Behavioral Consequences of Disrupted Circadian Clock Function within the Mouse Striatum

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ABSTRACT

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Daily rhythms are prominent in almost everything, from sleep/wake cycles, body temperature and hormone regulation to even cognition, motor coordination, attention and mood (McClung, 2007). Studies have shown that individuals with mood disorders and motor dysfunction exhibit alterations in the neural circuitry and neurochemistry in both the ventral and dorsal striatum (Del Donno et al., 2017) and recent research brought to the attention a potential involvement of the circadian system. There is evidence to suggest that the circadian clock gene *Period2* may be implicated in the vulnerability and development of striatum-related illnesses ((Lamont et al., 2007; Kim et al., 2018). However, the majority of these findings present mixed results and the mechanisms through which the core clock gene may influence these behaviors is still not understood. Hence, our aim is to elucidate how a conditional knockout of *Per2* in mood- and motor-related brain areas such as the striatum may contribute to circadian variation in striatum-related behaviors in male and female mice. Male and female mice of three representative genotypes: knockout (SKO), heterozygote (HET) and wild-type (WT), were used in a battery of behavioral tests to screen for striatum-related phenotypes. Animals were tested at two different time points (ZT2 or ZT14) for mood-related behaviors and ZT6 or ZT2 for motor function. Results indicated no differences in anxiety- and depressive-like behaviors across genotypes. However, SKO mice performed worse in the rotarod test in comparison to HET and WT mice, suggesting poorer motor coordination in mice where the circadian clock gene is conditionally knocked out from the striatum.

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Mood and anxiety disorders are among the most common types of mental illness in Canada and have been shown to have a major impact on the daily lives of those affected. According to the most recent Canadian survey, the prevalence of mood disorders was estimated at 5.4% (Leclerc et al., 2019). Psychiatric comorbidities are common in movement disorders (Miguel-Puga et al., 2017). Among individuals with mood disorders, about 55, 000 Canadians in private households aged 18 or older have been diagnosed with Parkinson's disease, a pathology characterized by severe motor dysfunction (Wong, Gilmour & Ramage-Morin, 2014). Past research has been devoted to elucidating the neural substrates involved in a variety of these and other conditions. Diverse literature has implicated abnormalities of striatal structure and function in mood disorders, severe motor dysfunction and addiction (Marchand & Yurgelun-Todd, 2010). One commonality in symptomatology among these illnesses is the presence of abnormal daily rhythms in physiology and behavior such as altered sleep-wake patterns, suggesting circadian dysregulation in patients (McClung, 2013). Many changes to physical, mental and behavioral states are governed by endogenous circadian clocks. Thus, disruption of clocks due to misalignment between the environment and internal clocks as well as clock gene expression alterations, can have pathologic consequences (Zhang & Sehgal, 2019). Clock genes and proteins exhibit daily variations in striatal structures (Bussi et al., 2014), and alterations in their expression play a significant role in the manifestation of mood disorders and substance abuse (Ketchesin et al., 2018; Spanagel et al., 2005). It has been proposed that disruptions in the circadian machinery may impact striatum-dependent functions and contribute to the pathophysiology of certain conditions such as affective disorders and motor dysfunction (Musiek & Holtzman, 2016). Thus, the present study aims to investigate the influence of striatal clock genes in striatum-related behaviors.

The environment we live in is rhythmic. To ensure adaptation, most living organisms evolved and developed clocks that generate rhythms in almost every aspect of their body, which allowed them to measure time intrinsically and anticipate important events (Kronfeld-Schor & Einat, 2011). Many critical events such as heart rate, blood pressure and sleep present temporal organization through the period of a day (Garmabi et al., 2016). A circadian rhythm is any

biological process that undergoes a period of about twenty-four hours and is modulated by both internal and external factors (Albrecht, 2012).

In mammals, the master clock is located in the suprachiasmatic nucleus (SCN) in the brain's hypothalamus. At the molecular level, it consists of an autonomic transcriptional/translational feedback loop involving a set of clock genes (Takahashi, 2017). This molecular feedback loop is mainly composed of brain and muscle arnt-like factor 1 (Bmal1), circadian locomotor output cycles kaput (Clock), neuronal per-arnt-sim domain protein 2 (Npas2), period (Per), and cryptochrome (Cry). BMAL1 and CLOCK form the positive loop, activating the transcription of *Per* and *Cry* through specific binding to E-boxes in the promoter of the aforementioned genes (Reppert & Weaver, 2001). Upon transcription in the nucleus and subsequent translation in the cytoplasm, PER and CRY proteins form a complex that re-enters the nucleus and inhibits the actions of their activators BMAL1/CLOCK, thereby generating a negative feedback loop. In the absence of any external or environmental input, this autoregulatory feedback loop cycles for about twenty-four hours (Albrecht, 2012). Nevertheless, the internal clock has the ability to synchronize itself in the presence of a zeitgeber. A zeitgeber refers to an environmental cue that synchronizes an organism's biological rhythm to the oscillations of the cue that is being presented (Aschoff, 1960). Thus, light and other environmental cues such as food and temperature influence the timing of the circadian rhythm.

Besides having a central clock residing in the SCN of the brain, peripheral clocks are also found in nearly every tissue and organ system throughout the body (Richards & Gumz, 2012). The relationship between the master clock and peripheral clocks has been the subject of much debate, giving rise to two major theories: the "master-slave" model and the "orchestra" model (Albrecht & Schibler, 2010). Nonetheless, recent studies provide increasing evidence for the latter. Instead of being fully synchronized by the circadian pacemaker, peripheral clocks can adjust to their own internal and external zeitgeber signals such as feeding cues for the liver, kidney and pancreas, while still being effectively coordinated by the cues sensed by the master clock (Albrecht & Schibler, 2010). This interplay between the master clock and peripheral clocks is necessary to maintain robust circadian rhythms and promote cellular homeostasis (Barclay, Tsang & Oster, 2012). Misalignment between central and peripheral oscillators resulting from disrupted clock function has been associated with the etiology of various diseases (Chauhan, Chen, Kent, and Crowther, 2017).

As previously stated, clock genes are expressed widely throughout the body and the mood- and reward-related centers of the brain are no exception. Several studies have reported rhythmic expression of molecular clock genes in rodents' striatum (Cai et al., 2009; Iijima et al., 2002). Different subdivisions of the striatum are assumed to be involved in distinct aspects of behavior. The dorsal striatum has been greatly implicated in movement control, decision making and some aspects of reward processing (Marche, Martel & Apicella, 2017). On the other hand, the ventral striatum is commonly implicated in reward, motivation, reinforcement and affect regulation (Haber et al., 2006).

Evidence supporting the relation between the circadian system and striatum-related behaviors comes from both human and animal studies. Previous studies have shown that altered circadian rhythms as well as disrupted clock gene expression contribute to the manifestation of striatum-related pathophysiology (Kim et al., 2017). Several human genetic studies have associated molecular clock genes in the manifestation of mood disorders. For instance, polymorphisms in PER2, NPAS2, RORA and ARNTL have been associated with depression vulnerability (Lavebratt et al., 2010). In parallel, other studies have documented associations between the circadian system and reward-related pathologies such as substance abuse. A study conducted by Li et al. (2009), observed disruptions in the rhythms of PER1 and PER2, cortisol, ACTH and other peptides in heroin-dependent individuals in comparison to age-matched healthy controls. It is noteworthy to mention that PER1 and PER2 have been found to be involved in reward processing, suggesting that alterations on their daily variation may contribute to relapse in patients (Abarca, Albrecht & Spanagel, 2002).

Given modest associations found in human studies, more direct lines of evidence in animals, such as gene expression and genetic manipulation studies, support clock genes' implication in striatum-related behaviors. Similar to human studies, extensive literature points out the role of clock genes in the vulnerability of mood-related disorders. A study conducted by Roybal et al. (2007), found that *Clock* mutant mice presented a behavioral profile similar to the manic state in bipolar disorder, involving hyperactivity, reduced sleep, decreased depression- and anxiety-like behavior and increased rewarding responses to cocaine and sucrose (Roybal et al., 2007). In another study, BMAL1, CRY1 and CRY2 deficient mice showed alterations in the amount of activity and the time to habituate to novel environments (Kondratova et al., 2010). Furthermore, selective deletion of *Bmal1* in the striatum resulted in altered voluntary alcohol

intake and preference, with opposite behaviors observed between knockout male and female mice (de Zavalia et al., 2020). In a Parkinson's disease model, alterations in the 24-hour rhythms of clock genes were identified in the dorsal striatum and master clock of the brain (Hayashi et al., 2010).

At the core of the circadian machinery in mammals is the *Period* family consisting of the genes *Per1*, *Per2* and *Per3* (von Schantz & Archer, 2003). This family of genes are not only widely expressed in the central nervous system (CNS) and in peripheral nervous systems (Wang et al., 2009). Over the years, researchers have been committed to identifying the distinct roles of these circadian genes. Notably, mPER2 has been found to play an important role in behavioral rhythmicity (Bae et al., 2001), and in the expression of pathophysiological conditions (Ripperger & Albrecht, 2012). For instance, Landgraf, Long and Welsh (2015) found a relation between PER2 expression and neural centers involved in mood and reward. Researchers looked at the rhythmicity of PER2 in the nucleus accumbens, a key structure involved in mediating motivational and emotional responses (Salgado & Kaplitt, 2015). After evoking depression-like behaviors in PER2 luciferase mice by use of a learned helplessness paradigm, results indicated that helpless mice showed disrupted rhythms of the circadian gene in the nucleus accumbens and periaqueductal grey (Landgraf, Long & Welsh, 2015). Moreover, Hampp et al. (2008), noted increased mania-like behaviors and preference towards drugs of abuse in $Per2^{Brdm1}$ mutant mice, indicating dysregulations in the reward system. $Per2^{Brdm1}$ mutant mice also displayed higher dopamine levels in the striatum, suggesting that *Per2* may play a critical role in regulating dopamine levels in the mesolimbic circuit (Hampp et al., 2008).

An ongoing question in neurobiology concerns the mechanisms through which clock genes possibly modulate the relation between circadian rhythms and physiopathologies in non-SCN areas. One proposed mechanism is through the central dopaminergic (DAergic) system, which is highly involved in motor functions, motivation and reward (Mendoza & Challet, 2014). DAergic neurons from the Substantia Nigra (SN) mainly project to the dorsal (caudate) and ventral striatum, which contain a high density of DA receptors (Francis & Lobo, 2017). Interestingly, DA-related parameters show robust daily rhythms that could be regulated by either the SCN or extra-SCN central nervous system oscillators (Mendoza & Challet, 2014). For instance, extracellular DA concentrations undergo circadian oscillations in the striatum of rodents suggesting that DA production and levels may be endogenously coordinated by a

circadian oscillator (Hood et al., 2010; Ferris et al., 2014). DA metabolism also appears to be modulated by the circadian clock. Sleipness et al. (2007), demonstrated that expression of tyrosine hydroxylase (TH), the rate-limiting enzyme of DA synthesis, as well as DA transporter proteins varies along the 24-h cycle in both the NAcc and the striatum, with higher levels at night. Conversely, Monoamine oxidase A (MAOA), an enzyme involved in DA degradation, was found to have a rhythmic expression in the ventral tegmental area (VTA) with highest expression in the light phase (ZT6) and lowest in the dark phase (ZT18) (Hampp et al., 2008). Results from these and other similar studies suggest that the dopaminergic system is under circadian clock influence.

