Plzeň. lék. Sborn., 83, 2017: 31–39

FUNCTIONAL SPLIT BETWEEN SOCIAL AND SPATIAL MEMORY ON A TGF 344–19 RAT MODEL OF ALZHEIMER'S DISEASE

S. L. Proskauer-Peña, K. Mallouppas, P. Flosman, K. Ježek

Biomedical Center, Medical Faculty in Pilsen, Charles University

Alzheimer's disease (AD) is a neurodegenerative disease with progressive character, ultimately leading to death. Its incidence is worldwide and the number of AD cases is estimated to double the actual rates by 2030. Despite intensive research our knowledge of AD etiology and pathophysiology is still insufficient and so far no effective medication is available. In order to achieve its better understanding, various animal models often based on transgenic techniques were developed. To map AD driven memory impairment we employed the new rat model of AD, TgF-344–19, which expresses two human genes for APP (Amyloid Precursor Protein) and PS1 (Presenilin 1) and emulates all the cytological characteristics of AD (1, 2). Our main goal is to study AD mechanisms on the level of neural networks engaged in spatial memory processing. This report shows a preliminary data on two memory systems in TgF-344–19 in a late stage of AD signs development.

The current hypothesis of AD development is primarily focused on the formation of Aβ-Amyloid plaques and Tau Neurofibrillary Tangles (NFTs) (3–7). Amyloid-plaques were found in many brain areas of AD patients extending practically across the whole neocortex and evolutionary older cortical regions. One of the first affected areas is the entorhino-hippocampal loop (HP), a circuit essential for spatial memory processing in animals and declarative memory in humans. It is of no surprise that spatial and episodic memories are among the first impaired cognitive abilities.

Hippocampus is a large palleocortical structure with different anatomy and functional characteristics on its dorsal and ventral poles. Whereas functions of dorsal hippocampus are relatively well understood, mnemonic functions of its ventral portion (vHP) are much less known. Recent work from Tonegawa lab suggests that vHP might be important for encoding and storage of the social memory (8). Other studies indicate that social memory is to large extent controlled by the dopaminergic system (DS), which endures considerable changes on the release of dopamine (DA) and its receptors (9–11). During the normal process of aging, the number of D-receptors decreases (12–13). In addition to this evidence, DA has been linked to an accelerated progression of the disease, a state known as fast progressive cognitive decline (14), a warning AD prognostic factor.

The functional split between the dorsal and ventral portion of hippocampus is further supported by the fact that they differ in their dopaminergic innervation. Whereas the substantial source of dopaminergic signaling for ventral hippocampus comes from ventral

tegmental area (VTA, 15), dorsal hippocampus gets projections from locus coeruleus (LC) besides those from VTA. Both projections then participate in hippocampal plasticity and are affected by AD (16, 17).

Objectives

The aim of this study is to describe and compare the progression of cognitive impairment in the AD rat model, TgF-344–19, across two types of memory: spatial navigation memory and social memory. Each of them represents a different memory system, but both are processed within the hippocampal circuitry. Spatial navigation memory engages the dorsal HP, whereas the social and emotional memory engages the ventral HP. Our goal is to test whether both deteriorate in the late stage of transgenic AD model in a comparable way, or, despite they both depend on one anatomical region, if their functional deterioration differs.

MATERIALS AND METHODS

Model

We employed a transgenic rat model of AD, TgF-344–19, containing the human mutant genes APP and PS1 for familial form of AD. This model provides a complete picture of all the pathological characteristics of AD: progressive deposition of amyloid plaques, neurofibrillary tangles and neurodegeneration including cell apoptosis (18).

The animals, of an average weight of 500 g, were housed in groups according to the Czech Animal Protection Law on a 12 hour dark/light cycle with food and water *ad libitum* in a pathogen free environment in the animal facility of the Biomedical Center of the Medical Faculty in Pilsen, Charles University.

We used 17 animals with an average age of 17–18 months (measured in 30-day months). They were handled prior the experiment up to three weeks for 10 minutes daily.

