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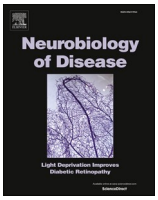

Cognitive comorbidities of experimental absence seizures are independent of anxiety

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ABSTRACT

Typical absence seizures (ASs) are brief periods of lack of consciousness, associated with 2.5–4 Hz spike-wave discharges (SWDs) in the EEG, which are highly prevalent in children and teenagers. The majority of probands in these young epileptic cohorts show neuropsychological comorbidities, including cognitive, memory and mood impairments, even after the seizures are pharmacologically controlled. Similar cognition and memory deficits have been reported in different, but not all, genetic animal models of ASs. However, since these impairments are subtle and highly task-specific their presence may be confounded by an anxiety-like phenotype and no study has tested anxiety and memory in the same animals. Moreover, the majority of studies used non-epileptic inbred animals as the only control strain and this may have contributed to a misinterpretation of these behavioural results. To overcome these issues, here we used a battery of behavioural tests to compare anxiety and memory in the *same* animals from the well-established inbred model of Genetic Absence Epilepsy Rats from Strasbourg (GAERS), their inbred strain of Non-Epileptic Control (NEC) strain (that lack ASs) and normal outbred Wistar rats. We found that GAERS do not exhibit increased anxiety-like behavior and neophobia compared to both NEC and Wistar rats. In contrast, GAERS show decreased spontaneous alternation, spatial working memory and cross-modal object recognition compared to both NEC and Wistar rats. Furthermore, GAERS preferentially used egocentric strategies to perform spatial memory tasks. In summary, these results provide solid evidence of memory deficits in GAERS rats that do not depend on an anxiety or neophobic phenotype. Moreover, the presence of differences between NEC and Wistar rats stresses the need of using both outbred and inbred control rats in behavioural studies involving genetic models of ASs.

1. Introduction

Absence seizures (ASs) are generalized non-convulsive seizures consisting of brief lapses of consciousness that are invariably accompanied by 2.5–4 Hz spike-and-wave discharges (SWDs) in the EEG (Crunelli et al., 2020; Crunelli and Leresche, 2002). Recent studies in Childhood Absence Epilepsy (CAE) cohorts, where ASs are the only seizure type, have shown that 60% of children display neuropsychological comorbidities (Masur et al., 2013) including emotional, cognitive, memory and linguistic deficits (Caplan et al., 2008). The cognitive deficits involve the attentional domain (35–40%), executive functions as well as verbal learning and visuospatial memory (Cheng et al., 2017; D'Agati et al., 2012; Henkin et al., 2005). Language and reading disabilities are also observed (Caplan et al., 2008; Vanasse et al., 2005), together with attention deficit hyperactivity disorder, and affective disorders, such as depression and anxiety (Caplan et al., 2008). Psychological comorbid conditions, in particular attention deficits, may precede the first absence seizure and diagnosis (Jones et al., 2007),

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persist after pharmacological control of the seizures and be exacerbated by some anti-seizure medications (ASMs) (Masur et al., 2013). Thus, there is a pressing clinical need to identify the neurobiological mechanisms of these comorbidities to develop appropriate therapeutic interventions.

To help with this endeavour, many studies have characterized the presence and features of AS comorbidities in genetic animal models. Whereas memory deficits have been shown to be present in different AS models, the presence of an anxiety-like behavior has been reported in some studies (Bouilleret et al., 2009; Jones et al., 2008; Powell et al., 2014) but others including GAERS (Genetic Absence Epilepsy rats from Strasbourg), and WAG/Rij (Wistar-Albino-Glaxo from Rijswijk (WAG/Rij) rats have failed to reproduce this anxiety phenotype (Brock et al., 1996; Cassar et al., 2022; De Deurwaerdère et al., 2022; José Eduardo Marques-Carneiro et al., 2014; Sarkisova and van Luijtelaar, 2011; Studer et al., 2019). Thus, it is still not clear whether this mood impairment underlies the deficient performance of AS models in memory tests. Moreover, though a few investigations have tested mood and cognition in the same study (De Deurwaerdère et al., 2022; Studer et al., 2019) only one anxiety test and one cognition test were carried out and anxiety and memory were not tested in the same animals. Furthermore, it is known that the control (often inbred) non-epileptic animals used in the behavioural analysis of AS comorbidities have a different behavioural phenotype than normal outbred rats. Thus, it is surprising that no study has so far used both inbred and normal outbred animals as control groups when assessing both anxiety and memory.

To overcome these issues, here we used three anxiety and seven memory tests (including allocentric/egocentric memory processing, object identity and cross-modal transfer tests) in the *same* animals from the Genetic Absence Epilepsy Rat from Strasbourg (GAERS), their relative inbred Non-Epileptic Control (NEC) rat strain and normal outbred Wistar rats. Our rationale was to a) investigate potential memory deficits of GAERS, b) establish whether such impairments are dependent on a high level of anxiety, and c) determine the learning strategies used by the epileptic and the two control strains. Our results showed that GAERS did not display exaggerated anxiety relative to controls with evidence of reduced anxiety in some tests, but showed deficits in spatial working and reference memory tasks and recognition memory (compared to both NEC and Wistar). Furthermore, assessment of learning strategies revealed that GAERS rats preferentially used egocentric strategies to perform spatial memory tasks. Finally, NEC rats showed reduced anxiety in the open field and a better performance in the Morris Water Maze (MWM), compared to Wistar. These results are discussed with reference to cognitive changes and potential networks alterations in GAERS rats.

2. Materials & methods

2.1. Animal subjects

Adult (3–6-month-old) GAERS and NEC male rats from the Cardiff (UK) colony and Wistar rats purchased from Charles River Laboratories (Lyon, France) were housed in groups of 3–4 animals in transparent plastic cages that contained cardboard tubes, nests, aspen woodblocks and bedding material. Animals were kept on a 12-h light-dark cycle (light on at 7.00 am) under constant temperature (22 °C) and humidity (80%) and were given ad libitum access to water and food (except in the preparatory phase of the tests that required mild food deprivation, see below). Animal age was equally distributed in the three strains in all tests. All experiments were conducted during the light phase of the light-dark cycle and in conformity with European Community Guidelines (Directive 2010/63/UE) and the UK Animal Scientific Act, and under general guidance for animal epilepsy experimentation (Lidster et al., 2016). Care was taken in minimizing the number and suffering of the animals.

All animals were acclimatized to the laboratory conditions for at least a week before the beginning of the experiments. Animals were then handled for 5 consecutive days (5 min/day) before the testing began (i.e. Figs. 1A, 3A, 4A). For each behavioural task, the rat testing order was randomized as was their starting position and the location of the object (where applicable). On the day of the experiments, the animals were placed for 1 h in the testing room for habituation and all equipment was cleaned with 30% ethanol between trials to remove olfactory cues. All trials were video-recorded using CamStudio and OBS studio® (V. 27.1.3, 480 × 640) and the video-tracking software, SMART PANLAB®, (V. 2.5 and V3.0.06, Panlab, Harvard Apparatus, Barcelona, Spain). The reference point to determine the position of the animal was the center of the rat dorsum. Off-line analysis of tracking was performed using Solomon Coder (V. beta 19.08.02).

2.2. Behavior tests

2.2.1. Elevated plus maze

The Elevated Plus Maze (EPM) was elevated 70 cm from the ground and consisted of two open arms (OA) crossing in the middle with two enclosed arms (CA) (Fig. 1 B1) (Pellow et al., 1985). Animals were placed in the centre of the maze facing an open arm and allowed to explore the maze for 5 min. Mild illumination was used (42 LUX). The total time spent in open arms (OA) and the total number of arm entries were measured. Twenty-four Wistar, 24 NEC and 26 GAERS were used in this test. The primary outcome was the time spent in open arms.

2.2.2. Emergence test

The apparatus of the Emergence Test (ET) consisted of a 50 × 50 × 50 cm floor arena with high black acrylic walls and a square box (20x20cm) in the middle with a cover and one lateral escape hole (Fig. 1 C1). Mild illumination (42 lx) was used. Rats were placed in the box and the box was then placed in the middle of the open field. The box was then closed and the time required to emerge completely from the box as well as the number of entries were recorded. Each animal performed a single trial of 10 min. Nineteen Wistar, 25 NEC and 26 GAERS were used in this test. The primary outcome was the latency to exit the box.

2.2.3. Open field test

The open field (OF) consisted of 50 × 50 x 50 cm arena with high black acrylic walls and the trials were 10 min long (Fig. 1 D1). Mild illumination was used (42 LUX). The arena was virtually divided into three different square zones – a peripheral zone, an intermediate zone, and a central zone. Each rat was placed in the center of the arena facing north, east, south or west in a randomly assigned manner. The percentage of time in the central zone, as well as entries in the central zone were measured, and the number of fecal boli counted. In addition, thigmotaxis, total distance covered, and average velocity were analysed as measures of locomotor activity. Twenty-four Wistar, 25 NEC and 26 GAERS were used in this test. The primary outcome was the permanence in the central zone.

2.2.4. Y-maze

Honig (1978) defined working memory as information retained on any single trial that is required for performance only for that trial (Honig, 1978).To test short-term spatial working memory, a Y-maze composed of three symmetric arms (a, b and c), with 120° between arms, was used (Fig. 2 A). The rat was placed at the end of a randomly assigned arm and allowed 8 min of free exploration of the apparatus. The total number of arm entries was recorded and the percentage of correct arm alternations (i.e., the animal visited a different arm than the one it arrived from) was quantified using the formula:

$$\%\text{correct alternations} = \frac{\text{Number of correct alternations}}{\text{Total entries}-2} \times 100.$$

Thirteen Wistar, 14 NEC and 14 GAERS were used in this test. The primary outcome was the percentage of correct alternations.

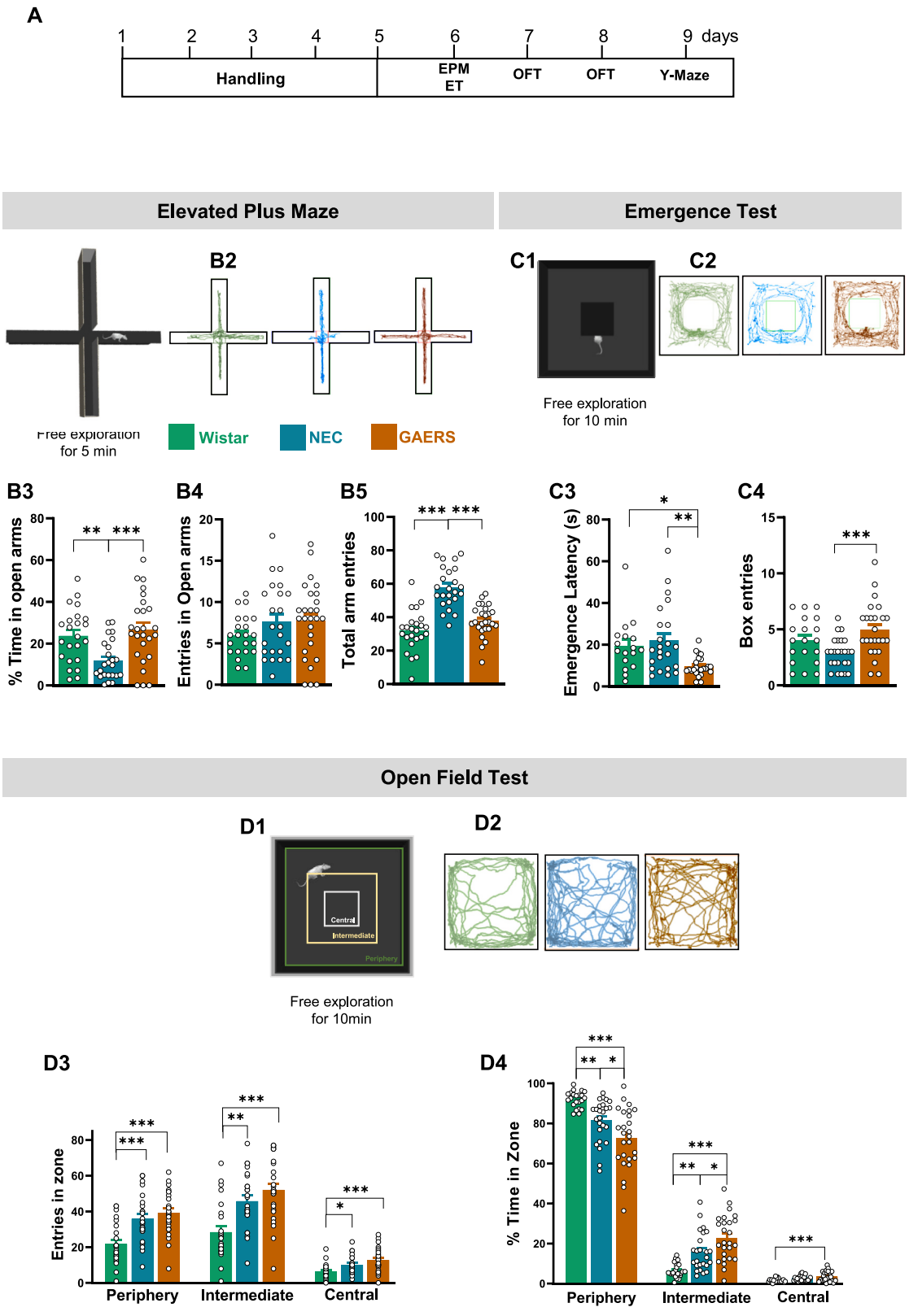


Fig. 1. Anxiety-like behavior in GAERS.
A) Timeline of the different tests. B1) Schematic drawing of the elevated plus maze (EPM). B2) Representative traces of the activity of a Wistar, a NEC and aGAERS rats during 5 min free exploration of the EPM. B3) Time spent in open arms as a percent of total testing time. B4) Number of entries in open arms. B5) Number of total entries. C1) Schematic drawing of the emergence test (ET) arena. C2) Representative traces of the activity of a Wistar, a NEC and a GAERS rat during 10 min free exploration of the arena and its central box. C3) Latency of emergence latency from the box. C4) Number of entries in the central box. D1) Schematic drawing of the open arena with indicated central, intermediate and peripheral zones that was used for the open field test (OFT). D2) Representative traces of the activity of a Wistar, a NEC and a GAERS rats during the 10 min free exploration of the arena. D3) Number of entries in different zones. D4) Time spent in each zone (Wistar $n = 24$, NEC $n = 25$, GAERS $n = 26$) (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, one-way ANOVA followed by Tukey pairwise multiple comparisons tests).