Rhythms in clock gene expression in the striatum are sensitive to changes in DA release (Hood et al., 2010; Ferris et al., 2014), which has potential implications for conditions such as mood disorders, Parkinson's disease and drug addiction (Kim et al., 2018). Hood et al. (2010), noted that PER2 daily fluctuations in the dorsal striatum of rats are dependent on the availability of extracellular DA. Specifically, when animals are treated with 6-hydroxydopamine (6-OHDA) in the medial fore brain bundle (MFB) eradicating the dopaminergic projections from the substantia nigra to the caudate putamen, the rhythms of PER2 expression in the dorsal striatum are severely blunted. Nevertheless, daily injections of quinpirole, a DA D_2 receptor agonist, in 6-OHDA treated animals restored and reversed the daily oscillations of PER2 expression (Hood et al., 2010). Daily variation of PER2 in the dorsal striatum is dependent on DA availability and may be mediated via the DA D_2 receptor pathway (Hood et al., 2010). Thus, DA may be crucial in modulating circadian activities of the striatum, including daily locomotion (Korshunov et al., 2017). A study conducted by Yamada and Martin-Iverson (1991), found that administration of D1 agonist in rodents increased the length of the period of free-running rhythms of locomotor activity in constant dark conditions. Moreover, mutations of clock genes lead to altered responses to drugs that affect the DAergic system (Abarca et el., 2002), linking clock genes in the modulation of behavioral responses to DA stimulation.

The goal of the present study is to assess clock gene expression's implications in striatum-related behaviors in male and female mice. Specifically, we aim to elucidate the effects of targeted deletion of the core clock gene *Per2* in the striatum in behaviors under the same neural substrate's control. Given that the central dopaminergic system plays a pivotal role in the expression of certain conditions such as affect-related disorders and motor dysfunction. Based on

this premise, we hypothesized that compromised clock function resulting from a plausible disrupted dopamine signaling within striatal neurons, may be a key factor in the expression of striatum-related phenotypes. To test this hypothesis, we generated a conditional mouse line in which *Per2* was selectively deleted from striatal medium spiny neurons (MSNs). MSNs are brain cells that account for about 95% of the total neuronal population in the striatum and have been identified as a modulatory circuit for stress-induced depression (Francis & Lobo, 2017). Male and female mice were screened for anxiety- and depression-like phenotypes, motor control and locomotor activity in response to DA stimulation in a battery of behavioral tests. Animals were tested at two specific time points: ZT2 (2 hours after lights on) and ZT14 (2 hours after lights off) to look at circadian variation in mood-related behaviors; and ZT2 or ZT6 for motor behavior.

Method

Mouse line

All experimental procedures were carried out according to the Concordia Animal Ethics Committee. Mice with a conditional deletion of the *Per2* gene from MSNs were generated via the Cre-lox recombination strategy (Perkins, 2002). Excision of the floxed alleles in the presence of Cre recombinase results in the removal of the PAS domain-coding exon 6 of *Per2*, yielding a truncated non-functional protein (Chavan et al., 2016). Floxed *Per2* (*Per2*$^{fl/fl}$) transgenic mice (European Mouse Mutant Archive, Strain ID:EM10599) were crossed with hemizygous transgenic *Gpr88*$^{cre/+}$ mice (JAX, stock number 22510), which express Cre recombinase and EGFP under the control of the *Gpr88*$^{cre/+}$ promoter yielding striatal specific *Per2* knockout mice (SKO; *Gpr88-Cre;Per2*$^{fl/fl}$), as well as heterozygotes with only one functional *Per2* copy (HET; *Gpr88-Cre;Per2*$^{fl/+}$) and wildtype controls (WT; *Gpr88;Per2*$^{fl/fl}$).

Subjects

All mice were (N = 50) grouped housed with a maximum of 4 mice per cage in the rooms of our laboratory at about 10 weeks of age. Mice were all tested at 12 weeks of age. Female (N = 21) and male (N = 29) mice were housed in different cages. Food and water were available *ad libitum*. With respect to illumination conditions, animals were put under a 12:12h light-dark cycle with lights on at 7hr and lights off at 19hr. This illumination schedule remained present throughout the rest of the experiment. As way to avoid novelty-induced stress, weekly cage changes were carried out right after behavioral tests were terminated giving animals a span of 1 week to habituate to the new cage before the next test was conducted.

Behavioral tests

Prior to testing, all animals went through a habituation period commencing 1 hour after lights were on (ZT1) or 1 hour after lights were off (ZT13). All mice were transferred to the testing room and were left in their home cage for one hour as a way to reduce stress due to neophobia and improve performance in behavioral tests (Gouveia & Hurst, 2017). All behavioral tests were conducted at two time points, starting at ZT2 for daytime testing and ZT14 for nighttime testing with the exception of the tail suspension test (TST) and motor-related tests, which took place at ZT8 and ZT6 respectively (8 hours and 6 hours after lights on). For mice tested at ZT14, the test was conducted under red dim light (1-2 lux) to control for the masking effects of light (Obrietan,Impey & Storm, 1998). The battery of behavioral tests was scheduled

one week after the other to eliminate any potential carry-over effects (Nakamura et al., 2009). Behavioral tests were conducted in the following order: Elevated Plus Maze (EPM), Open Field test (OFT), Light/Dark Box test (L/D Box), Marble Burying test (MBT), Tail Suspension test (TST), Horizontal Bar test (HBT), Rotarod, and SKF experiment.

Elevated Plus Maze Apparatus

The apparatus used for the EPM consists of a '+' shaped maze with two open arms (25 x 5 x .05 cm) across from each other and two perpendicular closed arms (25 x 5 x 16 cm) with a center platform. The entire apparatus is 50 cm above the floor and is placed in an empty rectangular perimeter to protect the mice from falling or attempting to escape during the experimental procedure. The entire apparatus is made of plastic materials with detachable pieces that facilitate cleaning. The walls of the maze were black, and the platforms were chosen to be white to maximize contrast within recordings.

Procedure & Measures

The EPM is a widely used behavioral assay to assess unconditioned anxiety-like phenotypes and anti-anxiety effects of pharmacological drugs in rodents (Walf & Frye, 2007). Prior to testing, all platforms were wiped out with 70% ethanol. After habituation, each mouse was placed in the center region of the maze with its head pointing toward one of the closed arms. Trails were counterbalanced between genotypes and animal behavior was recorded for a total of 10 minutes. The number of entries made by the rodent onto the open and closed arms as well as the time spent on each arm was recorded. Greater amount of time spent in the closed arms is taken as a measure of higher anxiety-like behavior compared to mice that spend more time in the open arms (Walf & Frye, 2007). The number of fecal boli on open and closed arms were also counted after each trial. Platforms were cleaned with 70% ethanol between trials.

Open Field Apparatus

Open-field testing took place inside sound insulated and ventilated chambers. The open field arena (45 cm x 45 cm x 40 cm) consisted of four transparent Plexiglas walls and a gray PVC floor. A period of 1 hour was given prior to testing for habituation. All arenas were wiped down with 70% ethanol before the experiment began.

Procedure & Measures

Given that mice have an innate aversion to large, open and unknown environments (Corey, 1978), the OFT was used to screen for anxiety-like behaviors. After habituation, animals

were tested at two different time points, ZT2 and ZT14. Animals were removed from their home cage by the tail and placed directly into the left corner of the open field. Tracking/recording was initiated upon first locomotion grid beam break. All testing sessions were 1 hour in duration. The horizontal and vertical movements within the open field arena were recorded for 60 minutes by the infrared motion detection system (Panlab, Barcelona, Spain). Total distance travelled, time spent in center, resting time, rearings (vertical activity) and the number of fecal boli were recorded. All parameters were scored automatically except for the number of fecal boli, which were counted manually during cleaning. Scores were analyzed via the Actitrack software package.

Light/Dark Box

The light/dark box test was carried out inside sound insulated and ventilated chambers. The L/D box apparatus consists of a testing arena (45 cm x 45 cm x 40 cm) divided into two sections of different dimensions divided by an insulated box. The two partitions consist of a light compartment and a dark compartment. One third of the arena is devoted to the dark compartment, while two thirds are devoted to the light compartment. All light compartments were illuminated with about 300 lux, whereas the other partition had almost no illumination (2 lux).

Procedure & Measures

The light/dark box is based on the premise that rodents exhibit an aversion to brightly illuminated areas and novel environments (Bourin & Hascoët, 2002). Thus, higher exploratory behavior as well as transitions to the light compartment are perceived as an index of anxiolytic activity (Bourin & Hascoët, 2002). Following habituation, mice were placed in the center of the dark compartment. Tracking/recording was initiated upon first locomotion grid beam break. Animals were allowed to move freely between the two chambers for a total of 15 minutes. Horizontal activity including total distance traveled in each partition, number of transitions to light and time spent in each chamber were recorded via the Actitrack software package.

Marble Burying

Standard polycarbonate mouse cages measuring approximately 25 x 18 x 15 cm, were used for experimental observations. The bottom of each cage was covered with 7cm of sanichip bedding and then manually pressed with an additional empty cage to ensure even distribution. Color glass marbles, measuring approximately 1.5 cm in diameter, were arranged in 4 rows of 5

marbles for a total of twenty marbles in the field arena. Marbles were evenly distributed and washed between trials.

Procedure & Measures

Animals were placed into a corner of the cage containing marbles. Each mouse was placed carefully in the cage as far from marbles as possible to avoid unwanted burying. A filter-top cover was then put on the cage so animals could not escape from the cage. Food and water were withheld during testing and animals were allowed to remain in the cage undisturbed for 30 minutes. After completion of the 30 minutes, mice were immediately removed from the testing arena and placed in their respective home cages. The number of marbles buried was counted for each mouse. With respect to marbles on the surface, at least two thirds of each marble had to be covered by bedding in order for it to be considered buried. The marble burying test has been used to look at anxiety, obsessive-compulsive or repetitive behavior in rodents (Lazic, 2015). Evidence suggesting that marble burying is an indicative of anxiety-related behavior comes from studies showing decreased burying after benzodiazepine administration (Broekkamp et al., 1986). Thus, increased marble burying is associated with anxiety-like phenotypes.

Tail Suspension

The suspension box consisted of a suspension shelf (50 x 60 cm) placed horizontally in the center of stable surface. A white cardboard was placed behind the shelf as a way to provide optimal contrast. White corrugated plastic cardboard was placed in the middle of the suspension shelf to prevent mice from observing or interacting with other animals during testing. A camera was placed in the middle of the testing room with a tripod capturing the complete suspension box for recordings.

Procedure & Measures

The tail suspension test is frequently used as a screening test for anti-depressant action (Stukalin, Lan & Einat, 2020). Habituation was carried out at ZT7, one hour before testing (ZT8). Mice were suspended by the tip of their tail to the suspension shelf that sit about 30 cm from the ground with adhesive tape. In order to prevent mice from tail-climbing behavior, a plastic tube was placed above their tails. In each trial, two animals in a different compartment were suspended for a total of 6 minutes while being video recorded. Time spent immobile was analyzed through the Stopwatch software. Greater time spent immobile is associated with higher depressive-like behaviors in rodents (Can et al., 2012).

Horizontal Bar Test

Metal bars of varying diameters (2 vs 4 mm) were raised 60 cm above the ground by cardboard boxes. A soft disposable cloth was placed at the bottom of each cardboard box as a way to soften the mice's fall. Disposable cloths were changed, and metal bars were cleaned with 70% alcohol across trials to control for any scent-related confounds. A camera was placed 1.5 meters above the ground and held with a tripod to capture the box perimeter.

Procedure & Measures

The horizontal bar test is commonly used to screen for motor coordination and muscle strength (Deacon, 2013). Habituation was performed at ZT5, one hour before testing (ZT6). Mice were raised by the base of their tail and placed on the center of the metal rod allowing the mice to grasp it with only their forepaws. Each trial consisted of 60s on each rod (2mm & 4mm), where animals could hold on for the entire 60s, fall or cross the rod till reaching one of the cardboard columns. An animal falling within the first 5s after being placed on the rod was regarded as a poor placement by the experimenter and the mouse was retested for that trial. Mice were first tested on the 2mm diameter rod and then tested on the ticker metal rod (4mm). Three trials consisting of 60s each were conducted for each metal rod, resulting in a total of 6 trials for each animal. Resting breaks of 30s each were allowed between trials to reduce fatigue. The time the mice remained on each of the bars was quantified and the average of the three trials for each rod was computed to generate an overall score. Higher scores are associated with better motor coordination and muscle strength (Jacquez, 2021).

