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Growth Hormone Secretagogue Receptor (GHS-R1a) Knockout Mice Exhibit Improved Spatial Memory and Deficits in Contextual Memory

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Abstract

Although the hormone ghrelin is best known for its stimulatory effect on appetite and regulation of growth hormone release, it is also reported to have beneficial effects on learning and memory formation in mice. Nevertheless, controversy exists about whether endogenous ghrelin acts on its receptors in extra-hypothalamic areas of the brain. The ghrelin receptor (GHS-R1a) is co-expressed in neurons that express dopamine receptor type-1 (DRD1a) and type-2 (DRD2), and we have shown that a subset of GHS-R1a, which are not occupied by the agonist (apo-GHSR1a), heterodimerize with these two receptors to regulate dopamine signaling *in vitro* and *in vivo*. To determine the consequences of *ghsr* ablation on brain function, congenic *ghsr*^{-/-} mice on the C57BL/6J background were subjected to a battery of behavioral tests. We show that the *ghsr*^{-/-} mice exhibit normal balance, movement, coordination, and pain sensation, outperform *ghsr*^{+/-} mice in the Morris water maze, but show deficits in contextual fear conditioning.

1. Introduction

The growth hormone secretagogue receptor (GHS-R1a) was initially identified by expression cloning using a synthetic agonist (MK-0677) that reversed aging of the growth hormone axis [1] [2]. Three years later this orphan G-protein coupled receptor was deorphanized by the discovery of the 28 amino acid octanoylated peptide ghrelin [3]. Presently, GHSR1a is the only known receptor for ghrelin, and is expressed in multiple tissues including pituitary gland, pancreas, brain and thymus [4–8]. Activation of the GHS-R1a by ghrelin stimulates feeding and growth hormone release among other functions [9]. Naturally occurring mutations of the GHS-R1a in humans have been connected to growth disorders, metabolic syndrome and obesity [10–13]. For instance, four mutations in the GHS-R1a have been found in a short stature Japanese population; at least one of these mutations resulted in a non-functional receptor and in two others a decreased response of the

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receptor to ghrelin [11]. Given ghrelin's role in regulation of glucose homeostasis and that GHS-R1a is co-expressed with dopamine receptors (DRD1a and DRD2) in the brain [8, 14], it is important to know if deletion of GHS-R1a is associated with altered behavior. Recent studies have connected metabolic disorders and diabetes with deficits in learning and memory [15, 16].

Diano, et al. showed that ghrelin treatment increased LTP in hippocampal slices from wildtype (WT) mice, suggesting that ghrelin promotes learning & memory by activating hippocampal neurons directly [17]. Indeed, they showed that exogenous ghrelin rescued deficits shown by *ghrelin*^{-/-} mice in a novel object recognition test. In addition, they showed that ghrelin treatment increased performance of rats and wildtype mice in multiple hippocampus-dependent learning and memory tests [17]. These results suggest that GHS-R1a agonists could serve as therapeutic agents for memory loss that accompanies aging as well as Parkinson's and Alzheimer's diseases. However, the ability of ghrelin to cross the blood brain barrier (BBB) when given systemically is controversial [18–20]. Diano and coworkers demonstrated that radioactive ghrelin (¹³¹I-Ghr) injected directly into the jugular vein reached hippocampal neurons [17]. However, in their learning and memory tasks, ghrelin was delivered by constant infusion using “subcutaneous mini-pumps” or intracerebroventricularly (icv) rather than intraperitoneally. These results could be misleading since at saturating concentrations (like the ones reached by the mini-pumps), ghrelin could cross the BBB through alternative transporter systems that exist for other peptides and hormones such as leptin [18, 19]. In addition, the GHS-R1a was reported by Holst, et al. to have constitutive activity *in vitro*, which has been hypothesized to be sufficient for its regulatory actions in the brain [21]. Additional studies indicate that apo-GHSR1a allosterically modifies dopamine signaling [14]. Taken together, these studies indicate that ghrelin availability might be less relevant in areas like the hippocampus and midbrain. Conversely, deficits in receptor activity could cause marked functional impairment.