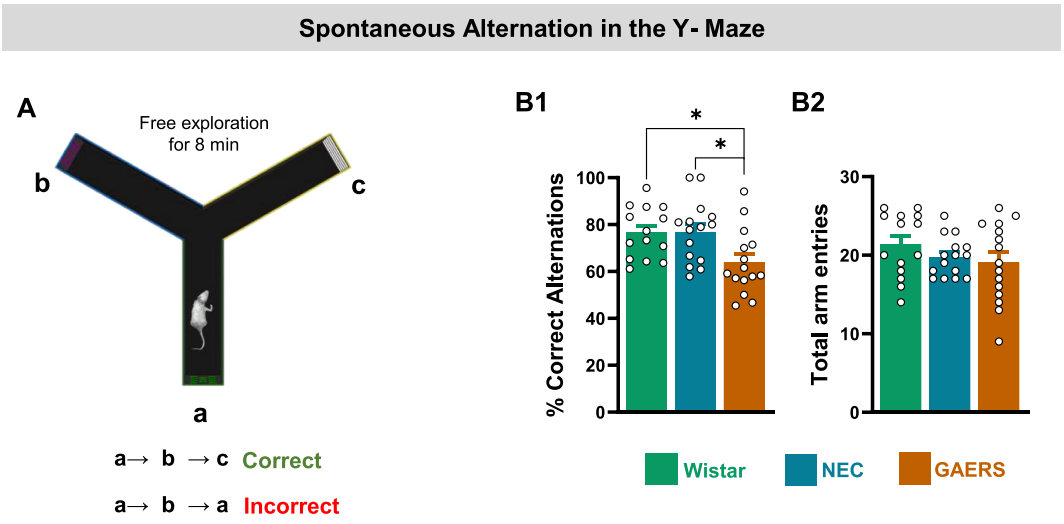


Fig. 2. Spatial working memory of GAERS in the Y-Maze.
A) Schematic drawing of the Y-Maze and types of alternations during the 8 min free exploration of the Y-Maze. B1) Number of correct alternations. B2) Total number of entries (Wistar $n = 13$, NEC $n = 14$, GAERS n = 14) (*p < 0.05, one-way ANOVA followed by Tukey pairwise multiple comparisons tests).

2.2.5. T-maze: Non-matching to place task (NMTP) and delayed non-matching to place task (DNMTP)

The T-maze was composed of a stem arm (50 cm) and two symmetric (40 cm) arms at 90° at one end (Fig. 3 B1). On day 3 of the handling period, food restriction began and body weight was recorded throughout the entire course of the experiments (Fig. 3 A). The animals were mildly food deprived to decrease their body weight to 85% of the initial free-feeding weight and were familiarize with the reward (chocolate biscuits cereals) during handling. On day 6, each rat was allowed two habituation periods in the T-maze in cage-mate pairs for 10 min, with rewards scattered all over the maze (approximately 1.5 treats/animal). On day 7, the animals received another habituation session in which the rewards were hidden in nests (constructed from newspaper sheets so that the animals were not able to see the reward) at the end of the goal arms. On days 8 and 9, spontaneous rewarded alternation was tested. The rats received 6 trials with an intertrial interval of 10 min, composed of 5 alternations. Initially, two rewards were hidden at the end of each goal arm, and the rat was allowed to choose: after eating from one arm the rat was removed and returned to the start arm and released. Spontaneous rewarded alternations were measured as Left (L) and Right (R) arm choices. If an animal entered an incorrect arm, it did not receive the reward and was removed from the maze.

The spatial non-matching to place task (NMTP) was performed on days 10, 11, and 12. In each session the animals received a total of 6 trials, 3 R arm trials, and 3 L arm trials. During the sample trial, access to one of the goal arms was blocked. After entering the open sample arm, the rat was contained in the arm for 10 s to consume the reward. The rat was then removed and immediately placed in the blocked start arm for 10 s before being released for the choice trial with both goal arms opened. A correct trial, i.e. the rat entered the opposite arm to the sample trial, was rewarded with access to two rewards at the end of the test arm. If the choice was incorrect, the animal was blocked in the incorrect arm for 15 s and then removed from the maze. The delayed non-matching place to task (DNMTP) was performed on day 13. A total of 6 trials, 3 R arms and 3 L arms were similarly performed but with a 2 min (instead of 10 s) interval. The intersection zone was considered as the mutual area between all arms. Latency to enter an arm was quantified as the time each animal took to reach the intersection zone. Six NEC and 6 GAERS were used in this test. The primary outcome was the percentage of correct choices.

2.2.6. Novel object recognition

The novel object recognition (NOR) test was conducted in an open field arena (65 × 65 cm) (Fig. 4 B1) The rats were habituated to the apparatus for 3 days, 10 min/day in the absence of any objects. Subsequently, all rats received an object sample trial (on day 9), and a novel/familiar object test trial (on day 10) (Fig. 4 A). The familiar or novel objects were randomized and the position relative to the other object was transposed on day 9. In the sample trial, the rat was placed in the arena with two familiar objects (*Pedras Salgadas*® water bottle and a *Cristal*® beer bottle) and allowed to freely explore the objects for 5 min. In the test trial (on Day 10), the rats were allowed to freely explore the open field containing a familiar object and a novel object. Exploratory behavior was quantified as the time touching or focusing an object (nose pointing in the direction of the object within a perimeter of 2 cm). The difference between time spent exploring novel and familiar objects in the total exploration time was defined as the novelty index (NI), and calculated as:

$$\text{Novelty index} = \frac{\text{Time exploring the novel object} - \text{Time exploring the familiar object}}{\text{Total time exploration (s)}}$$

Trials were rejected if the animal jumped on the object or explored each object for <10 s in the sample phase. The total time exploring each object and the frequency of interactions, as well as the percentage of novel object exploration were recorded. Nine Wistar, 6 NEC and 8 GAERS were used in this test. The primary outcome was the novelty index.

2.2.7. Cross-modal object recognition

A Y-Maze was used as the testing apparatus (Fig. 5 B1). Three distinct tests were used to assess tactile, visual, and cross-modal (CMOR)

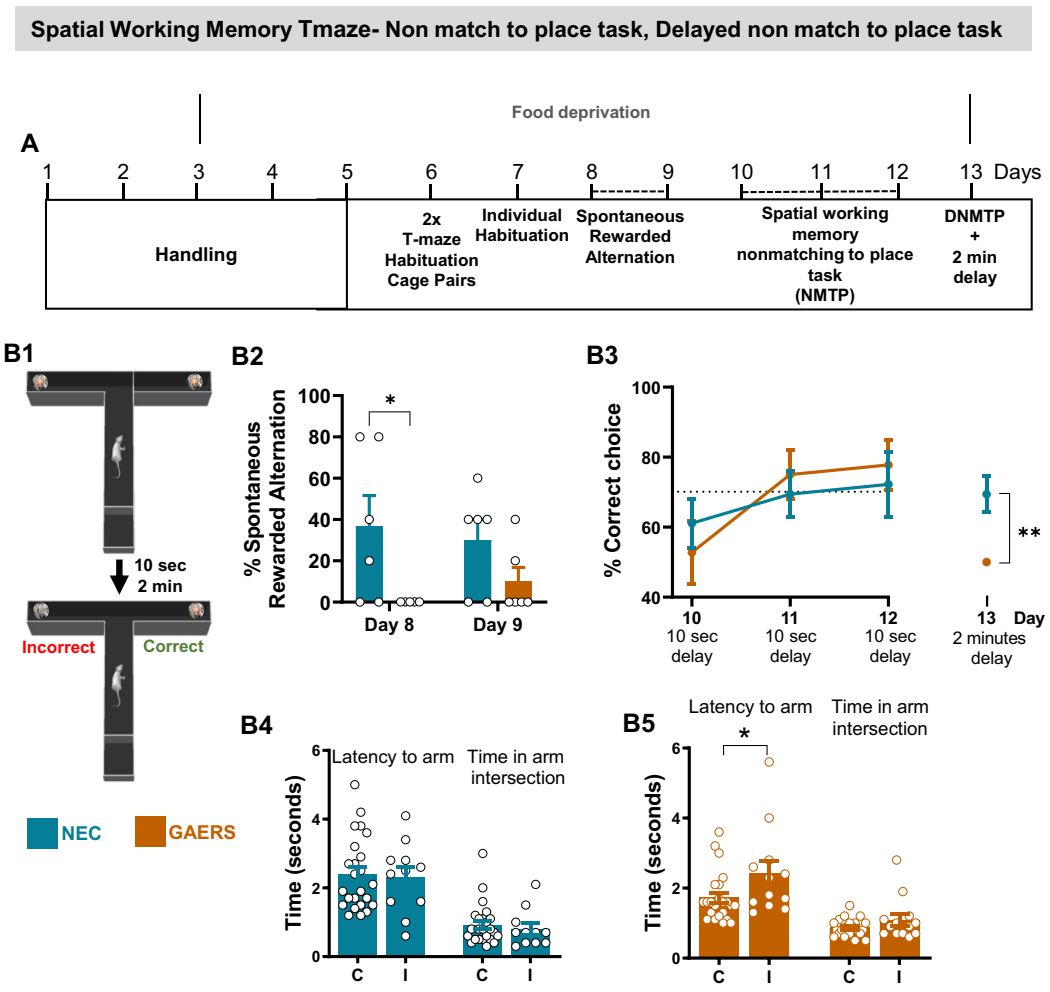


Fig. 3. Spatial working memory of GAERS in the DNMTP task.
A) Timeline of T-Maze experiments. B1) Schematic drawing of the T-Maze with delays between tests and illustrations of correct and incorrect choices. B2) Spontaneous rewarded alternations. B3) Correct alternations in different days with the indicated delays. B4) Latency to entry in an arm (left bars) and time spent in the intersection (right bars) for correct (C) and incorrect (I) choices by NEC. B5) As B4, but for GAERS (NEC $n = 6$, GAERS n = 6) (*p < 0.05, **p < 0.01; unpaired Students *t*-test (B2, B4, B5), two-way ANOVA followed by pairwise Tukey multiple comparisons test (B3).

memory, and for each trial two objects were placed at the end of two arms. Tactile exploration was conducted under red light illumination, thus preventing the rats from using the objects visual features (Marks et al., 2016). During the visual exploration (conducted under yellow light illumination), transparent acrylic barriers were inserted in front of the objects to avoid tactile exploration. Visual objects were previously validated for rats. Rats received 2 habituations, the first in pairs and the second individually for 10 min prior to the start of testing. For half of each habituation session, white and red illumination were separately presented. The day after the last habituation session, testing began. Each test was composed of a 3 min learning phase, followed by a 1 h delay and then a 2 min test phase. During the sample phase, the maze contained two identical objects at the end of each arm. In the test phase, the familiar object was paired with a novel object in the opposing arm (Fig. 5B 2). The order of testing was tactile, visual, and CMOR. The Novelty Index (NI) was calculated using the formula illustrated in the previous section. In this paradigm, visual recognition memory was described as more time spent with a novel object that had only been seen (not touched) during the sample phase. Tactile recognition memory is referenced as an increased tactile exploration of a novel object that had been previously touched (not seen) during the learning phase. Cross-modal recognition memory referred to a greater time spent looking at a novel object that had been touched but not seen, beforehand. The total exploration time in each trial, the NI, and the percentage of novel object exploration were analysed. Eight NEC and 8 GAERS were used in this test. The primary outcome was the novelty index for crossmodal variation.