In the social discrimination test, familiar and novel subjects were of the same age, gender and weight, in order to eliminate any bias of preferences towards one of the rat in the arena.

To minimize stress associated with transportation, animals were wheeled into testing rooms and rested there for at least 20 min prior to all tasks performed.

Social Discrimination Test

The experimental animals were exposed to Social Discrimination Test (SDT), described by Engelmann et al., 1995 (19). The test paradigm employed three rats at the time: the experimental, the familiar and the novel animal. The main measured parameter was the time the experimental rat spent exploring each of the other two subjects. This involved a direct encounter between conspecifics while the investigatory behavior of the experimental subject served to quantify the social discrimination performance.

Arena

A black, oval shaped arena was of 100 cm length, 50 cm width and 50 cm height. To achieve unbiased results and to ensure that the experimental rat can freely decide which rat to interact with, the familiar and novel subjects were kept in metal cages that prevented them from moving around the arena. The cages (16.5 cm in diameter and 24 cm in height) were positioned equidistantly from each other and from arena walls (Figure 1). Around each cage, 4 small white dots marked the "sniffing zones" for subsequent analysis. Later, the dots were digitally connected to form a circle before every video analysis. The circles indicated the sniffing zones and had a diameter of 26.4 cm. The experiment was performed in dimly lit room with evenly distributed light.

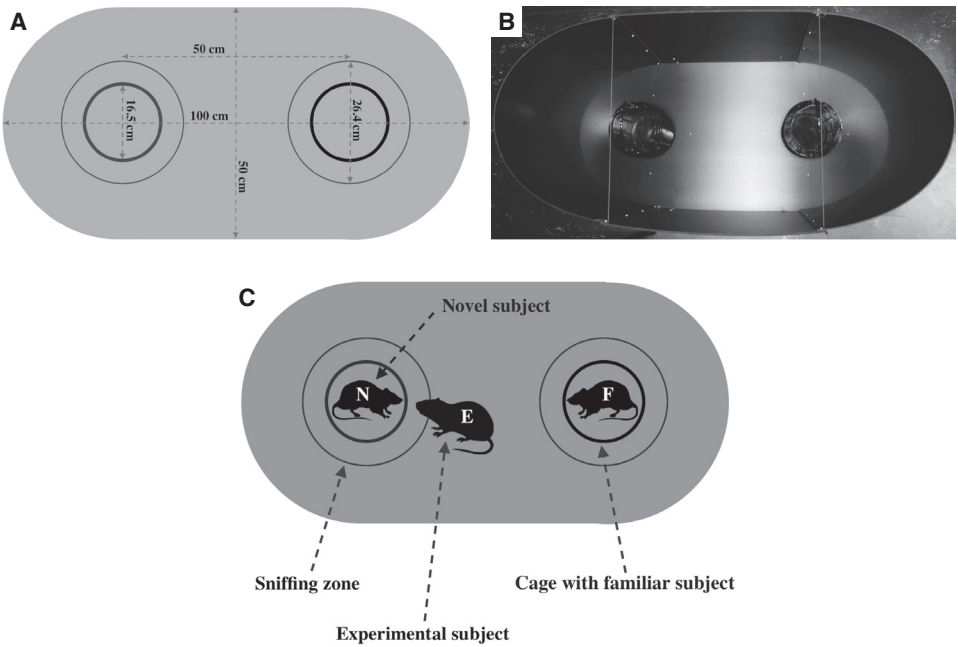

Fig. 1 A. Drawing of the arena. B. Photography of the arena with the cages. C. Schematic representation of the actual experiment.

Experiment

The experiment was performed on 6 experimental 17–18 old months rats. The whole procedure began with 2 days of habituation, which consisted of 10 minutes per day free movement in the experimental arena. After the second day of habituation, each experimental rat was housed with the animal that was assigned as a familiar for a period of 72 hours. During this period, both animals were living in the same cage, separated with a grid from each other.