GHS-R1a likely serves as a modifier of key neurotransmitters required for memory and learning formation such as glutamate and dopamine. Mechanisms of memory and learning formation involve activation of several receptors and cellular pathways by these neurotransmitters [22]. As a consequence of this activation, the transcription factor cAMP response element-binding (CREB) is turned on, which then induces the transcription of genes necessary for the learning process [23]. Since ghrelin, through the GHS-R1a, has been shown to enhance dopamine-induced cAMP accumulation *in vitro*, but not activate it directly, it is likely that the GHS-R1a has a modulatory role rather than a direct role on memory formation [8]. In this study, we examined the effects of *ghsr*^{-/-} on learning and memory. First, we conducted a thorough behavioral characterization of the congenic *ghsr*^{-/-} mouse using a standard phenotyping test battery. We then tested the mice in the Morris Water Maze task to look for effects on spatial learning and in a modified contextual fear conditioning protocol that would be sensitive to subtle effects of GHS-R1a deletion; consistent with the hypothesis that GHS-R1a has a modulatory role in learning and memory.

2. Material and Methods

2.1 Mice

The *ghsr*^{-/-} mice used in this study were created by our laboratory and backcrossed to C57BL/6J for at least 12 generations [9]. Mice were housed by genotype in groups of 3 to 5 mice per cage in standard laboratory conditions of 12:12 light:dark cycle. All behavioral tests were performed during the light period. Regular chow (Harlan 2920X) and water were available *ad libitum*, except during behavioral testing. All procedures were approved by the Scripps Florida Institutional Animal Care and Use Committee (IACUC) and were consistent

with the guidelines outlined in the NIH “Guide for the Care and Use of Laboratory Animals.”

All mice were handled once a day for three days prior to the start of behavioral testing in order to acclimate them to the experimenters. Handling included transportation of all mice in their home cage into the behavior core facility. On the morning of each day of behavioral testing, animals were moved from their holding room to the behavior core facility 30 minutes before starting experiments. At the conclusion of testing each day, mice were returned to their holding room.

Investigators were blind to the genotype of the mice throughout the duration of the test battery. The same cohorts of mice were used for all experiments (n = 9 per group). Mice were 6 months old at the beginning of the battery and 9–10 months old by the end. Experiments were performed in the order presented in this report and rest time was given between paradigms.

2.2 Rota-rod

The Rota-rod test was used to measure motor coordination and balance in *ghsr*^{-/-} and *ghsr*^{+/+} mice, as described previously [24]. To summarize, five mice were tested simultaneously on an accelerating Rota-rod (Med-Associates, St. Albans, VT) set to accelerate from 4 to 40 rpm over five minutes. Mice were tested for 4 trials with an inter-trial interval of 30 minutes. A beam break occurred when a mouse fell from the rotating rod, signaling the timer to stop automatically. The time and maximum rotational speed when an animal fell from the rod was recorded. A mouse was removed from the rotating rod if it clung to the rod and completed two “full passive rotations” [25]; the time and the speed at removal were recorded. Trials were averaged for data analysis.

2.3 Hot plate test

Nociception was measured using the hot plate test. The hot plate (Ugo Basile, Collegeville, PA) temperature was automatically controlled and set to 55°C. In addition, the hot plate had a removable clear acrylic cylinder surrounding the plate area. Mice were individually placed onto the hot plate, and latency to show a nociceptive response was manually recorded with a button press. The hot plate and acrylic cylinder were cleaned with 70% ethanol in between animals and verified that was dried before placing a new animal. A nociceptive response was defined as licking the rear paws (licking the front paws is a normal grooming activity), jumping and/or leaning of the body towards one side with one paw lifted in the air and served as a correlation of pain sensitivity or analgesia [26]. Mice were removed after 30 seconds or when they showed a nociceptive response, whichever came first (although all mice showed signs of nociception before the cutoff period).