2.2.8. *Morris water maze*

The Morris Water Maze (MWM) was a circular pool (180 cm in diameter and 60 cm in height) filled with warm water (24 ± 1 °C) (46 cm height) (Fig. 6 A1). A non-toxic water-based black paint was added to the water to render it opaque, and various visual cues were distributed over the walls of the room. The pool was virtually divided into four quadrants, and a 10 cm diameter black platform was hidden at the centre of one of the quadrants (1 cm below water level). Each animal was randomly allocated a training platform location that was maintained across training trials. Acquisition training was carried out over 4 days and was followed by a probe test on day 5 (Fig. 5 A1). During the acquisition phase, each animal received four trials per day. On each trial, the rat was placed in the pool, close to and facing the wall, and was never released from the same quadrant within a day. Individual trials lasted 60 s and finished when the animal reached the platform and remained on it for at least 10 s. If the animal did not find the platform or remained on it for 10 s, it was manually guided to the platform and remained on it for 20 s. A minimum interval of 30 min occurred between

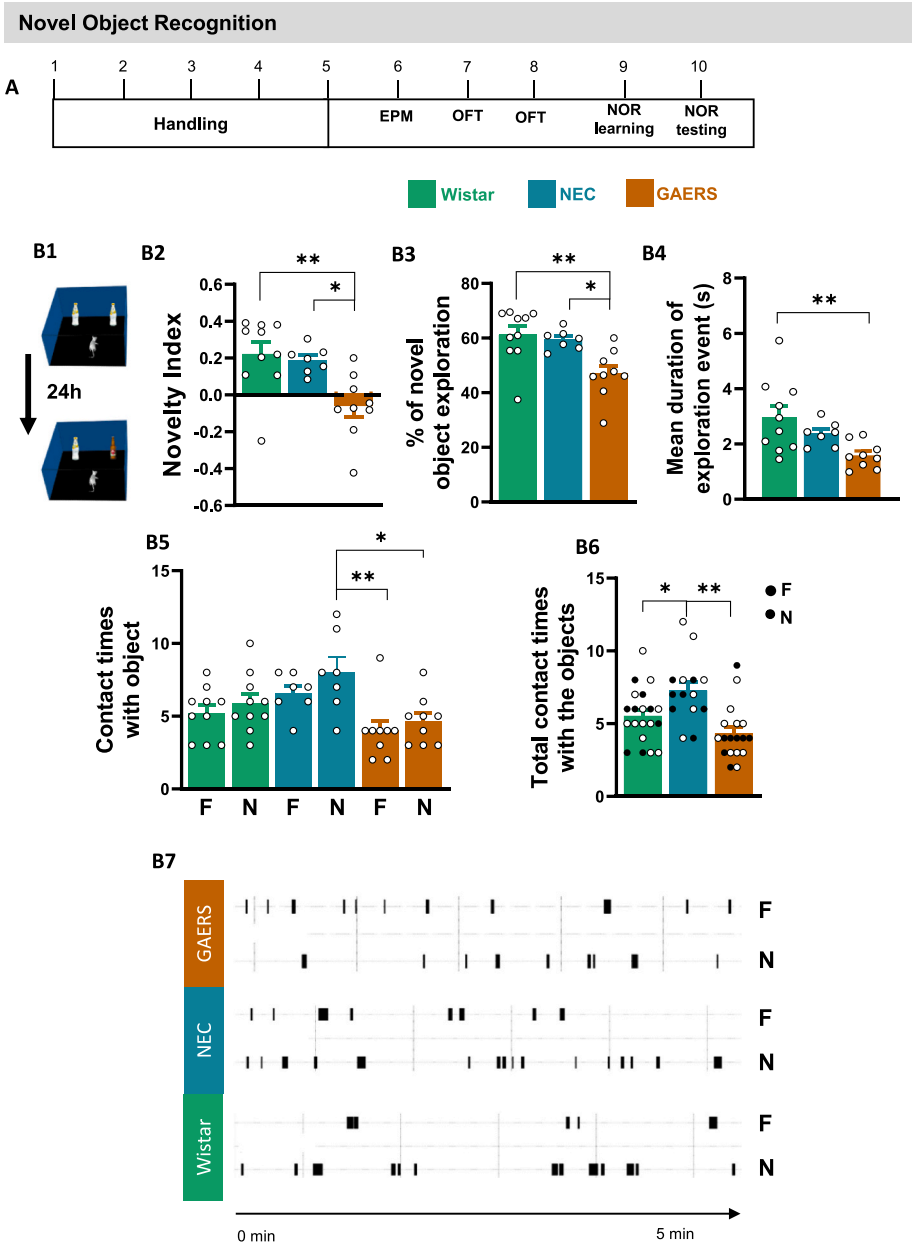


Fig. 4. Long-term recognition memory of GAERS in the NOR test.
A) Timeline of experiment. B1) Schematic drawing of the arena for the NOR test: rats were presented with a novel object 24 h after the learning session. B2) Novelty index (see Methods for details). B3) Time spent Percentage of time spent exploring the novel object. B4) Duration of individual exploration events of the novel object. B5) Contact times for novel or familial object for the different tested strains of animals. B6) Total contact times for the different tested strains of animals. B7) Full timeline of exploration of a representative GAERS, NEC and WISTAR rat (F: familiar object; N: novel object) (Wistar = 9, NEC n = 6, GAERS $n = 8$) (*p < 0.05, **p < 0.001; one-way ANOVA followed by Tukey pairwise multiple comparisons tests).

each trial. The time required for the rat to reach the platform in the 4 acquisition days was recorded. Upon being removed from the pool, the rats were towel-dried and remained in their home cages under a heat lamp to prevent hypothermia. For the single-trial probe test, the platform was removed and the animal was placed in the pool from a random start location and allowed to swim freely for 60 s. In the probe test, the time required to reach the location of the platform and the time the animal remained in that location was recorded.

Searching patterns during the acquisition and probe trials were analysed and seven swim strategies were differentiated: direct path, focal search, indirect search, chaining, scanning, random search and thigmotaxis. These patterns were grouped as allocentric search strategies: 1) direct (the rat performed nearly perfect trajectory to the platform with minimal deviation from a straight path); 2) focal (the search was focused around the platform); and 3) indirect (rat moved with a spatially directed search with a major directional error first). Non-spatial or egocentric-based strategies included: 4) chaining and/or scanning (rat perform a non-specific search at a fixed distance from the pool wall, or searched randomly but avoiding the pool walls); 5) random (rats moved with no directed spatial search); and 6) thigmotaxis (the rat moved along the pool wall) (Cooke et al., 2020; Curdt et al., 2022). Eight Wistar, 4 NEC and 11 GAERS were used in this test. The primary outcomes were the latency to target and the distance covered.

2.2.9. *Barnes maze*

The Barnes Maze consisted of a large (122 cm in diameter) circular platform placed under bright light (850 LUX) illumination with 20 holes around its circumference (Fig. 7 A1). During habituation, on the first day of the protocol, in the dark, the rat was placed in the middle of the maze in a start-box. After 10 s, the box was lifted, the light was turned on and the rat was trained to enter the escape (or target) hole without extra maze cues visible and remain in the underlying box for 1 min. Then, testing began: the rat was placed in the start-box for 10 s, then the box was lifted and the light was turned on, allowing maze exploration for 3

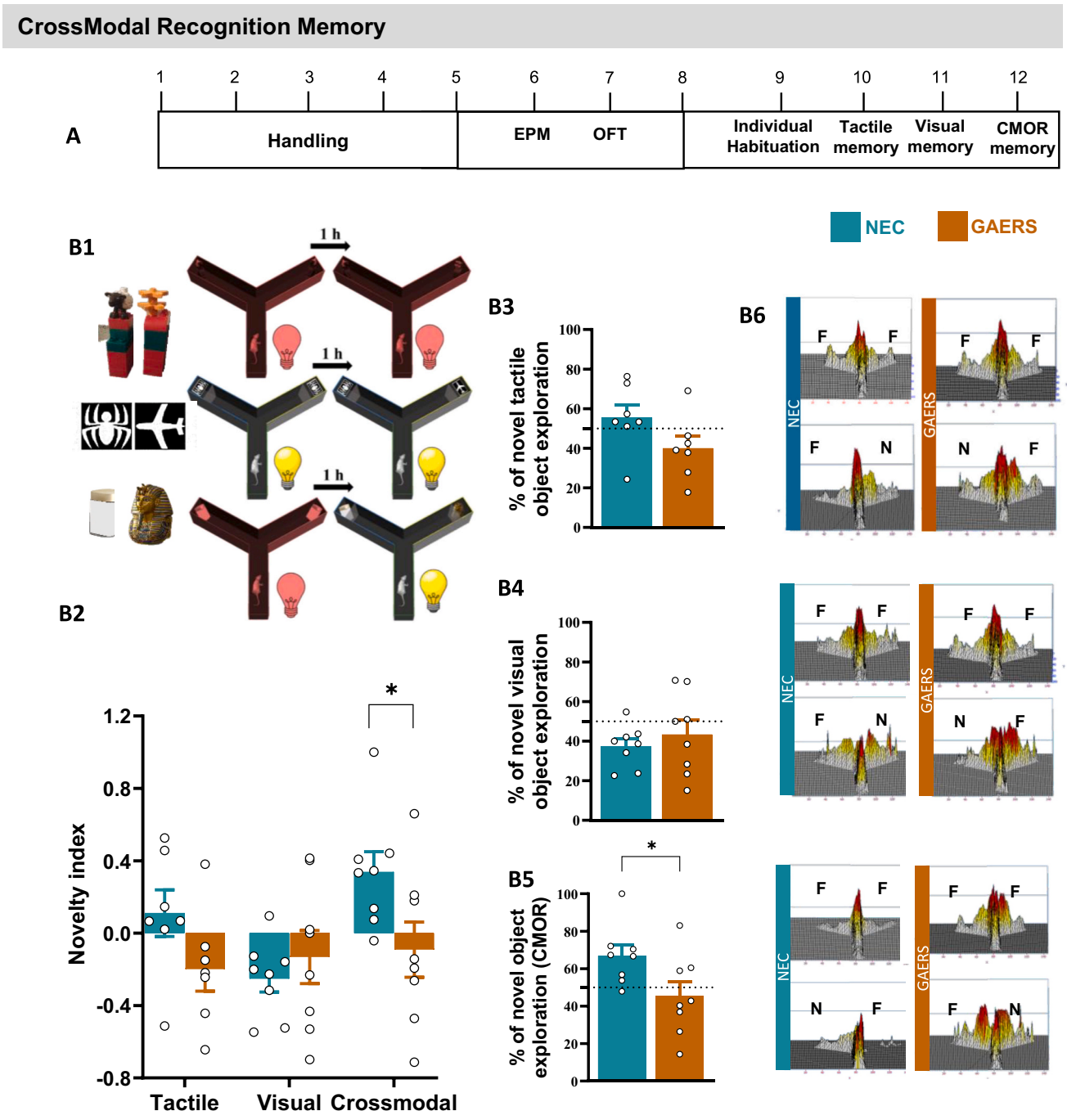


Fig. 5. Cross Modal recognition memory of GAERS.
A) Timeline of experiment. B1) Schematic diagram of texture discrimination test (top line, conducted under red illumination), visual discrimination (middle line, conducted under standard illumination) and crossmodal discrimination (bottom line) showing the objects used in the three tests. B2) Novelty index for 2 min test trial. B3–5) Percentage of novel tactile object, visual object and crossmodal exploration, respectively. B6) Heat-maps of the visual (top panels), texture (middle panels) and crossmodal (bottom panels) performance of a representative NEC and GAERS. Red represents increase activity of the animal in the maze, while white represents diminished activity of the animal in the maze (NEC n = 8, GAERS n = 8) (*p < 0.05; unpaired Students t-test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

min or until the rat entered the target hole. Immediately after entering the target hole, the light was turned off and the rat stayed inside for 1 min. The acquisition period lasted 4 days and consisted of 4 trials per day with a 15 min inter-trial interval (Fig. 7 A1).

The following were measured: latency to the target hole (i.e. time to find the hole), total path length (distance travelled to reach the target hole), speed, errors (i.e., nose pokes or head deflections over the non-target hole) and search strategy. To avoid confounding factors associated with exploration changes (e.g., if a rat learns the association between the spatial cue and target hole, but the number of errors and distance increase due to further exploration after visiting the correct location), we measured the primary latency, primary path length, and primary errors made to reach the target hole for the first time in a trial (Harrison et al., 2006).Navigation strategies were defined as either 1)

