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Modelling PTSD Diagnosis Using Sleep, Memory and Adrenergic Metabolites: An Exploratory Machine-Learning Study.

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Title: Modelling PTSD Diagnosis Using Sleep, Memory and Adrenergic Metabolites: An Exploratory Machine-Learning Study.

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ABSTRACT

Objective. Features of posttraumatic stress disorder (PTSD) typically include sleep disturbances, impaired declarative memory and hyperarousal. This study evaluated whether these combined features may accurately delineate pathophysiological changes associated with PTSD.

Method. We recruited a cohort of PTSD-diagnosed individuals (*N*=20), trauma survivors without PTSD (TE; *N*=20) and healthy controls (HC; *N*=20). Analyses of between-group differences and support vector machine (SVM)-learning were applied to participant features.

Results. Analyses of between-group differences replicated previous findings, indicating that PTSD diagnosed individuals self-reported poorer sleep quality, objectively demonstrated less sleep depth, and evidenced declarative memory deficits in comparison to HC. Integrative SVM-learning distinguished HC from trauma participants with 80% accuracy using a combination of five features, including subjective and objective sleep, neutral declarative memory and metabolite variables. PTSD and TE participants could be distinguished with 70% accuracy using a combination of subjective and objective sleep variables, but not by metabolite or declarative memory variables.

Conclusion. From among a broad range of sleep, cognitive and biochemical variables, sleep characteristics were the primary features that could differentiate those with PTSD from those without. Our exploratory SVM-learning analysis establishes a framework for future sleep- and memory-based PTSD investigations that could drive improvements in diagnostic accuracy and treatment.

1. BACKGROUND

Sleep disturbances and memory deficits are prominent features of posttraumatic stress disorder (PTSD), and are also strongly implicated in PTSD susceptibility and symptom development (Brewin, Kleiner, Vasterling, & Field, 2007; Germain, 2013). Although these disturbances and deficits are sometimes considered secondary symptoms of the disorder, disturbing dreams, insomnia and poor memory are reported by most diagnosed individuals (Agorastos, Kellner, Baker, & Otte, 2014; Pace-Schott, Germain, & Milad, 2015). Furthermore, recent evidence suggests that these sleep and memory-related alterations may be meaningfully associated, because of the role that sleep plays in memory consolidation (Feld & Diekelmann, 2015; Goerke, Muller, & Cohrs, 2017; Lipinska, Timol, Kaminer, & Thomas, 2014). A better understanding of the role of trauma-induced sleep and memory disturbances in PTSD development may help drive improved diagnostic and treatment approaches.

Data derived from structured clinical interviews and self-report symptom checklists and questionnaires suggest that PTSD-diagnosed individuals consistently experience numerous forms of sleep disruption. These include difficulties in falling asleep, frequent awakenings from sleep, shorter sleep duration, restless sleep,

daytime fatigue and nightmares (Buysse et al., 2008; Giosan et al., 2015; Werner, Griffin, & Galovski, 2016).

However, investigations using polysomnography (PSG) have produced inconsistent results regarding the nature and magnitude of sleep disturbances in PTSD. Some studies have found decreased rapid eye movement (REM) duration during sleep (Hefez, Metz, & Lavie, 1987; Lavie, Hefez, Halperin, & Enoch, 1979; Lipinska et al., 2014; Mikulincer, Glaubman, Wasserman, Porat, & Birger, 1989), whereas others have found either normal or prolonged REM durations (Dow, Kelsoe, & Gillin, 1996; Engdahl, Eberly, Hurwitz, Mahowald, & Blake, 2000; Glaubman, Miculincer, Porat, Wasserman, & Birger, 1990; Mellman, Kulick-Bell, Ashlock, & Nolan, 1995). Similarly, abnormalities in slow-wave sleep (SWS) are found inconsistently, with some studies reporting decreased SWS in PTSD-diagnosed individuals relative to controls (Fuller, Waters, & Scott, 1994; Glaubman et al., 1990; Habukawa, Uchimura, Maeda, Kotorii, & Maeda, 2007; Mikulincer et al., 1989; Yetkin, Aydin, & Ozgen, 2010), whereas others report no between-group differences (Dow et al., 1996; Hurwitz, Mahowald, Kuskowski, & Engdahl, 1998; Mellman, Kulick-Bell, et al., 1995; Ross et al., 1994; Woodward, Murburg, & Bliwise, 2000). Meta-analyses attribute these inconsistencies to between-study differences in methodology, as well as variability in controlling for moderating variables such as age, gender, trauma type, and psychiatric comorbidities that may

influence the nature and extent of sleep disruption (Kobayashi, Boarts, & Delahanty, 2007).

Inconsistences may also be related to the lack of comprehensive symptom descriptions. For instance, the frequency and magnitude of hyperarousal symptoms are rarely described, even though these have been linked with disturbed sleep (Kobayashi, Lavela, & Mellman, 2014; Mellman, Kumar, Kulick-Bell, Kumar, & Nolan, 1995; van Liempt et al., 2013). In these studies, hyperarousal symptoms have been indexed using self-report measures, psychophysiological measures (heart rate variability) and a number of biomarkers (levels of cortisol, adrenocorticotropic hormone and noradrenaline derived from plasma or urine metabolites). Measures of noradrenergic functioning are particularly useful in characterising the relationship between sleep disturbance and hyperarousal in PTSD, because noradrenaline is activated during sympathetic activation of the autonomic nervous system under conditions of perceived or actual threat. Furthermore, Mellman et al. (1995) found that PTSD-diagnosed participants, in comparison with healthy controls, had elevated night-time noradrenergic activity, and that the extent of this activation was correlated with the degree of sleep disturbance. However, the lack of consistent objective sleep findings indicates a need for improved biomarker approaches that might help differentiate reliably between those with and without a diagnosis of PTSD.

