Supplementary Material and Methods

Hindlimb clasping test

For each trial, mice were griped by their tale close to their body and held for 10 sec (3 trials in total). After each trial there was an (inter-trial interval) ITI of 5 min. During tail suspension, hindlimb clasping behavior was manually evaluated with following criterias: 0 - hindlimb were spread widely. 1 – one hindlimb pointed inwards for more than 50% of the time. 2 - both hindlimbs pointed inwards for more than 50% of the time in a repetitive manner. 3 - both hindlimbs were permanently pointing inwards for more than 50% of the time

Rope grip test

A 40 cm rope was tautly attached to a blue crate (40 x 30 x 26 cm). The blue crate was supplemented with padding on the bottom. Mice were gently griped by the tail close to the body and were allowed to grab the rope in the middle with both forepaws. Mice were then released and the time to reach one of the ends of the rope as well as the time to fall off the rope was measured. Mice were allowed to hang a maximum of 60 sec. The motoric behaviors were scored as followed: Score 0-Animal felt off. Score 1-Animal hang onto the string by one or both forepaws. Score 2-Animal hang onto the string by one or both forepaws and attempted to climb onto the string. Score 3-Animal hang onto the string by one or both forepaws plus one or both hindpaws. Score 4-Hang onto the string by forepaws and hindpaws plus tail was wrapped around string. Score-5-Animal escaped to the supports. Raw data acquisition was done manually by the experimenter.

Grip strength test

Each mouse was weighed before the test. Mice were griped by the tail close to the body and were gently lowered over the top of the bar that was connected to the TSE Grip Strength Meter apparatus. As soon as the mice attached the bar with both fore paws they were gently pulled away until they released the bar. Grip strength of the forelimbs only were recorded automatically and transferred to the computer by a (TSE) Grip Strength Meter. For each animal and type of measure, three values [g] were taken and averaged. Each animal of a batch

was tested consecutively in the same order the grip strength mean value in [g] that was then normalized to the weight of the mice.

Beam walking test

Wooden beams (100 cm) were placed horizontally, 50 cm above the bench surface, with one end mounted on a narrow support and the other end attached to an enclosed box into which the mouse could escape. The ground under the beam was supplemented with padding. Mice were put on the bright lit start position and trained for two days to traverse a 20-mm diameter beam and enter an escape box on the other side of the beam. For training, 4 trials per animal were performed. On the test day, mice had to traverse the beam in two consecutive trials on each beam (20 mm, 15 mm, 10 mm) progressing from the widest to the narrowest beam. Mice were allowed up to 60 sec to traverse each beam. The time that mice needed to traverse the beam and to enter the escape box was measured. In addition, the number of hind foot slips were count. Raw data acquisition was done manually by the experimenter.

Rotarod test

Mice were put in the TSE Systems Rotarod apparatus. This apparatus 3 cm diameter rod that had ~1 mm horizontal grooves on the surface, which are designed to provide the mouse with better grip whilst running the task. The rod length was 30 cm, sub-divided with four partitions to create separate compartments such that five mice could be run simultaneously. Each mouse completed 3 trials with an ITI of 15 minutes. After the start of the test the rotarod accelerated then from 4-40 rpm for 300 sec. The latency to fall of the rotarod was measured by the TSE Rotarod software.

Foot print test

For this test mice were held in a scruffed position in order to apply color paints to there feet (red-forelimbs, blue-hindlimbs) (Dr. Oetker, Food colors). After the color was applied, for both training and testing, mice traverse the horizontal lane (67 cm long, 5 cm wide) of a T-maze covered with a white sheet, for two consecutive trials each. After the test, different parameters on the sheet were measured: stride length-fore imbs/hindlimb (SL-FL, SL-HL) (distance for forward movement for hindlimb and forelimb), hindbase/forebase width (HBF,

FBW) (distance between left and right hind footprints for hindlimb and forelimb), S-(distance between hindlimb and forelimb or overlap between forepaw and hindpaw placement). Raw data acquisition was done manually by the experimenter.