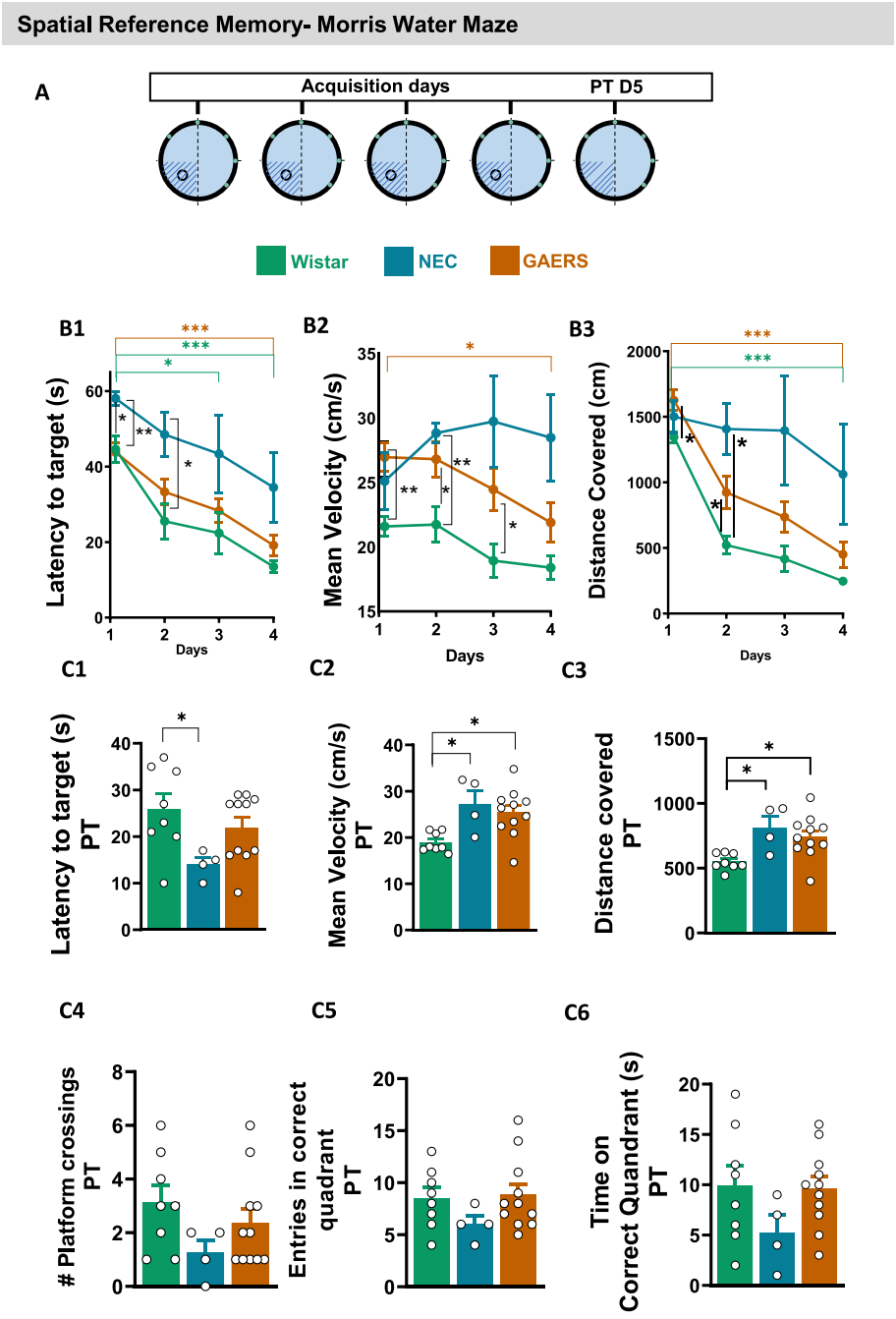


Fig. 6. Short-term spatial reference memory of GAERS in the MWM.
A) Timeline of the MWM test. B1-B3) Latency to target (i.e., time required to reach the platform), mean velocity and distance covered, respectively, during the acquisition phase (day 1 to day 4). C) Performance on the three strains during the probe test (PT) in day 5: latency to target (C1), mean velocity (C2), distance covered (C3), platform crossings (C4), entries in correct quadrant (C5) and time in the correct quadrant (C6) (Wistar n = 8, NEC $n = 4$, GAERS $n = 11$) (*p < 0.05, **p < 0.01, ***p < 0.001, two-way ANOVA for day and strain effect (B1-B3), one-way ANOVA followed by Tukey pairwise' multiple comparisons tests (C1-C6).

direct (the rat moved from the centre of the maze directly toward the target or initially moved away from the escape hole, but stopped after visiting one hole, and moved directly back to the escape hole; 2) serial (the rat moved around the periphery, making errors in holes adjacent to the escape hole; 3) random (the rat moved in an unsystematic manner, moving into the centre of the maze and visiting areas already visited). If the rat failed to enter the escape hole during the 3 min trial, it was considered a random strategy (Faraz et al., 2021).

The day after the last acquisition phase (i.e., day 5), the box underlying the target hole was removed and the rat performed a probe test for 90 s to measure 24 h retention of the spatial bias (day 5). Finally, long-term retention memory was tested on day 11 using the same protocol. Six Wistar, 6 NEC and 6 GAERS were used in this test. The primary outcomes were the primary latency, primary errors, and primary path length.

2.3. Statistics

Statistical significance was evaluated using GraphPad Prism (V. 8) for Windows® and Rstudio® (Version 2021.09.0 + 351). Data were reported in the text and figures as mean ± SEM of n independent observations in each experimental group. In behavior analysis, each rat's performance corresponds to an n value. The ROUT method was used for outlier identification. Unpaired two-tailed Student's *t*-test was used for independent samples, to perform two-sample comparisons. One-way ANOVA followed by Tukey's post-hoc test was used for multiple comparisons between more than two groups. Two-way ANOVA followed by Tukey's post-hoc test was used to detect interactions between strain and day (independent variables) on the dependent variables.

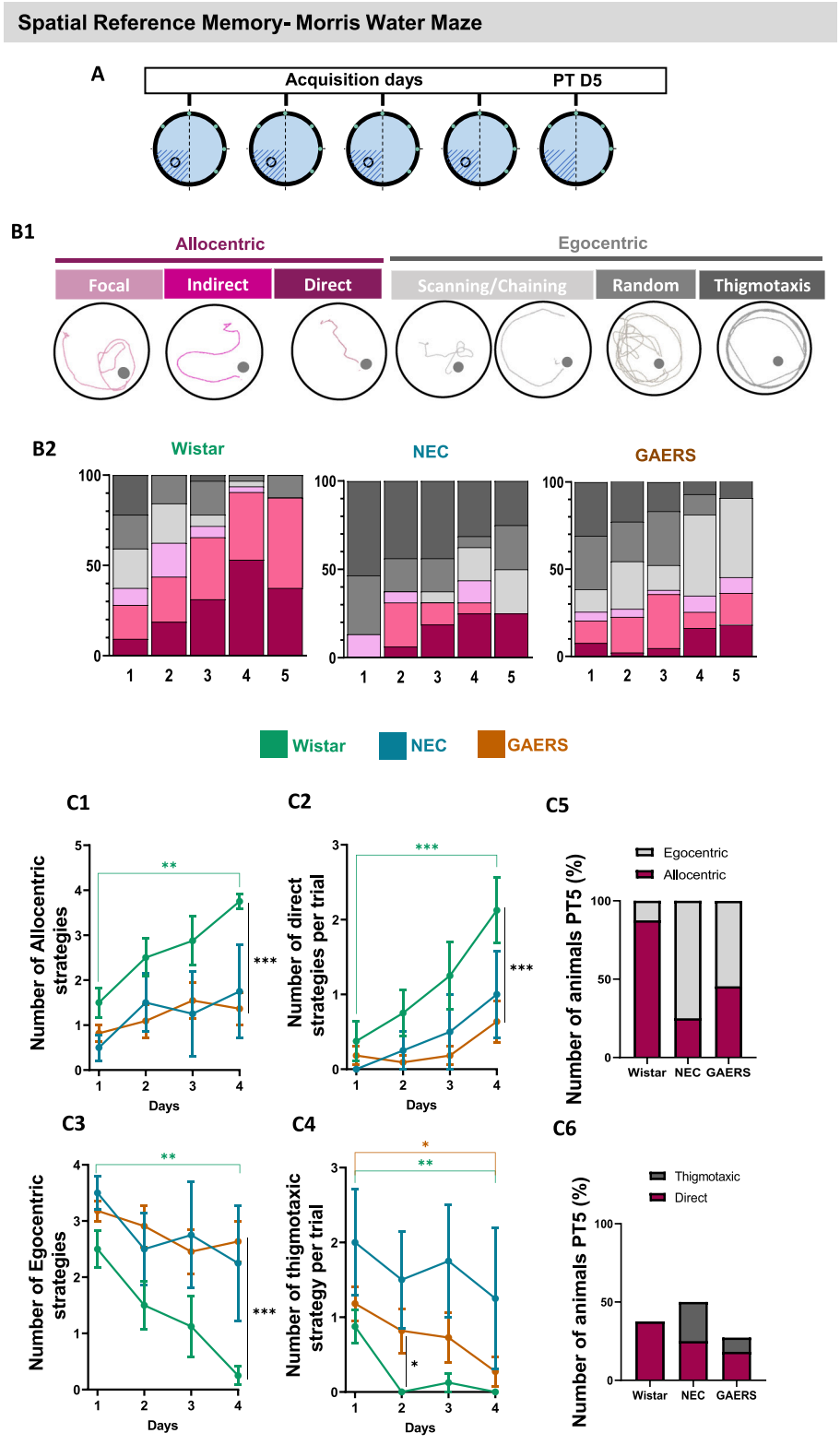


Fig. 7. Behavioural strategies of GAERS to reach the platform in the MWM.
A) Timeline of the MWM test. B1) Representative examples different allocentric and egocentric strategies used to reach the platform. B2) Percentage of rats employing different strategies (colour code as in B1) during the acquisition period (day 1–4) and in the probe test in day 5 (PT5). C1-C4) Strategies during the acquisition period: allocentric (C1), direct (C2), egocentric (C3) and thigmotaxic (C4). C5) Percentage of animals using allocentric or egocentric strategies in PT5. C6) Percentage of animals employing the thigmotaxic and direct strategies in PT5 (Wistar n = 8, NEC n = 4, GAERS n = 11) (*p < 0.05, **p < 0.01, ***p < 0.001, two-Way ANOVA for day and strain effect followed by Tukey pairwise' multiple comparisons tests).

3. Results

Since the performance in memory tests can be affected by anxiety and is highly test-sensitive (Ferreira et al., 2022), we first compared the anxiety-like phenotype of Wistar, NEC and GAERS rats using the EPM, the ET and the OFT.

3.1. Anxiety-like behavior of GAERS in the EPM

Following five days of handling (see Methods) (Fig. 1 A), analysis of the rat performance in the EPM (Fig. 1 B1), the most commonly used anxiety-like behavior test, showed a significant strain difference in the time spent in the OA (one-way ANOVA: $F_{2,70} = 8.51$; $p < 0.001$) (Fig. 1

B3) (see representative traces in Fig. 1 B2). Post-hoc comparisons indicated that the time spent in the OA by GAERS (26.8 ± 3.2%) was similar to that of Wistar (23.8 ± 2.7%; $p = 0.71$) and higher than that of NEC (11.9 ± 1.9%; $p < 0.001$). However, no strain difference was observed in the number of entries in the OA (one-way ANOVA: $F_{2,69} = 1.40$; $p = 0.252$) (Fig. 1 B4). In contrast, a significant strain effect was present for the total number of arm entries (one-way ANOVA: $F_{2,71} = 35.5$; $p < 0.001$), with post-hoc comparisons revealing a higher number of entries for NEC (57.7 ± 2.5) compared to both Wistar (31.8 ± 2.5; $p < 0.001$) and GAERS (37.7 ± 1.8; $p < 0.001$) (Fig. 1 B5). No difference was present between Wistar and GAERS in this parameter ($p = 0.16$).

In summary, in the EPM test GAERS exhibit a similar anxiety-like phenotype as Wistar, whereas no firm conclusion can be drawn about the NEC rats since they show a decreased time spent in the OA but more total arm entries.

3.2. Neophobia of GAERS in the ET

Before carrying out the OFT, we investigated the behavior of the three strains in the ET (see Methods) (Fig. 1 C1) which is a less anxiogenic adaptation of the OFT and allows to evaluate neophobia and exploratory behavior (see Fig. 1 C2 for representative exploration trace). One-way ANOVA found a significant difference between strains ($F_{2,61} = 7.02$; $p = 0.002$) for the latency to emerge from the central box, with GAERS having a significantly lower emergence latency (9.50 ± 1.0 s) than Wistar (19.5 ± 3.0; $p = 0.029$) and NEC rats (22.0 ± 3.4; $p = 0.002$) (Fig. 1 C3), indicating a decreased anxiety-like behavior and absence of neophobia. Moreover, the number of entries in the central box was significantly different among strains (one-way ANOVA: $F_{2,66} = 7.25$; $p = 0.001$), with the GAERS showing a higher number of entries in the central box 4.9 ± 0.5) compared to NEC (2.8 ± 0.3; $p = 0.001$) (Fig. 1 C4). No difference was observed between GAERS and Wistar rats ($p = 0.25$).

In summary, in the ET the GAERS rats show a lower anxiety-like behavior than Wistar and NEC rats, whereas NEC and Wistar have similar a similar anxiety phenotype.

3.3. Anxiety-like behavior of GAERS in the OFT

The OFT (Fig. 1 D1) was performed to measure spontaneous locomotor activity and anxiety-like behavior as a complement to the EPM on day 7 (Fig. 1 A) (representative traces are shown in Fig. 1 D2). One-way ANOVA for entries in the peripheral zone revealed differences between strains ($F_{2,72} = 14.0$; $p < 0.001$) with post-hoc comparisons showing that GAERS entered significantly more times in this zone than Wistar rats (39.3 ± 2.4 vs 21.8 ± 2.4; $p = 0.013$) (Fig. 1 D3). NEC also entered significantly more times in the periphery (35.9 ± 2.7 $p < 0.001$) compared to Wistar (Fig. 1 D4). For the intermediate zone, there were significant differences between strains ($F_{2,72} = 13.5$; $p < 0.001$) (Fig. 1 D3) with a larger number of intermediate zone entries for GAERS and NEC (52.1 ± 3.4 and 45.8 ± 3.1, respectively) compared to Wistar (6.2 ± 0.9; $p = 0.016$ and $p = 0.001$, respectively) (Fig. 1 D3). Finally, one-way ANOVA for entries in the central zone revealed a significant effect of strains ($F_{2,71} = 9.29$; $p < 0.001$) with GAERS and NEC showing a higher number of entries in the central zone (12.8 ± 1.3 and 10.2 ± 0.9, respectively) compared to Wistar (6.2 ± 0.9; $p < 0.001$ and $p = 0.032$, respectively) (Fig. 1 D3), which is indicative of a smaller anxiety-like behavior in the epileptic animals compared to normal Wistar rats.

