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Effect of lethal yellow (A^Y) mutation and photoperiod alterations on mouse behavior

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Decrease in natural illumination in fall/winter months causes depressive-like seasonal affective disorders in vulnerable individuals. Obesity is another risk factor of depression. The lethal yellow (A^Y) mutation causes ectopic expression of agouti protein in the brain. Mice heterozygous for A^Y mutation (A^Y/a) are obese compared to their wild-type littermates (a/a). The main aims of the study were to investigate the effects of A^Y mutation, photoperiod and the interaction between these factors on daily activity dynamics, feeding, locomotor and exploratory activities, anxiety-related and depressive-like behaviors in mild stress condition. Six weeks old mouse males of A^Y/a and a/a lines were divided into four groups eight animals each and exposed to long- (14 h light and 10 h darkness) or short- (4 h light and 20 h darkness) day conditions for 28 days. Then the behavior of these mice was successively investigated in the home cage, open field, elevated plus-maze and forced swim tests. We did not observed any effect of A^Y mutation on the general activity, water and food consumption in the home cage; locomotion and exploration in the open field test; anxiety-related behavior in the open field and elevated plus-maze tests. At the same time, A^Y mutation increased depressive-like immobility time in the forced swim test ($F_{1.28} = 20.03$, $p = 0.00012$). Short-day conditions decreased nocturnal activity in the home cage, as well as locomotion ($F_{1.28} = 16.33$, $p = 0.0004$) and exploration ($F_{1.28} = 16.24$, $p < 0.0004$) in the open field test. Moreover, short-day exposition decreased time spent in the center of the open field ($F_{1.28} = 6.57$, $p = 0.016$) and in the open arms of the elevated plus-maze ($F_{1.28} = 12.08$, $p = 0.0017$) tests and increased immobility time in the forced swim test ($F_{1.28} = 9.95$, $p = 0.0038$). However, no effect of the interaction between A^Y mutation and photoperiod on immobility time in the forced swim test was observed. Therefore, short-day photoperiod and A^Y mutation increased depressive-like behavior in the forced swim test by means of different mechanisms.

Key words: lethal yellow; photoperiod; activity; anxiety; depressive-like behavior; mice.

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Влияние мутации lethal yellow (A^Y) и изменений фотопериода на поведение мыши

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Снижение естественного освещения в осеннее/зимнее время вызывает депрессивно-подобные сезонные аффективные расстройства у предрасположенных индивидов. Ожирение является другим фактором риска депрессии. Мутация lethal yellow (A^Y) вызывает эктопическую экспрессию белка агути в мозге. Мыши, гетерозиготные по мутации A^Y (A^Y/a), страдают ожирением по сравнению с их однопометниками дикого типа (a/a). Основная цель работы – исследование влияния мутации A^Y, фотопериода и взаимодействия между этими факторами на суточную динамику активности, потребление пищи, локомоторную и исследовательскую активность, тревожность и депрессивно-подобное поведение в условиях умеренного стресса. Самцы A^Y/a и a/a возрастом 6 недель были разделены на четыре группы и содержались 28 дней при длинном (14 ч день:10 ч ночь) и коротком (4 ч день:20 ч ночь) фотопериоде. Затем поведение этих мышей последовательно исследовали в домашней клетке, тестах «открытое поле», «приподнятый крестообразный лабиринт» и «принудительное плавание». Мы не обнаружили влияния мутации A^Y на общую активность, потребление пищи и воды в домашней клетке; двигательную активность, исследовательское поведение в тесте «открытое поле»; тревожность в тестах «открытое поле» и «приподнятый крестообразный лабиринт». В то же время мутация A^Y усиливает депрессивно-подобное поведение в тесте «принудительное плавание» ($F_{1.28} = 20.03$, $p = 0.00012$). Короткий день снижал ночную активность мышей в домашней клетке, как и локомоторную ($F_{1.28} = 16.33$, $p = 0.0004$) и исследовательскую ($F_{1.28} = 16.24$, $p < 0.0004$) активность в тесте «открытое поле». Более того, короткий день снижал время в центре в тесте «открытое поле» ($F_{1.28} = 6.57$, $p = 0.016$) и в открытых рукавах в тесте «приподнятый крестообразный лабиринт» ($F_{1.28} = 12.08$, $p = 0.0017$), а также увеличивал время неподвижности в

тесте «принудительное плавание» ($F_{1.28}$ = 9.95, p = 0.0038). При этом не выявлено влияния взаимодействия генотип × фотопериод на выраженность этих признаков. Следовательно, мутация A^Y и короткий фотопериод усиливают депрессивно-подобное поведение в тесте «принудительное плавание» посредством различных механизмов.
Ключевые слова: lethal yellow; фотопериод; активность; тревожность; депрессивно-подобное поведение; мыши.

