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Activity changes and marked stereotypic behavior precede Aβ pathology in TgCRND8 Alzheimer mice

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Abstract

Alzheimer's disease (AD) is not only characterized by cognitive decline and neuropathological changes, but also by non-cognitive behavioral symptoms like restlessness, sleep disturbance, and wandering. These symptoms are categorized in the "Behavioral and Psychological Symptoms of Dementia" (BPSD). We investigated transgenic and wildtype mice of an APP transgenic mouse model of AD (TgCRND8) with respect to 24 h activity and spontaneous home cage behavior at 30, 60, 90 and 120 days of age. At all test days, transgenic and wildtype animals differed significantly with respect to activity patterns. In addition, activity rhythms changed distinctly in transgenic mice with increasing age. Transgenic mice also clearly showed more stereotypic behavior, which correlated significantly at 90 and 120 days of age with elevated corticosterone metabolite concentrations in fecal samples. Activity patterns in TgCRND8 mice resemble altered rhythms of activity in AD patients. Stereotypic behaviors may be caused by the same mechanisms as non-cognitive behavioral symptoms of AD. Thus, it is likely that analogies to BPSD that precede Aβ pathology are found in APP-overexpressing TgCRND8 mice.

Keywords: Alzheimer's disease; Activity rhythm; Stereotypic behavior; BPSD; Mice; TgCRND8; Transgenic; HPA; Corticosterone; Fecal corticosterone metabolites; SAM; Tyrosine hydroxylase

1. Introduction

Alzheimer's disease (AD) is the most common cause of senile dementia worldwide. Neuropathological hallmarks of AD include senile plaques and neurofibrillary tangles. Senile or neuritic plaques are insoluble extracellular protein aggregates, mainly made up of amyloid β-peptide (Aβ), a cleavage product of the amyloid precursor protein (APP). Neurofibrillary tangles consist of hyperphosphorylated tau protein forming paired helical filaments [6,21,51,59]. As it progresses, the disease affects brain regions critical for cognition and memory, e.g. cortex and hippocampus [2,51,59] resulting in a complex cognitive decline [52]. One of the earliest clinical manifestations of AD is memory loss [3]. But there are also non-cognitive behavioral symptoms such as physical aggression, screaming, restlessness, sleep disturbance, wandering, and agitation, belonging to the so called "Behavioral and Psychological Symptoms of Dementia" (BPSD), that are a major burden for relatives and care-givers [15,41,66].

In order for animal studies to contribute to the understanding and treatment of AD, it is important that such models exhibit specific symptoms of AD [12,26,51]. Ideally, a murine model of AD would develop neuropathological, cognitive, behavioral as well as hormonal changes that are comparable with those observed in human AD patients. Most studies using transgenic mouse models of AD concentrate on molecular and neuropathological changes as well as cognitive deficits [26]. Some studies also focus on standard behavioral tests for exploration and anxiety (e.g. [1,33,34]). Only a few

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studies have focussed on other Alzheimer-relevant features like glucocorticoid levels [19,47,68] or sleep–wake patterns [27,71,74]. In PDAPP mice, for example, carrying the Indiana mutation, age-dependent alterations in sleep–wake states are found [27]. In APP23 mice carrying the Swedish mutation, disturbed activity patterns appear long before amyloid plaques can be detected in the brain [71]. It was argued that such changes resemble sundowning behavior in AD: the appearance or exacerbation of behavioral disturbances during afternoon or evening [74].

Recently, we showed that TgCRND8 mice, a transgenic APP model of AD, do not differ from wildtypes in standard behavioral tests of anxiety, locomotor activity and exploration. However, they display an age- and sex-dependent hyperactivity of the hypothalamic pituitary adrenocortical (HPA) axis [68], another feature of Alzheimer's disease on the endocrinological level [23,35]. From qualitative investigations, the impression arose that so-called stereotypic behavior is displayed more frequently by transgenic mice. Stereotypic behavior is defined as repetitive, unvarying behavior patterns without any obvious function [38].

Since TgCRND8 mice develop an extensive Alzheimer-like pathology on neural, cognitive and hormonal levels, the aim of this study was to investigate these mice with respect to alterations in spontaneous behavior in the home cage assessing daily rhythms of activity and stereotypic behavior. The goal was to find possible analogies to BPSD in humans and to elucidate whether or not a relationship exists between alterations in spontaneous behavior and neural or hormonal manifestations.

2. Methods

2.1. Animals, breeding and housing conditions

In this study, we investigated male mice of the TgCRND8 line, a transgenic animal model of AD [7,28]. These mice carry a double mutated form of the human amyloid precursor protein 695 (APP), the 'Swedish' (K670N/M671L) and 'Indiana' (V717F) mutations, on a hybrid C57BL/6-C3H/HeJ genetic background. The transgene is regulated by the Syrian hamster prion promoter (PrP). The main characteristic of this model is a very rapid development of amyloid pathology in the brain. All mice display amyloid-plaques in the cortex and the hippocampus by three months of age [7]. These neuropathologic manifestations are accompanied by impaired acquisition and learning deficits in the Morris water-maze [7,28].

