Published in final edited form as:

Behav Brain Res. 2013 April 15; 243: 306–312. doi:10.1016/j.bbr.2013.01.003.

Automated Video Analysis System Reveals Distinct Diurnal Behaviors in C57BL/6 and C3H/HeN Mice

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Abstract

Advances in rodent behavior dissection using automated video recording and analysis allows detailed phenotyping. This study compared and contrasted 15 diurnal behaviors recorded continuously using an automated behavioral analysis system for a period of 14 days under a 14/10 light/dark cycle in single housed C3H/HeN (C3H) or C57BL/6 (C57) male mice. Diurnal behaviors, recorded with minimal experimental interference and analyzed using phenotypic array and temporal distribution analysis showed bimodal and unimodal profiles in the C57 and C3H mice, respectively. Phenotypic array analysis revealed distinct behavioral rhythms in *activity-like behaviors* (i.e. walk, hang, jump, come down) (*ALB*), *exploration-like behaviors* (i.e. dig, groom, rear up, sniff, stretch) (*ELB*), *ingestion-like behaviors* (i.e. drink, eat) (*ILB*) and *resting-like behaviors* (i.e. awake, remain low, rest, twitch) (*RLB*) of C3H and C57 mice. Temporal analysis demonstrated that strain and time of day affects the magnitude and distribution of the spontaneous homecage behaviors. Wheel running activity, water and food measurements correlated with timing of homecage behaviors. Subcutaneous (3 mg/kg, sc) or oral (0.02 mg/ml, oral) melatonin treatments in C57 mice did not modify either the total 24 hr magnitude or temporal distribution of homecage behaviors when compared with vehicle treatments. We conclude that C3H and C57 mice show different spontaneous activity and behavioral rhythms specifically during the night period which are not modulated by melatonin.

Keywords

C3H/HeN mouse; C57BL/6 mouse; homecage; automated behavior analysis; circadian rhythms

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Conflict of Interest

The authors declare that over the last three years MLD was a consultant for and received compensation from Takeda Pharmaceutical North America Inc.

Authors Contributions

All authors contributed to the conception and design, acquisition, and/or analysis and/or interpretation of data (EBAB, IS, RLH, MLD). EBAB and IS conducted all the in vivo experiments, and EBAB analyzed and interpreted data in consultation with MLD and RLH. RLH provided advices and tested the equipment as necessary. EBAB drafted the manuscript which was edited by MLD. All authors edited and approved the manuscript before submission.

1. Introduction

The laboratory mouse has become the primary model to study human disease and a tool to elucidate the molecular and neural mechanisms of behavior. Inbred strains and genetically modified mice are used to establish models of neurological and neuropsychiatric diseases including major depressive disorders (MDD), anxiety and Huntington disease among others [1–3]. Systematic phenotyping of inbred strains has emerged as an important tool in determining genetic control of behaviors. Inbred strains of mice have a uniform and stable genetic background with predictable phenotype that can be assessed over time. Hundreds of strains of mice have been described and new strains are continuously being developed due to the wide availability of genetic techniques and tools.

The availability of a wide range of mouse strains with diverse genetic background provides the scientific community with the opportunity to analyze and correlate these parameters with behavioral phenotypes. Phenotype is significantly affected by strain [4], sex [5], age [6] and circadian and/or diurnal phase [7]. To add to this complexity behavioral measurements performed with rodents, are often performed under conditions less than ideal as animals may be exposed to stressful situations due to extraneous noise, light exposure at inappropriate times [8, 9], novel environments and more important measurements when animals are resting (i.e., during the rodent inactive period). Thus, measuring rodent basal spontaneous behaviors in their homecage over the 24 hour period under well controlled experimental conditions is useful in experimental planning, data interpretation and choice of appropriate strain of mice for experimental use.

Two inbred laboratory mice, C57BL6J (C57) and C3H/HeN (C3H) mice are commonly used in neuropharmacology and circadian rhythm research [10–12]. C57 mice are widely used to generate transgenic mice due to its permissive background for maximal expression of most mutations and its ability to live longer with fewer tumors [13]. This strain of mice expresses melatonin receptors in different areas of the brain and the retina [11, 14]. C57 mice produce a low level of melatonin in the pineal gland [12, 15] due to a natural point mutation in the amino acid sequence of the aralkylamine N-acetyltransferase (AANAT) gene [16].