Introduction

Seasonal alterations of natural illumination in high or moderate latitudes trigger numerous adaptive changes in the nervous system and behavior of all wild animals. Although humans mainly live at constant illumination, its decrease during fall/winter months can cause seasonal affective disorders (SAD) or subsyndromal SAD characterized by carbohydrate craving, overeating, weight gain, decreased libido, hypersomnia and prominent fatigue (Levitan, 2007) in some vulnerable individuals. SAD and subsyndromal SAD are observed in 11–21 % of individuals and are a considerable social and economic problem due to high risk of disability (Miller, 2005).

Laboratory mice have been proposed as an animal model of SAD (Otsuka et al., 2014). However, the reported data about effect of photoperiod alteration on mouse behavior are contradictory (Otsuka et al., 2014; Young et al., 2018).

Obesity and concomitant type 2 diabetes increase risk of depressive disorders (Stunkard et al., 2003; Simon et al., 2006; Luppino et al., 2010; Lojko et al., 2015). Mice lacking leptin receptor are obese and show depressive-like features (Sharma et al., 2010). Another model of hereditary obesity is A^Y/a mice, heterozygous for lethal yellow (A^Y) mutation (Boston et al., 1997; Bazhan et al., 2013).

The main aims of the study were the following: to investigate the effects of (1) A^Y mutation, (2) short/long photoperiod and (3) these factors interaction on brain monoamines and behavior. Here we compared: the effect of the long- and short-day conditions on the daily activity in the home cage, the locomotor, exploratory activities, anxiety-related, depressive-like behavior in heterozygous A^Y/a mice and their wild-type littermates (a/a).

Materials and methods

Animals and experiments. The study was conducted at the Center for Genetic Resources of Laboratory Animals at the Institute of Cytology and Genetics Siberian Branch, Russian Academy of Sciences (Novosibirsk, Russia) using equipment supported by the Russian Ministry of Education and Science (project No. RFMEFI62117X0015). Experiments were carried out on SPF-state mouse males C57BL/6-A^Y (A^Y/a, n = 16) and their wild type littermates C57BL/6 (a/a, n = 16). These mouse genotypes were bred by crossing a/a females and A^Y/a males. Such breeding results only in obtaining A^Y/a and a/a mice (50 : 50) which have similar genetic background, are born and nurtured by the mothers of the a/a genotype. The genotype of mice is assayed by the fur color which is yellow in A^Y/a mice (Boston et al., 1997) and black in a/a mice. At the beginning of the experiment the A^Y/a and a/a mice were 6 weeks old, weighed 20.9 ± 0.3 g and 20.0 ± 0.3 g ($F_{1.30}$ = 5.03, p = 0.032), respectively. The males of the same genotype (A^Y/a or a/a) were kept for 28 days in groups of four in individually ventilated cages (Optimice, Animal Care Systems, Inc.) in rooms with 20-fold air exchange at temperature 24 ± 2 °C, humidity 45–50 %, and at long (14 h of light : 10 h of darkness, 14L : 10D, 40 lx) or short (4 h of light : 20 h of darkness, 4L : 20D, 40 lx) photoperiods with daybreak at 01:00 (14L : 10D) or 11:00 (4L : 20D) and sunset at 15:00 (14L : 10D and 4L : 20D). The 14L : 10D photoperiod is the standard photoperiod in our SPF-vivarium. Food and litter were autoclaved at 121 °C before use. Animals were provided with deionized water (produced in a Millipore device) with Severyanka (Eko-proekt, St. Petersburg, Russia) mineral supplement and feed *ad libitum*. There were four experimental groups of eight animals: (1) a/a kept at 14L : 10D, (2) a/a kept at 4L : 20D, (3) A^Y/a kept at 14L : 10D and (4) A^Y/a kept at 4L : 20D. Two days before the tests, the animals were isolated in the same cages to reduce group effect. The animals were kept at the same photoperiods during the tests. First, the dynamics of locomotion, sleep, water and food consumption in the home cage was tested using PhenoMaster (TSE, Germany). Then, the mice were tested for three successive days in open field (OF), elevated plus-maze (EPM) and forced swim (FS) tests.