We then analysed the time spent in the different zones. We found an effect of strain for the time in the peripheral zone ($F_{2,70} = 18.5$; $p < 0.001$) and post-hoc comparisons confirmed that GAERS spent less time (72.5 ± 2.9%) in the periphery compared to Wistar and NEC (92.0 ± 0.87%, $p < 0.001$ and 81.4 ± 2.1%, $p < 0.015$, respectively), with NEC to spending less time than Wistar ($p = 0.005$) (Fig. 1 D4). For the intermediate zone, one-way ANOVA revealed significant strain differences ($F_{2,70} = 19.6$; $p < 0.001$) with an increased time for GAERS (22.8 ± 2.2%) compared to Wistar (6.5 ± 0.8%; $p < 0.001$) and NEC (18.8 ± 1.9%; $p < 0.001$) (Fig. 1 D4). NEC also spent more time than Wistar in the intermediate zone ($p = 0.002$). For the percentage of time in the central zone, one-way ANOVA revealed a difference between strains ($F_{2,67} = 7.49$; $p = 0.001$), with GAERS spending more time in the central zone compared to Wistar (3.5 ± 0.5 vs 1.5 ± 0.2; $p < 0.001$) (Fig. 1 D4). The latter suggests reduced anxiety in epileptic animals. Finally, one-way ANOVA showed no strain effect for fecal boli ($F_{2,71} = 1.913$; $p = 0.155$), total distance travelled ($F_{2,69} = 0.388$, $p = 0.680$) and mean velocity ($F_{2,71} = 0.753$; $p = 0.475$) (Suppl. Fig. 1A1-3).

In summary, GAERS have a broadly similar pattern of neophobia as Wistar rats with some evidence of reduced anxiety as revealed by: 1) the longer time spent in the OA of the EPM (relative to NEC), 2) the shorter latency to emerge from the box and the larger number of box entries in the ET, and 3) the higher number of entries and the time spent in the central zone, as well as a lower time spent in the peripheral zone, of the OFT. No firm conclusion can be drawn for the anxiety-like behavior of NEC rats since they spent a smaller time in the OA of the EPM (indicative of higher anxiety) but show more entries in the intermediate and central zones than Wistar (suggestive of lower anxiety levels).

3.4. Spatial working memory

3.4.1. Spontaneous alternations of GAERS in the Y-maze

To determine spatial working memory, spontaneous alternations in the Y-Maze were investigated (Fig. 2 A) (see Methods). All strains (Fig. 2 A) showed a level of correct alternation performance that was above the chance level (i.e., > 50%). However, one-way ANOVA revealed significant differences between strains ($F_{2,41} = 5.17$; $p = 0.010$), with post-hoc comparisons showing a significant decrease in correct alternations for GAERS compared to Wistar (63.7 ± 3.58% vs 76.6 ± 2.94%; $p = 0.025$) and NEC (76.9 ± 3.36; $p = 0.019$) (Fig. 2 B1). Moreover, one-way ANOVA detected no difference in total arm entries between strains ($F_{2,41} = 1.23$; $p = 0.302$) (Fig. 2 B2).

In summary, GAERS rats showed a reduction in spontaneous alternation, a finding consistent with impaired working memory.

3.4.2. Spatial working memory of GAERS in the T-maze

To determine if the spatial working memory deficit of GAERS rats observed in the Y-Maze was not-test-dependent and if extended to other tasks within which alternation was explicitly rewarded, we investigated their performance in a T-Maze. The protocol was adapted from (Hussein et al., 2018) and carried out in mildly food-deprived animals with spontaneous alternations being reinforced by a reward present in the maze arms (see Methods) (Fig. 3 A).

The rat body weight was monitored daily to ensure that it remained at about 85% of the initial free-feeding weight (Suppl. Fig. 2A1,2) for the duration of the entire protocol, i.e., from day 3 to day 13 (Fig. 3 A). Two-way ANOVA of body weight revealed a significant main effect of day ($F_{12,142} = 38.04$; $p < 0.001$) and strain ($F_{1142} = 629.8$; $p < 0.001$). However, a strain-by-day interaction was not observed ($F_{12,142} = 1.735$; $p = 0.065$). Moreover, NEC had a higher body weight than GAERS throughout the test period ($p < 0.001$) (Suppl. Fig. 2A1).

During spontaneous rewarded alternation, GAERS showed a deficit in alternations compared to NEC for day 8 (Student *t*-test: $t_9 = 2.2$; $p = 0.05$) (Fig. 3 B2) but no difference was observed on day 9 ($t_{10} = 1.651$; $p = 0.13$) (Fig. 3 B2).

Performance on the NMTP task is shown in Fig. 3. Two-way ANOVA analysis of the NMTP task with a 10 s interval between day 10–12 indicated a significant effect of the day ($F_{1.8,18.03,29} = 4.774.091$; $p = 0.025$) but no significant effect of the strain ($F_{1,10} = 0.013$; $p = 0.$) or strain-by day interaction effect ($F_{2,20} = 0.812$; $p = 0.458$) (Fig. 3 B3). However, post-hoc tests did not show any significant differences between days. (Suppl. Table 1). When all animals had achieved a performance level above 70% of correct alternations, a retention interval of 2 min (between the sample and test runs, DNMTP) was applied on day 13 (Fig. 3 A). Unpaired Student's t-test revealed an impaired memory

retention in the GAERS compared to NEC (50 ± 0.0 vs 69.5 ± 5.1% correct choices; $p = 0.007$) (Fig. 3 B3) (Suppl. Table 1). Thus, increasing task difficulty with the introduction of a longer inter-trial delay revealed an impaired working memory in GAERS relative to NEC rats.

Notably, the strategy used by the two strains to perform the task was different. NEC displayed similar latency to the target arm in correct and incorrect trials (unpaired Student's t-test: $t_{33} = 0.215$; correct: 2.4 ± 0.22 s, incorrect: 2.3 ± 0.3 s; $p = 0.83$) and also spent the same amount of time in the intersection zone between the two arms for both correct and incorrect trials ($t_{33} = 0.543$; correct: 0.9 ± 0.1 s, incorrect: 0.8 ± 0.1 s; $p = 0.590$) (Fig. 3 B4). In contrast, GAERS showed a higher latency to arm entry on incorrect versus correct trials ($t_{33} = 2.099$; correct: 1.7 ± 0.2 s, incorrect: 2.4 ± 0.4 s; $p = 0.0436$). In contrast, no differences in the time spent in the intersection zone were found between correct and incorrect trials ($t_{32} = 1.471$; correct: 0.9 ± 0.1 s, incorrect: 1.1 ± 0.2 s; $p = 0.1510$) (Fig. 3 B5).

In summary, the GAERS showed more errors in a spatial DNMTP working memory task and displayed increased decision times on incorrect trials consistent with a poorer memory for recent arm entries.

3.5. Recognition memory of GAERS in the NOR

After 5 days of handling and 2 days of habitation to the OF arena (Fig. 4 A), the sample trial of the NOR test took place with two objects in the arena. This was then followed 24 h later by the familiar /novel test trial (Fig. 4 A,B) to assess long-term memory. In the testing phase, one-way ANOVA for total exploration time revealed significant strain differences ($F_{2,23} = 9.749$; $p < 0.001$), with post-hoc comparisons showing that GAERS exhibited a smaller total object exploration time compared to both NEC ($p < 0.001$) and Wistar rats ($p = 0.037$) (Suppl. Table 2). In the test trial, one-way ANOVA of the novelty index found a significant difference between strains ($F_{2,23} = 7.74$; $p = 0.003$), with post-hoc analysis showing that GAERS had a smaller novelty index (−0.06 ± 0.06) compared to both NEC (0.19 ± 0.03; $p = 0.019$) and Wistar (0.22 ± 0.06; $p = 0.003$) (Fig. 4 B2), not being able to discriminate the novel from the familiar object. This result was supported by a one-way ANOVA on the percentage of time spent in novel object exploration ($F_{2,23} = 7.74$; $p = 0.003$), with GAERS showing a smaller percentage of time exploring the novel object (47 ± 2.96%) than NEC (59.4 ± 1.38%; $p = 0.019$) and Wistar rats (61.1 ± 3.15%; $p = 0.003$) (Fig. 4 B3). Nevertheless, there was a difference between strains for the mean duration of novel object exploration ($F_{2,23} = 5.53$; $p = 0.01$) (Fig. 4 B4), with post-hoc comparisons showing that GAERS had smaller exploration time when they approached the novel object compared to Wistar ($p = 0.009$) (Fig. 4 B7). Notably, GAERS showed similar levels of contact with the familiar object as Wistar rats ($p = 0.077$) (Fig. 4 B5). NEC rats showed higher contact times (7.29 ± 0.6) than both Wistar (5.6 ± 0.42; $p = 0.04$) and GAERS (4.33 ± 0.43; $p < 0.001$) (Fig. 4 B6). Thus, the deficit in GAERS would not seem to simply reflect a global lack of interest in objects as contact times with the familiar were comparable to Wistar rat (Fig. 4 B6). Figure 4B7 shows a graphical representation of the time of exploration and contact times of the familiar and novel object for a rat from each strain.

In summary, GAERS rats exhibited poorer long-term object recognition memory relative to NEC and Wistar rats in the NOR test.

3.6. Cross-modal object recognition (CMOR) of GAERS

The CMOR test (Fig. 5 A) (adapted from (Winters and Reid, 2010)), included tactile-to-visual crossmodal object recognition (Fig. 5 B1) and representative 3D heat-maps of an animal's activity are shown in Fig. 5 B4). Unpaired Student's *t*-test analysis of total exploration time in the learning phase revealed no significant differences in total exploration time (Fig. 5 B2) (Suppl. Table 2). Nevertheless, unpaired Student's t-test revealed a significant strain difference in exploration time of the test phase for the visual (but not the tactile and the crossmodal) test (GAERS: 24.13 ± 3.17 s, NEC: 10.75 ± 0.70s; $t_{14} = 4.12$; $p = 0.001$) (Suppl. Table 2), with GAERS exploring objects more than NEC.

No significant differences between GAERS and NEC were observed in the novelty index and novel object exploration time for the tactile memory test (unpaired Student t-test: t_{12} 1.75; $p = 0.11$ for both) (Fig. 3 B2, B3). For the visual memory test, although it was assessed with validated objects, both strains were unable to distinguish between familiar and novel objects, displaying a negative novelty index (Fig. 3 B2) and a percentage of novel object exploration time < 50% (Fig. 5 B2, B4).

In contrast, GAERS showed a deficient CMOR memory compared to NEC. This was evident from their negative novelty index ($t_{14} = 2.26$; $p = 0.04$) (Fig. 5 B2) and the lower exploration time of the novel object (t_{14} 2.26; $p = 0.04$) (Fig. 5 B2, B5). Notably, GAERS did not perform above chance ($p = 0.56$) whereas NEC performed above chance level ($p = 0.009$) (Fig. 5 B5).

In summary, GAERS display deficits in short-term crossmodal recognition memory in the Y-Maze, which is not dependent on the exploration time.

3.7. Performance of GAERS in the Morris water maze

The Morris Water Maze (MWM) is one of the most widely used behavioural tasks for assessing allocentric spatial reference memory in rodents (Vorhees and Williams, 2006). After 4 days of training memory for the training quadrant was assessed in a probe test (with no escape platform) on day 5 (Fig. 6 A).

Two-way ANOVA of the latency to the platform during acquisition found significant effects of day ($F_{2.6,51.6} = 25.52$, $p < 0.001$) and strain ($F_{2,20} = 8.38$, $p = 0.002$), but no day x strain interaction was observed ($F_{6,60} = 0.43$; $p = 0.86$) (Fig. 6 B1). Post-hoc comparisons confirmed that the latency to the target significantly decreased during the four training days for all groups indicating that all animals improved their performance (Fig. 6 B1) (Suppl. Table 3). Post-hoc tests confirmed that the NEC exhibited the highest latency to target compared to Wistar and GAERS on day 1 ($p = 0.018$, $p = 0.002$, respectively) and compared to GAERS on day 2 ($p = 0.043$) (Fig. 6 B1) which was associated with higher maze exploration (Fig. 6 B3).

Regarding the mean velocity, two-way ANOVA found a significant effect for day ($F_{2.87,45.7} = 3.17$; $p = 0.045$) and strain ($F_{2,20} = 7.5$; $p = 0.004$) as well as an interaction of day × strain ($F_{6,60} = 2.75$; $p = 0.02$) (Fig. 6 B2) (Suppl. Table 3). Post-hoc comparisons confirmed that GAERS were significantly faster than Wistar rats on day 3 ($p < 0.044$) but there was no difference among strains on day 4 (Fig. 6 B2).

Concerning the distance covered, two-way ANOVA found a significant effect for day ($F_{2.49,49.7} = 40.55$; $p < 0.001$) and strain ($F_{2,20} = 8.8$; $p = 0.002$) as well as an interaction day x strain ($F_{6,60} = 3.44$; $p = 0.006$) (Fig. 6 B3). Post-hoc comparisons confirmed that the distance travelled differed between strains from day 1 to day 4 (Suppl. Table 3). However, on day 1 GAERS covered a longer distance to locate the platform compared to Wistar ($p = 0.016$) (Fig. 6 B3) (Suppl. Table 3). Post-hoc comparisons confirmed that a decrease in distance travelled was present in Wistar and GAERS from day 1 to day 4 ($p < 0.01$ for both strains) (Fig. 6 B3) (Suppl. Table 3).

In the probe test, there was a significant effect of strain ($F_{2,20} = 3,28$; $p = 0.059$) for the latency to the platform location, with NEC displaying a smaller latency than Wistar ($p = 0.047$) (Fig, 6C1). Moreover, there was a significant effect of strain for the mean velocity ($F_{2,20} = 6.5$; $p = 0.007$), with GAERS and NEC displaying higher velocity compared to Wistar ($p = 0.015$ and $p = 0.018$, respectively) (Fig. 6 C2). For total distance, there was a significant difference between strains ($F_{2,20} = 6.32$; $p = 0.007$), with post-hoc tests confirming distance was higher in NEC (812 ± 87.7 cm) and GAERS (741 ± 49.3 cm) compared to Wistar (550 ± 22.6 cm; $p = 0.016$ and $p = 0.020$, respectively) (Fig. 6 C3). No significant differences among strains were observed in the number of platform crossings (Fig. 6 C4) the number of entrances in the correct

quadrant (Fig. 6 C5) nor the time spent in the correct quadrant ($F_{2,20} = 1.59$; $p = 0.229$) (Fig. 6 C6).