Rotarod

The rotarod apparatus consists of a circular rod turning at a constant or increasing speed (Bohlen at al., 2009). Vertical barriers are used to separate animals from one another. The apparatus included 5 different speeds as measured by rotations per minute (rpm): 2, 4, 8, 12 & 15. A camera was placed in front of the apparatus to record animals. Prior to every trial, the circular rod as well as vertical barriers were wiped with 70% alcohol.

Procedure & Measures

The rotarod test is widely used to generally assess motor performance in rodents (Jurado-Arjona et al., 2019). Prior to the testing trial, animals were given a training session at ZT2 for them to habituate to the motion of the apparatus. The training session consisted of a 60s trial at the lowest speed (2rpm) and the number of falls for each animal was recorded to measure

baseline performance. Animals were habituated at ZT5, an hour before testing. During testing, mice were placed on the rotarod for 5 consecutive trials with varying speeds. Each trial consisted of 60 seconds and speeds were consecutively increased, starting with the lowest one (2rpm). The time and the RPM at which each mouse fell was recorded. If mice grabbed the rod and somersaulted around it, the time at which this happened was recorded and considered the fall time. If mice fell within the first 5 seconds of the trial, they were retested up to 3 times before giving it a score of zero. Animals placed on the rotating rod try to remain on it instead of falling onto a platform located at the bottom of the apparatus. Thus, poorer performance on the rotarod is perceived as an indicative for neuromuscular impairment (Cenci & Lundblad, 2005).

SKF Experiment

SKF81297 was dissolved in saline (0.9% NaCl) to produce an injectable volume of 1ml/kg at doses of 5mg/kg. Drug doses were based on previous studies (Pezze & Cassaday, 2016). Vials containing the diluted drug were sonicated for a total of 2 minutes to ensure that no residue was left on the walls of the vial.

Procedure & Measures

Prior to testing mice were habituated in the experimental room at ZT1 an hour before the experiment began (ZT2). Before administering the drug or vehicle, animals were placed in the open field test for 30 minutes as a way to collect locomotor baseline activity. Once baseline activity was recorded, animals were weighed and given a dose of either SKF81297 or saline relative to their respective weight. Then, mice were again placed on the left corner of the open field arena for a period of 60 minutes. The horizontal and vertical movements within the arena were recorded by the infrared motion detection system (Panlab, Barcelona, Spain). Total distance travelled, resting time, rearings (vertical activity) and the number of fecal boli were recorded. All parameters were automatically recorded via the ActiTrack software except for the fecal boli which were manually recorded.

Results

Data Integrity

Excel raw data files were saved and transformed into a SPSS file. All statistical analyses were run using SPSS Statistics, Version (IBM, 2011), while Prism 9 was used to generate graphs. Prior to assessing assumptions for univariate normality, all of the parameters were inspected for missing values. The assessment did not reveal any missing values for the observed variables. The presence of outliers can severely distort the data (Osborne & Overbay, 2004). Thus, the presence of extreme scores or outliers was checked for the distribution of scores in each variable of interest. The universal definition of an extreme score is non-existent (Kline, 2009); however, a commonly used criterion is that scores more than 3 standard deviations beyond the mean may be outliers (Kline, 2009). Univariate outliers were found by inspecting frequency distributions of z-scores. In order to do so, standardized values (z scores) for all variables were computed and sorted in ascending and descending order to detect extreme scores at the upper and lower ends of each distribution of scores. Based on this rule of thumb, we found two extreme scores in the horizontal bar test; however, we did not remove these scores from the analyses. Reasons for such are further explained in the discussion section.

Behavioral data

Elevated Plus Maze

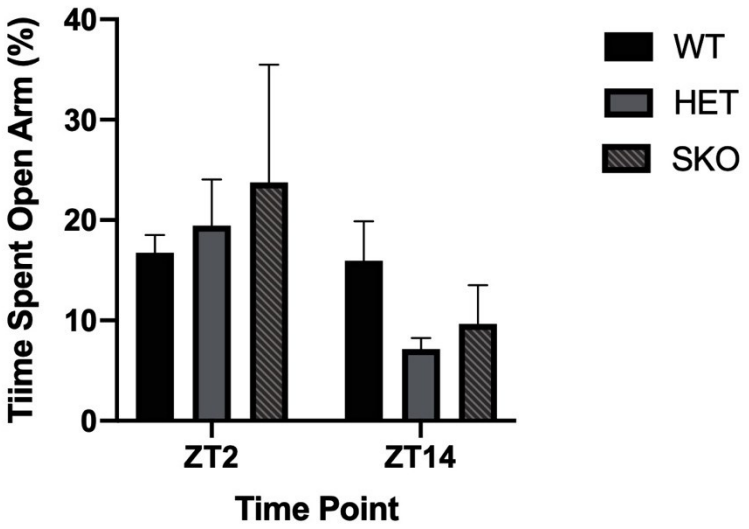
Percentage of time spent on open arms. A 3 x 2 between-subjects ANOVA was conducted to assess anxiety-like behaviors as measured by the percentage of time spent in the open arms of the EPM between genotypes (WT, HET & SKO) across two different time points (ZT2 vs ZT14) in both male and female mice. Reported in Table A1 and A2 are means and standard deviations on the percentage of time spent in the open arms for each genotype. The results for the 3 x 2 between-subjects ANOVAs are found in Table A3 for males and Table A4 for females.

For males, the two-way interaction effect between genotype and time point explains 9% of the total variability. No statistically significant differences were observed in the percentage of time spent in the open arms between control, heterozygotes and knockouts across the two different time points (ZT2 vs ZT14), $F(2, 23) = 1.357$, $p = .277$, $\eta 2 = .085$. Among the other effects, only a main effect of time point was statistically significant, which explains about 18%

of the total variance, F (1, 23) = 5.844, p = .024, η2 = .183. Descriptive statistics indicated that overall animals spend more time in the open arms of the maze at ZT2 (M = 19.97) than at ZT14 (M = 10.90) irrespective of genotype (see Figure 1A). Post-hoc results showed no statistically significant differences.

With respect to female mice, the two-way interaction effect between genotype and time point explains about 17% of the total variability. No statistically significant differences were observed in the percentage of time spent in the open arms between control, heterozygotes and knockouts across the two different time points (ZT2 vs ZT14), F (2, 15) = 2.389, p = .125, η2 = .179. The main effect of genotype was statistically significant, which explains about 35% of the total variance, F (2, 15) = 4.673, p = .026, η2 = .349. Descriptive statistics indicated that WT animals (M = 23.28) spent a higher percentage of time in the open arms followed by SKO mice (M = 18.53) and HET mice (M = 8.67) irrespective of time point (see Figure 1B).

A)



B)

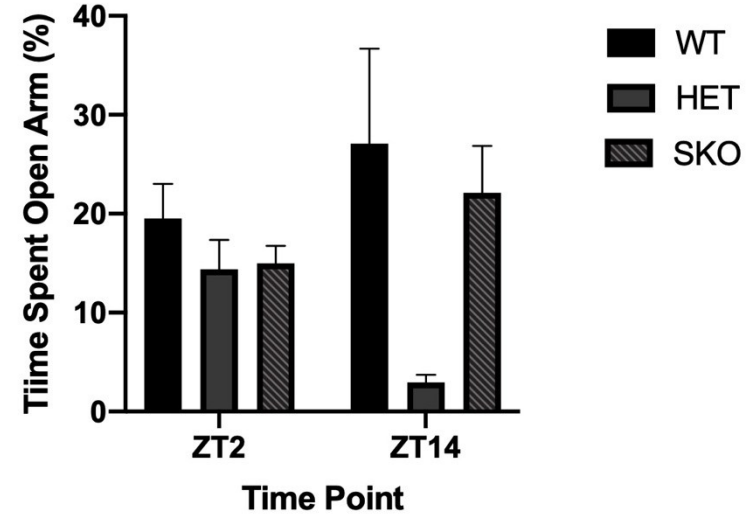


Figure 1. Percentage of time spent in the open arms of the elevated plus maze at two different time points (ZT2 & ZT14). A) In males, no differences were observed on the outcome variable between WT (n = 11), HET (n = 12) & SKO (n = 6) across the two time points. Animals spent more time in the open arms of the maze at ZT2 than at ZT4. B) In females, no differences were observed in the outcome variable between WT (n = 8), HET (n = 6) & SKO (n = 7) across the two time points. Error bars represent the standard error of the mean.

Percentage of open arm entries. A 3 x 2 between-subjects ANOVA was performed to look at the percentage of open arm entries between genotypes across two different time points (ZT2 vs ZT14) in both male and female mice. Reported in Table A5 and A6 are descriptives on the percentage of open arm entries for each genotype. The results for the 3 x 2 between-subjects ANOVAs are found in Table A7 for males and Table A8 for females.

For males, the two-way interaction effect between genotype and time point explains 11% of the total variance. Differences observed in the percentage of open arm entries between genotypes across the two time points were deemed to be non-significant $F(2, 23) = 1.619$, $p = .219$, $\eta 2 = .118$, indicating no differences in anxiety-related behaviors. All other effects were found to be non-significant.

In female mice, the two-way interaction effect between genotype and time point explains only 6% of the total variance. Differences observed in the percentage of open arm entries between genotypes across the two time points were deemed to be non-significant $F(2, 15) = 0.616$, $p = .553$, $\eta 2 = .063$, indicating no differences in anxiety-related behaviors. All other effects were found to be non-significant.

Open Field Test

Distance traveled (ZT2). A 6 x 3 mixed ANOVA was carried out to examine the effects of the between-subjects variable genotype (WT, HET, SKO) and the within-subjects variable being 10-minute intervals on the outcome variable distance traveled at ZT2. The within-subjects variable consisted of a total of six 10-min intervals. Analyses for both male and female mice were conducted separately. The results for the 6 x 3 mixed ANOVA are reported in Table B1 for males and Table B2 for females.

In males, Mauchly's test of sphericity indicated that the assumption of sphericity had been violated $\chi^2(14) = 28.12$, $p = .016$. Thus, the Greenhouse-Geisser epsilon value of 0.54 was used to correct the degrees of freedom used to evaluate the significance of the F ratio. The main effect of genotype was statistically significant, which explains about 31% of the total variance, $F(2, 11) = 4.526$, $p = .036$, $\eta 2 = .308$. Descriptive statistics indicated that for animals tested at ZT2, SKO animals ($M = 2516.9$) had a greater distance traveled followed by HET ($M = 2070.9$)

and WT (M = 1438.4) mice (see Figure 2A). The two-way interaction effect between genotype and interval explains only 1.5% of the total variance. No statistically significant interaction was observed between genotype and interval on the outcome variable F (5.32, 23.94) = 0.600, p = .712, η2 = .015.

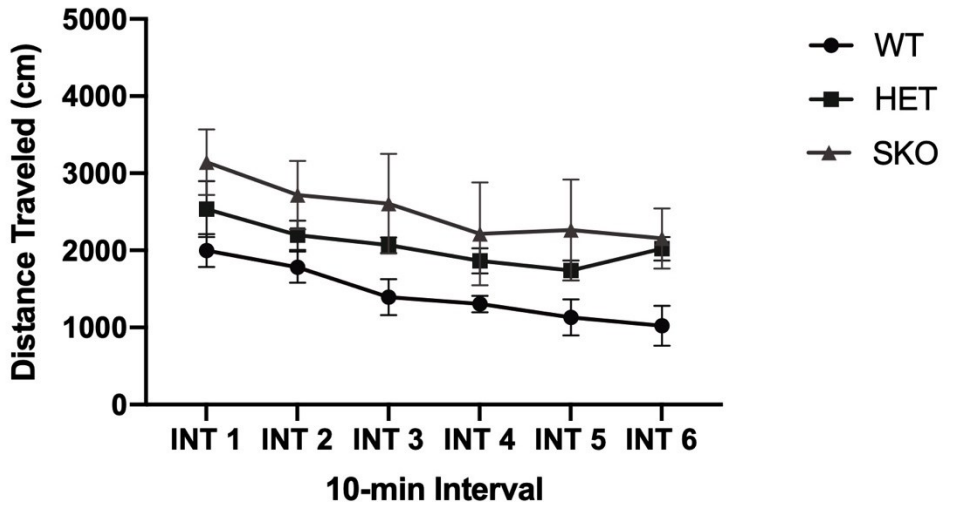
In females, Mauchly's test of sphericity indicated that the assumption of sphericity had been violated χ^2 (14) = 29.75, p =.011. Thus, the Greenhouse-Geisser epsilon value of 0.44 was used to correct the degrees of freedom used to evaluate the significance of the F ratio. The two-way interaction effect between genotype and interval explains only 2.6% of the total variance. No statistically significant interaction was observed between genotype and interval on the outcome variable F (4.42, 22.12) = 1.344, p = .284, η2 = .026, showing no differences in exploratory-related behavior.