Animals were bred in pairs of a wildtype (APP−/−) female and a transgenic (hemizygous, APP+/−) male. At 21 days of age, animals were weaned and transferred to the experimental housing conditions. A total of 18 male mice, 8 APP+/− and 10 APP−/− mice, were investigated. These mice were housed individually in transparent standard polycarbonate cages (Makrolon, type III, 38 cm × 22 cm × 15 cm) with sawdust as bedding material (Allspan, Höveler GmbH & Co. KG, Langenfeld, Germany). Individual housing was chosen to avoid socially induced effects on activity patterns as well as endocrine systems. All animals had free access to commercial mouse diet (Altromin no. 1324, Altromin GmbH, Lage, Germany) and bottled tap water. A photoperiod of 12 h light and 12 h dark, lights on at 7 a.m., temperature of 21 ± 1 °C, and relative humidity of 50 ± 10% were maintained in the animal housing room.

The presented work complies with current regulations covering animal experimentation in Germany and the EU (European Communities Council Directive 86/609/EEC). All experiments were announced to the local authority and were approved by the 'Animal Welfare Officer' of the University of Münster.

2.2. Behavioral analysis

The spontaneous behavior of all individuals was recorded in home cages at 30, 60, 90 and 120 days of age using a 24-h time-lapse VCR (Panasonic AG6730). Animals were tracked by a lightsensitive (0.5 lux) B/W CCD camera through the long side wall of the cage. A 25-W red light provided the necessary illumination for video recording during the dark phase. For behavioral analysis, individual data on activity patterns and stereotypic behavior were collected by focal animal sampling and continuous recording [37]. The entire 24 h of each recording was analysed using 'The Observer Video Pro 4.0' (Noldus). The frequency and duration of all bouts of the following behaviors were recorded for each of the 18 animals:

Activity patterns

Resting: Beginning with a bout of motionless lying for at least 10 s, ending when the animal moved at least one body length away. Changes of the resting position and intermediate bouts of grooming were considered as resting as long as the animal did not leave the resting place.

Activity: All behaviors other than *Resting*.

Stereotypies

Jumping: A bout of either repetitive jumping up and down or scratching with the paws along the cage wall.

Traversing-lid: A bout of repetitive climbing in a straight direction on the inside of the cage lid.

Circling-lid: A bout of repetitive climbing in a circular motion on the inside of the cage lid.

The definitions of *Resting*, *Activity* and *Jumping* were adopted from Würbel et al. [81]. *Resting* behavior was only recorded if bout length exceeded 60 s. Stereotypic behavior is defined as repeated and invariant movements without any obvious goal or function [38,39]. The stereotypic

behaviors *Jumping*, *Traversing-lid* and *Circling-lid* were only recorded if bout length exceeded 10 s. Thus, the repetitive character of stereotypic behaviors was assured and a reliable analysis of the videotapes at eightfold normal speed was possible. Intervals between two consecutive bouts of stereotypic behavior were defined to last for a minimum of 5 s. For statistical analysis, the three stereotypic behavior patterns *Jumping*, *Traversing-lid* and *Circling-lid* were summed to form the category *Stereotypies*.

Behavioral observations started at 11 a.m., 2 h after placing the animals in test cages identical to their home cage containing fresh bedding material. This was necessary because we wanted to collect the feces deposited by the mice following the 24 h of videotaping. Previous investigations demonstrated that this period was sufficient for the animals of both genotypes to habituate to the new cage.

2.3. Endocrinological analysis

2.3.1. Corticosterone metabolites in fecal samples

At intervals of 30 days (i.e. on days 30, 60, 90 and 120), the animals were weighed and placed for 26 h in a test cage (see above). All feces voided during these 26 h periods (starting at 9 a.m.) were collected and frozen at −30°C until assayed for corticosterone metabolites (corticosterone is the major glucocorticoid in mice [63]).