A large body of research applying memory measures (including list-learning, narrative recall tasks, and paired associated learning) to the study of PTSD has demonstrated prominent memory deficits, associated with small-to-moderate effect sizes, in affected individuals (Brewin et al., 2007; Johnsen & Asbjornsen, 2008). Such deficits are strongly related to reduced hippocampal volume in PTSD-diagnosed individuals (Bremner et al., 1995; Vythilingam et al., 2005). Recent research investigating the importance of healthy sleep (specifically, intact sequences of, and transitions between, early-night SWS and late-night REM sleep) for the processing and consolidation of memories encoded during waking has indicated that, in PTSD, disturbed sleep and poor performance on recall and recognition tasks may be closely related. Independent studies have demonstrated that either disrupted REM sleep (Lipinska et al., 2014) or fragmented early night SWS-rich sleep (van Liempt, Vermetten, Lentjes, Arends, & Westenberg, 2011) is associated with poor performance on post-sleep declarative memory tests. Irrespective of the kind of disruption in sleep architecture, these studies highlight that sleep and memory disruptions in PTSD are not isolated symptoms, but instead are meaningfully related, with potential mechanistic interaction. However, no study has investigated the potential utility of this constellation of features in exploring the correlates of PTSD diagnosis. Investigation of these features may

augment our understanding of current diagnostic criteria and provide deeper insight into the structure of post-traumatic symptoms.

The present study assessed the utility of combining 74 different features including subjectively and objectively measured sleep disturbance, declarative memory deficits and changes in biological markers of hyperarousal (noradrenergic and adrenergic metabolites) across those diagnosed with PTSD, those exposed to trauma and healthy controls. Our objective was to identify the minimum combination of features with the highest diagnostic accuracy to characterise PTSD. We conducted this exploration using supervised multivariate classification methods to integrate and identify a combination of features that may be capable of accurate PTSD classification.

2. METHODS

This study, which was part of a larger study investigating relations between cognition, affect, and sleep in PTSD-diagnosed individuals (Lipinska & Thomas, 2017), featured a cross-sectional quasi-experimental design.

2.1 Participants

Sixty women were enrolled into the study and assigned to three groups (*N* = 20 each): PTSD, trauma survivors without PTSD (TE), and healthy controls (HC). We opted to recruit participants in such a way as to be able to detect any significant results based on medium effect sizes, using a conservative estimate of *f* = 0.33 from a previous study using a similar cohort (effect sizes range: f = 0.33 to f = 0.73; Lipinska et al., 2014). A power analysis revealed that to achieve power of 0.8 with α set at 0.05, we needed to recruit at least 60 participants.

All participants in the PTSD and TE groups were adults ~~sexual assault survivors~~ who had experienced a single sexual assault, which was considered the index trauma. Participants were recruited through a local non-profit organisation that provides a counselling service to sexual assault survivors, and through advertisements placed in local newspapers. The three groups were aggregate-matched on age (for the entire sample, 25.3 ± 4.1 years), general intellectual functioning (for the entire sample, Wechsler IQ = 82.3 ± 14.2), level of income (for the entire sample, 4706.0 ± 2828.0 ZAR), and smoking habits (13% of all participants smoked cigarettes regularly). The sample included seven participants with asymptomatic HIV. The PTSD and TE groups were aggregate matched on time since trauma (overall, 1.3 ± 1.0 years) (**Table 1**).

All participants had no history of childhood sexual trauma and of alcohol or illicit substance abuse, and were free of psychoactive medication. Full ethical approval was obtained from the relevant bodies at the hosting institution, and all participants provided written informed consent.

A trained researcher used the Clinician-Administered PTSD Scale (CAPS; Blake et al., 1995) to measure the presence and severity of PTSD symptoms. We used a moderate scoring rule which stipulated that a CAPS score of at least 45 must be reached for a diagnosis of PTSD to be made, and that there must be the appropriate Diagnostic and Statistical Manual, Fourth Edition, Text Revision (DSM-IV TR) distribution of symptoms across clusters. PTSD diagnosis was confirmed using the Mini International Neuropsychiatric Inventory (MINI; Sheehan et al., 1998). Participants had to meet both CAPS and MINI PTSD diagnosis criteria to remain eligible for inclusion in the PTSD group. Presence and severity of depression was rated using the Beck Depression Inventory (BDI-II; Beck, Steer, & Brown, 1996). To provide an estimate of general intellectual functioning, participants completed the Performance subtests of the Wechsler Abbreviated Scale of Intelligence (WASI; D. Wechsler, 1999). Potential participants were excluded from the PTSD and TE groups if they had a current primary psychiatric diagnosis that was not secondary to trauma and were excluded from the HC group if they displayed any psychiatric diagnosis. Eligible participants were

scheduled for two night sessions and one day session, and were required to refrain from vigorous exercise, alcohol, nicotine, and caffeinated drinks for 12 h prior to testing.

2.2 Sleep-wake assessment and memory performance

A blocked randomisation approach assigned an equal number of participants from each group to either two consecutive night sessions followed by a day session, or a day session followed by two consecutive night sessions. The day and the night sessions were separated by at least 48 h and occurred within a week of each other.