The maintenance of mice was supported by the basic research project No. 0324-2018-0016. All procedures comply with the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes and were approved by the Committee on the Ethics of Animal Experiments of the Russian National Center of Genetic Resources of Laboratory Animals based in SPF-vivarium of Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia (protocol No. 32 of March 18, 2016).

Home cage activity. Daily dynamics of locomotor activity, sleep duration, water and food consumption were investigated in PhenoMaster (TSE, Germany) according to the manufacturer's instruction. The device consists of eight individual cages equipped with infrared sensors those tracing the animal movements. Drinking bowls and feeders were also equipped with sensors, allowing the accurate measurement of water and food consumption. The data from the sensors were recorded each minute and processed by the software from the manufacturer. The animals learned to use drinking bowls and feeders for two successive days and then they were isolated in PhenoMaster cages and their locomotion, sleep duration, water and food consumed were recorded for 48 h. The first 24-hour period (1–24 h) was considered as an adaptive one and was not taken into account. Therefore, the baseline animal activity was assessed only on the basis of data recorded for the second 24-hour period (25–48 h) (Khotskin et al., 2017). According to the manufacturer's instructions, the software determined the state of sleep as lack of mobility for 40 s or more. This sleep estimation correlates with the EEG estimation of the sleep state (Pack et al., 2007; Fisher et al., 2012; Bais et al., 2015). The dynamics of locomotor activity and sleep duration were respectively evaluated by the distance travelled (m) and accumulated sleep time (min) during one hour, while the water and food consumption were measured as water (ml) and food (g) quantity consumed during 24 h.

Other behavioral tests were held between 15:00 and 18:00 in the dark time. The EthoStudio software was applied for automatic tracking of mouse behavior in OF, EPM and FS tests (Kulikov et al., 2008, 2010, 2014). In OF and FS tests we used the transmitted (inverted) light for automatic tracking of mouse behavior when the light was transmitted through the arena to a WEB camera placed at 80 cm above the arena and connected to a computer via a USB 3.0 port. Since a mouse regardless of its color (white, agouti or black) is opaque, it contrasts with background in the transmitted light (Kulikov et al., 2008).

Open field test (OF). The OF test was carried out on a brightly illuminated white plastic arena of 55 cm in diameter with the wall of 30 cm in height. The 25 % zone in the center of the arena was selected as the center. The arena was made of opaque polyvinyl chloride and placed on a semitransparent platform. The arena was brightly illuminated (300 lx) with two halogen lamps (35 W) placed 40 cm below the platform. A mouse was place at the wall of the arena and its movement was automatically tracked for 5 min. The EthoStudio software automatically calculates two behavioral traits: the time spent in the center (%) is calculated as the ratio of mouse-associated pixels in the center to the total number of mouse-associated pixels and the distance travelled during the test (m), while the number of vertical postures were recorded by an experienced rater blind to experiment (Kulikov et al., 2008). The time in the OF center is negatively associated with fear (Carol et al., 2002; Prut, Belzung, 2003), while the numbers of vertical postures are associated with exploration (Crusio, 2001; Alves et al., 2012). The apparatus was cleaned with wet and dry napkins after each test.

