


# Obesity Impairs Mobility and Adult Hippocampal Neurogenesis

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Journal of Experimental Neuroscience  
Volume 13: 1–10  
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DOI: 10.1177/1179069519883580



**ABSTRACT:** Currently, it is controversially discussed whether a relationship between obesity and cognition exists. We here analyzed a mouse model of obesity (leptin-deficient mice) to study the effects of obesity on the morphology of the hippocampus (a brain structure involved in mechanisms related to learning and memory) and on behavior. Mice aged 4 to 6 months were analyzed. At this age, the obese mice have nearly double the body weight as controls, but display smaller brains (brain volume is about 10% smaller) as control animals of the same age. Adult hippocampal neurogenesis, a process that is linked to learning and memory, might be disturbed in the obese mice and contribute to the smaller brain volume. Adult hippocampal neurogenesis was examined using specific markers for cell proliferation (phosphohistone H3), neuronal differentiation (doublecortin), and apoptosis (caspase 3). The number of phosphohistone H3 and doublecortin-positive cells was markedly reduced in leptin-deficient mice, but not the number of apoptotic cells, indicating that adult hippocampal neurogenesis on the level of cell proliferation was affected. In addition, dendritic spine densities of pyramidal neurons in the hippocampal area CA1 were analyzed using Golgi impregnation. However, no significant change in dendritic spine densities was noted in the obese mice. Moreover, the performance of the mice was analyzed in the open field as well as in the Morris water maze. In the open field test, obese mice showed reduced locomotor activity, but in the Morris water maze they showed similar performance compared with control animals.

**KEYWORDS:** Leptin, mice, obesity, neuronal plasticity, Morris water maze, hippocampus

**RECEIVED:** August 15, 2019. **ACCEPTED:** September 24, 2019.

**TYPE:** Original Research

**FUNDING:** The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by a grant from the European Regional Development Fund (ERDF; UHGWM 21). The authors acknowledge the support for article processing charge from the DFG (German Research Foundation; 393148499) and the Open Access Publication Fund of the University of Greifswald.

**DECLARATION OF CONFLICTING INTERESTS:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Introduction

According to the estimates of the World Health Organization (WHO; <https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight>), about 2 billion of the world's population in 2016 were classified as overweight and more than 650 million of these were obese based on their body mass index (BMI).

In many countries of the world, obesity is a major health issue because it contributes to premature deaths by increasing the risk of diabetes mellitus type II, cardiovascular disease, and some forms of cancer.<sup>1,2</sup> Among others, a relationship between obesity and cognitive performance in humans has been described. A higher BMI was found to be associated with lower cognitive scores and a higher BMI at baseline was found also to be associated with a higher cognitive decline at follow-up.<sup>3</sup> In general, overweight and obese adults (BMI > 25) exhibited poorer executive function test performance than normal-weight adults, but the correlations were mainly modest in size.<sup>4</sup> Extremely obese individuals (BMI ≥ 40) performed poorly on a number of neuropsychological tests, indicating that the severity of obesity may have an impact on cognitive functions.<sup>5</sup>

Interpreting and comparing different studies in humans is highly challenging, because most studies used different methodological approaches and also the parameters the participants are controlled for, such as age, sex, educational level, or comorbidities, eg, diabetes mellitus, are not standardized. Diabetes may play important roles, especially in extremely obese individuals. Based on clinical, epidemiological, and experimental studies, a causative association between diabetes (diabetes mellitus type I as well as type II) and cognitive impairment has been suggested (see Riederer et al<sup>6</sup> for review).

To study the effects of obesity on brain morphology and brain function, leptin-deficient mice (termed ob/ob; ob = obese) or leptin-receptor-deficient mice (termed db/db; db = diabetic) are established animal models. Both mouse lines are characterized by a strong weight gain within the first postnatal months. As they grow up, the leptin-receptor-deficient mice (db/db) develop a permanent form of diabetes mellitus, whereas the leptin-deficient ob/ob mice do not.<sup>7</sup> Mouse models based on deficits in the leptin pathway, including ob/ob mice and db/db mice, as well as pro-opiomelanocortin (POMC)-deficient mice, or MCR3 or MCR4 knockout mice, display a variety of



phenotypic manifestations, including hyperphagia, decreased energy expenditure, hyperglycemia, or insulin resistance.<sup>7</sup> In contrast to, eg, the diabetic db/db mice, the blood glucose concentrations were not increased in the ob/ob mice, but the plasma insulin levels.<sup>8</sup> To avoid an impact of diabetes mellitus on brain morphology and/or function, we used the ob/ob mouse strain to analyze the effects of obesity on neuronal plasticity and hippocampal learning and memory. We focused on adult hippocampal neurogenesis, dendritic spine densities in hippocampal area CA1, and the behavior of the obese mice in the open field (OF) and Morris water maze (MWM).

## Materials and Methods

### Animals

The ob/ob mice as well as wild-type littermates (+/+) were generated by crossing of heterozygous B6.V-Lep<sup>ob</sup>/J (+/ob) animals, because homozygous female mice are infertile and male mice show reduced fertility. Mice were housed and bred in the “Zentrale Service- und Forschungseinrichtung für Versuchstiere” (ZSFV) at Universitätsmedizin Greifswald. The offspring of the heterozygous crosses were genotyped.

Animals were kept in a 12-hour day-night cycle with food and water access ad libitum. Leptin-deficient (ob/ob) and littermate wild-type (wt) control mice of both sexes were used in all subsequent experiments. For body weight analysis, mice aged between 60 and 200 days were used. For all other experiments, mice aged between 4 and 6 months were used. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted (“Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern,” LALLF M-V; 7221.3-1-016/16).