2.4 Open field: Activity and Anxiety-like Behavior

To test for baseline activity, locomotor behavior was measured in 17 × 17 inch square acrylic open field chambers. Prior to testing, uniformity of light across the arena was confirmed using a light intensity meter, and the chambers were cleaned with 70% ethanol before and between trials. Mice were placed into the center of the chamber to begin testing, and activity was recorded for 30 minutes. Data were analyzed in 10 minute blocks.

Activity during the first five minutes of the open field test was analyzed for differences in anxiety-like behavior. Thigmotaxis, or the tendency of a mouse to avoid the center of the open field (where ‘fear of predation’ potentially exists) and instead stay close to the sides, was measured as percent of time spent in the center of the open field [27, 28]. Latency to exit the center of the open field at the start of the test was also measured.

2.5 Morris Water Maze

The Morris Water Maze (MWM) was performed similarly to that previously described [29, 30]. Briefly, the water maze consisted of a 48-inch diameter white tank with a four-inch diameter platform submerged 0.75 cm below the surface of the water. The water was made opaque by adding non-toxic white tempera paint so that the platform was not visible during trials, and the temperature of the water was kept at 23°C with a heater. Visual cues were placed in the testing room around the tank for spatial reference.

Mice received a visual platform test where the spatial cues were removed and the platform elevated above the surface of the water and colored black so it could clearly be discerned. They were given four trials and the platform location was varied over trials. This served to verify the visual ability of the mice and to ensure that the mice had no deficits that would affect their ability to swim to the platform (no deficits were observed, data not shown). For the hidden platform test, mice were given four acquisition trials per day for seven consecutive days. The start location was varied for each trial, and mice were allowed 60 seconds to find the platform. Mice were left on the platform for 15 seconds before removing them from the water maze. If the mouse did not find the platform within 60 seconds, it was placed on or guided to the platform and kept there for 15 seconds. Mice were dried with a soft towel after each trial and placed into cages located atop a hot water heating plate to keep them warm, and an inter-trial interval of 20 minutes was used to space the acquisition trials and prevent hypothermia effects. Daily acquisition trials were averaged for analysis. Following acquisition, mice were given two probe trials, one at 24 hours and one at seven days post-training, during which the platform was not present. Total time spent in each quadrant and the number of entries into the target quadrant (northwest) was recorded.

2.6 Fear Conditioning

Because gross deficits in learning and memory were not expected (based on the results of the MWM test, see results), a mild contextual fear conditioning protocol was used in order to discern subtle genotypic effects. The fear conditioning chambers (Noldus Information Technology, Leesburg, VA) have been previously described [31]. Each chamber was cleaned with 70% ethanol prior to each trial. White light was used inside the chamber for training and testing, and white noise was played in the room to mask any unintended noise that might add to the context. Mice were placed individually into each chamber and allowed to explore it for two minutes. Mice then received a single two-second 0.75mA foot shock, and 30 seconds following the foot shock the mice were removed from the chambers and returned to their home cages. 24 hours later, mice were tested for contextual fear conditioning by placing them back into the chambers for five minutes. Percent of time spent freezing (immobility except for breathing) was recorded. Mice received a second fear-conditioning test 30 days after training, and again percent freezing was recorded.

2.7 Analysis and Statistics

Open field, water maze, and fear conditioning experiments were all conducted using the EthoVision XT 7.1 video tracking system (Noldus Information Technology, Leesburg, VA). Planned comparisons with two-tailed student's T-test were used across all experiments to determine statistical significance between genotypes ($p < 0.05$). One-way ANOVA were used to analyze open field locomotion activity over blocks of time and water maze acquisition over seven days ($p < 0.05$), both within groups to show an effect of time in activity and latency to find platform respectively. Prism software was used to analyze all data (GraphPad Software, Inc., La Jolla, Ca).