Elevated plus-maze test (EPM). The EPM test was carried out in the apparatus made of gray plastic including two closed and two open arms (30 cm in length × 5 cm in width). The close arms were framed by plastic walls of 30 cm in height. The apparatus was elevated at 60 cm above the floor and dimly illuminated with diffuse light (≈100 lx) of a halogen lamp (25 W) placed a meter above. Newly developed Microsoft Kinect 1 3-D sensor was applied for the first time for automatic tracking of mouse behavior in the EPM. This sensor was earlier successfully applied to track pig behavior (Kulikov et al., 2014). Microsoft Kinect 1 3-D sensor was placed 60 cm above the surface of the EPM and connected to a computer via a USB port. In contrast to standard digital video tracking systems based only on color or brightness of pixels, the present version of the EthoStudio software uses also the depth data provided by the 3-D sensor. Depth data contains the distance (m) from each pixel of the animal and arena to the sensor. The height threshold algorithm marks pixels higher or lower the threshold (1.5 cm in height) as associated with animal (1) or background (0), respectively (Kulikov et al., 2014). The main advantage of the 3-D sensor over standard digital video is that the former can track animal of any color in open and closed arms of the EPM.

A mouse was placed in the center of the EPM with the head targeted to a closed arm and its movement was automatically tracked for 5 min. Since the 3-D sensor can track animal both in open and closed arms the EthoStudio software can evaluate the following traits: the times (%) spent in the center, closed and open arms based on the ratio of the number of mouse-associated pixels in these parts of the maze to the total number of mouse-associated pixels. The time spent in the center and in the closed arms is, respectively, negatively and positively associated with fear (Carol et al., 2002; Prut, Belzung, 2003). The apparatus was cleaned with wet and dry napkins after each test.

Forced swim test (FS). Mice were placed for 6 min into a clear plastic tank (18 cm in diameter and 30 cm in height) filled with water at temperature 25 °C for 2/3 of the volume (20 cm). The water tank was placed on a semitransparent platform and illuminated (150 lx) with one halogen lamp (35 W) placed 40 cm below the platform (Kulikov et al., 2010). Behavior in the FS test was automatically evaluated for the last four minutes of the test using EthoStudio software by immobility time (s) and rate of alteration of the animal silhouette (%). The last parameter is the ratio of the number of mouse-associated pixels that have changed their position between two adjacent frames to the mean number of mouse-associated pixels in these frames (%). We used the mean rate calculated for the last four minutes of the test as the index of active resistance. The immobility time was calculated as the time (s) when the rate of the animal silhouette alteration was less than 17 %.

Statistics. The data were presented as the mean ± SEM. The mean values were compared using two way ANOVA ("genotype" and "photoperiod" factors). Daily dynamics of the locomotor activity and sleep in the home cage was compared using repeated measure ANOVA with "genotype" and "photoperiod" as the between and the "day time" as the within variables. The differences between groups were determined using the Fisher LSD post hoc multiple pairwise comparisons. Statistical significance was set at $p < 0.05$.

Results

Photoperiodic changes in weight of *a*/*a* and A^Y/*a* mice. The effect of the "genotype" factor on weight was observed: at the end of experiment mice of A^Y/*a* genotype (32.4 ± 0.4 g) were heavier than of *a*/*a* genotype (26.7 ± 0.4 g; $F_{1.28} = 110.81$, $p < 0.0001$). At the same time, no effect of the "photoperiod" factor ($F_{1.28} = 2.03$, n. s.) or the "genotype" × "photoperiod" interaction ($F_{1.28} < 1$, n. s.) on animals' weight was found.

Photoperiodic changes in home cage behavior in *a*/*a* and A^Y/*a* mice. No effect of the factors "genotype" (locomotion, $F_{1.24} < 1$, n. s.; sleep duration, $F_{1.24} = 1.2$, n. s.), "photoperiod" (locomotion, $F_{1.24} = 2.7$, n. s.; sleep duration, $F_{1.24} < 1$, n. s.) and the "genotype" × "photoperiod" interaction ($F_{1.24} < 1$, n. s., for both traits) on locomotion and sleep duration was observed. At the same time, the effects of the "day time" factor on locomotion ($F_{23.552} = 8.05$, $p < 0.00001$) and sleep duration ($F_{23.552} = 14.83$, $p < 0.00001$) were observed: in mice of both genotypes. The effects of "photoperiod" × "day time" interaction on the daily dynamics of locomotion ($F_{23.552} = 3.42$, $p < 0.00001$) and sleep duration ($F_{23.552} = 6.65$, $p < 0.00001$) were also observed. This indicates an influence of photoperiod on the daily dynamics of mouse activity. Mice exposed to long-day conditions were more active (199.54 ± 36.27 m/h in darkness vs 96.69 ± 21.01 m/h in light, $p = 0.000018$) in the dark-time period, while activity mice exposed to short-day conditions did not differ in the dark- and light-time periods (81.98 ± 36.27 m/h in darkness vs 33.89 ± 21.01 m/h in light, n. s.) (Fig. 1, *a*). At the same time, mice exposed to long-day (9.05 ± 1.44 min/h in darkness vs 26.20 ± 3.04 min/h in light,