On the day of the experiment, the familiar and the experimental animals were separated 30 minutes prior to data collection. During the last 5 minutes of separation time, the experimental animal was put in the arena in order to explore the apparatus freely without any other subject in it. Whole experiment was recorded using color digital video camera. When the experiment began, tested rat was put directly between the two cages (one with the novel animal and the other with the familiar animal) and the 10 minutes. recording began. Half of the experiments were performed with the novel animal in the right cage and the familiar animal in the left cage, whereas the other half of the experiments were arranged in the opposite way. At the end of each experiment all rats were removed and the arena was thoroughly cleaned using water with detergent. See table 1 for the timeline of the experiment.

Tab. 1 Timeline of the experiment.

Handling + Habituation	**Habituation**	**Experimental and familiar rat put together**	**Separation of experimental and familiar rat**	**Experimental rat placed in the arena to explore it prior to experimentation**	**All 3 rats in the arena discrimination test**
Day 1	Day 2	Day 2–5	Day 5	Day 5	Day 5
10 minutes	10 minutes	72 hours	25 minutes	5 minutes	10 minutes

Video analysis

Recordings were analyzed in a software environment called BORIS. The sniffing zones were digitally drawn using the 4 dots marked in the arena. The videos were played in slow motion (10 fps) and the time spent by each rat in the sniffing zone was measured only in the first 5 minutes of the experiment. The analysis was performed in a blind way, as the rats were randomly chosen for the analysis, and the positions of the novel and familiar rat in the arena weren't available during the analysis.

The nose of the rat was assigned as a tracking point used to calculate the time spent in each zone. Sniffing time was registered only when the nose of the experimental animal was clearly inside the sniffing zone. If other parts of the body than the nose entered the sniffing zone, sniffing time was not registered. In addition, in cases when the rat was not sniffing but rather sleeping, resting or grooming within the sniffing zone, such period was also discarded. The sniffing time in the vicinity of each presented subjects was recorded and the discrimination score for each experimental rat was calculated. After the analysis, the data was matched to the appropriate experimental rat and to the novel rat position. Calculation of the discrimination score followed.

$$\textbf{Discrimination Score} = (\mathbf{T}\text{Novel} - \mathbf{T}\text{Familiar}) / (\mathbf{T}\text{Novel} + \mathbf{T}\text{Familiar})$$

Active Allothetic Place Avoidance

A second task that the rodents have been tested on was the Active Allothetic Place Avoidance (AAPA), a hippocampal dependent task developed to functionally split the allocentric and egocentric navigation strategies. It has been shown that AAPA tends to be very sensitive even to hippocampal lesions that did not show any effect in conventional spatial memory tests as Morris water maze procedure (20, 21).

Briefly, the rat has to avoid a shock zone on circular arena that slowly rotates in one direction. The shock zone is defined in the allocentric orientation frame, e.g. it is stable in relation to room coordinates. The animal has to constantly check its own position in order not to enter the punished sector to avoid a mild footshock (0.3–0.7 mA). Various parameters are registered during the experiment. We assessed the maximum time the rat was avoiding the shock zone within each daily session.

A group of four TgF-344–19 rats was initially exposed to two habituation 20 minutes sessions on the arena without any shock. Then four days of training were performed under a protocol of one 20 minutes long session daily on a slowly rotating arena (one revolution per minute) with defined 60 degrees to-be-avoided shock zone in a room frame coordinate system.

RESULTS

SDT

Statistical analysis was made using MATLAB software. Because the proper non-transgenic age matched controls were not available, we tested the data against the hypothesis of fully random behavior (no memory expressed). One million randomly generated values of discrimination score between −1 to +1 were created in order to simulate the case of a complete impairment of the social discrimination (null hypothesis), during which the results are expected to have a normal distribution with a mean of 0 (Fig. 2). Fig. 2 illustrates that the experimental rats showed a relatively high positive discrimination scores with an average of +0.577 and a SEM of ±0.107. This shows their strong preference for the novel animal. The lowest discrimination score, observed within the group, was 0.338 and the highest was 0.968. These data were above 2σ (above 95% confidence interval) of the normal random distribution. This means the rats did show a significant social discrimination as they preferred to stay close to the novel animal.