3. Results

3.1 Hot plate test and Rotarod

The ability to respond to a painful stimulus (nociception) was measured in the hot plate test. We find *ghsr*^{-/-} and *ghsr*^{+/+} mice are indistinguishable in nociceptive response ($t_{18}=2.04$, $p>0.05$) (Figure 1A). Similarly, there are no differences between genotypes in latency to fall from an accelerating rotarod ($t_{18}=0.07$, $p>0.05$) (Figure 1B). From these observations, we can conclude that deletion of the GHS-R1a does not result in deficits in motor skill or nociception, an important pre-requisite to ensure subsequent behavioral testing is not skewed by differences in motor control and/or pain sensation.

3.2 Locomotor activity in open field

To assess baseline activity and differences in anxiety-like behavior we utilized the open field test. Total horizontal activity was measured as distance moved (cm) over a 30 minute period. Results are presented and analyzed in 10-minute blocks. *Ghsr*^{-/-} and *ghsr*^{+/+} mice show similar levels of activity during the first 10-minute interval, indicating that ablation of the GHS-R1a does not interfere with motivation to explore a novel environment (Figure 2). A One-Way ANOVA reveals that both groups decrease activity during the 30 minute test ($F_{2,36}=13.84$, $p<0.05$), as reported previously for C57BL/6J mice [32] [33]. However, this reduction in activity over time is greater for *ghsr*^{-/-} mice during the 10–20 minutes ($t_{18}=2.11$, $p<0.05$) and the 20–30 minute ($t_{18}=2.94$, $p<0.01$) blocks when compared to *ghsr*^{+/+} (Figure 2). This effect is not explained by sedation or freezing behavior, suggesting that *ghsr*^{-/-} mice habituate more quickly to a novel environment.

3.3 Anxiety-like behavior in open field

To test for differences in anxiety-like behavior between *ghsr*^{-/-} and *ghsr*^{+/+}, we analyzed time spent in the center of the open field during the first five minutes of the open field test (Figure 3A). Mice have shown a tendency to spend more time closer to the edges of the open field compared to the center, perhaps because the center of the open field represents an exposed site to 'predation' [27]. Thus, latency to first leave the center and total time spent in the center is used as a measure of anxiety-like behavior. We observe no differences between genotypes in the time spent in the center of the open field ($t_{18}=1.48$, $p>0.05$), suggesting that deletion of GHS-R1a does not affect this behavior (Figure 3A), however *ghsr*^{-/-} mice display a significantly shorter latency to initially leave the center compared to *ghsr*^{+/+} mice ($t_{18}=2.17$, $p<0.05$) (Figure 3B). Such behavior is usually interpreted as anxiety-like.

3.4 Morris Water Maze

The Morris water maze (MWM) is often used to measure spatial learning and memory in rodents, based on the principle that escaping from the water is a motivation and a positive reinforcement for mice [34]. Our testing reveals latency to find the platform to generally decrease in *ghsr*^{-/-} and *ghsr*^{+/+} groups over the 7-day acquisition period ($F_{6,108}=11.18$, $p<0.01$). However, on day four *ghsr*^{+/+} mice take longer to find the platform compared to *ghsr*^{-/-} mice (Figure 4A). This result is intriguing because on this day, a new start location, further from the platform than the previous one, was introduced. However, this difference disappears over subsequent acquisition days and both groups show a gradual decrease in latency, indicating that mice learn to find the hidden platform with each trial. Similarly, both groups showed a decrease in total distance traveled over the first 3 training days (Figure 4B). Traveled distance was stable for the last 3 days of the training period, indicating that mice were ready for probe trials.

