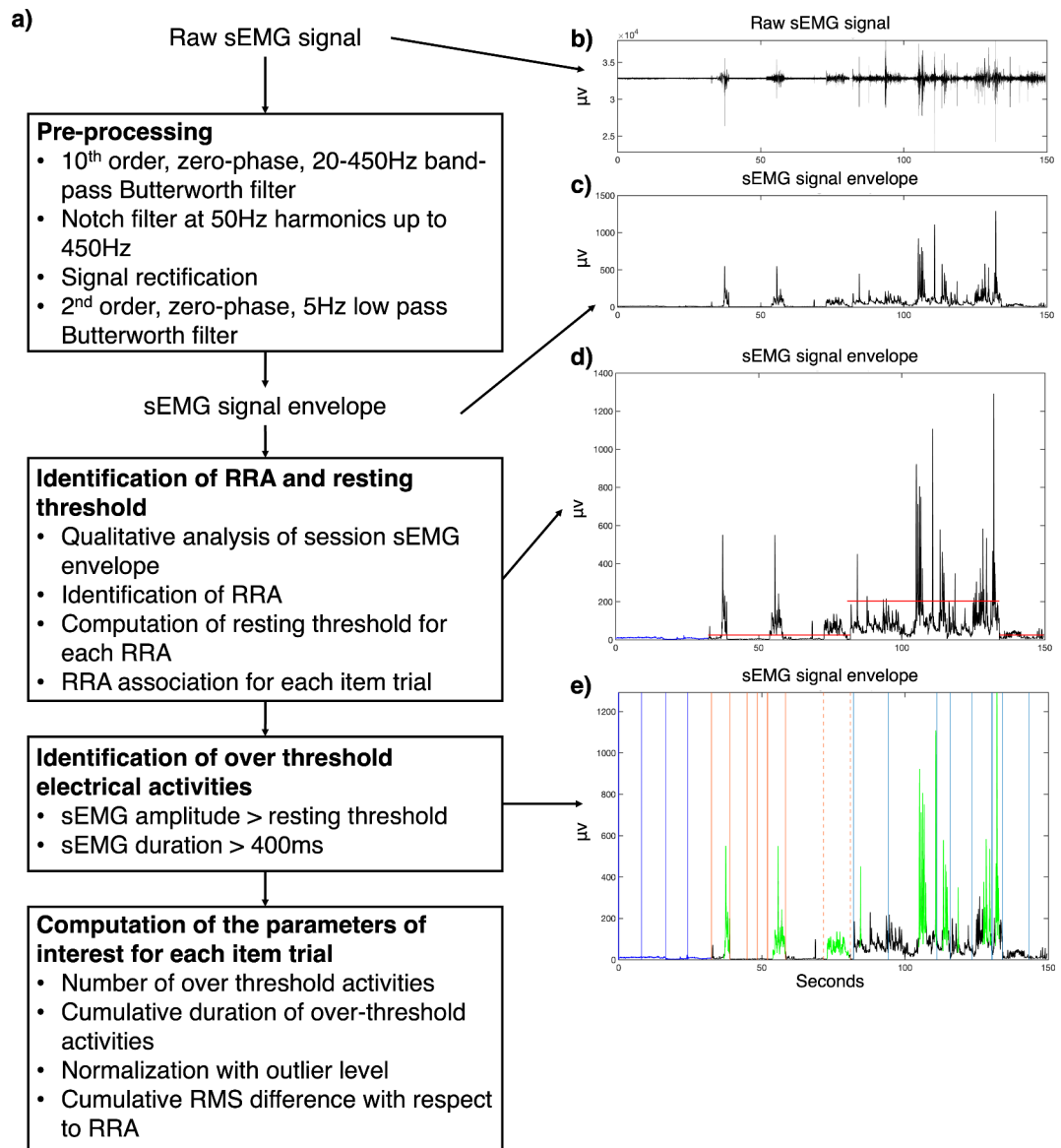


Fig. 4. (a) The four steps of the EMG analysis workflow. (b) sEMG raw signal recorded on the left flexor digitorum superficialis of patient 30 during the whole CRS-r session. (c) sEMG signal envelope. (d) Solid blue line: sEMG envelope during the calibration phase. Solid black line: sEMG envelope during CRS-r administration. Horizontal red lines: resting thresholds computed from RRA. (e) Solid green line: identified overthreshold activities. Each vertical line corresponds to the beginning of an item trial. Vertical orange lines: auditory subscale item trials. Vertical light blue lines: motor subscale item trials. Solid vertical lines: item trials rated as “YES”. Dashed vertical lines: item trials rated as “NO”. Here, the signal acquired in the calibration phase (solid blue line from 0 to 33 s) was considered as RRA for the sEMG acquisitions during the CRS-r (solid black line) in the ranges [33; 82) seconds and (135; 150] seconds. In the range [82; 135] seconds, a change in the resting activity was recognized. In this time interval, the signal itself was considered as RRA. The corresponding thresholds are reported as horizontal red lines.

process typically aims at finding elements that can exclude UWS, in favor of MCS or better diagnosis; an aspect that is particularly critical in countries where the UWS diagnosis implies, after a certain amount of time, the start of an end-of-life treatment. This is coupled with the intrinsic bias of the gold standard CRS-r, which is based on the detection of behavioral responses of patients whom behavior, in the sense of presence of perception, is most of the times absent. These are the reasons why support tools like STRIVE_{efc} are necessary. Their added value, that is the ability of detecting muscular activities/response attempts not recognizable by the naked eye of a trained clinical expert, must translate in a certain number of FPs with respect to the clinical gold standard: a low rate of FP would undermine the utility of the proposed approach, revealing a system that is not able to add enough information to what clinicians already identify. Thus, a FP in the proposed analysis should not always be interpreted as a diagnostic error, particularly if one considers that the consistency of the muscular response

across item trials was a key concept in the creation of the contingency table. Although establishing an “optimal” FP rate is not a straightforward task, the result of the current study is in line both with the misdiagnosis rate of the CRS-r⁴ and the percentage of FP found in Habbal et al.