Preserved memory capacities in aged Lou/C/Jall rats

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Abstract

Although memory impairments are a hallmark of aging, the degree of deficit varies across animal models, and is likely to reflect different states of deterioration in metabolic and endocrinological properties. This study investigated memory-related processes in young (3–4 months) and old (24 months) Sprague–Dawley rats (SD), which develop age-linked pathologies such as obesity or insulin-resistance and Lou/C/Jall rats, which do not develop such impairments. In short- and long-term memory recognition tasks, old Lou/C/Jall rats were never impaired whereas old SD rats were deficient at 1 and 24 h latencies. The expression of N-methyl-D-aspartate receptors (NMDAR)-mediated synaptic plasticity in CA1 hippocampal networks shifted towards lower activity values in old Lou/C/Jall rats whereas long-term potentiation was impaired in age-matched SD rats. Age-related decrease in NR2A subunits occurred in both strains, extended to NR2B, NR1 and GluR1 subunits in older animals (28 months) but only in SD rats. Therefore, the Lou/C/Jall rats can be considered as a model of healthy aging, not only in terms of its preserved metabolism, but also in terms of cognition and synaptic plasticity.

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Keywords: Aging; Memory; Synaptic plasticity; NMDA receptor; Hippocampus; Caloric restriction

1. Introduction

Aging is commonly associated with a wide variety of impaired biological functions. Among these, a decline in cognitive functions is generally observed. For example, elderly humans often display slower learning abilities and quicker forgetting (Grady and Craik, 2000). Accumulated evidence indicates that, on average, cognition and memory decline can occur during normal aging, i.e., in the absence of neurodegenerative diseases (Barnes, 1979; Barnes and McNaughton, 1985). Both pharmacological and behavioural interventions have been developed to reduce the cognitive decline in the elderly with, unfortunately, limited efficacy.

Diet and caloric intake are key to comfortable and healthy aging, since metabolic disorders such as increased adiposity and insulin-resistance occur with age (Frisard and Ravussin, 2006). Indeed, age-related brain dysfunctions induced by stress and inflammatory responses or enhanced neurotrophic expression, can be counteracted by a decreased caloric food intake via dietary restriction (Mattson et al., 2003). Although caloric intake restriction is now broadly used as a means of increasing lifespan, its efficacy in preventing memory deficits remains debated in the literature, likely reflecting differences in feeding schedules (Beatty et al., 1987; Bellush et al., 1996; Hansalik et al., 2006; Ingram et al., 1987; Markowska, 1999; Markowska and Savonenko, 2002; Pitsikas and Algeri, 1992; Stewart et al., 1989; Yanai et al., 2004). The inbred Lou/C rat, has recently been described as a model of healthy aging (Alliot et al., 2002) with a median lifespan (50% of survivors) of 29 months as compared to 24 months for Sprague–Dawley 1985). Both pharmacological and behavioural interventions have been developed to reduce the cognitive decline in the elderly with, unfortunately, limited efficacy. 

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rats (Alliot et al., 2002). This prolonged healthy lifespan is associated with a reduced and stable adipose-tissue mass due to a spontaneous decrease in caloric intake. In fact, *ad libitum* fed male Lou/C rats exhibit only 60% of the energy intake of other rat strains and do not develop obesity with age (Barnes, 1979; Boghossian et al., 2002).

Until now, laboratories have specifically investigated these rats for their spontaneous low-caloric intake and metabolic profile. Surprisingly, even though they have many characteristics (e.g., no insulin- or leptin-resistance, low growth hormone and IGF1 production) that constitute a model of healthy brain aging (Alliot et al., 2002; Boghossian et al., 2002; Veyrat-Dubreux and Alliot, 1997), their cognitive profile, as well as cerebral mechanisms underlying memory processing, remain to be determined.

2. Methods

Groups of male rats were assigned in this study according to their age: young (3–4 months old), aged (24 months old) and very old (>28 months old). Behavioural tests were performed on 10 young and 10 old SD rats, and 9 young and 7 old Lou/C/Jall rats. Electrophysiological tests (synaptic transmission and plasticity) were performed on 14 young, 10 old and 12 very old SD rats, and 12 young, 13 old and 8 very old Lou/C/Jall. For Western blot analyses, we used 15 young, 5 aged and 12 very old SD rats and 5 young, 4 old and 4 very old Lou/C/Jall.

The Lou/C/Jall rats were derived from the inbred Lou/C strain, originally imported in Louvain. They were bred at the Complexe scientifique des Cézeaux (Université Blaise-Pascal, Aubière, France) and weighed 225–275 g (young), 300–400 g (old) and 300–320 g (very old) at the beginning of the study. The SD rats (Charles River, France) weighed 300–350 g (young), 650–750 g (old) and 600–700 g (very old). They were housed in triplets in plexiglas cages, upon arrival, in a temperature-controlled (22 ± 1 °C) and humidity-controlled colony room with a 12:12-h light/dark cycle for at least 2 weeks before the experiments. All experimental procedures were performed during the light portion of the 12 h light/dark cycle. Food and water were available *ad libitum* throughout the experiment. As previously reported, Sprague–Dawley rats became obese with age, while Lou/C/Jall rats remained lean (Alliot et al., 2002). All experimental procedures involving animals and their care conformed with INSERM committee guidelines and to the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.1. Behavioural testing

2.1.1. Testing environment, apparatus, and objects

Behavioural testing took place in a rectangular room where the open field was visually separated from the experimenter. A square open-field box [60 cm × 60 cm × 50 cm (height)] constructed from white plywood was used for all testing. The objects were constructed from Duplo® (12 cm × 12 cm × 10 cm), and were placed on a fixed base located in a position that was symmetrically distant from the back and the side walls, and could not be moved by the rat during testing. Three identical copies of eight unique objects were used during memory tasks. These objects differed for shapes and for dark and clear colours contrast to keep the perceptive capacity of young and older explorer. The pairing, the order of presentation (new vs. familiar) and the position (right vs. left) were randomly presented, to avoid any preference bias.