We then investigated the strategies used by the three strains to find the platform during acquisition. There was a significant effect of allocentric strategies (i.e. direct, indirect and focal for day ($F_{4,109} = 20.16$; $p < 0.001$) and strain ($F_{2,109} = 17.32$; $p < 0.001$) as well as an interaction day x strain ($F_{8109} = 2.21$; $p = 0.032$). In particular, post-hoc analysis showed that from day 1 to day 4, Wistar increased the use of allocentric strategies ($p = 0.002$) (Fig. 7 C1) while decreasing the egocentric strategies (i.e. thigmotaxis, random and scanning/chaining) ($p < 0.001$) (Fig. 7 C3). Indeed, direct and thigmotaxic strategies, the most prominent of the allocentric and egocentric strategies, respectively, increased and decreased, respectively, during the acquisition period in Wistar ($p < 0.001$ and $p = 0.02$, respectively) (Fig. 7 C2, C4). In contrast, GAERS and NEC did not show any difference in the number of allocentric and egocentric strategies, which remained similar from day 1 to day 4 (Fig. 7 C1–C4). Indeed, on day 4, GAERS had a statistically smaller and larger number of allocentric and egocentric strategies to Wistar ($p < 0.001$ for both) (Fig 7C1, C3) as well as fewer direct strategies ($p < 0.001$) (Fig. 7 C2).

In the PT5, Wistar performed more allocentric strategies whereas NEC and GAERS mainly performed egocentric strategies (Fig. 7 C5). Moreover, both NEC and GAERS used a thigmotaxic strategy in the PT5 while Wistar did not (Fig. 7 C6).

In summary, the ability of the GAERS to remember the position of the platform is not different from both NEC and Wistar, as indicated by the similar latency to target, the number of entries and time in the correct quadrant (Fig. 6 C1, C5, C6), though they did display a higher velocity and distance travelled (Fig. 6 C2, C3). However, the performance of GAERS rats was not based on developing an efficient allocentric search strategy compared to Wistar, but similar to that of NEC, who, on the other hand, performed worse than Wistar. Thus, although the sample size was small, GAERS are aware of the existence of the platform but use a less effective, egocentric-based search strategy, suggestive of a reduced spatial reference memory to locate the platform.

3.7.1. Performance of GAERS in the Barnes maze

To determine if the changes in spatial navigation observed in the MWM extended to a different task with different motivational and motor requirements, we tested the animals on the Barnes maze.

Following four days of acquisition (see Methods), two probe tests were carried out, one on day 5 (to measure 24-h retention of the escape location) and a second on day 11 (to measure longer-term retention) (Fig. 8 A). During training, the primary latency (the time taken to initially reach the target hole location) was not different across strains (Fig. 8 B1). A two-way ANOVA of the primary latency found an effect for day ($F_{1.73,35.98} = 28.04$; $p < 0.001$) but not for strain ($F_{2,15} = 0.63$; $p = 0.55$) or interaction day x strain ($F_{6,45} = 0.82$; $p = 0.56$) (Fig. 8 B1) (Suppl. Table 4). Two-way ANOVA of the total latency (the total time taken to enter the hole) found significant main effects of day ($F_{3,60} = 6.6$; $p < 0.001$) and strain ($F_{2,60} = 32.91$; $p < 0.001$) but no interaction day x strain was observed ($F_{6,60} = 0.95$; $p = 0.46$). Post-hoc comparisons revealed that NEC exhibited the highest total latency compared to Wistar and GAERS on day 4 and GAERS exhibit highest latency compared to Wistar (Suppl. Table 4) (Fig. 8 B2).

Two-way ANOVA of primary path length (i.e. the path length required to initially reach the target hole) revealed a main effect of day ($F_{2,30} = 19.99$; $p < 0.001$) and an interaction of day x strain ($F_{6,45} = 2.64$;$p = 0.028$) but not an effect of strain ($F_{2,15} = 2.77$; $p < 0.095$). NEC showed a decreased primary path length between day 1 (604.34 ± 93.88) and day 4 (147.32 ± 14.86 cm, $p = 0.0173$) (Fig. 8 B3). For total path length (i.e. the length required to enter the hole), two-way ANOVA found significant effects of day ($F_{2.55,38.29} = 8.92$; $p < 0.001$) and strain ($F_{2,15} = 37.8$; $P < 0.001$) but no interaction of day x strain ($F_{6,45} = 1.77$; $p = 0.13$), with Wistar significantly decreased their total path length (Fig. 8 B4).

Regarding primary errors (i.e. the number of errors made before reaching the target hole on the first occasion), two-way ANOVA revealed a main effect of day ($F_{3,60} = 16.05$; $p < 0.001$) but not strain ($F_{2,60} = 0.54$; $p = 0.58$) or interaction of day x strain ($F_{6,60} = 0.38$; $p = 0.89$) with NEC being the only strain that significantly decreased their primary errors from day 1 to day 4 ($p < 0.001$) (Fig. 8 B5). Surprisingly, however, GAERS showed a trend of increased primary errors (suggestive of a worsened performance on the last day of acquisition), though this was not statistical significance due to the large variability (Fig. 8 B5). For the total errors (i.e., the number of errors before entering the target hole), two-way ANOVA revealed the main effect of day ($F_{2.8,52.7} = 10.44$; $p < 0.001$) and strain ($F_{2,15} = 13.33$; $p < 0.001$) but no interaction day x strain ($F_{6,45} = 1.05$; $p = 0.4$) (Fig. 8 B6). Post-hoc comparisons confirmed that NEC committed more total errors, (similar to their higher exploration in the MWM). Moreover, from day 1 to 4, Wistar total errors significantly decreased ($p < 0.01$), while this was not observed for GAERS ($p = 0.246$) (Fig. 8 B6).

For the hole deviation score (see Methods; the lower the score, the higher the proximity of the escape hole) two-way ANOVA found a main effect of day ($F_{3,60} = 3.24$; $p = 0.028$), but not for strain ($F_{2,60} = 2.95$; $p = 0.06$) or interaction day × strain ($F_{6,60} = 0.27$; $p = 0.95$). Moreover, NEC showed a significant decrease from day 1 to day 4 ($p = 0.033$) (Suppl. Fig. 3).

In the first (24 h) probe test (day 5; Fig. 8 A), one-way ANOVA of the primary latency did not find differences between strains ($F_{2,15} = 3.54$; $p = 0.55$), though there was a tendency of a higher latency for GAERS compared to NEC ($p = 0.059$) (Fig. 8 C1). On the second 6-day retention probe test (day 11), one-way ANOVA for primary latency found a difference between strains ($F_{2,12} = 8.92$; $p = 0.004$) with the post-hoc tests showing that GAERS took longer to reach the target hole compared to Wistar (28.2 ± 6.4 s vs 5.40 ± 0.7 s; $p = 0.027$) and NEC (5.3 ± 1.4 s; $p = 0.012$) (Fig. 8 C2). Moreover, on PT5 and PT11, there was no difference in primary length ($F_{2,15} = 1.65$; $p = 0.23$ and ($F_{2,15} = 0.458$; $p = 0.64$, respectively) (Fig. 8 C3, C4). On PT5, no differences were found between strains for primary errors ($F_{2,15} = 2.99$; $p = 0.08$) (Fig. 8 C5). However, there was a difference between strains in the primary errors on PT11 ($F_{2,13} = 8.06$; $p = 0.005$), with post-hoc tests showing that GAERS had a higher number or errors compared to both control strains (GAERS: 6.17 ± 1.62; Wistar: 0.20 ± 0.20; NEC 1.20 ± 0.80; $p = 0.007$ and $p = 0.022$, respectively) (Fig. 8 C6). On PT5, there was a significant effect of strain for total errors ($F_{2,15} = 9.9$; $p = 0.002$), with post-hoc tests revealing a higher number of errors for GAERS (12.0 ± 0.52) compared to Wistar (6.67 ± 1.43; $p = 0.002$) and NEC (7.50 ± 0.428; $p = 0.009$) (Fig. 8 C7) (with no difference between Wistar and NEC). The same was observed in the PT11 ($F_{2,14} = 8.30$; $p = 0.004$), indicating that GAERS performance is worse compared to both control strains (GAERS: 19.8 ± 0.74; Wistar:11 ± 1.63; NEC: 11.30 ± 2.09; $p = 0.007$ and $p = 0.009$, respectively) (Fig. 8 C8).

In PT5, two-way ANOVA found a main effect of pokes ($F_{19,300} = 41,47$; $p < 0.001$) and interaction of hole x strain ($F_{38,300} = 2.13$; $p < 0.001$) with post-hoc tests showing that Wistar and NEC performed significantly more visits to the target hole than GAERS ($p < 0.001$ for both) (Fig. 8 D1). In PT11, two-way ANOVA found a main effect of hole ($F_{19,300} = 7.09$; $p < 0.001$) and strain ($F_{2,300} = 6.69$; $p < 0.001$) but no interaction hole × strain ($F_{38,300} = 0.95$; $p = 0.56$) (Fig. 8 D2). Post-hoc comparisons confirmed that all strains had the same pokes in the target hole, though, unexpectedly, GAERS displayed a preference for hole 5 compared to NEC ($p = 0.044$) (Fig. 8 D2), the hole adjacent to the door of the experimental room.

We next investigated the strategies used to perform the task. During the acquisition phase, two-way ANOVA of direct strategies found a main effect of day ($F_{2.1,31.1} = 10.89$; $p < 0.001$): post-hoc comparisons showed that NEC, but not Wistar and GAERS, significantly improved the number of direct strategies from day 1 to 4 ($p = 0.01$) (Fig. 9 B2, C1). Regarding the use of egocentric strategies (i.e. serial and random), a significant effect of day was observed ($F_{2.1,31.1} = 10.89$; $p < 0.001$): post-hoc

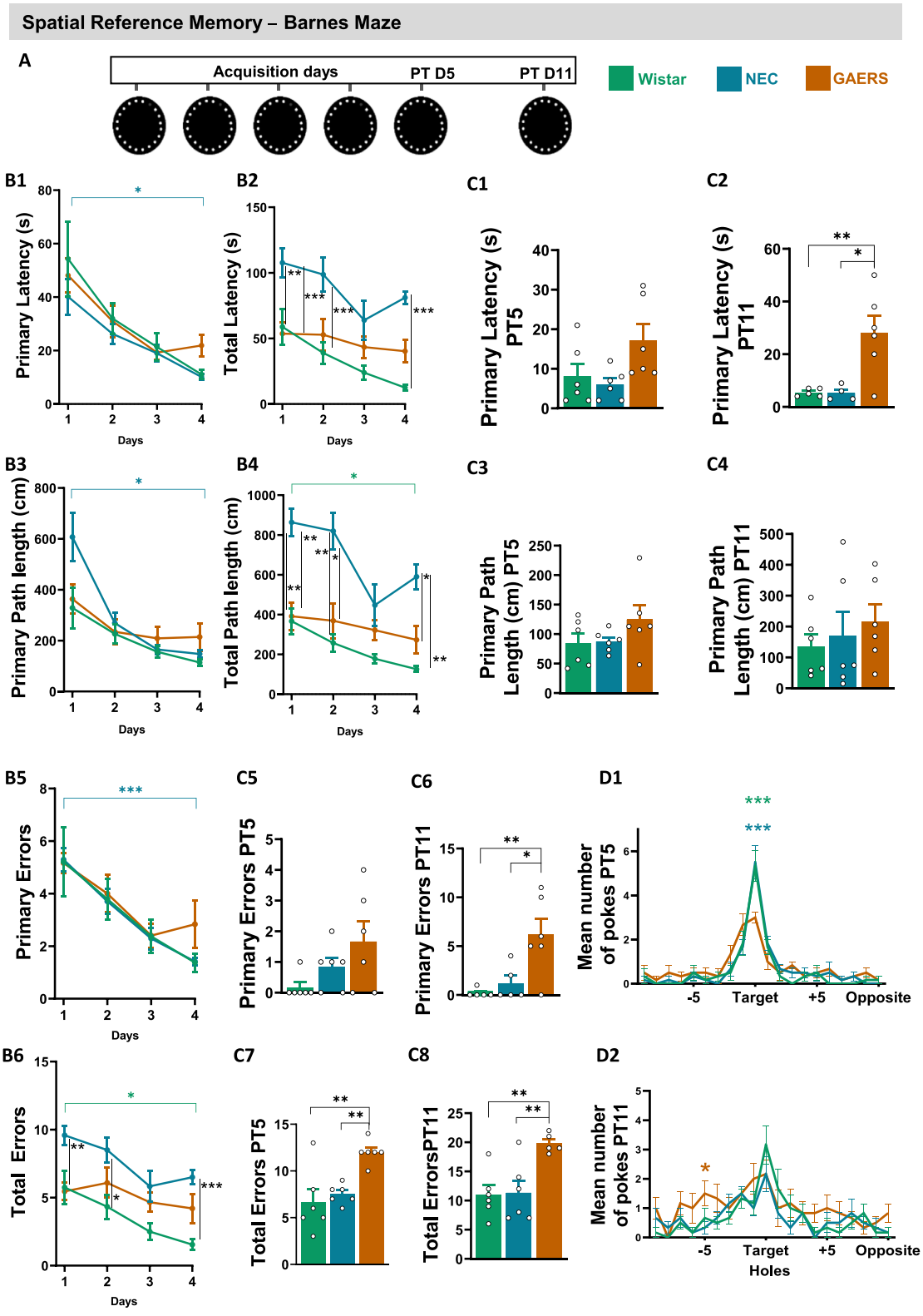


Fig. 8. Short term and long-term spatial reference memory of GAERS in the Barnes maze test.
A) Timeline of the experiment in the Barnes maze. B1-B6) Results of the acquisition period: primary latency (B1), total latency (B2), primary path length (B3), total path length (B4), primary errors (B5) and total errors (B6). C1-C8) Results of the probe test on day 5 (PT5) and on day 11 (PT11): primary latency (C1, C2), primary path length (C3, C4), primary errors (C5, C6) and total errors (C7, C8). D1, D2) Number of pokes in all holes of the maze for PT5 and PT11 (Wistar n = 6, NEC n = 6, GAERS n = 6) (*p < 0.05, **p < 0.01, ***p < 0.001, two-way ANOVA for day and strain effect (B3–6, D1–2), one-way ANOVA followed by Tukey pairwise' multiple comparisons tests (C1–8)).