¹⁶ Regarding FNs, their percentage was always the lowest when considering both the whole CRS-r and the single subscales (see Table 4), independently of the considered sample. This finding suggests that the system is, in general, likely to recognize the presence of behavioral responses detected by the examiner. A possible reason behind FNs could lie in the occurrence of reactions to CRS-r stimuli in body districts not measured by the sEMG probes. While, on one hand, a low FN rate is beneficial, on the other the presence of FNs should not be critical from a practical standpoint in the DoC context: being able to uncover hidden patterns should in principle be more valuable than confirming what the clinician can recognize behaviorally. When comparing separately UWS and MCS patients, interesting trends emerged: (i) the percentage of TP was greater in MCS than in UWS, given the better preserved volitional function in patients with less severe DoCs; (ii) the percentage of FN was greater in MCS than in UWS because the former are more reactive and they are likely to produce a higher number of consistent responses, possibly in districts that are not measured by the four sEMG channels; (iii) the percentage of FP in UWS is about the 50% of the whole EMG activities and higher than in MCS, underlying a high probability of consistent EMG activity generation in response to a stimulus, which is not translated into visible movements. In investigating FP and FN distributions in the single subscales, the motor one always showed the highest percentages of FPs and the lowest of FNs. This result is in line with the electrodes placement: being typically applied on arms and legs muscles, they were more suitable for the identification of EMG responses to the motor subscale stimuli, independently of the detection of a behavioral response. Relevant percentages of FPs were identified also in the auditory and visual (for UWS in particular) subscales, a trend that can be related to patient’s attempts of limbs activation in their high-level stimuli (e.g., reproducible movement to command or object localization). These findings seem to be supported by the results obtained at the muscle-level, where flexor and extensor carpi ulnaris, biceps brachii and tibialis anterior, all muscles that should be involved in the motor responses to the mentioned stimuli, accounted for around 60% of all the identified FPs. It is worth noting that the corrugator supercilii showed a relevant percentage of FP (22.22%) in the subscale where the muscle activation should be expected: the visual one. This suggests the potential utility of such a muscle for the characterization of responses related to visual items. False negatives were almost entirely located in the auditory and visual subscales, an outcome that is coherent with some of the behavioral responses which they try to elicit and the typically adopted electrodes configuration. Again, the result seems to be confirmed by the muscles present in the sessions characterized by FNs: in addition to the four mentioned for the FPs, abductor pollicis brevis and gastrocnemius medialis were the most common. Possible improvements regarding FNs are discussed in the paragraph dealing with the study limitations.