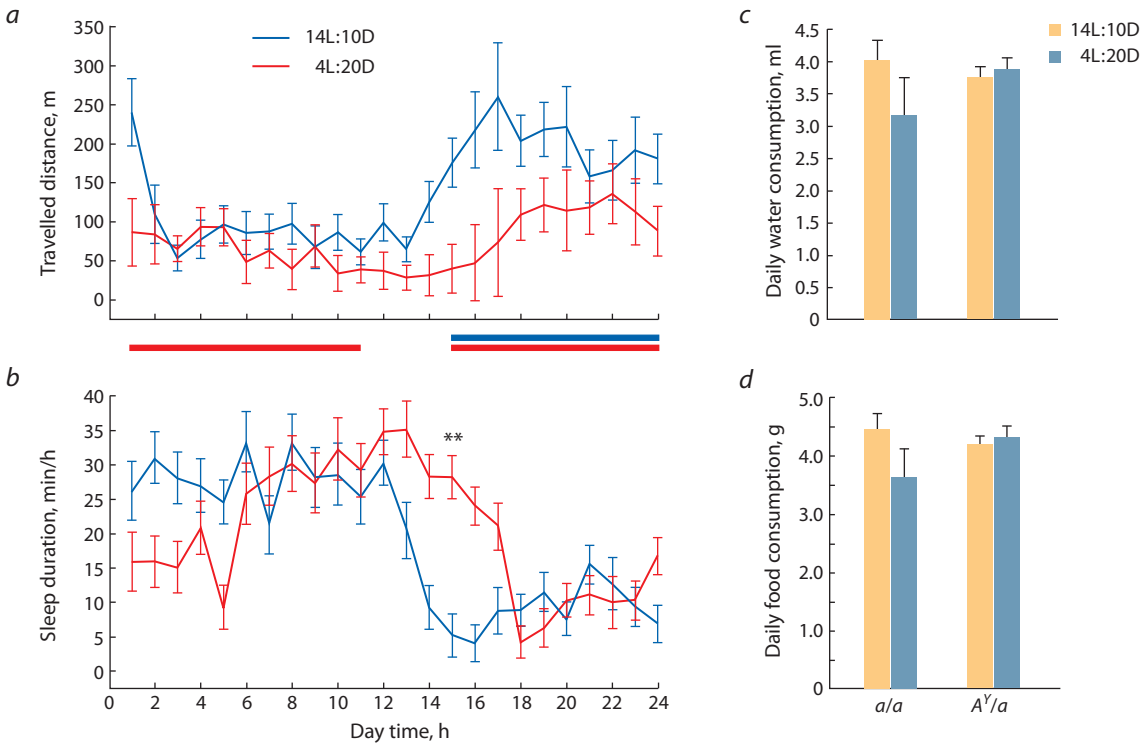

Fig 1. Daily dynamics of locomotor activity (distance travelled, m/h) (*a*), sleep duration (min/h) (*b*) as well as the daily consumption of water (ml) (*c*) and food (g) (*d*) in the home cage in *a*/*a* and A^Y/*a* mice exposed to long- and short-day conditions.

Blue and red bars under the panel (*a*) show the dark time for long- and short-day exposition, correspondingly. Since no effect of the "genotype" factor on the locomotor activity and sleep time is observed, the points of the corresponding curves are the means of values for *a*/*a* and A^Y/*a* mice exposed to the same photoperiod.

$^{**}p < 0.01$ difference between long- and short-day conditions.

