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PII: S1389-9457(20)30018-6

DOI: <https://doi.org/10.1016/j.sleep.2019.12.030>

Reference: SLEEP 4289

To appear in: *Sleep Medicine*

Received Date: 26 June 2019

Revised Date: 19 November 2019

Accepted Date: 23 December 2019

Please cite this article as: Betta M, Handjaras G, Ricciardi E, Pietrini P, Haba-Rubio J, Siclari F, Heinzer R, Bernardi G, Quantifying peripheral sympathetic activations during sleep by means of an automatic method for pulse wave amplitude drop detection, *Sleep Medicine*, <https://doi.org/10.1016/j.sleep.2019.12.030>.

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Author contributions (CRediT taxonomy)

Conceptualization, M.B., J.H.R., R.H.,G.B., F.S.;

Methodology, M.B.,G.H.;

Formal Analysis, M.B.;

Visualization, M.B.;

Resources, E.R., R.H.;

Software, M. B.;

Supervision, E.R., F.S., R.H., G.B.;

Writing – Original Draft, M.B., G.B.;

Writing – Review & Editing, M.B., G.H., P.P., E.R., J.H.R., F.S., R.H., G.B.

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1 **Quantifying peripheral sympathetic activations during sleep by means of an automatic method**
2 **for pulse wave amplitude drop detection**

3
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13
14 **Short Title:** Detection of sleep-related pulse wave amplitude drops

15
16 Abstract word count:250

17 Introduction word count:849

18 Discussion word count: 1334

19 Total word count:7926

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27 **Conflict of Interest**

28 The authors have no conflict of interest to declare.

29
30 **Author contributions (CRediT taxonomy)**

31 Conceptualization, M.B., J.H.R., R.H.,G.B., F.S.; Methodology, M.B.,G.H.; Formal Analysis,
32 M.B.; Visualization, M.B.; Resources, E.R., R.H.; Supervision, E.R., F.S., R.H., G.B.; Writing –
33 Original Draft, M.B., G.B.; Writing – Review & Editing, M.B., G.H., P.P., E.R., J.H.R., F.S., R.H.,
34 G.B.

35

36 **ABSTRACT**

37 Sudden drops in pulse wave amplitude (PWA) measured by finger photoplethysmography (PPG)
38 are known to reflect peripheral vasoconstriction resulting from sympathetic activation. Previous
39 work demonstrated that sympathetic activations during sleep typically accompany the occurrence of
40 pathological respiratory and motor events, and their alteration may be associated with the arising of
41 metabolic and cardiovascular diseases. Importantly, PWA-drops often occur in the absence of
42 visually identifiable cortical micro-arousals and may thus represent a more accurate marker of sleep
43 disruption/fragmentation. In this light, an objective and reproducible quantification and
44 characterization of sleep-related PWA-drops may offer a valuable, non-invasive approach for the
45 diagnostic and prognostic evaluation of patients with sleep disorders. However, the manual
46 identification of PWA-drops represents a time-consuming practice potentially associated with high
47 intra/inter-scorer variability. Since validated algorithms are not readily available for research and
48 clinical purposes, here we present a novel automated approach to detect and characterize significant
49 drops in the PWA-signal. The algorithm was tested against expert human scorers who visually
50 inspected corresponding PPG-recordings. Results demonstrated that the algorithm reliably detects
51 PWA-drops and is able to characterize them in terms of parameters with a potential physiological
52 and clinical relevance, including timing, amplitude, duration and slope. The method is completely
53 user-independent, processes all-night PSG-data, automatically dealing with potential artefacts, sensor
54 loss/displacements, and stage-dependent variability in PWA-time-series. Such characteristics make
55 this method a valuable candidate for the comparative investigation of large clinical datasets, to gain a
56 better insight into the reciprocal links between sympathetic activity, sleep-related alterations, and
57 metabolic and cardiovascular diseases.

58

59

60

61 **Keywords:** *autonomic nervous system, sympathetic activation, photoplethysmography, Pulse Wave*
62 *Amplitude, sleep, sleep disorders.*

63

64 1.INTRODUCTION

65 The autonomic nervous system (ANS) plays an integral role in the fine-tuning of a great number of
66 physiological processes through the complementary and synergic activity of its main divisions,
67 namely the sympathetic and parasympathetic nervous systems. Across a night of sleep, ANS
68 activity undergoes significant fluctuations in relation to sleep-stage transitions (Somers et al. 1993;
69 Trinder et al. 2001, 2012; Whitehurst et al. 2016) as well as phasic physiological and pathological
70 sleep-related events, including cortical arousal as well as respiratory (e.g., apnea/hypopnea) and
71 motor (e.g., limb-movement) events (Bosi et al. 2018; de Zambotti et al. 2018). Of note, abrupt and
72 transient autonomic activations, which lead to increased sympathetic activity and result in
73 peripheral vasoconstriction (Bartels et al. 2016; Catcheside et al. 2002; Grote et al. 2003a; Johnson
74 and Lubin 1967), have been shown to occur also in the absence of visually identifiable cortical EEG
75 arousals, and have thus been suggested to represent an even more accurate marker of sleep
76 fragmentation and disruption (Dresler et al. 2012; Haba-Rubio et al. 2005; Janackova and Sforza
77 2008; Lévy and Pépin 2003; Martin et al. 1997). Moreover, pathological conditions characterized by
78 recurrent autonomic arousals, such as obstructive sleep apnea syndrome (OSAS) and periodic leg-
79 movement syndrome (PLMS), are typically associated with an increased risk of cerebrovascular,
80 cardiovascular and metabolic disorders (e.g., hypertension, myocardial infarction, stroke) (Shimizu
81 et al. 1992) (Vargas-Pérez, Bagai, and Walters 2017). Indeed, evidence indicates that nocturnal
82 arousal-related autonomic and hemodynamic alterations may be associated with sustained daytime
83 sympathetic modifications (Biaggioni and Calhoun 2016; Carlson et al. 1993; Fletcher 2003; Hedner
84 et al. 1988; Somers et al. 1993, 1995) that are in turn implicated in the etiology of the above-
85 mentioned disorders (Brook and Julius 2000; Esler 2000; Fletcher 2003; Mark 1996; Sinski et al.
86 2006; Thorp and Schlaich 2015; Tsioufis et al. 2011; Vinik, Maser, and Ziegler 2011).

87 In light of these premises, the identification of a reliable, non-invasive approach for the
88 characterization of sleep-related autonomic activations would offer a new valuable tool for the
89 diagnostic and prognostic evaluation of patients with sleep disorders. Among many different
90 approaches for the assessment of sympathetic activity, finger photoplethysmography (PPG) emerged
91 as a very promising candidate, since it is typically recorded during both standard polysomnographic
92 (PSG) and polygraphic (PG) studies, and offers a portable, low-cost and non-obtrusive technology to
93 continuously monitor relative variations in peripheral blood flow in the microvascular bed of
94 tissue (Allen 2007). The PPG-signal comprises a pulsatile physiological waveform ('pulse wave')
95 attributed to cardiac synchronous changes, superimposed on a more slowly varying baseline with
96 various lower frequency components attributed to respiration, sympathetic nervous system activity
97 and thermoregulation (Nitzan et al. 1996, 1998, 2001). In particular, drops in pulse wave amplitude
98 (PWA) (Korpas, Hálek, and Dolezal 2009), as measured at each cardiac cycle by PPG, are known to
99 directly reflect changes in peripheral blood flow due to vasoconstriction (British Editorial Society
100 of Bone and Joint Surgery. 1954), and may thus offer a relatively simple index of
101 autonomic activation (Grote et al. 2003b). In line with this, several studies already demonstrated a
102 strong association of PWA-drops with obstructive respiratory events, like apneas and
103 hypopneas, (Bosi et al. 2018; Grote et al. 2003a; Haba-Rubio et al. 2005; Karmakar et al. 2014), as
104 well as with spontaneous and induced EEG arousals (Adler et al. 2013; Catcheside et al. 2002;
105 Delessert et al. 2010).

106 While most PWA-drops can be easily identified in the PPG-signal, their actual detection still largely
107 relies upon human scorers, who have to mark each event manually. Manual PWA-drops scoring is a
108 laborious and time-consuming process that inevitably limits the potential applicability of the PWA-
109 drop analysis to large databases of patients. Moreover, procedures based on manual scoring are
110 known to suffer from reproducibility issues related to intra- and inter-scorer variability. This latter
111 aspect is even more relevant in light of the fact that there is currently no consensus regarding the
112 minimum amplitude threshold for the definition of clinically relevant PWA-drops, and that such
113 parameters commonly differ across studies and laboratories. Finally, even if some PSG-software

114 recently started to offer automated PWA-drop detection methods, these are often not validated and
115 only allow the computation of simple, basic parameters, such as the overall number of drops per
116 hour(Pulse-wave-drop index, PDI).

