Cognitive abilities of Alzheimer's disease transgenic mice are modulated by social context and circadian rhythm

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Abstract

In the present study we used a new training paradigm in the IntelliCage automatic behavioral assessment system to investigate cognitive functions of the transgenic mice harboring London mutation of the human amyloid precursor protein (APP.V717I). Three groups of animals: 5-, 12- and 18-24-month old were subjected to both Water Maze training and the IntelliCage-based appetitive conditioning. The spatial memory deficit was observed in all three groups of transgenic mice in both behavioral paradigms. However, the APP mice were capable to learn normally when co-housed with the wild-type (WT) littermates, in contrast to clearly impaired learning observed when the transgenic mice were housed alone. Furthermore, in the transgenic mice kept in the Intellicage alone, the cognitive deficit of the young animals was modulated by the circadian rhythm, namely was prominent only during the active phase of the day. The novel approach to study the transgenic mice cognitive abilities presented in this paper offers new insight into cognitive dysfunctions of the Alzheimer's disease mouse model.

Introduction

Most of the transgenic mouse models of Alzheimer's Disese (AD) have been produced by introduction of human mutated genes, such as amyloid precursor protein (APP), presenilin (PS), and tau genes, either alone or in combination [1, 2, 3]. APP transgenic mice develop plaques and memory loss. According to the “cascade hypothesis,” accumulation of beta-amyloid protein firstly triggers synaptic dysfunction and then causes a cascade of reactions that lead to tau pathology and neuronal death [4]. In humans, it has been observed that AD has a long asymptomatic phase during which severe cognitive impairments are not observed even though the amyloid pathology is present. However, in this phase subtle cognitive deficits and abnormal patterns of brain activity are detected [3]. Since APP transgenic mice exhibit functional abnormalities similar to those observed in people at risk for AD, they are considered as useful models to study mechanisms of early changes in brain function that appear in the asymptomatic phase of AD and offer the possibilities of primary prevention.

Since early cognitive dysfunctions observed in AD patients are related to hippocampal function, the APP transgenic models have been tested mainly in the tasks relying on the integrity of the hippocampus. The majority of these studies showed deficits in navigation behavior and spatial learning in the Morris Water Maze (MWM). The decreased performance has been also observed in either spontaneous or reinforced spatial alternation task as well as in novel object recognition task [4]. However, large-scale efforts to phenotype mouse models of AD have proven to be difficult. Progress in the field has been hampered mainly by the lack of ethologically-relevant and “high-throughput” phenotyping strategies. Introduction of the “IntelliCage” behavioral assessment system [5] has provided a major breakthrough in this regard. In this system up to 16 mice can be studied simultaneously under conditions close to the regular animal housing, thus avoiding the stress of experimental manipulations that is the

major drawback of the vast majority of behavioral training/testing paradigms. However, so far only limited applications of the IntelliCage have been reported for the transgenic mice [6], supposedly because of the subtle consequences of many gene deficits.

The aim of the present study was to design and to perform a training paradigm in the IntelliCage system that would first reproduce early cognitive deficits reported previously [7] for the transgenic mice harboring London mutation of the human APP (APP.V717I) and then allowing to investigate the cognitive functions of those animals also in a greater detail. Unexpectedly, we have discovered that the mice are capable to learn normally when co-housed with the wild-type (WT) littermates, in contrast to clearly impaired learning observed when the transgenic mice were housed alone. Furthermore, we found that the learning impairment observed in the transgenic mice was circadian rhythm-dependent, being prominent only during the active phase of the day. The novel approach to study the transgenic mice cognitive abilities presented in this paper offers new insight into cognitive dysfunctions of the AD mouse model.

Materials and Methods

The animals

The First Warsaw Ethical Committee on Animal Research approved all experimental procedures on animals. All animals in this study were females, of common parentage, on a mixed, 50%/50%, FVB/N and C57BL/6 background. The mice were generated from a cross between male mice carrying human APP with a single point mutation at amino acid 717 (the 'London' mutation, APP.V717I) in the FVB genetic background and female wild-type C57BL/6 mice. The obtained female mice were housed in the litters they were born in, until the experiments started. The mice were housed under a 12/12 h light–dark cycle, with *ad libitum* access to rodent chow and water.

In behavioral experiments, performance of APP.V717I mice, carrying the 'London' mutation, was compared to that of littermate control mice (WT). A total of 87 female mice were categorized according to three age criteria: young (5 months; WT, n = 12; APP.V717I, n = 11), middle-aged (12 months; WT, n = 12; APP.V717I, n = 12) and old (18-24 months; WT n = 10, APP.V717I, n = 10). An additional group of old APP.V717I mice (n = 10) and their WT siblings (n = 10) was subjected to sucrose preference test.

Before the experiments, the mice were anesthetized (Isoflurane, Baxter) and injected with a glass-covered microtransponder (11.5 mm length, 2.2 mm diameter; Trovan, ID-100) with a unique code. After 3 days recovery, the mice were handled for 5 days and later tested in a 6-week battery of behavioral tests consisting of Morris water maze test and IntelliCage tests.

Morris water maze

Cued-navigation task: One day before the training, the mice were placed in a circular, white pool (1.4 m in diameter) filled with milky water (kept at 24-27°C) with a transparent platform (15 cm high, 11.5 cm X 11.5 cm) and the mice were familiarized with swimming in water, climbing the platform, and staying on the platform. The pool was surrounded by white curtains to cover extra-maze cues. The platform was made visible by a 10-cm high dark cylinder placed onto it, and was placed pseudo-randomly in the pool. Mice received four trials with the visible platform in four different locations of the pool. For all mice, a 15-s rest period in the separate cage was given before the start of the next trial.

Training: To train the mice, the same pool was divided into virtual quadrants and the platform was located in the target quadrant, invisibly 1.5 cm below the surface of the water. The curtains were removed and the platform was unmarked. The pool was surrounded by large, colorful visual cues. At the beginning, the mice were placed on the platform facing the wall of the pool for 1 min, before being given four trials, each from different starting position (north, south, east, west). The mice received one session consisting of four trials each day for 4 days, with a different quadrant starting pattern used for all days of acquisition. The latency to find the platform (2 min cut-off) was measured as the time elapsed between placing the animal at the starting position and climbing onto the hidden platform. Mice that searched for more than 2 min were gently led to the platform. All mice that reached the platform were allowed to stay there for 1 min.

