Sleep Disorders: Identifying Biomarkers and Clinical Applications

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Illnesses in Gulf War Veterans

• Complex set of symptoms that most resemble Chronic Fatigue Syndrome or Fibromyalgia:
  • fatigue, memory loss, sleep disturbances, joint pain, rash with depression/anxiety
• Sleep disorders such as insomnia, unrefreshing sleep are prevalent; in one small study mild sleep disorder breathing was found more frequently vs controls
• Psychological (anticipation, PTSD) and physical (desert, physostigmine, vaccines, CW, oil wells etc.) exposure were numerous, but none are constantly associated with illness
• No clear physical or biochemical abnormality. No consistent explanations, most agree it may be multifactorial
• Ascertainment/study results likely biased due to media attention
Why Study Sleep?

• Opportunity for major discovery: why we sleep is one of the last remaining biological mysteries.
• 70 million Americans complain of sleep problems:
  ✓ Sleep apnea (~20%)
  ✓ Insomnia (~10%)
  ✓ Restless Legs Syndrome/Periodic Leg Movements (~3%)
  ✓ Hypersomnia/Fatigue/Narcolepsy (~4%)
• Technology now exists to objectively analyze sleep data in ways that were previously impossible
• Move toward home based technology and therapies
Circadian Clock mechanisms well understood

4,256/7497=57% of genes are cycling
Sleep Homeostasis mechanisms not understood

- Sleep and circadian physiology interact to maintain wakefulness during the day and control sleep during the night.

- In the cortex and hypothalamus combined, 4811/11078=43% of genes are sleep debt dependent, with about 1,520 (13%) sleep debt specific (Mackiewicz et al, Physiological Genomics, 31(3): 441-457, 2007).

- No Core gene identified.
Sleep Disorders

• Obstructive sleep apnea (~20%)
  ✓ Sleepiness
  ✓ Cardiovascular risk

• Insomnia (~10%)
  ✓ Poor quality of life
  ✓ Anxiety/Depression

• Restless Legs Syndrome/Periodic Leg Movements (~3%)
  ✓ Depression
  ✓ Cardiovascular risk

• Hypersomnia/Fatigue/Narcolepsy (~4%)
  ✓ Link of long sleep with Bipolar disorder/Schizophrenia
All these new analyses are permitted thanks to novel signal analytic methods such as Wavelet, HHT in combination with various types of machine learning.

**Gold Standard:** Nocturnal Polysomnography (PSG)
Gold Standard: Nocturnal Polysomnography (PSG)

Electroencephalogram (EEG)

Chin Muscle Tone/Electromyogram (EMG)

Leg Movement (EMG)s

Electro-occulogram (EOG)

Breathing (flow)

Electrocardiogram (ECG)

Snoring

Breathing (effort)

Sleep hypnogram for one night of sleep

Wake
REM
Stage 1
Stage 2
Stage 3
Stage 4

time of night

first cycle
second
third
fourth
fifth

dreaming
deep sleep (slow wave)

Desynchronized EEG with sawtooth waves, atonia with EMG twitches, rapid eye movements.
EEG slows and alpha rhythm disappears, defining sleep onset and unconsciousness.
Appearance of K-complexes and sleep spindles in the alpha/sigma frequency range.
Increasing amounts of low frequency, high amplitude delta slow-waves.
Critical needs in the field and how we are solving them

- There is no single “on line” questionnaire that assess sleep habits and subjective sleep symptoms for all disorders
  - The field is still fragmented by discipline, with questionnaires that have been validated only in the context of single, pure sleep disorders populations
  - Creation of the on line Alliance Sleep Questionnaire (ASQ) and associated App

- Need for better analytics of PSG signals
  - Currently only summary statistics such as sleep stages, sleep efficiency, the Apnea Hyponea Index (AHI), periodic Leg movement Index (PLMI), REM sleep latency are provided, all scored by humans
  - Use of machine learning to reveal new biomarkers