Following the acquisition phase of the test, animals were subjected to two probe trials. The first probe trial, performed 24 hours following the acquisition phase, reveals similar time

spent in the target quadrant for both genotypes (northwest) ($t_{18}=1.23$, $p>0.05$) (Figure 5A). The second probe trial, performed one week later, assess 'lasting' long-term memory. On this day, *ghsr*^{-/-} mice spend significantly more time in the target quadrant, compared to *ghsr*^{+/+} ($t_{18}=2.66$, $p<0.05$) (Figure 5B). Frequency in platform crossings was also analyzed. There were no differences between groups in platform crossings at either probe trial (data not shown). However, latency to cross platform for the first time was significantly shorter for *ghsr*^{-/-} than *ghsr*^{+/+} (data not shown). These results suggest lasting spatial memory is improved in *ghsr*^{-/-} mice, as compared to *ghsr*^{+/+} mice, which spent approximately less than 25% time (chance) in the target quadrant.

3.5 Fear Conditioning

To further assess the effects of GHS-R1a deletion on learning and memory, a contextual fear-conditioning paradigm was used. We analyzed the pre-shock and post-shock freezing percentages. There were no differences between groups during pre-shock ($t_{18}=1.34$, $p>0.05$) and post-shock ($t_{18}=1.16$, $p>0.05$). As expected, both groups showed significantly higher post-shock freezing percentages than pre-shock percentages (wildtype $t_{20}=4.46$, $p<0.05$ and *ghsr*^{-/-} $t_{16}=4.32$, $p<0.05$). Similarly, no differences are observed between genotypes in freezing response during the 5-minutes test performed 24 hour post-training, however, 30 days later, *ghsr*^{-/-} mice show significantly less freezing at particular time points when freezing is analyzed by minutes of the test ($t_{18}=2.33$, $p<0.05$ for minute 2) ($t_{18}=2.18$, $p<0.05$ for minute 3) although they showed no significant difference overall (Figure 6). These results suggest that *ghsr*^{-/-} mice have an impaired memory in this model of mild contextual training 30 days after foot shock delivery compared to *ghsr*^{+/+}.

3.6 Weight

Body weights were measured at the end of the test battery. As reported previously [9], *ghsr*^{-/-} mice weighed significantly less than *ghsr*^{+/+} ($t_{18}=2.34$, $p<0.05$) (Figure 7).

4. Discussion

Ghrelin and GHS-R1a signaling are believed to play important roles in behavior; thus, it was critical to analyze the consequences of deletion of this receptor in learning and memory and to provide a detailed behavioral characterization of the *ghsr*^{-/-} mice created in our lab [9]. These mice perform identically to *ghsr*^{+/+} mice in both the rotarod and hot plate tests, suggesting normal locomotion, balance, coordination and nociception in the absence of GHS-R1a. Moreover, cerebellar function appears intact in *ghsr*^{-/-} mice, since the rotarod test mostly depends on the cerebellum [35]. When exposed to a novel open field, *ghsr*^{-/-} mice are neither hyperactive nor frozen, demonstrating that deletion of the GHS-R1a does not affect motivation and exploratory behaviors. Nevertheless, *ghsr*^{-/-} mice display significantly attenuated locomotor activity over time compared to *ghsr*^{+/+} mice in the open field, which suggests these animals habituate to the environment more quickly than control mice. In a previous study, neither *ghsr*^{-/-} or *ghrelin*^{-/-} mice showed differences in locomotor behavior when compared to wildtype mice [36]. However, to measure locomotion activity they used an open field setup with a grid created by intersecting laser beams and recorded beam disruptions instead of tracking constant activity by video recording. Thus, differences in these results could be explained by different experimental setups. In addition, *ghsr*^{-/-} mice initially move out of the center of the open field in significantly shorter time than *ghsr*^{+/+}, however, both genotypes spend similar times in the center of the open field. Thus, *ghsr*^{-/-} mice seem to show increased anxiety-like behavior initially (Figure 3A), but then display greater habituation to the novel environment over the entire testing period (Figure 2).