Testing (the probe trial): On the 5^{th} day, memory retention was evaluated. The mice were placed on the platform for 1 min and then they were subjected to a single 2 min probe trial without the platform. The starting position was directly opposite to the target quadrant. The time spent in each quadrant was analyzed by dedicated software (EthoVision, Noldus

Information Technology, Wageningen, The Netherlands). After completion of the Morris water maze tests the animals were moved to the experimental room for 7 days before starting adaptation to the IntelliCage system.

IntelliCage system

The IntelliCage system (NewBehavior Inc., Zurich, Switzerland) allows for observation of a group of animals coping with the same task (group learning) and for assessing learning behavior of individual mice living in a social context (individual learning task; [6, 8], see Fig. 1). The system was located inside of a large standard rat cage (Techniplast 2000) measuring 55.0 × 37.5 cm at the base and 58.0 × 40.0 cm at the top, with a height of 20.5 cm. In the corners of the housing cage were four operant learning chambers covering a triangular 15.0 × 15.0 × 21.0 cm area of floor space each. Mice had access to the chamber provided *via* a tubular antenna (50 mm outer diameter, 30 mm inner diameter) reading the transponder codes. Only a single mouse had access to one learning chamber at the same time. The chamber, equipped with a proximity sensor, contained two openings (13 mm diameter) that permitted access to the spouts of drinking bottles. These openings were crossed by photobeams that recorded nosepoke responses. Access to the bottles was blocked by small motorized doors. Additionally, each cage contained a sleeping shelter in the center, on which the animals could climb to reach the food. The entire set-up of four cages of the IntelliCage system was controlled by a computer that recognized visits, nosepokes, and tube-lickings of the individual mice and delivered rewards (by opening access to the water after a nosepoke) according to preprogrammed schedules depending on the assignment of the mice to different test groups within the same cage. All cages were located in an animal facility room. The system ran continuously for several days.

Group learning: To investigate the effect of the social interaction on learning, a group of mice had to cope with the same place-learning task. Two consecutive housing conditions were employed, *mixed:* WT and APP.V717I mice were housed, trained and tested together, and *separated:* a group of WT and a group of APP.V717I mice were housed, trained and tested separately in independent cages. In group learning tasks, the mice were monitored for 20 days (*mixed*: 5 days of adaptation to the cage and 5 days of learning; *separated*: 5 days of adaptation to the cage and 5 days of learning). In the first, *mixed* phase, APP.V717I mice with their WT siblings were introduced to the cage for a 5-day adaptation period, with an unlimited access to water in all the corners with the doors to the drinking bottles open. During that phase, the mice developed a spontaneous preference for one or two corners. In the corner that was the least preferred during the last 24 h of adaptation period by a group of mice, the plain water was replaced by sweetened water (10% sucrose in water). As a result a group of mice had access to sweetened water in 1 corner and to plain water in other 3 corners. During the next 5 days, we monitored visits to all corners. In the second, s*eparated* phase, APP.V717I mice were separated from WT mice, moved to new cages and the same procedure as in the *mixed* condition was employed.

Individual learning: Individual learning tasks consisted of 2 days adaptation period followed by 5 days of learning. At first, mice had to learn to operate, by nose-pokes, the doors barring access to water. After 2 days, for each mouse one corner was chosen where the mouse could obtain sweetened water, while visits to the other corners were not rewarded by drinking. The sweetened water-access doors remained closed and the mouse had to perform a nose-poke to drink. Plain water was unlimited and delivered from the top of the cage. In both paradigms, the number of visits to all corners was counted using IntelliCage software.

Sucrose preference test: A group of old APP.V717I mice and their wild-type siblings was subjected to modified two-bottle choice test for four days. Mice were housed singly and habituated to drink plain water from two identical bottles. Following this one-day habituation period, mice had a choice between plain water in one bottle and sweetened water (10% sucrose) in another bottle for 3 days. The positions of the two bottles were random and switched every 24 h. Fluid intakes were measured to the nearest of 0.1 ml every day in the middle of the light period. For each mouse on each day, sweetened water preference ratios were calculated by dividing the sweetened water intake per total (sweetened plus pain) water intake and expressed as percentage.

Following the behavioral testing, the brain pathology was investigated by staining with Thioflavin-S as described [9].

Data analysis

To study temporal dynamics of the learning process during group learning tests, the percentage of visits to the rewarded corner was calculated locally in time. A six-hours long sliding window was moved with 60 s resolution through the whole time domain (5 experimental days). In each window the number of visits to every corner was calculated. The number of visits in the corner with sweetened water divided by the total number of visits in the window was taken as an estimate of the probability of visiting the rewarded corner at this period. For the first 6 h of an experiment (i.e., for time shorter than the length of the sliding window) we set the probability of seeking reward to chance level (25%). This procedure rendered the probability of visits to the rewarded corner and total number of visits as functions of time, both numbers were attributed to the end of the window. To facilitate data analysis we further smoothed these curves with running averages over 50-min intervals. The mean percentage of visits in the rewarded corner and the total number of visits across all

animals in a group were computed for all experimental conditions. Error bars were estimated under assumption of normality (by the standard error of the mean, SEM). All above analysis were made using Matlab (The MathWorks Inc.).

We also compared the percentage of visits in the rewarded corner taking into account the phase of the day using as baseline the activity during the first 3 h. This was subsequently compared with three-hour period of active phase of the 4^{th} day (the time between 76.5 h and 79.5 h of the experiment when the light was off) and with 3 h period of inactive phase of the 4^{th} day (the time between 88.5 h and 91.5 h of the experiment when the light was on). These exact time intervals were taken because for such choice the difference between night and day was the most pronounced. In each of these three periods the percentage of visits to the corner with sweetened water was determined as described previously.