2.1.2. Spontaneous object recognition task

Object memory testing was achieved using previously reported tasks relying on spontaneous exploration of objects in an open field (Ennaceur and Delacour, 1988). After a daily habituation session for 4 days without any objects in the box, 3 testing sessions took place, every 3 days. Each session was composed of a 5-min training trial in which rats explored two copies of the same object, followed by a test trial in which rats explored a third copy of the original object (the “familiar” object) and a new one. On this test trial, non-deficient rats spend more time exploring the novel object than the familiar one, thereby demonstrating their ability to recognize the previously explored object (Ennaceur and Aggleton, 1997). Each animal was tested at three different delays, 5 min, 1 and 24 h. The order of the different delay sessions was counterbalanced between four groups of rats. For data acquisition, an overhead video camera was used to record the rats behaviour in the open field. Time spent exploring each of the two objects available on a given trial was scored from videotape with stopwatch, by at least two-independent experimentors. Object exploration was scored as the time that the rat faced an object less than 2 cm distant from the tip of its nose, but not during scratching the object. In the test trial, the novelty preference, taken as the main measure of recognition memory, was defined as the ratio of the time spent exploring the novel object over the total time spent exploring both objects.

2.2. Electrophysiological recordings

2.2.1. Slice preparation for electrophysiology

All rats were anaesthetized with halothane and decapitated. The hippocampus was quickly removed and placed in ice-cold artificial cerebrospinal fluid (aCSF) containing (mM) NaCl 124, KCl 3.5, MgSO4 1.5, CaCl2 2.5, NaHCO3 26.2, NaH2PO4 1.2, glucose 11. Transverse slices (~400-μm thick) were cut and placed in a holding chamber (at 27°C) for at least 1 h before recording. Each slice was individually transferred to a submersion-type recording chamber between two nylon holding nets, submerged with ACSF, and continuously superfused and equilibrated with 95% O2 and 5% CO2. Extracellular recordings were obtained at room temperature from apical dendritic layers of hippocampal
CA1 area using glass micropipettes filled with 2M NaCl and with a resistance of 2–6 MΩ. Field excitatory postsynaptic potentials (fEPSPs), resulting from activation of α-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionate (AMPA) receptors, were evoked by electrical stimulation (20 μs duration, bipolar electrode) of CA1 afferent Schaeffer’s collaterals and commissural fibres in the stratum radiatum. The magnitude of the fEPSP was determined by measuring its slope every 10 s.

2.2.3. Long-term depression and potentiation

Electrically induced long-term plasticity (LTD or LTP) was produced by application of different frequency stimulations in slices. The amplitude of three averaged presynaptic fibre volleys (PFVs) and the slope of fEPSPs were plotted as a function of stimulation intensity (100–500 μA). To record fEPSPs mediated by N-methyl-D-aspartate (NMDA) receptors, electrical stimulations (100 μs duration) were induced in low magnesium medium (0.1 mM) in the presence of non-NMDA receptor antagonist 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzoquinoxaline-7-sulfonamide (NBQX, 10 μM) and bicuculline (10 μM) to block inhibitory neurotransmission. A knife cut separating CA3 and CA1 was introduced to prevent the propagation of epileptiform discharges.

2.2.3. Long-term depression and potentiation

Curves were constructed to assess theresponsiveness of AMPA/kainate glutamate receptor subtype-dependent responses to electrical stimulation in slices. The amplitude of three averaged presynaptic fibre volleys (PFVs) and the slope of fEPSPs were plotted as a function of stimulation intensity (100–500 μA). To record fEPSPs mediated by N-methyl-D-aspartate (NMDA) receptors, electrical stimulations (100 μs duration) were induced in low magnesium medium (0.1 mM) in the presence of non-NMDA receptor antagonist 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzoquinoxaline-7-sulfonamide (NBQX, 10 μM) and bicuculline (10 μM) to block inhibitory neurotransmission. A knife cut separating CA3 and CA1 was introduced to prevent the propagation of epileptiform discharges.

2.2.2. Input/output

Field excitatory postsynaptic potentials (fEPSPs) were calculated, and the resulting average slope of fEPSPs were plotted as a function of stimulation intensity for 45 min after stimulation in ACSF medium. Three successive fEPSPs were calculated, and the resulting average slope was plotted online across time using Acquis 1 software (G Sadoc, Unic-CNRS, France).

Drugs, including NBQX and d-2-amino-5-phosphonovalerate (d-APV), were purchased from Tocris (Illkirch, France), while bicuculline was purchased from Sigma–Aldrich (Saint Quentin Fallavier, France). All drugs were dissolved in water, with the exception of NBQX, which was dissolved in DMSO (final concentration 0.01%).

