



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.

Bibliography

- 1) Adamovich Y, Aviram R, Asher G. 2014a. The emerging roles of lipids in circadian control. *Biochim Biophys Acta* Published online December 4, 2015.
- 2) Adamovich Y, Rouso-Noori L, Zwihaft Z, Neufeld-Cohen A, Golik M, Kraut-Cohen J, Wang M, Han X, Asher G. 2014b. Circadian clocks and feeding time regulate the oscillations and levels of hepatic triglycerides. *Cell Metab* 19: 319–330.
- 3) Arble DM, Bass J, Laposky AD, Vitaterna MH, and Turek FW 2009. Circadian timing of food intake contributes to weight gain. *Obesity (SilverSpring)* 17: 2100–2102.
- 4) Asher G and Sassone-Corsi P. 2015. Time for food: the intimate interplay between nutrition, metabolism, and the circadian clock. *Cell* 161: 84-92.
- 5) Aubrecht TG, Weil ZM, Magalang UJ, Nelson RJ. 2013. Dim light at night interacts with intermittent hypoxia to alter cognitive and affective responses. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 305(1): R78-R86.
- 6) Barclay JL, Husse J, Bode B, Naujokat N, Meyer-Kovac J, Schmid SM, *et al.* 2012. Circadian desynchrony promotes metabolic disruption in a mouse model of shiftwork. *PLoS ONE* 7: e37150.
- 7) Barf RP, Desprez T, Meerlo P, Scheurink AJ. 2012a. Increased food intake and changes in metabolic hormones in response to chronic sleep restriction alternated with short periods of sleep allowance. *Am J Physiol Regul Integr Comp Physiol* 302: R112–R117.
- 8) Barf RP, Meerlo P, Scheurink AJ. 2010. Chronic sleep disturbance impairs glucose homeostasis in rats. *Int. J. Endocrinol* 2010: 819414.
- 9) Barf RP, VanDijk G, Scheurink AJ, Hoffmann K, Novati A, Hulshof HJ, *et al.* 2012b. Metabolic consequences of chronic sleep restriction in rats: changes in bodyweight regulation and energy expenditure. *Physiol Behav* 107: 322–328.
- 10) Bedrosian TA, Fonken LK, Walton JC, Nelson RJ. 2011. Chronic exposure to dim light at night suppresses immune responses in Siberian hamsters. *Biol Lett* 7:468–471.
- 11) Bedrosian TA, Weil ZM, Nelson RJ. 2012. Chronic dim light at night provokes reversible depression-like phenotype: possible role for TNF. *Mol Psychiatry*
- 12) Bilbo SD, Drazen DL, Quan N, He L, Nelson RJ. 2002. Short day lengths attenuate the symptoms of infection in Siberian hamsters. *Proc R Soc Lond B* 269: 447–454.
- 13) Bilbo S. D., Nelson R. J. 2003. Sex differences in photoperiodic and stress-induced enhancement of immune function in Siberian hamsters. *Brain Behav. Immunol.* 17: 462–472.
- 14) Bray MS, Ratcliffe WF, Grenett MH, Brewer RA, Gamble KL, and Young ME 2013. Quantitative analysis of light-phase restricted feeding reveals metabolic dyssynchrony in mice. *Int J Obes (Lond)* 37: 843–852.