Statistical analysis

All statistical tests were performed using STATISTICA 6.0 software (StatSoft Inc.). One-way ANOVA was used for the Morris Water Maze tests. For multi-day tasks (place learning tasks in the IntelliCage) both one-way ANOVAs and two-way repeated measure ANOVAs were performed. Significant group effects were further analyzed using Fisher's PLSD tests. To reveal the influence of phase of the day on the probability of visit in the rewarded corner one-way ANOVA and Bonferroni *post-hoc* tests were used. In all group comparisons, $P < 0.05$ was used as a significance threshold.

Results

Morris Water Maze

The APP.V717I mice were previously shown to display early deficits in learning and memory [9]. To confirm the AD-related cognitive pathology in our animals, we have tested them in the MWM [10]. The initial cued-navigation task showed neither sensorimotor nor motivational abnormalities as APP.V717I and WT mice swam normally and climbed successfully onto the visible platform (Fig. 2A). Then, the animals were trained to locate the spatial position of the hidden platform. APP.V717I and WT mice received 4 trials per day for 4 days, and the probe trial was performed on the 5^{th} day. During the probe trial, the platform was removed and time spent in the platform quadrant was compared with time spent in other quadrants. WT mice learned the platform position successfully (Fig. 2B). In APP.V717I mice group, the probe trial revealed a reduced preference for the platform quadrant possibly indicating a spatial memory deficit, which is in line with previously published data [9]. The deficits appeared as early as at the age of 5 months and the results were similar across mice at all tested ages.

Pathological amyloid deposits

With ageing, APP.V717I mice exhibit amyloid pathology, recapitulating the diagnostic post-mortem pathology in the brain of Alzheimer's disease patients [9]. To confirm this phenotype in our animals, on the final day of behavioral testing, the brains were collected to detect pathological amyloid deposits in the APP transgenic mice of different ages. We observed no ThioflavinS-positive deposits in any structure of WT mice, irrespectively of their age (data not shown). Brain sections of middle-aged and old transgenic APP.V717 mice contained

diffuse and neuritic plaques as was described before [9, 11]. ThioflavinS staining of young APP.V717I did not show any amyloid deposits in brain (Fig. 3).

IntelliCage experiments

Before starting the IntelliCage experiments that relied on sweetened water preference we carried out neurological analyses that showed normal visual and olfaction reflexes among transgenic mice. To exclude an abnormal sweet taste perception, aversion to sweetened water and major sensory impairments, we performed a sucrose preference test in home cage on an additional group of old APP.V717I and WT mice. We found neither sensory nor motivational deficits, with all the mice developing a strong preference to sweetened water (Fig. 4).

In the IntelliCages we subjected young, middle-aged and old transgenic mice to the appetitively-motivated behavioral training with a spatial component. In *group learning* task, sweetened water was located in one corner while the other three corners delivered plain water. Mice could drink from all the corners. In *individual learning* task, plain water was freely available from the cage top, whereas sweetened water was located in four corners of the cage but each mouse was assigned to one specific corner where it could drink sweetened water [8]. Mouse behavior was measured continuously for 24 h a day for 5 days. Place learning was expressed as percentage of visits in the corner with sweetened water.

In *group learning*, when we compared the percentage of visits in the rewarded corner during the 1^{st} and 5^{th} day of the experiment conducted in *separated* conditions (different genotypes in different IntelliCages), no successful learning in APP.V717I animals was observed (Fig. 5). The similar results were obtained in the MWM. Thus, housing APP.V717I mice without WT siblings, revealed memory impairment of the transgenic mice. APP.V717I mice did not develop the preference to the rewarded corner (Fig. 5). On the other hand, when

APP.V717I mice were co-housed with WT mice in the same IntelliCages, the transgenic mice acquired the place preference response as efficiently, or almost as efficiently, as WT mice (Fig. 6).

In the next experiment, the *individual learning* task, we tested the APP.V717I and co-housed WT mice in the learning paradigm in which each mouse had access to sweetened water in one corner assigned individually, while nose-pokes to the other three corners were not rewarded by drinking. WT mice again learned the task irrespectively of their age (Fig. 7). In contrast, the APP.V717I mice were significantly deficient in acquisition of this task, when compared to the WT mice at all ages examined. Nevertheless, some improvement in performance over the 5 days of training could be noticed also in the transgenic mice (Fig. 7). It is also interesting to note that the probability to enter the rewarded corner for the APP.V717I mice was around one third and the same in all age groups, while for WT mice the success rate decreased with age.

In the next step, we subjected the data obtained in the group learning experiments to more thorough analyses of the patterns of individual mouse visits throughout each day (with special attention to the middle parts of active and non-active phases of the day) during the whole period of the investigation. The exemplary data of young WT and APP.V717I mice tested in separate condition are presented in figure 8. The gray histograms present the mean number of visits per hour with scale on the left vertical axis, whereas the solid black lines show the percentage of rewarded visits with scale on the right vertical axis. All data presented here are the means across all animals in a given group. The increases in the latter parameter from chance level (25%) reflect learning. Surprisingly, these analyses revealed that the APP.V717I mice housed separately from the WT displayed a preference (albeit lower than in the case of WT mice) to the reinforced, sweetened water corner during the light, i.e., non-

active phase of the day, when number of visits is dropping (Fig. 8, top panel). Remarkably, this preference was entirely lost during the active, dark phase. This effect was not visible when data from the whole days were pooled, as the higher number of visits to the reinforced corner during the active phase dominated the whole 24 h data and concealed the apparent memory shown during the light phase (Fig. 8, top panel). The effect of apparent memory for the reinforced corner containing sweetened water was particularly clear for the young and middle–age animals, and even in the old ones, there was a tendency to visit the reinforced corner more frequently during the light phase of a day when compared with the dark phase (data not shown). In the case of the WT mice, the preference for the reinforced corner was evident irrespectively of the phase of the day and age of the animals (Fig. 8, bottom panel). To summarize the differences between learning in the active (light off) and inactive (light on) phases of the day we compared the baseline probability of entering the corner with the reward during the first three hours of the experiment (in the active phase) with the middle three hour periods of the active and inactive phases of the 4^{th} day (Fig. 9).