2.3. Semiquantitative immunoblotting analysis

Fifteen young (3–4 months), 5 old (24 months) and 12 very old (28 months) SD rats as well as 5 young, 4 old and 4 very old Lou/Jc/Jall rats were used for the Western blot analysis. Rat hippocampi were extracted immediately after decapitation of the animal. The anterior halves of hippocampi were cut into small pieces and homogenized by sonication in an ice-cold buffer consisting of 20 mM sodium phosphate pH 7.2, 150 mM NaCl, 10 mM EDTA, 10 mM EGTA, 150 mM sodium fluoride, 0.5% Triton X100, also containing protease inhibitors (Protease inhibitors set III, Sigma). Aliquots of the extracts were assayed for protein using the Bradford method and equal amounts of proteins (60 μg/lane) were loaded on sodium dodecyl sulfate-polyacrylamide gels (6.5% acrylamide), electrophoresed and transferred onto nitrocellulose membranes (Transblot, Biorad) by semi-dry blotting. The blots were blocked in 5% defatted milk in 10 mM Tris–HCl (pH 8.3), 150 mM NaCl containing 0.05% Tween-20 (TBST), and then incubated overnight at 4 °C with appropriate antiserum dilution (NR2A antibody, 1/750; NR2B antibody, 1/750 (rabbit, Upstate); GluR1, 1/6000 (rabbit, Upstate); NR1 antibody, 1/5000 (mousy, Chemicon); and Neuron Specific Enolase, 1/6000 (rabbit, TEBU) in the TBST containing 0.5% defatted milk. After washing, they were incubated with goat or rabbit horseradish peroxidase-conjugated secondary antiserum (1/10,000 for rabbit or goat, P.A.R.I.S). Immunoblots were developed by enhanced chemiluminescence (ECL Western blotting detection kit, Amersham). Protein bands of interest were analyzed by measuring optical density (OD) by scanning densitometry (Quantity One, Biorad). Densitometric results for each protein of interest were normalized to NSE density. Levels of NSE, which were not altered in aging, were used as a control to correct for variations in protein loading in the gels. To limit blot variability all extracts from young and old animals were loaded onto the same gel and each sample was run three times in different western blots. To assess the specificity and linear range of responses in immunoblots for the antibodies used in this study, preliminary experiments were performed with different protein concentrations ranging from 20 to 100 μg per lane of young and aged rat hippocampus. Densitometric quantitation of the band intensities corresponding to the immunoreactivity of the antibodies showed linear relationship between signal intensity for each antibody and amount of total protein, within the range from 40 to 80 μg of protein loaded.

2.4. Data analysis

All results are expressed as mean ± S.E.M. In all cases, differences were considered significant when p ≤ 0.05. Bonferroni correction was applied when multiple comparisons were conducted on the same data family. The significant criterion was modified from α = 0.05 to α = 0.05/k where k is the number of statistical tests conducted on our data.

In behavioural experiments, series of one-tailed t-tests were performed on the novelty preference to test whether the mean scores differ from 50%, indicating that the rats explore more the novel object than the other one. Statistical analysis of exploration times across trials and novelty preference during aging were analyzed using a two-ways ANOVA with delay and age as factors. To appreciate the compared effect of age on novelty preference between strains, repeated measures two-ways ANOVA for delays with age and strain as factors were used. These analyses were performed during the total 5 min of the test phase. The correlation between the exploration time and the novelty preference was evaluated with a simple regression analysis. Comparisons between young
and aged SD rats of repartition of object exploration were performed using chi-square ($\chi^2$) tests. In electrophysiological recordings, the mean values of the synaptic plasticity were measured between the 35th and 45th minute after the end of the conditioning stimulation. The synaptic plasticity after stimulations at different frequency were analyzed using analyses of one-way ANOVA for the fEPSPs obtained pre- vs. post-stimulation. In order to compare the frequency-response curves, repeated-measures one-way or two-ways ANOVA with age and/or strain as factors were performed. For synaptic transmission, statistical differences were assessed using one-way ANOVA for amplitude AMPA and NMDA-mediated fEPSP with age as factor. For western blot, statistical differences were assessed using one-way ANOVA for intensity units of the different receptors with age as factor.

3. Results

3.1. Comparison between young (3–4 months) and old (24 months) SD and Lou/C/Jall rats

3.1.1. Spontaneous object recognition task

The effect of age on object recognition was investigated in group of SD and Lou/C/Jall rats of 3–4 and 24 months of age. Fig. 1A shows the percent novelty preference measured across the total time of object exploration during the test trial at three different delays. One sample $t$-tests revealed that 3–4-month-old SD rats showed significantly greater novelty preference than would be expected by chance on all of the delays ($p < 0.05$). In contrast, 24-month-old rats showed preference at the level expected by chance on all delays ($p > 0.05$), suggesting a significant object recognition deficit compared to their young counterparts. Moreover, a repeated measure ANOVA with delay interval and age as factors revealed a main effect of age ($F(1, 17) = 9.556, p < 0.017$ after Bonferroni corrections) but no significant effect of delays ($F(2, 17) = 0.102, ns$) and no significant delay $\times$ age interaction ($F(2, 17) = 0.294, ns$). Therefore old SD rats present a novelty preference deficit regardless of the delay. The total object exploration time across training and test trials in each group is shown collapsed across delays in Fig. 1B1. Overall, the aged group spent markedly less time exploring the object as compared to the young group (ANOVA with age as main factor, training trial: $F(1, 18) = 4.89, p < 0.05$; and test trial: $F(1, 18) = 4.85, p < 0.05$; Fig. 1B1, left panel). Because the decrease in exploratory activity could interfere with novelty recognition performance, we statistically analyzed the correlation between the two parameters (Fig. 1B1, right panel). ANOVA for regression analyses showed no significant effect.
between the amount of exploratory behaviour and the percentage of the novelty preference whatever the SD age groups (young: $F(1, 28) = 0.265, \text{ns}$; old $F(1, 27) = 0.109, \text{ns}$). Since some sensory deficits related to age processes could interfere with memory capacity, we analyzed the total exploration time spend on each object proportionally to total time of exploration during the training phase. This analysis revealed that aged SD rats do not differ from young rats in the rate of exploration on each single object ($\chi^2 = 1.744, \text{ns}$) suggesting that both groups of animals present an equivalent sensory perception. Thus, these results indicate that, independently of a decrease in exploratory behaviour, old SD rats displayed a significant recognition memory deficit.