Nest Construction Test

Each Mouse was singly housed in a new home cage with fresh bedding that included a nestlet (5x5 cm pressed white cotton square). After 16 hours the nest built out of the cotton was scored manually with following criterias: Score 1-The nestlet was largely untouched (>90% intact). Score 2-The nestlet was partially torn up (50-90% remaining intact). Score 3-The nestlet was mostly shredded but often there was no identifiable nest site: < 50% of the nestlet remained intact but < 90% was within a quarter of the cage floor area, i.e. the cotton was not gathered into a nest but spread around the cage. Score 4- > 90% of the nestlet was torn up, the material was gathered into a nest within a quarter of the cage floor area, but the nest was flat, with walls higher than mouse body height on less than 50% of its circumference. Score 5- > 90% of the nestlet was torn up, the nest was a crater, with walls higher than mouse body height on more than 50% of its circumference. Furthermore, the nestlet was weighed before the test and afterwards. The difference between both time points was calculated in [%].

Home cage scan and computational analysis

Mice were put in a home cage separated from each other and monitored for 23h. For the quantification of natural animal behavior in the familiar environment of its home cage, we used the home cage scen (HCS) video and analytic software (CleverSys) which detects automatically different behaviors of freely moving mouse in its home cage (35 × 20 × 15 cm). Nineteen different behavior patterns of the mice were detected during a 23 h period (11 h of light phase and 12 h of dark phase/mouse). Number of occurrences and duration of each behavior were analyzed over time and on average per hour using custom-made R scripts.

Social activity monitoring and computational analysis

Mice were monitored for 14 days (first approach) with mixed genotypes in their home cage. For the second approach we monitored mice for 4 days with mixed and separated (non-mixed) genotypes. During this time the animal cages were placed on the social activity monitor

(SAM) system (Phenosys. Germany). Each cage is placed on an ID-Grid sensor plate that detects the individual animals and tracks them as they move throughout the cage. Each ID-Grid sensor plate contains eight RFID sensors that records data on the location of the animals at every time point. The data is collected using Phenosoft Control software, preprocessed by Phenosoft analytics software and analyzed in R (R Core Team, 2017).

Reference: R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Barnes maze test

During the training phase animals were given 4 trials a day for 4 days with an ITI of 15 minutes between trials. For each trial the animals were placed on a table with a 92cm diameter, containing 20 holes along the peripheral in which one of the holes contained a nest that the animal could go into. The animal was allowed 3 minutes to find the nest. To encourage the animal to search for the nest a light white noise was played. Once the animal found the nest the white noise was stopped and the animal was allowed to stay in the nest for 1 minute. If the animal did not find the nest in the allotted 3 minutes it was shown the nest and the white noise was stopped. The animal was then allowed to stay in the nest for 1 minute. During the probe trials on day 5 and day 12 animals were returned to the table containing the 20 holes with the nest removed. During the probe trials the animal's behavior was observed for 90s. For the evaluation of the search strategies 33.3% of the trials were analyzed for each animal. All parameters were recorded by the video-tracking system Viewer (Biobserve). Two animals had to be excluded from the analysis, since they were not able to learn the location of the exit hole during day 1-4.

Buried food test

For 3 consecutive days before testing, the mice were given sweet pellets (Purified rodent tablets, 45 mg, TestDiet®, at least 3 times). All mice had to consume the pellets before test or be excluded from the test. Four hours before testing, mice were deprived of food with water ad libitum. Before testing, each mouse was given 5 min of habituation in the testing cage (regular home cage; 35 × 20 × 15 cm) that contained 4cm of fresh bedding. After this habituation, the mouse was temporarily removed from the testing cage, while, a sweet pellet was hidden 1 cm deep and 5 cm away from the cage rear. For testing, the mouse was

positioned in the center of the opposite end of the cage, and the latency to find the food, i.e., the time from the moment the mouse was placed into the cage to the time it located the sweet pellet and initiated digging, was recorded. Whether the pellet was eaten or not and other spontaneous behavior (rearing, climbing on the lid, grooming and extensive digging) were also scored. The mouse was then removed from the cage. A new cage and fresh bedding were used for each mouse. Raw data acquisition was done manually by the experimenter.