For the night sessions, participants arrived at the sleep laboratory approximately 2 h before their normal bedtime. The first night (N1) was an adaptation night to familiarise them with the environment and with the equipment used to monitor their sleep. Prior to bedtime during N1, participants completed the Pittsburgh Sleep Quality Index (PSQI; Buysse, Reynolds, Monk, Berman, & Kupfer, 1989) to characterise subjective sleep quality in their home environment. Prior to bedtime on the second night (N2), participants completed the Logical Memory (LM) and Word List (WL) subtests of the Wechsler Memory Scale-Third Edition (WMS-III; D. Wechsler, 1997) and the Story Memory (SM) subtest of the Wide Range Assessment of Memory and Learning (WRAML; Sheslow & Adams, 2003). Administration of these tests followed standardised procedures. That

is to say, an immediate recall trial followed initial presentation of each stimulus set (LM and SM: a one-paragraph story in each case; WL: a list of 12 semantically unrelated words), and an uncued delayed recall trial followed some time after that. The only exception to the standard administration procedures was that the delayed recall trial followed a period of sleep, rather than the usual 25-35 mins of filled activity. After completing that set of cognitive tests, participants prepared for bedtime and were attached to a 16-channel Nihon Kohden NeuroFax EEG9000 electroencephalograph (EEG) adapted for sleep research.

Upon waking after an 8-hour sleep opportunity, an adapted version of the PSQI (Laboratory PSQI; Supplementary Appendix A) assessed subjective laboratory sleep during the night, and delayed free and cued recall trials of the three memory tasks (LM, WL, SM) were administered. Both the PSQI and Laboratory PSQI were scored, following convention, to calculate a Global Score capturing overall sleep quality over the reporting period (Buysse et al., 1989).

For the day session, participants arrived at the laboratory at approximately 08h00 and completed parallel versions of the LM, WL, and SM immediate recall tasks. After 8 h of waking activity, they returned to the lab so that delayed free tasks could be administered. Participants were instructed to carry out their ordinary daily activities during the 8 h wake period, but not to nap. To estimate declarative

memory performance, immediate recall, delayed recall, and retention (i.e., comparison of immediate with delayed recall, expressed as a percentage) scores were derived from each of the LM, WL, and SM tasks. Three composite scores were also calculated averaging immediate recall, delayed recall, and retention *Z*-scores across tasks. Retention was the primary variable of interest.

2.3 PSG acquisition, analysis and data pre-processing

To ensure reliable PSG recording, we applied a combination referential and bipolar montage with the bipolar derivations being F3-C3, C3-P3, P3-O1, and F4-C4, C4-P4, P4-O2, and the referential derivations being F3-A2, C3-A2, O3-A2, and F4-A1, C4-A1, O4-A1. Eye movements, muscle tonus, and heart rate were recorded on two electrooculography (EOG) channels, and on one electromyography (EMG) and one electrocardiography (ECG) channel, respectively. Standardised filters for recording sleep were employed for the EEG and EOG (0.5-35Hz), EMG (10-70Hz) and ECG (1-70Hz) leads to ensure integrity of the signal. The ground electrode was placed on the middle of the forehead.

PSG records were analysed according to Rechtschaffen and Kales (1968) criteria, for twelve sleep-related outcome variables: sleep latency; sleep efficiency; number of awakenings (defined as a period of wakefulness longer than 1.5 min after sleep onset); number of

spontaneous arousals (defined as a period of abrupt PSG shift during the night, usually an increase in PSG frequency lasting at least 3s but less than 1.5 min); number of minutes spent awake after sleep onset (WASO); non-rapid eye movement (NREM) stage 1 percentage (NREM 1%); NREM stage 2 percentage (NREM 2%); SWS percentage (SWS%); REM percentage (REM%); REM latency; REM spontaneous arousals (REM arousals); and REM arousals leading to waking or NREM 1 sleep (REM NREM1/W). G.L. scored all sleep records while blind to group assignment.

2.4 Measurement of noradrenergic and adrenergic metabolites

Participants were asked to micturate into containers holding 10ml 6M HCl preservative during three periods: (1) 08h00-16h00, (2) 16h00-24h00, and (3) 24h00-08h00. The first two collection periods occurred within the day session, whereas the third occurred during the N2 session. During each collection period, participants voided into a receptacle and transferred their urine to a convenient and appropriately marked small cooler box or kept directly at 4°C. Eventually, each bottle was transferred to the local National Health Laboratory Service where volumes for the three collections were measured and aliquots obtained. The samples were then frozen at -80º for assay. The samples were then analysed using gas chromatography (GC) and mass spectrometry (MS). An Agilent Technology 7890A gas chromatographer and 5975C mass

spectrometer were used for analysis. Samples were analysed according to standard GC-MS procedures described elsewhere (Burtis, Ashwood, & Bruns, 2012; Naccarato, Gionfriddo, Sindona, & Tagarelli, 2014). Such analysis of urinary-derived normetadrenaline and metadrenaline metabolites is considered a robust, valid, and reliable method to estimate noradrenergic and adrenergic activity respectively. Furthermore, this analytic procedure provides adequate protection against interference from drugs, drug metabolites, exercise, and stress (Peaston & Weinkove, 2004)

2.5 Statistical Analyses

Prior to SVM analysis, we conducted a number of conventional analyses to examine between-group differences with respect to sleep, memory and metabolite measures. Data related to these variables were assessed for normality with the Shapiro-Wilk test, and then either a one-way analysis of variance (ANOVA) or a Kruskal-Wallis ANOVA with Tukey HSD *post hoc* correction was implemented, respectively. Tukey's method was used to create confidence intervals for all pairwise differences between-group level means while controlling for 95% family error rate. Models were adjusted for age, nicotine, and HIV status to control for potential confounding effects on PSG. Correlations among clinical assessment measures (CAPS and PSQI) across PTSD and TE groups were estimated using Spearman coefficient. Significant

correlations were inspected for outlying data points, which were removed accordingly.