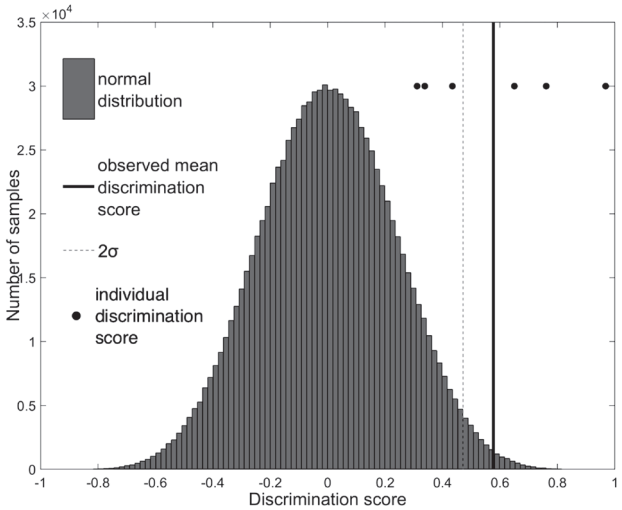

Fig. 2 Normal distribution curve of the social discrimination score, assuming total social discrimination impairment, simulated with a million randomly generated values from –1 to +1. The dotted line represents standard deviation of 2σ. The black dots represent Discrimination scores of each individual experimental rat on a scale from +1 to –1 and the bold line represents the average discrimination score value.

AAPA

We analyzed the maximum time each animal was able to avoid entrance to the shock zone. Across all days however, the tested animals showed flat learning curve. ANOVA with repeated measures (STATISTICA software) returned no significant effect. This indicates that the rats were unable to learn the AAPA task (Fig. 3).

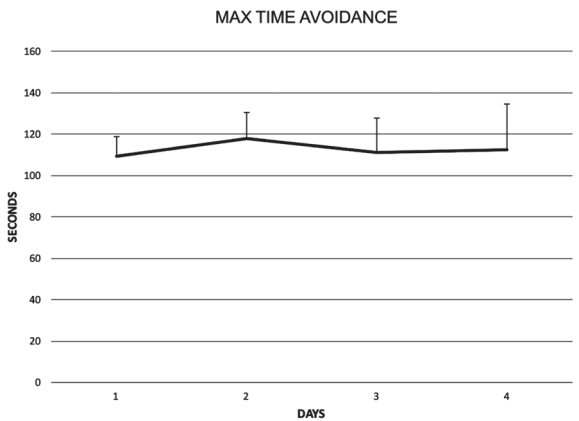

Fig. 3 Shows the results of 18-month-old age group on AAPA test. Animals of this age showed absent learning and memory abilities through 4 days of training.

DISCUSSION

In this study we compared functionality of two independent memory systems in a late stage development of Alzheimer disease-related signs in a transgenic rat model TgF-344–19 AD. We aimed at assessing social discrimination and spatial memory performance.

Social memory in rodents is essential for adaptive social behavior and reproduction. Recent work has shown ventral hippocampus (8) is part of the circuitry that includes portion of brain dopaminergic system and controls the encoding and expression of social memory (22, 23).

On the other hand, another part of hippocampus – its dorsal portion, is crucial for spatial memory processing. This system, entorhino-hippocampal loop was shown to be among the first impaired by AD. In the TgF-344–19 AD model, rats in our pilot experiments showed spatial memory deficit beginning the 12–15 months in the AAPA task.

In this work we found a substantial split between social and spatial memory performance. While the allocentric spatial memory performance in AAPA test was practically absent, the social memory was largely spared as its scores were considerably high and significantly differed from the random data even at relatively low number of subjects. In social discrimination the subjects showed consistent results as all of them displayed more or less clear discrimination favoring the novel rat over the familiar one.

