

Analysis of dynamic gene expression during sleep/wake cycles and following sleep deprivation in the mouse brain

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INTRODUCTION

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 suggest that thousands of genes appear to be regulated by the 24-hour circadian rhythm.

The **Allen Institute**, in collaboration with **SRI International**, generated a comprehensive collection of cellular resolution data for gene expression in the mouse brain in response to sleep state. Using a high throughput nonradioactive in situ hybridization methodology, gene expression has been examined under five sleep conditions, and the high resolution images are presented online similar to the Allen Brain Atlas database as a resource for sleep researchers. This dataset provides exceptional utility with its complete coverage of anatomical areas in the mouse brain.

The current set of images includes data for 224 genes that have either been reported to change with sleep state in mammals, are known to be involved in sleep regulation, or exhibited state-dependent changes in expression in an in-house microarray data set. Please refer to the "Gene List" for the currently available genes.

ANIMAL GROUPS AND RECORDINGS.

C57BL/6 male mice approximately 2 months of age were implanted for electroencephalogram (EEG)/electromyogram (EMG) recording in the Neurobiology Laboratory at SRI International (Menlo Park, CA). EEG/EMG recordings were used to validate the efficacy of the sleep deprivation in the first 50 animals (10 animals per condition) and thus tissue sections exhibit some cortical damage for this subset. Subsequent animals received the appropriate handling procedures per condition but were not implanted for EEG.

Sleep Deprivation (SD). 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.

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).

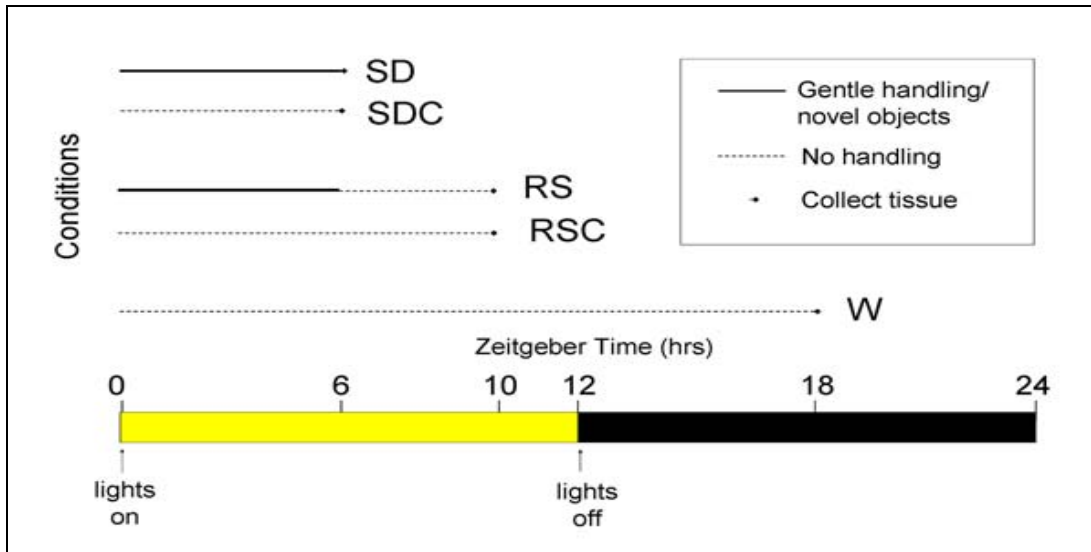


Figure 1. Schematic illustrating the five experimental conditions.

IN SITU HYBRIDIZATION (ISH)

ISH was performed on 25 um sagittal sections with sampling at 200 um per gene, and 100 um for Nissl stained sections in a high-throughput format similar to the Allen Brain Atlas project (Lein et al, *Nature* 445:168-76, 2007).

DATA MODALITIES

Image data. The original image data for the ISH experiments is available for 224 genes in the sagittal plane, and 56 genes have additional replicates.

Difference grids. A 3D representation of changes in gene expression for the comparison of pairs of experimental conditions are available at <http://download.alleninstitute.org/sleep/>. Documentation describes the generation and use of the difference grids.

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