2.5.1 Machine-learning analyses

Our dataset comprised 74 different features of PTSD, including subjective sleep, objective sleep, and memory and metabolite variables, across three groups (PTSD, TE and HC). Our objective was to identify the minimum combination of features with the highest diagnostic accuracy. Data pre-processing handled missing data using the kNNimputation package in R (Torgo, 2010), applying a non-parametric nearest neighbour method, akin to previous reports (Galatzer-Levy, Karstoft, Statnikov, & Shalev, 2014).[1] Categorical variables were then treatment coded (e.g. 0, 1) and continuous variables were *Z*-transformed in order to normalise the range and variance of all data measured by disparate technologies and clinical nosology. All variables were then subjected to BRB-Array Tools (Simon et al., 2007) supervised classification methods to construct PTSD classifiers. Two separate models were specified: (1) HC *vs.* trauma-exposed participants (PTSD and TE), and (2) PTSD *vs.* TE participants. Each model consisted of three steps. First, to ensure a fair comparison, all variables were subjected to classifier construction. This heuristic approach was used to cast a wide net to

[1]A total of 31 missing data points across 20 different measures were imputed. This represents ~0.6% of the total data used in our analyses

catch all potentially informative measurements, while false positives would be pared off by subsequent optimisation and cross-validation steps.[2] Second, classifiers composed of different numbers of measurements were constructed by recursive feature elimination (RFE), which provided feature selection, model fitting, and performance evaluation through identifying the optimal number of features with maximum predictive accuracy.[3] ~~RFE is a feature selection method that fits a model and removes the weakest feature (or features) until the specified number of features is reached. Features are ranked by the model's feature importance attributes, and by recursively eliminating a small number of features per loop, RFE attempts to eliminate dependencies and collinearity that may exist in the model.~~ Third, the ability of RFE to predict group outcome was assessed by support vector machines and compared with four different multivariate classification methods (that is, diagonal linear discriminant analysis, nearest centroid, first-nearest neighbours and three-nearest neighbours) in a nested leave-one-out cross-validation approach. Three notable advantages of this approach, include: i) its

[2]In cross-validation, data are broken up into *K* partitions and then, *K* times in turn, one partition is selected for testing while the the remaining ones are used for training. We used a nested cross-validation, which uses an outer k-fold cross-validation loop to split the data into training and test folds, while an inner loop is used to select the model via k-fold cross-validation on the training fold only. After model selection, the test fold is then used to evaluate the model performance. Supplemental Figure 1 is a graphic depiction of these points.

[3]RFE is a feature selection method that fits a model and removes the weakest feature (or features) until the specified number of features is reached. Features are ranked by the model's feature importance attributes, and by recursively eliminating a small number of features per loop, RFE attempts to eliminate dependencies and collinearity that may exist in the model.

use of maximum-margin hyper-planes in order to maximise the SVMs ability to predict the correct classification of previously unseen samples using small incremental changes; ii) the use of nested leave-one-out cross validation; and iii) the use of RFE to identify a unique set of features with maximum predictive value. The comparison between and amongst these four machine learning methods have been reviewed elsewhere (Simon et al., 2007). A detailed schematic of the current approach is summarized in **Supplemental Figure 1.**

3. RESULTS

3.1 Clinical characteristics of PTSD and TE participants

Table 2 shows the correlations between PSQI Global Score and CAPS Total Score with other clinical measures. Participants' BDI-II Score, PSQI Global Score and CAPS total score showed medium to strong positive correlations with each other, indicating that higher severities of depression, sleep disruption and PTSD were all associated with one another.

3.2 PSG and declarative memory analysis

Between-group analyses on PSG data from N2 showed that, compared to HC participants, PTSD and TE participants displayed

prolonged NREM 1% (p=0.04 and p=0.03, respectively), and decreased SWS% (p=0.01 and p=0.02, respectively**; see Supplementary Table 1)**

Declarative memory performance was measured during the day and N2 sessions (**Supplementary Table 2)**. Regarding data from the *night* sessions, PTSD participants performed significantly more poorly than HC on *a*) LM delayed recall, *b*) LM retention, *c*) WL delayed recall, *d*) delayed recall composite, and e) retention composite (p = .05, p = .04, p = .01, p = .02, and p = .001 respectively). TE participants performed more poorly than HC on *a*) LM delayed recall, *b*) retention composite and *c*) WL delayed recall (p = .01, p = .001, and p = .02 respectively). Analyses of data from the *day* session detected no significant between-group differences.

3.3 Noradrenergic and adrenergic metabolite analysis

No significant differences were observed in metabolite levels either between groups or at any of the specified collection times (**Supplementary Table 3**). Additionally, no metabolite measurement was significantly associated with PSQI Global Score or with the CAPS Total Score.

3.4 Exploring PTSD classification with machine-learning

Using SVM-learning two separate models were specified to distinguish between: 1) HC *vs.* trauma groups (PTSD and TE); and 2) PTSD *vs.* TE. We identified five features able to discriminate HC from PTSD and TE participants with 80% accuracy (**Figure 1A-C and Supplementary Table 4A and 4B**). The composition of this classifier was populated with one memory feature (WL delayed recall, night session), three sleep features (PSQI, SWS% N2, awakenings N2), and one biological marker (metanephrine 16h00-24h00) (**Figure 1B and Supplementary Table 4A**). The second model identified five features able to discriminate PTSD from TE participants with 70% accuracy using SVM classification (**Figure 1D-F and Supplementary Table 4B**). The composition of this classifier was populated with five sleep features (PSQI, subjective sleep N2, normal bedtime, arousals N2, and awakenings N2) (**Figure 1E**).

