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INCREASE IN ALZHEIMER'S RELATED MARKERS PRECEEDS MEMORY DISTURBANCES: STUDIES IN VASOPRESSIN-DEFICIENT BRATTLEBORO RAT

Running title: Early changes in Alzheimer's disease

János Varga[1], Barbara Klausz[1], Ágnes Domokos[1,2], Sára Kálmán[2], Magdolna Pákáski[2], Szabina Szűcs[2], Dénes Garab[2], Ágnes Zvara[3], László Puskás[3], János Kálmán[2], Júlia Tímár[4], György Bagdy[5], Dóra Zelena[1]

[1]Department of Behavioural Neurobiology, Institute of Experimental Medicine, Budapest, Hungary

[2]Department of Psychiatry, Alzheimer Research group, University of Szeged, Szeged, Hungary

[3]Laboratory of Functional Genomics, Biological Research Centre, Szeged, Hungary

[4]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary

[5]Department of Pharmacodynamics, Semmelweis University, Budapest, Hungary

Corresponding author:

Dóra Zelena

1083 Budapest

Szigony 43.

Hungary

Tel.: +36-1-210-9400/290

Fax.: +36-1-210-9954

e-mail: zelena.dora@koki.mta.hu

Abstract

Alzheimer's disease (AD) is the most common form of dementia in the elderly. For more effective therapy early diagnostic markers could be beneficial. Therefore we compared one year old rats with adults and examined if changes in possible brain markers of AD preceeded memory decline. We also tested if vasopressin-deficient animals were useful model of AD as vasopressin has well known positive effect on memory and AD patient has decreased vasopressin production.

We compared adult (3 month) and old (12 month), normal and vasopressin-deficient Brattleboro rats. To receive a comprehensive picture about their memory we examined their social discrimination, object discrimination and conditioned learning abilities (shuttle box). Amyloid precursor protein (APP), mitogen-activated protein kinase 1 (MAPK1), β-actin and tryptophan 2,3-dioxygenase 2 (TDO2) mRNA levels was measured by quantitative PCR.

There was no difference between the memory of adult and aged groups. The vasopressin-deficient rats at both ages showed a weaker performance in the course of social and object discrimination tests and a higher escape failure during the shuttle box experiment. The brain marker mRNAs of the elder animals were higher than the levels of the adults, but the absence of vasopressin had no influence on them.

Thus, the one year old rats showed elevated levels of AD-related markers, but memory deficits were observable only in vasopressin deficient animals. Vasopressin does not seem to have pathogenic role in AD. Changes in the studied markers might predict later symptoms, although further studies are required for confirmation.

Keywords: APP, β-actin, MAPK1, memory, object discrimination, shuttle box, social discrimination, TDO2

1. Introduction

Alzheimer's disease (AD) is a heterogenous neurodegenerative disorder and the most common form of dementia worldwide. It is characterized pathologically by senile plaques, neurofibrillary tangles, neuronal loss, and clinically by a progressive cognitive decline. There is a growing body of evidence that the most beneficial effects of treatment might only be achieved in the preclinical stage of dementia, prior to the immense hallmarks of neurodegeneration [36]. Therefore early biological markers are very important [24]. The amyloid hypothesis of AD postulates that the initiating molecule of the disease is amyloid-β, a proteolytic derivative of the amyloid precursor protein (APP) [51], ultimately leading to neuronal degeneration and dementia [25]. It is assumed that APP is an important protein for tissue repair and its expression is up-regulated following nerve damage [64]. Interestingly, the APP level of the human cerebrospinal liquor shows a positive correlation with the progress of age independently from any illness [10]. Beside amyloid-β, neurofibrillary tangles (NFT) are other hallmarks of AD [11]. NFT production is a result of abnormal hyperphosphorylation of the microtubule-associated protein tau and the mitogen-activated protein kinase (MAPK) plays a leading role in this abnormal hyperphosphorylation [27]. The β-actin has a relatively stable expression, but this molecule is considered to be involved in synaptogenesis, neuronal plasticity and clinical conditions like depression and AD, too [23]. The tryptophan 2,3-dioxygenase (TDO2) enzyme is a rate limiting factor in the metabolism of the neuro-immune modulator quinolinic acid [50] and this way promotes the neurodegenerative processes.

During the course of aging some important changes are found in the human hypothalamus. Among others, the normal circadian fluctuations seen in the number of vasopressin (AVP) neurons in the nucleus suprachiasmaticus of young subjects diminished in elderly subjects [38], thus an important role of AVP during the course of aging could be

supposed [14]. Although the main function of AVP is the antidiuresis in kidney, it plays a key role in hypothalamo-pituitary-adrenocortical (HPA) axis regulation, thereby in stress-related psychiatric diseases, like depression [31]. Depression can also be one of the earliest symptoms of AD [2]. Half of the AD patients suffer from minor or major depressive disorder, too [47, 56]. As the leading symptom of AD is the memory deficit [28], molecules that influence memory formation might have a pathogenic role. In connection with its role in circadian regulation [15] AVP might be such a molecule. Indeed, administration of AVP receptor antagonists resulted in memory impairment both in mice [3] and in rats [39]. The adult AVP-deficient Brattleboro rats showed reduced social discrimination ability which was improved by AVP administration [16]. Moreover, a significant AVP decrease has been found in many brain regions of AD patients [20, 34]. A recent study in rats found centrally administered AVP protective against amyloid-β protein-induced memory decline in the Morris water maze test [42], supporting the beneficial effect of central AVP in the prevention and treatment of AD.