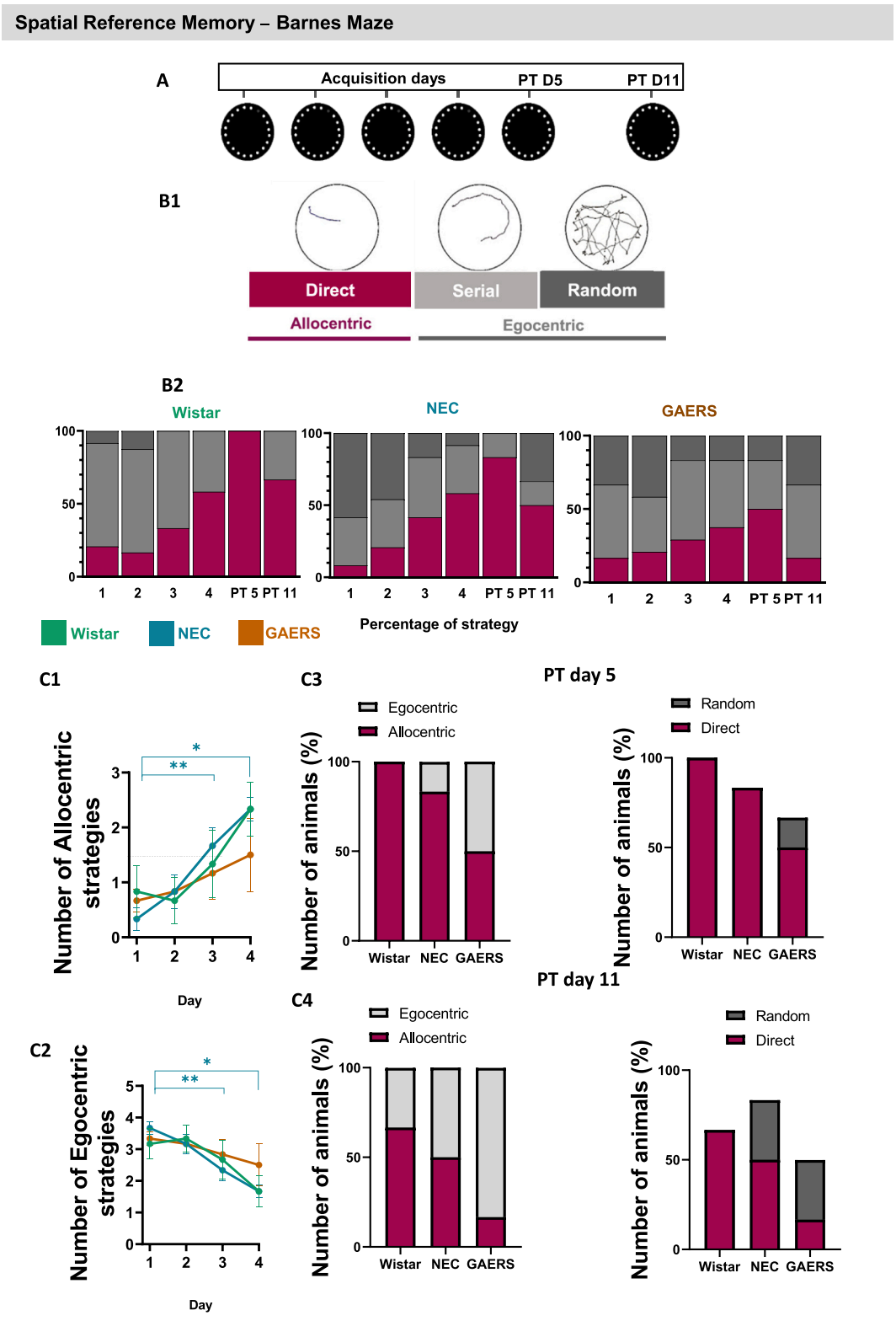


Fig. 9. Behavioural strategies of GAERS in the Barnes Maze.
A) Timeline of the experiment in the Barnes maze. B1) Strategies used in the Barnes maze. B2) Percentage of animals performing different strategies (colour codes as in B1). C1, C2) Allocentric and egocentric strategies, respectively, during the acquisition period. C3, C4) Percentage of animals using allocentric and egocentric strategies (left plots) and random and direct strategies (right plots) during probe test (PT) in day 5 (top plot) and day 11 (bottom plots) (Wistar n = 6, NEC n = 6, GAERS n = 6) (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, two-way ANOVA for day and strain effect followed by Tukey pairwise' multiple comparisons tests.

comparisons showed that a significant decrease for NEC($p = 0.01$) (Fig. 9 C2). On PT5, Wistar only performed direct strategies and NEC displayed both direct and serial strategies. In contrast, GAERS exhibited all three types of strategies and were the only strain exhibiting random strategy (Fig. 9 B2, C3). On PT11, a higher number of Wistar and NEC rats used a direct strategy, whereas most GAERS employed egocentric strategies (Fig. 9 B2, C4). Moreover, no Wistar used a random strategy whereas the same number of GAERS and NEC employed a random strategy (Fig. 9 C4).

In summary, short-term (i.e., 24 h) and 6 day retention of memory for the escape location was impaired in GAERS compared to Wistar and NEC as indicated by a larger number of total errors and pokes in the target hole. Moreover, GAERS used both allocentric and egocentric strategies whereas Wistar only used a direct (i.e. allocentric) strategy. Long-term (i.e., 6 days) memory of GAERS rats was also compromised as indicated by a larger primary latency and a higher number of primary and total errors compared to both Wistar and NEC rats. Moreover, more GAERS rats used egocentric strategies to find the target hole compared to Wistar and NEC rats which used a similar proportion of allocentric and egocentric strategies.

4. Discussion

The major findings of this study, which investigated anxiety as well as short and long-term memory in the *same* animals, is that GAERS show deficits in working, spatial reference and recognition memory (compared to both NEC and Wistar) and use different spatial learning strategies. Furthermore, GAERS rats do not show an exaggerated anxiety-like phenotype (Fig. 10A, B). Moreover, in tests requiring spatial cues, GAERS preferentially use egocentric strategies both during the acquisition period and in the test trial. NEC rats show higher anxiety-like behavior in one (out of three) tests, a better memory performance in one (out of seven) memory tests and mainly use egocentric and allocentric strategies in the MWM and the Barnes Maze test, respectively, compared to Wistar (Fig. 10C).

4.1. Anxiety

Since anxiety, neophobia and motor deficits may impact performance in memory tests (Ferreira et al., 2022), we investigated these traits using three behavioural tests, the EPM, the ET and the OFT, before testing the animals in the memory tests. In the OF test, the GAERS locomotion parameters were similar to both control strains, indicating the absence of any deficit in motor behavior. The greater time and number of entries into the central zone of the OF indicate that the anxiety level in these epileptic animals is lower than that in NEC and Wistar. This was also confirmed by GAERS' increased time in the open arms of the EPM compared to Wistar. Finally, the shorter emergence latency in the ET, compared to the control strains, supports the lack of neophobia in these animals. Overall, the results indicate that GAERS rats do not display exaggerated anxiety. Indeed, there was evidence of a reduction in anxiety in the OFT and ET tests, which may reflect disinhibition or impulsivity in GAERS rats. A similar disinhibition syndrome has been described in children with childhood absence epilepsy together with deficits in attention (Cerminara et al., 2013; J. E. Marques-Carneiro et al., 2016), as well as it has been reported no substantial changes in attention (or response inhibition) in GAERS rats using the 5-choice serial reaction time task. Nevertheless, further experiments are required to establish whether putative changes in impulsivity are observed in impulsive-choice tasks, such as the delayed discounting procedure (Cerminara et al., 2013; Doremus-Fitzwater et al., 2012; Marques-Carneiro et al., 2016), to establish whether GAERS rats show similar issues in impulsivity control as displayed by children with absence seizures.

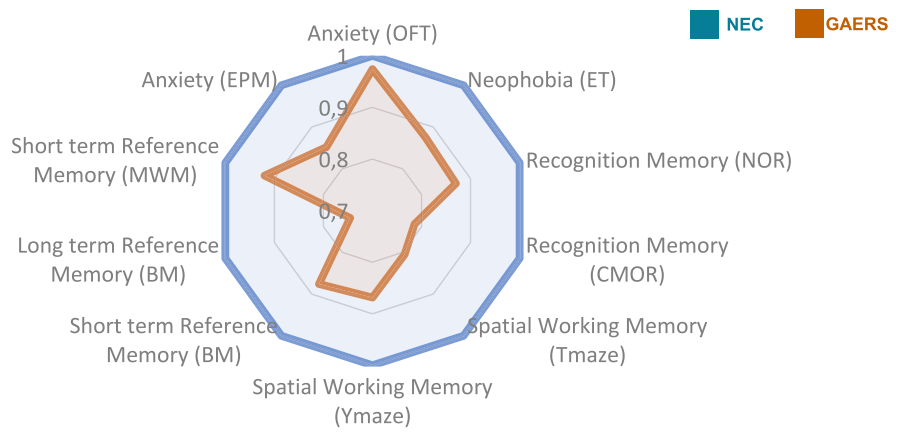
It is important to note that genetic drifts occur in an inbred colony and the level of environmental enrichment of the housing cage can affect the results of behavioural experiments (Bouwknecht et al., 2007; Powell et al., 2014). Indeed, studies in other GAERS colonies has shown contrasting results for anxiety-like behavior. A higher anxiety (compared to NEC) was reported in the GAERS Melbourne colony tested in EPM, a square and a circular OFT, the original Strasbourg colony tested in the EPM and the Canadian colony tested in the EPM (Bouilleret et al., 2009; Jones et al., 2008; Marks et al., 2016; Marques-Carneiro et al., 2014; Studer et al., 2019). In contrast, the Maltese GAERS colony tested in the EPM and the hole-board show no anxiety-like phenotype (Cassar et al., 2022; De Deurwaerdère et al., 2022; Marques-Carneiro et al., 2014; Studer et al., 2019). Notably, although two control strains were used in two previous publications by (Marques-Carneiro et al., 2014, 2016), these authors used different groups of animals to test anxiety and memory while in this study we used the *same* animals to test anxiety and memory/cognition.

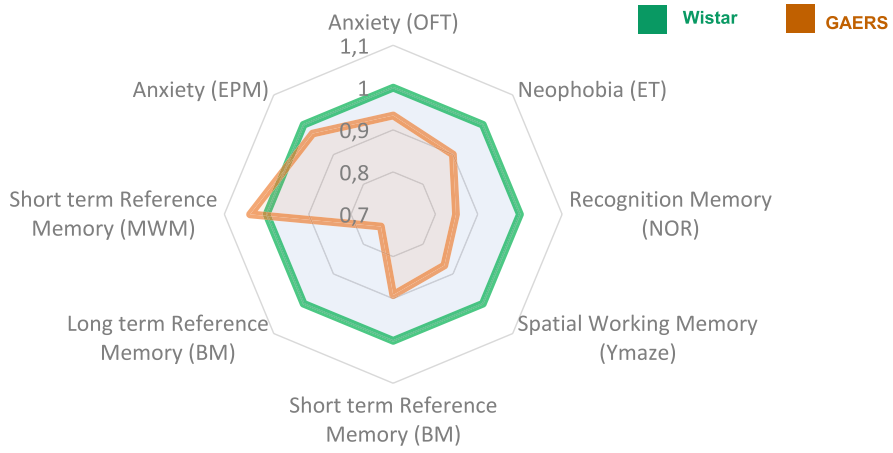
Moreover, the WAG/Rij rats (Sarkisova and van Luijtelaar, 2011) are less anxious than their respective non-epileptic controls (Brock et al., 1996). Thus, except the Melbourne, Strasbourg and Canadian GAERS colonies, all other colonies of this epileptic strain and other mouse and rat AS models have either similar or lower anxiety levels than their relative control strains. This is in agreement with the present results showing that there is a lower anxiety-like behavior in our GAERS compared to both NEC rats (where genetic drifts may be present) and outbred Wistar rats. Moreover, this provides strong support to our conclusion that the memory deficits observed in the very same GAERS of our colony (see below) do not depend on an anxiety-like phenotype. The performance of GAERS rats on the battery of memory tasks is not confounded by exaggerated fear/anxiety, though the reduction in some measures of anxiety might have an impact on their performance in exploration-based tasks (see below).