In contrast to SD rats, the novelty preference exhibited by the old Lou/C/Jall rats (Fig. 1A2) was similar to their young counterparts. The preferences were always significantly higher than what would be expected by chance regardless of the delay ($t$-test, $p < 0.05$). Here again, the aged Lou/C/Jall rats explored less than the young Lou/C/Jall rats (Fig. 1B2, left panel) (ANOVA with age as main factor, training trial $F(1, 4) = 2.05, p < 0.05$ and the test trial, $F(1, 14) = 1.98, p < 0.05$). ANOVA for regression analyses showed a significant correlation between exploration time and novelty preference for young rats ($F(1, 24) = 7.85, p < 0.05$) but not for old rats ($F(1, 18) = 0.009, \text{ns}$) (Fig. 1B2, right panel).

In order to appreciate the comparative memory recognition performance in age-matched SD and Lou/C/Jall rats, we performed repeated measures ANOVA with delay interval and strain as factors. These analyses revealed no effect of strain in young rats ($F(1, 16) = 0.743, \text{ns}$) and no significant delay $\times$ strain interaction ($F(2, 16) = 0.171, \text{ns}$). In old rats these ANOVA revealed a no effect of strain ($F(1, 15) = 6.997, p = 0.02 > 0.017$ after Bonferroni corrections) and no significant delay $\times$ age interaction ($F(2, 13) = 0.231, \text{ns}$).

The comparison between strains showed a significant difference when the novelty preference during the test trial was compared with age and strain factors (age $\times$ strain interaction $F(1, 101) = 5.66, p < 0.017$ after Bonferroni corrections). Moreover time of object exploration was significantly longer for the Lou/C/Jall rats as compared to age-matched SD rats whatever the age of the animals (young: $F(1, 54) = 21.39, p < 0.001$; old $F(1, 47) = 21.99; p < 0.001$). This result underscored the different memory ability across age in the two strains.

3.1.2. Electrophysiological analysis

In order to compare the frequency–response curves of synaptic plasticity (or Bienenstock–Cooper–Munroe BCM curves; see (Bienenstock et al., 1982)) in young and old SD and Lou/C/Jall rats, synaptic plasticity was examined in the

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**Table 1**

Synaptic plasticity in SD and Lou/C/Jall rats of different ages, as a function of stimulation frequencies

<table>
<thead>
<tr>
<th>Frequency</th>
<th>SD 3 months old</th>
<th>SD 24 months old</th>
<th>SD 28 months old</th>
<th>Lou/C/Jall 3 months old</th>
<th>Lou/C/Jall 24 months old</th>
<th>Lou/C/Jall 28 months old</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Hz</td>
<td>80.2 ± 4.8 [10 (5)]</td>
<td>80.0 ± 4.9 [6 (6)]</td>
<td>80.4 ± 4.8 [9 (9)]</td>
<td>82.6 ± 5.8 [10 (7)]</td>
<td>93.5 ± 6.1 [8 (7)]</td>
<td>96.5 ± 4.7 [5 (2)]</td>
</tr>
<tr>
<td>2 Hz</td>
<td>86.9 ± 4.4 [9 (7)]</td>
<td>88.7 ± 4.3 [6 (6)]</td>
<td>94.4 ± 4.5 [12 (10)]</td>
<td>79.6 ± 6.5 [9 (6)]</td>
<td>88.5 ± 5.3 [11 (9)]</td>
<td>107.8 ± 9.0 [7 (4)]</td>
</tr>
<tr>
<td>10 Hz</td>
<td>89.7 ± 3.7 [8 (6)]</td>
<td>95.3 ± 6.0 [8 (5)]</td>
<td>94.4 ± 4.5 [12 (10)]</td>
<td>96.4 ± 2.3 [9 (7)]</td>
<td>105.4 ± 3.0 [7 (5)]</td>
<td>107.8 ± 9.0 [7 (4)]</td>
</tr>
<tr>
<td>50 Hz</td>
<td>111.7 ± 6.1 [9 (6)]</td>
<td>107.0 ± 1.0 [6 (5)]</td>
<td>121.8 ± 8.9 [10 (9)]</td>
<td>113.7 ± 1.5 [8 (8)]</td>
<td>130.9 ± 8.4 [8 (6)]</td>
<td>140.3 ± 9.7 [6 (5)]</td>
</tr>
<tr>
<td>100 Hz</td>
<td>120.9 ± 6.0 [11 (8)]</td>
<td>111.1 ± 8.3 [8 (7)]</td>
<td>131.7 ± 8.0 [12 (8)]</td>
<td>130.9 ± 5.8 [9 (6)]</td>
<td>140.3 ± 9.7 [6 (5)]</td>
<td>140.3 ± 9.7 [6 (5)]</td>
</tr>
</tbody>
</table>

Values are expressed as percent of baseline values ± S.E.M. Numbers in square bracket represent the number of slices used and the number between parentheses represent the number of rats.
Fig. 2. Synaptic plasticity in SD (left) and Lou/C/Jall (right) rats. Comparison of synaptic plasticity induced by (A) 2 Hz stimulation for 7.5 min, (B) 10 Hz stimulation for 1.5 min and (C) 100 Hz for 1 s in 3- (open circles) and 24-month-old (dark squares) rats of both strains. The recording is stopped during the period of stimulation. Error bars are S.E.M. Black frames indicate the time window of measurement of mean fEPSP slope (over 10 min starting 35 min after stimulation). (D) Mean amplitude of synaptic plasticity measured for 10 min starting 35 min after stimulation across frequency of stimulation. The point of the curve crossing the X-axis is referred as $\theta$ m. Error bars are S.E.M. *$p<0.05$ (repeated measure one-way ANOVA). With age, the BCM curve is shifted to the left in Lou/C/Jall strain.
hippocampal CA1 area across a wide range of stimulation frequencies.

3.1.2.1. LTD. In SD rats, low frequency stimulations (1 and 2 Hz) significantly reduced fEPSPs in slices of young rats (Tables 1 and 2 and Fig. 2A1). Similar depressions were observed in slices from old rats compared to young rats (for 1 Hz: F(1, 14) = 0.001, ns and for 2 Hz: F(1, 13) = 0.08, ns; Tables 1 and 2 and Fig. 2A1).