### Body weight analysis

For body weight analysis, 48 control and 48 ob/ob animals (from postnatal day 60 up to postnatal day 200) were weighed once a week using a Beurer KS 36 scale (Beurer, Germany).

### Microvolumetry

Brain volume was determined using microvolumetry ( $\mu$ -VM) as described previously.<sup>9</sup> In brief, a 5-mL syringe was used as a sample container with a 1-mL syringe attached as a measurement device. 3 mL of 4% paraformaldehyde (PFA) were used as the fluid in the sample container in which the mouse brain was placed. Then the initial level of 3 mL was restored using the measurement device in which the displaced volume could be measured. Measurements were repeated 3 times for each brain ( $n = 19$  per group) and averaged.

### Immunohistochemical analysis

Animals were euthanized and transcardially perfused with phosphate-buffered saline (PBS) and 4% PFA. The brains were explanted and stored in 4% PFA at 4°C for at least 4 days. 30- $\mu$ m coronal sections were made using a vibration blade microtome (VT 1000 S; Leica, Germany). Sections were mounted on superfrost slides (R. Langenbrinck GmbH, Germany) and dried overnight at 37°C. Rehydration and antigen retrieval were achieved via microwave treatment (Samsung Electronics GmbH, Germany; 20 minutes and 800W).

For phosphohistone H3 (PH3) staining, sections were rinsed with distilled water (A. dest.) and PBS. Unspecific binding sites were blocked using 5% normal goat serum (NGS) + 5% bovine serum albumin (BSA) + 0.1% Triton X-100 in PBS for 60 minutes at room temperature (RT). After washing with PBS, sections were incubated in a solution (1% NGS + 0.1% Triton X-100 in PBS) containing rabbit anti-phosphohistone H3 antibody (Santa Cruz, USA; 1:100) for 120 minutes at 4°C. After washing with PBS, sections were incubated in a solution (1% NGS + 0.1% Triton X-100 in PBS) containing Cy3-conjugated goat anti-rabbit-IgG (Dianova, Germany; 1:2.000 in PBS) for 60 minutes at RT. Thereafter, sections were rinsed in PBS, counterstained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI; 1:10.000), washed in A. dest., and embedded in Mowiol (Merck KGaA, Germany).

For doublecortin (DCX) staining, sections were permeabilized with 0.4% Triton X-100 in PBS for 30 minutes. After rinsing in PBS, sections were incubated in a blocking solution (3% horse serum + 0.3% Triton X-100 in PBS) for 60 minutes at RT. After rinsing in PBS, sections were incubated in a solution (3% horse serum + 0.1% Triton X-100 in PBS) containing the primary goat anti-DCX antibody (Santa Cruz Biotechnology, Germany; 1:100) for 3 days at 4°C. Rinsing with PBS was followed by incubation in a solution (3% horse serum + 0.1% Triton X-100 in PBS) containing biotin-conjugated horse anti-goat IgG (Vector Laboratories, USA; 1:200) for 2 hours at RT. After washing in PBS, sections were incubated with the secondary antibody (Cy3-conjugated streptavidin [Dianova]; 1:2.000 in PBS) for 2 hours at RT. Sections were rinsed in PBS and counterstained with DAPI (1:10.000), washed, and embedded in Mowiol (Merck KGaA).

For caspase 3 (Casp3) staining, sections were rinsed and transferred to a blocking solution (3% NGS + 0.1% Triton X-100 in PBS) for 60 minutes at RT. After rinsing in PBS, sections were incubated in a solution (3% NGS + 0.1% Triton X-100 in PBS) containing the primary rabbit anti-active Casp3 antibody (Merck Millipore, Germany; 1:100) at 4°C overnight. Rinsing the sections with PBS was followed by the application of the secondary antibody (Cy3-conjugated goat anti-rabbit-IgG [Dianova]; 1:400) for 90 minutes at RT. Afterwards, sections were rinsed in PBS and counterstained with DAPI (1:10.000 in A. dest.), washed with A. dest., and embedded in Mowiol (Merck KGaA).

Analysis was conducted within the dentate gyrus (DG) of the hippocampus using an Axioplan 2 imaging microscope (Zeiss, Germany). Immunopositive cells were counted in each animal analyzed (PH3: wt  $n=9$ ; ob/ob  $n=10$ ; DCX: wt  $n=13$ ; ob/ob  $n=9$ ; Casp3: wt  $n=12$ ; ob/ob  $n=8$ ) and the estimated total cell count for each immunostaining was corrected as described previously<sup>9</sup> using the Linderstrom-Lang/Abercrombie equation

$$N = n \times \frac{t}{t+H} \text{ or } \frac{N}{n} = f = \frac{t}{t+H}$$

where  $N$  is the estimated number of objects in the defined region,  $n$  is the total count of objects,  $t$  is the mean thickness of the virtual section,  $H$  is the mean object height, and  $f$  is the conversion factor for converting  $n$  to  $N$ . In the first step,  $n$  was determined. Thereafter, the height of the cells ( $H$ ) was estimated using a computer-driven motorized stage (Merzhäuser, Germany) connected to an Axioplan 2 imaging microscope under the control of Stereo Investigator (MBF Bioscience, USA).

#### Analysis of dendritic spines

Animals were sacrificed and transcardially perfused with PBS and 4% PFA. For post-fixation, the brain tissue was stored in 4% PFA at 4°C for at least 3 days. Golgi silver impregnation was conducted using the FD Rapid GolgiStain Kit (FD Neuro Technologies, USA). 120- $\mu\text{m}$ -thick coronal sections were made using a vibration blade microtome (VT 1000 S, Leica). Sections were mounted on gelatinized glass slides, dehydrated, and exposed to xylol. The mounted sections were coverslipped with Merckoglas (Merck Millipore).