- Need for better ‘at home” data collecting systems but what sensors should they have?
  - EEG, triaxial accelerometer, O2 sat, transcutaneous or expiratory Co2, snoring sound, cardioballistography, nasal or oral pressure cannula, Respiratory belts, EMG, Photo Pletysmography (PPG), EKG etc

- Need for biological biomarkers
  - Could we find biological markers of sleep disorders, hypoxia (sleep apnea), sleep deprivation/debt, circadian phase?
  - Genetics and proteonomics

- Need for at home, on line interventions with monitoring
  - cBTI on line
  - EEG with feedback therapies (rythm)
ASQ Uses Branching Logic:

Restless Legs Syndrome Section

**Negative Responses**

- Have you ever had unpleasant feelings or sensations in your legs that occurred on a regular basis, and mainly while sitting or lying down?
- Yes, it is still a problem for me now
- Yes, in the past, but I don't have it now
- No
- Don't know

- Have you ever felt the need or urge to move your legs that occurred on a regular basis, and mainly while sitting or lying down?
- Yes, it is still a problem for me now
- Yes, in the past, but I don't have it now
- No
- Don't know

**Positive Responses**

- Have you ever had unpleasant feelings or sensations in your legs that occurred on a regular basis, and mainly while sitting or lying down?
- Yes, it is still a problem for me now
- Yes, in the past, but I don't have it now
- No
- Don't know

- Have you ever felt the need or urge to move your legs that occurred on a regular basis, and mainly while sitting or lying down?
- Yes, it is still a problem for me now
- Yes, in the past, but I don't have it now
- No
- Don't know

**Additional questions**

- Are there unpleasant sensations in your legs or the urge to move your legs always due to a “Charlie horse” or muscle cramp?
- Yes
- No
- Don't know

- If you get up and move around, do these unpleasant sensations in your legs get any better with moving around?
- Yes
- No
- Don't know

- Are these sensations worse in the evening or right before you go to sleep or in the morning?
- Yes
- No
- Don't know

- When you actually experience these unpleasant sensations in your legs or the urge to move your legs, how distressing are they?
- Not at all distressing
- A little distressing
- Moderately distressing
- Very distressing
- Extremely distressing
<table>
<thead>
<tr>
<th>Insomnia Symptoms:</th>
<th>Test, Full, MRN n/a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insomnia Scale (ISI):</td>
<td>28</td>
</tr>
<tr>
<td>Meets ISQ diagnostic criteria:</td>
<td>No</td>
</tr>
<tr>
<td>Difficulty falling asleep:</td>
<td>Very severe</td>
</tr>
<tr>
<td>Problems waking too early:</td>
<td>Very severe</td>
</tr>
<tr>
<td>Difficulty staying asleep:</td>
<td>Very severe</td>
</tr>
<tr>
<td>Frequent wakenings:</td>
<td>Always (5-7 times per week)</td>
</tr>
<tr>
<td>Satisfaction with current sleep pattern:</td>
<td>Very dissatisfied</td>
</tr>
<tr>
<td>Extent prob interferes with daily functioning:</td>
<td>Very much interfering</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Restless Legs Symptoms:</th>
<th>Test, Full, MRN n/a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current Probability:</td>
<td>Unlikely</td>
</tr>
<tr>
<td>Current Severity Level:</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Limb discomfort/ unpleasant sensation while sitting/ lying down:</td>
<td>Yes, it is still a problem for me now</td>
</tr>
<tr>
<td>Frequency of symptoms:</td>
<td>n/a</td>
</tr>
<tr>
<td>Urge to move with relief upon movement:</td>
<td>Yes, it is still a problem for me now</td>
</tr>
<tr>
<td>Level of distress:</td>
<td>n/a</td>
</tr>
<tr>
<td>Unpleasant sensations assoc with muscle cramps:</td>
<td>Don't know</td>
</tr>
<tr>
<td>Age of onset:</td>
<td>5</td>
</tr>
<tr>
<td>Began with pregnancy:</td>
<td>n/a</td>
</tr>
<tr>
<td>Ended with same pregnancy:</td>
<td>n/a</td>
</tr>
</tbody>
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(validated in about 10,000 subjects @ Stanford sleep clinic)
Improved PSG analytics: Machine learning Convolutional Neural Network (ConvNet)

- A supervised prediction model in which the input data is low-level e.g. an image or a spectrogram.
- Features are constructed through a network of filter and subsampling layers.
- Deeper networks may construct more complex features.
- Resulting features depend on input data, as parameters change iteratively during training.
- Often used in computer vision or speech recognition tasks.

