Neural plasticity is impaired in cold-exposed hippocampal slices from senescent but not from age-matched presenescent F344 rats

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Abstract

Near the end of their natural life, many mammals enter a terminal state identifiable by a rapid loss of body weight resulting from hypophagia. This study extends characterization of this senescent state by comparing viability of metabolic mechanisms supporting neural plasticity in hippocampal slices from 24 to 30 month old senescent and age-matched presenescent (body-weight stable) F344 male rats. Half of the slices from each rat were incubated at 22–23 °C, and half were immersed in cool incubation medium (12–15 °C) immediately after slicing and allowed to passively warm to room temperature over ~50 min to impose a cold stressor on recovery mechanisms. Following incubation, CA1 pyramidal cell population spike (PS) amplitudes were measured before and after tetanus. In slices incubated at 22–23 °C, the 221.0 ± 24.2 % increase in PS amplitude following tetanus in seven slices from five senescent rats was not significantly different from the 202.5 ± 23.8% increase in six slices from five age-matched presenescent rats. In contrast, in cold-exposed slices, the 133.8 ± 13.1% increase in PS amplitude following tetanus in 14 slices from 10 senescent rats was significantly smaller (p < 0.05) than the 184.7 ± 10.2% increase in 13 slices from seven age-matched presenescent rats. This smaller PS enhancement in senescent rats cannot be attributed to weight loss because robust potentiation was induced in cold-exposed slices from five food-restricted presenescent rats having a weight loss comparable to their senescent counterparts. Thus, the blunted enhancement observed in cold-exposed slices appears to be a characteristic of senescence.

1. Introduction

Mammals often display a relatively stable or slowly declining maintenance of physiological function until abruptly entering, near the end of their natural life, a distinctive terminal stage that we have termed senescence [20]. Rats in the senescent state exhibit a rapid loss in weight reflecting hypophagia [3,4,11], reduced ability to maintain homeothermy during cold exposure, and blunted circadian rhythmicity of body temperature [20,21]. Senescence is part of the aging process that rats eventually enter if they avoid fatal attacks, lethal disease, or adverse environmental conditions that prematurely shorten their lives. These observations suggest changes in hypothalamic and suprachiasmatic neural processing; but the extent to which neural metabolic mechanisms may be altered in other brain regions has not been studied.

Since the studies by Bliss and Gardner-Medwin [5], population spike measurements have been used to provide a measure of overall plasticity in hippocampal networks. Changes in overall plasticity can be attributed to changes at several sites in the circuit including excitatory synapses, E-S coupling, and GABAergic inhibitory pathways. A form of synaptic plasticity, long term potentiation (LTP) seen as a change in slope of field EPSPs, has been most thoroughly investigated both as a cellular exemplar of neural plasticity and as a potential mechanism involved in learning and memory [5,19]. Studies on old rats of the same chronological age (potentially a mix of both presenescent and senescent rats) have further established dissimilar effects of age in different regions of the hippocampus [1]. Within the CA1
region, impairment in the establishment of LTP is often not evident in chronologically old animals using supra-threshold stimulation, although partial impairments have been identified in experiments using peri-threshold stimulation [2].

While mild cold exposure is generally considered neuro-protective, more severe cold exposure during slice incubation imposes a stressor on metabolic recovery mechanisms [29]. Cold exposure was used in this study to facilitate detection of metabolic differences in CA1 neurons of presenescent and senescent rats. We found that population spike potentiation (PSP) established by suprathreshold stimulation of CA1 pyramidal cells remained robust in slices from both senescent and age-matched presenescent rats when slices were incubated at room temperature. However, when slices were placed in cool artificial cerebral spinal fluid (ACSF) and allowed to warm to room temperature during incubation [29], PSP and fEPSP slope increases were significantly smaller in slices from senescent rats but not in those from either weight stable or from food restricted presenescent controls. These observations are consistent with the hypothesis that hippocampal neural mechanisms are impaired in the senescent state.

2. Methods

2.1. Animal groups

Male F344 rats, obtained from the National Institute of Aging colony maintained by Harlan Sprague–Dawley laboratory (Indianapolis, IN), were housed at thermoneutrality (25–26 °C) and 50% humidity on a light:dark 12:12 cycle (lights on at 6:00 am) for a minimum of three weeks prior to slice experiments. Animals were provided with NIH-31 laboratory chow (Teklad Research Diets, Indianapolis, IN) and distilled water ad libitum. Experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996.