Most importantly, FPs were used to create the ICRS-r score. Sessions behaviorally evaluated as UWS were characterized by ICRS-r scores exceeding the CRS-r ones in 16/16 cases, a trend reaching statistical significance. Notably, a considerable part of such sessions seemed to hide a relevant higher level of functioning. Indeed, the ICRS-r score was suggestive of a potential change of diagnosis from UWS to MCS in nine out of 16 sessions evaluated in our sample. The existing literature underlines that up to 40% of UWS patients are wrongly classified and that new instrumental tools such as STRIVEfc that can enhance responses not detectable by the examiner could be very ground-breaking in the diagnostic work-up of DoCs, especially when a diagnosis between UWS and MCS- is required^{15,24,25}. Furthermore, the overall ICRS-r score was significantly greater than the overall CRS-r score also in the MCS group. Therefore, our data endorsed the hypothesis that all DoCs patients may have a higher level of functioning, especially UWS patients who have hidden (to the examiner’s eye) behaviors that can be decisive for diagnostic change, if revealed. Overall, the use of systems such as STRIVEfc appears useful to better understand the patient’s real functional picture, even when the diagnosis of MCS is not reasonably in doubt. Although our data must be confirmed by a larger sample of patients, the use of the STRIVEfc system is promising, because it would support and refine the ability to detect muscular responses in DoCs patients. Therefore, its use in clinical practice could also have a great impact on the choice of personalized rehabilitation or clinical pathways.

The protocol was not immune from shortcomings. Given the pool of heterogeneous behaviors investigated by the CRS-r, ranging from auditory/visual startle to reproducible response to command, four sEMG signals were not sufficient on their own for the characterization of the entire scale. For example, the addition of the sEMG recording on upper and lower limbs was not relevant when assessing items in which the muscular response on the target muscles was not prevalent (e.g., sound localization, visual fixation and pursuit, verbal subscale). This aspect was considered during the STRIVEfc development, when system usability during the standard routine was given priority over the amount of information provided. Indeed, the system was designed to enrich the traditional assessment, rather than to replace it. The placement of the four couples of electrodes was then chosen by the examiners according to the patient’s clinical history, their behavior at the time of acquisition with STRIVEfc, and what the examiners were interested in characterizing. The collected data revealed that the electrodes were placed mainly on flexor carpi ulnaris, extensor carpi ulnaris, biceps brachii and tibialis anterior. Therefore, limbs activation, involved in the motor subscale but also in the high-level items of auditory and visual subscales, were of the highest interest for the examiners. Despite excluding the CRS-r items for which the electrodes placement could not allow the identification of muscular activity (e.g., visual pursuit without electrodes mounted near the eye), the presence of behavioral responses not identified by sEMG analysis (FNs) occurred, particularly in the auditory and visual subscales. This was likely due to the generation of responses on non-mounted body districts. Given these outcomes, further improvements can be envisaged. Probes can be added to reduce the occurrence of FNs due to activations in body segments where the electrode is not present, which is in principle possible because the adopted architecture is modular. Other wearable technologies can be added, such as the electro-oculography²⁶ for the characterization of the visual subscale or the EEG, providing info on cerebral activation during the auditory, visual and verbal subscales. Such additions should reduce the FN

rate found in the study, particularly in the auditory and visual subscales, while further improving the capability to identify behavioral responses or at least an attempt to generate them, possibly turning into a higher number of FPs. Finally, the proposed approach cannot solve the misdiagnoses due to cognitive motor dissociation¹⁸, since it is able to investigate patient's motor activation patterns only.

The main limitation of the EMG data analysis was the identification of a reliable RRA when baseline oscillations or changes in posture prevented the use of the initial calibration acquisition. On one hand, the duration of the available baseline recordings could have been not sufficient to capture the full range of resting activity fluctuations. Therefore, using the baseline directly would have led to inaccuracies in threshold computation. On the other, despite necessary for the entire analysis, RRAs were derived by visual inspection, also considering sEMG signals recorded during the administration of CRS-r stimuli. This operation could have introduced a bias in the threshold setting, as in these instances patients were explicitly asked to produce a behavioral response. To mitigate this pitfall, the duration of baseline recordings should be increased and/or relaxation phases coupled with sEMG recording should be added before the beginning of each subscale or each item. This change would make the process completely automatic, even if the impossibility for the patient of performing a real relaxation phase when requested remains an open point. Moreover, caution should be put on the increase in the administration time, as the patients' level of attention could be subject to fluctuations, thus undermining the validity of the CRS-r assessment.