Dendritic spines on secondary or tertiary dendrites of hippocampal CA1 pyramidal cells were analyzed, as described previously.<sup>10</sup> Only 1 segment per dendritic branch was chosen and approximately 20 apical and 20 basal dendrites were analyzed. Dendrites were reconstructed 3-dimensionally using the NeuroLucida 9.12 software (MBF Bioscience) to control the  $x$ - $y$ - $z$  axis of the microscope (Axioplan 2 imaging; Zeiss) and a digital camera attached to it (AxioCam HRC; Zeiss). The reconstructed dendrites were then analyzed using the software NeuroExplorer (MBF Bioscience). Statistical analysis was based on the animal numbers ( $n=6$  for both groups) and not on the numbers of reconstructed dendritic spines ( $4827 \pm 475.18$  dendritic spines of apical and  $4653 \pm 110.31$  dendritic spines of basal dendrites of hippocampal CA1 pyramidal neurons per group were analyzed).

#### Behavioral analysis

For behavioral analyses, mice were subjected to 2 different tests:

**OF.** A quadratic  $45 \times 45 \text{ cm}^2$  test arena (Panlab, Spain) was used for the OF test, with illumination set to 25 lux. To start

a trial, the animal (wt  $n=29$ ; ob/ob  $n=32$ ) was placed in the center of the area for 7 minutes to explore the environment. The tracking started automatically and movements were recorded via webcam (Logitech C300; Logitech, Switzerland). Parameters characterizing the OF behavior, namely, total distance covered (cm) and velocity (cm/s), were analyzed from recorded sessions using SmartJunior 1.0.0.7 (Panlab). Between trials, the arena was cleaned with 70% ethanol.

**MWM.** Animals (wt  $n=26$ ; ob/ob  $n=26$ ) were trained to localize a circular, hidden platform ( $\varnothing=11 \text{ cm}$ , acrylic glass; 2 cm underneath the water surface) within a water-filled circular pool ( $\varnothing=140 \text{ cm}$ ). Therefore, animals could only use visual cues of simple geometric figures attached to the surrounding walls of the room. The water (renewed daily) was rendered opaque with milk powder (550 g in 740 L water; BASU Mineralfutter GmbH, Deutschland) and kept at  $24^\circ\text{C} \pm 1^\circ\text{C}$ . Illumination was set to 25 lux. Animals were trained in 20 trials, with 4 trials on 5 consecutive days (inter-trial interval = 30 minutes) during which the position of the platform was kept unchanged (acquisition phase). The starting position for each animal was changed between trials. During the acquisition phase, mice were allowed to explore the pool for a maximum of 120 seconds or until finding the platform. If they failed, they were guided onto the platform. On the sixth day (probe trial), the platform was removed from the water maze. Mice were allowed to explore the water maze for 120 seconds. After 1 day of resting, animals had to perform a spatial reversal task.<sup>11,12</sup> The platform position was moved to the opposite side of the pool to test the ability of the animals to adapt to the new situation. The training phase and probe trial were then performed as described above (4 training sessions on 5 consecutive days, probe trial on the sixth day). Swimming tracks of each individual were recorded via webcam (Logitech C905) centered above the pool and analyzed using Smart 3.0 (Panlab). The following parameters were recorded during the acquisition phase: swimming speed (cm/s) and time to target platform (seconds); during the probe trial, the software additionally recorded the parameters: platform position crossings and time in platform quadrant (seconds). The tracking data also allowed to calculate an additional parameter, which was independent from the generally mobility-impaired velocity of the animals. The parameter “swimming direction” was defined as the swimming accuracy, by measuring the speed-independent movement toward the platform position.

#### Statistics

GraphPad Prism version 5 for Windows (GraphPad Software, USA; www.graphpad.com) was used for statistical analysis of all data (unpaired  $t$  test; level of significance set to  $P \leq .05$ ). Data presented in the figures were either expressed as boxplot with median line and whiskers for the lowest and highest



values or as mean  $\pm$  standard deviation (SD). Significant changes are labeled as  $*P \leq .05$ ,  $**P \leq .01$ , and  $***P \leq .001$ .

## Results

### *Weight nearly doubles in ob/ob mice during the first 120 postnatal days*

Leptin-deficient mice show an obese phenotype that continuously develops over time. Therefore, we monitored body weight of the mice from postnatal day 60 until postnatal day 200 (Figure 1A). We observed that, at postnatal days 120 to 180, the mean weight of the obese mice strongly increased ( $59.35 \pm 4.84$  g) compared with the control mice ( $26.19 \pm 4.58$  g; Figure 1B).

### *Brain volume is reduced in ob/ob mice*

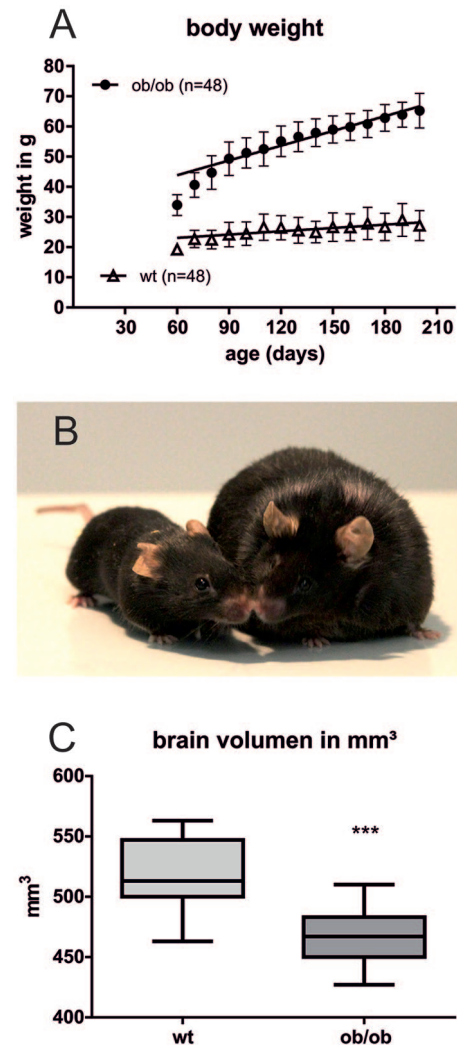
For analyzing the whole brain volume of ob/ob mice and wt animals,  $\mu$ -VM was used. Adult ob/ob mice display a significantly ( $P < .0001$ ;  $t = 5.72$ ;  $df = 36$ ) reduced brain volume of about 10.7% compared with age-matched wt controls (Figure 1C).