117 To overcome the above-mentioned issues, here we describe a novel, automated approach for the
118 detection and characterization of PWA-drops from whole-night PPG data. The proposed approach
119 allows the extraction of various parameters of potential interest, including relative timing (used to
120 compute PDI or the association with other scored events of interest), amplitude, descending slope,
121 ascending slope and duration. Several data-quality checks are included in the procedure in order to
122 automatically deal with potential artefacts caused by movement and/or sensor loss/displacement
123 throughout the night. The performance of this PWA-drop detection approach was evaluated by
124 comparing the detections of the algorithm with those performed manually by two expert scorers
125 board-certified in sleep medicine. In order to further showcase the possible advantages of the
126 automated procedure for PWA-drop detection, we also applied the algorithm to investigate relative
127 differences across sleep stages in terms of PWA-drop number and morphological properties.

128

129 **2. MATERIALS AND METHODS**

130

131 **2.1 The PWA-drop detection algorithm**

132 The PWA-drop detection algorithm includes three main 'steps' that are described in detail in the
133 following paragraphs and are graphically summarized in Figure 1. First, the PWA-time-series is
134 extracted from the raw PPG-signal and potential artifactual segments are identified and excluded
135 from subsequent evaluations. Then, candidate PWA-drops corresponding to local peaks in the
136 variance of the PWA time-series are identified. Finally, significant drops are selected among all
137 candidates, based on a-priori defined criteria, and their main characteristics (e.g., timing, amplitude,
138 slopes, duration, etc.) are stored for further evaluation.

139

140 *2.1.1 PWA-signal extraction and preliminary artifact detection*

141 The PPG-signal obtained from conventional pulse-oximeters, measuring pulsatile blood volume in
142 the fingertip, is used as primary input for the algorithm. The Pulse Wave Amplitude ($PWA(i)$)
143 time-series is then defined, at each cardiac cycle (i), as the difference between the maximum (peak)
144 and minimum (nadir) values of the correspondent blood volume pulse-wave. Prior to PWA-signal
145 extraction, the PPG-signal is smoothed (Savitzky-Golay filter with order 2 and 200 msspan) and
146 constant and linear trends are removed.

147 Prior to the actual PWA-drop detection, segments of the PWA-signal containing potential artefacts
148 are automatically identified and excluded from further evaluations. Specifically, an automatic
149 procedure is used to exclude time-points for which: i) the PPG-waveform does not clearly show a
150 consecutive maximum and minimum couple, but only two or more consecutive local maxima or
151 minima (in other words, the blood volume pulse-wave of a particular cardiac cycle is not clearly
152 identified); ii) the temporal distance from the previous PWA-time-point is not consistent with the
153 physiologically plausible range of values for the heart-rate (a fixed threshold corresponding to 250
154 heart-beats per minute is used); iii) the PWA-value at a specific time-point shows differences with
155 respect to both the previous and the following PWA-time-points that differ significantly from the
156 total distribution of difference values computed between all consecutive PWA-points (Modified
157 Thompson Tau Test (Thompson 1985)). Moreover, in order to take into account the possibility of
158 sensor loss or temporary displacement (e.g., due to subject movements throughout the night), the
159 time-course of the root-mean-square (RMS) envelope of the PPG-signal is evaluated using a 100

160 samples moving-window, and PWA-drop detections are prevented within tracts in which the RMS-
 161 value falls below a predefined arbitrary threshold that should be adjusted depending on the
 162 particular instrument used (here it was empirically set to $5 \mu V$).

163

164 2.1.2 Definition of the baseline mask and identification of candidate PWA-drops

165 The obtained PWA-signal is smoothed using a moving average filter with a 5 heart-beats span.
 166 Then, the time-varying PWA local variance and first derivative are computed using a 5 heart-beats
 167 width window moving with steps of one heart-beat. The time-courses of these PWA-features
 168 are used both for the definition of a ‘baseline mask’ (i.e., stable segments of PWA-signal used as
 169 reference for the detection of relative variations) and the identification of candidate time-points
 170 including potential PWA-drops.

171 The baseline mask corresponds to the ensemble of all the ‘stable’ PWA-signal tracts lasting at least
 172 2 consecutive heart beats, and is obtained by excluding all time-points corresponding to local PWA-
 173 variance-outliers, as computed with respect to the whole-night local variance distribution (Modified
 174 Thompson Tau Test (Thompson 1985)). Of note, the baseline mask includes no drops or
 175 discontinuities and is also progressively updated during the PWA-drop detection procedure by
 176 removing periods containing confirmed PWA-drops.

177 Candidate time-points corresponding to potential PWA-drops are instead defined as local peaks in
 178 the time-course of the PWA-local-variance that simultaneously display correspondent negative
 179 values for the first-derivative estimates. As detailed below, these candidate PWA-drops are
 180 subsequently confirmed only if they fulfil specific a-priori conditions. For each candidate time-point
 181 (i_c) an observation interval is defined, ranging from the closest previous (i_{obs1}) to the closest
 182 following (i_{obs2}) maxima of the smoothed PWA-signal (PWA_f). Absolute percent signal
 183 decreases $P_{\%}$ are then computed for each time-point within the observation interval with respect to a
 184 baseline value (b), defined as the mean of the closest previous 5 PWA-points belonging to the
 185 baseline mask ($PWA(i_{obs1}), PWA(i_{obs2}), \dots, PWA(i_{obs5})$). Of note, in the case of consecutive
 186 PWA-drops, stable baseline points may be available only far from the considered candidate drop, and
 187 this could result in an inaccurate estimate of the PWA-drop amplitude. For this reason, if the
 188 distance between the candidate point and the baseline points (N) is greater than 10 heart beats, all the
 189 points belonging to this separating interval are considered, in addition to baseline points, in order to
 190 compute b .

191 *Baseline definition:*

$$192 \text{ if } N < 10, b = \frac{\sum_{i=1}^5 PWA(i_{b1})}{5}$$

$$193 \text{ if } N > 10, b = \frac{\sum_{k=1}^5 PWA(i_{bk}) + \sum_{k=1}^N PWA(i_{b5+k})}{5+N} \quad (1)$$

194 Where $i_{b1}, i_{b2} \dots i_{b5}$ represent the closest time-points belonging to the baseline mask and smaller
 195 than i_{obs} , and $N = i_{obs1} - i_{b5} - 1$ (number of time-points included between the baseline points
 196 and the current drop). Thus, the percent signal decrease is then computed as:

197

$$198 P_{\%}(i) = \left| \frac{PWA(i) - b}{b} \right|, i = i_{obs1}, i_{obs1} + 1, \dots, i_{obs2}; \quad (2)$$