We aimed to test if aging influenced the effect of AVP on memory and if the possible behavioral changes were underlined by alteration in Alzheimer's related molecules. In this case AVP-deficient animals could be a useful model of Alzheimer`s disease. We compared adult (10-12 weeks old) and older (1 year old) AVP-deficient (di/di) and control (+/+) animals of the Brattleboro strain. This strain is due to a spontaneous mutation in the neurophysin region of the AVP gene resulting in abnormal AVP synthesis [53]. Previous studies on memory did not use older Brattleboro rats and the control animals were also questionable (see later) [4]. Our hypothesis was that aging will further enhance the impaired learning ability of AVP-deficient rats and it will be accompanied by enhanced mRNA level of APP, β-actin, MAPK1 and TDO2, which – according to our knowledge - were never examined in one-year-old rats. Thus, at the same time we tested also the possibility that

changes in possible brain markers of AD preceeded memory decline. As parietal lobe dysfunction is an important characteristic of early AD we took tissue samples from the parietal cortex [32].

2. Material and methods

2.1. Animals

Male Brattleboro rats, naturally deficient in AVP were used in their adult (~330 g, 10-12 weeks old) or older (~500 g, 1 year old) age. Rats were obtained from the local breeding colony of the Institute of Experimental Medicine in Budapest, Hungary started from breeder rats from Harlan, Indianapolis, IN, USA. The animals were kept under controlled laboratory conditions (21±1 ◦C, 50–70% humidity, 12 h light starting at 07:00 h) with commercial rat chow (Charles River, Budapest, Hungary) and tap water ad libitum.

We compared the AVP deficient homozygous recessive (di/di) rats with diabetes insipidus to normal homozygous dominant (+/+) control rats [68]. As Brattleboro rats originated from Long Evans strain approx. 50 years ago, it is not a proper control for AVP-deficiency. Our control, +/+ rats were bread out locally [68]. They are in close relationship with the AVP-deficient ones (heterozygous mothers are the daughters of +/+ mothers). The only difference is the point mutation in the AVP gene.

The adult animals were sexually naive, and the older animals were sexually experienced breeder rats. To avoid possible confounding effect of social contact with other rats (e.g. hierarchy [61]) all experiments were performed on single-housed animals (approx. 1 week isolation).

All manipulations of the animals were approved by the local committee for animal health and care and performed according to the European Communities Council Directive recommendations for the care and use of laboratory animals (86/609/CEE).

2.2. Behavior

2.2.1. Social discrimination test

Olfactory memory was tested during the light phase between 9:00 am and 1:00 pm using the social discrimination procedure [17]. First, the rats were transferred to a new cage (41.3 x 26 x 29.8 cm, GeoMaxi, Ferplast, Italy) with fresh bedding 1 h before starting the test. Each social discrimination test consisted of two 4 min exposures to juveniles in the new cage. During the first, sampling phase the adult animal was allowed to acquire the olfactory signal of a given juvenile (Juvenile 1). The little male or female Wistar rats were 25-30 days old. The observed animals do not regard them as rival, or prey in this age and body size. After 4 min Juvenile 1 was removed and kept individually in a fresh cage with food and water ad libitum. After 30 minutes interval the adult was re-exposed to Juvenile 1 (old) together with a second, novel juvenile (Juvenile 2) (choice phase). The duration of the investigatory behavior of the adult towards each juvenile was measured separately by a trained observer blind to the animal's age and genotype. To allow the observer to distinguish between the two juveniles, one of them was marked with green lines (Edding 30 permanent marker, odorless, green, Edding AG, Germany) at least 30 min before testing. To exclude the opportunity of preference or aversive reaction toward the marked animals we randomised the marking between Juvenile 1 and Juvenile 2. The tests were videotaped and the investigatory behavior was analysed by events recorder (H77). Investigatory behavior was defined as direct action towards the juvenile rat including anogenital sniffing, licking, hunting, pawing and close pursuing. A significantly longer investigation duration of Juvenile 2 versus Juvenile 1 during choice phase is taken as an evidence for an intact recognition memory [17]. The discrimination index was calculated as follows: Discrimination index = time percentage Juvenile 2 / (time percentage Juvenile 1 + time percentage Juvenile 2) The result of the index changes between 0 and 1, where 0.5 = no discrimination. Normally the animals spend more

time with the new stimulus (novelty effect), thus the index below 0.5 is a sign of memory deficit.

2.2.2. Object discrimination test

For the object related equivalent of social discrimination we developed an experimental design comparable to the social paradigm in both time course and test settings. Two different objects were used instead of juveniles. A cat bedding filled blue round iron box (diameter: 9.5 cm, height: 4 cm) and comparable size transparent glass bottle (diameter: 7 cm, height 7.5 cm) were used. One of this objects (Object 1) was presented for 4 min (sampling phase), than removed, and 30 min later the same (Object 1) and a different object (Object 2) were introduced to the rat for 4 min. The objects were thoroughly cleaned with water before each test. The tests were videotaped and analysed as mentioned before. In behavior directed towards the object we distinguished sniffing and gnawing as an important component. To exclude object preference the two objects were randomly used as Object 1 or Object 2, and to exclude place preference the place of Object 1 and Object 2 were also randomised. Discrimination index was calculated as previously.

