Universität Zürich[UZH]

Sleep & Health Zurich

Towards Identification of Metabolic Biomarkers of Sleepiness for Risk Prevention and Traffic Safety

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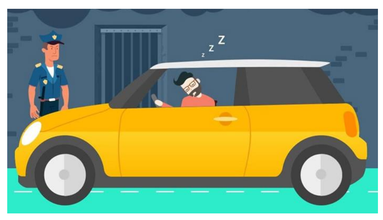
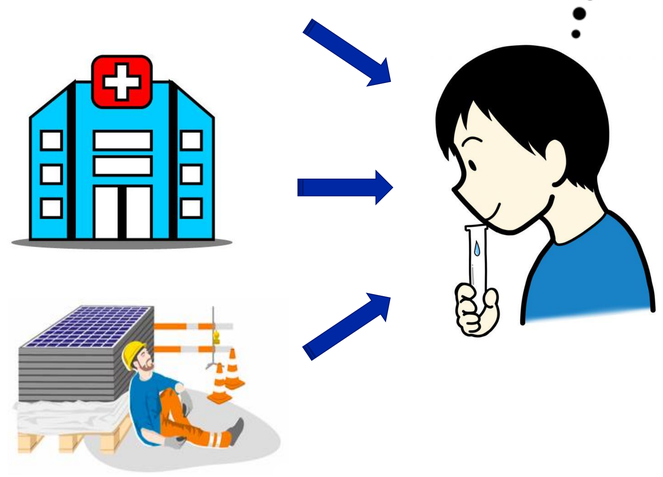
Background

Sleep loss and sleepiness are a multifaceted and ever-growing problem in modern societies, leading to high costs by affecting public health, safety, productivity and general well-being.
To date, there is no reliable objective measurement for the detection of acute sleepiness or for post-hoc analysis. For instance, a roadside sleepiness detection test could improve traffic safety both for prosecution and prevention settings. Nowadays, driving impairment can be measured and quantified securely in highly immersive simulators with customizable scenarios that adapt to changing day and night situations.
Forensic toxicology potentially provides powerful methodologies to identify and target biomarkers for sleepiness detection in routine analyses by unraveling the complex relationship between sleepiness-induced performance impairments and the metabolome.





Aim: An oral fluid test for the detection of sleepiness would be helpful in road safety, clinical, and occupational settings.

In a nutshell…

One night of acute sleep deprivation affected vigilance, simulated driving performance, and the metabolic content of oral fluid stronger than consecutive sleep restriction with equal sleep loss.
The driving impairment was increased beyond the clinical threshold after sleep deprivation only.
This study further presents the first predictive model for classifying sleep deprivation against sleep restriction and controlled sleep in oral fluid metabolites under realistic conditions.
A logistic regression model was built to identify sleep-deprived donors in unseen oral fluid test samples based on 12 molecular features. This model could classify the samples with high accuracy and precision, even if compared with sleep-restricted donors. It does not require a reference sample after controlled sleep and loses classification performance after recovery sleep, making it suitable for a forensic use case.

Study

ClinicalTrials.gov identifier: NCT05585515

Population
20 young, healthy-sleeping men (age 22.4 years [median], habitually sleeping ~8 hrs per night)

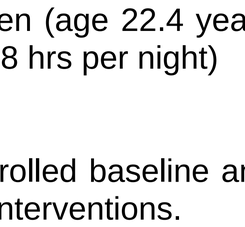
Design
Randomized, cross-over, controlled baseline and recovery sleep before & after interventions.

Interventions
Sleep deprivation (SD): 1x 8 hrs sleep deficit
Sleep restriction (SR): 4x 2 hrs sleep deficit
Control (C): no sleep deficit

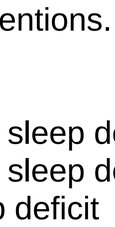
Methods

Driving simulation (30 km)
custom-modified BMW i3
360° immersive environment
➢ 12 min night scenario
➢ 10 min morning scenario
Focus on Standard Deviation of Lateral Position (SDLP), directly associated with crash risk. [1]

Psychomotor Vigilance Test (PVT)
10 min, 100 stimuli
1 x at baseline
5 x after interventions
1 x after recovery sleep



Oral fluid metabolomics
collected in Salivette® device
1 x at baseline
6 x after intervention
1 x after recovery sleep



The collected oral fluid samples were screened for metabolites in an untargeted metabolomics approach (multi-column liquid chromatography coupled to high-resolution tandem mass spectrometry, i.e. LC-HRMS/MS).
Data analysis and machine learning modelling was conducted using scikit-learn package in Python 3.9.12.

The full study protocol was peer-reviewed and registered before the start of the study. [2]

Results

Driving simulation

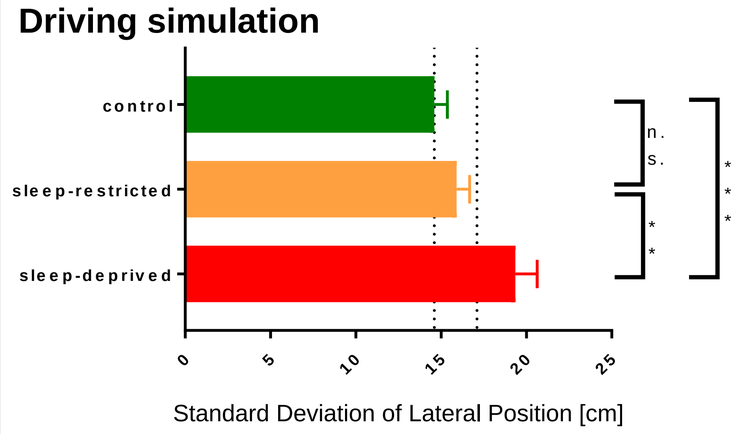


Fig.1: SDLP results. Mean and standard error values. The clinical threshold (indicated by dotted lines between control mean + 2.5 cm) is equal to a driving impairment under the influence of 0.5 ‰ blood alcohol concentration. [1] Asterisks show result of Friedman test and subsequent Wilcoxon test: ** p < 0.01, *** p < 0.001