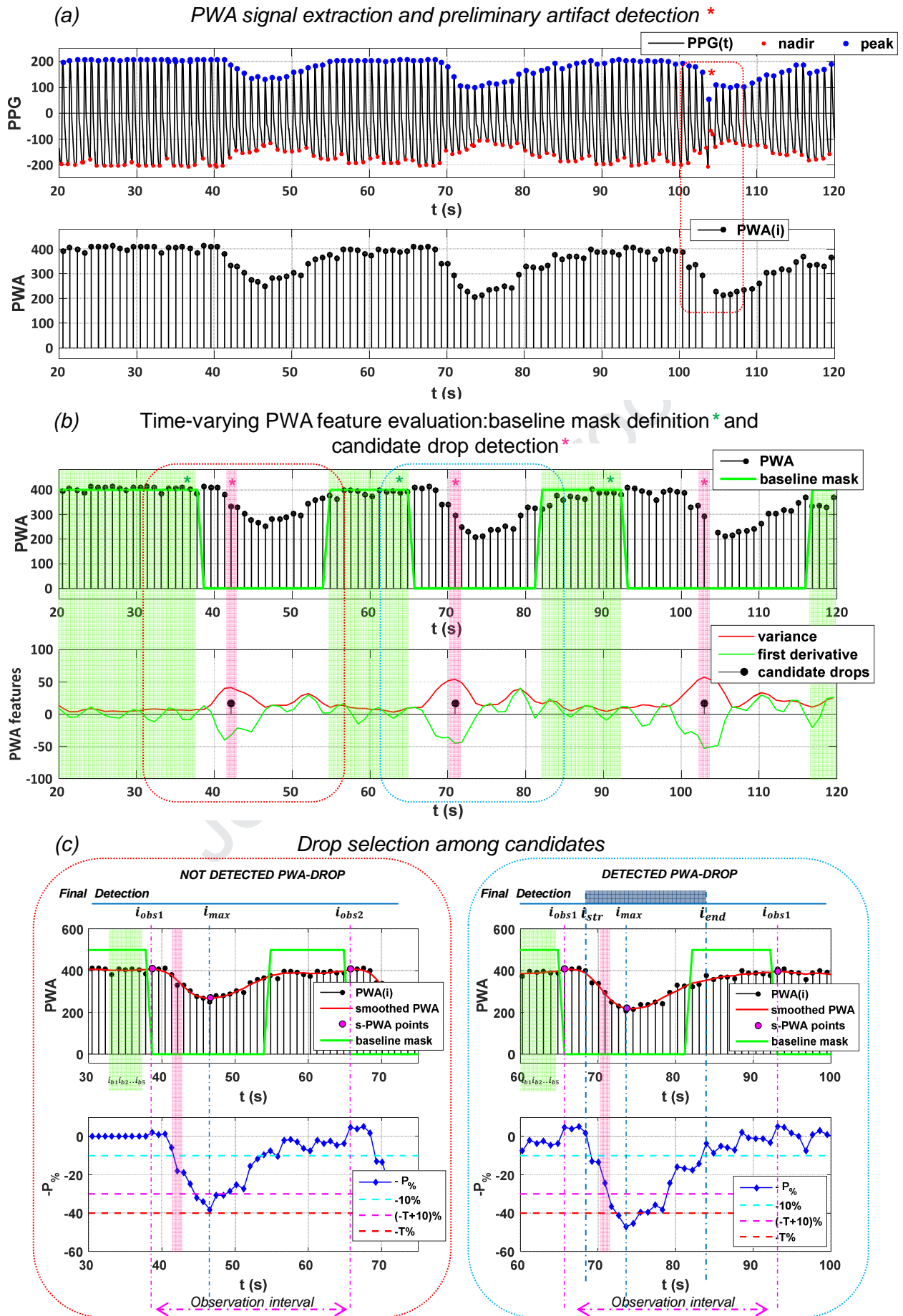
199

200 *2.1.3 Selection and characterization of the 'significant' PWA-drops*

201 A 'candidate' PWA-drop is ultimately confirmed as a significant drop if the following empirical
202 criteria are simultaneously fulfilled for the corresponding observation interval: i) at least two time
203 points separated by less than 2 heart-beats show a percent decrease $P_{\%}$ greater than a
204 specific threshold T , selected by the user ($P_{\%} > T$); ii) at least four time points separated by less than
205 2 heart-beats show a percent signal decrease $P_{\%}$ greater than $T - 10$. Indirect implication of these
206 criteria is that, in order to be eventually detected, a PWA-drop must be at least 4-heart-beats long.

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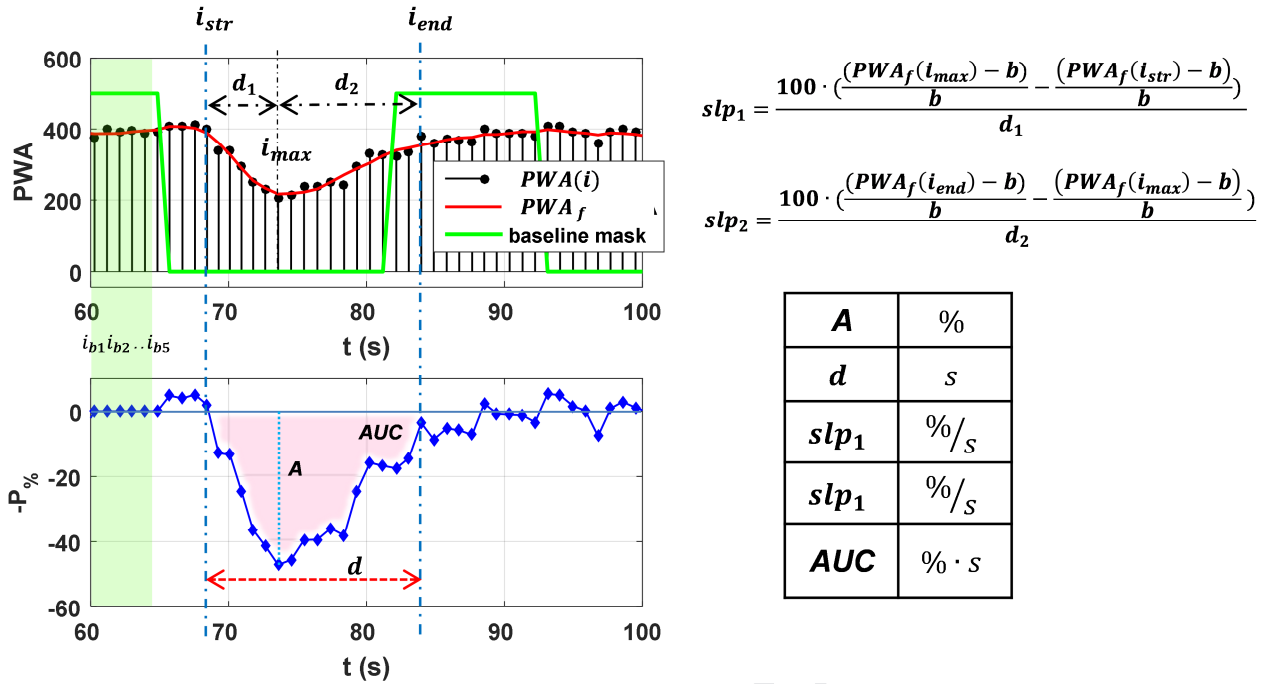
210 *Figure 1. PWA-drop detection algorithm. Panels (a), (b) and (c) report representative examples of the*
 211 *main steps of the PWA-drop detection algorithm. (a) First, the PWA(i) time-series (lower row) is*
 212 *extracted from the raw PPG-data (upper row). The red * indicates a representative example of an*
 213 *artefactual signal-change that is automatically identified and removed from the final PWA-time-series.*
 214 *(b) Local variance and first derivative (respectively drawn in red and green in the second row) are then*
 215 *evaluated for the PWA-signal in order to define a baseline mask (green line in the upper row) and*
 216 *detect candidate time-points for possible PWA-drops (indicated with magenta color). Finally, in panel*
 217 *(c), two examples of candidate drops are reported, with, below, the correspondent values of percent*
 218 *signal decrease (blue diamonds) in respect to the current baseline (mean value within the green shaded*
 219 *area). Only the candidate in the right panel fulfills the given conditions and it is eventually detected.*

220

221 Once a PWA-drop is finally detected its temporal extension is more precisely re-defined within the
 222 observation interval by using as a reference the time-point corresponding to the greatest percent
 223 decrease (i_{max} , local minima in the smoothed PWA-signal). In particular, the drop starting point is
 224 defined within the $i_{obs1}:i_{max}$ range as the first time point i_{str} after which $P_{\%}$ continuously remains
 225 greater than 10% ($P_{\%}(i) \geq 10\% \forall i_{str} \leq i \leq i_{max}$). The ending point of each drop i_{end} is
 226 instead selected in the range $i_{max}:i_{obs2}$, in correspondence of the first time-point for which at least
 227 one of the following conditions becomes true: i) $P_{\%}$ falls below 10%; ii) the PWA-first-derivative
 228 approaches zero; iii) the duration of the ascending tract of the PWA-drop becomes greater than 30
 229 heart-beats. The reason for posing multiple conditions for defining the drop ending-point is related
 230 to the fact that the PWA-signal often does not return to previous baseline values, but rather ‘resets’
 231 to a different stable value after the drop. Moreover, mainly in correspondence of sleep-stage
 232 transitions, the PWA-signal may undergo slow variations that could reflect a stage-dependent
 233 adaptation of the basal sympathetic activation level, rather than a sudden variation.

234 For each confirmed PWA-drop a set of properties are estimated as shown in Figure 2. These
 235 properties include: the total duration d (time-interval comprised between i_{str} and i_{end}), the amplitude
 236 A (defined as the maximum absolute signal percent decrease within the drop), and the descending
 237 and ascending slopes (defined respectively as the decrement and increment in $P_{\%}$ values divided by
 238 the time expressed in seconds). The area under the curve (AUC) of the PWA-drop (computed
 239 approximating the integral of the absolute instantaneous percent decrease over the time expressed in
 240 seconds via the trapezoidal method), which depends on both the duration and the amplitude, is also
 241 computed for each event.

242



243

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Figure 2 .Description of the parameters extracted for each PWA-drop detected by the algorithm. The amplitude **A**, the duration **d** and the area under the curve **AUC** are graphically represented on the left, while the descending **slp₁** and ascending **slp₂** slopes are mathematically defined on the right. Finally, the table on the right summarizes the unit of measurement for each parameter.