2.2.3. Shuttle box test

The shuttle-box apparatus consisted of two identical compartments (30 x 30 x 30 cm each) separated by a barrier with a gate in the middle [5]. It was placed in an isolated room. Animals were trained to cross the barrier under the duration of a flashlight (conditioned stimulus, CS). If they fail to do so they were punished with a footshock (unconditioned stimulus, US) of 0.3 mA delivered via the grid floor of the shuttle-box. Each trial consisted of three periods: (1) Resting period (intertrial interval) was constant**.** Intertrial crossings (IC) were not punished. (2) CS period when CS was presented for a maximum of 10 sec, signalling the impending footshock. If the rat responded within this 10 sec period (referred to conditioned avoidance response, CAR), the CS was terminated and the next trial started again

without delivery of US. (3) If the animal failed to respond to CS, the footshock (US) was applied together with the light until the rat responded or for a maximum of 5 sec (CS + US period). In case the rat failed to escape the US within 5 sec, it was considered as escape failure (EF). The rats got 100 trials in daily sessions for 5 consecutive days. The number of CAR, EF and IC were automatically recorded during the five daily sessions. On the days of the experiments animals were transported in their home cages to the laboratory 60 min prior to the shuttle-box session. All parts of the experiment were made at comparable periods of the day during the light period. On the first day the animals were allowed to habituate to the environment for 5 minutes.

2.3. Molecular changes

Samples and RNA preparation

Naive animals were anaesthetized intraperitoneally by 10% chloralhydrat and after transcardial perfusion by 2-4°C physiological saline both hemispherium were removed and samples were taken from the parietal cortex (30 mg/animal) on wet ice and were stored immediately on -80°C until processing.

Total RNAs were extracted and purified using NucleoSpin RNA purification kit (Macherey-Nagel, Durren, Germany) according to the manufacturer's instructions. We homogenized 30 mg brain tissue with 350 µl RA1 buffer and then we lysed the cells using 3.5 µl mercaptoethanol. We filtered the lysatum on the first filter by centrifugation (60 s, 1000 g), then we added 350 µl 70% ethanol to it and centrifuged again through the second filter (30 s, 11000 g). At this time the nucleic acids tied on the filter. We salted out the silica membrane with 350 µl membrane salt buffer (desalting buffer, MDB). Than we gave 10 µl rDNase, 90 µl rDNase reaction buffer and 95 µl rDNase reaction mix to the solution, incubated on room temperature for 15 minutes. Then we washed it and desiccated the membrane by spin drying with 200 µl RA2 buffer (30 s, 11000 g), then with 600 µl RA3 buffer (30 s, 11000 g), and

finally again 250 µl RA3 buffer (2 minutes, 11000 g). We dissolved the solution containing full cell RNA from the membrane with 60 µl ribonuclease free water (1 minute, 11000 g). Finally we added 0.5 µl ribonuclease inhibitor to the solutions and stored it on –80 °C until use.

Reverse transcriptase polymerase chain reaction (RT-PCR)

We measured the full RNA concentration of the solutions with a spectrophotometric method, nanodrop apparatus. We added 15 µl reverse transcription cocktail [reverse transcriptase (1.5 µl), DNA-polimerase, monomer (1.2 µl), random primer one (3 µl), RT buffer (3 µl), ribonuclease inhibitor and bidistilled water (4.8 µl)] onto the 2 µg/15 µl concentration diluted RNA solutions. The primers were the follows:

APP forward: 5'-CCC CAA GAT CCG GTT AAA CT-3'

reverse: 5'-TAC TTG TCG ACT GCG TCA GG-3'

MAPK1 forward: 5'-CCA AGC TCA ACC GTC TCA TC-3'

reverse: 5'-GGC TGG TAG GGT AGT TGA TGC-3'

β-actin forward: 5'-CCC GCG GAG TAC AAC CTT CT-3'

reverse: 5'-CGT CAT CCA TGG CGA ACT-3'

TDO2 forward: 5'-TGT AGC CGT GAC TGA TGT TCA GA-3'

reverse: 5'-ACA GGT ACA AGG TGT TCG TGG AT-3'

GAPDH forward: 5'-AGA TCC ACA ACG GAT ACA TT-3'

reverse: 5'-TCC CTC AAG ATT GTC AGC AA-3'

The reverse transcriptase synthetized copy DNA (cDNA) from the full cell mRNS during the polymerase chain reaction, then we gave 10 µl Roche SYBR Green Mix, 1 µl primer to 9 µl template cDNA. The RT-PCR machine (Corbett 3000 RT-PCR) defined the mRNS quantities in the original solutions.

In the course of data evaluation we applied the ΔΔCt method [33]. The reference gene was the glyceraldehyde 3-phosphate dehydrogenase (GAPDH). All results are normalized to the values of adult +/+ rats.

2.4. Statistical analysis

The results are expressed as mean ± S.E.M. Statistical significance was evaluated by two way analysis of variance (ANOVA; discrimination sample phase, discrimination index, brain markers; levels age and genotype) or repeated measure ANOVA (discrimination tests, shuttle box; time as within factor) followed by Neumann Keuls post hoc test, or evaluated by single sample t-test (in case of discrimination index) using Statistica 9.0 software (StatSoft, Tulsa, USA). A p value of less than 0.05 is considered to be significant.

3. Results

3.1. Behavior

3.1.1. Social discrimination test

During the whole social discrimination test (sampling + choice) the adult rats spent significantly more time with the juveniles, than the old ones based on repeated measure ANOVA (age $F_{(1,37)}$=6.21, $p<0.05$) (Fig.1A). During the sample phase there was only a marginally significant effect of age by factorial ANOVA (age $F_{(1,45)}$=3.08 p=0.08). At the same time during the choice phase the age had a significant effect by repeated measure ANOVA (age $F_{(1,37)}$=11.97 $p<0.01$). In point of discrimination the time spent with old (juvenile 1) or new (juvenile 2) juveniles was significantly different by repeated measure ANOVA (old-new $F_{(1,37)}$=13.44 $p<0.01$), but the genotype just marginally influenced this parameter (old-new x genotype $F_{(1,37)}$=3.54 p=0.07).