C3H mice are widely used as general purpose mice in a variety of research ranging from cardiovascular to cancer research [13]. This strain of mice has a propensity to develop tumors, carries a mutation in the retinal degeneration (rd) gene that makes them blind six weeks after birth [17], and expresses melatonin receptors in the brain and the retina [11, 14]. C3H mice produce a high level of melatonin in the pineal gland in a circadian manner [12, 15].

These two strains have been the mice of choice in circadian biology and for assessing the effect of melatonin on phase shifts and re-entrainment of circadian activity rhythms [10]. Both C3H and C57 mice entrain locomotor activity rhythm to a light/dark cycle, with the C57 mice adapting faster to a phase shift [18, 19]. These strains also show differences in canonical behavioral tests. For example C57 mice exhibited more pronounced depression-like behaviors in the force swim test (FST) than C3H mice [20, 21]. C3H mice expressed more anxiety-like behaviors than C57 mice after exposure to a novel open space using a 3D spatial navigation task [22]. In the light/dark box and free exploratory paradigm [23], C3H and C57 mice expressed the same level of anxiety like behaviors as previously stated while the opposite was found with the elevated plus maze [24]. These results show marked differences in behavioral phenotype between these two strains of mice.

Assessment of basal spontaneous behaviors parameters should aid in the interpretation and analysis of behavioral phenotype of different strain of mice. Collection of basal spontaneous behaviors over extended periods of time in mice has been hindered by the lack of appropriate equipment. The advent of automated behavioral analysis systems made possible continuous and non-disruptive acquisition of single mouse behavioral data in their homecage and allows dissection of diurnal patterns, behavioral rhythms and quantification of multiple behaviors magnitude [25–28].

The goal of this study was to acquire data on basal spontaneous behaviors in C57 and C3H mice over extended periods of time to allow assessment of consecutive behavioral diurnal patterns. We compared and contrasted the magnitude and the diurnal distribution of basal spontaneous homecage behaviors of C3H and C57 mice as well as determined the ability of exogenous melatonin to modulate homecage behaviors over a 24 hour period in C57 mice using the automated behavioral analysis system. These two strains of mice showed distinct phenotypic behavioral arrays and temporal profiles for 15 spontaneous behaviors.

2. Methods

2.1. Animal Husbandry

Male C57 and C3H mice were bred in house at our Laboratory Animal Facility at the University at Buffalo, School of Medicine and Biomedical Sciences. Mice were kept in a 14/10 light/dark cycle and have access to food and water *ad libitum*. The animals were group-housed (3 to 5 per cage) and maintained in a temperature ($22 \pm 1^\circ\text{C}$) and humidity (20 – 23%) controlled room. The light intensity on the shelf where cages were placed was 150 to 200 lux. All experiments were approved and performed according to the guideline of the National Institute of Health and the Institutional Animal Care Use Committee of the University at Buffalo.

2.2. Homecage Monitoring

Male C3H (32.8 ± 1.1 g, $n = 14$) and C57 (30.8 ± 0.7 g, $n = 8$) mice aged 3 to 4 months were individually housed in acrylic cages ($33 \times 12 \times 13$ cm). C57 and C3H mice were placed in single compartments inside light-tight cabinets with constant temperature and humidity. Each compartment was equipped with infrared cameras to monitor mouse activity continuously over 14/10 light/dark cycles. Food and water was available *ad libitum*. Cameras were connected to computers (Dell Precision T3500, W503 @ 2.4 GHz) loaded with acquisition software Capture Star Ver. 1 from CleverSys Inc. (Reston, VA). After one week of acclimation to the new environment, the mice behaviors were recorded for a period of one week and analyzed using the automated behavioral analysis system (HomeCageScan 3.0) software from CleverSys Inc. (Reston, VA) available on a main computer (Dell Precision T5500, W5580 @ 3.20GHz). The automated behavioral analysis system called HomeCageScan System is capable of visualizing and analyzing more than 15 distinct mice behaviors in their natural environment [28].

2.3. Food and Water Measurements

Food and water consumption were measured weekly. Each mouse received 75 g of chow and a glass bottle containing 200 ml of sterile water. Amount of food and water consumed was determined by weighing food and water bottles at the beginning and end of the seven day period. Spillage and evaporation from drinking containers rarely exceeds 1% per day, and average food spillage does not exceed 0.1 g/day/mouse on food provided *ad libitum* [29, 30].