Distance traveled (ZT14). A 6 x 3 mixed ANOVA was carried out to examine the effects of the between-subjects variable genotype (WT, HET, SKO) and the within-subjects variable being 10-minute intervals on the outcome variable distance traveled at ZT14. The within-subjects variable consisted of a total of six 10-min intervals. Analyses for both male and female mice were conducted separately. The results for the 6 x 3 mixed ANOVA are reported in Table B3 for males and Table B4 for females.

In males, Mauchly's test of sphericity indicated that the assumption of sphericity had been violated χ^2 (14) = 31.67, p =.005. The two-way interaction effect between genotype and interval explains about 6% of the total variation. A significant interaction was observed between genotype and interval in distance traveled F (4.23, 25.25) = 2.818, p = .044, η2 = .058. A Tukey post hoc reveled no statistically significant differences between genotypes in the outcome variable (see Figure 2B).

In females, Mauchly's test of sphericity indicated that the assumption of sphericity had not been violated χ^2 (14) = 36.07, p =.062. The two-way interaction effect between genotype and interval explain about 3% of the total variation. No significant interaction was observed between genotype and interval in distance traveled F (10, 25) = 1.592, p = .167, η2 = .033. No significant main effect of genotype was found.

A)



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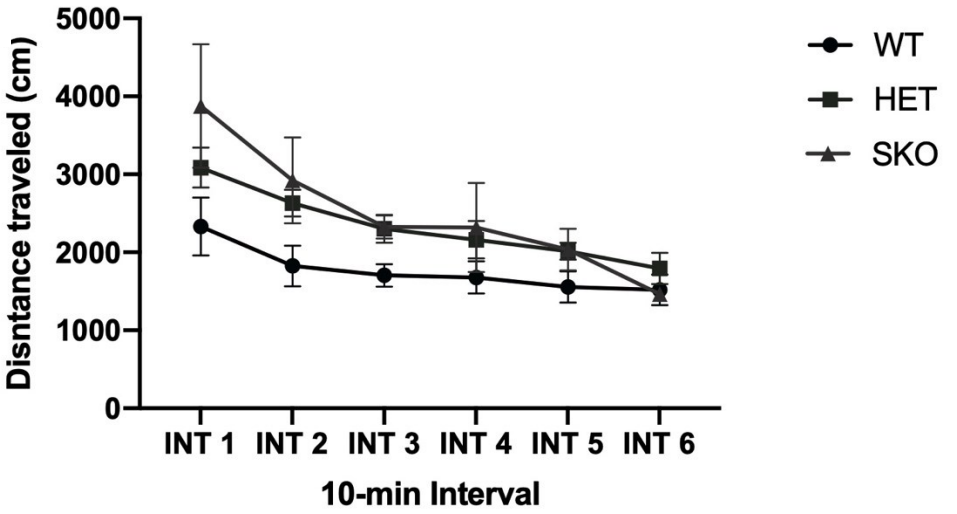


Figure 2. Distance traveled in the OFT between genotypes across two different time points (ZT2 & ZT14) in male mice. A) Male mice tested at ZT2, no differences were observed between WT (n = 5), HET (n = 6) & SKO (n = 5) across the 10-min intervals in distance traveled. There was a statistically significant effect of genotype, where a post-hoc analysis revealed a significant difference between HET and WT mice (p = .032). B) Male mice tested at ZT14, there was a statistically significant two-way interaction where SKO mice (n = 3) had a greater distance traveled during the first 10 minutes of the OFT in comparison to the other genotypes.

Percentage of time spent in center. A 3 x 2 between-subjects ANOVA was conducted to assess the percentage of time spent in the center of the open field arena between genotypes (WT, HET & SKO) across two different time points (ZT2 vs ZT14) in both male and female mice. The results for the 3 x 2 between-subjects ANOVAs are found in Table B7 for males and Table B8 for females.

With respect to males, the two-way interaction effect between genotype and time point explains only 1.5% of the total variability. No statistically significant differences were observed in the percentage of time spent in the center between control, heterozygotes and knockouts across the two different time points (ZT2 vs ZT14), F (2, 23) = 0.282, p = .756, η2 = .015. Among the other effects, only a main effect of time point was statistically significant, which explains about 30% of the total variance, F (1, 23) = 11.060, p = .003, η2 = .298. Descriptive statistics indicated that animals spend more time in the center of the open field arena at ZT14 (M = 17.81) than at ZT2 (M = 9.14) irrespective of genotype (see Figure 3A).

In females, a main effect of time point was statistically significant, which explains about 17% of the total variance, F (1, 15) = 4.797, p = .044, η2 = .170. The analysis indicated that female mice spend a higher percentage of time in the center of the open field at ZT14 (M = 9.24) than at ZT2 (M = 5.46) regardless of genotype (see Figure 3B). The two-way interaction effect between genotype and time point explains about 19% of the total variability. No statistically significant differences were observed in the percentage of time spent in the center between genotypes across the two different time points (ZT2 vs ZT14), F (2, 15) = 2.789, p = .093, η2 = .198, showing no differences in anxiety-related behaviors.

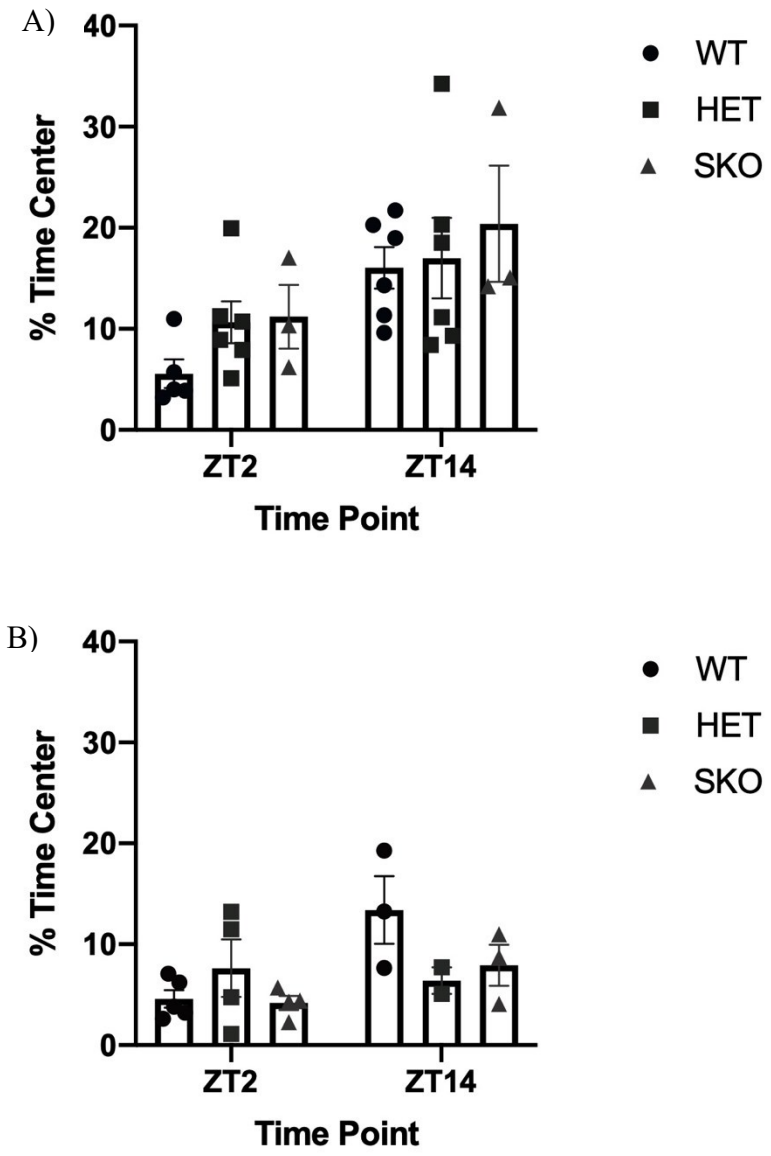


Figure 3. Percentage of time spent in the center of the OFT between genotypes across two different time points (ZT2 & ZT14). A) Male mice, no significant effect was observed in the interaction between WT (*n* = 11), HET (*n* = 12) & SKO (*n* = 6) mice across the two time points. There was a statistically significant effect of time point, where mice spent a greater percentage of time in the center of the OFT at ZT14 than at ZT2. B) Female mice, no significant interaction was observed between WT (*n* = 8), HET (*n* = 6) & SKO (*n* = 6) mice across the two time points. There was also a main effect of time point where mice spent a greater percentage of time in the center at ZT14 than at ZT2. Error bars represent the standard error of the mean.

Light/Dark Box

Percentage of time spent in light. A 3 x 2 between-subjects ANOVA was conducted to assess anxiety-like behaviors as measured by the percentage of time spent in the light portion of the light/dark box between genotypes (WT, HET & SKO) across two different time points (ZT2 vs ZT14) in both male and female mice. The results for the 3 x 2 between-subjects ANOVAs are found in Table C1 for males and Table C2 for females.

In males, the two-way interaction effect between genotype and time point explains only 2% of the total variability. No statistically significant differences were observed in the percentage of time spent in the illuminated area of the box between genotypes across the two different time points (ZT2 vs ZT14), $F(2, 23) = 0.296$, $p = .746$, $\eta 2 = .025$. No differences were observed in anxiety-related behaviors between genotypes across the two time points.

Regarding females, the two-way interaction effect between genotype and time point explains 6% of the total variation in the outcome variable. Differences observed in the percentage of spent in the bright area of the box between genotypes across time points were not statistically significant, $F(2, 15) = 0.819$, $p = .459$, $\eta 2 = .062$. Nevertheless, there was a marginally statistically significant main effect of time point $F(1, 15) = 3.170$, $p = .095$, $\eta 2 = .119$. The main effect of time point accounted for about 12% of the total variability in the outcome variable (see Figure 4B, Appendix C).

Marble Burying Test

Number of buried marbles. A 3 x 2 between-subjects ANOVA was conducted to assess anxiety-like behaviors as measured by the number of buried marbles between genotypes (WT, HET & SKO) across two different time points (ZT2 vs ZT14) in both male and female mice. The results for the 3 x 2 between-subjects ANOVAs are found in Table D1 for males and Table D2 for females.

Concerning males, the two-way interaction effect between genotype and time point explains roughly 1% of the total variation in the outcome variable. No statically significant differences were observed in the number of buried marbles between genotypes across time

points, F (2, 23) = 0.027, p = .974, η2 = .002, indicating no differences in anxiety-related behaviors. No other effects were observed.

With respect to females, the two-way interaction effect between genotype and time point explains 10% of the total variation in the outcome variable. No statically significant differences were observed in the number of buried marbles between genotypes across time points in female mice, F (2, 15) = 0.899, p = .427, η2 = .104, showing no differences in the outcome variable between genotypes across the two time points.

Horizontal Bar Test

Motor coordination score. A 3 x 2 mixed ANOVA was carried out to examine the effects of the between-subjects variable genotype (WT, HET, SKO) and the within-subjects variable being rod thickness (thin vs thick) on motor coordination scores as measured by the time taken to fall off the bar. Analyses for both male and female mice were conducted separately. The results for the 2 x 3 mixed ANOVA are reported in Table E1 for males and Table E2 for females.

Regarding males, the assumption of sphericity was assumed to be met due to the fact that the within-subjects variable had only two levels. The two-way interaction effect between genotype and rod thickness explains about 3% of the total variation in the outcome variable. No statically significant differences were observed in the time taken to fall between genotypes across the two different rod thickness, F (2, 26) = 1.510, p = .240, η2 = .027, showing no differences in motor coordination scores.