- 15) Bray, MS, Tsai, JY, Villegas-Montoya, C, Boland, BB, Blasier, Z, Egbejimi, O, *et al.* 2010. Time-of-day-dependent dietary fat consumption influences multiple cardiometabolic syndrome parameters in mice. *Int J Obes (Lond)* 34: 1589–1598.
- 16) Briaud SA, Zhang BL, and Sannajust F. 2004. Continuous light exposure and sympathectomy suppress circadian rhythm of blood pressure in rats. *Journal of Cardiovascular Pharmacology and Therapeutics*, 9(2): 97–105.
- 17) Campos LA, Plehm R, Cipolla-Neto J, Bader M, Baltatu OC. 2006. Altered circadian rhythm reentrainment to light phase shifts in rats with low levels of brain angiotensinogen. *Am J Physiol Regul Integr Comp Physiol*. 290: R1122–R1127.
- 18) Castanon-Cervantes O, Wu M, Ehlen JC, Paul K, Gamble KL, Johnson RL, Besing RC, Menaker M, Gewirtz AT, Davidson AJ. 2010. Dysregulation of inflammatory responses by chronic circadian disruption. *J Immunol* 185: 5796–5805
- 19) Chaix, A, Zarrinpar, A, Miu, P, and Panda, S. 2014. Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges. *Cell Metab* 20: 991–1005.
- 20) Craig LA, McDonald RJ. 2008. Chronic disruption of circadian rhythms impairs hippocampal memory in the rat. *Brain Res Bull*. 76:141–151.
- 21) Damiola F, LeMinh N, Preitner N, Kornmann B, Fleury-Olela F, Schibler U. 2000. Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev* 14:2950–2961.
- 22) Davidson AJ, Sellix MT, Daniel J, *et al.* 2006. Chronic jet-lag increases mortality in aged mice. *Curr Biol*: 16: R914–R916.
- 23) Davidson, AJ, Sellix MT, Daniel J, Yamazaki S, Menaker M, Block GD. 2006. Chronic jet-lag increases mortality in aged mice. *Curr. Biol*. 16: R914–R916.
- 24) Devan BD, Goad EH, Petri HL, *et al.* 2001. Circadian phase-shifted rats show normal acquisition but impaired long-term retention of place information in the water task. *Neurobiol Learn Mem*. 75: 51–62.
- 25) Evans JA and Davidson AJ. 2013. Health consequences of circadian disruption in humans and animal models. In: *Progress in Molecular Biology and Translational Science*, vol. 119: pp. 283-323.
- 26) Fekete M, van Ree JM, Niesink RJ, de Wied D. 1985. Disrupting circadian rhythms in rats induces retrograde amnesia. *Physiol Behav* 34: 883–887.
- 27) Fonken LK, Nelson RJ. 2013. Dim light at night increases depressive-like responses in male C3H/HeNHsd mice. *Behav Brain Res* 243C: 74–78.
- 28) Fujioka A, Fujioka T, Tsuruta R, *et al.* 2011. Effects of a constant light environment on hippocampal neurogenesis and memory in mice. *Neurosci Lett* 488:41–44.
- 29) Fuse, Y, Hirao, A, Kuroda, H, Otsuka, M, Tahara, Y, and Shibata, S. 2012. Differential roles of breakfast only (one meal per day) and a bigger breakfast with a small dinner (two meals per day) in mice fed a high-fat diet with regard to induced obesity and lipid metabolism. *J. Circadian Rhythms* 10: 4.
- 30) Gibson EM, Wang C, Tjho S, Khattar N, Kriegsfeld LJ. 2010. Experimental ‘jet lag’ inhibits adult neurogenesis and produces long-term cognitive deficits in female hamsters. *PLoS One*. 5: e15267.

- 31) Guerrero-Vargas et al. Escobar. 2015. Shift work in Rats Results in Increased Inflammatory Response after Lipopolysaccharide Administration: A Role for Food Consumption. *J Biol Rhythms* 30(4): 318-330.
- 32) Hagino N, Sako T, Nakamoto O, Kunz Y, Saito H. 1983. Prevention of continuous light induced anovulation in rats by early exposure to continuous light. *Biol Reprod* 29(3): 55–361.
- 33) Hatori, M., Vollmers, C., Zarrinpar, A., DiTacchio, L., Bushong, E.A., Gill, S., Leblanc, M., Chaix, A., Joens, M., Fitzpatrick, J.A., *et al.* 2012. Time restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metab* 15: 848–860.
- 34) Hsieh, W.H., Escobar, C., Yugay, T., Lo, M.T., Pittman-Polletta, B., Salgado-Delgado, R., *et al.* 2014. Simulated shift work in rats perturbs multiscale regulation of locomotor activity. *J.R. Soc. Interface* 11: 20140318.
- 35) Husse, J., Hintze, S.C., Eichele, G., Lehnert, H. and Oster, H. 2012. Circadian clock genes *Per1* and *Per2* regulate the response of metabolism-associated transcripts to sleep disruption. *PLoS ONE* 7: e52983.
- 36) Jang H, Lee G, Kong J, Choi G, Park YJ, and Kim JB 2012. Feeding period restriction alters the expression of peripheral circadian rhythm genes without changing body weight in mice. *PLoS ONE* 7: 49993.
- 37) Kirby JD, Froman DP. 1991. Research note: evaluation of humoral and delayed hypersensitivity responses in cockerels reared under constant light or a twelve hour light:twelve hour dark photoperiod. *Poult Sci* 70: 2375–2378.
- 38) Kishi T, Sunagawa K. 2011. Experimental ‘jet lag’ causes sympathoexcitation via oxidative stress through AT1 receptor in the brainstem. *Conf Proc IEEE Eng Med Biol Soc* 2011: 1969–1972.
- 39) Kort WJ, Weijma JM. 1982. Effect of chronic light-dark shift stress on the immune response of the rat. *Physiol Behav* 29: 1083–1087.
- 40) Kott J, Leach G, Yan L. 2012. Direction-dependent effects of chronic “jet-lag” on hippocampal neurogenesis. *Neurosci Lett* 515: 177–180.
- 41) Leenaars CH, Kalsbeek A, Hanegraaf MA, Foppen E, Joosten RN, Post G, *et al.* 2012. Unaltered instrumental learning and attenuated body-weight gain in rats during non-rotating simulated shift work. *Chronobiol. Int* 29: 344–355.
- 42) Loh DH, Navarro J, Hagopian A, *et al.* 2010). Rapid changes in the light/dark cycle disrupt memory of conditioned fear in mice. *PLoS One* 5: e12546.
- 43) Ma WP, Cao J, Tian M, *et al.* 2007. Exposure to chronic constant light impairs spatial memory and influences long-term depression in rats. *Neurosci Res* 59: 224–230.
- 44) McGowan and Coogan 2013. *Journal of Molecular Psychiatry* 1: 7
- 45) Moore CB, Siopes TD. 2000. Effects of lighting conditions and melatonin supplementation on the cellular and humoral immune responses in Japanese quail *Coturnix coturnix japonica*. *Gen Comp Endocrinol* 119: 95–104.
- 46) Murphy. 2003. A laboratory animal model of human shiftwork. *Integr Physiol Behav Sci* 38: 316–328.