The collected fecal samples were analyzed for immunoreactive corticosterone metabolites (CM) using a recently established 5α-pregnane-3β,11β,21-triol-20-one enzyme-immunoassay (EIA). Details regarding development, biochemical characteristics and biological validation of this assay have been described [69,70]. Before EIA analysis, the fecal samples were homogenized and aliquots of 0.05 g were extracted with 1 ml of 80% methanol. A detailed description of assay performance has been published [70]. To be brief, the EIA used a double-antibody technique and was performed on anti-rabbit-IgG-coated microtiter plates. After overnight incubation (at 4 °C) of standards (range: 0.8–200 pg/well) and samples (in duplicate) with steroid antibody and biotinylated label, the plates were emptied, washed and blotted dry, before a streptavidin horseradish peroxidase conjugate was added. After 45 min incubation, plates were emptied, washed and blotted dry. The substrate (tetramethylbenzidine) was added and incubated for another 45 min at 4 °C before the enzymatic reaction was stopped with 2 M sulphuric acid. Then the optical density (at 450 nm) was recorded with an automatic plate reader and the hormone concentrations were calculated. The intra- and interassay coefficients of variation were 9.1 and 14.0%, respectively.

2.3.2. Corticosterone concentrations in blood samples

At day 123, 3 days after the last behavioral recording, the animals were decapitated at 9 a.m. and trunk blood (about 0.2 ml) was collected in heparinised capillaries to determine circulating corticosterone concentrations. After separation of cellular constituents by centrifugation (5 min at 14,800 × *g*), plasma was frozen at −30°C until analysis. To avoid stress effects of the handling procedure on the investigated endocrine parameters, blood sampling was performed in a separate room adjacent to the animal housing room and within a maximum time of three minutes from disturbing the animal's cage [4,18,25,63].

Blood samples were also analyzed by EIA. Specifications of the corticosterone EIA used have been described [45]. Assay performance was similar to the EIA described above. However, the standard curve ranged from 2–500 pg/well and the plasma samples (0.02 ml) were extracted with diethyl ether (5 ml) before EIA analysis. The intra- and interassay coefficients of variation were 10.0 and 13.4%, respectively.

2.3.3. Adrenal tyrosine hydroxylase activity

As an indicator of the activity of the sympathetic adrenomedullary (SAM) system [13,56,76], adrenals were analyzed for tyrosine hydroxylase (TH) activity. Immediately after decapitation, both adrenals were dissected, cleaned from fat and adherent tissue, and snap-frozen in 5 mM Tris–HCl buffer (pH 7.2). Adrenals were stored at −70°C until assayed for TH activity using a radio-enzymatic technique [43,77] with slight modifications. To be brief, adrenals were thawed on ice and homogenised in 0.15 ml 5 mM Tris–HCl buffer (pH 7.2). After centrifugation (30 min at 20,000 × *g* and 4°C), TH activity was determined in triplicate from the supernatant. Aliquots were incubated (for 30 min at 37°C) with ^{14}C-labelled tyrosine (^{14}C-Tyr) as substrate, before stopping the enzymatic reaction by adding 10% trichloroacetic acid and Dopa-carrier solution. Precipitated protein was eliminated by centrifugation and subsequently ^{14}C-Tyr and ^{14}C-Dopa were separated by column-chromatography. Columns of equilibrated Al_2O_3 slurry (in Pasteur pipettes closed with glass wool) were used and washed successively with NH_4CH_3COOH buffer (pH 6.1), demineralized water, and acetic acid before eluting ^{14}C-Dopa with HCl-MeOH (1:1 mixture). Then the amount of recovered ^{14}C-Dopa was quantified by liquid scintillation counting.

2.4. Neuropathological analysis

Immediately after decapitation, brains were removed and bisected in the mid-sagittal plane. One hemisphere was fixed in 4% buffered formaldehyde for 2 days followed by paraffin-embedding, whereas the other half was snap-frozen and stored at −80°C.

Sagittal brain sections of 2 μm were cut using a microtome. Two serial sections (immediately lateral from the mid-sagittal fissure) and two further ones at a distance of 140 μm from the first sections were affixed to Dako ChemMateTM Capillary Gap Microscope Slides. All samples were pretreated with formic acid and automatically stained in a TechMate Instrument (DAKO) with 6F/3D anti-Aβ monoclonal

antibody to residues 8–17 (Dako, antibody dilution 1:100). For further steps, the Dako StreptABC complex-horseradish peroxidase conjugated "Duet" anti mouse/rabbit antibody kit was used and developed with 3,3′-diaminobenzidine (DAB) as chromogen. Counterstaining was performed with hematoxylin. The whole staining procedure was performed simultaneously for all sections.

To quantify amyloid deposition, plaques were counted in four different brain regions (neocortex, corpus callosum, hippocampus and basal ganglia including striatum and thalamus) of all four sections. Total plaque burden was calculated by addition of the sixteen single values obtained for each animal.

Since Ingram and coworkers showed that glia inclusions, present in normal brain aging, might be mistaken for amyloid plaques [30], the brains of two APP−/− animals were also immunostained for Aβ in order to achieve a baseline of non-specific staining. As expected , no plaques or plaque-like structures were observed in the brains of any wild-type mice investigated.