In Lou/C/Jall rats, 1 Hz stimulation induced LTD in slices of young rats but not in old rats (Tables 1 and 2 and Fig. 2A2). Nevertheless, the difference between slices from young and old rats was not statistically significant (F(1, 16) = 1.62, ns). When stimulation was raised to 2 Hz, significant and similar LTD was observed in slices from both young and old rats (Tables 1 and 2 and Fig. 2A2, F(1, 17) = 0.61, ns).

3.1.2.2. LTD/LTP threshold. We next examined whether age would impact synaptic plasticity at frequencies near the LTD/LTP modification threshold by stimulating Schaffer collaterals at intermediate frequencies.

In SD rats, the 10 Hz stimulation elicited depression in slices from young but not from old rats (F(1, 14) = 0.61, ns; Tables 1 and 2 and Fig. 2B1) whereas stimulation at 50 Hz induced potentiation in both groups of animals to a similar extent (Tables 1 and 2, F(1, 13) = 0.40, ns).

In Lou/C/Jall rats, stimulation at 10 Hz elicited a slight depression in young slices but a slight potentiation in aged slices (Tables 1 and 2 and Fig. 2B2). The differences between young and old are statistically significant (F(1, 14) = 5.66, p < 0.05; Tables 1 and 2 and Fig. 2B2). At 50 Hz, fEPSPs were potentiated to a similar extent in both groups (F(1, 15) = 1.96, ns; Tables 1 and 2).

3.1.2.3. LTP. In SD rats, 1 train of 100 Hz induced a significant potentiation in slices from young animals, but not in the old group (Tables 1 and 2). The difference between the two groups is nevertheless not statistically significant (F(1, 17) = 0.65, ns; Fig. 2C1). At stronger tetanic stimulation (2 × 100 Hz), a similar potentiation was elicited in both groups (Tables 1 and 2 and Fig. 2D1, F(1, 14) = 0.099, ns). In old SD rats, the NMDA receptor antagonist d-APV only partially decreased the LTP magnitude induced by 2 × 100 Hz stimulation (control: 132.5 ± 5.8% of baseline in n = 4 slices from 3 rats; d-APV: 116.7 ± 3% of baseline in n = 8 slices from 5 rats) while it completely suppressed the potentiation in young rats (control: 127.7 ± 5.4% of baseline in n = 9 slices from 6 rats; d-APV: 100.2 ± 2.4% of baseline in n = 10 slices from 6 rats) (Fig. 1 Suppl.). These results indicate that, in 24-month-old SD rats, when high-intensity stimulation is used, a non-NMDA LTP component is induced.

In Lou/C/Jall rats, 100 Hz and 2 × 100 Hz stimulations induced significant potentiation in slices from both young and old rats with a slightly more robust LTP for aged slices compared to young ones (100 Hz: F(1, 14) = 3.1, ns; 2 × 100 Hz: F(1, 19) = 0.015, ns; Tables 1 and 2 and Fig. 2D2).

When all these results are plotted on a frequency–response curve, old Lou/C/Jall rats display a clear response shift towards potentiation compared to young ones (F(1, 13) = 12.5, p < 0.01; Fig. 2D2). No significant difference is observed with age in SD rats (F(1, 12) = 0.005, ns; Fig. 2D1). Interestingly, young SD and Lou/C/Jall BCM curves have the same profile (F(1, 14) = 0.005, ns) while differences appear with aging (F(1, 11) = 9.5, p < 0.017 after Bonferroni corrections). This significant interaction between ages and strains (F(1, 125) = 4.73, p < 0.05) suggests that synaptic plasticity characteristics are differentially affected across the lifespan in these two strains.

3.1.2.4. Synaptic AMPA and NMDA transmission. In order to investigate possible alterations in glutamate receptor properties which could underlie the age-related differences in BCM curves between SD and Lou/C/Jall rats, AMPA and NMDA synaptic potentials were determined from single stimulations. Input-output curves of synaptic strength in AMPA and NMDA-mediated fEPSPs were measured in both strains (data not shown). Both responses increased as a function of stimulus intensity regardless of the age of the rats. However, the magnitudes of AMPA and NMDA fEPSPs were significantly reduced in slices from old SD rats as compared to those from young rats (i.e., for 400 µA stimulation, AMPA fEPSPs: 0.26 ± 0.02 V/s (young rats) vs. 0.15 ± 0.01 V/s (old rats), p < 0.001; NMDA fEPSPs: 0.10 ± 0.01 V/s (young rats) vs. 0.05 ± 0.01 V/s (old rats), p < 0.05). In contrast, AMPA and NMDA fEPSPs slope were comparable in young and old Lou/C/Jall rats (AMPA fEPSPs: 0.25 ± 0.02 V/s (young rats) vs. 0.24 ± 0.02 V/s (old rats), ns; NMDA fEPSPs: 0.09 ± 0.01 V/s (young rats) vs. 0.07 ± 0.01 V/s (old rats), ns) (Fig. 3).

3.1.3. Protein expression of NMDA and AMPA subunits

As illustrated in Fig. 4, the levels of NMDA receptor subunits NR1, NR2B and AMPA receptor subunit GluR1 were similar in both young and aged tissues regardless of the strain considered. The protein level of NR2A subunit of the NMDA receptor was significantly decreased with age in both SD and Lou/C/Jall rats (SD: 27% decrease, p < 0.05; Lou/C/Jall: 48% decrease, p < 0.001; Fig. 4A and B).

3.2. Comparison between 24- and 28-month-old SD and Lou/C/Jall rats

As Lou/C/Jall rats live longer than SD rats (Alliot et al., 2002), the alterations already observed in 24-month-old SD rats might appear later in Lou/C/Jall rats. Thus, synaptic properties were compared in 24- and 28-month-old animals from both strains.