Psychomotor Vigilance Test

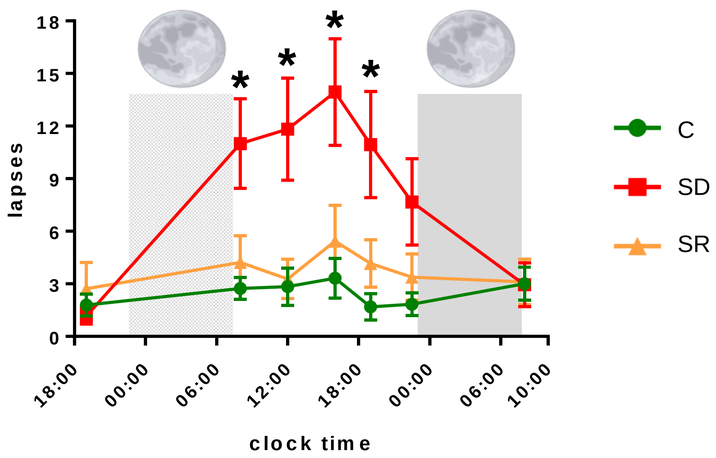


Fig.2: PVT lapses. Left night in light grey represents interventional night, right night in dark grey recovery night. Points indicate mean values, whiskers indicate standard error of the mean. Asterisks show FDR-adjusted result of repeated-measures ANOVA and subsequent Wilcoxon signed rank test (SD/SR vs C): * q < 0.05

Oral fluid metabolomics

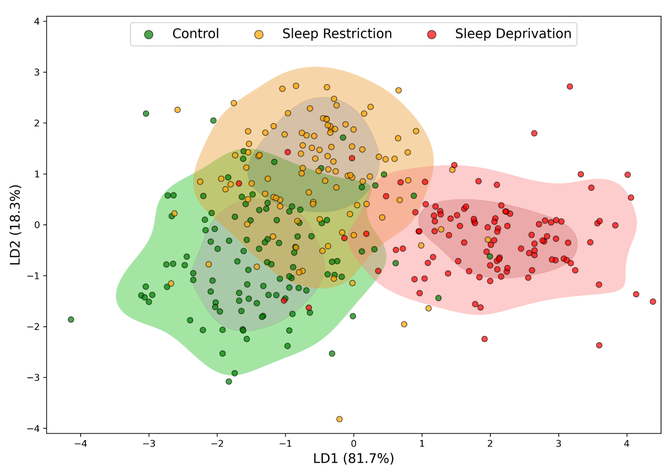


Fig.3: Linear discriminant analysis. Scatter plot of all 440 study samples, each consisting of 6034 molecular features (after data pre-processing)

Predictive model

Based on the oral fluid metabolomics data, a logistic regression (LR) classifier was trained to detect sleep deprivation, and iteratively refined using recursive feature elimination (RFE) with an 8-fold cross-validation procedure:

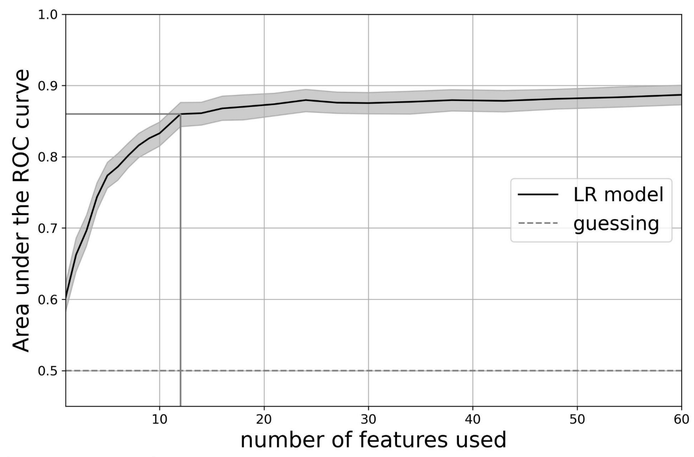


Fig.4: RFE results
Mean and 95% confidence interval of LR model compared with guessing

The results indicate that the LR model requires at least 12 molecular features to reach a stable and acceptable classification performance.
After reduction to the 12 most important molecular features and post-tuning of the decision threshold, the evaluation on classifying the unseen test samples (SD vs. C & SR) yielded these results:

Area under the ROC curve	0.86
Accuracy	0.92
Precision	0.91
F_1 score	0.88
Matthew's correlation coefficient (MCC)	0.82

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References

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2 Scholz, M., Lakaemper, S., Keller, K. *et al.* Metabolomics-based Sleepiness Markers for Risk Prevention and Traffic Safety (ME-SMART): a monocentric, controlled, randomized, crossover trial. *Trials* **24**, 131 (2023).