Our study presented the STRIVEfc system, a wearable device able to record the sEMG activity of four muscles in total, chosen by the clinician according to the single patient's conditions during the administration of the CRS-r behavioral assessment. This system has the potential to provide a more comprehensive characterization of the patient's motor responsiveness, without altering how the gold standard clinical scale is administered in clinical practice. The combined information would in turn enhance the possibility to identify muscular responses not observable to the naked eye of the clinician, thus improving the capability of a correct diagnosis mainly based on the classification of the patient as MCS or UWS. Moreover, the assessment supported by STRIVEfc is also less affected by the inter-rater reliability characterizing the CRS-r scoring, particularly when administered by non-expert clinicians. The use of STRIVEfc in the clinical routine would have a great impact on the diagnosis and, consequently, on the rehabilitative strategy of DoCs patients.

Materials and Methods

Participants

Patients were enrolled according to the following inclusion criteria: (i) being older than 18 years; (ii) confirmed diagnosis of DoCs following acquired brain injury. Patients suffering of major neurological and/or psychiatric disorders before the brain injury were excluded from the study. Patients were evaluated with the CRS-r, administered with the STRIVEfc system, and their diagnosis was determined accordingly as UWS, MCS-, MCS+, or eMCS. The study was part of a national multicentric clinical trial (EudraCT Number: 2019-001898-87, start date 26/09/2019) aimed at increasing the diagnostic accuracy in the DoCs and evaluating the tolerance and the efficacy of sleep disorders' treatments in patients suffering from DoCs after severe acquired brain injury.

The study was conducted in accordance with the Declaration of Helsinki, and it received approval from the ethics committee of the Fondazione-IRCCS-Istituto Neurologico "Carlo Besta" of Milan (approval number 51/2018). The informed consent was obtained from the patient's legal representative prior to the study enrolment.

STRIVEfc system

The STRIVEfc system allows the administration of the CRS-r, combined with the simultaneous recording of four sEMG signals, able to characterize patient's motor responses. The system consists of four main components: (i) two 2-channel bipolar sEMG probes (for a total of four channels), for the acquisition of the sEMG signals; (ii) the STRIVEapp, an iOS application running on an Apple iPad*, which manages the CRS-r administration and coordinates the entire system; (iii) a Raspberry Pi 3* (www.raspberrypi.com) system, acting as a middleware for the communication between the EMG probes and the STRIVEapp; (iv) a Firebase cloud database (firebase.google.com/docs/database), for data storage.

sEMG probes

The selected sEMG probe is the DueLite™, produced by OTBioelettronica (www.otbioelettronica.it), a wireless wearable device that allows the safe recording of sEMG at the patient's bedside. A single probe (47 × 47 × 11 mm) includes two channels to be used with disposable, bipolar surface Ag/AgCl electrodes. The probes record the sEMG signal with a 2048 Hz sampling rate in the 10–500 Hz bandwidth, and store the data for their successive wireless communication, exploiting a USB dongle and Bluetooth 4.0 protocol.

Middleware

Given that the DueLite™ is not configured to communicate with Apple* devices, a Raspberry Pi 3* is introduced as a middleware: it mediates the communication between the probes and the STRIVEapp during the CRS-r administration, as described later. Moreover, it receives the data from the DueLite™ probes and transmits them to the Firebase cloud database. The middleware works as a background device, requiring internet and USB dongle connection but no user inputs.

STRIVEapp

The STRIVEapp was designed as a graphical user interface (GUI), guiding the clinical user through the CRS-r administration combined with EMG acquisition, while managing the communication with the middleware and the cloud database. The GUI was developed in collaboration with the clinical staff involved in the study, to adapt to the clinical needs and guarantee ease of use.