2.4. Melatonin Treatment in the Homecage

Male C57 wild type mice (n=16) aged 3 to 4 months were housed individually in the automated behavioral analysis system as described above. After one week acclimation, mice received daily injections (0.1 ml) of either vehicle (3% ethanol in saline, sc) or melatonin (3mg/kg in vehicle, sc) at ZT10 for three consecutive days. Mice behaviors were recorded continuously during treatment and for four more days afterward. Following a recovery period of one week, mice were treated with vehicle (0.1% ethanol in water, oral) or melatonin (0.02 mg/ml in vehicle, oral) in the drinking water for a period of 7 days. Treatments were prepared using sterile distilled water and were replaced every three days. Bottles containing vehicle or melatonin treatments were wrapped with duct tape to prevent melatonin degradation. The total amount of liquid consumed was determined by weighing the bottles at the beginning and at the end of each three day periods. Mice behaviors were recorded before, during and after the subcutaneous and oral treatment periods.

2.5. Wheel Running Activity

Male C3H (n = 9) and male C57 (n = 12) mice, were individually housed in cages (33 × 12 × 13 cm) equipped with wheels. Food and water were provided *ad libitum*. Wheel running activity was recorded by micro-switches attached to the wheels, which detected wheel revolutions online via a computer (Colbat Computers OPK, Pentium R @ 3.2 GHz) across the 24 hour period. Data were collected with ClockLab (*Actimetrics, IL*) and analyzed with MatLab R2012a (*Math Works, MA*) as previously described [10].

2. 6. Data Representation

Phenotypic arrays represent each behavior counts scaled and plotted over the 24 h period as a function of color. Scaling was performed by dividing each one hour bin value by the root mean square value of the 24 hour as described in Van den Berg et al., 2006. A value of 1 (light red) indicates that the behavior is occurring at very low magnitude. Increasing the intensity of the red color (2 to 4 value), indicates the behavior is occurring at higher magnitude. Each square represent one hour of the 24 hour period. Together the array allows visualization of the ensemble of diurnal behaviors.

Temporal distribution plots represent the distribution of homecage behaviors as function of time expressed as Zeitgeber time (ZT)(ZT0 is define as lights on). These plots allow visualization of the magnitude of diurnal rhythms of single spontaneous behaviors between mouse strains as well as assessment of drug effects when compared to vehicle treatments.

2.7. Statistical Analysis

The outputs of the behavioral analysis performed on the recorded videos were the counts and the time spent of each of the 15 behaviors over the 24 hour period. The data was exported in one hour bins. Phenotypic array analysis of the 15 behaviors was performed using R 2.15.1. The data was scaled and plotted as a function of color to visualize the repartition and the profile of the 15 behaviors as an ensemble. Temporal distribution analysis was performed using PASW Statistics 16 and GraphPad 5.2. Two-way analysis of variance (2-Way ANOVA) was used to compare the shape, differences between temporal distributions of specific behaviors in the two strains of mice or to assess the effect of melatonin as compared to vehicle on the behavioral rhythms of homecage behaviors over the 24hr period. Strain and Time (ZT) were the two factors used in the 2-Way ANOVA analysis.

2.8. Behavioral Classification

Table 1 shows the behavioral parameters measured using the automated behavioral analysis system as previously defined [27, 28]. These behaviors include coming down (Come Down), hanging (Hang), jumping (Jump), walking (Walk), digging (Dig), grooming (Groom), rearing (Rear Up), sniffing (Sniff), stretching (Stretch), drinking (Drink), eating (Eat), awaking (Awake), remaining low (Remain Low), resting (Rest) and twitching (Twitch).

We grouped these behaviors into classes of behaviors based on data from the literature. Psychomotility and locomotion related behaviors that are measured in canonical testings such as gait analysis [31], open field test [32, 33], and hang wire test [34] were called *Activity-Like Behaviors* (ALB). Self-maintenance and exploration related behaviors that are measured in tests such as open field [33], grooming [35] and burrowing [36] tests were grouped under *Exploration-Like Behaviors* (ELB). Behaviors that are associated with food and water consumption were called *Ingestion-Like Behaviors* (ILB). Behaviors related to resting including immobility (e.g. remain low, awake) were called *Resting-Like Behaviors* (RLB). It is to be noted that these behaviors reflect endogenous level of these classes of behaviors and are not induced behaviors due to stress or drug treatment.