248

249 2.2 Validation of the algorithm in a clinical dataset

250 2.2.1 PSG recordings and manual scoring

251 The detection algorithm was applied to the PPG-data extracted from the PSG overnight recordings
 252 of 16 patients (age 50.9 ± 6.33 yrs, 13F) randomly sampled from the *HypnoLausSleep* Cohort
 253 database, collected between 2009 and 2013 in Lausanne, Switzerland (Heinzer et al. 2015). The
 254 following selection constrains were imposed: absence of excessive daytime sleepiness (as measured
 255 using the Epworth Sleepiness Scale); BMI < 25 kg/m²; absence of hypertension, diabetes,
 256 metabolic syndrome and current or past cardiovascular diseases in the last 4 years; absence of self
 257 reported traffic accident in the last 4 years; absence of depression in the last 4 years. All the 16
 258 analyzed subjects were not under psychotropic medicament affecting the central nervous system,
 259 and none of them had a prior diagnosis of a central nervous system disease. As described in
 260 previous work, all recordings took place in the patients' home environment in accordance with the
 261 2007 AASM recommended setup specifications, using a portable PSG recorder (Titanium, Embla
 262 Flaga, Reykjavik, Iceland). Specifically, an Embla Titanium 8000J Nonin adult oximeter was used
 263 to simultaneously collect SpO₂ and PPG data. SpO₂ data was acquired with a sampling rate of
 264 16Hz and a low-pass filter of 1.99Hz, while the PPG signal was collected with a sampling rate of 32
 265 Hz and a low-pass filter of 15.9 Hz. Two trained sleep technicians manually scored the PSG
 266 recordings using the Somnologica software (version 5.1.1, Embla Flaga, Reykjavik, Iceland),
 267 according to the 2007 AASM recommendations (Silber et al. 2007) (Table 1). Moreover, two
 268 physicians board-certified in sleep medicine (hereinafter referred to as 'scorer 1' and 'scorer 2'),
 269 blind to algorithm detections, visually inspected the PPG data of each subject and manually marked
 270 individual PWA-drops (Somnologica software, 3-min windows) presenting a minimum percent
 271 signal decrease of 30%. The scorers relied on an electronic ruler provided in the software GUI next
 272 to the PPG-signal in order to assess the percent amplitude decrease of each PWA-drop. Information
 273 provided by non-PPG signals, including EEG, EMG and EOG were not taken into account during

274 the visual detection of PWA-drops. Obtained visual detections, were used to evaluate the accuracy
 275 of the automated PWA-drop detection algorithm, as detailed below.

276

PARAMETER	AVG	SD
Age	50.9	6.3
Gender	13F/3M	-
BMI	22.7	1.8
RDI	8.2	7.9
AHI	5.6	6.7
ODI	6.3	7.2
SAT	95.5	1.6
T90%	1.1	3.7
PLM	3.3	7.7

277 **Table 1. Demographic and clinical characteristics (average and standard deviation) of included**
 278 **subjects. BMI = body mass index (kg/m²); RDI = respiratory disturbance index (events/h); AHI =**
 279 **apnea-hypopnea index (events/h); oxygen desaturation index (events/h); SAT = mean pulse oxygen**
 280 **saturation; T90% = percentage of total sleep time under a 90% oxygen saturation threshold; PLM =**
 281 **Periodic limb movement index during sleep (events/h). These parameters were calculated based on the**
 282 **AASM 2013 criteria. Based on AHI,10 subjects had no sleep disordered breathing (SBD), 5 subjects**
 283 **had mild SBD and 1 subject had moderate SBD.:**

284

PARAMETER	AVG	SD
Total Sleeptime (min)	382.45	65.35
N1 time (min)	44.50	25.40
N2 Time (min)	152.44	36.30
N3 Time (min)	93.72	23.98
REM time (min)	91.81	29.55
N1 proportion (%)	11.66	6.37
N2 proportion (%)	39.81	5.34
N3 proportion (%)	24.92	6.82
REMproportion (%)	23.61	5.83
WASO (min)	31.56	18.92
ArousalIndex (n/h)	15.00	7.20

285

286 **Table 2. Sleep structure of the 16 subjects included in the analysis. The table includes both the total**
 287 **duration (min) and the relative proportion (%) of each sleep stage (N1,N2,N3,REM), as well as the**
 288 **number of arousals per hour and total time spent awake after the sleep onset (WASO; min). Group**
 289 **average (AVG) and standard deviation (SD) values for each property are reported in the first and the**
 290 **second column, respectively.**

291

292 2.2.2 Application of the PWA-drop detection algorithm

293 For each subject, the PWA-drop detection algorithm was repeatedly applied using different
 294 thresholds for the absolute PWA-signal decrease, with values (T) that varied between 10 and 80%,
 295 with 10% steps. All other algorithm parameters were set to fixed values, as described above. For
 296 each detected PWA-drop, the following properties were computed and stored for further evaluation
 297 (Figure 2): maximum absolute amplitude A (%), descending slopes sp_1 (%/s), ascending slopes sp_2

298 (%/s), duration in seconds (s) and area under the curve $AUC(\% \cdot s)$. The PWA-drop index (PDI),
 299 corresponding to the number of detected drops per hour, was also calculated.

300

301 2.2.3 Comparison of automated and manual scoring

302 Each PWA-drop detected by the algorithm was defined as either a true positive (TP) or a false positive
 303 (FP) depending on whether (TP) or not (FP) it overlapped for at least 10% of its length with the
 304 human scorer's detections. Cases in which a PWA-drop was detected by the human scorer but did
 305 not overlap for at least 10% of its duration with algorithm detections were marked as false negatives
 306 (FN). In this context, true negative (TN) cases (generally used for computation of 'specificity')
 307 could have been expressed as the total length of the recording that was free from both human and
 308 algorithm detections divided by the mean duration of the PWA-drops detected by the
 309 scorers. However, this kind of definition can lead to inflated specificity values, in particular when
 310 target events are very rare with respect to the total duration of the recording, as could be expected
 311 for PWA-drops (Yetton et al. 2016). For this reason, we opted for quantifying the performance of the
 312 algorithm in terms of sensitivity (or recall) and precision, separately for each human scorer, at the
 313 varying of the amplitude threshold T (10-80%). Global values of sensitivity and precision across all
 314 the analyzed subjects were obtained by 'concatenating' all subjects' recordings. In order to describe
 315 with a single measure the overall accuracy of the algorithm with respect to the two scorers, the F-
 316 score was also computed on the whole recordings. Adopted definitions for sensitivity, precision and
 317 F-score are reported below.

$$318 \text{ Sensitivity} = \frac{TP}{TP+FN}, \text{ Precision} = \frac{TP}{TP+FP}, \text{ F-score} = 2 * \frac{\text{Sensitivity} * \text{Precision}}{\text{Sensitivity} + \text{Precision}}$$

319

320 Analyses were performed both considering the whole period of sleep independently of the sleep-
 321 stage ('ALL SLEEP') as well as separately for 'NREM' (N1+N2+N3) and 'REM' periods.

322

323 2.2.4 Evaluation of stage-dependent differences in PWA-drop properties

324 In order to provide an example of how the present algorithm could be applied to investigate changes
 325 in the properties and distribution of PWA-drops in physiological or pathological conditions, a
 326 specific analysis was conducted by evaluating stage-dependent differences in the characteristics of
 327 PWA-drops. As described below (see section 3.1), this analysis was conducted using a minimum
 328 absolute amplitude threshold of 40%. For each of the 16 subject included in the validation
 329 procedure, the following parameters were calculated within each individual sleep stage (N1, N2,
 330 N3, REM): PDI, amplitude, duration, slope-1, slope-2, AUC. Then, potential effects of the sleep
 331 stage on the examined properties were investigated using a repeated measures (rm) ANOVA (the
 332 sleep stage was considered as within-subjects factor; N=16).

