National Toxicology Program
U.S. Department of Health and Human Services

Shift work at Night, Artificial Light at Night, and Circadian Disruption Workshop

Appendix D

Animal studies: Non-cancer health outcomes

March 10-11, 2016

Office of the Report on Carcinogens (ORoC)
Office of Health Assessment and Translation (OHAT)
Division of the National Toxicology Program
National Institute of Environmental Health Sciences
U.S. Department of Health and Human Services

Abstract

A variety of animal model systems were employed to disrupt the central and peripheral circadian clocks using four main categories of exposures (Table 1). Alterations in the intensity and timing of light were employed to mimic shift work, jet lag, or exposure to artificial light at night (ALAN) via shifts of varying duration and frequency in the light-dark cycle (LD), continuous bright light (LL), or bright light during the day and dim light at night (Ldim). These light exposure studies were used to directly disrupt the circadian clock and several measures of circadian disruption were reported including activity, body temperature, melatonin and corticosterone levels, and expression of circadian-related genes such as clock genes. Numerous measures of the effects of light studies were also reported including metabolic, cardiovascular, immune, neurological, reproductive, and mental health (Tables 1 and 2). The effects of light on many different species of animals were studied including lab strains of rat and mouse as well as hamsters, gerbils, cockerels, quail, and wild rodents.

Three additional categories of animal models were used to probe the entrainment of peripheral circadian clocks: timing of activity, timing of food, and timing of sleep. Similar measures of circadian disruption were reported in these studies with the exception of melatonin, which was only measured in light studies. The measures of effects were more limited for peripheral disruption and focused solely on metabolic effects. Of the studies reviewed by OHAT, only laboratory strains of rat or mouse were employed.

Alterations in the timing of food availability were the most numerous of the three additional categories of studies and among the first to investigate the effects of disruption of the peripheral circadian clocks. The effects of food timing on metabolic risk factors were numerous and included measures of bodyweight, adiposity, food intake, energy expenditure, glucose and lipid metabolism, leptin and ghrelin levels, and expression of many metabolism-related genes. Evidence from food availability studies indicates that food is the strongest entrainment signal, or zeitgeber, of the peripheral clocks and may actually uncouple central and peripheral circadian rhythms, which may be protective against short-term disruptions such as jet lag.

Alterations in the timing of activity or sleep were far fewer than for light exposures or food availability. During activity studies, rats were placed in slowly rotating wheels for regular shifts of 8 hours per day, 5 days per week, or for rotating 12 hour shifts; these studies were often paired with shifts in the timing of light and/or food to more closely mimic human shift work. For timing of sleep studies, several scenarios were used to limit sleep duration or disrupt the normal sleep cycle from complete deprivation of sleep via slowly rotating wheels, restriction of sleep to specific times of day, or interruption of sleep using gentle probing or similar methods. Due to the limited number of studies, the impact of these zeitgebers on entrainment of the peripheral clocks and downstream metabolic effects are not as clear as those for food.

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Table 1. Animal models of SW, ALAN and CD: metabolic outcomes

	Light*	**Timing of Activity**	**Timing of Food**	**Timing of Sleep**
Types of exposure models	Shifts in light-dark (LD) cycle: vary in length of shift (1-12h), frequency of shift (every day vs. once per week), duration of shift (once vs. repeated), direction of shift (forward vs. backward) Continuous light exposure: Constant 24h bright light (LL); Bright light during day with dim light at night (LDim)	Forced activity: rats housed in slowly rotating wheels for shifts (e.g., 8h per day, 5 days per week; or rotating 12h shift); able to lie down and eat, but can't sleep Often paired with timing of food availability and/or light	Restricted food availability: restricted to light or dark phase; duration of exposure varies (e.g., 12L:12D, 16L:8D, 8L:16D, etc.)	Shift in timing of sleep to dark phase for nocturnal rodents: variety of models including disruption, restriction, complete deprivation May use slowly rotating wheels, gentle probing upon falling asleep, etc.
Measures of circadian disruption	Activity; body temperature; melatonin; corticosterone; gene expression (e.g. clock genes)	Activity; corticosterone	Activity; body temperature; corticosterone; gene expression	Activity; corticosterone ; gene expression
Metabolic				
Measures of effect	Bodyweight; adiposity; food (caloric) intake; energy expenditure (total EE, RER); glucose metabolism (plasma glucose, plasma insulin, plasma glucagon, glucose tolerance, glycogen levels); leptin levels; lipid metabolism (plasma cholesterol, plasma triglycerides); gene expression (e.g. liver genes)	Bodyweight; adiposity; food intake; glucose metabolism; lipid metabolism; gene expression (e.g. liver genes)	Bodyweight; adiposity; food (caloric) intake; energy expenditure (total EE, RER); glucose metabolism; leptin levels; ghrelin levels; lipid metabolism; gene expression	Bodyweight; food intake; total energy expenditure; glucose metabolism; lipid metabolism; leptin; gene expression

*Excluded non-24h LD cycles

Table 2. Animal models of SW, ALAN and CD: non-cancer health outcomes

	Cardiovascular	Immune	Neurological	Reproductive	Mental Health
Measures of effects	Blood pressure; heart rate; Plasminogen activator inhibitor-1 (PAI-1); angiotensin; survival time in aged, cardiomyopathic, or hypertensive animals; cardiac gene expression; epigenetic factors (miRNA expression); whole heart morphology; cardiomyocyte morphology	Cellular immune response: various challenges including delayed-type hypersensitivity (DTH), LPS-induced fever, bactericide activity of blood; Concanavalin A stimulation of peripheral blood (Con A) and a Popliteal Lymph Node Assay (PLNA); dextran sodium sulfate to induce colitis; cutaneous basophil hypersensitivity reaction to phytohemagglutinin (PHA-P); MPO; NK cell activity Inflammatory response: cytokines Humoral immune response: primary antibody titers Sickness behaviors (anorexia, decreased activity, weight loss)	Learning and memory: conditioned place preference (CPP) ; water maze; contextual fear conditioning; Barnes maze Histology: hippocampal cell proliferation and neurogenesis Endocrine factors: glucorticoid, sex steroids	Estrous cycling: plasma estrogen, luteinizing hormone, ovulation Pregnancy outcomes: pregnancy to term; birth weight Male fertility: sperm count, ejaculation, reproductive organ weight and morphology	Depression-like behaviors: activity, forced swim test, sucrose preference Anxiety-like behaviors: blood pressure, heart rate, elevated plus maze, risk assessment, grooming