3. Results

Table 1 lists the fifteen behaviors reordered using the automated behavioral analysis system in mice single housed in the home cage. These behaviors are grouped in four major classes (ALB, ELB, ILB, RLB) based on correlations with canonical behaviors. Individual mouse behaviors recorded simultaneously and continuously over 24 h periods were analyzed and grouped into phenotypic arrays. The phenotypic arrays showed in Fig. 1 revealed distinct diurnal profiles of spontaneous homecage behaviors in C3H and C57 mice with higher behavioral activity during the dark period (Fig. 1 A and B). In the C3H mice all active behaviors are restricted to the dark phase following a unimodal profile. The active behaviors decrease one hour before lights on except for hang and jump behaviors that decreased three hours earlier (Fig. 1A). C57 mice displayed a bimodal profile of spontaneous homecage behaviors (Fig. 1B), which are partitioned into two modes restricted to ZT15–17 and ZT22–1 (Fig. 1B). Active behaviors of C57 mice extended one to two hours into the light period. Resting-Like Behaviors (i.e., awake, rest, twitch) in both strains of mice were observed during the light period. C57 mice on the other hand displayed a resting period from ZT18 and ZT21 (Fig. 1B).

The diurnal unimodal and bimodal profiles are more easily visualized in the individual behaviors plotted over the 24 h period (ZT). These plots called temporal distributions of homecage behaviors, allowed us to determine differences in diurnal rhythms of activities between the C3H and C57 mice (Fig. 2). The maximal peak of behaviors for C3H mice was at ZT 16 (i.e. come down, hang, jump, eat, dig, rear up, sniff) or ZT 21 (i.e. remain low, groom) except for awake, rest, twitch, drink and that did not display the unimodal pattern of behavior. C57 mice on the contrary, exhibited the first activity peak at ZT16 for all behaviors except for awake, rest, twitch, drink and stretch behaviors that were not organized in the two modes during the dark period. The second peak of behaviors for C57 mice were at ZT23 (i.e. hang, jump, eat), ZT0 (i.e. come down, dig, rear up, sniff) or ZT1 (i.e. walk, groom, sniff). The temporal distribution of each spontaneous homecage behaviors for each strain of mice was very similar from day to day during the one week recording period (data not shown).

3.1. Activity-Like Behaviors (ALB) and Wheel Running Activity

In *Activity-Like Behaviors (ALB)*, a main effect of strain was found in comedown ($F = 5.42$, $p = 0.024$), hang ($F = 442.66$, $p < 0.0001$) and jump ($F = 61.75$, $p < 0.0001$) but not walk ($F = 0.77$, $p = 0.3801$) behaviors (Fig. 1A–D). However, Bonferoni post test showed that compared to C57 mice, C3H mice have higher magnitude of walk behavior similarly to comedown behaviors at ZT18–21 during the dark period. At the onset of the light period, C57 mice have higher magnitude of comedown (ZT23–1) and walk (ZT0–1). Bonferoni posttest in hang and jump showed that the main strain effect was restricted to the dark period (ZT15–ZT20) where C3H mice have higher magnitude of both behaviors (Fig. 1A–D). These results indicate that C3H mice might be more active than the C57 mice during the dark period while their behavioral responses in *ALB* to the onset of the light period are different. There was also a significant main effect of time in the *ALB* such as comedown ($F = 17.55$, $p < 0.0001$), hang ($F = 52.40$, $p < 0.0001$), jump ($F = 11.63$, $p < 0.0001$) and walk ($F = 31.05$, $p < 0.0001$). The effect of time was coupled with significant interactions in comedown ($F = 9.09$, $p < 0.0001$), hang ($F = 38.17$, $p < 0.0001$), jump ($F = 61.75$, $p < 0.0001$) and walk ($F = 19.63$, $p < 0.0001$) indicating that the mice might have different threshold of behavior magnitudes at different time over the 24 hour period. Interestingly, wheel running activity in C3H and C57 mice offered the same temporal distribution of *ALB* in the homecage and correlated well with walk behavior in term of timing and not magnitude (Fig. 2D vs. Fig. 3A). Locomotion in the homecage is assessed based on lateral movement that is spontaneous rather than stimulated by external events. In contrast, running wheel activity has both reinforcing and rewarding properties that increase its own magnitude over time [37]. C3H mice exhibited a unimodal temporal distribution with a peak at ZT16 while C57 mice exhibited a bimodal temporal distribution profile with peaks at ZT16 and ZT1 (Fig. 3A). A significant main effect of strain was found using two-way ANOVA ($F = 6.65$, $p < 0.01$) with Bonferoni post-tests showing higher wheel revolution counts for C3H mice during the middle of the night (ZT19–21) and C57 mice at the onset of the light (ZT0–1). There was also a main significance of time ($F = 34.88$, $p < 0.0001$) coupled with significant interactions ($F = 7.67$, $p < 0.0001$) indicating that C3H and C57 mice are active at different times of the 24 hour day (Fig. 3A).