333

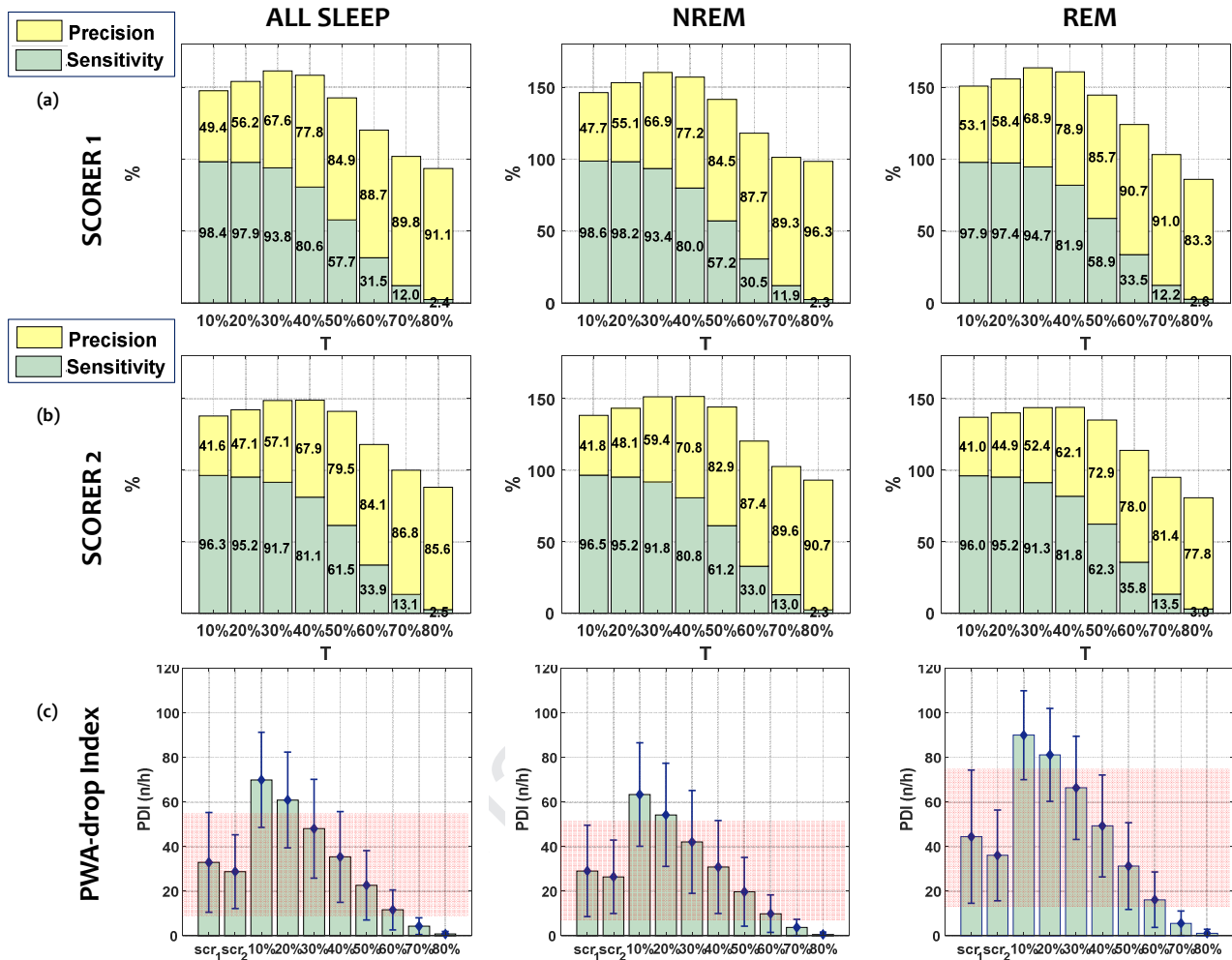
334 3. RESULTS

335 3.1 Performance of the algorithm with respect to human scorers

336 The performance of the PWA-drop detection algorithm, expressed in terms of both sensitivity and
 337 precision, are reported separately for each of the two human scorers, in panel (a) and panel (b) of
 338 Figure 3, respectively. Panel (c) of Figure 3 directly compares the mean number of detections per
 339 hour (PWA-drop index; PDI), for the two scorers and for the automated algorithm. All results are
 340 reported as a function of the amplitude threshold ($T = 10\%, 20\%, \dots, 80\%$) used for the detection

341 algorithm. Of note, the human scorers applied instead a rough amplitude threshold of about 30% to
 342 mark PWA-drops.

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345 **Figure 3.** Performance of the algorithm with respect to human scorers. Panel (a) and panel (b) show the
 346 sensitivity and precision of the detection algorithm with respect to scorer1 and scorer2, respectively.
 347 Values are expressed as a function of the amplitude threshold used by the algorithm (10-80% range, 10%
 348 steps). In panel (c) are instead reported the mean number of detections per hour for the two human
 349 scorers (scr_1 and scr_2) and for the algorithm. The red shaded area indicates the maximum confidence
 350 interval of the mean PDI obtained across the two human scorers and is reported to facilitate the
 351 comparison with values obtained for the automated algorithm at different threshold levels. Results are
 352 reported for the whole sleep period and separately for NREM and REM stages.

353

354 On the whole, our results indicate that the amplitude threshold of 40% was associated with the best overall
 355 performance (best compromise between sensitivity and precision values) and resulted in
 356 a number of detections comparable to those of the two scorers. Mean values across scorers were
 357 80.9% and 72.9%, for sensitivity and precision respectively (as reported in detail in Table 2). Similar
 358 values were found also when NREM and REM sleep were analyzed separately: mean sensitivity
 359 values were in fact 80.4% and 81.8% for NREM and REM respectively, while corresponding mean
 360 precision values were 74.0% for NREM and 70.5% for REM. In light of these observations, the
 361 40% amplitude threshold was used for further evaluations aimed at comparing PWA-drops detected
 362 by the algorithm with those identified upon visual scoring.

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	<i>ALL SLEEP</i>	<i>REM</i>	<i>NREM</i>
Total length (min)	382.5 ± 65.3	290.7 ± 47.1	91.8 ± 29.6
N of PWA-drops (Scorer1)	3449 (215.6 ± 160.9)	2292 (143.2 ± 110.7)	1157 (72.3 ± 57.1)
N of PWA-drops (Scorer2)	3053 (190.8 ± 117.9)	2115 (132.2 ± 89.3)	938 (58.6 ± 40.5)
N of PWA-drops (Algorithm)	3696 (231 ± 143.3)	2455 (153.4 ± 111.8)	1241 (77.6 ± 44.6)
Inter-Scorer Agreement (F-score)	70.2 %	71.6 %	67.4 %
Accuracy Vs. Scorer1 (F-score)	78.9 %	78.3 %	80.1 %
Accuracy Vs. Scorer2 (F-score)	73.8 %	75.3 %	70.6 %
	80.9 %	80.4 %	81.8 %
Mean Sensitivity	Scorer1: TP = 2875, FN = 691 Scorer2: TP = 2510, FN = 589	Scorer1: TP = 1896, FN = 474 Scorer2: TP = 1739, FN = 414	Scorer1: TP = 979, FN = 217 Scorer2: TP = 771, FN = 172
Mean Specificity	96.3 % Scorer1: TN = 18825, FP = 821 Scorer2: TN = 35967, FP = 1186	96.9 % Scorer1: TN = 14741, FP = 559 Scorer2: TN = 28011, FP = 716	94.2 % Scorer1: TN = 4077, FP = 262 Scorer2: TN = 7948, FP = 470
Mean Precision	72.9 % Scorer1: TP = 2875, FP = 821 Scorer2: TP = 2510, FP = 1186	74.0 % Scorer1: TP = 1896, FP = 559 Scorer2: TP = 1739, FP = 716	70.5 % Scorer1: TP = 979, FP = 262 Scorer2: TP = 771, FP = 470

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Table 3. Inter-scorer agreement and detailed performance evaluation of the PWA-drop detection algorithm for $T = 40$ %. The algorithm-scorer agreement and the inter-scorer agreement levels are reported in terms of F-score. In addition to mean sensitivity and precision, the table also shows mean values of specificity (see section 2.2.3 of main text). For each parameter, the total number of true positive (TP) and false positive (FP) PWA-drops computed with respect to each scorer are reported. Results are divided in “ALL-SLEEP”, “NREM” and “REM”, according to whether they were computed on the whole sleep period, or specifically within a single sleep stage. The overall length of each considered condition is reported in the first row, while rows from 2 to 5 report the respective number of detections performed by the two scorers and the algorithm.

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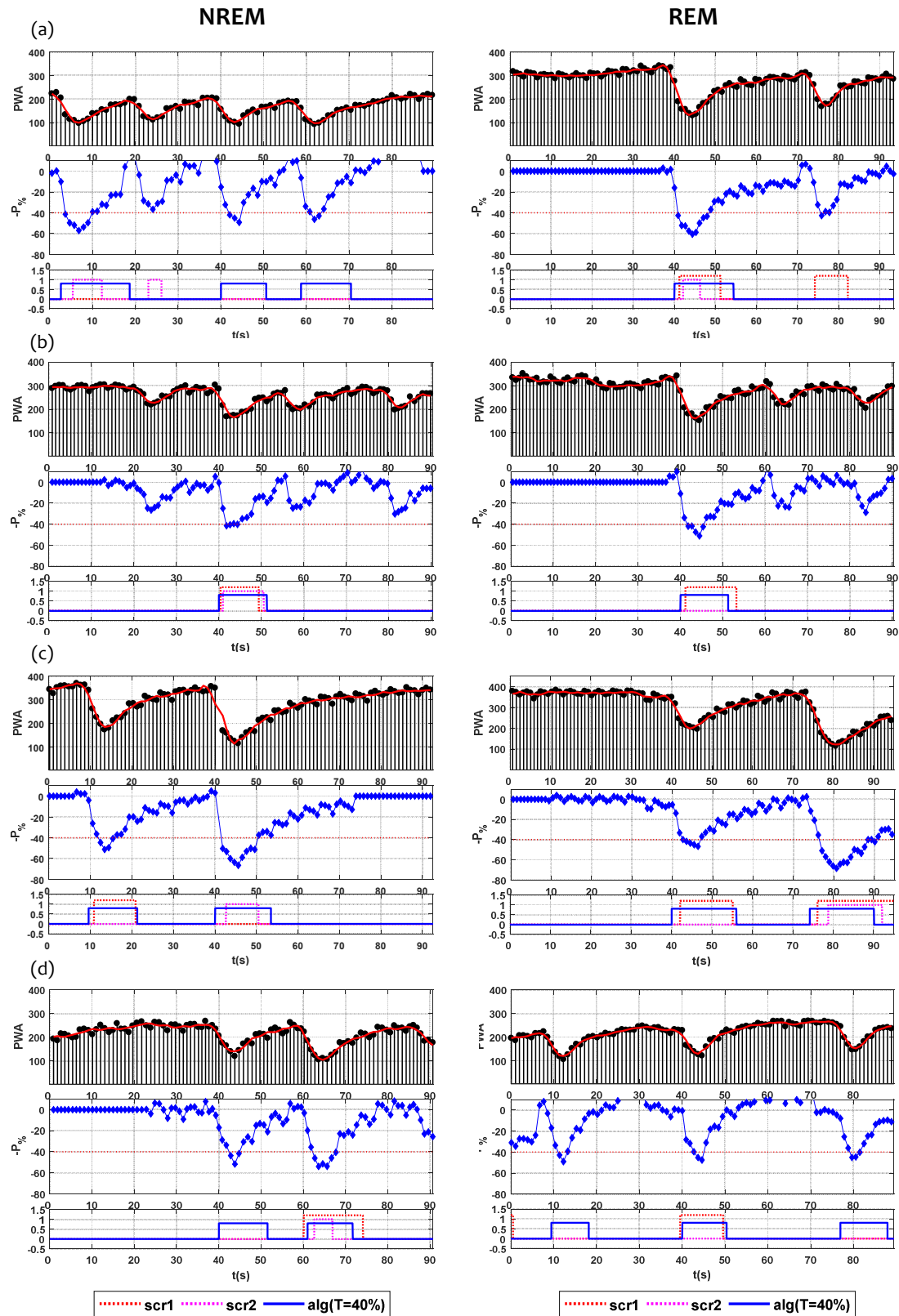
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As shown in Table 2, the overall accuracy of the algorithm with respect to the human scorers, expressed in terms of F-score, reached values of 78.9% and 73.8% for scorer 1 and scorer 2, respectively. Both these values were greater than the inter-scorer agreement, which corresponded to 70.2%. In other words, the overlaps between the algorithm's detections and the detections of each one of the two scorers were greater than the overlap between the detection of the two scorers. Again, similar results were obtained when NREM and REM sleep were analyzed separately.