There is no information regarding the existence of a specific GAERS subgroup that exhibits higher anxiety levels, similar to the subgroup of WAG/Rij rats that are susceptible to audiogenic seizures and have a higher level of anxiety (Sarkisova and Kulikov, 2006). Consequently, a potential approach might be to initially compare GAERS with Wistar and/or NEC in terms of anxiety-like behavior and categorize them on the basis of their high and low anxiety-like traits. This comparative analysis may help establish a correlation between anxiety-like behavior and cognitive performance, such as a cognitive score. Moreover, our results about GAERS having a less anxious phenotype in three out of three tests compared to NEC and two out of three tests compared to Wistar indicate that in this respect this model does not reproduce this comorbidity observed in children.

4.2. Working memory

Deficits in working memory were detected in the spontaneous alternation in the Y-Maze and the spontaneous rewarded alternation in the T-Maze of GAERS compared to Wistar and NEC, in agreement with their lower performance in the acquisition trials of the Barnes Maze. These results contrast with those of Marques-Carneiro et al. (2016) who found no working memory impairments of GAERS rats in spontaneous alternation in the T-maze, though these authors did not test their performance in the Y-maze. Whereas this difference may be related to different GAERS colonies, it is more likely that the inability of Marques-Carneiro et al. (2016) to observe a working memory deficit in the Y maze could be due to the smaller number of alternations performed in their tests compared to ours or the decreased number of alternations, indicative of a reduced exploration. We also found that GAERS were deficient (compared to NEC) when task difficulty was increased by the introduction of a 2 min delay in the T-maze DNMTP task which requires memory retrieval (Barnes et al., 2004). GAERS rats also showed a higher latency to perform the choice response on trials when a wrong response was made, in contrast to NEC that showed no difference in latency between correct and wrong trials. Notwithstanding, we cannot exclude the possibility that the animals smelling the reward might be a limitation of this test. Moreover, GAERS spend more time in the intersection between





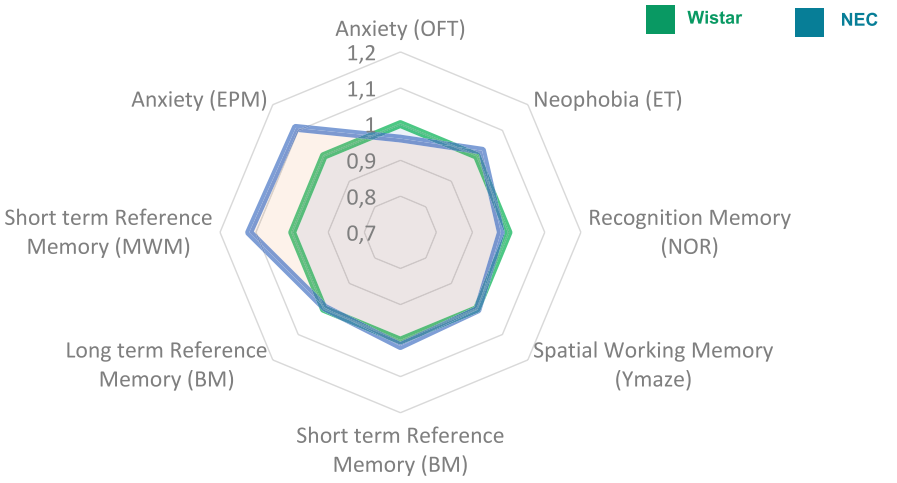


Fig. 10. Schematic summary of the anxiety and memory in GAERS, NEC and Wistar rats.
Schematic radial plots comparing the results of different anxiety and memory tests between NEC and GAERS (A), Wistar and GAERS (B) and Wistar and NEC (C) rats. Data are normalized to NEC (A) and Wistar (B and C). Each point corresponds to either an anxiety or a memory test as illustrated. Data points $<$ 1 and $>$ 1 indicate a lower and a higher performance, respectively. A) Compared to NEC, GAERS are deficient in all memory tests and show a lower anxiety-like behavior in two out of 3 anxiety tests. B) Compared to Wistar, GAERS show a deficit in six out of 7 memory tests and a lower anxiety-like phenotype in two out of 3 anxiety tests. C) Compared to Wistar, NEC show an increase performance in one out of 7 memory tests and a higher anxiety-like behavior in one out of 3 anxiety tests.

arms in the trials when performing incorrectly. This pattern of behavior could reflect inattention or less confidence in memory for the preceding sample trial (Lee and Yoon, 2023). Indeed, previous studies have shown that mice display an increased reaction time when performing incorrect trials, which was correlated with impaired attention (Fitzpatrick et al., 2017).

4.3. Recognition memory

To investigate recognition memory, we first studied the GAERS performance in the NOR test which has never been used in GAERS, although cognitive impairments in the WAG/Rij rats were evaluated by NOR as well as MWM and passive avoidance (Leo et al., 2019). Notably, the GAERS showed a smaller novel object exploration compared to both controls as well as reduced exploration of the novel object, indicating a recognition memory impairment.

The CMOR has the advantage over the NOR test since the time of object exploration is controlled by confining the animal being the arms of the Y-maze. Moreover, the CMOR helps to narrow down the specific cues used by the animals to identify object novelty/familiarity, i.e., would identify whether the deficit observed in the NOR test is visual- or texture-dependent and strengthen the results of other memory tests that rely on spatial cues (i.e. the MWM and BM, see below). GAERS showed recognition memory deficits in visual-texture crossmodal variation and texture recognition memory was moderately decreased, relative to NEC, and was not dependent on reduced exploration. Notably, we could not investigate visual recognition memory as the NEC rats performed below chance level. The decreased texture and crossmodal memory deficits agree with the results of Marks et al. (2016) who, on the other hand, were able to carry out visual memory tests but found no difference between GAERS and NEC. Notably, GAERS are able to discriminate different textures (Studer et al., 2019).

4.4. Spatial reference memory

In agreement with the deficits reported by Marques-Carneiro et al. (2016) in the MWM probe test, we found that despite similar latencies to reach the platform, the GAERS covered considerably longer distances with a higher swim velocity than Wistar. Thus, since GAERS are aware of the existence of the platform and do indeed search for and reach it, these data might suggest that these animals have difficulties in creating a reference map of the pool and navigating it. These differences in exploration patterns, despite accurate performance suggest that GAERS may be using a different strategy to locate the platform. Indeed, our comprehensive analysis of the strategy used by the three strains revealed that the GAERS preferentially used egocentric strategies in contrast to Wistar and NEC, suggesting an impairment in allocentric behavior. GAERS displayed a lower number of direct paths and more thigmotaxic strategies on the last day of acquisition compared to Wistar. This is objectively described in the literature (Cooke et al., 2020; Curdt et al., 2022), and strongly suggests disruption of spatial reference memory in GAERS based on allocentric information. Interestingly, NEC displayed increased latency to target, velocity and distance covered during the first 2 days of training, behaving similar to GAERS on the probe test parameters. Notably, due to logistical constraints, a small number of NEC was used in the MWM: though this does impact on the strength of the above conclusions regarding the comparison between GAERS and NEC, it does not affect those regarding the comparison between GAERS and Wistar.

Although the MWM test is designed for testing spatial memory, it requires the coordination of multiple higher cognitive functions, any of which could be impaired. To address this issue (and that related to the few number of NEC rats used in this test), and thus to obtain more conclusive data, we performed the Barnes maze test, another behavioural test based on spatial reference memory (Pitts, 2018). In this test, the GAERS were able to achieve similar performance levels as Wistar and NEC for primary parameters, since no difference was found for day 4 of acquisition, but made more errors in locating the target hole on probe test. Similar to the MWM test, assessment of the navigation strategies in Barnes maze showed that GAERS preferentially used egocentric, random and serial strategies with fewer allocentric, direct strategies compared to both control strains.

Although we did not assess directly the integrity of hippocampal cellular function, it is noteworthy that animals with hippocampal lesions fail to form allocentric representations and rely on egocentric-based strategies (e.g., scanning, chaining)(Arns et al., 1999; Ramos and Morón, 2022), and display decreased escape latencies and path lengths in the MWM (Eichenbaum et al., 1990). Although non-spatial strategies such as 'random search' and 'scanning' can be successful and result in lower escape latencies, these strategies are not indicators of spatial allocentric memory (Curdt et al., 2022). Performance in this task relies on hippocampal place cells (Dupret et al., 2010; Grieves et al., 2016) and further experiments are required to evaluate the physiological properties of the hippocampus in GAERS rats.

One potential limitation of behavioural studies in epileptic animals is that the occurrence of seizures during the tests could markedly affect the animal's performance. However, it is well established that ASs do not occur when an animal is involved in active exploration but are present almost exclusively when the animal is in a behavioural state of quite wakefulness (Coenen et al., 1991; Danober et al., 1998). Indeed, analysis of the video recordings of all tests performed in the present study provided no evidence of AS in GAERS rats. Notably, a few outbred Wistar rats can also exhibit brief, small-amplitude SWDs (Crunelli et al., 2020), but no ASs were observed in those used in this study

4.5. Potential pathophysiological mechanisms underlying the GAERS memory deficits

Many genetic, cellular and neuronal network abnormalities have been described in GAERS (compared to NEC and Wistar) (see recent reviews by Crunelli et al., 2020, 2023; Lindquist et al., 2023), which have been shown to underlie the expression of ASs. However, the effects of the vast majority of these deficits on comorbidities have not been investigated since these neuropsychological deficits of absence models have only relatively recently been reported. Indeed, changes in, for example, NMDA, mGlu and endocannabinoid receptors, that are known to modulate the cellular and synaptic components of behavioural memory and learning (Alkadhi, 2021; Collingridge and Abraham, 2022; Piette et al., 2020), have been reported in absence seizure models (Celli et al., 2022; Koerner et al., 1996; Roebuck et al., 2022) but their effect on comorbidities has not been tested. In contrast, the missense gain-of-function mutation in CaV3.2 T-type Ca^{2+} channels of GAERS (Powell et al., 2009), which is also present in some children with absences (Y. Chen et al., 2003) might be critical in the behavioural deficits reported in this study, since these channels are expressed not only across the entire cortical mantle but also at high levels in the thalamic reticular nucleus that plays a critical role in attention (Crabtree, 2018; McAlonan et al., 2008). Moreover, the involvement of T-type Ca^{2+} channels in memory is supported by their contribution to intrathalamic long-term depression of inhibitory synapses (Pigeat et al., 2015) and the rescue of GAERS learning and memory deficits by Z944, a selective T-type Ca^{2+} channel blocker (Marks et al., 2016). Moreover, the non-genetic loss-of-function of the GABA transporter GAT1 in GAERS (and other genetic models of absence seizures) (Cope et al., 2009) would also contribute to the allocentric behavioural deficits observed in this study, since GAT1 KO mice have ASs, a deficient long-term potentiation and memory deficits (L. Chen et al., 2015; Cope et al., 2009; Gong et al., 2009). Indeed, a deficient long-term potentiation is present at the CA3-CA1 synapses of the GAERS hippocampus compared to NEC rats (unpublished results).

Together with these "genetic" deficits, there will undoubtedly be additional deficits resulting from the ASs when networks of cortical, thalamic and basal ganglia neurons fire in phase with the EEG SWDs (Crunelli et al., 2020). Notably, recent work in non-anesthetized rat and mouse AS models has shown that each neuron in cortical and thalamic territories does not fire at every cycle of the SWD and thus may show either a decrease, an increase, or no change in firing from one seizure to the next (McCafferty et al., 2018a, 2018b; Meyer et al., 2018). Notwithstanding, however, there is invariably a consistent and solid output from these networks at each cycle of the paroxysm (Crunelli et al., 2020). Following their start in the primary somatosensory cortex, SWDs then engulf the rest of the neocortex, the thalamus and the basal ganglia (Meeren et al., 2002). Since many ASs occurs daily, a rhythmic barrage of high-frequency firing is imposed to neurons of the above networks and other interconnected brain areas, including regions involved in memory, cognition and mood disorders (e.g., the medial prefrontal and perirhinal cortices, the dorsal and ventral hippocampus, the amygdala and the thalamic reticular, mediodorsal and reuniens nuclei (Aggleton and Brown, 1999; Aggleton et al., 2010; Aggleton and

Nelson, 2015; Barone et al., 2020; Bolkan et al., 2017a, 2017b; Chudasama and Robbins, 2004; Dolleman-Van Der Weel et al., 2019; Jankowski et al., 2013) Thus, together with primary cortical areas, association cortices will also bear the brunt of such regular synaptic bombardment as will both anterior, intralaminar and sensory thalamic nuclei, including the mediodorsal nucleus and its connections with the prefrontal cortex, that are involved in cognitive flexibility, spatial navigation, and working and recognition memory (Aggleton and Brown, 1999; Bolkan et al., 2017a; Jankowski et al., 2013; Saalmann, 2014; Wolff and Vann, 2019). As a result, cellular and synaptic processes involved in these functions are remodelled by the periodic firing bombardment associated with the SWDs. Notably, the frequency of this paroxysmal rhythmic activity (2–4 Hz in humans, 5–7 Hz in animals) well overlaps with the theta frequency band which is critical for the expression of long-term potentiation(Larson and Munkácsy, 2015; Tsanov and Manahan-Vaughan, 2009).

Author contribution

MG, AMS, GDG, VC and SHV conceived the research; MNS, CCP, TPM and SHV planned the experiments; MNS, CCP, TPM, MFF, DA, FS and FM conducted the experiments; MNS, CCP and TPM analysed the data and prepared the figures and tables; MNS, TPM, GDG, VC and SHV wrote the manuscript which was revised by all authors before submission.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2023.106275.

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