Neurobiology of Aging 36 (2015) 380–393

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Genetic background influences age-related decline in visual and nonvisual retinal responses, circadian rhythms, and sleep☆

Gareth Banks [a], Ines Heise [a], Becky Starbuck [a], Tamzin Osborne [a], Laura Wisby [a], Paul Potter [a], Ian J. Jackson [b], Russell G. Foster [c], Stuart N. Peirson [c], Patrick M. Nolan [a,*]

[a] MRC Harwell, Harwell Science and Innovation Campus, Oxfordshire, UK
[b] MRC Human Genetics Unit, MRC IGMM, University of Edinburgh, Western General Hospital, Edinburgh, UK
[c] Nuffield Laboratory of Ophthalmology (Nuffield Department of Clinical Neurosciences), University of Oxford, John Radcliffe Hospital, Oxford, UK

ARTICLE INFO

Article history:
Received 9 May 2014
Received in revised form 23 July 2014
Accepted 28 July 2014
Available online 2 August 2014

Keywords:
Aging
Circadian
Sleep
Light input
Mouse strain

ABSTRACT

The circadian system is entrained to the environmental light/dark cycle via retinal photoreceptors and regulates numerous aspects of physiology and behavior, including sleep. These processes are all key factors in healthy aging showing a gradual decline with age. Despite their importance, the exact mechanisms underlying this decline are yet to be fully understood. One of the most effective tools we have to understand the genetic factors underlying these processes are genetically inbred mouse strains. The most commonly used reference mouse strain is C57BL/6J, but recently, resources such as the International Knockout Mouse Consortium have started producing large numbers of mouse mutant lines on a pure genetic background, C57BL/6N. Considering the substantial genetic diversity between mouse strains we expect there to be phenotypic differences, including differential effects of aging, in these and other strains. Such differences need to be characterized not only to establish how different mouse strains may model the aging process but also to understand how genetic background might modify age-related phenotypes. To ascertain the effects of aging on sleep/wake behavior, circadian rhythms, and light input and whether these effects are mouse strain-dependent, we have screened C57BL/6J, C57BL/6N, C3H-HeH, and C3H-Pde6b+ mouse strains at 5 ages throughout their life span. Our data show that sleep, circadian, and light input parameters are all disrupted by the aging process. Moreover, we have cataloged a number of strain-specific aging effects, including the rate of cataract development, decline in the pupillary light response, and changes in sleep fragmentation and the proportion of time spent asleep.

1. Introduction

In healthy individuals, a rhythmic sleep/wake cycle is maintained through the interaction of homeostatic and circadian mechanisms, as well as being modulated by external cues. The homeostatic process refers to an increase of sleep pressure that accumulates during wakefulness and is relieved by sleep (Borbely, 1982). The timing of the sleep/wake cycle is also regulated by the internal circadian clock, which provides an innate biological rhythm exerting control over a wide range of physiological and behavioral processes. The circadian clock is defined by its ability to maintain free running rhythms in the absence of external timing cues. However, to be of use, this clock must be synchronized to external cues—a process known as entrainment. In mammals, the most influential external cue for both sleep and circadian rhythms is light. Light is detected by the retina and, as well as its familiar role in image-forming vision, also acts to entrain circadian rhythms and directly modulate sleep by promoting sleep or alertness (Hubbard et al., 2013). Although the interactions between retinal light input pathways, the circadian clock, and the sleep homeostat are well maintained in healthy individuals, aging is known to have a negative impact on all these processes, leading ultimately to disrupted circadian rhythms and sleep/wake cycles in older individuals. In humans, the reported effects of aging include an increased occurrence of cataracts (Klein and Klein, 2013), loss of retinal photoreceptors (Gao and Hollyfield, 1992), reduced circadian regulation of melatonin and temperature (Pandi-Perumal et al., 2005; Weinert, 2010), a reduction in sleep duration and

* Corresponding author at: MRC Harwell, Harwell Science and Innovation Campus, Oxfordshire, OX11 0RD, UK. Tel./fax: +44 (0)1235 841091.
E-mail address: p.nolan@har.mrc.ac.uk (P.M. Nolan).

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http://dx.doi.org/10.1016/j.neurobiolaging.2014.07.040

consolidation, and an increased susceptibility to misalignments in circadian phase (Dijk et al., 1999) and increased fragmentation of sleep and time spent asleep during the day (Huang et al., 2002).

Animal models have been used widely to understand the mechanisms underlying not only rhythmic behavior but also aging. Prominent among these models are inbred mouse strains. Inbred mice are commonly used in a range of biological research areas as their genetic homogeneity allows researchers in different laboratories to independently replicate results without the genetic background of the model being a confounding factor. They are also used extensively in genetic studies in which specific genes can be selectively knocked out or mutated to study their effects on the whole organism. A large number of different inbred mouse strains currently exist and through a combination of spontaneous mutation and genetic drift each inbred strain carries its own combination of mutations within its genome (Stevens et al., 2007). Two of the most commonly used mouse strains are C57BL/6J and C3H-HeH. The C57BL/6J strain has been used to characterize the influence of aging on both circadian rhythms and sleep (Hasan et al., 2012; Possidente et al., 1995; Valentinuzzi et al., 1997), whereas the C3H-HeH strain has been used in studies into how aging alters light inputs (Lupi et al., 2012; Semo et al., 2003a, 2003b).