Discussion

The major findings of this study can be summarized as follows. The IntelliCage system allows for detection of memory deficit as efficiently as the Morris Water Maze. Moreover, we show that the observed impairments are modulated by social context and circadian rhythm, since the deficit in young mice is prominent only when the AD mice do not have access to the information provided by their WT siblings, and only during the active phase of the day.

The IntelliCage system allowed for revealing the memory deficits that were previously documented in the MWM in most of the strains of APP transgenic mice (for review see [3]), thus proving the usefulness of the system for such studies. Hence, appliction of the system to other AD mouse strains could be of great interest. Of particular importance would be to investigate whether the described cognitive peculiarities of APP.V717I transgenics could be also observed in other mouse models of AD. The IntelliCage system provides a less labor-intense and, at the same time, more ethologically-relevant experimental context. Moreover, our study has revealed several new features of cognitive function and dysfunction of APP.V717I transgenic mice that were not reported before. In fact, the results of our study raise questions whether the memory deficit is a major hallmark of impaired behavioral performance in the young AD mice. Our finding is particularly surprising when one considers that the APP transgenic mice bearing the same mutation were shown to be impaired in basic synaptic functions at the age of 5 months and it could be inferred that the learning deficits were related to those synaptic functional alterations [12].

The results of the group learning experiment in the *mixed* condition, when the same corner containing sweetened water was assigned to both APP.V717I and WT mice, suggest

that the transgenic mice are able to effectively acquire information about the availability and location of the appetitive stimulus from their WT cage-mates. Our results support the notion that social learning is an adaptive behavior allowing an animal (observer) to execute its response using the experience of others (demonstrators; [13, 14]). In the IntelliCage, social transmission occurred when observer animals obtained information about the location of an appetitive stimulus from demonstrators who had already gained such knowledge. We indexed transfer of information by measuring the performance of APP.V717I mice in the presence of WT siblings. The information about familiar, palatable liquid available in the environment was socially acquired by the transgenic mice.

Social transfer of a food-preference is a well known adaptive response among rodents [15]. However, in our study the familiarization of the mice with sweetened water preceded spatial experiments with appetitive reinforcement. Therefore, the present findings suggest that the transgenic mice obtained complex information about familiar tasty liquid availability and its location in the environment. The acquisition of the spatial component of information appeared to be crucial in getting access to appetitive stimulus. Mimic learning cannot be excluded, especially since a neurological analysis preceding memory tests showed normal visual and olfaction reflexes and sweet taste perception among transgenic animals. However, elucidation of the channels of social transmission demands further studies.

The results presented herein show that learning of APP transgenic mice can be improved by co-housing with healthy individuals. It has been shown previously that cognitive enhancement in AD transgenic mice is possible to achieve by long-term exposure to an enriched environment [16]. However, here we report a more direct and immediate effect on learning caused by social interaction with healthy individuals. The reduction of APP-induced deficits cannot be considered solely the result of enriched housing conditions, *i.e.*, new social

relations and cage equipment, as we did not observe a better performance of a group of transgenic mice housed alone. Significant advantageous effect on learning of the AD transgenic mice was observed only when these animals were housed with their WT siblings.

It remains difficult to explain the reasons of significant differences in the ability to find the rewarded corner demonstrated in our analyses between the active *vs.* non-active phases of a day. Clearly, during the non-active phase of a day, i.e., when the animals performed less visits, the AD mice displayed good memory for the location of rewarded corner. It can be suggested that enhanced animal activity during the active phase of the day may act as a distractor, impairing, for example, attention [17]. This hypothesis is supported by the results of Bronfman et al. [18], who showed basal forebrain cholinergic neurons shrinkage in transgenic APP mice with London mutation. The function of cholinergic neurons is closely related to attention as well as to sleep and circadian rhytm abnormalities [19]. As an alternative explanation one may also consider differential strategies to remember and approach the rewarded corner, depending on the access to the ambient light available and thus also to the visual cues.

It is noteworthy that our findings would be difficult to obtain using standard behavioral tests. Firstly, a majority of behavioral tests analyze behavior of individual mice in spite of the fact that these rodents are social animals. Secondly, most animal studies involve short time observations during the light phase. The multidimensional, 24-h a day for several days analysis of mice' behavior including individual and group learning in social context was achievable only in the IntelliCage system. Our novel approach shows advantageous effects of housing the demented mice with the WT ones on learning the spatial location of a reward.

Finally, it should be mentioned that rich social interactions as well as intellectual and physical activity have been emphasized in reducing the AD risk and progression in experimental animals and humans [20, 21]. One of the possible mechanisms of such positive

social influence is observational learning. It has been shown that procedural memory (based on the acquisition of skills or procedures) is spared in AD patients, in contrast to declarative memory, which is affected at the early stages of the disease. The results of our study show that learning by observation is still present in mice that already have difficulties in spatial learning. In this context, employing therapies based on observational learning of healthy persons could appear beneficial for AD patients.

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Figure Captions

Figure 1. The IntelliCage set-up for automated testing of spontaneous behavior and cognition of mice living in social groups within their home cage. Behavior and learning of individual mice is controlled and measured by a controlling computer (modified after [22]).

Figure 2. Morris Water Maze. *A:* Cued-navigation task in the MWM. APP.V717I mice were indistinguishable from WT mice with respect to the time of swimming to visible platform; young, $F(1,19) = 0.01$, $P > 0.9$; middle-aged, $F(1,18) = 0.03$, $P > 0.8$; old, $F(1,21) = 0.06$, $P > 0.8$. *B:* In the probe trial, the time spent searching for the platform in the target quadrant was analyzed. Mice from WT groups, displayed much more selective memory when probed 24 h after training as they were spending more time in the target quadrant when compared to APP.V717I mice; young WT (n=12) *vs.* young APP.V717I (n=11), $F(1,21) = 4.38$, $P < 0.01$; middle-aged WT (n=12) *vs.* APP.V717I (n=12), $F(1,22) = 4.42$, $P < 0.01$; old WT (n=10) *vs.* APP.V717I (n=10), $F(1,18) = 4.32$, $P < 0.01$, one-way ANOVA.