3.2.1. Synaptic plasticity

Synaptic plasticity was tested in 28-month-old rats at three different stimulations: 1 Hz, 2 × 100 Hz and 10 Hz. These frequencies were selected according to their capacity...
to induce LTD, LTP, or to be near the LTD/LTP modification threshold. No change was observed in synaptic plasticity magnitude after 1 or 10 Hz stimulation in slices from old or very old SD rats (at 1 Hz: $F(1, 13) = 0.003$, ns; at 10 Hz: $F(1, 18) = 0.03$, ns) (Tables 1 and 2 and Fig. 5). However, at 2 × 100 Hz stimulation, the potentiation was significantly decreased in the oldest group as compared to slices from 24-month-old SD rats ($F(1, 13) = 8.82, p = 0.01$) (Tables 1 and 2).

In 28-month-old Lou/C/Jall rats, synaptic plasticity remained shifted toward LTP induction and is comparable to that observed in 24-month-old Lou/C/Jall rats (1 Hz: $F(1, 11) = 0.13$, ns; 10 Hz: $F(1, 12) = 0.07$, ns; 2 × 100 Hz: $F(1, 13) = 0.47$, ns). (Tables 1 and 2 and Fig. 5).

Fig. 3. AMPA receptor and NMDA receptor-mediated synaptic potentials. (A-top) Superimposed examples of AMPA receptor-mediated fEPSPs recorded in a young, and an aged, SD (left) and Lou/C/Jall (right) rat in normal ringer after stimulation of pyramidal afferents in stratum radiatum. (A-bottom) Bar graphs of mean AMPA receptor-dependent fEPSPs recorded in each group for a current intensity of 400 μA. These events are statistically smaller in 24-month-old as compared to 3-month-old SD rats (A1) (**$p < 0.01$, one-way ANOVA), but are unaffected by the aging process in the Lou/C/Jall strain (A2). Error bars are S.E.M. The number of slices followed by the number of animals is indicated in parentheses. (B-top) Superimposed examples of NMDA receptor-mediated fEPSP recorded in a young and an aged SD (left) and Lou/C/Jall (right) rat in the presence of bicuculline (10 μM) and DNQX (10 μM). (B-bottom) Bar graphs of mean NMDA receptor-dependent potentials recorded in each group for a current intensity of 400 μA. Synaptic responses are significantly reduced in aged SDs compared to young SDs (B1, *$p < 0.05$, one-way ANOVA) but are not affected in the old Lou/C/Jall strain (B2). Error bars are S.E.M. The number of slices followed by the number of animals is indicated in parentheses.
3.2.2. Synaptic transmission

In 28-month-old SD rats, NMDA and AMPA fEPSPs remained altered (AMPA fEPSPs: 0.20 ± 0.02 V/s from 28-month-old rats compared to those of 24-month-old rats, ns; NMDA fEPSP: 0.048 ± 0.007 V/s from 28-month-old rats, compared to those of 24-month-old rats, ns; Fig. 6A2 and B2). They are unchanged in Lou/C/Jall rats (AMPA fEPSPs: 0.22 ± 0.02 V/s from 28-month-old rats compared to those of 24-month-old rats, ns; NMDA fEPSPs: 0.08 ± 0.005 from 28-month-old rats, compared to those of 24-month-old rats, Fig. 6A1 and B1).

3.2.3. Protein expression of AMPA and NMDA subunits

In SD rats, in addition to the persistent decrease in NR2A subunit expression already observed in 24-month-old rats, the levels of the NR1, NR2B subunits (45%, p < 0.01 and 52%, p < 0.001 decrease, respectively) as well as GluR1 AMPA subunits (26%, p < 0.05) decreased, in comparison to 24- and 3-month-old SD rats (Fig. 6C1). In 28-month-old Lou/C/Jall rats, no further alteration was revealed in addition to the decrease in the level of NR2A protein expression already observed in 24-month-old rats (Fig. 6C2).

4. Discussion

The main observation of the present study is that in addition to a stable metabolic profile, Lou/C/Jall rats maintain intact memory profile and robust synaptic plasticity profiles with age.

The behavioural analysis indicates that the mnestic abilities of Lou/C/Jall rats are unchanged with age, as opposed to SD rats, that present impairment in object recognition memory during aging. This deficit in SD rats does not seem dependent on sensory processing deficit since young and aged rats display similar rates exploration for each single object during the training phase of the task. The object recognition task is a non-spatial, non-stressful memory task increasingly used in rodents (Ennaceur and Delacour, 1988) in the investigation of...
neurobiological mechanisms of learning and memory. In the present study, rats were allowed to explore the two identical objects only briefly, (5 min of training). This short exploration time and long-term retention (≥1 h) were chosen in order to increase the involvement of the hippocampal formation in the task, as previously reported (Ainge et al., 2006; Clark et al., 2000; Hammond et al., 2004). This paradigm tends to reflect the episodic memory component of the task (Mumby et al., 2005). During the behavioural task, Lou/C/Jall rats spent a greater amount of time exploring objects compared to age-matched SD rats. This may be related to the high locomotor activity already described in this strain (Garait et al., 2005). However, in both strains, old rats spent significantly less time exploring objects relative to young adults, as previously observed by Liu et al. (2004) in SD rats. The idea that the deficit being demonstrated in aged rats directly reflects the reduction in initial exploratory activity may appear as a plausible interpretation; however, such a direct link has already been refuted in previous studies (Liu et al., 2004; Pitsikas et al., 2005). Indeed, our present results clearly demonstrated, from a statistical point of view, the absence of correlation between the amount of exploratory activity and memory performance in any conditions of our experimental paradigms. These observations suggest that the absence of age-related memory deficits in Lou/C/Jall rats is not due to their increased exploratory behaviour. Thus, protective cellular mechanisms effective in the Lou/C/Jall rats (Garait et al., 2005) may play a role in preventing age-associated cognitive decline usually occurring in other strains.