The discrimination index, originated from the time percentages of the choice phase, significantly differed between the genotypes (genotype $F_{(1,37)}$=4.85 $p<0.05$), but the age had no effect by factorial ANOVA (Fig.1B). If we examine the difference of the discrimination

index from 0.5 (0.5 = not discrimination) by single sample t-test, we observed that the AVP-deficient animals (di/di) were unable to discriminate (adult +/+: $p<0.05$; old +/+: $p<0.05$; adult di/di: $p=0.78$; old di/di: $p=0.19$).

3.1.2. Object discrimination test

During the whole object discrimination test (sampling + choice) the AVP-deficient rats spent significantly more time with the objects, than the +/+ ones based on repeated measure ANOVA (genotype $F_{(1,30)}=16.94$, $p<0.01$) (Fig.2A). Both during the sampling phase (genotype by factorial ANOVA $F_{(1,35)}=16.34$, $p<0.01$) and the choice phase alone (genotype by repeated measure ANOVA $F_{(1,30)}=11.83$ $p<0.01$) the effect of genotype remained significant. In point of the discrimination there was a significant difference between the time spent with old (object 1) and new (object 2) object during the choice phase by repeated measure ANOVA (old-new $F_{(1,30)}=18.03$ $p<0.01$) and this effect was different between the two genotypes (old-new x genotype $F_{(1,30)}=9.26$ $p<0.01$).

The discrimination index significantly differed between the two genotypes (genotype by factorial ANOVA $F_{(1,30)}=28.84$ $p<0.01$) without any influence of age (Fig.2B). Based on the difference of the discrimination index from 0.5 by single sample t-test the AVP-deficient (di/di) animals were unable to discriminate (adult +/+: $p<0.01$; old +/+: $p<0.01$; adult di/di: $p=0.94$; old di/di: $p=0.33$).

3.1.3. Shuttle box test

In case of intertrial crossing (IC) the two genotypes showed significant difference from each other (genotype $F_{(1,35)}=20.73$, $p<0.01$) (Fig.3A). This parameter was higher in the AVP-deficient rats. The effect of the time was also significant (time $F_{(4,140)}=11.78$, $p<0.01$) being the IC levels higher at later time-points. The effect of genotype was even more pronounced in the course of the experiment (genotype x time $F_{(4,140)}=6.80$, $p<0.01$).

The conditioned avoidance response (CAR) differed between the two genotypes, too (genotype $F_{(1,35)}$=15.30, p<0.01), being higher in the AVP-deficient rats (Fig.3B). The effect of the time was also significant (time $F_{(4,140)}$=11.60, p<0.01).

The escape failure (EF) differed also between the two genotypes (genotype $F_{(1,35)}$=9.31, p<0.01), being higher in AVP-deficient rats (Fig.3C). The effect of the time was also significant (time $F_{(4,140)}$=19.04, p<0.01), but the effect of genotype became smaller with time (genotype x time $F_{(4,140)}$=3.22, p<0.01). There was a marginally significant effect of age x time interaction (age x time $F_{(4,140)}$=2.02, p=0.09), with higher levels in old animals.

3.2. Molecular changes

The relative quantity of all the measured mRNA (APP, MAPK1, β-actin and TDO2) were significantly higher in the cerebral cortex of the 1 year old than adult animals (age APP $F_{(1,23)}$= 62.81, p<0.01; MAPK1 $F_{(1,26)}$= 4.33, p<0.05; β-actin $F_{(1,24)}$= 35.20, p<0.01; TDO2 $F_{(1,26)}$= 48.24, p<0.01) without any effect of the genotype or interaction between age and genotype (Fig.4A-D).

4. Discussion

During the memory tests the AVP-deficient rats showed weaker performance independently of the age, while AD-related molecular markers were higher in older rats independently from the genotype. Thus, we were unable to support our first hypothesis, that AVP-deficient animals are good AD models, but we found that in 1 year old rats the changes in examined brain markers preceeded memory decline.

Some authors reported memory deficits already in 1 year old rats [46]. As we assumed a further decrease in AVP-deficient rats, we did not use older animals with more pronounced memory disturbances. In our hand the 1 year old rats did not show any decline in memory traces compared to the adult population, although the results of the studied AD markers were already pathological. This could prompt further studies on an early diagnosis.

The social and object discrimination tests investigate short term, stressless memory processes, while shuttle box tests long term, stressfull learning. In accordance with the literature the AVP-deficient animals showed weaker discrimination abilities, than the normal ones [7, 16]. Previous studies used only adult animals, but – despite our expectations – aging did not enhance the memory disturbances of the AVP-deficient rats. Data in the literature on the results of avoidance tests are not consistent (e.g. no effect: [9]; memory deficit: [63]; prolonged extinction: [29]). The findings obtained in the shuttle box test in the present experiments were also contradictory. Both the adult and aged di/di rats showed worse escape failure, however their conditioned avoidance response (CAR) was superior to control animals. Take into consideration the ubiquiter role of AVP in our body [19], many other processes may confound the shuttle box performance of Brattleboro rats. (1) Although decline in activity is an important problem of human aging and can be found also in animals [45, 55], it is not a core symptom of AD and cannot be always detected in older rats [46]. On the contrary, higher physical activity seems to be protective against AD [6]. Enhanced activity of the di/di animals was reflected by enhanced sampling time in the object discrimination task and increased intertrial crossing (IC) during shuttle box experiment. Although in our previous experiments we could not detect any hyperactivity [37], some other authors found Brattleboro rats to be hyperactive in a novel environment already in their 10-day-old age [12, 54]. Enhanced locomotion can lead to higher CAR values independently from any memory processes. (2) AVP is an important regulator of the HPA axis, which consist from corticotropin-releasing hormone (CRH) in the hypothalamus, adrenocorticotropin in the pituitary and glucocorticoids in the adrenal cortex. The glucocorticoid hypothesis of aging and AD proposed that chronic exposure to glucocorticoids induced hippocampal atrophy with a decline in learning abilities [30]. Aged-superior learners had lower expression of glucocorticoid receptor and CRH mRNA in the hypothalamus