Regarding females, sphericity was also assumed to be met. There was a marginally significant effect of rod thickness, accounting for about 12% of the total variation (η2 = .122). The interaction between genotype and rod thickness explains about 6% of the total variation in the outcome variable. No statistically significant differences were observed in the dependent variable between genotypes across the rod thicknesses, F (2, 18) = 0.870, p = .433, η2 = .056.

Rotarod

Motor coordination. A 3 x 5 mixed ANOVA was performed to look at the influence of the between-subjects variable genotype (WT, HET, SKO) and the within-subjects variable being speed on motor coordination as measured by the time taken to fall from the rotarod. Animals were tested at five different levels of the within-subjects variable: 2RPM, 4RPM, 8RPM, 12RPM and 15RPM. Analyses for both male and female mice were conducted separately. The results for the 2 x 3 mixed ANOVA are reported in Table F2 for males and Table F3 for females.

In males, Mauchly's test indicated that the assumption of sphericity had been violated χ^2 (9) = 34.87, $p < .001$. There was a statically significant main effect of speed, F (2.57, 66.88) = 39.329, $p < .001$, $\eta 2 = .408$. Animals performed worse as speed increased. The two-way interaction effect between genotype and speed explains about 8% of the total variation. There was a statistically significant effect in the time taken to fall off from the rotarod between genotypes across the different speeds, F (5.15, 66.88) = 3.734, $p = .004$, $\eta 2 = .078$. A Tukey post hoc revealed that the time taken to fall off from the rotarod was significantly lower in SKO mice at 15RPM ($M = 14.26$, $p = .005$) in comparison to WT animals ($M = 40.45$) (see Figure 5).

Regarding females, Mauchly's test indicated that the assumption of sphericity had been violated χ^2 (9) = 31.23, $p < .001$. There was a statically significant main effect of speed, F (2.36, 42.49) = 20.260, $p < .001$, $\eta 2 = .367$, where mice performed worse as speed increased. The two-way interaction effect between genotype and speed explains about 7% of the total variation. No statistically significant effect was found in the time taken to fall off from the rotarod between genotypes across the different speeds, F (4.72, 42.49) = 1.939, $p = .111$, $\eta 2 = .070$.

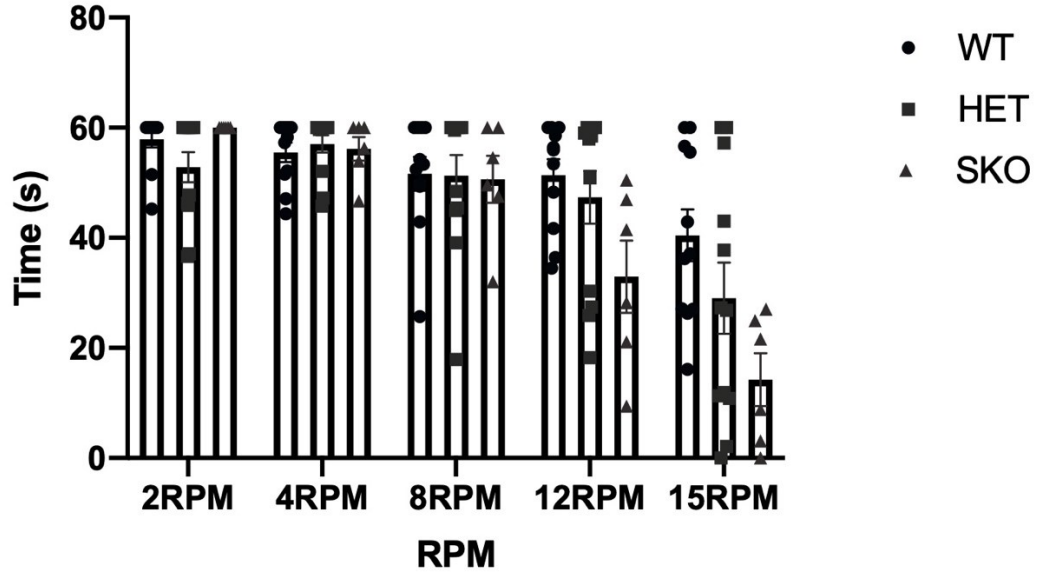


Figure 5. Time taken to fall off from the rotarod across different speeds between genotypes in male mice. There was a statistically significant effect in the time take to fall off from the rotarod between WT (*n* = 11), HET (*n* = 12) & SKO (*n* = 6) mice across the different speeds. Specifically, the time taken to fall off from the rotarod was significantly lower in SKO mice than in WT mice as speed increased. This effect was mas more pronounced at the highest speeds (12 RPM & 15 RPM). Error bars represent the standard error of the mean.

Tail Suspension Test

Time spent immobile (s). A one-way between-subjects ANOVA was conducted to look at depressive-like behaviors as measured by the time spent immobile between genotypes (WT, HET, SKO) at ZT8. Analyses for both male and female were conducted separately. Results for the one-way ANOVA are found in Table G1 for males and Table G2 for females. In males, there was not a statistically significant difference in the time spent immobile between the three different genotypes, F (2, 26) = 0.141, p = .869. Similarly, no statistically significant effect was observed in the outcome variable in female mice, F (2, 18) = 0.632, p = .543.

SKF D1 receptor agonist

Distance traveled (au). A mixed ANOVA was performed to examine the effects of the between-subjects variable genotype (WT, HET, SKO) and the within-subjects variable being 10-minute intervals on the outcome variable distance traveled for each condition (SKF vs Saline). The scores for distance traveled were normalized to the baseline activity of the animals. The within-subjects variable consisted of a total of six 10-min intervals. The results of the mixed ANOVA are found in Table H1and H2 for males and Table H3 and H4 for females.

With respect to males in the SKF condition, Mauchly's test indicated that the assumption of sphericity had been violated χ^2 (20) = 65.26, p < .001. No statistically significant interaction was observed, F (3.75, 20.60) = 1.662, p = .119, η2 = .094. Among all other effects, the main effect of genotype was statistically significant explaining about 32% of the total variation, F (2, 11) = 7.960, p = .007, η2 = .316. Overall, WT mice had a higher distance traveled (au) (M = 1.43) in comparison to HET (M = 0.77) and SKO mice (M = 0.87). For male mice in the saline condition, no statistically significant interaction was observed on the outcome variable, F (3.18, 38.27) = 1.963, p = .096, η2 = .097 (see Figure 6).

Regarding females in the SKF condition, Mauchly's test indicated that the assumption of sphericity had been violated χ^2 (20) = 62.23, p < .001. No statistically significant interaction was observed, F (1.88, 15.08) = 1.230, p = .292, η2 = .076, indicating no differences in the outcome variable between genotypes that received the D1 agonist. For female mice in the saline condition,

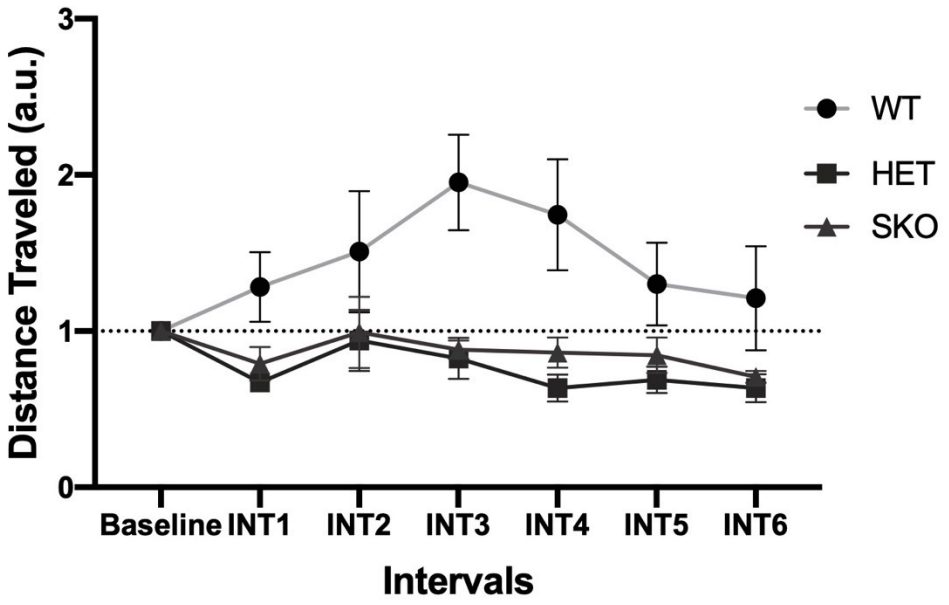
no statistically significant interaction was observed on the outcome variable, F (2.20, 15.41) = 0.281, p = .989, η2 = .028 (see Figure 7, Appendix H).

Rearing Activity. A 3 x 2 between-subjects ANOVA was conducted to assess rearing or vertical activity between genotypes (WT, HET & SKO) across the two different treatment conditions (Saline & SKF) in both male and female mice.

Concerning males, there was main effect of condition which accounted for 36% of the total variability F (1, 24) = 14.34, p = .000, η2 = .363, where animals that received the D1 agonist reared more (M = 338) than those that received saline (M = 150). No statistically significant interaction was observed in rearing activity between genotypes across the two different treatments, F (2, 24) = 1.501, p = .243, η2 = .076 (see Figure 8A).

Regarding female mice, no main effect of condition was observed F (1, 15) = 0.232, p = .637, η2 = .013, contrary to what was observed in male mice. No statistically significant interaction was observed in rearing activity between genotypes across the two different treatments, F (2, 15) = 0.859, p = .444, η2 = .094 (see Figure 8B).

A)



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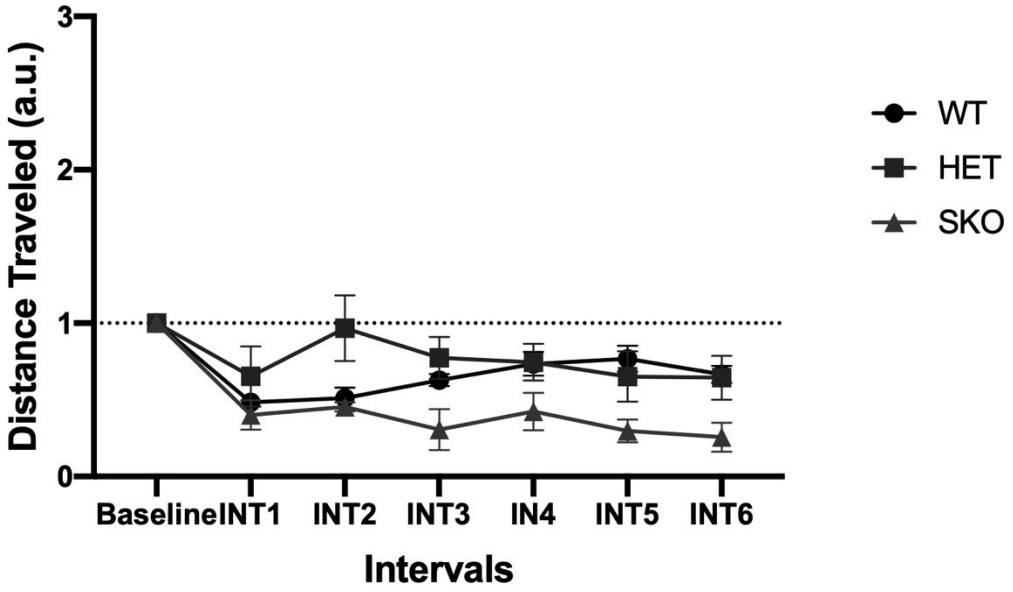

Figure 6. Distance traveled (normalized to baseline activity) in the open field between genotypes after saline or SKF injection in male mice. A) After SKF injection, no statistically significant interaction was observed between WT (n = 5), HET (n = 6) & SKO (n = 3) across intervals. A main effect of genotype was found, where WT mice had a higher distance traveled (au) I comparison to HET and SKO mice. B) After saline injection, no significant effects were observed in the outcome variable between genotypes across intervals. Error bars represent the standard error of the mean.