2.5. Statistical analysis

Data were analysed using non-parametric statistics [61] because portions of the data were not normally distributed. To compare two independent samples, the Mann–Whitney *U*-test was used. Differences between more than two paired samples were assessed using the Friedman two-way ANOVA. Wilcoxon–Wilcox rank sum tests for multiple testing were performed to assess statistically significant differences between paired samples in post-hoc analyses. To evaluate the strength of association between two variables, Spearman's rank correlation coefficient was calculated. All tests were applied two-tailed using the software package SPSS (version 11.0.1) except for the Wilcoxon–Wilcox rank sum test, which was performed as described by Sachs [54]. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Descriptive analysis of activity patterns

Distinct activity patterns were observed in APP+/− and APP−/− mice as depicted in Fig. 1. APP−/− mice generally showed a kind of two-phased activity rhythm with an activity peak at the beginning, and another peak at the end, of the dark phase. They displayed only very few stereotypies, and the activity rhythm did not change substantially over the time-course of the experiment, except for a mild decline in total activity (see Fig. 2).

APP+/− animals, however, showed a very long activity bout during the first hours of the dark period at all test days. Stereotypic behavior also occurred mainly during the first hours of the dark period. At the age of 30 days the duration of stereotypic behavior in APP+/− mice was relatively short, but from day 60 on, the duration increased such that up to 40% of the activity time was spent in stereotypic behavior (cf. Figs. 1 and 3). With increasing age, prolonged activity and bouts of stereotypic behavior were more widely spread over the day including the light phase.

3.2. Total activity and stereotypic behaviors

Regarding total activity, significant differences between APP+/− and APP−/− mice were found at all test days (Fig. 2). At day 30 APP+/− mice already were active much longer than APP−/− mice, by a median difference of about 2 h. This difference remained stable at days 60 and 90, and increased to more than a 5 h median at day 120. This was due to a slight increase of activity in APP+/− mice and a marked decrease in APP−/− animals compared to the previous test days. Over the time-course, significant variation occurred in APP−/− mice only, with differences between the highest activity at day 60 (median: 9.1 h) and the lowest at day 120 (median: 6.6 h; Friedman two-way ANOVA, $n = 10, df = 3, \chi^2_R = 14.040, p < 0.01$; Wilcoxon–Wilcox, $D_{60–120} = 21, p < 0.01$).

We investigated three different stereotypic behavior patterns. While *Circling-lid* was performed by both, wildtype and transgenic animals, but more frequently by transgenics, *Jumping* appeared exclusively in transgenic mice and *Traversing-lid* was only shown by wildtype animals. Since there is a relation between the duration of total activity and stereotypic behaviors, stereotypic behavior data are also given as percentage of total activity in order to normalize for differences in total activity (see above). Nevertheless, there still were significant differences between APP+/− and APP −/− mice at all test days (Fig. 3). At the age of 30 days APP+/− mice showed slightly more stereotypies (median: 1.2%) than APP−/− animals (median: 0%). This difference became much clearer at day 60, when APP+/− mice spent a median of about 19% of their activity time performing stereotypic behaviors, while only few APP−/− mice showed stereotypic behaviors at all (1.4% of total activity). The same difference between groups was found at 90 days of age, the range in APP+/− mice being greater than at day 60. At day 120, APP+/− mice showed somewhat less stereotypic behaviors than at days 60 and 90, while the percentage of stereotypic behaviors in APP−/− mice increased slightly. Across different test days, there was a significant difference in APP+/− mice only, that can be attributed to the significant increase of stereotypies between test days 30 and 60, as well as 30 and 90 (Friedman two-way ANOVA, $n = 8, df = 3, \chi^2_R = 11.400, p < 0.01$; Wilcoxon–Wilcox, $D_{30–60} = 14, D_{30–90} = 16, p < 0.05$).

3.3. Correlation of different parameters with stereotypic behaviors

Regarding plasma corticosterone concentrations, tyrosine hydroxylase activity and number of amyloid plaques that