compared with other groups [35]. During the critical perinatal period the Brattleboro rats have higher resting glucocorticoid levels [66], which could contribute to their worse discrimination abilities later in life. On the contrary, different stressors, like the footshock during the shuttle box testing, may induce lower HPA axis reactivity [67]. Lower glucocorticoid levels can be accompanied by reduced inactivity in the shuttle box [1]. (3) The time spent in inactive posture (immobility) depends also on the subject`s temperament and coping style. The well-known depression test, forced swimming measures also the coping of the animals, however some authors suggested that it reflected effects of learning and memory rather than effects of despair or depression [65]. According to these assumptions the diminished immobility time of AVP-deficient rats during the forced swimming test [37] could be interpreted as changes in temperament [8, 26], more active coping style or even learning disabilities. Other depressive-like symptoms, like anhedonia, which is also changed in the Brattleboro rat [48], may also influence memory processes.

As far as we know AD-related markers were never tested in AVP-deficient Brattleboro rats, and the studied markers were not examined in 1 year old „normal" rats either. According to the amyloid hypothesis APP is the initiating molecule of AD. Although several authors emphasize the involvement of APP metabolism in normal aging and AD [40], our results suggest that altered APP expression may also contribute. Literary data referred to AVP and APP interaction [41, 59], which we failed to confirm.

The MAPK pathway is a key regulator of pro-inflammatory cytokine biosynthesis, which may contribute to the chronic low-grade inflammation observed with aging [43]. We found a small increase in the MAPK mRNA level in old rats, which was more pronounced in AVP-deficient animals. Signalisation cascade of AVP may include the MAPK pathway as well [44]. Although an elevated MAPK expression in old di/di rats seems to be contradictory, this

is not the only pathway for AVP and many other molecules may manipulate the MAPK cascade.

The β-actin, a non-muscle cytoskeletal protein, has a relatively stable expression, therefore it is worldwide applied as a reference gene in the course of PCR [49]. Newer data indicated that β-actin is differentially expressed in the brain specimens of both AD and control subjects [23]. Indeed, the results of recent research in the pathomechanism of AD emphasize the significance of cytoskeletal changes [18]. Remodelling of the cytoskeletal architecture is a basic phenomenon in neuronal plasticity [58]. Despite previous report on constant β-actin gene expression in aging [60], we found an elevated level in old rats, perhaps due to some methodological differences. Changes in actin remodelling may influence the neurotransmitter release, among others the secretion of AVP [62].

The TDO2 enzyme plays a role in the transformation of the tryptophan to kynurenine [57] and increased concentrations of kynurenine metabolics leads to neurotoxic effects [52]. Regarding AD pathomechanism post mortem immunocytochemistry in the hippocampus of AD patients revealed elevated TDO2 levels [21] and amyloid-β treatment increased TDO2 expression [22]. Thus, elevated TDO2 level in one year old rats might anticipate pathological changes. Although Comai et al. found a decline in the enzyme activities involved in tryptophan metabolism in old Sprague-Dawley rats compared to 2-3 month old ones [13], it may be explained with methodological differences.

5. Conclusions

Our finding on early appearance of specific markers before appearance of any memory decline may prompt further studies and in the long run can be helpful in early diagnosis. The positive impact of AVP on memory was confirmed, thus it can be a useful cognitive enhancer. AVP-related other psychological functions like circadian clock, activity, stress and coping may also contribute to its positive effect on AD. As the absence or presence

of AVP did not influence the levels of AD-related markers, AVP decrease in AD patients seems to be more likely the result rather than the cause of AD development. However, we cannot close out the possibility that AVP effects the processing/metabolism of these factors rather that their mRNA levels.

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7. References:

[1] M. Baez, I. Siriczman, M. Volosin, Corticosterone is involved in foot shock-induced inactivity in rats, Physiology & behavior, 60 (1996) 795-801.

[2] A.K. Berger, L. Fratiglioni, Y. Forsell, B. Winblad, L. Backman, The occurrence of depressive symptoms in the preclinical phase of AD: a population-based study, Neurology, 53 (1999) 1998-2002.

[3] I.F. Bielsky, S.B. Hu, K.L. Szegda, H. Westphal, L.J. Young, Profound impairment in social recognition and reduction in anxiety-like behavior in vasopressin V1a receptor knockout mice, Neuropsychopharmacology, 29 (2004) 483-493.

[4] B. Bohus, D. de Wied, The vasopressin deficient Brattleboro rats: a natural knockout model used in the search for CNS effects of vasopressin, Prog Brain Res, 119 (1998) 555-573.