CRS-r administration and sEMG acquisition

The STRIVEapp workflow is reported in Fig. 3. In the flowchart, the word “User” always refers to the clinician administering the CRS-r, while the “DB” blocks refer to information being stored on the cloud database. In the following, the procedure of CRS-r administration and sEMG acquisition is described referring to Fig. 3 flowchart, starting from the “Muscles Selection” block. Detailed explanations of the previous steps can be found in the Supplementary Methods.

Muscles selection

The selection of the four muscles for applying sEMG electrodes was made by the clinician considering the spontaneous movements exhibited by the patients and their clinical conditions at the time of acquisition, as the STRIVEfc system does not impose any restriction. Specifically, clinicians selected the muscles of major interest according to the patient’s residual motor activity they were interested in characterizing during the CRS-r. Before electrodes application, the skin was properly cleaned using an alcohol swab to remove surface oils or other contaminants and reduce the impedance.

sEMG test

After connecting the electrodes to the probes and placing them on the selected muscles, their functioning in real time was tested through the app, which displays the acquired sEMG signals, separately for each channel. This operation could be repeated until the signal quality was considered appropriate by the examiner. If not the case, the electrodes could be removed, and new ones could be applied and tested. Once all the sEMG channels were successfully tested, the following step could take place.

sEMG calibration

The STRIVEapp required a calibration acquisition. Specifically, the sEMG signal for each channel was recorded in resting conditions, without providing any stimuli to the patient and without duration limits. A minimum duration of at least 10 s was recommended. The sEMG signals collected in this phase were stored and subsequently used as a baseline for the data analysis.

Start CRS-r administration

After the hardware set-up and the recording of the baseline signals, the actual administration of the CRS-r could start. Usually, the CRS-r includes the evaluation of six subscales (arousal, auditory, visual, motor, verbal, and communication), each made up of several items requiring the standardized administration of sensory stimuli to elicit a specific behavior. The inverted administration procedure described by Sattin et al.^{27,28} was adopted, starting from items representing lower reflexive behaviors and moving upwards to the higher cognitively mediated items. Each CRS-r item was administered taking into account the patients’ arousal fluctuations: the bedside evaluation was conducted by two examiners expert in the field (F. G. M., M. C., C. I. or F. B.) while the patient was awake (open eyes), and without environmental interference or factors affecting and modulating the patient’s arousal. Sensory stimulation for each item was repeated several times (varying according to the item, as stated into the CRS-r manual) to assess consistency in the behavioral response. The single repetition of the same sensory stimulation within an item is called “item trial” in the following. The absence of behavioral responses in two consecutive items of a given subscale caused the subscale to end. The examiner then moved on to the following one.

CRS-r item trial administration and sEMG item trial acquisition

For each item trial, as soon as one operator started the sEMG acquisition of all four channels on the STRIVEapp, the other examiner delivered the stimulus. For the administration of an item trial, the recorded data consisted of the examiner’s answer (“YES” in presence of a behavioral response, “NO” otherwise) and the four-channel sEMG recordings. Once completed the item administration, the examiner noted on the STRIVEapp whether the item was credited or not (encoded as 1 or 0, respectively), following the CRS-r guidelines². For each subscale, when all the items belonging to it were assessed, the subscale score was automatically assigned by the STRIVEapp considering the highest credited item within the subscale.

End CRS-r

After the examiner’s confirmation of the assigned scores (the app allows their revision), the session could be closed and the CRS-r total score and modified score²⁷ could be obtained. All the data generated during the procedure were stored in the cloud database in a single object, containing:

- Information regarding the session (patient’s ID, session ID, timestamp, and muscles considered for sEMG acquisition).
- CRS-r scores, including the CRS-r total score, the subscales scores, the information on item crediting and the item trial answers.
- sEMG recordings (divided in test, calibration, and CRS-r recordings).

Data analysis

Following the literature^{15–17}, data analysis focused on the identification and quantitative characterization of over threshold sEMG activities, interpreted as the subject’s volitional tentative to produce a movement in response to a CRS-r stimulus. The aim was to identify potentially relevant characteristics which were not detected through the standard behavioral assessment. Importantly, item trials in which the combination of electrodes placement and observed CRS-r response made the identification of sEMG activity meaningless (e.g., items in the visual

subscale which look for visual pursuit and electrodes placed on the biceps brachii) were excluded from the analysis.