Example borrowed from:
http://www.computervisionblog.com/2015/03/deep-learning-vs-machine-learning-vs.html

Example borrowed from:
http://cs231n.github.io/convolutional-networks/
Feed-forward neural network (FFNN):
- Process each observation independently

Recurrent neural network (RNN):
- Introduces a temporal dimension
- Used to model change in frequency

*Long Short-Term Memory (LSTM) network*
- Introduces support for long-term dependencies
- Context-aware decisions

Machine Learning
Sleep data representation. Overlapping autocorrelations: Wake

EEG - C3

EEG - F3

EEG - O1

EEG L/R

EOG

EMG - Chin
Three networks models are used:

- **Network 1**:
  - Conv 7x7 / 2 64
  - Max Pool 3x3 / 2
  - Conv 3x3 x 128
  - Max Pool 2x2
  - Avg. Pool 24x7
  - Concatenate
  - Fully connected layer - 512 nodes
  - Softmax output - 5 nodes
  - Total nr. of parameters: -2.8M

- **Network 2**:
  - Conv 7x7 / 3 64
  - Max Pool 3x3
  - Conv 3x3 x 64
  - Max Pool 2x2
  - Avg. Pool 33x5
  - Concatenate
  - Fully connected layer - 512 nodes
  - Softmax output - 5 nodes
  - Total nr. of parameters: -2.8M

- **Network 3**:
  - Conv 7x7 / 3.1 64
  - Max Pool 4x2
  - Conv 3x3 x 64
  - Max Pool 2x2
  - Avg. Pool 16x5
  - Concatenate
  - Fully connected layer - 512 nodes
  - Softmax output - 5 nodes
  - Total nr. of parameters: -22.3M
Comparaison with the AASM Inter-Scorer Reliability

Performance of networks used

Distribution of scorers

Net1

Net2

Net3

| P(y|x) |
|--------|
| 0.5    |
| 1      |

Minutes

<table>
<thead>
<tr>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
</tr>
</thead>
</table>
Selection of narcolepsy features using Lasso regression

<table>
<thead>
<tr>
<th>#</th>
<th>Description</th>
<th>Relative importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>REM latency (Automatically extracted)</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Mean co-occurrence of REM and wake</td>
<td>0.80</td>
</tr>
<tr>
<td>3</td>
<td>Mean value of REM x the time till 10 % of the cumulative fraction of REM is reached.</td>
<td>0.68</td>
</tr>
<tr>
<td>4</td>
<td>The maximum value of co-occurrence of N2 and REM.</td>
<td>0.66</td>
</tr>
<tr>
<td>5</td>
<td>The mean value of N2 x the time till 10 % of the cumulative fraction of N2 is reached.</td>
<td>0.61</td>
</tr>
<tr>
<td>6</td>
<td>Time till 10 % of the cumulative fraction of the co-occurrence of all 5 stages is reached.</td>
<td>0.55</td>
</tr>
<tr>
<td>7</td>
<td>Mean value x the Shannon entropy of the co-occurrence of N2 and N3.</td>
<td>0.40</td>
</tr>
<tr>
<td>8</td>
<td>Time till 10 % of the cumulative fraction of the co-occurrence of N1 and N2 is reached.</td>
<td>0.37</td>
</tr>
<tr>
<td>9</td>
<td>Maximum value of co-occurrence of wake and N1</td>
<td>0.36</td>
</tr>
<tr>
<td>10</td>
<td>Maximum value of co-occurrence of wake and REM</td>
<td>0.35</td>
</tr>
</tbody>
</table>
Validation to diagnose narcolepsy