### *Adult hippocampal neurogenesis is altered in ob/ob mice*

As neurogenesis can be classified into different phases such as, eg, stages of proliferation, differentiation, migration, maturation, and synaptic integration, we used immunofluorescence staining of marker proteins that are expressed in distinct phases of neuronal development.<sup>13</sup> Antibodies directed against PH3 label proliferating cells in the m-phase. The number of PH3-positive cells (Figure 2A) in the DG was significantly ( $P = .044$ ;  $t = 2.171$ ;  $df = 17$ ) reduced by  $\sim 18\%$  in ob/ob mice ( $n = 10$ ) compared with control littermates ( $n = 9$ ). As PH3 is specific for mitotic cells, but does not differentiate between neuronal and non-neuronal cells, the population of newly formed immature neuronal cells (mitotic as well as postmitotic) was visualized using antibodies directed against DCX. The analysis revealed that ob/ob mice ( $n = 9$ ) showed significantly ( $P = .0003$ ;  $t = 4.371$ ;  $df = 19$ ) less ( $\sim 32\%$ ) DCX-positive cells in the DG compared with controls ( $n = 12$ ; Figure 2B and B'). As proliferation and differentiation during adult neurogenesis are accompanied by apoptosis, we next analyzed whether differences in apoptosis could be noted in the DG using antibodies directed against active (cleaved) Casp3. The analysis revealed no significant differences between wt ( $n = 12$ ) and ob/ob ( $n = 8$ ) mice (Figure 2C).

### *Dendritic spine densities in area CA1 are not altered in ob/ob mice*

We analyzed densities of dendritic spines on apical and basal dendrites of CA1 pyramidal hippocampal neurons. Although elevated spine densities were noted on apical dendrites (wt:  $1.193 \pm 0.036$  vs ob/ob:  $1.367 \pm 0.074$ ; Figure 2D) and basal