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Figure 4. Examples of PWA-drops detected by the algorithm (using $T=40\%$) and by the two human scorers, during NREM (left) and REM (right) sleep periods. Each panel includes three figures: the first row shows the PWA time-series (black dots) and its smoothed version (red line); the second row shows the correspondent time-varying percent signal decrease with respect to the preceding baseline period (blue dots); the third row shows the detections of the two human scorers (in red and magenta respectively) and the ones of the automated algorithm (in blue).

390

391 The lack of a full consistency between the algorithm and the scorers, especially in terms of
 392 precision, suggested that the detection procedure may have led to a relatively high number of false
 393 positive (FP) detections. However, a visual inspection of the PWA-signals allowed us to confirm
 394 that quite all detections performed by the algorithm corresponded to ‘true’ PWA-drops, and that
 395 most of the inconsistencies likely emerged from the tendency of the human scorers to detect larger,
 396 more evident drops, while neglecting the smaller ones. This is clearly illustrated by the
 397 representative examples reported in Figure 4, and by the comparison of the properties of TP and FP
 398 PWA-drops shown in Figure 5. Indeed, paired t-tests comparing the properties of true positive (TP)
 399 and false positive (FP) detections across subjects revealed a significant difference in the area under
 400 the curve (AUC), with greater values for TP relative to FP detections. Moreover, the human scorers
 401 tended to detect longer and more abrupt PWA-drops, characterized by a steeper descending slope
 402 (slp_1) and a shallower ascending slope (slp_2), as compared with those detected by the algorithm. Of
 403 note, however, substantial inter-scorer and inter-stage variability were also observed. For instance, a
 404 significant difference in PWA-drop duration between TP and FP detections was found only for
 405 scorer 2, while a very similar duration of TP and FP drops was observed for scorer 1.

406 Finally, Table 4 displays the values of accuracy, sensitivity, specificity and precision of the
 407 algorithm obtained after manual re-classification of all PWA-drops that were identified by the
 408 algorithm but were missed by at one or both the human scorers. All indices increased considerably
 409 and reached values above 90%. Indeed, we observed that 83.4% and 86.1% of algorithm detections
 410 missed respectively by scorer1 and scorer2 resulted to represent true PWA-drops. The true false
 411 positive cases were mainly caused by artefacts in the PPG signal, mostly in correspondence with
 412 movements and arousals

413

	<i>ALL SLEEP</i>	<i>REM</i>	<i>NREM</i>
Accuracy Vs. Scorer1 (F-score)	90.3 %	90.1 %	90.5 %
Accuracy Vs. Scorer2 (F-score)	91.3 %	91.0 %	91.9 %
Mean Sensitivity	90.8 %	90.6 %	91.2 %
Mean Specificity	99.6 %	99.7 %	99.3 %
Mean Precision	95.2 %	95.1 %	95.2 %

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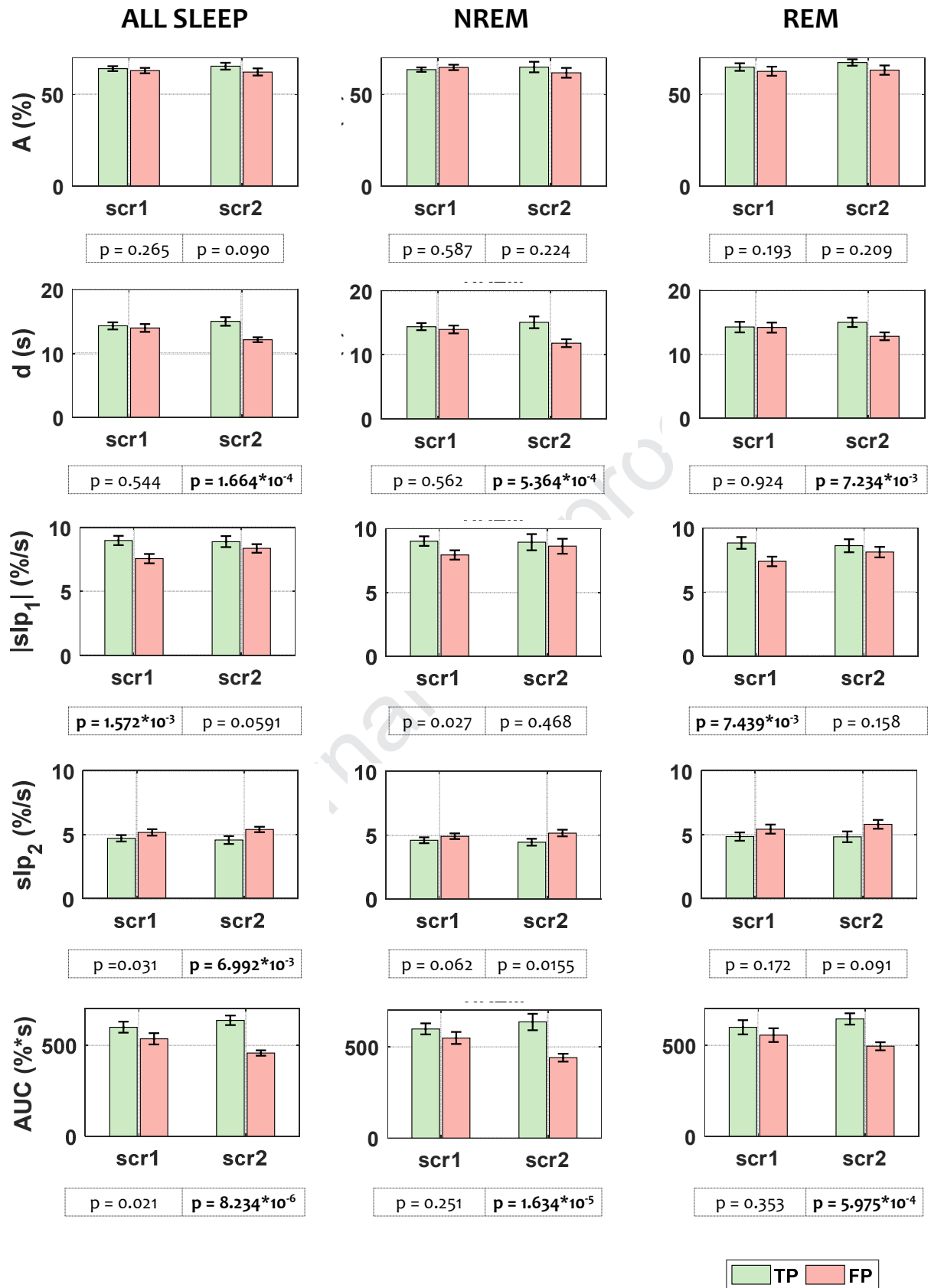
415 *Table 4. table reports performance statistics (the same of Table 3) for the algorithm (T = 40%) re-*
 416 *evaluated after visual reclassification of all algorithm detections marked as wrong by one or both of*
 417 *the two scorers. In order to re-evaluate the statistics, we have added algorithm detections reclassified as*
 418 *“true” to the pool of single scorer detections.*