Body weights of 24–30 month old rats were measured several times each week. If their weights were stable or slowly declining over a 15-day period, they were classified as presenescent (the period p to s in Fig. 1A for each rat). Entry into senescence (on day s) could spontaneously occur at any time over a period of months and was identified by an abrupt change in slope of the curve for body weight versus time. Rats in senescence (the period s to e in Fig. 1A) were sacrificed for slice experiments (on day e) when their body weight had rapidly declined 10% or more over a 5–10 day period.

The 18 senescent rats used in the slice experiments had a mean age of 26.3 ± 0.4 months and had lost approximately 13% of their body weight. A control group of weight-stable presenescent rats (n = 14) had a mean age of 27.0 ± 0.2 months; while a food-restricted group (n = 5) consisted of old, presenescent male F344 rats with a mean age of 27.0 ± 0.2 months. Rats in this latter group were fed 55–70% of their (ad libitum) normal daily intake over a period of about 2 weeks and sacrificed when their weight loss was comparable to the average weight loss of the senescent rats. A fourth group from which slices were obtained consisted of young adult male F344 rats (n = 3; mean age = 7.4 ± 0.1 months).

2.2. Slice preparation

Hippocampi were removed bilaterally, chilled to approximately 4 °C and sectioned (400 µm thick slices) using a McIlwain tissue chopper. Every other slice was incubated at room temperature (22–23 °C). Remaining slices from the
same animal were placed in cool ACSF (13–15 °C) and allowed to passively warm to room temperature over approximately 50 min (the cold-exposure group). Slices in both groups were incubated in a 300 ± 5 mosM ACSF aerated with 95% O2/5% CO2 and composed of (in mM): NaCl 124, KCl 2.5, NaH2PO4 1.5, MgSO4 2, NaHCO3 26, CaCl2 2, dextrose 10.

2.3. Recording

Slices transferred to the recording chamber were superfused with aerated ACSF at 28–30 °C at a flow rate of 8 ml/min. Bipolar tungsten stimulating electrodes were placed in the stratum radiatum of area CA3. Evoked responses were measured with glass pipettes which were filled with 3M NaCl, had resistances of 2–8 MΩ, and were placed in the CA1 pyramidal layer for population spike (PS) measurements and in the stratum radiatum for fEPSP measurements. Electrical activity was recorded as described previously [27].

In preliminary experiments, input/output (I/O) curves were generated by increasing stimulus intensity from threshold to saturation. While I/O curves recorded in slices from age-matched presenescent rats (four slices from two rats) showed a more robust enhancement after tetanus than the I/O curves recorded from slices from senescent rats (three slices, two rats), all I/O plots had smooth sigmoidal curves, and no differences were seen that would be missed by using only a midlevel stimulus [14]. Therefore, data in this study were obtained using a 100 μs pulse adjusted to evoke a half-maximal PS at the beginning of the experiment and thereafter held constant. Evoked responses for five pulses (with an interstimulus interval of 15 s) were averaged for each record saved on disk. PS amplitude was calculated from peak voltages (labeled a, b, and c on the PS in Fig. 1B) using the equation \( \left( \frac{V_a - V_b}{C_0} \right) + \left( \frac{V_c - V_b}{C_0} \right) / 2 \). Once the response was stable (a variation in PS amplitude on the records of less than 15% over a fifteen minute interval), a control record was obtained and tetanus applied (100 Hz for 1 s repeated five times at 15 s intervals). Following tetanus, PS amplitudes in old presenescent F344 and

2.4. Statistical analysis

Slice groups including only warm-exposed slices (young, senescent and presenescent) or only cold-exposed slices (presenescent, senescent and food restricted) were analyzed utilizing a one factor ANOVA. When the ANOVA indicated significant differences existed between groups, a Fisher LSD post hoc test was utilized to compare the means of interest. The effects of temperature of slice incubation and aged state (senescent or presenescent) were analyzed using a two factor ANOVA. A protected LSD test was utilized to compare the specific means of interest. Comparison of fEPSPs and of pre-tetanus half-maximal PS amplitudes between cold exposed slices from senescent and presenescent rats was made using the Student’s two-tailed t-test. All values are reported as mean ± SEM. and all differences were considered significant at \( p < 0.05 \).