From the cloud database, data were imported in MATLAB R2021b[®] for the analysis. As detailed in Fig. 4, panel (a), the EMG analysis is composed of four steps: (1) pre-processing, (2) identification of representative resting activities (RRA) and resting thresholds, (3) identification of over threshold electrical activities, (4) computation of the parameters of interest.

Pre-processing

In the pre-processing, all the sEMG signals were filtered with a 10th order, zero-phase, band-pass Butterworth filter between 20 and 450 Hz. The frequency band was chosen to preserve most of the signal energy, while removing low frequency oscillations due to motion artefacts and the high frequency noise. Moreover, a notch filter bank was applied to remove the net interference at 50 Hz and at its harmonics up to 450 Hz. The harmonics were taken into account in the filtering as the patient could have been connected to other electronic devices at the bedside, but the EMG probes did not include a reference electrode. After filtering, the signal was rectified, the mean removed and the envelope extracted applying a 5 Hz, zero-phase, 2nd order low pass Butterworth filter. An example of the acquired raw signal and the resulting envelope are depicted in Figure 4 panels b) and c), respectively.

Identification of RRA and resting threshold

The second step of the EMG processing was devoted to the identification of RRA and resting thresholds, basing on the signals acquired during the whole procedure (from the calibration phase to the last administered item trial Fig. 4, panel c), which will be referred to as “session sEMG”. The signal acquired in the calibration phase was intended to be exploited as a baseline for the identification of over threshold electrical activity in each channel separately. This was deemed necessary as the traditional procedure, relying on the identification of a relaxation phase and a maximal voluntary contraction (MVC), could not be applied in DoCs patients.

However, changes in the baseline values over time occurred repeatedly, making the identified threshold not always representative of the subject's resting activity. Thus, considering only the calibration signal would have led to the identification of over threshold segments representing fluctuations of the resting activity, rather than responses to a CRS-r stimulation. For this reason, the data were analyzed as follows to extract RRA:

- Visual inspection of the session sEMG in order to exclude epochs severely polluted by artefacts.
- Identification of representative resting activities, featuring only random fluctuations around a mean value. RRA were identified within the session sEMG, either in the calibration phase or during CRS-r administration. In the latter case, they could consist of a single item trial or the concatenation of multiple, consecutive item trials. Importantly, within a session sEMG, different RRA could be identified, but each item trial could be assigned only one RRA.
- Computation of the resting threshold for each RRA. The threshold T was computed as: $T = \mu + J \cdot S$, where μ and S represent the mean and standard deviation of the RRA samples lower than or equal to the RRA median value, respectively. This choice allowed excluding relevant sEMG peaks when the RRA was selected within the CRS-r item trials. The constant J determines the tolerance for over threshold signal detection. After reviewing the literature^{29,30} and examining the available data, J was set equal to 10.

Figure 4, panel d shows an example of RRA identification.

Identification of over threshold electrical activities

The third step of the EMG processing was the identification of the over threshold activities, as a possible indicator of residual motor ability. An over threshold activity was identified if the sEMG envelope was higher than the corresponding RRA threshold for at least 400 ms. This constraint was imposed to remove involuntary, reflexive activities and/or movement disorders from the analysis as they did not represent a voluntary behavioral response³¹. In Fig. 4, panel (e), the identified over threshold activities are shown.

Computation of the parameters of interest for each item trial

Finally, the fourth step of the EMG processing consisted in the computation of the following parameters for each identified over threshold activity:

- The activity duration (*Duration*) in seconds.
- The difference between the root mean square (RMS) of the over threshold activity and the RMS of the corresponding RRA (*RMS_Diff*), as measured in¹⁷.

To allow inter-muscles and inter-subjects comparisons, normalization was necessary for the *RMS_Diff* parameter. Given the unavailability of the MVC, the following procedure was adopted:

- The Outlier Level ($[\max(envelope) - RRA\ threshold] / standard\ deviation(envelope)$) was computed for each over threshold activity. This quantity measures the over threshold activity deviation from its corresponding threshold.
- The envelope was divided by its maximum value, yielding a signal bounded in the [0; 1] range, independently of the original envelope waveform.
- The parameter was computed on the normalized envelope.
- Within a given channel, the parameter was normalized by the highest Outlier Level throughout the session sEMG (not considering the calibration phase), yielding the *nRMS_Diff* parameter.