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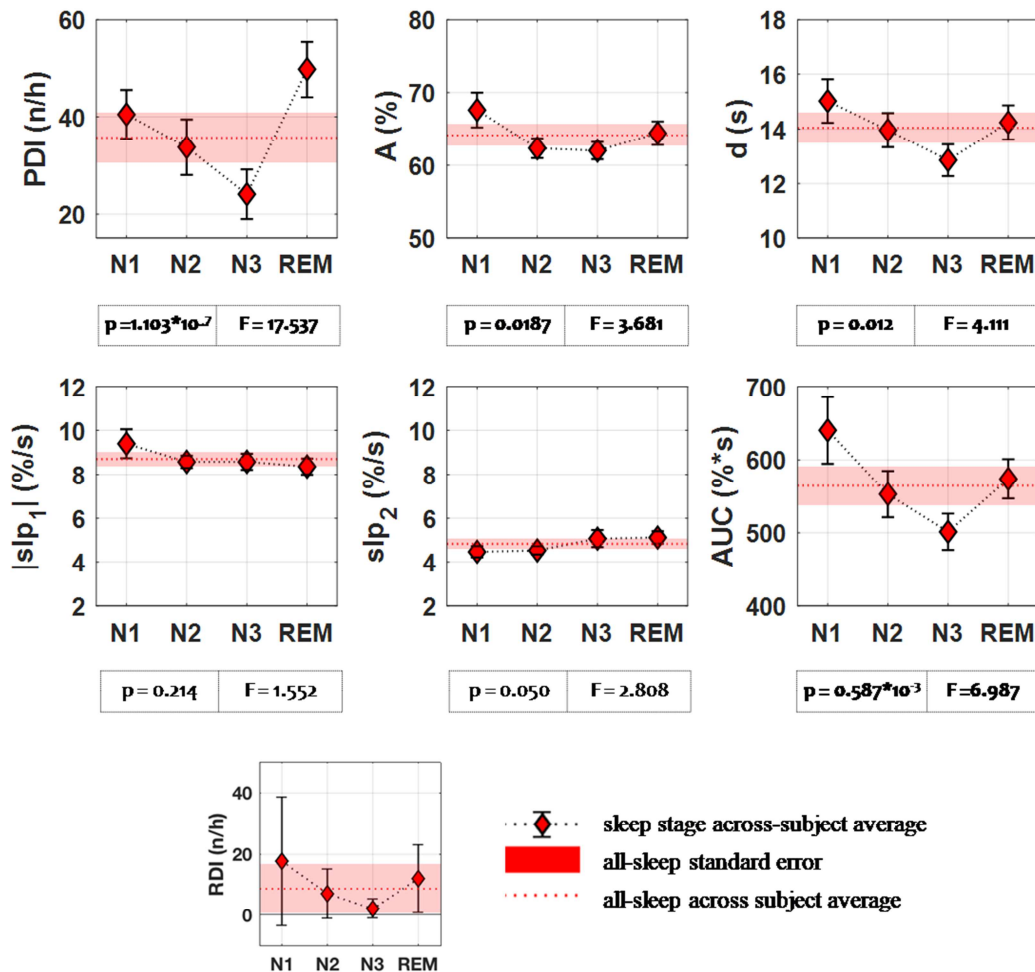
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Figure 5. Properties of the PWA-drops corresponding to true positive (TP) and false positive (FP) detections (amplitude threshold $T = 40\%$). Shown properties include amplitude (A), duration (d), descending and ascending slopes (slp_1 and slp_2), and the area under the curve (AUC). Comparisons were performed using paired t-tests across subjects, separately for the two human scorers (scr1 and scr2, respectively). For each property, mean values across subjects (TP in green and FP in magenta)

429 *are reported in separate bar-plots. P-values obtained for each comparison are reported in boxes below*
430 *the bar-graphs. The three columns of the graph report results obtained for the whole sleep period as*
431 *well as for NREM and REM episodes separately. Significant results ($p < 0.01$) are indicated with*
432 *bold text.*

433
434 *3.2 Analysis of PWA-drops properties and distribution across sleep stages*

435 In order to further highlight potential applications and advantages of the present automated procedure
436 for PWA-drop detection, we evaluated the properties and distribution of PWA-drops across
437 different sleep stages, investigating the presence of potential differences with a repeated measures
438 (rm)ANOVA performed across subjects. As in previous analyses, PWA-drops were detected using a
439 40% amplitude threshold. Figure 6 shows in distinct panels the mean number of PWA-drops per
440 hour (PDI), and the mean values of their amplitude (A), duration (d), descending slope (slp_1),
441 ascending slope (slp_2), and area under the curve (AUC), across N1, N2, N3 and REM sleep.



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Figure 6. Distribution of PWA-dropindex and main characteristics (A , d , slp_1 , slp_2 and AUC) across different sleep stages. Red diamonds indicate mean values (across subjects) for N1, N2, N3 and REM episodes. The red shaded area indicates corresponding mean values \pm SD computed for the whole sleep period. In order to evaluate the effect of the sleep stage on PWA-drop properties an rmANOVA was performed ($N=16$, STAGE as within subject factor). Correspondent p and $F(3,45)$ values are reported in the boxes below each graph. Significant results ($p < 0.05$) are indicated with bold text. Given the known association between PWA-drops and respiratory events, the bottom panel shows the respiratory disturbance index (RDI) computed for each sleep stage.

The PDI was found to differ significantly across sleep stages (rmANOVA; $p=10^{-7}$, $F_{(3,45)}=17.54$), with the lowest value (24.1 ± 5.1 n/h) in N3 sleep and the highest in REM sleep (49.7 ± 5.7 n/h). Significant, but less robust effects, were also observed for amplitude ($p=0.02$, $F_{(3,45)}=3.68$), duration ($p=0.01$, $F_{(3,45)}=4.11$) and AUC $p=0.0006$, $F_{(3,45)}=6.99$). In these cases, the lowest values were again observed for N3, while the highest were found for N1. No significant effects of stage were found for descending ($p>0.2$) and ascending ($p>0.05$) slopes, although a statistical trend was observed for this latter parameter. In fact, the ascending slope tended to be lowest in N1 and highest in REM-sleep.

460

461 4. DISCUSSION

462 A growing body of evidence indicates that drops in pulse wave amplitude (PWA) resulting from
463 autonomic vasoconstriction, may represent a sensitive marker of autonomic activations, whose

464 alterations are in turn associated with several pathological conditions. While PWA-drops can be
465 easily measured non-obtrusively through photo-plethysmography (PPG), their actual detection still
466 largely relies upon human operators, who visually inspect the whole-night PPG-trace and manually
467 mark each event. Importantly, this approach strongly limits reproducibility, due to intra- and inter-
468 scorer variability, and prevents the possibility to investigate the potential predictive role of PWA-
469 related indices in large cohorts of patients. Of note, most of the automated detection tools available
470 in commercial software suites are not scientifically validated, nor freely available, and only allow
471 the computation of simple, basic parameters, such as the PWA-drop index. To overcome these
472 issues, here we developed and validated an automated approach for the detection and
473 characterization of PWA-drops in whole night PPG-signals. We demonstrated that the algorithm
474 reliably detects PWA-drops with an accuracy that appears to be even higher than the one of expert
475 human operators. Finally, we showed that the algorithm may allow to easily investigate not only the
476 number of events per hour, but also several other parameters with a potential physiological and
477 clinical relevance, including timing, amplitude, duration and slopes. These properties can be used to
478 study how the PWA-drops change as a function of different sleep stages, or in relation to their
479 association with other physiological or pathological events (e.g., cortical arousals, motor or
480 respiratory events). This kind of investigation could gain an important role in clarifying the
481 pathogenesis of many cardiovascular and metabolic diseases, as well as in the definition of tools for
482 the diagnostic and prognostic evaluation of patients with sleep disorders.

483

484 *Performance of the PWA-drop detection procedure*

485 In order to evaluate the performance of the PWA-drop detection procedure, a comparison was
486 performed with the gold standard represented by the manual scoring of two independent human
487 experts. In line with previous work (Adler et al. 2013; Haba-Rubio et al. 2005), the human scorers
488 were instructed to mark events characterized by a minimum absolute PWA-signal reduction
489 corresponding to the 30%. Of note, however, there is currently no consensus, nor a well-defined
490 pathophysiological knowledge, to guide the selection of this important parameter, that has been in
491 turn indicated as potentially informative about the intensity of the underlying sympathetic
492 activation. Moreover, during visual scoring, the quantification of the actual signal percent variation
493 may be subject to a great within- and between-scorer variability. For these reasons, we incorporated
494 in the automated detection procedure the possibility for the user to modulate the amplitude threshold
495 and we performed the present validation procedure by testing different values of this parameter,
496 between 10% and 80% (with 10% steps).