Figure 3. Histopathology of APP.V717I mice. The occurrence of amyloid plaques stained with ThioflavinS in Middle-aged (middle panel) and Old (right panel) APP.V717I mice brain. Brain section of APP.V717I mice at the age of 12 months (Middle-aged mice) revealed neuritic and some diffuse plaques. Number of ß-amyloid deposits increased with age. No histological changes were found in Young APP.V717I (left panel) and WT (not shown) mouse brains at any age.

Figure 4. Neurological analysis of APP.V717I and WT mice. Daily water intake measured using modified two-bottle test (sweetened water *vs.* plain water) in old APP.V717I and WT mice. The test showed high preference of both groups to sweetened water. The intake of sweetened water was consistently higher than that of plain water from the first day of the

experiment and the effect was independent of genotype (APP.V717I, n=10, preference ratio = 76%; WT, n=10, preference ratio = 72%, P > 0.4). All mice developed strong preference to sweetened water as at the third day of the test both groups achieved 84% of preference ratio, APP.V717I *vs.* WT, F(1,18) = 2.02E-05, P > 0.9.

Figure 5. *Group learning* in the IntelliCage system, separated. In separated version of experiment, a group of APP.V717I mice did not show any significant difference in the percentage of visits to the rewarded corner on the first and the last day of the place learning. In contrast, WT groups developed a strong preference for the corner with sweetened water during the 5-day training. Results are presented as a ratio of visit in the rewarded corner to visits to all corners of the IntelliCage. Sweetened water was placed in the corner that was the least frequented during adaptation. Error bars represent S.E.M., *P < 0.05, **P < 0.01, ***P < 0.001, one-way ANOVA.

Figure 6. *Group learning* in the IntelliCage system, mixed. Similarly to separated conditions, WT group showed the increasing preference for the rewarded corner during the 5-day training. Surprisingly, transgenic mice, at all ages examined, were able to acquire the information about the sweetened water location when co-housed with WT siblings.

Figure 7. *Individual learning* in the IntelliCage system. In *individual learning*, mice were assigned to corners individually and each mouse had an access to one corner with sweetened water with no access to other corners. The mice had access to plain water from the top of the cage. For each mouse the least visited corner during the 24 h before the test was chosen and then plain water was replaced by sweetened water in this corner. During the next 5 days, each mouse could obtain sweetened water only from this one corner. Place learning was evaluated as a preference for visiting the rewarded corner on the 1^{st} and the 5^{th} day of learning. Compared with WT mice (black bars), APP.V717I mice (empty bars) performed a lower

number of correct responses. The graph shows the percentage of visits to the rewarded corner. Error bars represent S.E.M., *P < 0.05, **P< 0.01, ***P < 0.001, one-way ANOVA.

Figure 8. Circadian activity and group learning in the IntelliCage system. Activity measured as a number of visits to all corners (grey bar plot with scale on the left vertical axis) and preference for visiting of rewarded corner (black line with scale on the right vertical axis) in group learning task. Example shows young WT (top panel) and APP.V717I (bottom panel) mice tested in separate condition showing averages across all animals in a given group. In both cases we observe *increase* of the probability of entering the corner with reward during inactive phase characterised by the trough of activity plot. In case of WT mice these oscillations are on top of steadily increasing trend. For transgenic mice the baseline probability does not change indicating lack of long-term memory consolidation.

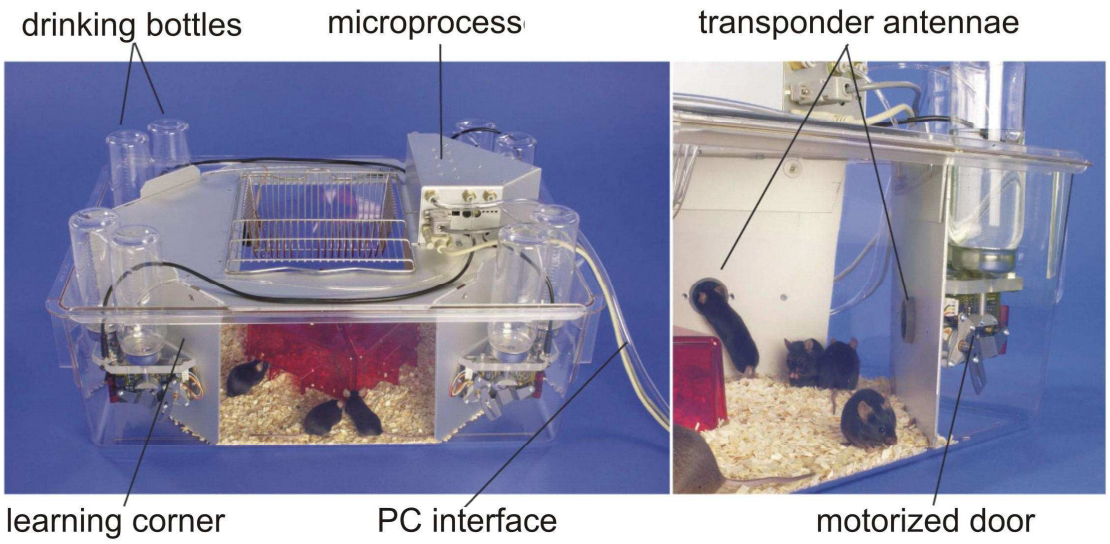
Figure 9. Group learning in the IntelliCage system, separeted. Circadian rhythm dependencies - summary plot. Probability of entering the corner with the reward for WT and APP.V717I was calculated for three, non-overlapping intervals of time. The first period was chosen as a baseline and span the first three hours of the active phase of the experiment. The second and third periods were selected as the middle three hours of the active and inactive phases of the 4th day of experiment. For all age groups of WT animals (black bars) there is statistically significant effect of learning between the 1st and the 4th day of the experiment in the active phase (Bonferroni *post-hoc* tests: young – P<0,001; middle-age – P<0,01; old – P<0,001). We do not observe such dependencies for any groups of the transgenic animals (empty bars). What is common for both mice types is enhanced probability to reach for reward during the inactive phase when compared with basic level (Bonferroni *post-hoc* tests: WT young – P<0,001; WT middle-age – P<0,01; WT old – P<0,001 and APP.V717I young – P<0,01). The differences in test performance during the