[5] D. Bovet, P. Renzi, A. Oliverio, Transfer of responding between different stimuli in rats trained in two avoidance tasks, Life Sci, 8 (1969) 575-582.

[6] T.L. Briones, Environment, physical activity, and neurogenesis: implications for prevention and treatment of Alzhemier's disease, Curr Alzheimer Res, 3 (2006) 49-54.

[7] G.N. Brito, The behavior of vasopressin-deficient rats (Brattleboro strain), Physiology & behavior, 30 (1983) 29-34.

[8] G.N. Brito, G.J. Thomas, D.M. Gash, J.H. Kitchen, The behavior of Brattleboro rats, Ann N Y Acad Sci, 394 (1982) 740-748.

[9] R.J. Carey, M. Miller, Absence of learning and memory deficits in the vasopressin-deficient rat (Brattleboro strain), Behav Brain Res, 6 (1982) 1-13.

[10] R.T. Carroll, M.R. Lust, K.S. Kim, P.D. Doyle, M.R. Emmerling, An age-related correlation between levels of beta-amyloid precursor protein and beta-amyloid in human cerebrospinal fluid, Biochem Biophys Res Commun, 210 (1995) 345-349.

[11] R. Ceravolo, D. Borghetti, L. Kiferle, G. Tognoni, A. Giorgetti, D. Neglia, N. Sassi, D. Frosini, C. Rossi, L. Petrozzi, G. Siciliano, L. Murri, CSF phosporylated TAU protein levels correlate with cerebral glucose metabolism assessed with PET in Alzheimer's disease, Brain research bulletin, 76 (2008) 80-84.

[12] J. Cilia, J.E. Gartlon, C. Shilliam, L.A. Dawson, S.H. Moore, D.N. Jones, Further neurochemical and behavioural investigation of Brattleboro rats as a putative model of schizophrenia, J Psychopharmacol, 24 (2010) 407-419.

[13] S. Comai, C.V. Costa, E. Ragazzi, A. Bertazzo, G. Allegri, The effect of age on the enzyme activities of tryptophan metabolism along the kynurenine pathway in rats, Clin Chim Acta, 360 (2005) 67-80.

[14] R.L. Cooper, M.C. McNamara, W.G. Thompson, Vasopressin and conditioned flavor aversion in aged rats, Neurobiol Aging, 1 (1980) 53-57.

[15] L.A. Craig, R.J. McDonald, Chronic disruption of circadian rhythms impairs hippocampal memory in the rat, Brain research bulletin, 76 (2008) 141-151.

[16] M. Engelmann, R. Landgraf, Microdialysis administration of vasopressin into the septum improves social recognition in Brattleboro rats, Physiology & behavior, 55 (1994) 145-149.

[17] M. Engelmann, C.T. Wotjak, R. Landgraf, Social discrimination procedure: an alternative method to investigate juvenile recognition abilities in rats, Physiology & behavior, 58 (1995) 315-321.

[18] K.E. Fodor, M. Pakaski, P. Santha, Z. Janka, J. Kalman, [Cytoskeletal alterations in Alzheimer's disease: the "skeleton" of therapeutic hope?], Neuropsychopharmacol Hung, 13 (2011) 163-171.

[19] E. Frank, R. Landgraf, The vasopressin system--from antidiuresis to psychopathology, Eur J Pharmacol, 583 (2008) 226-242.

[20] E. Goudsmit, A. Neijmeijer-Leloux, D.F. Swaab, The human hypothalamo-neurohypophyseal system in relation to development, aging and Alzheimer's disease, Prog Brain Res, 93 (1992) 237-247; discussion 247-238.

[21] G.J. Guillemin, B.J. Brew, C.E. Noonan, O. Takikawa, K.M. Cullen, Indoleamine 2,3 dioxygenase and quinolinic acid immunoreactivity in Alzheimer's disease hippocampus, Neuropathol Appl Neurobiol, 31 (2005) 395-404.

[22] G.J. Guillemin, K.R. Williams, D.G. Smith, G.A. Smythe, J. Croitoru-Lamoury, B.J. Brew, Quinolinic acid in the pathogenesis of Alzheimer's disease, Adv Exp Med Biol, 527 (2003) 167-176.

[23] R.V. Gutala, P.H. Reddy, The use of real-time PCR analysis in a gene expression study of Alzheimer's disease post-mortem brains, J Neurosci Methods, 132 (2004) 101-107.

[24] H. Hampel, A. Goernitz, K. Buerger, Advances in the development of biomarkers for Alzheimer's disease: from CSF total tau and Abeta(1-42) proteins to phosphorylated tau protein, Brain research bulletin, 61 (2003) 243-253.

[25] J.A. Hardy, G.A. Higgins, Alzheimer's disease: the amyloid cascade hypothesis, Science, 256 (1992) 184-185.

[26] J.P. Herman, G.J. Thomas, J.F. Laycock, I.B. Gartside, D.M. Gash, Behavioral variability within the Brattleboro and Long-Evans rat strains, Physiology & behavior, 36 (1986) 713-721.

[27] G.V. Johnson, C.D. Bailey, The p38 MAP kinase signaling pathway in Alzheimer's disease, Exp Neurol, 183 (2003) 263-268.

[28] H.W. Klafki, M. Staufenbiel, J. Kornhuber, J. Wiltfang, Therapeutic approaches to Alzheimer's disease, Brain, 129 (2006) 2840-2855.

[29] G.F. Koob, R. Dantzer, R.M. Bluthe, C. Lebrun, F.E. Bloom, M. Le Moal, Central injections of arginine vasopressin prolong extinction of active avoidance, Peptides, 7 (1986) 213-218.