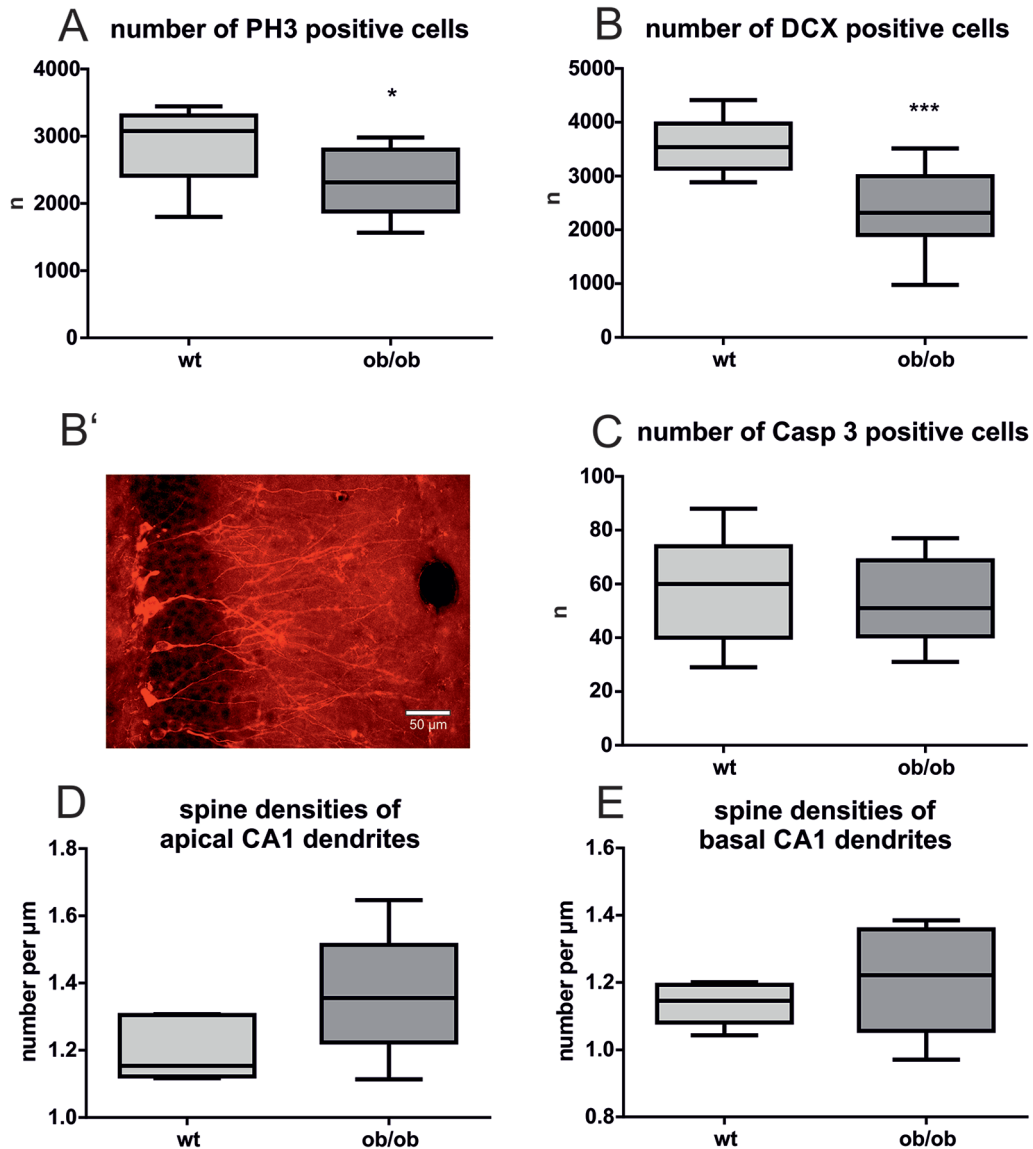


**Figure 1.** Body weight and brain volumes. (A) Body weights of wild-type (wt) and ob/ob mice were measured from postnatal day 60 until postnatal day 200. Data from 10 consecutive days were pooled ( $n = 48$  per group). Data are represented as mean  $\pm$  SD. (B) A leptin-deficient ob/ob mouse in comparison to an age-matched wt mouse. (C) Whole brain volumes were analyzed using microvolumetry. Brains of leptin-deficient mice (ob/ob) are significantly smaller compared with wt controls ( $n = 19$  per group). Data are represented as boxplot with median line and whiskers for the lowest and highest values.

dendrites (wt:  $1.137 \pm 0.027$  vs ob/ob:  $1.206 \pm 0.064$ ; Figure 2E) of ob/ob mice, these differences did not reach statistical significance.

### *Locomotor activity is reduced in ob/ob mice*

The OF test is a common and standardized test to analyze general locomotor activity. Locomotor activity was determined in 4- to 6-month-old ob/ob mice and their wt controls by analyzing the animals in a time window of 7 minutes. The wt animals ( $n = 29$ ) traveled an average of  $3817 \pm 153.3$  cm, whereas ob/ob mice ( $n = 32$ ) only traveled  $1648 \pm 116$  cm within that time window. Thus, the total distance covered by the obese mice was 43% less compared with age-matched



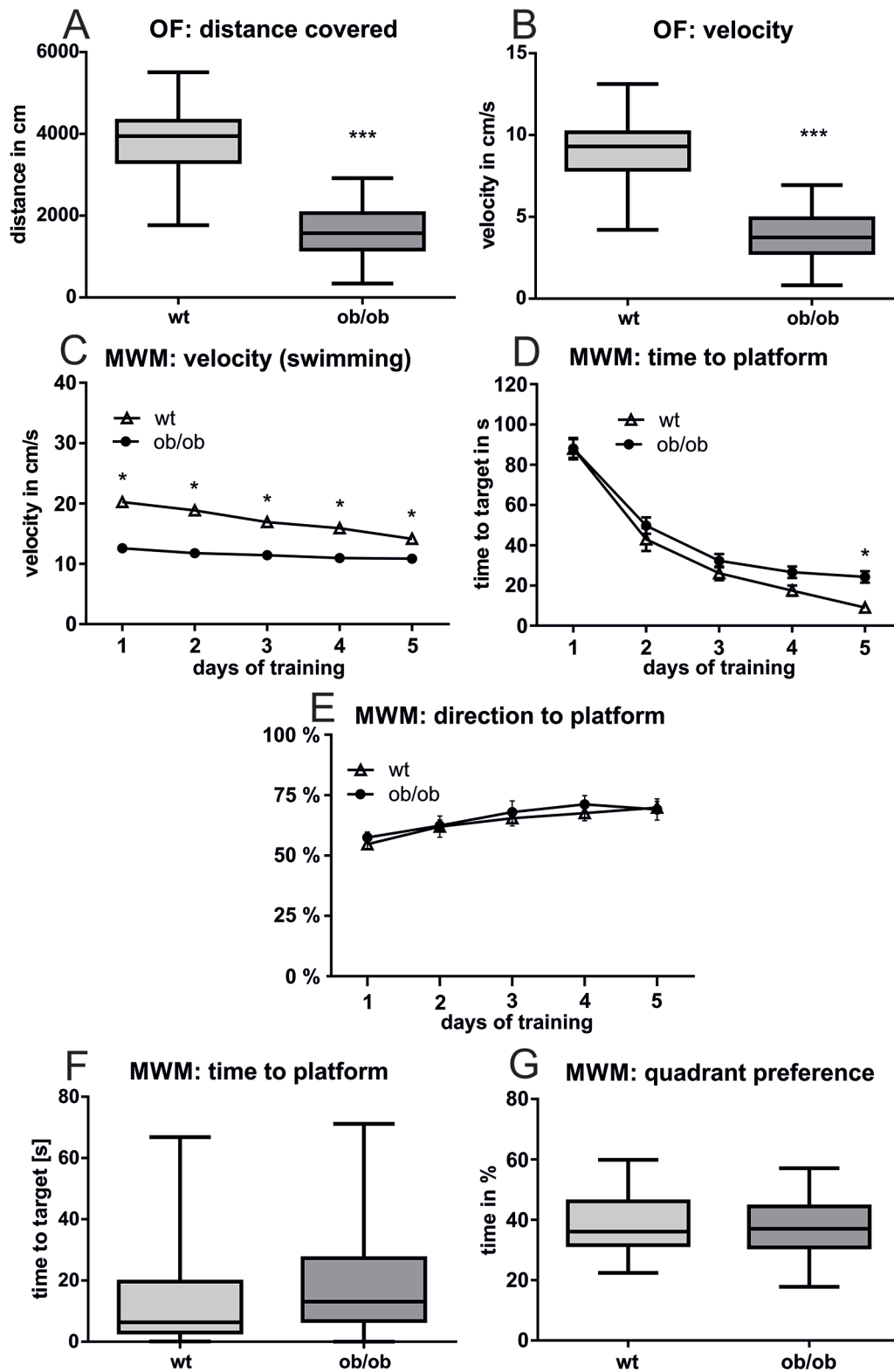
**Figure 2.** Adult hippocampal neurogenesis was analyzed using immunofluorescence staining within the dentate gyrus (DG) of the hippocampus. Cell counts were made using Abercrombie correction. Golgi-impregnated material was used to reconstruct dendrites in area CA1 and to analyze dendritic spine densities. (A) Leptin-deficient (ob/ob) mice showed a reduced amount of PH3-positive proliferating cells. (B) The ob/ob mice show a reduced number of DCX-positive cells in the DG compared with wild-type (wt) controls. (B') Example of DCX-stained neurons within the DG. Images were observed using an Olympus BX63 microscope with a 40× objective. (C) As an apoptosis marker for apoptotic events, caspase3 (Casp3) was used. The number of Casp3-positive cells did not differ between the 2 groups of mice. (D) No difference in spine densities of apical dendrites of hippocampal CA1 pyramidal neurons was noted by comparing age-matched control (wt) or obese (ob/ob) mice. (E) Spine densities of basal dendrites of hippocampal CA1 pyramidal neurons were also not significantly different. DCX indicates doublecortin; PH3, phosphohistone H3.