4. DISCUSSION

We set out to investigate relationships between sleep, memory, and noradrenergic and adrenergic metabolites in PTSD using conventional parametric/non-parametric analyses and exploratory supervised multivariate classification. Analyses of data from a primary cohort of 20 PTSD, 20 trauma-exposed, and 20 healthy control participants controlled for age, smoking and HIV status, as

these variables have the potential to confound outcomes related to sleep and memory (Lo, Groeger, Cheng, Dijk, & Chee, 2016; Watkins & Treisman, 2015). Initial between-group and correlational analyses replicated previous findings, indicating that PTSD diagnosed individuals self-reported poorer sleep quality, objectively demonstrated less sleep depth and evidenced deficits in neutral declarative memory in comparison to healthy controls. The novelty of the current paper, however, lies in findings from exploratory SVM-learning analysis. That analysis suggests that a combination of sleep (measured both objectively and subjectively), metabolite and neutral declarative memory variables could differentiate those with trauma exposure from healthy controls with 80% accuracy. Furthermore, PTSD and TE participants could be differentiated from one another, with 70% accuracy, by a combination of subjective and objective sleep variables (but not metabolite or neutral declarative memory variables). Thus, from among a broad range of clinical features, sleep characteristics were the primary features that differentiated those with PTSD from those without.

Our findings are consistent with previous reports suggesting sleep disruption in PTSD is central to the disorder, rather than merely a secondary symptom (Agorastos et al., 2014; Pigeon & Gallegos, 2015; Spoormaker & Montgomery, 2008). Indeed, some studies indicate that sleep disruption, specifically that related to REM fragmentation, is predictive of development of PTSD following

trauma (Mellman, Bustamante, Fins, Pigeon, & Nolan, 2002). In other words, there are strong suggestions that sleep quality is a key factor in the transition between trauma experience and resilience or development of psychopathology. Furthermore, several randomised control trials have demonstrated that both psychotherapeutic and pharmacological treatments aimed specifically at sleep disruption in PTSD are effective not only in treating sleep disturbance, but also ameliorate other symptoms of the disorder (Davis & Wright, 2007; Germain et al., 2012; Raskind et al., 2013; Talbot et al., 2014). Together, these strands of the literature suggest that sleep disruption is not an isolated symptom of PTSD, and that it may, in fact, be mechanistically associated with other cognitive, emotional, and endocrine functions within the disorder.

4.1 Features differentiating PTSD, TE and HC participants

Five features (three related to subjective measures of sleep quality, and two related to PSG measures) distinguished participants as belonging to either the PTSD or the TE group (**Figure 1 D-F**): subjective laboratory sleep quality, subjective home sleep quality, normal bedtime, number of spontaneous arousals during laboratory sleep, and number of awakenings during laboratory sleep. Regarding the three subjective sleep features, better subjective laboratory sleep quality, poorer subjective home sleep, and earlier bedtime were associated with a PTSD diagnosis rather than with

trauma exposure alone. Although these classifiers contrast with respect to subjective sleep quality between the laboratory and home environment, previous studies have found that PTSD-diagnosed individuals self-report better sleep quality in the laboratory than in the home environment (Hurwitz et al., 1998; Kobayashi, Huntley, Lavela, & Mellman, 2012; Lipinska & Thomas, 2017; Spoormaker & Montgomery, 2008). Furthermore, although at first glance it might be surprising that PTSD-diagnosed individuals tended to have earlier bed-times, we speculate that the nature of their home environments might explain this. Specifically, those carrying the diagnosis may go to be earlier in an attempt to avoid amplified feelings of nighttime insecurity and vulnerability associated with the objective reality of danger present in South African townships.

Regarding PSG measures, increased spontaneous arousals and awakenings were also associated with a PTSD diagnosis, rather than with trauma exposure alone. This finding suggests that despite fair-quality self-reported sleep in the laboratory, PTSD-diagnosed individuals objectively have more fragmented sleep than TE participants. Several recent studies have demonstrated that fragmentation of sleep is an important characteristic of sleep disruption in PTSD (Mellman et al., 2002; Mellman, Kobayashi, Lavela, Wilson, & Hall Brown, 2014; van Liempt et al., 2011).

Classifiers that differentiated between those with trauma exposure

(PTSD and TE participants) and those without (HC participants) indicated that exposure was associated with poorer subjective and objective sleep quality (that is, a higher PSQI score and less SWS percentage), elevated evening adrenaline, and poorer neutral declarative memory at post–sleep awakening (**Figure 1 A-C**). These findings are important because previous research has shown that activation of the sympathetic branch of the autonomic nervous system in the evening - proposed in this study by descriptively elevated adrenaline in trauma-exposed individuals - is associated with poor sleep quality, including disrupted early-night SWS (Mitchell & Weinshenker, 2010). Because SWS is critical for memory consolidation (Feld & Diekelmann, 2015; Marshall, Helgadottir, Molle, & Born, 2006; Walker, 2009), the implication here is that lowered SWS percentage in trauma-exposed individuals may contribute to poor neutral declarative memory, a conjecture consistent with data reported in one other study (van Liempt et al., 2011).