We could not compare the abovementioned behavior with the proper non-transgenic controls as such animals were not available. Therefore we could not empirically assess an eventual effect of AD condition. But the functional split between these memory types strongly suggests the underlying neural circuits are affected by AD in a different time course. Moreover, in a late stage of the disease model we face a situation where one system seems collapsed while the other shows surprisingly decent functional reliability.

We might speculate to what degree this functional split involves hippocampus – its ventral part is considered to be part of the circuitry controlling the social memory functions while its dorsal portion is responsible for spatial memory and orientation. If this is correct, then it would be of an outstanding importance to explore the degree of tau- and beta-amyloid pathology across its ventral and dorsal portion. Considering the results presented here, we would hypothesize that the ventral portion would be damaged much less than the dorsal one.

An alternative interpretation would consider similar morphological changes under the assumption that the two tests used are not comparable in their demands on the underlying circuits. Such alterative is hard to disprove in behavioural testing. Certainly, the suggested morphological evaluation of the tissue damage would show in a more straightforward way whether or not the structures constituting different memory systems are affected in the late stage of this new transgenic AD model in a similar extent.

SUMMARY

TgF 344–19 animal model shows that its spatial orientation is literally devastated by AD at the age of 18 months. However, the social memory system seems functionally unaffected at the same age. This finding shows a different progression of functional deterioration across two memory systems that seems of a particular interest given they share the same anatomical structure, hippocampus.

Disociace paměti pro prostorové a pro sociální vztahy u transgenního modelu Alzheimerovy choroby TgF 344–19

SOUHRN

Tato práce si klade za cíl porovnat dva různé druhy paměti, prostorovou a sociální, na potkaním modelu Alzheimerovy choroby TgF 344–19 v pokročilé fázi této choroby. Ve zpracování obou těchto druhů paměti hraje významnou roli hippocampus. K testování prostorové paměti jsme použili metodu zvanou Active Allothetic Place Avoidance a k testování sociální paměti jsme použili test sociální diskriminace mezi familiárním a neznámým potkanem. Zvířecí model TgF 344–19 ukázal, že jeho schopnost prostorové orientace je ve věku 18 měsíců již výrazně poškozena, kdežto ve stejném věku vykazuje model výraznou schopnost sociální diskriminace. Tento výsledek naznačuje rozdílnou funkční deterioraci těchto dvou paměťových systémů, a to i přes jejich anatomickou blízkost.

AUTHOR CONTRIBUTIONS

SP, CM, PF and KJ planned the experiments and wrote the manuscript. CM and PF performed the experiments and analyzed the data.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be considered as a potential conflict of interest.

ACKNOWLEDGEMENTS

The authors wish to thank Mgr. Lenka Sýkorová for the technical support, Mgr. Eva Kralovcová and Dr. Pavel Pitule for supervising the genotyping techniques, and Elena Dacheva for proofreading and correcting the text.

Supported by GAUK 1114216, SVV 260281, PROGRES Q39 and NPU I LO1503 of Czech Republic.