3.2. Exploration-Like Behaviors (ELB)

In *Exploration-Like Behaviors (ELB)*, a main effect of strain was found in dig ($F = 27.72$, $p < 0.0001$), sniff ($F = 12.42$, $p < 0.0005$) and stretch ($F = 57.43$, $p < 0.0001$). Bonferoni posttest indicated that C3H mice have higher magnitude of dig, sniff and stretch, respectively at ZT20, ZT19 and ZT15–23 during the middle of the dark period. At the onset of the light period C57 mice have higher magnitude of dig and sniff behavior than C3H mice, respectively at ZT0–1 and ZT23–1 (Fig. 2E, G and H). No main significant effect of strain was found in groom ($F = 0.11$, $p = 0.7460$) and rear up ($F = 3.14$, $p = 0.0771$). However, Bonferoni posttest showed that the C3H mice expressed more groom (ZT19–21) and rear up (ZT18–20) behaviors than C57 mice for three hour time during the dark phase whereas C57 mice expressed more groom and rear up activity for 3 hours' time (ZT23–1) at the light/dark transition (Fig. 2F and G). A significant effect of time was found in dig ($F = 6.58$, $p < 0.0001$), groom ($F = 15.75$, $p < 0.0001$), rear up ($F = 17.62$, $p < 0.0001$), sniff ($F = 12.77$, $p < 0.0001$), stretch ($F = 5.99$, $p < 0.0001$). This effect of time was accompanied by significant interaction in dig ($F = 2.96$, $p < 0.0001$), groom ($F = 6.45$, $p < 0.0001$) rear up ($F = 9.01$, $p < 0.0001$), sniff ($F = 10.21$, $p < 0.0001$) and stretch ($F = 9.22$, $p < 0.0001$) indicating opposite behaviors at different time of day by C3H and C57 mice.

3.3. Ingestion-Like Behaviors (ILB), Food and Water Consumption

In *Ingestion-Like Behaviors (ILB)*, a significant effect of strain was found in eat ($F = 11.03$, $p < 0.0010$) and drink ($F = 8.76$, $p < 0.0033$) behaviors (Fig. 2J–K). Bonferoni posttest

indicated that C3H mice spend significantly more time in eat and drink behaviors than C57 mice in the middle of the night at ZT17–21 while the opposite occurs at the onset of the light period respectively at ZT23–0 and ZT1. Overall C3H mice consumed more water and food than C57 mice during the dark period (Fig. 2 J and K). Interestingly, measurement of water and food consumption during the length of the experiment corroborated this finding in the homecage (Fig. 3B). C3H compared to C57 mice consumed (29.25 ± 0.62 vs. 21.00 ± 0.72 g/week; $p < 0.0001$) and drunk (31.69 ± 1.05 vs. 27.21 ± 0.87 g/week; $p < 0.005$) more food and water, respectively, during the one week of the experiment. Mouse body weight did not significantly change during the one week recording period (Fig. 3C). Time affected eat ($F = 16.85$, $p < 0.0001$) and drink ($F = 3.77$, $p < 0.0001$) behaviors in the homecage. This effect is followed by a significant interaction in both eat ($F = 4.46$, $p < 0.001$) and drink ($F = 3.34$, $p < 0.0001$) indicating that C3H mice needs high consumption of water and chow to maintain their high activity while C57 mice do not, possibly due to their low activity during the middle of the dark period

3.4. Resting-Like Activity

In Resting-Like Behaviors (RLB), a significant main effect of strain was found in of awake ($F = 9.95$, $p < 0.0017$), rest ($F = 52.71$, $p < 0.0001$) and twitch ($F = 18.48$, $p < 0.0001$) and remain low ($F = 3.91$, $p = 0.0486$) (Fig. 2L–O). Bonferoni post-test showed higher level of rest and twitch behavior for C57 compared to C3H mice during the dark phase respectively at ZT15–21 and ZT20–21. In the remain low behavior, Bonferoni posttest indicated a higher magnitude for C3H compared to C57 mice for three hours (ZT18–21) during the dark period and a higher magnitude for C57 compared to C3H mice for two hours (ZT0–1) at the onset of the light period. These results demonstrate that C3H mice are more active and pause more between activities than C57 mice during the dark phase. A significant effect of time was found in the RLB behaviors that included awake ($F = 3.29$, $p < 0.0001$), remain low ($F = 21.95$, $p < 0.0001$), rest ($F = 58.91$, $p < 0.0001$) and twitch ($F = 4.30$, $p < 0.0001$). This effect is accompanied by significant interaction in remain low ($F = 14.12$, $p < 0.0001$), rest ($F = 23.83$, $p < 0.0001$) and twitch ($F = 2.69$, $p < 0.0001$) but not in awake ($F = 1.24$, $p < 0.2065$) emphasizing the opposite behaviors pattern during the middle of the dark period and at the onset of the light period in C57 and C3H mice.