497 As expected, sensitivity and precision showed opposite trends as a function of the amplitude
498 threshold, irrespective of the sleep stage or the human scorer. In particular, the sensitivity of the
499 algorithm was found to reach a maximum in correspondence of the minimum amplitude threshold
500 ($T = 10\%$), when the algorithm detected the greatest number of drops. Vice-versa, values of
501 precision increased with higher amplitude thresholds, while the number of algorithm detections
502 decreased. Among the tested amplitude thresholds, the one corresponding to 40% produced the best
503 compromise between sensitivity ($\sim 80\%$) and precision ($\sim 70\%$) and led to an overall number of
504 detections consistent with those provided by the two human scorers. The detections obtained by the
505 algorithm with a 40% threshold are thus more similar to those obtained based on visual inspection
506 and a (visually estimated) 30% amplitude threshold. This observation suggests that the operators
507 may actually tend to miss PWA-drops for which the amplitude value is higher but relatively close to
508 the selected threshold, probably because of a low precision in visually estimating the drop's relative
509 amplitude. Of note, this effect could be also expected to result in relative variations across scorers,
510 with a negative impact on the reproducibility of results. In line with this, we found that the
511 agreement between the two scorers (expressed in terms of F-score) only reached 70%, a value that

512 was lower than those expressing the agreement between the algorithm and each of the two scorers
513 (~75%). This result implies the existence of a greater variability between the two human scorers,
514 compared to the one between each human scorer and the automated algorithm and points to a
515 greater reliability and reproducibility of the latter.

516 Importantly, while the sensitivity of the algorithm reached values above 80%, the precision
517 remained relatively low (~70%), indicating the possibility of false positive detections by the
518 algorithm. However, a visual inspection of PWA-drops detected by the algorithm but not by the
519 scorers (apparent false positives), confirmed that these were actual changes in the amplitude of the
520 PWA-signal, very similar in shape to true positive drops, even if sometimes less pronounced and
521 with their amplitude close (but not below) the selected threshold (see examples in Figure 4).
522 Moreover, in many cases, even when a PWA-drop detected by the algorithm was not recognized by
523 a scorer, it was instead identified by the other scorer. A more detailed analysis of the differences
524 between true positive (i.e., drops marked by both the algorithm and the scorers) and false positive
525 PWA-drops confirmed that such events tended to have similar amplitudes, but different slopes and
526 areas under the curve (AUC). However, these differences were not consistent across the two scorers
527 and could then be driven by subjective criteria. Globally, our results suggest that the human scorers
528 tended to miss relatively small and shallow events, even though they actually passed the selected
529 (30%) amplitude threshold. This happened especially when several PWA-drops occurred in
530 sequence, thus reflecting difficulties in precisely and reproducibly estimating upon visual
531 inspection the maximum amplitude of each drop in relation to preceding baseline values. The same
532 issue related to the within and between scorer variability in amplitude estimation may also contribute
533 to explain the apparent 'false negative' detections, corresponding to cases in which the algorithm
534 failed to detect a drop marked by the human scorer (Figure 4).

535 Overall, while the inclusion of only two human scorers is not sufficient to draw clear conclusions
536 regarding the reproducibility of a human-based evaluation of PWA-drops, our results suggest that
537 this process may be affected by a non-negligible inter-scorer variability. Such variability may alter
538 the reproducibility of results in individual studies, thus limiting the possibility to compare findings
539 obtained in different cohorts and by different research groups. This issue is made even worse by the
540 lack of a consensus regarding the criteria –and in particular the amplitude threshold– that should be
541 applied for the correct identification of clinically relevant PWA-drops. Our results suggest that these
542 limitations could be overcome by using automated approaches based on standardized criteria.

543

544 *Potential applications in physiological and pathological states*

545 A clear advantage of the automated detection algorithm over manual scoring lies in the possibility
546 to rapidly and easily measure several properties of each PWA-drop, even in large samples. In
547 addition to the timing of each event, which could be used to evaluate the number of drops per hour
548 (PDI) across the whole sleep period or within particular stages, or the association with other specific
549 events (e.g., apnea or hypopnea events), other measured parameters include amplitude, duration,
550 descending slope, ascending slope and area under the curve. Here we showed that these properties
551 of the PWA-drops tend to differ significantly across sleep stages. In particular, the number,
552 amplitude, duration and area under the curve of PWA-drops share a common trend, decreasing from
553 N1 to the deepest stage of NREM sleep (N3). Most properties of PWA-drops occurring during
554 REM-sleep were similar to those observed in N2, with the notable exception of the number of
555 events per hour, which was found to be higher in REM than in any other sleep stage. This
556 observation is consistent with the known intrinsic variability of most autonomic signals during REM
557 sleep. While, at present, the possible clinical value of described morphological properties of the
558 PWA-drops is unknown, future studies in clinical populations will allow to investigate their possible
559 alterations and predictive role in relation to distinct physiological and pathological processes.

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5. CONCLUSION

Evidence indicates that the analysis of drops in pulse wave amplitude during sleep may offer a very promising marker of changes in autonomic activation, which are potentially associated with sleep fragmentation and disruption in pathological states (Delessert et al. 2010; Grote et al. 2011; Karmakar et al. 2014; Sommermeier et al. 2014, 2016). Here we developed an automated algorithm for the detection and characterization of PWA-drops that aims at overcoming most of the limitations associated with user-dependent analyses, including long processing time and high intra- and inter-scanner variability. The proposed algorithm is supposed to work in a completely user-independent fashion, starting from all-night PSG-data, and automatically dealing with the potential presence of movement artifacts and/or the possibility of sensor loss/displacement throughout the night, and self-adapting to stage-dependent variability in the PWA-signal. In light of these properties, it may allow to analyze large databases in a relatively short time and provides the opportunity to efficiently evaluate the potential impact of different PWA-drop amplitude thresholds on other parameters of interest. The algorithm, written in MATLAB (The Mathworks Inc 2009), is freely available for download in the OSF repository (https://osf.io/c2eup/?view_only=a2890a0f06704cf1a281eee5727d8790). We hope that the use of automated detection approaches by future studies will lead to a better understanding of physiological changes triggered by autonomic activations during sleep and of the possible value of PWA-derived parameters in predicting the risk of cardiovascular and metabolic diseases associated with sleep disorders.

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755

Dr. S. Chokroverty
Editor-in-Chief
Sleep Medicine

Dear Dr. Chokroverty,

please find enclosed the manuscript entitled “*Quantifying peripheral sympathetic activations during sleep by means of an automatic method for pulse wave amplitude drop detection*”, that we would like to submit for consideration in the Special Issue “*Artificial Intelligence and Sleep*”.

The present work describes a novel automated algorithm for the identification and characterization of drops in pulse-wave-amplitude (PWA), as measured in whole-night finger plethysmographic (PPG) signals recorded during sleep. In fact, previous work demonstrated that PPG PWA-drops, which are assumed to reflect peripheral vasoconstriction resulting from sympathetic activation, typically accompany the occurrence of pathological respiratory (apneas, hypopneas) and motor events (periodic limb movement). Moreover, deviations from normality in overnight sympathetic activity (and thus in the number/amplitude of PWA-drops) have been also shown to be associated with the development of metabolic and cardiovascular diseases.

The present work is motivated by the fact that, despite the potential clinical relevance of sleep-related PWA-drops, their analysis still largely relies upon human operators, who have to visually inspect the whole-night PPG-traces and manually mark each event. This approach strongly limits reproducibility, due to intra- and inter-scorer variability, and prevents the possibility to investigate the potential predictive role of PWA-related indices in large cohorts of patients. Moreover, while few commercial software suites started to offer automated detection tools these are typically not validated, nor freely available, and only allow the computation of simple, basic parameters, such as the number of PWA-drops per hour.

The algorithm described in this work has been developed with the aim of overcoming these limitations. Indeed, the algorithm works in a completely user-independent fashion, thus minimizing issues related to intra- and inter-scorer variability, and automatically deals with the potential presence of movement artifacts and/or the possibility of sensor loss/displacement throughout the night. The performance of the automated detection procedure has been tested against those of two expert human operators, with results suggesting a higher accuracy of the algorithm with respect to each individual human scorer. Of note, the algorithm allows the automated computation not only of the number of events per hour, but also of several properties of each PWA-drop, such as its amplitude, descending/ascending slopes, duration and area under the curve. The algorithm, fully developed in MATLAB, is made freely available for download through the OSF repository.

We believe that the use of automated algorithms for the analysis of PWA-drops by future clinical studies will lead to a better understanding of physiological changes triggered by autonomic activations during sleep and of the possible value of PWA-derived parameters in predicting the risk of cardiovascular and metabolic diseases associated with sleep disorders.

For these reasons, we are convinced that our work could be of great interest for the audience of *Sleep Medicine* and we kindly ask you to consider this manuscript for publication.

Many thanks in advance for your time and attention,

Sincerely,



Giulio Bernardi, M.D., Ph.D.