ALLEN INSTITUTE *for* BRAIN SCIENCE

Microarray profiling of gene expression after sleep deprivation and recovery sleep

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1. OVERVIEW

Sleep deprivation leads to a repertoire of cognitive, attentional and emotional deficits that are seriously detrimental in occupations requiring alertness. Presumably, these deficits involve altered gene expression within brain regions related to sleep regulation or higher-level brain functions. Consistent with this idea, several recent lines of research have demonstrated region-specific regulation of gene expression during the sleep-wake cycle and following sleep deprivation. About 200 genes have been reported in the literature to respond to sleep state or sleep deprivation in fruit fly, mouse, rat or human, and microarray studies indicate thousands of genes appear to be regulated by the 24-hour circadian rhythm.

The Allen Institute, in collaboration with SRI, has undertaken a project to characterize gene expression in response to sleep deprivation, recovery sleep, and sleep-wake or time of day. A major part of this study involved a discovery science approach to genetically profile regions of the brain that are involved in sleep/wake regulation, circadian rhythmicity, and deficits associated with sleep deprivation. Two methodologies were used: 1) laser microdissection/microarray to allow genome-scale expression profiling for seven brain regions implicated either in sleep regulation or in higher level functions, and 2) high throughput non-radioactive in situ hybridization for validation of microarray findings, allowing comprehensive neuroanatomical characterization of >200 genes as presented at http://sleep.alleninstitute.org.

This document details the creation of the microarray dataset, which was used to select some of the genes for the high throughput in situ hybridization screen.

2. BEHAVIORAL CONDITIONS

Sleep Deprivation (SD). C57BL/6 male mice between 2-3 months of age were implanted for electroencephalogram (EEG)/electromyogram (EMG) recording in the Neurobiology Laboratory at SRI International (Menlo Park, CA). After baseline recordings for sleep and wake were generated, the mice were sleep deprived for 6 hours by tapping the cage, introduction of novel objects, and disturbing the cage bedding between ZT0-ZT6. Mice were sacrificed by cervical dislocation and brains were dissected and frozen in OCT embedding compound. The EEG/EMG recordings were used to validate the efficacy of the sleep deprivation.

Recovery Sleep (RS). Following sleep-deprivation (as described above), mice were allowed 4 hours of undisturbed time for recovery sleep. Mice were sacrificed by cervical dislocation and brains were dissected and frozen in OCT embedding compound.

Waking (W). Mice were sacrificed at ZT18, a time at which animals are predominantly awake.

Controls (SDC, RSC). Appropriate cage controls were generated at ZT6 and ZT10 to correspond to the time of sacrifice of sleep-deprived (SDC) or recovery sleep animals (RSC).