Stephansen et al., in preparation
LSTM detection of arousals

Performance per individual (dot size=n observation)

- Precision = 0.76
- Recall = 0.82
- F1 = 0.79
- auPR = 0.84
Goal 1: A single, multimodal PSG analyzer

- Doing classic and novel sleep stage identification
- Detecting macro and microarousals (autonomic and EEG)
- Detecting all breathing abnormalities during sleep, including subtypes of sleep apneas
- Detecting periodic leg movements during sleep, including subtypes
- The detector would reveal a complex multi-dimensional phenotype for each individual
- To apply in the context of the Stanford Technology Analytics and Genomics of sleep (STAGES) study
# Need to Measure Phenotypes Across Disciplines

<table>
<thead>
<tr>
<th>Insomnia, hypersomnia/narcolepsy, and psychiatric comorbidity</th>
<th>EEG power spectra features per sleep stage*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Delta power during wake, Slow Wave Sleep during sleep corrected for habitual sleep amounts</td>
</tr>
<tr>
<td></td>
<td>Hilbert Huang Transform analysis of the EEG (see letter of support of Dr. Huang)†</td>
</tr>
<tr>
<td></td>
<td>Sleep stage transition analysis*</td>
</tr>
<tr>
<td></td>
<td>Sleep stage space analysis and automatic sleep/wake scoring by 10 sec epochs*</td>
</tr>
<tr>
<td></td>
<td>Slow wave/delta power dynamics across the night and sleep cycle analysis</td>
</tr>
<tr>
<td></td>
<td>Coherence analysis (inter- and intra- hemispheric, per sleep stage)</td>
</tr>
<tr>
<td></td>
<td>Microarchitecture analysis (spindles*, k-complex, saw tooth waves detection)</td>
</tr>
<tr>
<td></td>
<td>REM sleep features (REM density, REM sleep atonia)*</td>
</tr>
<tr>
<td></td>
<td>Microarousal detections out of various sleep stages†</td>
</tr>
<tr>
<td></td>
<td>Measures of circadian phase through analysis of all signals in correlation with body temperature†</td>
</tr>
</tbody>
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<thead>
<tr>
<th>RLS and PLMs (may predispose to depression and cardiovascular disease)</th>
<th>PLMI, periodicity index, dynamics across the night and by sleep stage, with and without arousal (frequency of PLMs)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time locked analysis of EEG, EMG, ECG locked on PLM (impact of PLMs)*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sleep disordered breathing (SDB) (predisposes to cardiovascular disease and sleepiness)</th>
<th>Apnea Hypopnea Index and other derivatives (by sleep stage, with various definitions with and without oxygen desaturation and arousal, central or obstructive) (frequency of respiratory events)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time locked analysis of Oxygen saturation, EEG, EMG, ECG locked on SDB events per sleep stage (severity of respiratory events)*</td>
</tr>
<tr>
<td></td>
<td>Oxygen saturation at baseline and end of the night, per sleep stage, time spent at various levels of Oxygen saturation*</td>
</tr>
<tr>
<td></td>
<td>Breathing frequency, inspiration and expiration time per sleep stage†</td>
</tr>
</tbody>
</table>

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<tr>
<th>Parkinson’s disease early biomarkers</th>
<th>REM sleep without atonia*</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Sleep fragmentation, loss of spindles*</td>
</tr>
<tr>
<td></td>
<td>ECG-power spectra changes across sleep stages*</td>
</tr>
</tbody>
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<tr>
<th>Alzheimer’s disease early biomarkers</th>
<th>Power spectra in REM sleep*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coherence analysis</td>
</tr>
</tbody>
</table>

| Seizure activity during sleep | Analysis of shape, PR, QT, arrhythmia, and conduction defects across sleep stages |

| ECG | Analysis of shape, PR, QT, arrhythmia, and conduction defects across sleep stages |
Analytical Flow Chart of large scale PSG analysis

1. Manually scored text files
   - Validation
   - Improvement
2. European Data Format (EDF) - files
   - Automatic scoring of Sleep Stages, PLM, SDB and hypoxia events (with flagging or unusual cases)
3. Clinical Diagnosis Symptoms (ASQ)
4. Development of new biomarkers with physiological or pathological significance
5. Demonstration of clinical value in Wisconsin Sleep (WSC) and Stanford Sleep Cohorts (SSC)
6. Incorporation of salient biomarkers in automatic sleep scoring outputs (with flagging or unusual cases)
7. Genetic Data
The whole thing is more than the sum of its parts –
tell me, is that your feeling only or more than that?