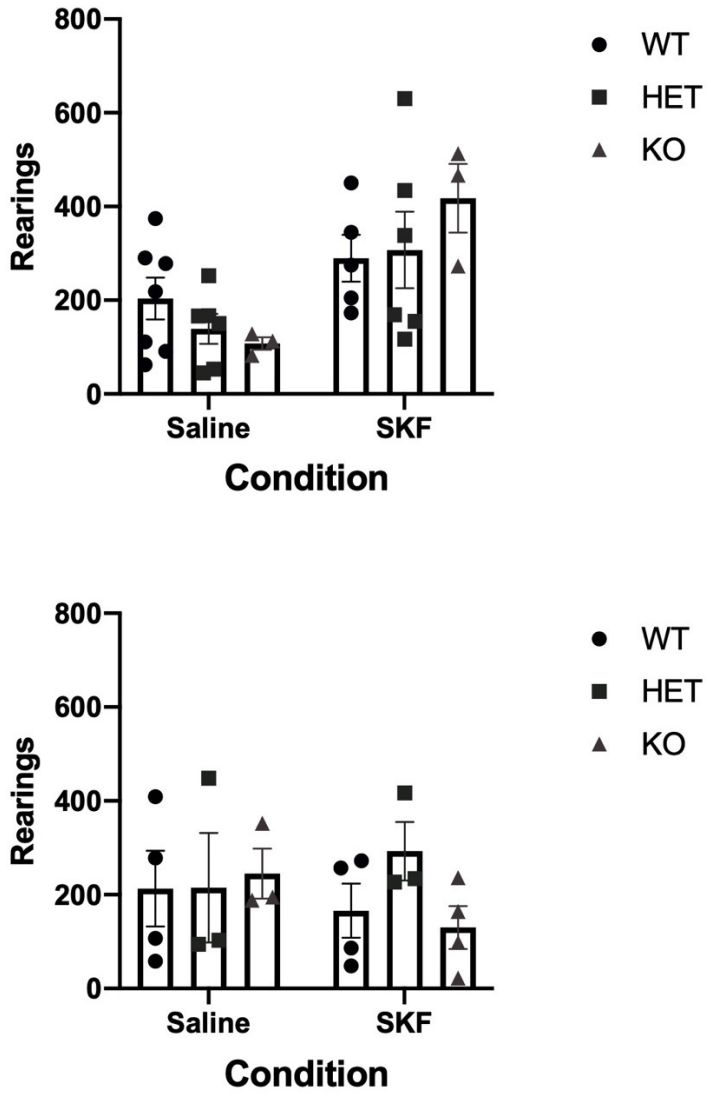

Figure 8. Rearing activity after administration of Saline or SKF between genotypes in male and female mice. A) In male mice, no statistically significant interaction was observed between WT (n = 12), HET (n = 11) & SKO (n = 6) across conditions. A main effect of condition was observed, where overall mice that received SKF had greater vertical activity than those that received saline. B) In female mice, no differences were observed in rearing activity between WT (n = 9), HET (n = 6) & SKO (n = 7) across conditions. Depicted are arithmetic means and standard error of the mean.

Discussion

The purpose of the present thesis project was to assess the implications of striatal clock gene expression in striatum-related behaviors in male and female mice. Specifically, we aimed to investigate the effects of targeted deletion of the core clock gene *Per2* in the striatum in affect-related and motor behaviors, which are believed to be under the control of this brain region. Based on previous research demonstrating that expression of the *Per2* gene in the dorsal striatum is controlled by dopamine (Hood et al., 2010), we hypothesized that dysregulation of striatal clock gene expression may have an effect on striatum-related behaviors. The results of the present project partially supported our hypothesis, where an effect of genotype was predominantly observed in motor-related tasks, but not as predominantly observed in mood-related tasks.

Concerning mood-related behaviors, we observed that male mice seem to spend a greater percentage of time in the open arms of the EPM during their inactive phase (ZT2) than their active phase (ZT14). In contrast, male mice spent a higher percentage of time in the center of the OFT during their active phase (ZT14) than their inactive phase (ZT2) and the same trend was observed in female mice. This diurnal variation in test-specific anxiety-like behaviors is similar to what has been observed in previous literature. A study conducted by Nakano et al. (2016), noted that anxiety-like behaviors in WT mice are under tight circadian regulation. Researchers observed that rhythms in anxiety-like behaviors were anti-phasic to each other. Specifically, anxiety-like phenotypes in the EPM were higher during the animal's active phase and lower during the resting phase, while anxiety-like behaviors in the OFT were more prominent during the animal's resting phase. It is important to consider that the majority of mood disorders fall within a spectrum instead of being limited to a set of specific symptoms (Lang & McTeague, 2009). Therefore, it is plausible that different behavioral tests may be capturing distinct aspects of the anxiety spectrum mediated by distinct neural substrates (Nakano et al., 2016). For example, recent research has pinpointed various important brain regions and circuitries underlying fear and anxiety responses (Tovote, Fadok & Luthi, 2015). Such regions include the basolateral amygdala (BLA) and the bed nucleus of the stria terminalis (BNST), which are characterized by their connections with other limbic structures and could potentially mediate overlapping anxiety circuitries (Tovote, Fadok & Luthi, 2015).

Results from the anxiety-related behavioral tests indicated that HET female mice spent a lower percentage of time spent in the open arms of the EPM, suggesting anxiety-like behaviors in these animals. Furthermore, although not significant, OFT results showed that SKO male mice had a greater distance traveled followed by HET and WT mice throughout the whole one-hour period propounding hyperactivity and decreased habituation to novel environments (Kondratova et al., 2010). Despite not finding genotype differences in the rest of the mood-related tests, these results support the notion that circadian clock gene manipulations outside of the master clock of the brain lead to some anxiogenic effects. A study conducted by Spencer et al. (2013), noted that *Per1* and *Per2* knockdown in the NAc, using RNA interference, increased anxiety-like behaviors in mice. Similarly, global mutant animals lacking *Per1* and *Per2* exhibited a robust increase in anxiety-like phenotypes; however, this increase in anxiety-related behaviors was not observed when either of the genes alone was targeted (Spencer et al., 2013). Thus, the manifestation of anxiety-like phenotypes might become more likely when expression of both *Per* genes is altered. Having one intact clock gene and its protein may be able to make up for the loss of the other protein (Tamiya et al. 2016), which in turn could also dampen any effects at the behavioral level. This could explain why we did not see differences in anxiety-like behaviors between genotypes in the majority of mood-related tests.

Disruption of circadian rhythm as well as clock gene expression play a role in the development of depressive-like behaviors (Kim et al., 2018). In our study, we did not find any significant differences in depressive-like behaviors as measured by the time spent immobile in the TST between genotypes in both male and female mice. These results successfully replicated what had been observed in a different group of animals that were previously tested in our laboratory (see Figure 9, Appendix I). In contrast, Landgraf et al. (2015) found that learned helplessness in mice is associated with absence of circadian rhythms in the nucleus accumbens and increased depression-like behaviors. A recent study conducted by Russell et al. (2021), demonstrated that *Per2* knockout mice showed disrupted corticosterone secretion and increased depressive-like behaviors, suggesting that the vulnerability of developing affect-related behaviors is related with dysregulation of the HPA axis involved in the stress response. Depression is a heterogeneous disorder; however, it is well known that stressful life events could induce a series of psychological and physiological changes that contribute to the development of mood disorders (Yang et al. 2015). Thus, our discrepancy in depressive-like behaviors could be

attributed to the methods used in this study given that we did not use any kind of unpredictable stress paradigm to elicit a stress response prior to testing. Overall, these results indicate that disrupted Per*2* expression in the striatum is not sufficient to induce depressive-like behaviors.

Given the interaction between *Per2* and DA via DAD2 receptors (Hood et al. 2010) and the role of dopaminergic signalling in motor deficits and circadian disruption (Brichta et al., 2015), we investigated the role of *Per2* in motor coordination. Our results indicated that *Per2* SKO male mice performed worse in the rotarod as speed increased in comparison to the other genotypes. These results suggest that disrupting striatal *Per2* expression is associated with motor coordination deficits in male mice. Severe motor abnormalities are a core symptom of several neurodegenerative disorders such as Parkinson's Disease (PD), where depletion of dopaminergic neurons in the substantia nigra is believed to contribute to motor dysfunction (Brichta et al., 2015). The majority of animal models of PD involve lesioning the nigrostriatal dopaminergic system (Simola, Morelli & Carta, 2007); thus, disentangling the effects of particular variables of interest is increasingly challenging. Given that our approach is not detrimental to the nigrostriatal pathway, the current SKO model used in this study allows us to attribute any effects seen in motor coordination to alterations in clock gene expression in striatal MSNs. Hence, poorer motor coordination observed in knockout mice supports the involvement of a circadian mediated pathway in the expression of motor dysfunction.

Regarding the SKF experiment, where animals were stimulated with a DAD1 receptor agonist, we found that WT males mice exhibited a robust increase in distance traveled normalized to baseline activity. However, this increase was not observed in both HET and SKO mice, indicating decreased sensitivity to the locomotor activating effects of the D1 agonist SKF81297. In females, locomotor activity was not significantly different between genotypes after exposure to the drug. These results seem to be opposite to what has been previously found. Schindler & Carmona (2002), noted that female rats displayed increased locomotor activity following D1 agonist administration, suggesting increased sensitivity in D1 receptor function. However, the type of receptor agonist used by Schindler & Carmona (2002), was different to the one we used in our experiment. It has been shown that receptor responsiveness is dependent on agonist efficacy or the strength of signalling (Charlton, 2009); thus, this could explain why we did not see a robust effect of the drug in female mice.

The present study had some limitations. Given that animals were housed in groups, circadian parameters such as light/dark phase activity as well as activity onsets and offsets were not assessed via running-wheel activity in this sample. Therefore, we cannot tell whether the distinct genotypes presented any differences in circadian rhythms. Furthermore, due to COVID-19 restrictions we could not validate the *Per2* knockout model used in this project. Nevertheless, previous work using the same Cre/lox recombination system in *Per2* floxed mice in the laboratory and protein expression analyses revealed successful verification of the model (de Zavalia et al., 2020). Therefore, there is evidence to believe that the knockout was successful. Since the current pandemic situation did not allow us to increase the number of animals in this study, some of the sample sizes per genotype, sex and time point were quite small ($n = 2$). Given our limited sample size, we did not remove any of the values from the analyses. Lastly, a potential confounding factor in female mice is the stage of the estrus cycle during which the tests were conducted (Schindler & Carmona, 2002). Even though smear samples were collected during testing, it was not possible to determine the estrus stage of female mice. As a result, we cannot rule out an effect specific to a particular phase of the estrus cycle.

Despite limitations this study may have some implications and future directions. Although we did not see substantial differences in anxiety- and depression-related phenotypes between genotypes at the behavioral level, further investigation is warranted to examine differences at the molecular level. Interestingly, *Per2* plays a crucial role in coordinating DA levels in the mesolimbic circuit via enzymatic activity (Hampp et al., 2008; Bussi et al., 2014). *Per2* mutant mice exhibited a blunted daily expression and reduced activity of MAOA, which resulted in higher levels of DA release and activity in the striatum (Hampp et al., 2008). Thus, future research could be devoted to elucidating the influence of *Per2* in mood-related behaviors and DA metabolism via enzymatic activity in our model. Also, the use of stress-induced paradigms can help determine if observable behavior differences can be reduced or attenuated. Moreover, the results of this study suggested a potential role of *Per2* in motor coordination. It is important to note that the conditional knockout of *Per2* does not completely compromise rhythmic clock output (Albrecht et al., 2007), suggesting that the effects seen in behavior cannot be ascribed to the clock or clock-controlled genes. Subsequent studies can shed light on the role of striatal clock gene in mediating circadian and motor abnormalities in motor-related or neurodegenerative disorders such as Parkinson's Disease.