REFERENCES

1. The 2015 Ageing Report. Underlying Assumptions and Projection Methodologies. European Economy 8, 2014. – 2. Alzheimer's Association*. Alzheimer's Association Report 2015 Alzheimer's disease facts and figures. Alzheimer's & Dementia 11, 2015: 332–384. – 3. Selkoe D. J. , Mandelkow E., Holtzman D.: Deciphering Alzheimer Disease. Cold Spring Harb. Perspect. Med. 2, 2012: a011460. – 4. Selkoe D. J.: Alzheimer's disease: genes, proteins, and therapy. Physiol. Rev. 81, 2001: 741–766. – 5. Swerdlow R. H., Khan S. M.: The Alzheimer's Disease Mitochondrial Cascade Hypothesis: An Update. Exp. Neurol. 218 (2), 2009: 308–315. – 6. Swerdlow R. H.: Pathogenesis of Alzheimer's disease. Review. Clinical Interventions in Aging 2 (3), 2007: 347–359. – 7. Stancu I. C., Ris L., Vasconcelos B. et al.: Tauopathy contributes to synaptic and cognitive deficits in a murine model for Alzheimer's disease. The FASEB J. 28 (6), 2014: 2620–2631. – 8. Okuyama T., Kitamura T., Roy D. S. et al.: Ventral CA1 neurons store social memory. Science. Vol 353, Issue 6307. 30 September 2016. – 9. Attems J., Quass M., Jellinger K. A.: Tauandalpha-synuclein brain-stem pathology in Alzheimer disease :relation with extrapyramidal signs. Acta Neuropathol. 113, 53–62. doi:10.1007/s00401–006–0146–9. – 10. Portet F., Scarmeas N., Cosentino S. et al.: Extrapyramidal signs before and after diagnosis of incident Alzheimer disease inaprospective population study. Arch. Neurol. 66, 2009: 1120–1126. – 11. Trillo L., Das D., Hsieh W. et al.: Ascending monoaminergic systems alterations in Alzheimer's disease.translating basic science into clinical care. Neurosci. Biobehav. Rev. 37, 2013: 1363–1379. – 12. Volkow N. D., Fowler J. S., Wang G. J. et al.: Decreased dopamine transporters with age in health human subjects. Ann. Neurol. 36, 237–239. doi:10.1002/ana.410360218. – 13. Bäckman L., Lindenberger U., Li S. C. et al.: Linking cognitive aging to alterations in dopamine neurotransmitter functioning: recent data and future avenues. Neurosci. Biobehav. Rev. 34, 2009: 670–677. – 14. Becker J. A., Hedden T., Carmasin J. et al.: Amyloid-β associated cortical thinning in clinically normal elderly. Ann. Neurol. 69, 1032–1042. doi:10.1002/ana. 22333. – 15. Chowdhury R., Guitart-Masip M., Bunzeck N. et al.: Dopamine modulates episodic memory persistence in old age. J. Neurosci. 32 (41), 14193–14204. – 16. Koch G., Di Lorenzo F., Bonnì S. et al.: Dopaminergic modulation of cortical plasticity in Alzheimer's disease patients. Neuropsychopharmacol., 39 (11), 2654–2661. – 17. Kempadoo K. A., Mosharov E. V., Choi S. J. et al.: Dopamine release from the locus coeruleus to the dorsal hippocampus promotes spatial learning and memory. Proc. Nat. Acad. Sci. 113 (51): 14835–14840. – 18. Cohen R. M., Rezai-Zadeh K., Weitz T. M. et al.: A Transgenic Alzheimer Rat with Plaques, Tau Pathology, Behavioral Impairment, Oligomeric A, and Frank Neuronal Loss. J. Neurosci. April 10, 33 (15), 2013: 6245–6256. – 19. Engelmann M., Hädicke J., Noack J.: Testing declarative memory in laboratory rats and mice using the nonconditioned social discrimination procedure. Nature protocols, 6 (8), 1152–1162. – 20. Cimadevilla J. M., Kaminsky Y., Fenton A. et al.: Passive and active place avoidance as a tool of spatial memory research in rats. J. Neurosci. Methods 102, 2000: 155–164. – 21. Wesierska M., Dockery C., Fenton A. A.: Beyond memory, navigation, and inhibition: behavioral evidence for hippocampus-dependent cognitive coordination in the rat. J. Neurosci. 25 (9), 2005: 2413–9. – 22. Zinn C. G., Clairis N., Cavalcante L. E. et al.: Major neurotransmitter systems in dorsal hippocampus and basolateral amygdala control social recognition memory. Proc. Nat. Acad. Sci., 113 (33): E4914–E4919. – 23. Gray C. L., Norvelle A., Larkin T. et al.: Dopamine in the nucleus accumbens modulates the memory of social defeat in Syrian hamsters (Mesocricetus auratus). Behav. Brain Res., 286: 22–28.

Author's address: S. L. P.-P., Alej Svobody 76, 323 00 Plzeň, Czech Republic