[30] P.W. Landfield, E.M. Blalock, K.C. Chen, N.M. Porter, A new glucocorticoid hypothesis of brain aging: implications for Alzheimer's disease, Curr Alzheimer Res, 4 (2007) 205-212.

[31] R. Landgraf, I.D. Neumann, Vasopressin and oxytocin release within the brain: a dynamic concept of multiple and variable modes of neuropeptide communication, Front Neuroendocrinol, 25 (2004) 150-176.

[32] G. Leuba, A. Vernay, V. Zimmermann, K. Saini, R. Kraftsik, A. Savioz, Differential damage in the frontal cortex with aging, sporadic and familial Alzheimer's disease, Brain research bulletin, 80 (2009) 196-202.

[33] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods, 25 (2001) 402-408.

[34] M.F. Mazurek, M.F. Beal, E.D. Bird, J.B. Martin, Vasopressin in Alzheimer's disease: a study of postmortem brain concentrations, Ann Neurol, 20 (1986) 665-670.

[35] O.C. Meijer, B. Topic, P.J. Steenbergen, G. Jocham, J.P. Huston, M.S. Oitzl, Correlations between hypothalamus-pituitary-adrenal axis parameters depend on age and learning capacity, Endocrinology, 146 (2005) 1372-1381.

[36] B. Misiak, J. Leszek, A. Kiejna, Metabolic syndrome, mild cognitive impairment and Alzheimer's disease--the emerging role of systemic low-grade inflammation and adiposity, Brain research bulletin, 89 (2012) 144-149.

[37] M. Mlynarik, D. Zelena, G. Bagdy, G.B. Makara, D. Jezova, Signs of attenuated depression-like behavior in vasopressin deficient Brattleboro rats, Horm Behav, 51 (2007) 395-405.

[38] D.G. Moon, M.H. Jin, J.G. Lee, J.J. Kim, M.G. Kim, D.R. Cha, Antidiuretic hormone in elderly male patients with severe nocturia: a circadian study, BJU Int, 94 (2004) 571-575.

[39] B.C. Nephew, R.S. Bridges, Arginine vasopressin V1a receptor antagonist impairs maternal memory in rats, Physiology & behavior, 95 (2008) 182-186.

[40] M.R. Palmert, M. Usiak, R. Mayeux, M. Raskind, W.W. Tourtellotte, S.G. Younkin, Soluble derivatives of the beta amyloid protein precursor in cerebrospinal fluid: alterations in normal aging and in Alzheimer's disease, Neurology, 40 (1990) 1028-1034.

[41] Y.F. Pan, X.R. Chen, M.N. Wu, C.G. Ma, J.S. Qi, Arginine vasopressin prevents against Abeta(25-35)-induced impairment of spatial learning and memory in rats, Horm Behav, 57 (2010) 448-454.

[42] Y.F. Pan, X.T. Jia, X.H. Wang, X.R. Chen, Q.S. Li, X.P. Gao, J.S. Qi, Arginine vasopressin remolds the spontaneous discharges disturbed by amyloid beta protein in hippocampal CA1 region of rats, Regulatory peptides, 183C (2013) 7-12.

[43] R. Panarsky, L. Luques, M. Weinstock, Anti-Inflammatory Effects of Ladostigil and Its Metabolites in Aged Rat Brain and in Microglial Cells, J Neuroimmune Pharmacol, (2012).

[44] A. Pena, B. Murat, M. Trueba, M.A. Ventura, G. Bertrand, L.L. Cheng, S. Stoev, H.H. Szeto, N. Wo, G. Brossard, C. Serradeil-Le Gal, M. Manning, G. Guillon, Pharmacological and physiological characterization of d[Leu4, Lys8]vasopressin, the first V1b-selective agonist for rat vasopressin/oxytocin receptors, Endocrinology, 148 (2007) 4136-4146.

[45] Y.I. Peng, S.H. Lin, T.J. Chen, M.Y. Tai, Y.F. Tsai, Effects of age on open-field behavior of male rats, Chin J Physiol, 37 (1994) 233-236.

[46] R.D. Prediger, L.C. Batista, R.N. Takahashi, Caffeine reverses age-related deficits in olfactory discrimination and social recognition memory in rats. Involvement of adenosine A1 and A2A receptors, Neurobiol Aging, 26 (2005) 957-964.

[47] P. Proitsi, J.F. Powell, Missense substitutions associated with behavioural disturbances in Alzheimer's disease (AD), Brain research bulletin, 88 (2012) 394-405.

[48] M. Riediger, C. Wrzus, F. Schmiedek, G.G. Wagner, U. Lindenberger, Is seeking bad mood cognitively demanding? Contra-hedonic orientation and working-memory capacity in everyday life, Emotion, 11 (2011) 656-665.

[49] W. Ruan, M. Lai, Actin, a reliable marker of internal control?, Clin Chim Acta, 385 (2007) 1-5.

[50] K. Saito, S. Fujigaki, M.P. Heyes, K. Shibata, M. Takemura, H. Fujii, H. Wada, A. Noma, M. Seishima, Mechanism of increases in L-kynurenine and quinolinic acid in renal insufficiency, Am J Physiol Renal Physiol, 279 (2000) F565-572.

[51] E.I. Samarova, N.I. Bravarenko, T.A. Korshunova, N.V. Gulyaeva, A. Palotas, P.M. Balaban, Effect of beta-amyloid peptide on behavior and synaptic plasticity in terrestrial snail, Brain research bulletin, 67 (2005) 40-45.