3.5. Melatonin Treatment in C57 Mice

We next determine the role of exogenous melatonin administration via subcutaneous or oral administration on the magnitude and temporal distribution of home cage behaviors in C57 mice. C57 mice were first treated for three consecutive days with either vehicle (3% ethanol in saline, sc) or melatonin (3mg/kg in vehicle, sc) (Fig. S1). The temporal distribution profiles for vehicle treated C57 mice were identical to those observed in untreated C57 mice (compared Fig 2 vs. Fig.S1). This dose of melatonin, known to phase shift running wheel activity rhythms, did not affect basal spontaneous behaviors of C57 mice in the 2 hours after treatment or along the 24 hour diurnal cycle in any of the three consecutive treatment days when compared with vehicle (Fig. S1). The temporal distribution of spontaneous activity was not changed during the four days recovery period (data not shown).

C57 mice treated with melatonin in drinking water (0.02 mg/ml in vehicle, oral), provided to the mice *ad libitum*, for a 7 day period did not modify the temporal distribution nor the magnitude of activity-, exploration-, ingestion- and resting-like behaviors in the home cage when compared to the behaviors of mice treated with vehicle (0.1% ethanol in water, oral) (Fig 4). Note that the temporal distribution profiles for vehicle treated C57 mice were identical to those observed in untreated C57 mice (compared Fig 2 vs. Fig. 4). Melatonin treatment in the homecage did not significantly affect mouse body weight (VEH: 31.1 ± 1.2 g, $n = 8$; MLT: 30.5 ± 0.8 g, $n = 8$) (Fig. 3C). The total consumption of water (VEH: $35.8 \pm$

1.6 g, n = 8; MLT: 31.5 ± 0.7 g, n = 8) and food (VEH: 42.3 ± 1.4 , n=8; MLT: 43.3 ± 0.6 , n=8) was also similar in the two treatment groups (Fig. 3D). The temporal distribution profiles for vehicle treated C57 mice on the last day of treatment were identical to those observed for the untreated C57 mice in Fig. 2.

4. Discussion

This study shows significant differences in total magnitude and temporal distribution of basal spontaneous homecage behaviors in the C3H and C57 mice measured using an automated behavioral analysis system. The differences in behavioral magnitude were more pronounced during the dark period when mice are naturally active [38] and at the onset of the light period suggesting distinct behavioral phenotype and diurnal rhythms for the two strains of mice. Locomotion and ingestion in the homecage respectively correlated well with wheel running activity in terms of timing, food and water ingestion, indicating that the homecage is well suited to assess differences in the magnitude of spontaneous behaviors and diurnal rhythms. Subcutaneous or oral melatonin treatments did not affect either the magnitude or the temporal distribution of homecage spontaneous behaviors in C57 mice, at doses known to modulate phase shifts of circadian rhythms, accelerate re-entrainment to a new dark onset and potentiate running wheel induced neurogenesis [10, 39]. These results strongly suggest that the differences in behavioral profiles between the C3H and C57 mice, observed primarily during the dark period, may be due to genetic background rather than to higher levels of endogenous pineal melatonin in the C3H mice.

Phenotypic array analysis showed distinct behavioral profile for C3H and C57 mice. All active behaviors in the C3H mice were unimodal and restricted to the dark phase, and in the C57 mice, were bimodal extending two hours after lights on (Fig. 1B). C3H compared to C57 mice have higher magnitude of behaviors during the dark period in the activity, exploratory, ingestion and resting classes with the exception of rest and twitch in the latter class group that express during the day. The effect of strain was restricted to specific parts of the dark or the light periods as previously shown with other tests [40, 41]. Interestingly most of the studies comparing behaviors in the C3H and C57 mice are generally conducted during the resting period [18–22] when often basal spontaneous behaviors are low and of the same magnitude for these two strains. These studies applied stress to animals tested in a variety of canonical behavioral paradigms to determine strain differences. Stress can either increase or decrease the magnitude of the homecage behaviors along the 24hr period [42, 43]. Understanding the effect of stressors on the two strains of mouse can facilitate assessment of stressor effectiveness and the effect of time of day on the behavioral tests. Temporal distribution analysis predicts that stress can differentially affect spontaneous behaviors in C3H and C57 mice during the night period when the spontaneous behaviors are distinct. During the light period, stress should have the same effect since their activity level is similar.