Three-chamber Test

The social testing arena was a rectangular, three-chamber box (60 x 40 x 22 cm, Stoelting apparatus) with white non transparent external walls. Each chamber was (20 x 40 x 22 cm) in size. Dividing walls were made from clear Plexiglas, with rectangular openings (50 cm x 80 mm) allowing access into each chamber. The light condition was kept dim and uniform over the 3 chambers. In the experimental room, the test mouse was first placed in the middle chamber of the arena and allowed to explore for ten minutes. To test sociability, after this habituation the mouse was trapped in the center chamber and an stranger mouse, that had no prior contact with the subject mouse, was placed in one of the side chambers. The location of the stranger mouse in the left vs. right side chamber was systematically alternated between test animals. The stranger mouse was enclosed in a round wire cage with grey Plexiglas covers (7 x 7 x 15 cm), which allowed nose contact through the bars but prevented fighting. The animals serving as stranger mice had previously been habituated to the small cage (at least 3 times before test, 5 min each time). An identical empty wire cage was placed in the opposite chamber. Both openings to the side chambers were then unblocked and the test mouse was allowed to explore the entire social arena for a 10min session. The amount of time spent in each chamber and in close proximity to the grid were recorded by the video-tracking system Viewer (Biobserve). Number of sniffs at the wire cage was recorded manually as an index of close investigation. At the end of the first 10 min, each mouse was tested in a second 10 min session to quantify social recognition for a new stranger mouse after a 5 min break (similar condition as the habituation phase). A second, stranger mouse was placed into the previously empty wire cage. The test mouse had a choice between the first, already-investigated mouse (familiar mouse=stranger 1), and the novel stranger mouse (stranger 2). As described above, measures were taken of the amount of time spent in each chamber and in close proximity with the grid during the second 10 min session. The position of stranger 1 and 2 was for each mouse changed in order to avoid side bias. Stranger mice had the same age and

sex as the testing animal. One animal was excluded from the analysis since during habituation it only explored one side (chamber) of the maze.

Y-maze

Mice were placed in a y-maze which has 3 identical arms (34 x 6 x 17 cm) placed at 120o from each and allowed to explore for 5 minutes. The animals were placed facing the end of one arm of the maze. The starting arm was alternated between mice. The percentage of spontaneous alternation (SAP), same arm returns (SAR), and alternate arm returns (AAR) were recorded and measured using the video-tracking system Viewer (Biobserve), as well as distance traveled and time spent in each arm.

Marble burying test

In a mouse cage (35 × 20 × 15 cm), twenty clean and identical glass marbles (green) were placed, equally spaced in five rows of four marbles on a 4 cm layer bedding. For each animal a different cage with fresh bedding was used but illumination was kept homogeneous. The animal was placed in the testing cage close to a wall and allowed to explore the cage for 30 min. The latency for the first marble to be buried was manually scored. At the end of the test the mouse was rapidly removed from the cage and the total number of marbles buried was counted manually. A marble was considered “buried” when two thirds of its volume was covered by bedding. The marbles were cleaned with 5 % alcohol and dried for approximately 10 min before being used again.

Social proximity test

Mice were put in a transparent testing cage (size: 4x17 cm, 98cm^2) together with a stranger mouse with same age and sex. The behavior of the mice were recorded for 20 minutes. The video was manually evaluated after all experiments were finished. Number of different social traits were count: nose tip-to-nose-tip contact: The scored animal nose-tip and/or vibrissae contact with the nose-tip and/or vibrissae of the other animal. Nose-to-head contact: The scored animal nose-tip and/or vibrissae contact the dorsal, lateral, or ventral surface of the other animals head. Nose-to-anogenital contact: The scored animal nose-tip or vibrissae contact with the base of the tail and/or anus of the other animal. Crawl over: The scored

animal forelimbs cross the midline of the dorsal surface of the other animal. Crawl under: The scored animal head goes under the ventral surface of the other animal. Rear up: Mice stretch their body vertically towards the cage. jump escape: the scored animal makes a vertical jump with all four feet leaving the ground.

Dark/light Box

Within the TSE multi-conditioning system mice were placed in the light chamber of a two compartment box that consisted of a light chamber and a dark chamber (30 x 22 cm for each chamber). Mice were then allowed to explore both chambers for a total of 10 minutes. The time that mice explored each the light and dark chambers was measured and recorded by the TSE software.

Elevated Plus Maze

The mice were placed into a white PVC (50x50x53) Elevated plus maze with two open arms and two closed arms, and allowed to explore for 5 minutes. Activity in the elevated plus maze was tracked and recorded using the Viewer software (Biobserve). The total distance, average velocity, and the number of visits to each arm were recorded.