[52] A. Santamaria, S. Galvan-Arzate, V. Lisy, S.F. Ali, H.M. Duhart, L. Osorio-Rico, C. Rios, F. St'astny, Quinolinic acid induces oxidative stress in rat brain synaptosomes, Neuroreport, 12 (2001) 871-874.

[53] W.H. Sawyer, H. Valtin, H.W. Sokol, Neurohypophysial Principles in Rats with Familial Hypothalamic Diabetes Insipidus (Brattleboro Strain), Endocrinology, 74 (1964) 153-155.

[54] J.C. Schank, Early locomotor and social effects in vasopressin deficient neonatal rats, Behav Brain Res, 197 (2009) 166-177.

[55] C.T. Siwak, H.L. Murphey, B.A. Muggenburg, N.W. Milgram, Age-dependent decline in locomotor activity in dogs is environment specific, Physiology & behavior, 75 (2002) 65-70.

[56] S.E. Starkstein, R. Jorge, R. Mizrahi, R.G. Robinson, The construct of minor and major depression in Alzheimer's disease, Am J Psychiatry, 162 (2005) 2086-2093.

[57] Y. Sun, Indoleamine 2,3-dioxygenase--a new antioxidant enzyme, Mater Med Pol, 21 (1989) 244-250.

[58] T. Svitkina, W.H. Lin, D.J. Webb, R. Yasuda, G.A. Wayman, L. Van Aelst, S.H. Soderling, Regulation of the postsynaptic cytoskeleton: roles in development, plasticity, and disorders, J Neurosci, 30 (2010) 14937-14942.

[59] T. Tanaka, K. Yamada, K. Senzaki, H. Narimatsu, K. Nishimura, T. Kameyama, T. Nabeshima, NC-1900, an active fragment analog of arginine vasopressin, improves learning and memory deficits induced by beta-amyloid protein in rats, Eur J Pharmacol, 352 (1998) 135-142.

[60] N. Tanic, M. Perovic, A. Mladenovic, S. Ruzdijic, S. Kanazir, Effects of aging, dietary restriction and glucocorticoid treatment on housekeeping gene expression in rat cortex and hippocampus-evaluation by real time RT-PCR, J Mol Neurosci, 32 (2007) 38-46.

[61] M. Timmer, C. Sandi, A role for glucocorticoids in the long-term establishment of a social hierarchy, Psychoneuroendocrinology, 35 (2010) 1543-1552.

[62] V.A. Tobin, M. Ludwig, The actin filament and dendritic peptide release, Biochem Soc Trans, 35 (2007) 1243-1246.

[63] T.B. van Wimersma Greidanus, Disturbed behavior and memory of the Brattleboro rat, Ann N Y Acad Sci, 394 (1982) 655-662.

[64] Z. Wang, L. Yang, H. Zheng, Role of APP and Abeta in Synaptic Physiology, Curr Alzheimer Res, 9 (2012) 217-226.

[65] A.P. West, Neurobehavioral studies of forced swimming: the role of learning and memory in the forced swim test, Prog Neuropsychopharmacol Biol Psychiatry, 14 (1990) 863-877.

[66] D. Zelena, A. Domokos, I. Barna, Z. Mergl, J. Haller, G.B. Makara, Control of the hypothalamo-pituitary-adrenal axis in the neonatal period: adrenocorticotropin and corticosterone stress responses dissociate in vasopressin-deficient brattleboro rats, Endocrinology, 149 (2008) 2576-2583.

[67] D. Zelena, A. Domokos, S.K. Jain, R. Jankord, L. Filaretova, The stimuli-specific role of vasopressin in the hypothalamus-pituitary-adrenal axis response to stress, J Endocrinol, 202 (2009) 263-278.

[68] D. Zelena, K. Langnaese, A. Domokos, O. Pinter, R. Landgraf, G.B. Makara, M. Engelmann, Vasopressin administration into the paraventricular nucleus normalizes plasma oxytocin and corticosterone levels in Brattleboro rats, Endocrinology, 150 (2009) 2791-2798.

Figure legend

Fig.1

Social discrimination test. n=7-13. A. Time percent spent with the juveniles during the sample and the choice phases. B. Discrimination index = time percentage with juvenile 2 / (time percentage with juvenile 1 + time percentage with juvenile 2). Abbreviations: *p<0.05, **p<0.01 vs. juvenile 1., #p<0.05 vs. 0.5 (no choice).

Fig.2

Object discrimination test. n=7-10. A. Time percent spent with the objects during the sample and the choice phases. B. Discrimination index (= time percentage Object 2 / (time percentage Object 1 + time percentage Object 2)). Abbreviations: $p<0.05 vs. +/+; **p<0.01 vs. object 1.; ##p<0.01 vs. 0.5 (no choice).

Fig.3

Shuttle box test. n=7-12. A. Intertrial crossing. B. Conditioned avoidance response. C. Escape failure. Abbreviations: $p<0.05, $$p<0.01 vs. +/+; +p<0.05, ++p<0.01 vs. day 1.; **p<0.01 vs. adult

Fig.4

Alzheimer`s markers' mRNA levels in comparison to adult, 3 month old +/+ animals calculated by the $2^{-\Delta\Delta Ct}$ method with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) standard. n=6-10. A. Amyloid precursor protein (APP). B. Mitogen-activated protein kinase 1 (MAPK1). C. β-actin. D. Tryptophan 2,3-dioxygenase 2 (TDO2). Abbreviations: *p<0.05, **p<0.01 vs. adult; $p=0.05 vs. +/+.