controls ( $P \leq .0001$ ;  $t = 11.42$ ;  $df = 59$ ; Figure 3A). In addition, the ob/ob mice displayed a significantly ( $P \leq .0001$ ;  $t = 11.33$ ;  $df = 59$ ) reduced average velocity of about 43.5% over time (wt:  $9.023 \pm 0.3613$  cm/s vs ob/ob:  $3.925 \pm 0.2716$  cm/s; Figure 3B).

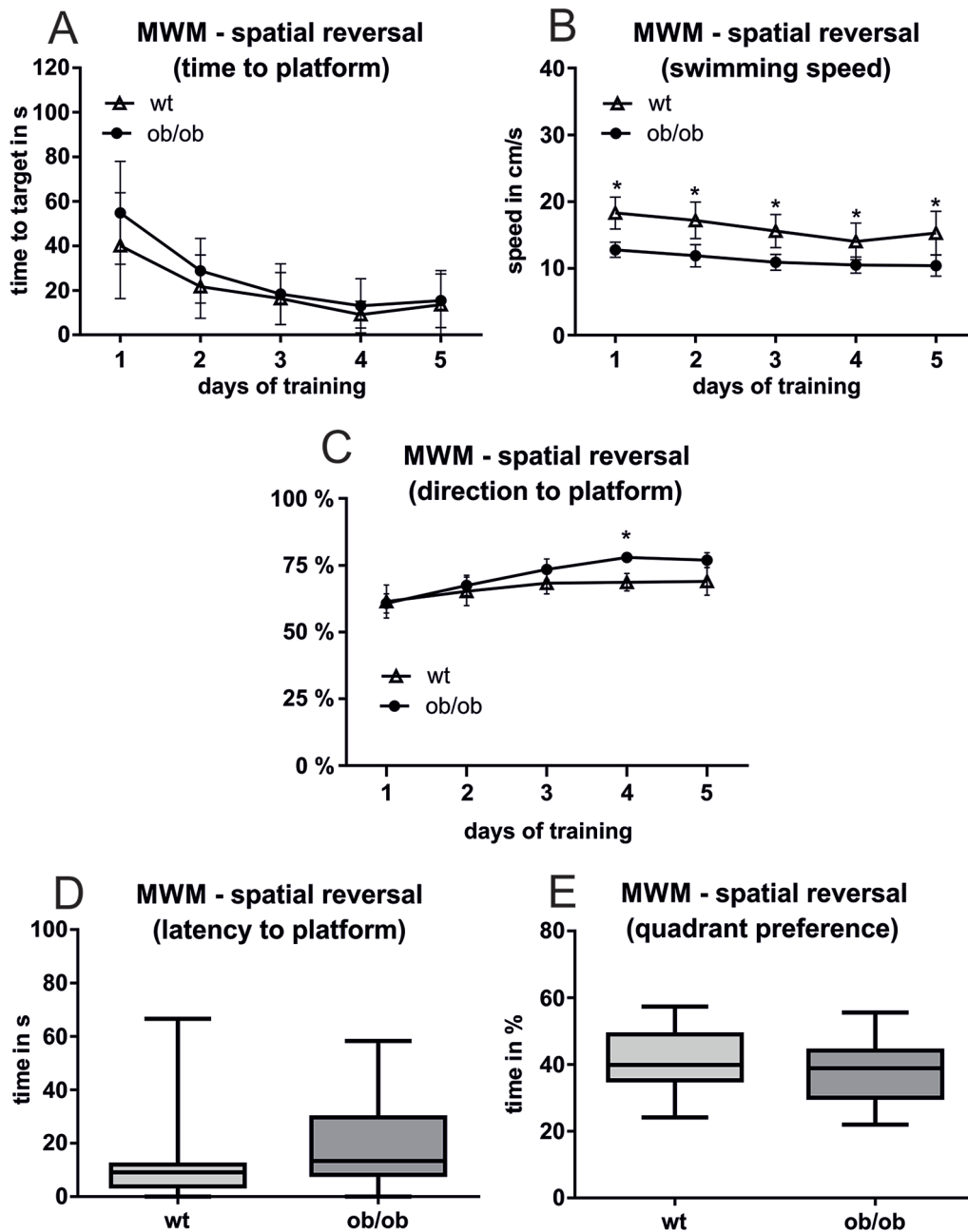
#### *The ob/ob and wt mice show comparable performances in the MWM test*