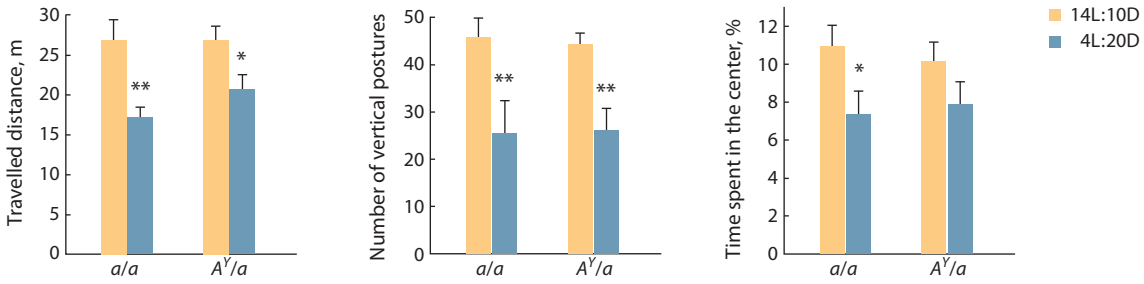

Fig. 2. Travelled distance (m), number of vertical postures and time spent in the center (%) in the OF test in *a*/*a* and A^Y/*a* mice exposed to long- and short-day conditions.

$^{*}p < 0.05$; $^{**}p < 0.01$ vs mice of the same genotype exposed to long-day conditions.

$p < 10^{-6}$) and short-day (18.16±1.44 min/h in darkness vs 31.87±3.04 min/h in light, $p = 0.000018$) conditions slept more time in the light phase than in the dark phase (see Fig. 1, *b*). So, the observed difference in daily dynamics of sleep between mice exposed to long- and short-day conditions results from the shift of light phase (long-day, between 01:00 and 15:00; short-day, between 11:00 and 15:00).

We did not observed any effect of "genotype" (water, food, $F_{1.24} < 1$, n. s.), "photoperiod" (water, $F_{1.24} = 1.16$, $p = 0.29$, n. s.; food, $F_{1.24} = 1.41$, $p = 0.25$, n. s.) factors and their interaction (water, $F_{1.24} = 2.11$, $p = 0.16$, n. s.; food, $F_{1.24} = 2.66$, $p = 0.12$, n. s.) on water and food consumption (see Fig. 1, *c* and *d*).

Photoperiodic changes in locomotor activity, anxiety-related and depression-like behavior in *a*/*a* and A^Y/*a* mice. In the OF test we revealed effects of the "photoperiod" factor on the traveled distance ($F_{1.28} = 16.33$, $p = 0.0004$), time spent in the center ($F_{1.28} = 6.57$, $p = 0.016$) and the numbers of vertical postures ($F_{1.28} = 16.24$, $p < 0.0004$). However, no effect of the "genotype" factor ($F_{1.28} < 1$, n. s.) or the interaction "genotype" × "photoperiod" ($F_{1.28} < 1$, n. s.) on these traits was observed. Short-day conditions decreased horizontal (traveled distance) (*a*/*a*, $p = 0.0016$; A^Y/*a*, $p = 0.035$) and vertical (vertical postures) (*a*/*a*, $p = 0.0053$; A^Y/*a*, $p = 0.012$) activities, while anxiety-related time in the center was decreased only in *a*/*a* mice ($p = 0.035$), but not in A^Y/*a* ones ($p = 0.17$) (Fig. 2).

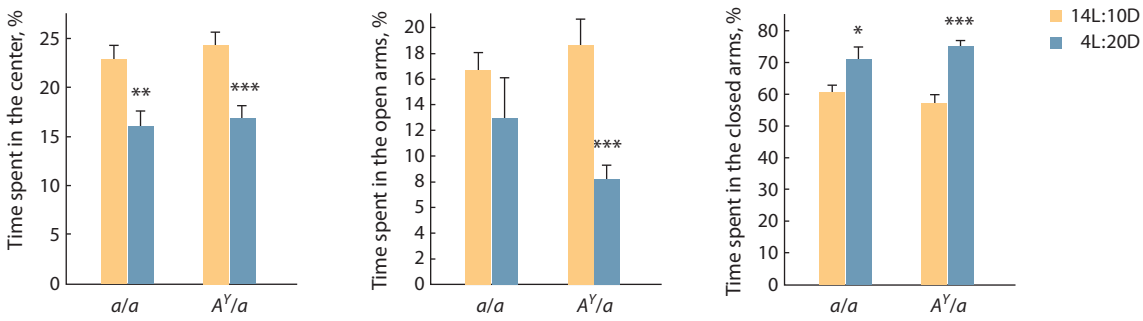

Fig. 3. Time spent (%) in the center, open and closed arms in the EPM test in *a/a* and A^Y/a mice exposed to long- and short-day conditions.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs mice of the same genotype exposed to long-day conditions.