Given that multiple over threshold activities could be detected within a single item trial, aggregated results were computed: *Duration_Trial*, computed by summing the *Duration* of the single over threshold activities, and *nRMS_Diff_Trial*, computed as the weighted temporal average of *nRMS_Diff* over the single over threshold activities. The last parameter was the number of over threshold activities (*OT_Number*) for each patient.

Statistical analysis

The statistical analysis was conducted in MATLAB R2021b*.

Aim (i)

To achieve the first aim of the study, i.e., the comparison between patients diagnosed as UWS and the ones diagnosed as MCS (either MCS−, MCS+, or eMCS), three analyses were performed. At first, their demographic and clinical characteristics, namely age and education in years and time since injury (TSI) in months, were compared with the appropriate statistical test for independent samples (either the two-sample *t*-test or the Mann-Whitney *U* test) after assessing whether they were normally distributed with the Shapiro-Wilk test. Sex distribution was studied with the chi-squared test.

Then, the two groups were compared in terms of the scores obtained in the CRS-r and its subscales. Finally, a comparison of the parameters extracted by the EMG signal was performed. After assessing whether the parameters were normally distributed using the Shapiro-Wilk test, the two-sample *t*-test was applied for normally distributed indicators, while the Mann-Whitney test was considered otherwise. The UWS/MCS comparisons were conducted considering the CRS-r total score and the single subscales score, when the sample size made the analysis possible, including all examined muscles, as the STRIVEfc system was conceived to be used during the standard CRS-r assessment. Given the different clinical conditions each patient presented with, the focus was the characterization of the muscles preserving the highest amount of motor activity. Such an assessment, independently of the specific muscles, would provide information about the two diagnostic groups' capability to produce a volitional response to the CRS-r stimuli.

Aim (ii)

To achieve the second aim of the study, i.e., to evaluate the impact of the sEMG analysis on the patients' diagnosis, the instrumented CRS-r score (ICRS-r) was computed using the information provided by the sEMG activities recorded by the STRIVEfc system. Specifically, each item trial was assigned score 1 if an over threshold sEMG activity was detected, 0 otherwise. Then, similarly to what is done with items crediting in the CRS-r, each item was either "instrumentally" credited or not credited, according to the number of item trials scored 1 required to credit the specific item. A contingency table on the CRS-r items was constructed, the CRS-r score acting as the gold standard. The table yielded: True Positives (TP) and True Negatives (TN) when the item was credited or not, respectively, by both CRS-r and sEMG analysis; False Positives (FP) when the item was credited by sEMG activities but not by the operator; False Negatives (FN) if the opposite happened. The distribution of TPs, TNs, FPs and FNs was evaluated also across the CRS-r subscales. For FPs and FNs, a muscle-level investigation was conducted, considering the global CRS-r. To compute the ICRS-r score, the number of FP was added to the available CRS-r score, to combine both behavioral and instrumented information.

A paired-samples hypothesis test – either the paired-sample *t*-test or the Wilcoxon signed rank test – was run to statistically compare the CRS-r and ICRS-r scores, both on the whole sample and separately for UWS and MCS patients. Finally, the number of potential changes in diagnosis due to the use of the ICRS-r score was evaluated, together with the comparison of the *OT_number* parameter on the whole session between ICRS-r-diagnosed UWS and MCS patients.

All statistical tests were two-tailed and conducted with the significance level set at 5%.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

Conceptualization, D.S., D.S.R., and S.F.; methodology, S.F., M.M., F.L., S.T., D.S., and D.S.R.; software, S.F., M.M., F.L., and S.T.; formal analysis, S.T.; investigation, F.G.M., and M.C.; project administration, D.S., F.G.M., and M.C.; resources, J.N., G.D., V.P., and M.L.; data curation, M.M., S.T., F.G.M., and M.C.; visualization: S.T., F.G.M., M.C., C.I., and F.B.; writing—original draft preparation, F.G.M. and S.T.; writing—review and editing, M.C., C.I., F.B., J.N., G.D., M.L., V.P., S.M., L.F.S., M.L., D.S.R., D.S., and S.F.; supervision, D.S.R., D.S., S.F., and M.L.; funding acquisition, D.S., D.S.R., S.F., S. M., L.F.S., and M.L. All authors have read and agreed to the published version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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