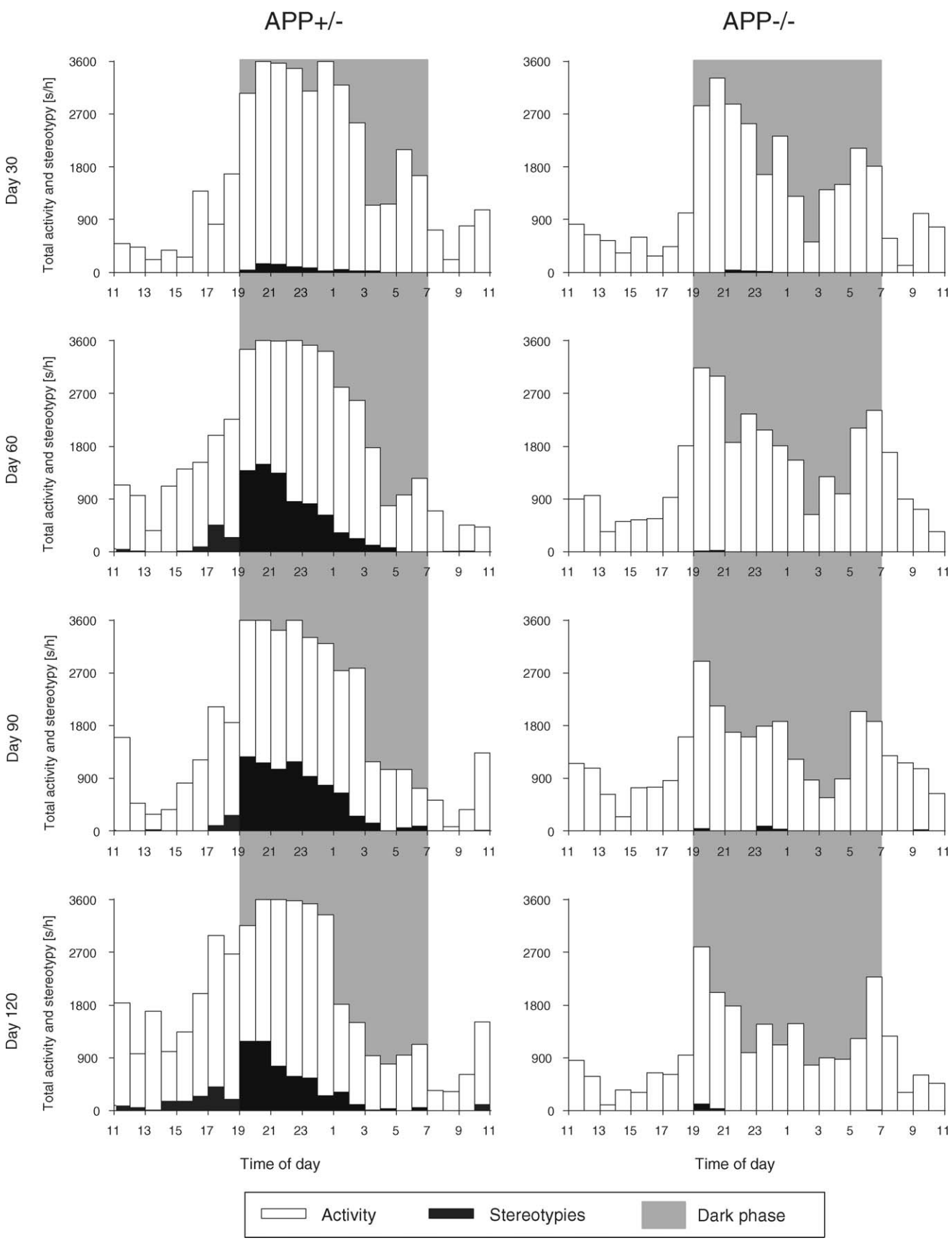

Fig. 1. Diurnal patterns of total activity (white) and total duration of stereotypic behavior (black) in APP+/− (left column) and APP−/− mice (right column) at 30, 60, 90 and 120 days of age. The dark phase is indicated by grey background. Data are given as means of 8 APP+/− and 10 APP−/− animals, respectively.

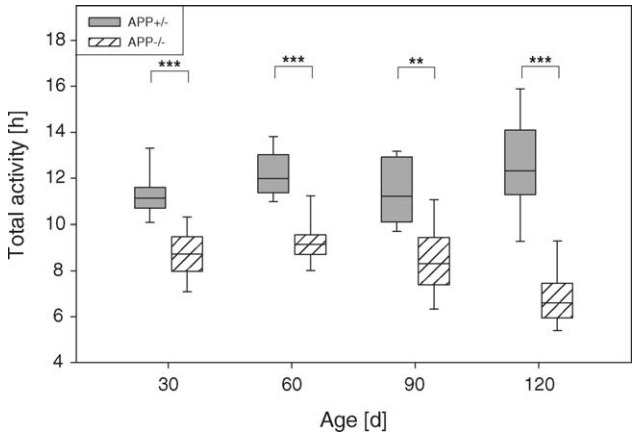

Fig. 2. Total activity of APP+/− and APP−/− male mice during 24 h periods at 30, 60, 90 and 120 days of age. Data are given as box-whisker plots showing medians (lines in the boxes), 25th and 75th percentiles (boxes), and 10 and 90% ranges (whiskers). Statistics: Mann–Whitney *U*-test (two-tailed), $n_{APP+/-} = 8$, $n_{APP-/-} = 10$, $U_{30} = U_{60} = U_{120} = 1$, $U_{90} = 6$, $^{**}p < 0.01$, $^{***}p < 0.001$.

were found in all four brain regions [68], no significant correlations with stereotypic behaviors were found for either APP−/− or APP+/− mice. In APP−/− mice, there was no significant correlation between the total duration of stereotypic behavior and corticosterone metabolite (CM) concentrations at any test day. However, in APP+/− animals, significant positive correlations between stereotypic behavior and CM concentrations were found at 2 test days (Fig. 4). While at day 30 no clear relationship between stereotypies and CM concentrations could be confirmed ($r_s = 0.343$, n.s.), a strong tendency was found at day 60 ($r_s = 0.643$, $p < 0.1$). At days 90 and 120 there were significant positive correlations between stereotypic behavior and CM concentrations (both $r_s = 0.714$, $p < 0.05$).