Here we show that *ghsr*^{-/-} mice have improved spatial memory in the water maze and a deficit in contextual memory in the fear conditioning. These results were surprising because Abizaïd *et al* showed that ghrelin has been shown to improve memory in rodents [4] and, *ghsr*^{-/-} mice showed deficits in water maze in separate studies performed in different line of *ghsr*^{-/-} mice [37] [38]. To decrease probability of gene expression originating from the donor strain, our mice have been backcrossed >12 times [39], thus genetic background may account for some of the discrepancies observed between studies [35, 40, 41]. Backcrossing the animals for multiple generations is important because the majority of embryonic stem cells used for gene recombination and creation of knockout and transgenic mice come from the 129Sv mouse strain and genes flanking the disrupted gene are likely to recombine as well [39] [42], which leads to incongruent results in behavioral studies between laboratories, even when the same gene has been deleted [35, 42, 43]. Indeed, deficits in water maze have been observed for 129Sv mouse strain [43].

Mouse age is another important factor to consider in behavior studies. Studies that compared 8–12 month old to 3 month old wildtype C57Bl/6 mice have shown deficits in older mice in rotarod and water maze, but not fear conditioning [41] [44, 45]. In our study, mice were 10 month old during the water maze testing and 11 month old for fear conditioning. Thus, age could also explain part of the discrepancies between studies.

A probe trial with a visual platform does not reveal deficits in vision (data not shown). *Ghsr*^{-/-} mice clearly outperform *ghsr*^{+/+} mice in this test a week after the acquisition period. Our *ghsr*^{+/+} mice actually spent less than 25%, or chance, of the test in the target quadrant showing a deficit in remembering the exact platform location. Performance of *ghsr*^{-/-} mice in both the MWM and open field is consistent with better spatial memory. Hippocampal neurons known as 'place cells' have been shown to fire in specific patterns as mice explore novel environments, such as the open field, creating a 'cognitive spatial map' [46]. Thus, we suggest that the significant decrease in locomotor activity shown by *ghsr*^{-/-} mice could be explained by the ability to create stronger spatial memories more quickly than *ghsr*^{+/+}. In addition, the MWM task is dependent on an intact dorsal hippocampus, whereas, contextual fear conditioning is dependent on an intact ventral hippocampus [35]. Because *ghsr*^{-/-} mice showed deficits in fear conditioning 30 days after training, we speculate that GHS-R1a is more important in the ventral hippocampus than in the dorsal hippocampus. Further experiments will be necessary to clarify whether the presence of the GHS-R1a is more relevant in certain parts of the brain than others.

Multiple studies have linked a deficit in spine number with a deficit in learning and memory [47, 48]. In a recent article, two different mouse models of Alzheimer's disease, the TG2576 and APP/LO, showed a decrease in hippocampal spines in conjunction with impaired responses in fear conditioning and water maze tests, respectively [47]. Interestingly, Diano et al. showed that *ghrelin*^{-/-} mice had fewer numbers of spines compared to wildtype mice and ghrelin treatment rescued this phenotype [17]. In addition, they showed that ghrelin treatment increased the number or density of spines in the CA1 area of the hippocampi of wildtype mice [17]. Thus, it would be interesting to study spine density in the *ghsr*^{-/-} mice during aging and determine any correlation with the observed changes in performance in the MWM and fear conditioning.