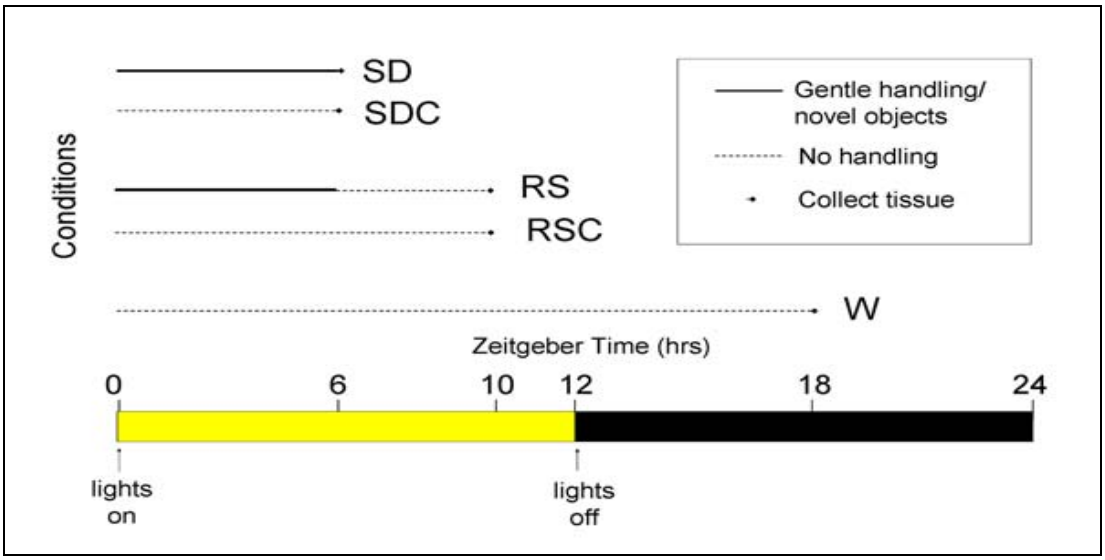

Figure 1. Schematic illustrating the five experimental conditions.

3. LASER MICRODISSECTION/MICROARRAY

3.1. Regions of interest

The goal of this study is to genetically profile regions of the brain that are involved in sleep/wake regulation, circadian rhythmicity, and deficits associated with sleep deprivation. Four regions associated with sleep/wake regulation and circadian rhythmicity were chosen: the locus coeruleus, suprachiasmatic nucleus, hypocretin area (lateral hypothalamus), and the tuberomammillary nucleus.

Deficits resulting from sleep deprivation are associated with large, heterogeneous structures including the hippocampus, amygdala and neocortex, and thus the precise subdivisions to be analyzed were to be determined based on the behavioral state-dependent upregulation of immediate early genes described above. As predicted, the immediate early gene mapping indicated that alterations in gene expression occur in highly discrete subnuclei of these structures, although these regions differ somewhat from those previously described in the literature. For example, the input region for the hippocampus, the entorhinal cortex, showed much more dramatic activation than the hippocampus proper. The corticoamygdalar nuclei showed increased expression, and the frontal (orbitofrontal) cortex showed selective cortical activation with sleep deprivation, consistent with the role of frontal cortex in higher executive brain function. The final list of structures isolated for microarray analysis is shown in Table 1.

Table 1. Regions for laser microdissection

Region	Abbreviation
Locus coeruleus	LC
Suprachiasmatic nucleus	SCN
Hypocretin area	HCRT

Tuberomammillary nucleus	TMN
Posteromedial cortical amygdala	COApm
Dorsal medial entorhinal cortex	ENT
Orbital cortex	ORB

3.2. Laser microdissection

Laser microdissection was carried out on tissue sections (10 um) which were lightly stained with cresyl violet to aid in identification of anatomical regions. Cresyl violet was sufficient to identify most target regions, with the exception of the hypocretin neurons. Hypocretin ISH on an adjacent section was sufficient to allow identification of the appropriate region for laser microdissection on the cresyl violet-stained tissue.

The Leica LMD6000 laser microdissection system was used to isolate tissue into a cap filled with the RLT buffer from the Qiagen RNeasy kit. The Qiagen kit was used to isolate RNA, followed by BioAnalyzer Picochip (Agilent) analysis to confirm RNA yield and quality based upon 28S and 18S rRNA peaks.

3.3. Two-round RNA amplification

The Qiagen RNeasy kit was used to isolate RNA, followed by BioAnalyzer Picochip (Agilent) analysis to confirm RNA yield and quality based upon 28S and 18S rRNA peaks.

Using 5 ng total RNA as starting material, a two-round RNA amplification (Ambion MessageAmp II kit) was used to produce sufficient cDNA for biotin-labeling and hybridization to microarrays.

3.4. Codelink microarrays

The amplified, biotin-labeled cRNA was hybridized to mouse whole genome Codelink microarrays (GE Healthcare) by GenUS BioSystems (Northbrook, Illinois).

4. ACCESSING FILES

Three file formats are available for download. Choose the format that is most appropriate for your microarray analysis software.

Raw .txt files. The raw text files include the raw information exported by the Codelink software.

Codelink .xls files. These files contain the processed data from the Codelink software. This version is appropriate for most software programs, including Agilent Genespring.

Resolver files. Files exported in a special format specifically for the Rosetta Resolver software. If using Resolver, this format is necessary to allow error modeling.

5. ACKNOWLEDGEMENTS

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2) Two-round amplification, probe labeling, and microarray hybridization was performed by GenUS BioSystems (Northbrook, Illinois).