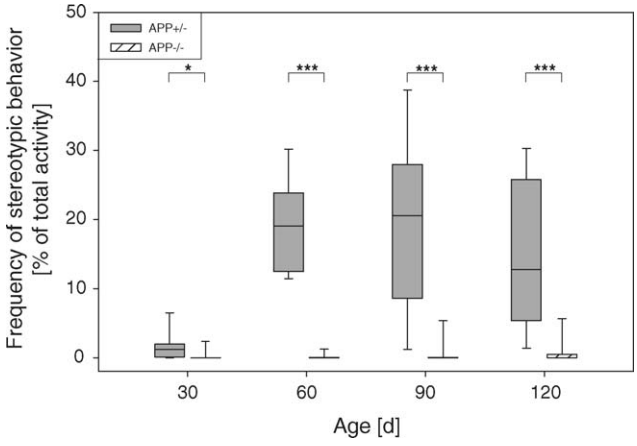

Fig. 3. Frequency of stereotypic behavior of APP+/− and APP−/− male mice during 24 h periods at 30, 60, 90 and 120 days of age. Data are given as box-whisker plots (for a description see legend of Fig. 2). Statistics: Mann–Whitney *U*-test (two-tailed), $n_{APP+/-} = 8$, $n_{APP-/-} = 10$, $U_{30} = 19$, $U_{60} = 0$, $U_{90} = 1$, $U_{120} = 2$, $^{*}p < 0.05$, $^{***}p < 0.001$.

4. Discussion

4.1. 24 h activity patterns

The objective of this study was to detect alterations in spontaneous behavior in the home cage of TgCRND8 Alzheimer mice with respect to possible analogies to the "Behavioral and Psychological Symptoms of Dementia" in humans. We found greater activity in transgenic mice of the TgCRND8 line as compared to wildtypes. The differences could already be detected at the age of 30 days, i.e. long before amyloid plaques in the brain are found (routinely around 90 days of age [7]). Activity rhythms of wildtype mice can be considered normal [24,32,74]. In contrast, activity patterns of transgenic animals deviated from what is usually described for laboratory mice. The activity rhythm of these animals changed with increasing age and progressing pathology. This does not appear due to differences in visual function, as both genotypes appear visually competent under bright light [68]. It should not be surprising that the greater activity of the transgenic animals could not be detected in the open-field and elevated plus-maze test [68]. While these tasks study a behavioral response to an unfamiliar situation, in this study we observed the spontaneous behavior in the familiar environment of the home cage.

Several other studies of APP transgenic mice have also reported alterations in circadian rhythms [27,71,74]. Moreover, rats with Aβ-overexpressing brain grafts in the suprachiasmatic nucleus (SCN) displayed disrupted activity patterns [67]. Syrian hamsters that received Aβ microinjections into the SCN exhibited significantly greater variability in onset time of wheel running activity [16]. Taken together, these data suggest that alterations of circadian rhythms might be a specific effect associated with altered APP metabolism or enhanced Aβ levels.

In human Alzheimer patients, disturbances of circadian rhythms and sleep, as well as sundowning are common symptoms [22,72,73,75,78]. In fact, sleep disturbances are the primary reason for institutionalization of AD patients [36,62]. It is interesting to note that AD patients show less locomotor activity in the diurnal phase but more in the nocturnal phase than healthy controls [22]. This is in accordance with our findings in TgCRND8 mice. As nocturnal animals, they were more active during the light hours than wildtypes, i.e. they showed a shifted activity phase. It seems that alterations of 24 h activity rhythms are a common symptom in humans and APP transgenic mice. However, AD patients usually do not differ from healthy controls concerning total activity [22,72]. In contrast, TgCRND8 mice displayed significantly increased total activity compared to wildtypes in this study.