Functional decline has long been described in tasks associated with changes in encoding and neural representations in the hippocampus (Tanila et al., 1997a,b), and tasks involving associative, aversive or spatial aspects, such as inhibitory avoidance (Egger and Livesey, 1972; Riccio
and Schulenburg, 1969), fear conditioning (Powell et al., 1991), radial arm maze (Caprioli et al., 1991; Winocur and Gagnon, 1998) or Morris water maze tasks (Gage et al., 1989; Gallagher and Burwell, 1989; van der Staay, 1997). The age-associated deficits in memory recognition in SD rats, described herein, are also in line with previous reports in which aged rodents exhibited poor recognition memory abilities (de Lima et al., 2005; Flicker et al., 1987; Liu et al., 2004; Pitsikas et al., 2005; Schiavetto et al., 2002). Moreover, the within group variability observed in recognition performance of aged SD rats, that have been previously described by other authors (Zyzyk et al., 1995), appeared only with increasing age. This observation highlights the individual adaptation to the deterioration occurring over time in this susceptible strain. The weaker within group variability in aged Lou/C/Jall rats suggests, in contrast, that this strain of rats is not confronted to the same challenging brain deterioration over time.

One of the major differences already described between SD and Lou/C/Jall rats is the ability of Lou/C/Jall rats to exhibit low-caloric intake and a preference for fat with no development of obesity with age (Garait et al., 2005; Veyrat-Durebex and Alliot, 1997; Veyrat-Durebex et al., 1999). This strain has been described as a model of successful aging, since Lou/C/Jall rats have a median lifespan (50% of survivors) of approximately 28–29 months, whereas more common strains such as the SD rats have a median lifespan of approximately 24 months. So far, caloric restriction is probably the most consistently successful method of ameliorating or preventing age-related changes. This paradigm, in which, adult animals have their diet limited to 60–80% of the average caloric intake, extends lifespan in a wide variety of species (Weindruch, 2003). However, if dietary restriction consistently shows beneficial effects on physiological aspects, including prolonged lifespan, it does produce inconsistent effects concerning age-related learning impairments in both aversive and appetitive tasks (Beatty et al., 1987; Bellush et al., 1996; Hansalik et al., 2006; Ingram et al., 1987; Markowska, 1999; Markowska and Savonenko, 2002; Pitsikas and Algeri, 1992; Stewart et al., 1989; Yanai et al., 2004). Differences in the tasks, and the motivational level, differences in the extent of dietary restriction (ranging from 35 to 60% of the maximum body weight of ad libitum rats), differences in feeding schedules, etc., are likely to account for these discrepancies. In this respect, Lou/C/Jall rats probably constitute a new and more suitable model to study the metabolic contribution to age-related cognitive decline.

Given the differences in mnesic abilities between both strains, it was of interest to test whether synaptic plasticity was also differentially affected during aging. We paid particular attention to bidirectional plasticity, a property of synaptic circuits thought to be essential for information processing and memory storage. In young Lou/C/Jall rats, the $\theta$ m (breaking point between LTD and LTP induction) of the BCM curve is between 10 and 50 Hz, similar to previous observation in adult albino rats (Dudek and Bear, 1992), and comparable to the data obtained with young SD rats. However, in contrast to SD rats, 24- and also 28-month-old Lou/C/Jall rats do not exhibit any deficits of synaptic plasticity. On the contrary, they even present a shift towards potentiation. The facilitated ability of aged Lou/C/Jall rats to discriminate the characteristics of novel objects is consistent with the facilitated potentiation mechanisms observed in the hippocampus. In keeping with the behavioural data, the present results indicate that deficit in LTP properties in SD rats is already evident at 24 months of age. Intact hippocampal LTP can still be induced when robust, high-intensity stimulation protocols are used (2 × 100 Hz), but deficits occur when lower-intensity stimulation is applied (1 × 100 Hz) (Deupree et al., 1993; Moore et al., 1993; Rosenzweig et al., 1997). Aging has been reported to cause a shift in synaptic plasticity from NMDA receptor-dependent mechanisms to voltage-dependent calcium channel (VDCC)-dependent mechanisms (Junjaud et al., 2006; Shankar et al., 1998), probably via an increase in L-type calcium channel expression (Veng and Browning, 2002; Veng et al., 2003). While LTP is induced by 2 × 100 Hz in the presence of d-APV, an NMDA receptor antagonist, in both young and old rats, a modest potentiation remained only in old rats (Fig. I Suppl.). Since the total magnitude of LTP (the sum of NMDA-dependent and VDCC-dependent LTP) was similar in young and old rats, this suggests an age-related decline in NMDA dependent LTP with age “compensated” by VDCC-dependent mechanisms (see also (Junjaud et al., 2006)). At an older age (28 months group), LTP magnitude is significantly decreased suggesting that both NMDA and VDCC-dependent LTP are altered. Interestingly, NMDA receptors and VDCCs trigger processes that underlie different aspects of memory formation (Woodside et al., 2004). Blocking Ca$^{2+}$ influx via NMDA receptors impairs the animals’ ability to acquire the task, while blocking VDCCs has no effect on acquisition, but does impair retention. An excessive Ca$^{2+}$ influx through L-VDCC may even be detrimental to memory formation (Veng et al., 2003). Taken together, these data are compatible with the age-related memory deficits described above, and with previous studies reporting a correlation between memory impairment and loss of LTP (Bach et al., 1999; Barnes, 1979; Landfield et al., 1978).