Our results indicate that deletion of GHS-R1a has opposing regulatory effects on learning and memory. While spatial memory was improved in the *ghsr*^{-/-} mice, contextual memory was impaired by the lack of this receptor. One plausible explanation is that ghrelin is acting as a modulator of other neurotransmitters such as glutamate or dopamine. Numerous studies have shown that dopamine can regulate synaptic plasticity, memory and learning [49, 50]. Of particular relevance, studies have shown that antagonism of dopamine receptor type-1

(D1R) in the hippocampus blocks formation of long-term memory and that blockage of D1R signaling transmission in the pre-frontal cortex (PFC) impairs working memory [51, 52]. In addition, dopamine receptor type-1a knockout mice show deficits in contextual fear conditioning 24 hours after training [50]. Furthermore, Rossato et al. showed that in the hippocampus, D1R activation of cAMP and PKA is required for long-term memory acquisition in a step-down avoidance test [52]. Preliminary studies in our laboratory are showing that ghrelin augments dopamine-induced cAMP mediated signaling and transcription in a neuroblastoma cell line expressing both D1R and GHS-R1a (unpublished observations). Moreover, recently published data from our laboratory show that GHS-R1a is required for DRD2-induced feeding suppression in mice [14]. It is thus very likely that there is similar importance of the GHS-R1a for effects of dopamine signaling *in vivo* on learning and memory.

In conclusion, our results show evidence of GHS-R1a requirement in contextual memory. In addition, we show that ablation of GHS-R1a may help in acquisition of spatial memory in the open field and Morris water maze. Our study is further proof of the many actions that ghrelin and GHS-R1a have in the brain besides regulation of energy metabolism.

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Highlights

- *Ghsr*^{-/-} mice showed a deficit in fear conditioning 30 days after training vs wt.
- *Ghsr*^{-/-} mice spent more time in target quadrant of the water maze vs wt.
- *Ghsr*^{-/-} mice habituated faster to novel environments.
- Performance in the rotarod and hot plate test were not different between genotypes.

Figure 1.

Ghsr -/- and *ghsr* +/+ mice display normal pain sensation and motor coordination. A) Mice were placed on a hot plate set to 55°C and removed when signs of discomfort were shown. The mean latency to nociceptive response is shown for each group ($p > 0.05$). B) Mice were placed on a Rota-rod accelerating from 4 to 40 rpm over 5 minutes. Four trials were averaged for each mouse. The mean latency to fall from the rotating rod is shown for each group ($p > 0.05$).

Figure 2.

Ghsr -/- mice habituated faster to novel environments. Both *ghsr* -/- and *ghsr* +/+ mice showed reduction in total locomotor activity over time in the open field, *ghsr* -/- showed a greater reduction compared to *ghsr* +/+ at time points 2 and 3 (t-test * $p < 0.05$; ** $p < 0.01$).

Figure 3.

Ghsr^{-/-} mice show anxiety-like behavior. A) Percentage of time spent in the center of the arena during 0–5 min in open field. B) *Ghsr*^{-/-} mice translated from center to sides of the arena in significantly less time than *ghsr*^{+/+} (**p*<0.05 compared to *ghsr*^{+/+}).

Figure 4. *Ghsr*^{-/-} and *ghsr*^{+/+} mice performed similarly during acquisition in the Morris water maze. A) Both genotypes showed similar decline in latency time to find the hidden platform. B) Both genotypes swam similar distances towards platform.

Figure 5.

Ghsr -/- performed better than *ghsr* +/+ in the Morris water maze 7 days after acquisition. A) Both groups spent similar amount of time in the target quadrant 24 hours after acquisition (probe trial 1), ($p > 0.05$). B) *Ghsr* -/- mice spent significantly more time in the target quadrant than *ghsr* +/+ mice (* $p < 0.05$ compared to *ghsr* +/+).

Figure 6.

Ghnr^{-/-} mice displayed a deficit in 'lasting' contextual fear-conditioning. In both figures, 0–5 minutes represent the total percent freezing during the test. Additional bars depict the data in 1-minute intervals. A) The percent freezing response was similar for both genotypes on the first test performed 24 hours after training. B) *Ghnr*^{-/-} mice percent freezing was significantly lower during minutes 2 (**p*<0.05) and 3 (**p*<0.05) of the second test, performed 30 days after training.

Figure 7.
Ghsr^{-/-} mice weigh less than *ghsr*^{+/+} mice (*p<0.05).