In the EPM test we did not observed any effect of the "genotype" factor ($F_{1.28} < 1$, n. s.) and the interaction "genotype" × × "photoperiod" ($F_{1.28} < 1$, n. s.) on time spent in the center, open and closed arms. At the same time, significant effects of the "photoperiod" factor on the time spent in the center ($F_{1.28} = 27.04$, $p = 0.000016$), open ($F_{1.28} = 12.08$, $p = 0.0017$) and closed ($F_{1.28} = 25.02$, $p = 0.000026$) arms were revealed. Mice of both genotypes exposed to short-day spent less time in the center (A^Y/a, $p = 0.0006$; *a/a*, $p = 0.0016$) and open arms (A^Y/a, $p = 0.001$; *a/a*, $p = 0.21$, n. s.), but spent more time in the closed arms (A^Y/a, $p = 0.0001$; *a/a*, $p = 0.014$) of the EPM compared to mice exposed to long-day (Fig. 3).

In the FS test the influences of factors "genotype" (rate of silhouette alteration, $F_{1.28} = 21.54$, $p = 0.00007$; immobility time, $F_{1.28} = 20.03$, $p = 0.00012$) and "photoperiod" (rate of silhouette alteration, $F_{1.28} = 13.36$, $p = 0.0011$; immobility time, $F_{1.28} = 9.95$, $p = 0.0038$), but not the factors' interaction ($F_{1.28} < 1$, n. s. for both traits) on the rate of silhouette alteration and immobility time were revealed. The rate of silhouette alteration was lower ($p = 0.0005$) and immobility time was higher ($p = 0.0004$) in A^Y/a mice exposed to the long-day conditions compared to *a/a* animals exposed to the same photoperiod (Fig. 4). Short photoperiod decreased the rate of silhouette alteration ($p = 0.003$) and increased immobility time ($p = 0.0045$) in *a/a*, but not in A^Y/a mice (see Fig. 4).

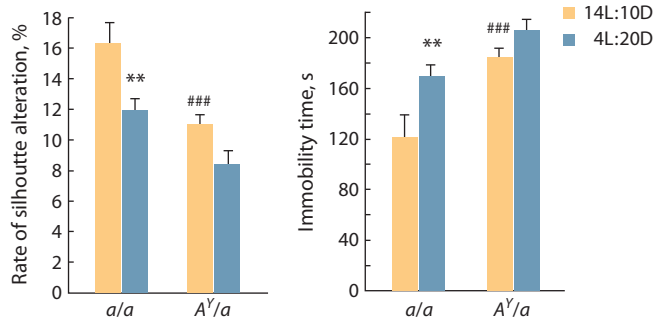

Fig 4. Rate of mouse silhouette alteration (%) and immobility time (s) for the last 4 min in the FS test in *a/a* and A^Y/a mice exposed to long- and short-day conditions.

**$p < 0.01$ vs mice of the same genotype exposed to long-day conditions; ###$p < 0.001$ vs *a/a* mice exposed to long-day conditions.

Discussion

In the present study, new information about the effects of the "genotype" (A^Y mutation), "photoperiod" and "genotype" × × "photoperiod" interaction was obtained.

The A^Y mutation results from large deletion in the promotor of the mouse *agouti* gene that puts the *agouti* gene under control of the promotor of an ubiquitously expressed *Raly* gene (Perry et al., 1994). Although, normally, agouty protein is expressed only in hair follicles, this deletion causes ectopic expression of agouti protein in many tissues including the brain, adipose and other tissues (Boston et al., 1997). Agouti protein inhibits melanocortin-4 receptors (Lu et al., 1994) involved in the regulation of total metabolism, feeding, anxiety and depressive-like behavior (Caruso et al., 2014; Gragnoli, 2014). However, since its creation, A^Y/a mice are mainly used to study the effect of obesity and type 2 diabetes on the peripheral control of metabolism, immunity, reproduction etc. This is the first systematic study of the effect of the A^Y mutation on the mouse behavior as well as on the vulnerability to altered photoperiod.