This conjecture is supported, in part, by the observation that poorer neutral declarative memory performance was associated with trauma exposure only at post-sleep awakening, rather than before sleep or across waking according to the SVM model. This interpretation is tempered somewhat because the reported measure of neutral declarative memory did not control for pre-sleep learning. Between-group comparisons of several measures of neutral

declarative memory performance also demonstrated post-sleep patterns of deficits in trauma-exposed individuals. Collectively, these findings permit the cautious speculation that sleep-dependent memory consolidation is impaired in individuals with trauma exposure. We are currently analyzing data from a study designed to test that speculation.

Interestingly, this cluster of variables only differentiated trauma-exposed individuals from HC participants – it did not distinguish those with trauma exposure but no PTSD from those with PTSD. One possible explanation is that although poor sleep quality (measured both subjectively and objectively), elevated evening adrenaline and poor neutral declarative memory at post–sleep awakening are all associated with trauma, sleep fragmentation is a core feature distinguishing trauma-exposed individuals with and without PTSD. An alternative explanation with respect to sexual assault survivors, who comprised the entirety of this sample, is that trauma-exposed individuals without PTSD still bear a high load of trauma symptomology that makes them more similar to their counterparts with PTSD than to HC participants. Support for this speculation emerges from a meta-analysis of neutral declarative memory deficits in PTSD which found that PTSD-diagnosed participants had poorer memory in comparison with TE participants, except for survivors of sexual assault, where there were no such between-group differences (Johnsen & Asbjornsen, 2008).

4.2 Strengths and limitations

A strength of the study design is that it controlled for a series of variables (time since trauma, type of trauma, childhood trauma exposure, and a history of alcohol or substance abuse) that might otherwise have offset results and interpretation of the main findings. Such confounds have been identified across the literature, and constitute a significant barrier towards identifying reproducible objective markers of sleep and memory in PTSD. Additionally, most studies investigating the underlying neurobiology and disturbances of circadian rhythm in PTSD have been conducted using male combat veterans; few PTSD studies focus on women. Because women are more likely to develop PTSD (Christiansen & Hansen, 2015), this highlights an important aspect of the design. Moreover, although many studies investigating sleep and memory deficits in PTSD have included trauma survivors, diagnosed with or without PTSD, they often do not also incorporate a trauma-free healthy control group. Finally, studies of the application of bio-statistical tools for integrating and cross-validating such measures for predicting PTSD diagnosis are scarce. Advantages of the SVM technique over conventional linear regression include: (*a*) the quality of out-of-sample generalisation and ease of training; (*b*) the use of maximum-margin hyper-planes in order to maximise the SVMs ability to predict the correct classification of previously unseen samples; (*c*) the use

of hold-out cross validation, which are capable of achieving superior accuracies than those derived from conventional approaches; and (*iv*) the use of RFE to identify a unique set of features with maximum predictive value (*i.e.* classifiers). We anticipate this framework can be extended for future studies collecting a range of clinical, PSG, and/or biological markers from samples of individuals with psychiatric diagnoses.

One limitation is that participants in the PTSD and TE groups had unmatched depression severity. Previous studies show that depression severity increases proportionally with PTSD symptomology in sexual assault survivors (Au, Dickstein, Comer, Salters-Pedneault, & Litz, 2013). Given this clinical finding, our sample represents the clinical 'norm'. However, we are unable to fully delineate the specific contributions of PTSD and depression symptomology. Furthermore, although our results suggest features that distinguish PTSD, TE, and HC participants, our study does not provide direct evidence of relationships, causal or otherwise, among these measures. Moreover, the current work characterises these data with the use of *a priori* defined clinical diagnoses, which may potentially limit prediction/classification accuracies. Finally, this exploratory study applies leave-one-out cross validation in a moderately sized cohort, thus necessitating split sample cross-validation in a larger, non-overlapping sample of participants.

4.3 Conclusion

Using machine learning techniques, we were able to differentiate between individuals with (a) trauma exposure versus no trauma exposure or psychopathology, and (b) those with a PTSD diagnosis versus those with trauma exposure but without PTSD. The analyses yielded moderate-to-high classification accuracies. With respect to (a), the findings showed that sleep variables (measured both subjectively and objectively), evening metadrenaline metabolites, and neutral declarative memory performance after a period of sleep differentiated between those with trauma exposure and those without. With respect to (b), the findings showed that only sleep variables differentiated those with a PTSD diagnosis from those with trauma exposure but without PTSD. Our findings lend support to the hypothesis that sleep disruption is a core feature of PTSD, and provide a framework for future sleep- and memory-based investigations into the pathophysiology of the disorder. Specifically, we hope to inspire future, larger sleep- and memory-based research to apply multivariate classification techniques to model PTSD diagnosis. Regarding clinical implications of the present findings, understanding sleep disruption as a central component of PTSD, and consequently testing whether targeted sleep interventions alleviate other symptoms of the disorder, may pave the way for novel treatment approaches.

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Table 1. Demographic and clinical characteristics of all participants (N=60).