Regarding the mechanism underlying rhythm disturbances, several studies reveal that people suffering from AD exhibit lower melatonin levels than age matched controls [42,44,62,79]. The lower melatonin levels may be related to a reduction of vasopressin-expressing neurons in the SCN, which is observed in normal aging but is even

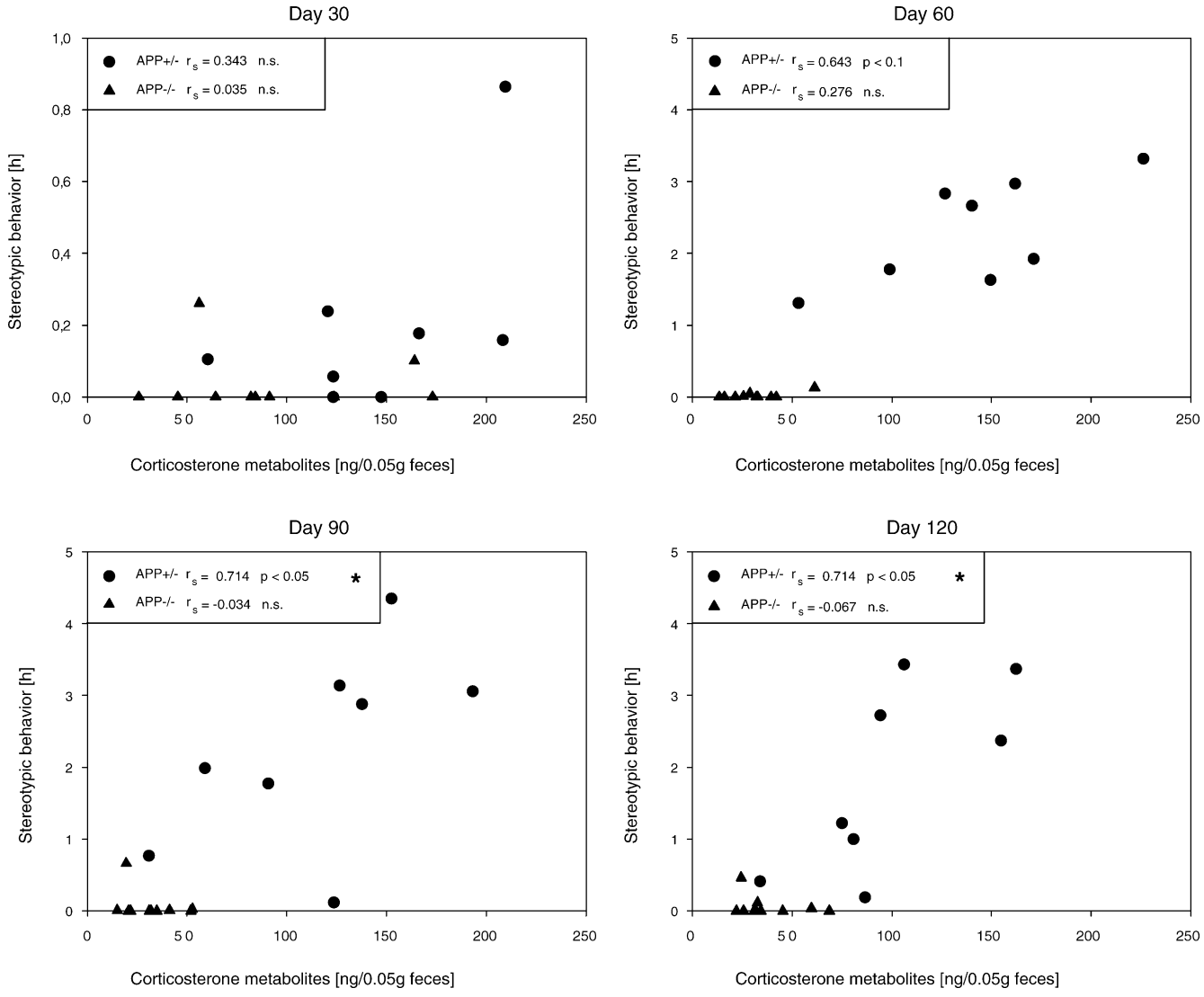

Fig. 4. Correlation between stereotypies and corticosterone metabolite concentrations of APP+/− and APP−/− male mice at 30, 60, 90 and 120 days of age. Note the different *Y*-axis scale at day 30. Statistics: Spearman's rank correlation coefficient.

more pronounced in AD [64]. It is remarkable that melatonin administered in the drinking water could also neutralize the effect of Aβ microinjections in hamsters [16]. In addition, it has been shown that melatonin administration reduces the degree of the Alzheimer related pathology and improves learning abilities in APP transgenic mice [14,40]. Thus, several studies on melatonin in humans as well as in animals provide evidence that the same mechanisms may underlie rhythm disturbances in AD patients and in animal models as described above.

4.2. Stereotypic behavior, endocrinological parameters and brain pathology

The second part of this study comprised the assessment of stereotypic behavior in the home cage. This was carried out in a transgenic mouse model of AD for the first time. We observed that TgCRND8 mice exhibit significantly more stereotypic behavior than wildtype littermates. This difference was apparent by the age of 30 days and became even more pronounced with increasing age.