Temporal distribution analysis showed that C3H mice are more active than C57 mice during the nighttime while the opposite occurred at the onset of the light period (Fig. 2). Wheel running activity results (Fig. 3A) provided similar results. C3H and C57 mice, respectively, have high and low wheel revolution counts during the dark period while the opposite occurs at the onset of the light period (Fig. 3A). These results lead us to conclude that C3H mice are more active than C57 mice during the night period while the opposite occurs at the onset of the light period.

Food and water measurements also correlated with the time spent in eat and drink in the homecage. C3H drink and eat more than C57 mice during the 24 hour period. The results add support to the fact that C3H overall are more active than C57 mice in the homecage.

C3H mice consuming more food and water than C57 mice might correlate directly with the energy requirements to maintain high activity level during the dark period [44].

It is worth noting that the profile of rest and twitch were inverse to that of all the other behaviors in both strain of mice during the dark period and at the onset of the light period. C57 mice have high magnitude of rest and twitch during the dark period while the inverse occurs with C3H mice indicating that C57 mice have a resting phase during the middle of the night. This rest period is called “siesta sleep” and has been previously reported in the C57 mice [45].

Behavioral differences including home cage behaviors have been demonstrated for other strains (i.e. BALBc, 129P3, DBA, C57). In the study 129 P3 mice exhibited less locomotion but more rearing than C57 mice [41]. Strains also showed significant differences in homecage activity including walking which was more intensive in the BALB followed by the C57 and the DBA mice [26, 40].

Subcutaneous or oral melatonin treatment of C57 mice did not modify the temporal distribution or magnitude of homecage activity-, exploration-, ingestion-, and resting-like behaviors. Our laboratory previously showed that subcutaneous doses of melatonin ranging from 0.01 to 3 mg/kg at specific times of sensitivity phase shift (i.e., advance or delay) the onset of wheel running activity in C3H or C57 mice maintained in constant conditions [10, 46–48]. Further, melatonin (1 and 3 mg/kg, sc) administration at the new dark onset facilitates re-entrainment after an abrupt 6–8 hours phase advance of dark onset in rodents kept in a light/dark cycle [46, 47, 49]. Additionally the oral dose used in this study (0.02 mg/ml, oral) under the same treatment regimen potentiated running wheel-induced neurogenesis in the dentate gyrus of the hippocampus of the C3H mice but did not affect this phenomenon in animals with fixed wheel [39]. Thus our present results show that exogenous melatonin did not alter the temporal distribution or magnitude of spontaneous home cage behaviors even when administered via doses and routes that produced changes in behavior and neurogenesis in previous studies.

C3H/HeN and C57BL/6 mice exhibit distinct total magnitude and temporal distribution of most behaviors in the activity, exploration, ingestion and resting classes over a 24 hr period in the homecage when data was collected continuously using an automated video recording and analysis system. Melatonin did not affect basal spontaneous behaviors of C57 mice, suggesting it may not contribute to the differences in magnitude and diurnal rhythms of basal spontaneous behaviors observed between the melatonin proficient (C3H) and deficient (C57) mice. These differences, however, may be attributed to differences in genetic background rather than the level of melatonin in C3H mice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are indebted to Dr. Fraser J. Sim for his insightful comments and expert guidance on data analysis and interpretation. The authors would like to thank Dr. Anthony J. Hutchinson for helpful discussions and advice on this research project, and Dr. Jun-Xu Li for constructive comments on the manuscript.

The authors declare that this work was funded by US Public Health Service Grants NS 061068 and DA 21870 to MLD.

ABBREVIATIONS

C3H/HeN	C3H
C57BL/6	C57
ZT	Zeitgeber Time
ALB	Exploration-Like Behaviors
ELB	Exploration-Like Behaviors
ILB	Ingestion-Like Behaviors
RLB	Resting-Like Behaviors

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Highlights

C3H/HeN and C57BL/6 mice have distinct diurnal homecage behaviors.

Melatonin does not alter the magnitude and/or behavioral rhythms in C57BL/6 mice.

Diurnal differences in spontaneous behaviors appear to be genetically regulated.