Acute slice preparation

Acute hippocampal brain slices were prepared as described by Stempel et al. 2016. Adult mice were sacrificed by cervical dislocation. The brain was quickly removed and chilled in ice-cold sucrose - based artificial cerebrospinal fluid (sACSF) containing (in mM): NaCl 87, NaHCO3 26, sucrose 50, glucose 10, KCl 2.5, NaH2PO4 1.25, MgCl2 3, CaCl2 0.5, continuously oxygenated. Horizontal slices (300 μm) were cut and stored submerged in sACSF for 30 min at 35 °C and subsequently stored in ACSF containing (in mM): NaCl 119, NaHCO3 26, glucose 10, KCl 2.5, NaH2PO4 1, CaCl2 2.5 and MgCl2 1.3 saturated with 95% (vol/vol) O2/5% (vol/vol) CO2, pH 7.4, at room temperature. Experiments were started 1 to 6 h after the preparation.

Preparation of paraffin-embedded sections

Animals were deeply anesthetized with Ketamin/Rompun and subsequently transcardially perfused with 20 ml 1x DPBS and with 20 ml paraformaldehyde (PFA) (4%) in 1x PBS with a 23G needle. The brain was removed and fixated in 4% PFA at room temperature over night. Thereafter, the brain was washed in 1x PBS twice for 30 min and finally in 50% and 70% Ethanol at room temperature for 30 min in each solution. The brain was stored in 70% ethanol at 4°C or further processed. Paraffin infiltration was performed with the tissue processor (Leica). Afterwards, paraffin-infiltrated brains were embedded in paraffin and sectioned (coronal) with a thickness of 5 microns using the paraffin microtome (Leica). Paraffin-embedded sections were deparaffinized in Xylene twice for 5 min. Afterwards, sections were rehydrated by washing them in 100% (twice), 90%, 70%, and 50% Ethanol for 5 min in each solution. Sections were then immediately processed for NISSL staining or synaptophysin-staining.

NISSL staining

Paraffin-embedded sections were deparaffinized and rehydrated as described above. Sections were then washed twice in ddH_2O for 5 min. Afterwards, sections were maintained in Cresyl Violet buffered in Acetate Buffer (NISSL-staining solution) (pH 3,8-4) for 10 min. After NISSL-staining sections were washed twice for 3 sec in 100% Ethanol. Finally, the sections were embedded in Entellan. Images of sections were taken at 4x magnification.

Isolation of hippocampal cells

The animals were deeply anesthetized with Ketamin/Rompun and transcardially perfused with 20 ml 1x DPBS (pH 7,4) via a 20 ml syringe and a 23 G needle. The brains were removed and the hippocampus (2 hippocampi, each animal) were surgically taken out.

All procedures conducted for hippocampal cell isolation were performed on ice or at 4°C and all solutions were pre-cooled to 4°C. We pooled in total 8 hippocampi of 4 male animals per genotype. Hippocampi were dissociated in 1,5 ml Hibernate A (BrainBits) with a Dounce homogenizer (Active Motif) using the loose mortal. Single-cell suspension was transferred through a 70 µm strainer into a falcon. Afterwards, the Dounce homogenizer was washed twice with 1 ml Hibernate A. Remaining cells were collected from the washing step, passed through the 70 µm strainer and transferred into the falcon. Single-cell suspension was

centrifuged at 500g for 10 min. Thereafter, pellet was resuspended in 1,55 ml DPBS (pH 7,4). Afterwards, 450µl isotonic Percoll (10x DPBS diluted 1:10 in Percoll, pH 7,4) was added and single-cell suspension was pipetted up and down. 2 ml of 1x DPBS was very gently transferred on the single-cell suspension. The layered solution was then centrifuged for 10 min at 3250g. The supernatant with the myelin disk was removed immediately and the remaining pellet was washed in 4ml 1x DPBS (pH 7,4) and centrifuged for 10 min at 400g. Finally, the pellet was resuspended in 1x DPBS (pH 7.4) and the volume was adjusted to get a final concentration of 10000 cells per 10 µl. Single-cell suspension was then further processed for single cell RNA sequencing-library preparation.