Dirk Meissner
Goal 2: A simplified, usable portable PSG system

- The brain during sleep is going through an automatic program uninfluenced by sensory inputs and motor activity
- We believe that longitudinal studies could be a method to check brain health periodically
- What minimal number of sensors must be included to capture all the useful information a full PSG can provide
  - Old markers such as AHI, new markers like EEG biomarkers of depression or neurodegenerative disease
- Must be portable and comfortable
- Signals could be sent over the internet and automatically processed
- Potentially, feedback could be given to the subject to correct abnormality (like for CBTi)
Consumer Sleep Tracking Devices

- Beddit Sleep Tracker
- Basis
- Fitbit
- Tory Burch FitBit
- Whitings Aura pad
- ResMed S+
Comfortable EEGs

8U7187 with Headband 579
76% signal quality.

AVAILABLE IN SEPTEMBER '17
What do we hope to achieve?

The future: at home therapies
The STAGES study: Genetics and Technology in 30,000 subjects

Supplementary sleep data
- Sleep schedule evaluation by actigraphy
- Sleep anatomy evaluation by photography

Subjective sleep data (ASQ)
- Sleep habits
- Sleep symptoms (e.g. sleepiness)
- Sleep Disorders (insomnia, OSA, Restless leg syndrome, narcolepsy, parasomnia)

Computerized Neurocognitive Battery (CNB)
- Attention, executive functions
- Performance Vigilance Tests

Objective Sleep Nocturnal Polysomnography (Sleep analytics, Machine learning)

Open source

Genetic data GWAS, sequencing
Why Use Genetic Analysis?

• Genetics and molecular biology are exploding fields and their applications are growing exponentially
• With genetic analysis, it is possible to find the mechanism behind a disease or a physiological process even if there are no clues on where to look
• Once genes and/or pathways have been identified, new treatment strategies and diagnostic tools can be developed

Sleep is an objectively measurable biology with strong genetic effects, therefore it will be tractable by genetic analysis
Sleep Phenotype

Gene Variant (SNP)

Systematic measurement of single nucleotide polymorphisms (SNPs) across the entire human genome.

Genome-wide Association Study (GWAS) or Exome / Whole Genome Sequencing*

*Need large sample size

*Need large sample size for genetic studies
GWAS Hits

- Height
- Body mass index
- QT interval
- HDL cholesterol
- Bone mineral density

Discovery sample size vs. # GWAS hits
Example Of How Genetics Have Been Used To Identify Underlying Genes/Pathways In A Disease Of Unknown Mechanism

Analysis of SNP genotypes across genome indicates pathways

Genes identified
- complement factor H (CFH)
- complement component 2 (C2)
- complement component 3 (C3)
- complement factor I (CFI)
- HTRA1/ARMS2

Pathway identified
Complement is involved

Age Related Macular Degeneration (AMD)

Six new treatment in pipelines
Complement-Based Therapies
Pathway Analysis of Genetic Markers
Large samples GWA on subjective sleep

- Overlapping circadian genes found through GWA and basic biology (Kalmbach et al., 2017)
- Tight relationships with neuropsychiatry:
  - Genetic architecture of Long sleep duration overlaps with that of Schizophrenia/bipolar (Lane et al. 2017)
    - This complements our finding on KLS and TRANK1
- May redefine some pathologies:
  - Insomnia shares genetic architecture with anxiety
  - Restless leg syndrome shares MEIS1 and genetic architecture with insomnia (Lane et al. 2017)

>>> Mandates studies with objective sleep