Stereotypies are commonly thought to be abnormal behaviors that mainly occur in captive animals as a sign of suboptimal housing conditions [39]. Stereotypic behavior can often be prevented or reduced by additional stimulation provided by environmental enrichment (e.g. [8,50,55,80]). Even if single housing in barren standard cages might not be considered optimal, this cannot explain the huge difference between APP+/− and APP−/− animals. Qualitative observations in our laboratory indicate that the stereotypic behavior of APP+/− mice also occurs under other housing conditions (i.e. group housing and enrichment). Stereotypic behavior may in fact be a specific phenotype of APP-overexpression. This is supported by a qualitative observation of Lalonde et al. [33], who report APP23 mice often rear and jump in a continuous fashion against the walls

of their home cage. This behavior pattern appears to be similar to the *Jumping* behavior observed in TgCRND8 mice.

Diverse neurotransmitters, such as dopamine, serotonin and opioids have been proposed to be involved in the generation of stereotypies [5,17]. Based on several pharmacological studies in rodents, dopaminergic mechanisms in the striatum appear to play a key role in this context [5,9,53]. It has been shown that stereotypies can be induced by direct or indirect dopamine (DA) agonists (e.g. apomorphine or amphetamine) while they can be inhibited by DA antagonists like haloperidol [5,53]. However, it still has to be determined if dopaminergic mechanisms underlie the described stereotypic behaviors in TgCRND8 mice as well.

In Alzheimer patients, subtle changes in DA content are found in different brain regions. An increase in DA content has been observed in Brodmann area 4 of the primary motor cortex [20]. This may underlie the clinical observation of increased motoric restlessness and wandering in Alzheimer patients [20]. DA receptor densities are lowered in the striatum of AD brains [10,29,31,49,58]. A recent PET study found a correlation between reduced numbers of D_2 receptors in the striatum of Alzheimer patients and behavioral symptoms using the "Behavioral Pathology in AD Frequency Weighted Severity Scale" [66]. While our findings suggest that TgCRND8 mice might serve as a useful model to study BPSD, it remains to be determined whether there are changes in DA content and reduced striatal D_2 receptors that correspond to those seen in the human neurobiology of AD.

The significant correlations between the duration of stereotypic behavior and corticosterone metabolite (CM) concentrations at the age of 90 and 120 days provide evidence for an involvement of the HPA axis in the occurrence of stereotypic behavior. It is well known that different stressors lead to both increased HPA axis activity [76] as well as increased extracellular dopamine concentrations in the mesoprefrontal cortex, nucleus accumbens and striatum [46]. And even physiological doses of peripherally administered corticosterone lead to elevated extracellular concentrations of dopamine [48]. Therefore, elevated DA levels might be indirectly caused by AD pathology as an effect of HPA axis hyperactivity. This may result from pathologic changes in the hippocampus [68], followed by the glucocorticoid cascade as formulated by Sapolsky et al. [57]. It is of interest that there was no correlation between stereotypic behavior and plasma corticosterone concentrations. This might be due to the fact, that a blood sample provides hormone concentrations at a certain time-point only, while fecal samples were collected over the 24 h periods, when behavior was recorded. Thus CM concentrations mirror the activity of the HPA axis over a major part of the observation period [68].

Concerning the organisms' second stress axis, the sympathetic adrenomedullary (SAM) system, no significant correlation was found between adrenal tyrosine hydroxylase (TH) activity and stereotypic behavior. This is surprising because increased locomotor activity usually correlates with increased activity of the SAM system [76]. Hence, our current data suggest that the SAM system is not affected by APP overexpression.

In addition, there was no correlation between plaque numbers in different brain regions and the duration of stereotypies. On the one hand, this might be because plaques were quantified in different brain regions than those involved in the neurobiology of stereotypic behavior. On the other hand, plaque burden has not always been shown to correlate with dementia severity and functional impairments in AD [11,60,65].

4.3. Conclusion

In summary, this study revealed significantly higher total activity and evidence for altered activity patterns in male transgenic mice of the TgCRND8 model. These findings may result from altered APP metabolism or Aβ levels, as it confirms previous studies using animal models of AD and resemble comparable symptoms in AD patients. In addition, we report for the first time, that transgenic mice displayed significantly more stereotypic behavior than wildtype animals. The high amount of stereotypies correlated significantly with elevated CM concentrations. It is suggested that stereotypy and BPSD may share underlying mechanisms. Thus, this study provides strong evidence that BPSD has behavioral analogies in TgCRND8 mice. As behavioral changes in this study appeared very early, before Aβ pathology can be detected, some of the non-cognitive symptoms may potentially occur in Alzheimer patients before the manifestation of amyloid deposits.

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