active and inactive phases of the fourth day of experiment were statistically significant for young and old control mice (Bonferroni *post-hoc* tests: young – $P<0,001$; old – $P<0,05$) and young transgenic mice (Bonferroni *post-hoc* tests: young – $P<0,01$). Additional one–way ANOVA for repeated measurements revealed influence of the phase of the day on the percent of visits in the rewarded corner for WT mice: young ($F(2,22)=78,27$; $P<0,001$), middle-age ($F(2,16)=9,74$; $P<0,01$) and old ($F(2,18)=36,43$; $P<0,001$) and for APP.V717I mice: young ($F(2,20)=11,51$; $P<0,001$).

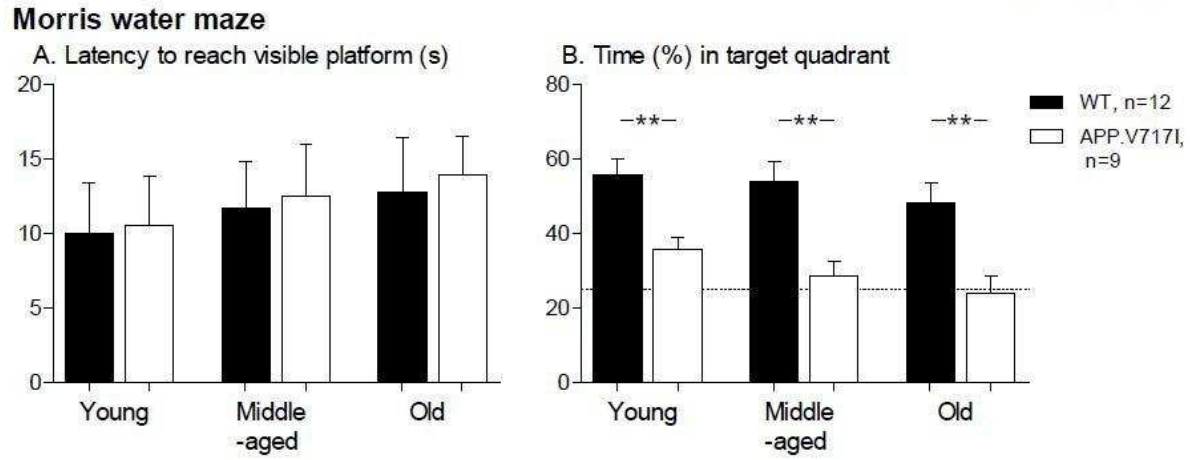
Table 1. Group learning of Young, Middle-aged and Old mice in the IntelliCage system. The percentage of visits in rewarded corner at the beginning and the end of training as well as the difference between those two parameters depending on the type of *group learning* setup is shown. APP.V717I mice at all ages showed learning impairment (*separated*) which was reversed by co-housing with WT siblings (*mixed*). The WT mice were able to fulfill the task independently of housing conditions.

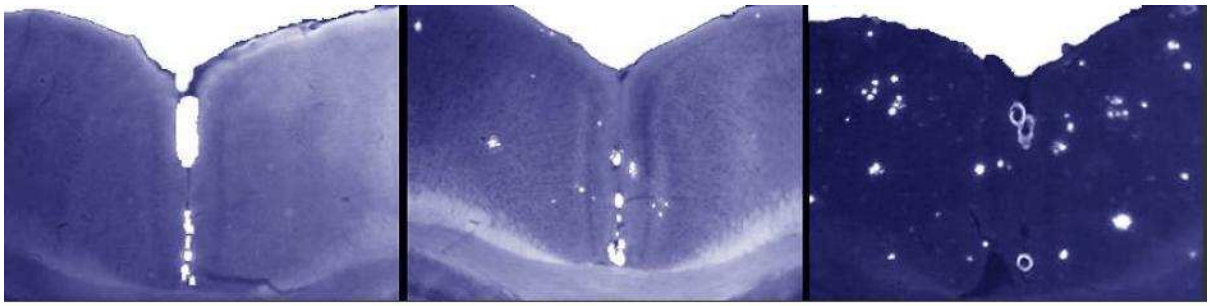
GROUP LEARNING	GROUP	AGE	% visits in corner with sugar water		STATISTICS
			1st day	5th day	1st day vs. 5th day of training
mixed	WT	YOUNG, n=12	19.3 ± 4.4	57.3 ± 4.8	F(1.22) = 34.57 P < 0.001
		MIDDLE-AGED, n=12	24.7 ± 5.1	59.5 ± 6.8	F(1.24) = 16.92 P < 0.001
		OLD, n=10	22.9 ± 5.8	44.4 ± 5.7	F(1.18) = 6.89 P < 0.05
	APP.V717I	YOUNG, n=11	15.9 ± 5.2	51.0 ± 5.7	F(1.22) = 20.70 P < 0.001
		MIDDLE-AGED, n=12	14.9 ± 5.6	43.2 ± 7.3	F(1.22) = 9.44 P < 0.01
		OLD, n=10	13.4 ± 5.1	39.3 ± 5.2	F(1.18) = 12.59 P < 0.01
separated	WT	YOUNG, n=12	24.3 ± 5.2	69.3 ± 5.2	F(1.22) = 37.22 P < 0.001
		MIDDLE-AGED, n=12	21.6 ± 4.0	60.1 ± 4.5	F(1.24) = 41.00 P < 0.001
		OLD, n=10	24.4 ± 5.2	56.3 ± 4.9	F(1.18) = 20.07 P < 0.001
	APP.V717I	YOUNG, n=11	22.9 ± 5.1	29.8 ± 5.4	F(1.22) = 0.86 P = 0.362
		MIDDLE-AGED, n=12	16.5 ± 6.1	27.4 ± 5.8	F(1.22) = 1.66 P = 0.211
		OLD, n=10	13.3 ± 4.5	21.8 ± 4.3	F(1.18) = 1.86 P = 0.189