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Appendix A: Summary Statistics and Analysis of Variance Source Table

Table A1

Means and standard deviations of % time spent in the open arms in males, 2x2 factorial design

Genotype	Time Point	*M*	*SD*	*N*
WT	ZT2	16.74	3.97	5
	ZT14	15.93	9.69	6
HET	ZT2	19.43	11.33	6
	ZT14	7.13	2.74	6
SKO	ZT2	23.73	20.36	3
	ZT14	9.63	6.70	3
Total	ZT2	19.39	11.18	14
	ZT14	11.15	7.74	15

Note. M & *SD* represent the mean and standard deviation of each genotype at each time point, respectively.

Table A2

Means and standard deviations of % time spent in the open arms in females, 2x2 factorial design

Genotype	Time Point	*M*	*SD*	*N*
WT	ZT2	19.50	7.88	5
	ZT14	27.07	16.66	3
HET	ZT2	14.40	5.87	4
	ZT14	2.95	1.06	2
SKO	ZT2	14.98	3.52	4
	ZT14	22.10	8.22	3
Total	ZT2	16.54	6.19	13
	ZT14	19.18	14.29	8

Note. M & *SD* represent the mean and standard deviation of each genotype at each time point, respectively.

Appendix A: Summary Statistics and Analysis of Variance Source Table

Table A3

Analysis of Variance Results for % Time Spent in the Open Arms, Males

Source	*SS*	*df*	*MS*	F	*p*	η^2
Genotype	71.28	2	35.64	0.385	.685	.024
Time Point	541.61	1	541.61	5.844	.024	.183
Genotype*Time Point	251.59	2	125.79	1.357	.277	.085
Error	2131.69	23	92.68			
Total model	9595.50	29				

Note. WT (*n* = 11), HET (*n* = 12) & SKO (*n* = 6)

Table A4

Analysis of Variance Results for % Time Spent in the Open Arms, Females

Source	*SS*	*df*	*MS*	F	*p*	η^2
Genotype	673	2	336.50	4.673	.026.	.349
Time Point	5.63	1	5.63	.078	.784	.003
Genotype*Time Point	343.99	2	171.99	2.389	.126	.179
Error	1080.12	15	72.01			
Total model	8388.26	21				

Note. WT (*n* = 8), HET (*n* = 6) & SKO (*n* = 7)

Table A5

Means and Standard Deviations in Open Arm Entries in Males, 2x2 factorial design

Genotype	Time Point	*M*	*SD*	*N*
WT	ZT2	9.80	4.55	5
	ZT14	10.33	6.22	6
HET	ZT2	13..33	4.63	6
	ZT14	9.33	4.68	6
SKO	ZT2	12	8.19	3
	ZT14	11.67	4.04	3
Total	ZT2	11.79	5.25	14
	ZT14	10.20	4.97	15

Note. M & *SD* represent the mean and standard deviation of each genotype at each time point, respectively.

Appendix A: Summary Statistics and Analysis of Variance Source Table

Table A6

Means and Standard Deviations in Open Arm Entries in Females, 2x2 factorial design

Genotype	Time Point	*M*	*SD*	*N*
WT	ZT2	15.60	5.03	5
	ZT14	12	4.58	3
HET	ZT2	17.50	5.32	4
	ZT14	7.50	0.71	2
SKO	ZT2	12.75	3.59	4
	ZT14	9.33	1.53	3
Total	ZT2	15.31	4.75	13
	ZT14	9.87	3.23	8

Note. M & *SD* represent the mean and standard deviation of each genotype at each time point, respectively.

Table A7

Analysis of Variance Results for Open Arms Entries, Males

Source	*SS*	*df*	*MS*	F	*p*	η^2
Genotype	20.18	2	10.09	0.075	0.927	.006
Time Point	24.59	1	24.59	0.185	0.672	.007
Genotype*Time Point	431.60	2	215.80	1.619	0.220	.118
Error	3066	23	28.67			
Total model	4210	29				

Note. WT (n = 11), HET (n = 12) & SKO (n = 6)

Table A8

Analysis of Variance Results for Open Arms Entries, Females

Source	*SS*	*df*	*MS*	F	*p*	η^2
Genotype	484.90	2	242.40	1.517	.251	.158
Time Point	7.03	1	7.03	0.044	.837	.002
Genotype*Time Point	197	2	98.52	0.616	.553	.064
Error	2397	15	159.80			
Total model	4170	21				

Note. WT (n = 8), HET (n = 6) & SKO (n = 7)

Appendix B: Analysis of Variance Source Table

Table B1

Analysis of Variance Results for Distance Traveled at ZT2, Males

Source	*SS*	*df*	*MS*	F	*p*	η^2
Within-subjects effect						
Interval	7237238	2.66	1447448	12.31	.000	.158
Interval x Genotype	705409	5.32	70541	0.600	.712	.015
Error	5652725	23.94	236101			
Between-subjects effect						
Genotype	13531675	2	7078964	4.526	.036	.308
Error	16784054	9	1563973			

Table B2

Analysis of Variance Results for Distance Traveled at ZT2, Females

Source	*SS*	*df*	*MS*	F	*p*	η^2
Within-subjects effect						
Interval	9110840	2.21	4118618	26.12	.000	.257
Interval x Genotype	937510	4.42	211903	1.344	.284	.026
Error	3487827	22.12	69756.50			
Between-subjects effect						
Genotype	3683654	2	1841827	1.037	.390	.104
Error	11765855	10	1776585.50			

Appendix B: Analysis of Variance Source Table

Table B3

Analysis of Variance Results for Distance Traveled at ZT14, Males

Source	*SS*	*df*	*MS*	F	*p*	η^2
Within-subjects effect						
Interval	18654068	2.11	8828120	33.84	.000	.349
Interval x Genotype	3107017	4.23	310701	2.818	.044	.058
Error	6615277	25.25	260892			
Between-subjects effect						
Genotype	8478778	2	4239389	2.687	.109	.159
Error	18930987	12	1577582			

Table B4

Analysis of Variance Results for Distance Traveled at ZT14, Females

Source	*SS*	*df*	*MS*	F	*p*	η^2
Within-subjects effect						
Interval	11106190	5	4123027	44.54	.000	.465
Interval x Genotype	793937	10	147369	1.592	.167	.033
Error	1246777	25	92569			
Between-subjects effect						
Genotype	4159695	2	2079847	1.847	.251	.174
Error	5631445	5	1126289			

Appendix B: Summary Statistics and Analysis of Variance Source Table

Table B5

Means and standard deviations of % time spent in the center in males, 2x2 factorial design

Genotype	Time Point	*M*	*SD*	*N*
WT	ZT2	5.56	3.17	5
	ZT14	16.04	5.01	6
HET	ZT2	10.65	5.06	6
	ZT14	16.99	9.78	6
SKO	ZT2	11.21	5.47	3
	ZT14	20.39	9.96	3
Total	ZT2	8.95	4.94	14
	ZT14	17.29	7.75	15

Note. M & *SD* represent the mean and standard deviation of each genotype at each time point, respectively.

Table B6

Means and standard deviations of % time spent in the center in females, 2x2 factorial design

Genotype	Time Point	*M*	*SD*	*N*
WT	ZT2	4.30	2.43	5
	ZT14	13.39	5.81	3
HET	ZT2	7.63	5.68	4
	ZT14	6.39	1.85	2
SKO	ZT2	3.67	1.22	4
	ZT14	7.92	3.51	3
Total	ZT2	5.45	4.00	13
	ZT14	9.59	4.89	8

Note. M & *SD* represent the mean and standard deviation of each genotype at each time point, respectively.

Appendix B: Summary Statistics and Analysis of Variance Source Table

Table B7

Analysis of Variance Results for % Time Spent in Center, Males

Source	*SS*	*df*	*MS*	F	*p*	η^2
Genotype	107.60	2	53.82	0.282	.757	.070
Time Point	494.90	1	494.90	11.060	.003	.298
Genotype*Time Point	25.25	2	12.64	1.203	.319	.015
Error	1029	23	44.75			
Total model	6765.22	29				

Note. WT (n = 11), HET (n = 12) & SKO (n = 6)

Table B8

Analysis of Variance Results for % Time Spent in Center, Females

Source	*SS*	*df*	*MS*	F	*p*	η^2
Genotype	28.26	2	14.13	1.128	.350	.080
Time Point	70.31	1	70.31	4.797	.045	.170
Genotype*Time Point	75.41	2	37.71	2.789	.093	.198
Error	207.35	15	17.28			
Total model	1344.57	21				

Note. WT (n = 8), HET (n = 6) & SKO (n = 7)

Appendix C: Analysis of Variance Source Table

Table C1

Analysis of Variance Results for % Time Spent in the Light Portion, Males

Source	*SS*	*df*	*MS*	F	*p*	η^2
Genotype	31.34	2	76.06	0.061	.941	.005
Time Point	15.60	1	15.60	0.061	.808	.003
Genotype*Time Point	152.10	2	15.67	0.296	.746	.025
Error	5911	23	257			
Total model	6110.04	29				

Note. WT (n = 11), HET (n = 12) & SKO (n = 6)

Table C2

Analysis of Variance Results for % Time Spent in the Light Portion, Females

Source	*SS*	*df*	*MS*	F	*p*	η^2
Genotype	596	2	298	2.464	.119	.185
Time Point	383.50	1	383.50	3.170	.095	.119
Genotype*Time Point	198.30	2	99.16	0.820	.459	.061
Error	1814	15	121			
Total model	2991.3	21				

Note. WT (n = 8), HET (n = 6) & SKO (n = 7)

Appendix C: Percentage of Time Spent in the Light Portion in L/D Box

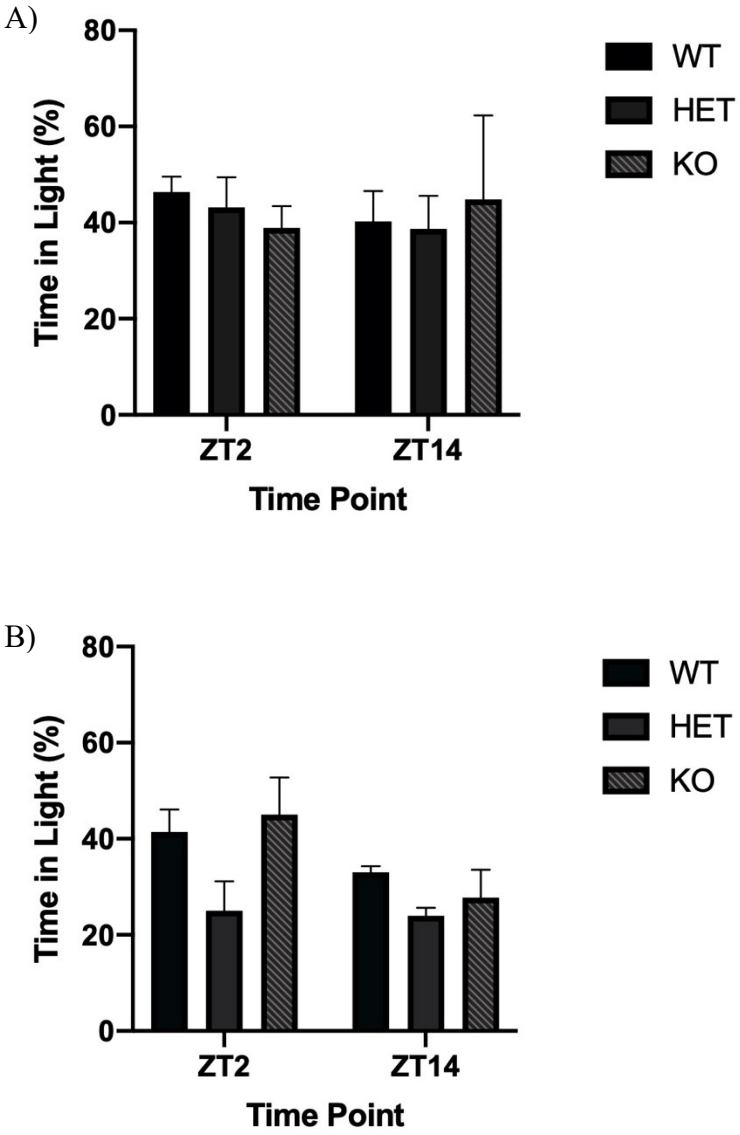


Figure 4. Percentage of time spent in the illuminated/light portion of the light/dark box test at two different time points (ZT2 & ZT14). A) In male mice, no differences were observed in the percentage of time spent in the illuminated area of the L/D test between WT (n = 11), HET (n = 12) & SKO (n = 6) across the two different time points. B) In females, no differences were observed in the outcome variable between WT (n = 8), HET (n = 6) & SKO (n = 7) across the two time points. The main effect of time point accounted for about 12% of the total variability in the outcome variable