3. Results

3.1. Robust PSP was observed in slices from senescent, presenescent and young adult rats incubated at room temperature

Fig. 1 shows the establishment of substantial PSP in slices from two rats that were in senescence when sacrificed (day e in Fig. 1A), as determined by their rapid fall in body weight over the preceding week. Population spikes recorded from slices for each of these rats before and after tetanus are shown in columns B and C in Fig. 1. At 15 min following tetanus (B2 and C2), PS amplitude was increased, an enhancement that was still present 30 min after tetanus (B3 and C3). These room temperature incubated slices from both senescent F344 rats show PSP following tetanus and illustrate the variability between slices/animals.

Following the same protocol (incubation at room temperature), PS amplitudes in old presenescent F344 and
young adult rats typically showed an increase in amplitude following tetanus (Fig. 2) similar to that seen in senescent rats (Fig. 1). In seven slices (from five senescent rats) incubated at 22–23 °C, the 221.0 ± 64.2% increase in PS amplitude following tetanus was not significantly different from the 202.5 ± 23.8% increase in six slices from five age-matched presenescent rats, nor from the 202.6 ± 19.7% increase recorded in five slices from three young rats. Thus, robust tetanus-induced PSP can be established in slices incubated at room temperature from young adult, presenescence, and senescent rats.

3.2. Blunted potentiation was observed in cold-shocked slices from senescent but not presenescence rats

To impose a cold stressor, slices were placed in cool (13–15 °C) ACSF and allowed to rewarm toward room temperature (Fig. 3A, lower trace). Representative responses following incubation are shown in Fig. 3B (from a presenescent rat) and Fig. 3C (from a senescent rat). Prior to tetanus, population spikes measured in cold-exposed slices from both old presenescent and senescent rats had similar pre-tetanus half-maximal PS amplitudes. These averaged 6.04 ± 0.62 mV (n = 10 slices from 10 old presenescent animals) and 6.11 ± 0.83 mV (n = 9 slices from 9 senescent rats) (p = 0.94). The average change in PS amplitude following tetanus (expressed as a percentage of control) for 14 cold-exposed slices from 10 senescent rats was 133.8 ± 13.1%. This value was significantly smaller (p < 0.05) than the average change in PS amplitude of 184.7 ± 10.2% for 13 slices from seven presenescent rats.

Fig. 4 summarizes the PSP enhancement in slices from senescent and presenescence animals. The only significant difference between the two aged groups was the decrease in potentiation seen in slices from senescent rats incubated in cool media.

fEPSP slopes increased following tetanus (expressed as percent of pre-tetanus control) by 135.6 ± 7.1 % in six cold-exposed slices from five senescent rats and by 208 ± 35.9% in six slices from five presenescent rats. Paralleling the...
decrease in PSP, this slope increase was significantly smaller ($p < 0.05$) in slices from senescent rats.

3.3. PSP is not attenuated in slices from food-restricted, age-matched presenescent rats

Evoked responses in slices from food-restricted presenescent rats (body weight-loss- and age-matched to senescent rats) were measured to determine if weight loss itself led to attenuated PSP in senescent rats. As shown in Fig. 5, following incubation in cool media, robust PSP could be obtained from presenescent, food-restricted rats. The average increase in PS amplitude for seven slices from three food-restricted animals was 179.9 ± 14.6%, a value that did not differ from that of slices from presenescent ad libitum fed controls. Thus, the blunted PSP in cold-exposed slices from senescent rats cannot be attributed to weight loss per se.

4. Discussion

In studies on aging, animals having similar chronological ages, e.g. 30 ± 3 months, have been commonly regarded as being physiologically similar [1,2,15,17]. We have shown, however, that even in in-bred strains of animals, this is not the case (e.g., Refs. [3,4,11,20,21]). In our experiments, chronologically old rats have been classified as either senescent (exhibiting rapid weight loss) or presenescent (body weight stable) [3,4,11,20,21]. Studies on regulatory systems [3,4,20,21] show that senescence may be accompanied by additional impairment of brain function compared to that seen in comparably aged presenescent rats. Our major finding in this study is that slices from both senescent and presenescent rats showed no impairment in PSP when incubated in warm media; but there was impaired PSP in cold-exposed slices from senescent rats but not presenescent rats (Fig. 5). In addition, comparison of PSP in similarly aged ad lib versus food-restricted senescent rats showed that this blunted neural plasticity could not be attributed to food restriction. The finding that fEPSP slopes were blunted in slices from senescent rats compared to those from presenescent rats further suggests that one site of impairment in senescent animals is synaptic LTP.