	HC (N=20)	**TE (N=20)**	**PTSD (N=20)**			
	Mean ± SD	*Mean ± SD*	*Mean ± SD*	*F/H (df = 2,57)*	*P*	*Post-Hoc Significance*
Age	25.30 ± 4.62	24.50 ± 4.41	25.50 ± 4.46	0.793	0.673	*n.s.*
Education (years)	12.90 ± 1.99	12.50 ± 2.04	11.65 ± 1.63	4.880	0.087	*n.s.*
WASI PIQ†	83.80 ± 14.21	83.00 ± 12.12	78.05 ± 14.77	2.891	0.236	*n.s.*
MAST	0.70 ± 1.12	0.85 ± 1.39	1.00 ± 1.02	1.737	0.420	*n.s.*
PSQI	4.15 ± 2.65	6.22 ± 3.25	9.75 ± 4.11	19.354	<0.001	PTSD > TE; PTSD > HC; TE > HC
BDI-II	6.20 ± 3.41	17.89 ± 7.80	30.60 ± 6.05	43.130	<0.001	PTSD > TE; PTSD > HC; TE > HC
Normal bedtime	22h50 ± 0.9h	22h55 ± 1.0h	21h10 ± 1.0h	6.812	0.049	TE > PTSD; HC > PTSD
Nicotine: *N* (%)	5 (25%)	1 (5%)	6 (30%)	-	-	*n.s.*
Time since trauma	-	1.26 ± 1.06	1.26 ± 1.03	1.800	0.892	*n.s.*
Total counsel sessions	-	4.20 ± 3.00	3.94 ± 1.85	1.039	0.308	*n.s.*
CAPS	-	29.50 ± 11.65	68.80 ± 13.46	29.293	<0.001	PTSD > TE
MINI total	-	2.05 ± 1.32	3.65 ± 1.53	9.270	0.002	PTSD > TE

Shapiro-Wilk test was used to assess normality of variables and either a one-way analysis of variance (ANOVA) or Kruskal-Wallis ANOVA with post hoc Tukey correction was implemented accordingly. Abbreviations: WASI PIQ, Wechsler Abbreviated Scale of Intelligence Performance Intelligence Quotient; MAST, Michigan Alcohol Screening Test; PSQI, Pittsburgh Sleep Quality Index; BDI-II, Beck Depression Inventory-Second Edition; Nicotine, number of smoking participants; Time since trauma, given in years; CAPS, Clinician-Administered PTSD Scale; MINI, Mini-International Neuropsychiatric Interview; MINI total, number of MINI diagnoses; n.s., not significant.

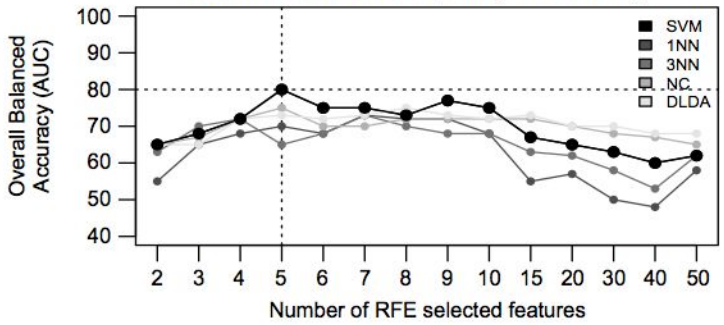
†IQ scores are in the average range for non-first language English speaking South African adults with 12 years of poor quality education (Pienaar, Shuttleworth-Edwards, Klopper, & Radloff, 2016)

Table 2. Correlations between PTSD severity (CAPS Total) and subjective sleep (PSQI) with clinical measurements across PTSD and TE groups (N=40).

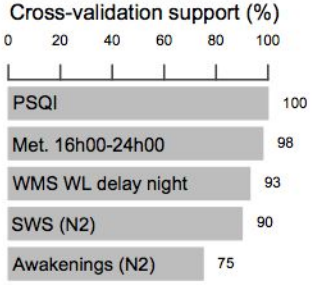
	PTSD and TE (*n*=40)		PTSD (*n*=20)		TE (*n*=20)	
	PSQI R-value (*P*-value)	CAPS Total R-value (*P*-value)	PSQI R-value (*P*-value)	CAPS Total R-value (*P*-value)	PSQI R-value (*P*-value)	CAPS Total R-value (*P*-value)
Time since trauma	0.230 (0.150)	0.063 (0.700)	0.39 (0.089)	0.293 (0.21)	-0.037 (0.876)	-0.199 (0.400)
BDI	0.553 (< 0.001)*	0.804 (< 0.001)*	0.501 (0.024)*	0.542 (0.013)*	0.436 (0.055)	0.584 (< 0.01)*
PSQI	-	0.654 (< 0.001)*	-	0.588 (< 0.01)*	-	0.654 (< 0.01)*
CAPS Total	0.654 (< 0.001)*	-	0.588 (< 0.01)*	-	0.654 (< 0.01)*	-
CAPS B	0.550 (< 0.001)*	0.827 (< 0.001)*	0.465 (0.039)*	0.762 (< 0.001)*	0.258 (0.271)	0.462 (0.040)*
CAPS C	0.581 (< 0.001)*	0.900 (< 0.001)*	0.462 (0.040)*	0.79 (< 0.001)*	0.463 (0.040)*	0.698 (< 0.001)*
CAPS D	0.576 (< 0.001)*	0.816 (< 0.001)*	0.304 (0.193)	0.533 (0.015)*	0.511 (0.021)*	0.751 (< 0.001)*
MINI Total	0.381 (0.015)*	0.604 (< 0.001)*	-0.059 (0.805)	0.201 (0.396)	0.632 (< 0.01)*	0.839 (< 0.001)*
MINI depression	0.379 (0.016)*	0.496 (< 0.01)*	-0.199 (0.401)	-0.06 (0.802)	0.665 (< 0.01)*	0.872 (< 0.001)*
MINI suicidality	0.128 (0.430)	0.098 (0.550)	0.072 (0.762)	-0.015 (0.949)	0.172 (0.468)	0.314 (0.177)
MINI panic disorder	-0.017 (0.923)	0.073 (0.661)	-0.15 (0.527)	-0.157 (0.510)	-0.009 (0.969)	-0.253 (0.281)
MINI agoraphobia	-0.066 (0.694)	0.325 (0.041)*	-0.229 (0.332)	0.184 (0.439)	-0.183 (0.441)	0.117 (0.623)
MINI PTSD	0.550 (< 0.001)*	0.655 (< 0.001)*	0.079 (0.740)	0.154 (0.517)	0.665 (< 0.01)*	0.872 (< 0.001)*
MINI hypomanic episode	0.359 (0.023)*	0.298 (0.062)	0.458 (0.042)*	0.234 (0.321)	*NA*	*NA*
MINI dysthymia	0.130 (0.421)	0.222 (0.174)	-0.145 (0.541)	-0.098 (0.681)	0.293 (0.210)	0.176 (0.457)
MINI OCD	0.244 (0.130)	0.179 (0.270)	0.315 (0.176)	0.336 (0.148)	0.206 (0.384)	0.273 (0.244)
MINI social phobia	0.145 (0.373)	0.318 (0.045)*	0.012 (0.959)	0.386 (0.093)	*NA*	*NA*
MINI mood disorders	-0.014 (0.934)	0.257 (0.113)	-0.135 (0.569)	0.301 (0.198)	*NA*	*NA*