Both ob/ob mice and their wt controls were able to learn the task in the MWM during the training phase. Comparable to the OF, the ob/ob mice ( $n = 26$ ) moved with a significantly

reduced velocity ( $P = .0001$ ;  $t = 12.15$ ;  $df = 40.81$ ) in the MWM compared with controls ( $n = 26$ ; Figure 3C). However, during the first 4 days, the mean time to the target platform was not significantly different between both groups (Figure 3D). Keeping in mind the reduced mobility of ob/ob mice, as seen, eg, in the OF or already demonstrated by activity measures by others,<sup>14</sup> we calculated an additional parameter ("swimming direction"), which is independent from the velocity of the animals. The analysis of this parameter revealed that the performance in localizing the hidden platform of ob/ob mice was comparable to that of lean wt controls (Figure 3E). After 5 consecutive days of training, the animals were tested in the



**Figure 3.** To analyze the behavior of leptin-deficient (*ob/ob*) mice, the open field (OF) test as well as the Morris water maze (MWM) test was used. (A) In the OF, *ob/ob* mice covered significantly shorter distances compared with wild-type (*wt*) mice. (B) In the OF, the *ob/ob* mice displayed significantly reduced velocity compared with the *wt* mice. (C) The *ob/ob* mice displayed a significantly reduced swimming speed in the MWM. (D) Concerning the time to reach the platform, *ob/ob* mice showed overall comparable results to their controls. Only on day 5, the *ob/ob* mice even performed better than the control mice. (E) In the MWM, both groups of mice did not differ in their accuracy to find the platform. (F) In the probe trial, the *ob/ob* mice showed no significant differences regarding the time to reach the platform position. (G) In the probe trial, the leptin-deficient (*ob/ob*) mice nearly spent the same time in the platform quadrant as the mice of the control group (*wt*).



**Figure 4.** In the spatial reversal experiment of the MWM, the platform was positioned on the opposite side of the maze. (A) Leptin-deficient (ob/ob) and wild-type (wt) mice showed comparable latencies to reach the platform. (B) The ob/ob mice showed reduced swimming speed in the spatial reversal task. (C) The ob/ob mice were able to reach higher accuracy concerning the swimming direction toward the platform position. (D) In the probe trail, both groups did not differ in the latency to reach the platform. (E) The ob/ob mice and wt mice showed comparable platform quadrant preferences. MWM indicates Morris water maze.

probe trial. The latency to find the former platform position (wt:  $13.78 \pm 3.251$  seconds vs ob/ob:  $19.28 \pm 3.428$  seconds; Figure 3F), as well as the time animals spent in the quadrant, where the platform used to be (wt:  $38.8 \pm 1.85\%$  vs ob/ob:  $37.71 \pm 1.871\%$ ; Figure 3G), was not significantly different between the both groups analyzed.

Thereafter, the animals had to perform in the spatial reversal task.<sup>11,15</sup> This test varies the already known behavioral task by placing the platform on the opposite side in the maze. When testing the animals again, it is possible to evaluate their ability

to adapt to the new situation. The spatial reversal test also consists of a training for 5 days and probe trial on the 6th day. The time to reach the platform was not significantly altered between both genotypes (Figure 4A). The swimming speed was still significantly different in the second training phase (wt:  $15.29 \pm 0.638$  cm/s vs ob/ob:  $10.42 \pm 0.308$  cm/s; Figure 4B). Surprisingly, ob/ob mice showed a slightly more accurate swimming direction in the spatial reversal task (Figure 4C). During the probe trial of the spatial reversal, the ob/ob mice did not show any obvious behavioral alteration, concerning

neither their latency to platform (Figure 4D) nor the platform quadrant preference (Figure 4E).

## Discussion

Several genetic mouse models of obesity are available (eg, ob/ob mice and db/db mice) as well as diet-induced approaches.<sup>7</sup> We have chosen a mouse model of obesity, based on leptin deficiency (ob/ob), because at 4 months of age the ob/ob mice weighed approximately twice as much as the lean littermate controls (see Figure 1). In this study, we analyzed 4- to 6-month-old mice to investigate the possible effects of obesity on neuronal plasticity, learning, and memory.

A related mouse model is that of the obese and diabetic leptin-receptor-deficient (db/db) mice.<sup>7</sup> In contrast to the ob/ob mice, young db/db mice display not only deficits in the MWM, but also impairments in long-term potentiation (LTP) and long-term depression (LTD) in the hippocampal area CA1, and administration of leptin does not ameliorate these deficits seen on the electrophysiological level.<sup>16</sup> Moreover, when they grow up, the leptin-receptor-deficient db/db mice develop a permanent form of diabetes mellitus, whereas the leptin-deficient ob/ob mice do not.<sup>7</sup> Interestingly, it was shown using adult db/db mice that diabetes impairs hippocampus-dependent memory, synaptic plasticity, and adult neurogenesis, and that these changes in hippocampal plasticity and function are reversed when normal physiological levels of corticosterone are maintained, suggesting that cognitive impairment in diabetes may result from glucocorticoid-mediated deficits.<sup>17</sup> This suggests that diabetes is a strong risk for the normal functioning and maintenance of neuronal processes.