- 47) Oishi K, Ohkura N. 2013. Chronic circadian clock disruption induces expression of the cardiovascular risk factor plasminogen activator inhibitor-1 in mice. *Blood Coagul Fibrinolysis* 24: 106–108.
- 48) Oishi K., Shibusawa K., Kakazu H., Kuriyama T., Ohkura N., Machida K. 2006. Extended light exposure suppresses nocturnal increases in cytotoxic activity of splenic natural killer cells in rats. *Biol Rhyth Res* 37: 21–35.
- 49) Opperhuizen AL, van Kerkhof LWM, Proper KI, Rodenburg W and Kalsbeek A 2015. Rodent models to study the metabolic effects of shiftwork in humans. *Front. Pharmacol* 6: 50.
- 50) Oyama, Y., Iwasaka, H., Koga, H., Shingu, C., Matsumoto, S., and Noguchi, T. 2014. Uncoupling of peripheral and master clock gene rhythms by reversed feeding leads to an exacerbated inflammatory response after polymicrobial sepsis in mice. *Shock* 41: 214–221.
- 51) Penev PD, Kolker DE, Zee PC, Turek FW. 1998. Chronic circadian desynchronization decreases the survival of animals with cardiomyopathic heart disease. *Am J Physiol* 275: H2334–H2337.
- 52) Prendergast BJ, Bilbo SD, Nelson RJ. 2005. Short day lengths enhance skin immune responses in gonadectomised Siberian hamsters. *J Neuroendocrinol* 17: 18–21.
- 53) Prendergast BJ. 2008). Behavioral tolerance to endotoxin is enhanced by adaptation to winter photoperiods. *Psychoneuroendocrinology* 33: 540–545.
- 54) Preuss F, Tang Y, Laposky AD, Turek. 2008. Adverse effects of chronic circadian desynchronization in animals in a “challenging” environment. *Am J Physiol Regul Integr Comp Physiol* 295: R2034–R2040.
- 55) Reznick, J., Preston, E., Wilks, D.L., Beale, S.M., Turner, N., and Cooney, G.J. 2013). Altered feeding differentially regulates circadian rhythms and energy metabolism in liver and muscle of rats. *Biochim. Biophys. Acta* 1832: 228–238.
- 56) Salgado-Delgado, R., Angeles-Castellanos, M., Buijs, M.R., and Escobar, C. 2008). Internal desynchronization in a model of night-work by forced activity in rats. *Neuroscience* 154: 922–931.
- 57) Salgado-Delgado, R., Angeles-Castellanos, M., Saderi, N., Buijs, R.M., and Escobar, C. 2010a. Food intake during the normal activity phase prevents obesity and circadian desynchrony in a rat model of nightwork. *Endocrinology* 151: 1019–1029.
- 58) Salgado-Delgado, R.C., Saderi, N., Basualdo Mdel, C., Guerrero-Vargas, N.N., Escobar, C., and Buijs, R.M. 2013. Shiftwork or food intake during the rest phase promotes metabolic disruption and desynchrony of liver genes in male rats. *PLoS ONE* 8: e60052.
- 59) Schroder, E.A., Burgess, D.E., Manning, C.L., Zhao, Y., Moss, A.J., Patwardhan, A.R., *et al.* 2014. Light-phase restricted feeding slows basal heart rate to exaggerate the type 3 long QT syndrome phenotype in mice. *Am J Physiol Heart Circ Physiol* 341: 2014.