Spearman's correlation was used to assess relationships of PSQI and CAPS to clinical diagnostic criteria. Abbreviations: BDI, Beck Depression Inventory; PSQI, Pittsburgh Sleep Quality Index; CAPS, Clinician Administered PTSD Scale; MINI, Mini-International Neuropsychiatric Interview. *P*-value < 0.05(*) is considered significant.

a.



b.

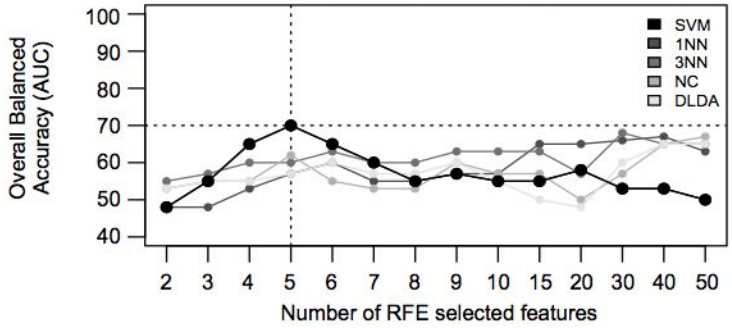


c.

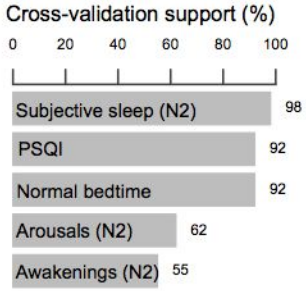
Model 1: Top 5 Features

	NC	1NN	3NN	DLDA	SVM
Sensitivity	0.75	0.78	0.80	0.75	0.87
Specificity	0.65	0.45	0.43	0.67	0.65
AUC	0.72	0.68	0.68	0.72	0.80
95% C.I.	0.59-0.84	0.52-0.80	0.52-0.82	0.57-0.83	0.69-0.91

e.



e.



f.

Model 2: Top 5 Features

	NC	1NN	3NN	DLDA	SVM
Sensitivity	0.76	0.70	0.72	0.75	0.80
Specificity	0.33	0.36	0.38	0.39	0.61
AUC	0.55	0.60	0.63	0.60	0.70
95% C.I.	0.49-0.60	0.48-0.66	500.-0.68	0.53-0.67	0.66-0.74

Figure 1. Supervised machine-learning integrating clinical, sleep, memory, and biological data to predict group outcomes. Panel (**a**): ~~Classifier~~ classifier accuracies achieved when discriminating HC from PTSD and TE groups; ~~and~~ Panel (**b**): cross-validation support (i.e., the percentage of times a feature was used in the predictor for a leave-one-out cross-validation procedure); ~~and~~ Panel (**c**): results of the top performing model, which ~~containing~~ contains 5 features ~~are displayed.~~; Panel (**d**): ~~Classifier~~ classifier accuracies achieved when discriminating PTSD participants from TE participants; Panel ~~and~~ (**e**): cross-validation support; Panel ~~and~~ (**f**): results from the ~~top~~ top-performing model ~~containing 5 features are displayed~~. ~~Cross-validation support is the percentage of times when a feature was used in the predictor for a leave-one-out cross-validation procedure.~~ In each case, supervised class prediction was performed ~~using different combinations of genes with~~using Recursive Feature Elimination (RFE) and evaluated with four different multivariate classification methods. For Panels (c) and (f), ~~*Sensitivity*~~ *sensitivity* is ~~defined as~~ the rate of true positives over the rate of true positives plus false negatives, and ~~.~~ *S*~~*S*~~*pecificity* is ~~defined as~~ the rate of true negatives over the rate of true negatives plus false positives. Abbreviations: AUC, overall balanced accuracy; C.I., confidence interval; NC, nearest centroid; 1NN, nearest neighbour; 3NN, three-nearest neighbours; SVM, support vector machines; DLDA, diagonal linear discriminate analysis.

Supplementary Figure 1. Schematic of the implemented machine-learning and cross-validation pipeline. Leave-one-out cross-validation is initiated by randomly sub-setting one sample (test) and the remaining samples (training set) are subjected to recursive feature elimination feature selection (FS) and internal nested 10-fold cross-validation performed 10 times. The final classifier (C) is applied to predict classification for the original left-out sample. This process is iterated until all samples are left out and a contingency table is populated for each prediction, entailing true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN). These values are used to compute measures of sensitivity, specificity and overall balanced accuracy (AUC).