Until recently, phenotypic analyses of most of the mouse genetic knockout models have been carried out on undefined mixtures of C57BL/6J and 129S7 mouse backgrounds. It is notable that a lack of consideration of the influence of these 2 strains on a phenotype has lead to confounding results. For example, initial studies using a mouse model of Fragile X syndrome on a mixed C57BL/6-129 background reported only mild learning deficits (D'Hooge et al., 1997). However, later work demonstrated that the influence of C57BL/6 could rescue a more severe learning deficit found using the same mice line on a 129 background (Dobkin et al., 2000). The latest mouse knockout resources such as the International Knockout Mouse Consortium are now producing knockout mouse models exclusively on the C57BL/6N background. Therefore, in the future most of the mouse phenotyping studies will use the C57BL/6N background rather than C57BL/6J or 129S7. The C57BL/6J and C57BL/6N strains have been separated by around 220 generations, and a comprehensive genotype comparison has demonstrated significant genetic differences between the 2 lines (Simon et al., 2013). Given these differences and the prominent use of C57BL/6N animals in knockout studies there is a requirement for baseline phenotyping and longitudinal studies that establish how this strain can be used as an animal model and exactly what phenotypic differences are evident among these 2 substrains. To date, differences have been reported between C57BL/6J and C57BL/6N in locomotor activity, anxiety measures, prepulse inhibition of acoustic startle response, grip strength, motor learning, and visual acuity (Matsuo et al., 2010; Simon et al., 2013). However, longitudinal studies exploring circadian rhythms, light responsiveness, and sleep activity differences between the 2 substrains have yet to be determined.

As noted previously, the C3H-HeH strain has been used extensively for light input studies. However, this strain is notable as it carries the *rd1* mutation in the *Pde6b* gene (*Pde6b*^{rd1}), which causes a rapid degeneration of photoreceptors in the retina (Pittler and Baehr, 1991). Although this mutation is useful in modeling retinal degeneration, other phenotypes (such as light responses) are likely to be confounded by the presence of *Pde6b*^{rd1}, and so a new substrain called C3H-Pde6b+ was created to remove the *Pde6b*^{rd1} mutation. This was achieved by introducing the wild-type *Pde6b* allele from the BALB/c strain and backcrossing to congenic status (10 generations) (Hart et al., 2005). Although this approach successfully removed the retinal degeneration phenotype from the C3H-Pde6b+ strain, it also has introduced regions of BALB/c genome into C3H-Pde6b+. Given these genetic differences, the C3H-Pde6b+ line cannot be considered as merely the C3H-HeH line with the *Pde6b*^{rd1} mutation removed but as a genetically similar but distinct strain, and we therefore expect phenotypic differences between the 2 strains that cannot be explained as simply because of the *Pde6b*^{rd1} mutation. It is also notable that once phenotypic differences between the C3H-HeH and C3H-Pde6b+ strains have been established we can use techniques such as haplotype analysis to map regions of the BALB/c genome that are causative for the divergent phenotypes and thus identify the genes which underlie these differences. However, such studies again require baseline phenotyping comparisons of the 2 strains to identify the differences needed to undertake these more long term genetic investigations.

To address the need for more comprehensive phenotyping of these mouse strains we constructed a phenotyping pipeline that allows the study of both visual and nonvisual retinal responses, in addition to circadian rhythms and sleep in the same cohort of animals. This approach combines classic circadian wheel running activity monitoring (Banks and Nolan, 2011) and visual phenotyping assays (slit lamp and optokinetic drum) with 2 novel methods of phenotyping—assessment of the nonvisual pupillary light response (PLR) and immobility-defined sleep (Fisher et al., 2012). Using this pipeline of phenotyping techniques, we report here on the effect of aging on all these processes and furthermore show that several of these changes are strain specific.

2. Methods

2.1. Mice and test pipeline

All animal studies described in this article were performed under the guidance issued by the Medical Research Council in Responsibility in the Use of Animals for Medical Research (July 1993) and Home Office Project Licences 30/2686 and 30/3070. When not being tested, mice were housed in individually ventilated cages under 12/12 hours light/dark (LD) conditions with food and water available *ad libitum*. Four different inbred mouse strains were used: C57BL/6J, C57BL/6N, C3H-HeH (abbreviated to C3H), and C3H-Pde6b+ (abbreviated to C3PDE—see Hart et al., 2005). Female mice were used for all cohorts. Phenotyping tests were performed in the following order: pupillometry, circadian wheel running, sleep analysis by video tracking, and visual phenotyping. The animals had 1 week rest intervals between tests. Animal cohorts began testing at 5 different ages: approximately 3, 6, 9, 12, and 18 months. A separate cohort was bred and aged for each time point. 8–10 animals were used for each experiment with the following exceptions: 6 months C57BL/6J, n = 7 and 18 months C3PDE, n = 6.

2.2. Visual phenotyping

Visual acuity was assessed by head tracking response to a virtual-reality optokinetic system as described by Douglas et al. (2005) and manufactured by CerebralMechanics Inc (Alberta, Canada). Briefly, mice were placed onto a podium in an area comprising computer monitors as walls and a mirrored floor. The mouse was monitored by a camera built into the lid of the arena. A vertical sine wave rotates around the monitors, and the head and neck movements of the mouse are used to assess how well the mouse tracks the sine wave rotation. The spatial frequency of the lines is increased until there is no longer a response from the animal, indicating that the stimulus is no longer perceived. Grating is measured in cycles per degree. Illuminance within the apparatus was 30 lux.