Appendix D: Analysis of Variance Source Table

Table D1

Analysis of Variance Results for Number of Buried Marbles, Males

Source	*SS*	*df*	*MS*	F	*p*	η^2
Genotype	141.28	2	70.64	2.023	.155	.149
Time Point	.000	1	.000	.000	.999	.000
Genotype*Time Point	1.86	2	.931	.027	.974	.002
Error	803.33	23	34.93			
Total model	3989	29				

Note. WT (n = 11), HET (n = 12) & SKO (n = 6)

Table D2

Analysis of Variance Results for Number of Buried Marbles, Females

Source	*SS*	*df*	*MS*	F	*p*	η^2
Genotype	8.23	2	4.12	0.136	.874	.020
Time Point	0.65	1	0.65	0.021	.885	.001
Genotype*Time Point	54.27	2	27.13	0.899	.427	.104
Error	452.53	15	30.17			
Total model	2187	21				

Note. WT (n = 8), HET (n = 6) & SKO (n = 7)

Appendix E: Analysis of Variance Source Table

Table E1

Analysis of Variance Results for Time taken to Fall, Males

Source	*SS*	*df*	*MS*	F	*p*	η^2
Within-subjects effect						
Thickness	32.34	1	32.34	0.048	.829	.000
Thickness x Genotype	2042	2	1021	1.510	.240	.027
Error	17580	26	1976			
Between-subjects effect						
Genotype	2741	2	1371	0.694	.508	.040
Error	5211	26	676.20			

Table E2

Analysis of Variance Results for Time Taken to Fall, Females

Source	*SS*	*df*	*MS*	F	*p*	η^2
Within-subjects effect						
Thickness	3274	1	3274	3.851	.065	.122
Thickness x Genotype	1493	2	746.40	0.870	.433	.056
Error	5211	18	289.50			
Between-subjects effect						
Genotype	1758	2	878.80	3.035	.073	.065
Error	15303	18	850.10			

Appendix F: Analysis of Variance Source Table

Table F1

Means & Standard Deviations in Time Taken to Fall in Rotarod, Male Mice

	WT $N = 11$		HET $N = 12$		SKO $N = 6$	
	M	*SD*	*M*	*SD*	*M*	*SD*
Within-subjects						
2RPM	57.88	4.91	52.81	9.47	60	.00
4RPM	55.53	5.78	57.06	5.44	56.17	5.25
8RPM	51.62	10.23	51.28	13.04	50.63	10.47
12RPM	51.38	9.68	47.37	16.57	32.95	16.08
15RPM	40.44	15.61	29.03	22.36	14.25	11.73

Table F2

Analysis of Variance Results for Time taken to Fall, Males

Source	*SS*	*df*	*MS*	F	*p*	η^2
Within-subjects effect						
Speed	15175.06	2.57	5899.25	39.329	.000	.408
Speed x Genotype	2881.75	5.15	560.14	3.734	.004	.078
Error	10032	66.88	150			
Between-subjects effect						
Genotype	1451.59	2	725.79	2.021	.153	.135
Error	9337.33	26	359.13			

Table F3

Analysis of Variance Results for Time taken to Fall, Females

Source	*SS*	*df*	*MS*	F	*p*	η^2
Within-subjects effect						
Speed	10183.63	2.36	4314.17	20.260	.000	.367
Speed x Genotype	1949.49	4.72	412.94	1.939	.111	.070
Error	9047.38	42.49	212.93			
Between-subjects effect						
Genotype	468.45	2	234.22	.628	.545	.065
Error	6710.54	18	372.81			

Appendix G: Analysis of Variance Source Table

Table G1

Analysis of Variance Results for % Time Spent Immobile, Males

Source	*SS*	*df*	*MS*	F	*p*	r^2
Genotype	48.99	2	24.49	0.141	.869	0.011
Error	4524	26	174			
Total model	4573	28				

Note. WT (*n* = 11), HET (*n* = 12) & SKO (*n* = 6)

Table G2

Analysis of Variance Results for % Time Spent Immobile, Females

Source	*SS*	*df*	*MS*	F	*p*	r^2
Genotype	166.8	2	83.41	0.632	0.543	0.066
Error	2375	18	131.9			
Total model	2542	20				

Note. WT (*n* = 8), HET (*n* = 6) & SKO (*n* = 7)

Appendix H: Analysis of Variance Source Table

Table H1

Analysis of Variance Results for Distance Traveled (au), Males SKF

Source	*SS*	*df*	*MS*	F	*p*	η^2
Within-subjects effect						
Interval	1.36	1.87	0.72	1.687	.210	.048
Interval x Genotype	2.67	3.75	0.71	1.662	.119	.094
Error	8.84	20.60	0.43			
Between-subjects effect						
Genotype	8.93	2	4.47	7.960	.007	.316
Error	6.17	11	0.56			

Table H2

Analysis of Variance Results for Distance Traveled (au), Males Saline

Source	*SS*	*df*	*MS*	F	*p*	η^2
Within-subjects effect						
Interval	2.28	1.87	0.38	9.302	.000	.215
Interval x Genotype	0.96	3.18	0.08	1.963	.096	.097
Error	2.94	38.26	0.04			
Between-subjects effect						
Genotype	1.51	2	0.76	2.973	.093	.143
Error	3.09	12	0.26			

Table H3

Analysis of Variance Results for Distance Traveled (au), Females SKF

Source	*SS*	*df*	*MS*	F	*p*	η^2
Within-subjects effect						
Interval	1.41	1.87	0.23	5.927	.013	.184
Interval x Genotype	0.58	1.88	0.05	1.230	.292	.076
Error	1.89	15.08	0.04			
Between-subjects effect						
Genotype	0.52		0.26	0.667	.539	.070
Error	3.13		0.39			

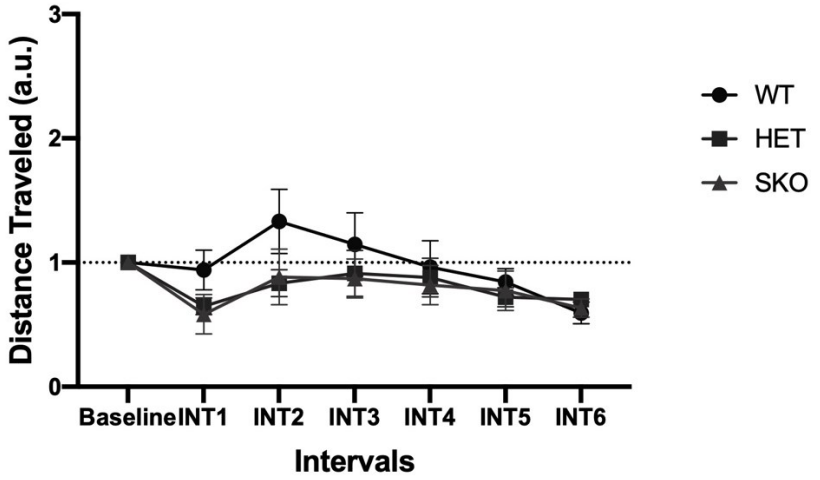
Appendix H: Analysis of Variance Source Table & SKF in female mice

Table H4

Analysis of Variance Results for Distance Traveled (au), Females Saline

Source	*SS*	*df*	*MS*	F	*p*	η^2
Within-subjects effect						
Interval	1.77	1.80	0.29	3.528	.051	.176
Interval x Genotype	0.28	2.20	0.02	0.281	.989	.076
Error	3.51	15.41	0.08			
Between-subjects effect						
Genotype	0.44	2	0.22	0.376	.699	.043
Error	4.05	7	0.58			

A)



B)

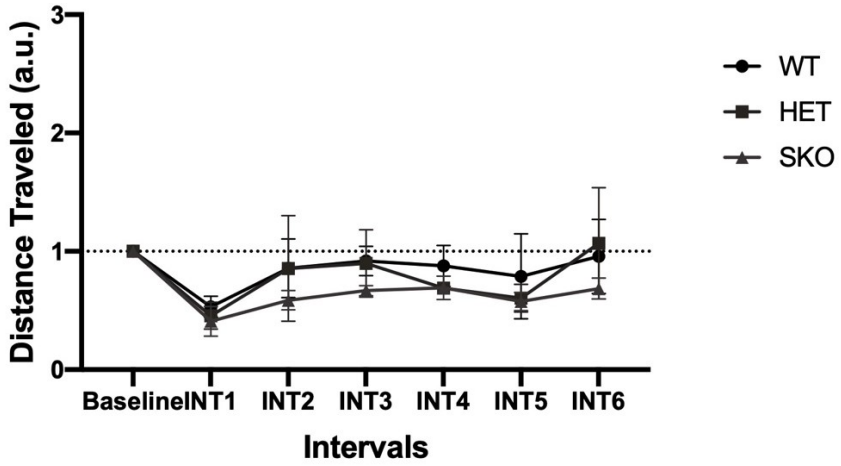


Figure 7. Distance traveled (normalized to baseline activity) in the open field between genotypes after saline or SKF injection in female mice. A) After SKF injection, no statistically significant interaction was observed between genotype across intervals. B) No differences were observed in the outcome variable after saline injection. Error bars represent the standard error of the mean.

Appendix I: Tail Suspension Test Results

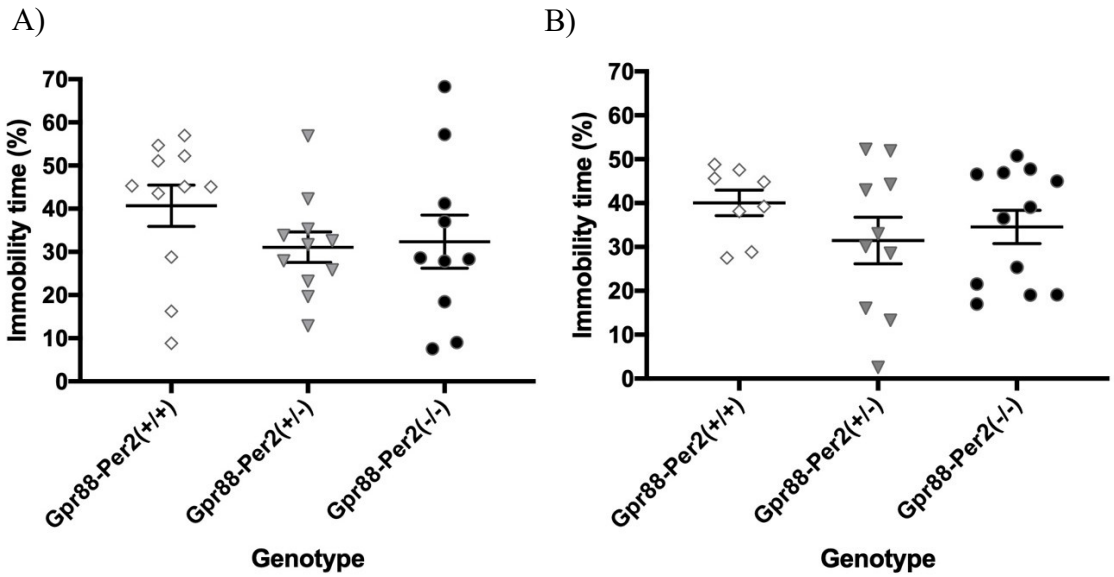


Figure 9. Percentage of time spent immobile in the tail suspension test. A) In male mice, no statistically significant differences were observed in depressive-like behaviors between genotypes. B) Regarding female mice, no significant differences were observed in the outcome variable between genotypes. Error bars depict the standard error of the mean.