Kiryk et al., Fig. 1



Kiryk et al., Fig. 2

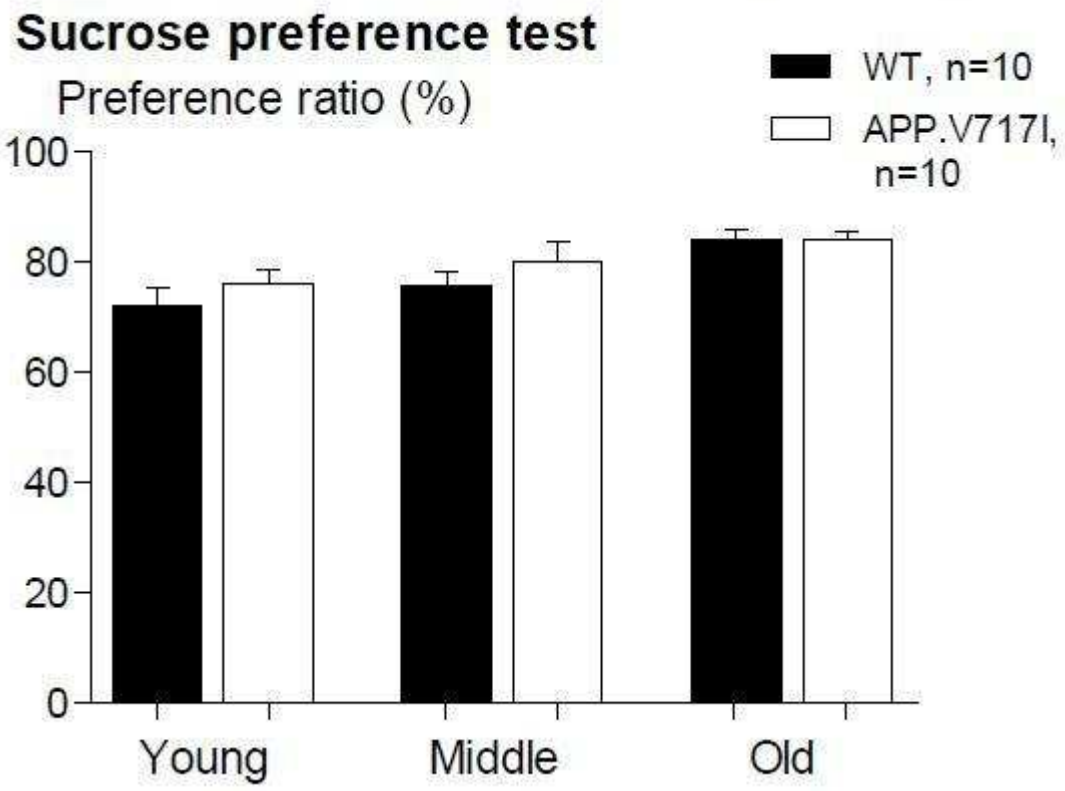


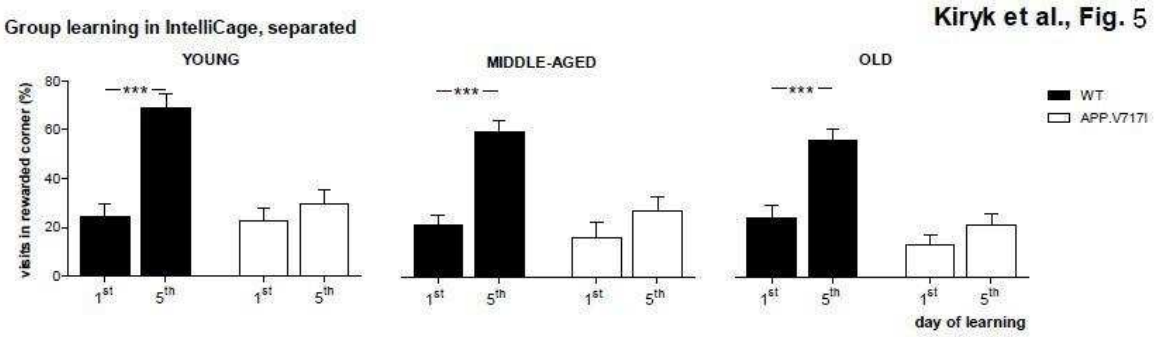


Young Middle-age Old

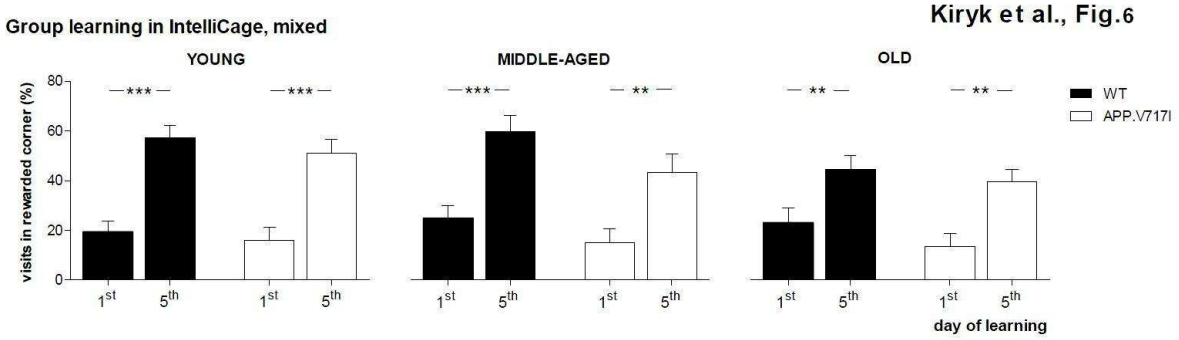
Kiryk et al. Fig. 3

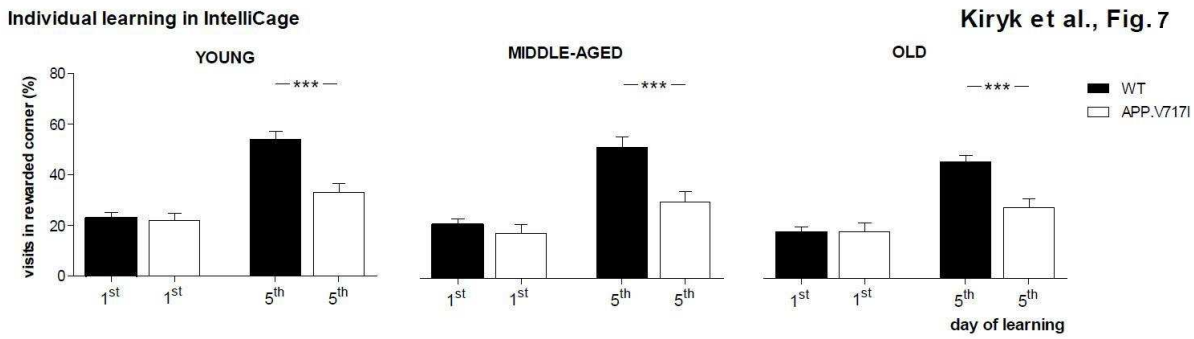
Kiryk et al., Fig. 4



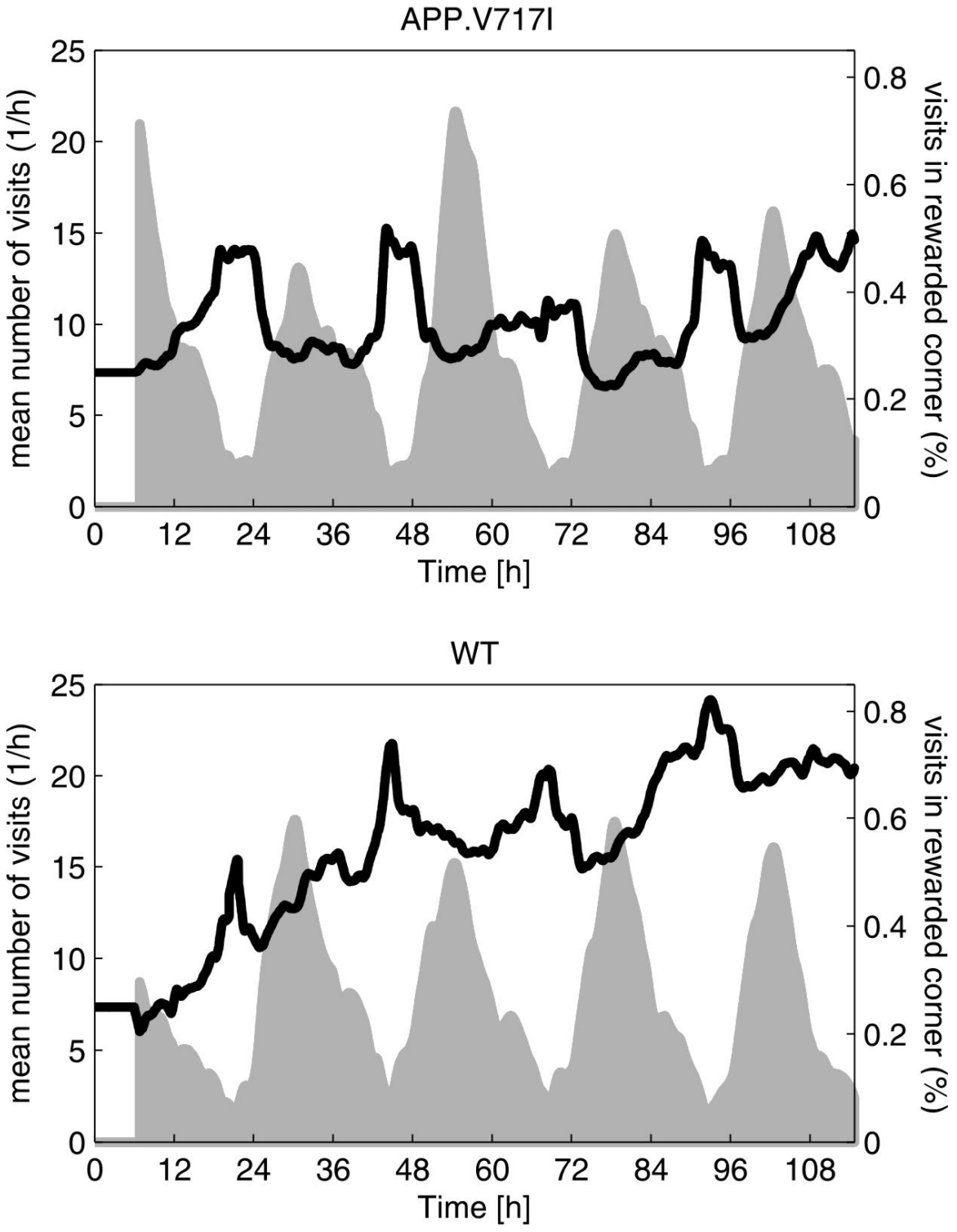

Group learning in IntelliCage, separated
Kiryk et al., Fig. 5
YOUNG
MIDDLE-AGED
OLD
visits in rewarded corner (%)
80
60
40
20
0
***
***
***
1st
5th
1st
5th
1st
5th
1st
5th
1st
5th
1st
5th
day of learning
WT
APP.V717I

Kiryk et al., Fig.6




Individual learning in IntelliCage
Kiryk et al., Fig. 7
YOUNG
MIDDLE-AGED
OLD
***
***
***
WT
APP.V717I
visits in rewarded corner (%)
0
20
40
60
80
1st
1st
5th
5th
day of learning

Kiryk et al., Fig. 8



Group learning in IntelliCage, separated **Kiryk et al., Fig. 9**