- 60) Shamsi NA, Salkeld MD, Rattanatray L, Voultzios A, Varcoe TJ, Boden MJ, *et al.* 2014. Metabolic consequences of timed feeding in mice. *Physiol Behav* 128: 188–201.
- 61) Sherman H, Genzer Y, Cohen R, Chapnik N, Madar Z, Froy O. 2012. Timed high-fat diet resets circadian metabolism and prevents obesity. *FASEB J* 26, 3493–3502.
- 62) Summa KC, Vitaterna MH, Turek FW. 2012. Environmental perturbation of the circadian clock disrupts pregnancy in the mouse. *PLoS One* 7:e37668.
- 63) Tapp WN, Holloway FA. 1981. Phase shifting circadian rhythms produces retrograde amnesia. *Science* 211:1056–1058.
- 64) Thomas BB, Oommen MM, Ashadevi JS. 2001. Constant light and blinding effects on reproduction of male South Indian gerbils. *J Exp Zool* 289: 59–65.
- 65) Tsai LL, Tsai YC. 2007. The effect of scheduled forced wheel activity on bodyweight in male F344 rats undergoing chronic circadian desynchronization. *Int J Obes (Lond)* 31: 1368–1377.
- 66) Varcoe TJ, Wight N, Voultzios A, Salkeld MD, Kennaway DJ 2011. Chronic phase shifts of the photoperiod throughout pregnancy programs glucose intolerance and insulin resistance in the rat. *PLoS One* 6: e18504.
- 67) Wen JC, Dhabhar FS, Prendergast BJ. 2007. Pineal-dependent and -independent effects of photoperiod on immune function in Siberian hamsters (*Phodopus sungorus*). *Horm Behav* 51: 31–39.
- 68) Wen JC, Prendergast BJ. 2007. Photoperiodic regulation of behavioral responsiveness to proinflammatory cytokines. *Physiol Behav* 90: 717–725.
- 69) Wu T, Sun L, ZhuGe F, Guo X, Zhao Z, Tang R, Chen Q, Chen L, Kato H, Fu Z. 2011. Differential roles of breakfast and supper in rats of a daily three-meal schedule upon circadian regulation and physiology. *Chronobiol Int* 28: 890–903.
- 70) Yoon JA, Han DH, Noh JY, Kim MH, Son GH, Kim K, *et al.* 2012. Meal time shift disturbs circadian rhythmicity along with metabolic and behavioral alterations in mice. *PLoS ONE* 7:e44053.
- 71) Yoshida C, Shikata N, Seki S, Koyama N, Noguchi Y. 2012. Early nocturnal meal skipping alters the peripheral clock and increases lipogenesis in mice. *Nutr Metab (Lond)* 9: 78.

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	<p>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</p>	<p>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</p> <p>Inflammatory response: cytokines</p> <p>Humoral immune response: primary antibody titers</p> <p>Sickness behaviors (anorexia, decreased activity, weight loss)</p>	<p>Learning and memory: conditioned place preference (CPP) ; water maze; contextual fear conditioning; Barnes maze</p> <p>Histology: hippocampal cell proliferation and neurogenesis</p> <p>Endocrine factors: glucocorticoid, sex steroids</p>	<p>Estrous cycling: plasma estrogen, luteinizing hormone, ovulation</p> <p>Pregnancy outcomes: pregnancy to term; birth weight</p> <p>Male fertility: sperm count, ejaculation, reproductive organ weight and morphology</p>	<p>Depression-like behaviors: activity, forced swim test, sucrose preference</p> <p>Anxiety-like behaviors: blood pressure, heart rate, elevated plus maze, risk assessment, grooming</p>