In the present study we did not find any effect of the "genotype" factor (A^Y mutation) on the general activity, sleep duration and feeding in the home cage; locomotor and exploratory activity in the OF test, anxiety-related behavior at mild stress conditions in the OF and EPM tests. At the same time, the A^Y mutation dramatically reduced active escape behavior (evaluated by the rate of silhouette alteration) and increased depressive-like immobility in the FS test. There are two possible mechanisms of the depressant-like effect of A^Y mutation: a direct via blockade of melanocortin mechanism of mood regulation or an indirect via obesity. The first hypothesis does not seem to be true, since melanocortin-4 receptor inhibitors show antidepressant effect (Chaki, Okuyama, 2005; Chaki et al., 2005; Chaki, Okubo, 2007). The second hypothesis seems to be more correct, since the leptin receptor deficiency results in obesity and increased depressive-like behavior in the FS test in $Lepr^{db}/Lepr^{db}$ mice (Sharma et al., 2010).

We first showed marked effect of photoperiod on daily dynamics of mouse activity in the home cage. Mice are nocturnal animals and under "normal", long-day conditions, they showed "normal" daily dynamics: they were more active in the darkness. At the same time, no such daily dynamics was observed in mice exposed to short-day condition: they did not increase their locomotor activity in the darkness.

There are contradictory data about the effect of photoperiod alterations on anxiety-related and depressive-like behavior. Some authors reported that exposition of C57BL/6 mice for 21 days to short-day condition (8L : 16D) increased anxiety-related and depressive-like immobility in the EPM and FS tests, respectively (Otsuka et al., 2014). Other authors reported that exposition of C57BL/6 mice for 14 days to long-day conditions (19L : 5D) increased anxiety-related and depressive-like immobility in the EPM and FS tests, respectively (Young et al., 2018).

Here we first showed that exposition to short-day conditions reduced locomotion (travelled distance), exploration (numbers of vertical postures), time spent in the center in the OF test, time spent in the center and open arms in the EPM test, as well as increased time spent in the closed arms in the EPM test. These findings can be interpreted as increase of anxiety-related behavior. We also found that the prolonged exposition to short photoperiod increased depressive-like behavior evaluated by decrease in the rate of silhouette alteration and increase in immobility time in the FS test in mice exposed to short-day conditions. Therefore, our results indicate that prolonged exposition to short-day condition increased anxiety-related and depressive-like behavior in mice. This results and conclusion agree with those of T. Otsuka with coauthors (2014), but contradict to those of J.W. Young and coauthors (2018). We think that these discrepancies result from the difference in experimental protocols. We and T. Otsuka with coworkers (2014) used a mild lighting conditions for long-day exposition 14 h, 40 lx and 16 h, 50 lx, respectively. At the same time, J.W. Young and coauthors (2018) used more intensive lighting, 19 h, 130 lum (≈150 lx) which is stressful for mice and frequently used as a stressor in the chronic unpredictable stress model (Monteiro et al., 2015) and in the OF test. Therefore, the protocol of J.W. Young and coauthors (2018) is a model for study the effect of intensive lighting rather than photoperiod alteration.

A key problem of the present study was to study the effect of genetic obesity on the vulnerability to short-day photoperiod. We did not find any effect of the "genotype" × "photoperiod" interaction on the studied behavioral traits in the home cage, OF, EPM and FS tests. Therefore, genetic obesity cause by A^Y mutation does not seem to increase vulnerability to short-day photoperiod. Although exposition to short day conditions and A^Y mutation separately increase immobility time in the FS test, they increase this depressive-like behavior by means of different mechanisms.

Conclusions

One practical conclusion can be based on the results. Obese A^Y/a and $Lepr^{db}/Lepr^{db}$ mice show similar elevated depressive-like behavior in the FS test. At the same time, breeding of A^Y/a mice is more simple than that of $Lepr^{db}/Lepr^{db}$ mice. Moreover, being a dominant mutation that can be easily determined by the fur color of its carriers, A^Y mutation is a very useful tool for studying the interaction between hereditary obesity and other neurological mutations.

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