It has been reported that obesity has an impact on human brain functions, eg, by impairing performance, when executive functions were tested.<sup>18,19</sup> In addition, several studies have linked obesity to morphological alterations within the human brain. For example, a linear association between higher BMI and smaller brain volume as well as reduced hippocampal volume has been shown (for details, see Carnell et al<sup>20</sup>). Comparable to the situation in humans, the ob/ob mice display a reduction of ~10% in total brain volumes (Figure 1). A comparable difference has also been noted by analyzing total brain weight of the obese mice in comparison with their controls.<sup>21,22</sup> Among others, changes in adult neurogenesis, as well as in the morphology of individual neurons, might contribute to obesity-induced brain volume reductions. Interestingly, ob/ob mice showed deficits in adult hippocampal neurogenesis. The ob/ob mice display significantly reduced numbers of PH3- as well as DCX-positive cells, indicating that not only cell proliferation but also the differentiation into new neurons is affected. Therefore, it can be suggested that obesity may have an impact on adult neurogenesis. Indeed, rodents (mice<sup>23</sup> as well as rats<sup>24</sup>) that become obese due to a high-fat diet (HFD) also display impairments in hippocampal neurogenesis. Furthermore, in diabetic mice (db/db mice), hippocampal neurogenesis is not only impaired, but also dramatically reduced by up to 50%.<sup>25</sup> Thus, obesity may represent a risk factor for a reduction in

adult hippocampal neurogenesis. This obesity-induced reduction in adult neurogenesis may be attributed directly to changes in brain leptin levels. Chronic, but not short-term, administration of leptin to adult mice (intraperitoneal leptin injections twice a day over a time period of 14 days) increases cell proliferation in the DG without affecting the survival of newly proliferated cells.<sup>26</sup> The effect of leptin on proliferation within the adult hippocampus is thought to be mediated by the AMP-activated protein kinase (AMPK) and extracellular-signal-regulated kinase (ERK) signaling pathways.<sup>27</sup> Moreover, in neurospheres, leptin is thought to maintain the self-renewal ability and epidermal growth factor reactivity of immature neural lineage cells.<sup>28</sup> Interestingly, a key role of leptin in mild-exercise-induced effects on adult hippocampal neurogenesis has been discovered recently.<sup>29</sup> Therefore, leptin might play a role in the obesity-induced effects on adult neurogenesis.

In addition to changes in adult neurogenesis, alterations in dendritic spines represent another morphological correlate of changes in neuronal plasticity.<sup>30</sup> We noted slightly elevated spine densities in the ob/ob mice in apical as well as basal dendrites of CA1 pyramidal neurons. Diabetic db/db mice display a strong increase in dendritic spine densities in the DG<sup>25</sup> and diabetic rats display significantly reduced spine densities of apical as well as basal dendrites of CA1 pyramidal neurons,<sup>31</sup> indicating that obesity in combination with diabetes results in a different neuromorphological phenotype. As the db/db mice did not only display striking morphological differences compared with controls, it is not surprising that they also showed impairments in their behavior, as observed in the MWM test.<sup>16</sup>

Based on this, we were interested in analyzing whether adult ob/ob mice also display altered behavior. In the OF, the ob/ob mice covered less distance in the arena compared with their lean controls. Likewise, db/db mice traveled less than age-matched controls.<sup>32</sup> The ob/ob mice not only traveled less than control animals in the OF, but also displayed a reduced velocity, possibly due to their increased body weight—a comparable behavior seen in humans, as, eg, in overweight children.<sup>33</sup> In the MWM, the obese ob/ob mice differed in their behavior from db/db mice that were found to display impaired spatial learning.<sup>16</sup> The average swimming speed of ob/ob mice in the MWM was significantly reduced, somewhat comparable to the velocity recorded in the OF. The standard parameters evaluated in the MWM, such as distance or time to target, are highly dependent on mobility (“swimming speed”). The parameter “swimming direction” is independent of the speed of this movement and allows monitoring movement toward the platform position. The analysis of this parameter clearly demonstrates a precise course toward the platform position for both groups of mice, indicating that the obese mice display no deficits in spatial orientation. Thus, at least in the present mouse model of obesity, and comparable to the data obtained in humans,<sup>34</sup> obesity does not seem to interfere with hippocampal-dependent spatial learning. To test the obese mice in an even more demanding setting of the MWM, the animals had to perform in a spatial reversal task, where the platform was moved to the opposite quadrant. This requires the mice to



extinguish the previously learned goal position and acquire a path to the new position.<sup>12</sup> The ob/ob mice were also able to solve the spatial reversal task of the MWM, even showing a more accurate movement toward the platform position (Figure 4C), combined with reduced swimming speed (Figure 4B). These results further provide support for the notion that obesity does not interfere with hippocampal-dependent spatial learning.

Addressing the causality of obesity and cognition in humans, previous studies yielded largely different results,<sup>35,36</sup> which may be due to the very different methodological approaches as well as due to the different parameters controlled for (eg, age, sex, education, and comorbidities; for more details, see Prickett et al<sup>36</sup>). Among others, an inverse relationship between obesity and intellectual abilities has been postulated. Along this line, a correlation between academic success and obesity has been reported.<sup>37,38</sup> However, by controlling the study for the social and economic background of the participants, no correlation between academic success and obesity was evident.<sup>39</sup> Moreover, in some studies, impaired spatial learning of obese humans was reported,<sup>35,40</sup> whereas in another study even a better performance of obese humans in spatial learning tasks has been observed.<sup>34</sup>

The results obtained using ob/ob mice suggest that obesity has an impact on adult neurogenesis but not on dendritic spine densities in the hippocampal area CA1 and not on the spatial memory performance in the MWM. Obesity is a risk factor for diabetes and that there is strong evidence for an association of diabetes with cognitive decline and dementia in humans.<sup>41</sup> Thus, based on these data, obesity does not affect learning, memory, and cognition directly, but is a risk factor for developing such disturbances.


## Acknowledgements

The authors wish to thank Mrs Hanisch and Mrs Kaiser for their excellent technical assistance.

## Author Contributions

SH and OvBuH planned and designed most parts of the study. AB performed most of the experiments and some behavioral studies were conducted by KB, GD, JvdB, B B were involved in animal housing, breeding and genotyping. AB and OvBuH performed the statistical analysis of the data.

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