Overall, the clear difference between aged SD and Lou/C/Jall rats in changes in synaptic plasticity corroborates the behavioural data, and is consistent with the healthier metabolic profile of Lou/C/Jall rats. Since caloric restriction restores age-related changes in synaptic plasticity (Eckles-Smith et al., 2000; Hori et al., 1992; Okada et al., 2003), probably via neuronal protection against excitotoxic, oxidative and metabolic insults (Garait et al., 2005; Guo et al., 2001; Ingram et al., 1987), it is very likely that the metabolic homeostasis of Lou/C/Jall rats across the lifespan is a major factor in their preserved cognitive and electrophysiological performance. In keeping with this finding, it has been demonstrated that Lou/C/Jall rats do not develop insulin-resistance (Perrin et al., 2003) or changes in blood leptin levels (Kappeler et al., 2004) across their life spans. This could contribute to the intact LTP induction observed in aged Lou/C/Jall rats,
since leptin has been reported to modulate hippocampal synaptic plasticity via enhancing NMDA receptor activation (Harvey et al., 2006). Moreover, the low-caloric intake of the Lou/C/Jall strain reduces oxidative stress (Garait et al., 2005), and it is known that oxidation decreases NMDAR activity (Herin and Aizenman, 2004). Reduction of oxidative stress also limits age-related impairments in synaptic plasticity (McGahon et al., 1999; Watson et al., 2006).

NMDA receptors are essential for LTP induction and maintenance (Clayton et al., 2002; Malinow and Malenka, 2002) and are required for spatial learning and memory (Adams et al., 2001; McHugh et al., 1996; Tsien et al., 1996). Therefore, differential alterations of glutamate receptor activation might underlie the respective changes in synaptic plasticity occurring in aged SD and Lou/C/Jall rats.

In SD rats, synaptic potentials mediated by NMDA receptor activation were significantly depressed in 24- and 28-month-olds as previously reported (Barnes et al., 1997; Clayton et al., 2002; Mothet et al., 2006; Potier et al., 2000). In line with these results, NR2A subunits decreased in aged animals while all NMDA subunits were affected in the senescent groups. Earlier reports also described reduced density of both NR2A and NR2B receptor subunits in hippocampi of 21–30-month-old F344 × Brown Norway rats (Shi et al., 2007; Sonntag et al., 2000), a decrease in NR2B-receptor subunits in hippocampus of 24-month-old Fischer 344 (Clayton and Browning, 2001), or in CA3 and, to a lesser degree, in the dentate gyrus of 29-month-old Fischer 344 × Brown Norway rats (Newton et al., 2007). Paradoxically, some authors observed a decrease in NMDA receptors evident as early as 18 months of age, in contrast with the present data. Such discrepancies could be due to differences in rat strain (SD vs. Fischer 344 × Brown-Norway) or region sampled (whole hippocampus vs. dissected hippocampal subregions) (Mesches et al., 2004; Shi et al., 2007).

Synaptic potentials mediated by NMDAR activation were not affected in Lou/C/Jall rats, despite a decrease in the NR2A receptor subunits expression in 24-month-old rats. Recent data have shown that NR2A and NR2B subunits are not similarly involved in synaptic plasticity with a preferential role of NR2A in LTD induction (Berberich et al., 2007; Morishita et al., 2007). This decrease in NR2A could contribute to the reduction in LTD magnitude observed in 24- and 28-month-old Lou/C/Jall rats. This lack of important alteration of NMDA receptor in Lou/C/Jall rats during aging may not be solely associated with their spontaneous caloric restriction, since the effects of caloric restriction on NMDA receptor expression during aging remains somewhat controversial (Eckles-Smith et al., 2000; Monti et al., 2004).

Since on one side, the functionality of NMDA receptors is already significantly altered in 24-month-old SD rats, while their expression is only slightly affected at the same age while on another side, Lou/C/Jall rats display a decrease in NR2A expression without any functional alteration in NMDA synaptic transmission, factors other than receptor density must be involved in accounting for the impairment of NMDA receptor activation in aging. Accordingly, this deficit is rescued in aged rats after either saturating the receptor with a partial agonist, d-cycloserine (Billard and Rouaud, 2007) or the endogenous agonist d-serine (Junjaud et al., 2006) both acting at the glycine modulatory site. d-serine predominantly derives from astrocytes and its synthesis is decreased during aging (Mothet et al., 2006) indicating that glial cells are involved in the age-related deficit of NMDAR activation. Therefore, although declines in NMDA subunits have been associated with cognitive decline in old rats (Mesches et al., 2004; Newcomer and Krystal, 2001), results from the present study suggest that such a relationship is either anecdotal, or more complex than, a simple correlation.

In conclusion, Lou/C/Jall rats display remarkable cognitive capacities and CA1 hippocampal synaptic plasticity in aging. These performances are likely linked to the exceptional metabolic homeostasis observed in these rats under a spontaneous caloric intake restriction. Since caloric restriction is well known to improve lifespan and hippocampal synaptic plasticity, Lou/C/Jall rats are relevant tools to determine why and how metabolic homeostasis can skew the elderly brain towards improved capacities.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j. neurobiolaging.2008.03.010.

References


Boghossian, S., Ntang Nguema, G., Jourdan, D., Alliot, J., 2002. Old as young? The role of NMDA receptor subtypes and charge transport during hippocampal LTP induction. Neupe...


Veng, L.M., Browning, M.D., 2002. Regionally selective alterations in expression of the alpha (1D) subunit (Ca (v)1.3) of L-type calcium channels in the hippocampus of aged rats. Brain Res. Mol. Brain Res. 107 (2), 120–127.

Veng, L.M., Mesches, M.H., Browning, M.D., 2003. Age-related working memory impairment is correlated with increases in the L-type calcium channel protein alpha1D (Cav1.3) in area CA1 of the hippocampus and both are ameliorated by chronic nimodipine treatment. Brain Res. Mol. Brain Res. 110 (2), 193–202.