Fig. 1. Phenotypic Arrays of C3H and C57 Mice Homecage Behaviors over a 24 hour Period
Mean values for behavior magnitudes in the C3H (A) and C57 (B) mice were scaled and plotted over the 24 h period as function of color (0 to 4). The fold increase is indicated by the increase in the color red intensity from baseline represented by 1. The gray bar represents the light period (14h) while the dark bar represents the dark period (10h).

Fig. 2. Effects of C3H and C57 Mice Strain on the Temporal Distribution of Homecage Behaviors

The ordinates represent the temporal duration of each behavior over 24 h period expressed as seconds (s) per hour for behaviors included in the following classes: ALB (A–D), ELB (E–I), ILB (J–K) and RLB (L–O). The abscissae represent Zeitgeber time (ZT) with ZT0 indicating Lights on. Shaded area on the graph indicates the dark period (10 h). The total 24 h duration of each behavior in each strain was compared using two-way ANOVA. The main effect of strain on total behavioral magnitude is indicated by a p value at the upper right corner of each graph. n.s.: non-significant. * p<0.05 when compared the behavior magnitude in C3H with C57 mice at each ZT (Bonferoni post-test).

Fig. 3. Effects of Mice Strain on Wheel Running Activity, Ingestion and Weight Parameters

A. The ordinate represents C3H and C57 mice wheel running activity expressed as counts per minute and plotted as a function of Zeitgeber time (ZT) (ZT0: Light onset). Shaded area indicates the dark period. The C3H and C57 mouse behavioral profiles were compared using two-way ANOVA ($p < 0.01$). * $p < 0.05$ when comparing running wheel activity in C3H with C57 mice at each ZT (Bonferroni post-test).

B. Columns represent total ingestion of water and food by C3H and C57 mice over a week-long period during recording of basal homecage behaviors. ** $p < 0.001$, **** $p < 0.0001$ when compared with C3H (Student t-test).

C. Body weight determined during basal spontaneous behaviors recording in the C3H and C57 mice, and on the last day of the seven day treatment with vehicle (VEH) (0.1 ethanol in water, oral) or melatonin (0.02 mg/ml in vehicle, oral) in C57 mice.

(D) Columns represent total ingestion of water and food by C57 mice over a week period during recording of homecage behaviors of C57 mice treated with either oral vehicle or melatonin.

Fig. 4. Effects of Oral Melatonin Treatment on the Temporal Distribution of C57 Mice Behaviors in the Homecage

The ordinates represent the temporal profile of each behavior over a 24 h period expressed as seconds (s) per hour for behaviors included in the following classes: ALB (A–D), ELB (E–I), ILB (J–K) and RLB (L–O). The abscissae represent Zeitgeber time (ZT) with ZT0 indicating Light on. Shaded area on the graph indicates the dark period (10 hrs.).

C57 mice were treated with VEH (0.1% ethanol in water, oral) or melatonin (0.02 mg/ml in vehicle, oral). The effect of treatment on the total behavioral magnitude was compared using two-way ANOVA. ns.: non-significant.

Shaded area on the graph indicates the dark period (10 h) (ZT0: Lights on). Data represents behavioral values for the last day of treatment (1 week). The profile for each day of treatment was identical.

Table 1
Classes of Spontaneous Homecage Behaviors

The table lists fifteen individual spontaneous homecage mouse behaviors recorded using an automated behavioral analysis system, and the corresponding name we use to refer to the behaviors in this study. The 15 home cage behaviors were grouped in four classes [ALB, ELB, ILB, RLB] to correlate with comparable behaviors measured using canonical behaviors tests.

Homecage		Canonical Behaviors	Classes of Behaviors
Behaviors	Name		
Coming Down Jumping Hanging Walking	Come Down Jump Hang Walk	Gait Analysis (33) Open Field (34, 35) Hang Wire (36)	Activity-Like Behaviors (<i>ALB</i>)
Digging Grooming Rearing Up Sniffing Stretching	Dig Groom Rear Up Sniff Stretch	Open Field (35) Social Investigation (35) Grooming Test (37) Burrowing Test (38)	Exploration-Like Behaviors (<i>ELB</i>)
Drinking Eating	Drink Eat	Food and Water Consumption	Ingestion-Like Behaviors (<i>ILB</i>)
Awaking Remaining Low Resting Twitching	Awake Remain Low Rest Twitch	Homecage Recording (24, 25)	Resting-Like Behaviors (<i>RLB</i>)