4.1. Robust PSP is established in room-temperature-incubated slices from presenescent and senescent F344 rats

Our data demonstrate that under favorable conditions for slice recovery, supra-threshold tetanus elicited substantial PSP in slices from presenescent, senescent, and young adult rats (Figs. 1 and 2). In slices incubated at 22–23 °C, PS amplitude increased 221.0 ± 24.2% in senescent rats, 202.5 ± 23.8% in age-matched presenescent, and 202.6 ± 19.7% in young adult rats. This result is consistent with the general finding that supra-threshold stimulation can elicit potentiation in CA1 pyramidal cells over the lifespan of the animal [1,2,15,17] (but see Refs. [12,18]).

When stimulus intensity is lowered from supra-threshold to peri-threshold levels, effects of aging on LTP establishment in CA1 pyramidal cells become more apparent, with the minimal stimuli needed to establish LTP being higher in old rats [7,22]. By clamping transmembrane potential (signal B in Fig. 6), Barnes et al. [2] localized this increased threshold in old rats to steps in LTP induction prior to the entry of Ca$^{2+}$ through NMDA channels. The sparser Schaffer collateral synaptic contacts to CA1 pyramidal cells in old rats, as inferred from electrophysiology studies [1], could result in smaller cell depolarization and hence, less effective relief of the Mg$^{2+}$ block of NMDA receptor channels [1,24]. Sparser synaptic contacts could also account for another experimental observation: the small but significant decrease in EPSP amplitude seen in chronologically old rats at all stimulus intensities.

While synaptic sparseness has clear effects on the establishment of LTP in peri-threshold stimulation experiments, most studies have shown that at supra-threshold, LTP in CA1 pyramidal cells remains robust. Thus, the slight reduction in EPSP amplitude in old rats is apparently compensated for by increased neuron excitability [16,24]. Our experiments show that potentiation established by supra-threshold stimulation remained robust in room temperature-incubated slices from senescent as well as presenescent rats. It thus appears unlikely that the variability in LTP amplitude
noted in various studies using rats of the same chronological age [8,18] can be attributed simply to a mix of presenescent and senescent rats.

4.2. PSP is impaired in cold-exposed hippocampal slices from senescent but not from age-matched presenescent rats

When subjected to cold temperatures during incubation, slices from senescent rats exhibited only an 133.8±13.1% increase in PS amplitude following tetanus (Fig. 3), a relatively minor enhancement compared to that observed for slices incubated at room temperature. The cold incubation protocols in our experiments were similar to those used by Watson et al. for slices from young animals [29]. They found differences in evoked responses, including population spike recordings, between slices incubated at room temperature and those placed in chilled ACSF (13–15 °C) and allowed to rewarm to room temperature (an exponential rise in temperature over a two hour period).

Watson et al. [29] attributed the differing responses of cold-shocked and room-temperature incubated slices to cold or some related factor(s), such as altered pH, that may have inhibited mechanisms required for slice recovery. Because PS recordings have also been used to provide an overall measure of plasticity in CA1 neurons [including changes in E-S coupling [6] as well as changes at the synapse], we focused on PS amplitudes. One modification in our protocol from that of Watson et al. [29] was the imposition of a less severe cold shock so that we could consistently evoke PSs and fEPSPs in slices from old animals. As a result, in our experiments incubation temperature returned to room temperature sooner (in approximately 50 min) than in the experiments reported by Watson et al. [29].

In control records (prior to tetanus), single-shock stimulation of Schaffer collaterals evoked population spikes in cold-exposed slices that had approximately the same amplitude in both senescent and presenescent rats. Thus, the blunted PSP evoked by supra-threshold stimulation of slices from senescent versus presenescent rats (Fig. 3) does not appear to be due to insufficient depolarization. Following tetanus, the reduced augmentation in fEPSP slopes in slices from senescent rats indicates that one part of the overall network that is altered is the Schaffer collateral—CA1 pyramidal cells synapse (some portion of the process following depolarizing current through AMPA channels indicated by bolded lines in Fig. 6).

Further studies are required to precisely localize this synaptic deficit in senescent rats. Lynch’s review [17] of age-dependent changes in LTP lists four general, interrelated theories of aging that have received significant attention (the membrane, free radical, Ca²⁺ homeostasis and glucocorticoid hypotheses). This general framework, together with more recent reviews on aging [9,10], provide an overview of the constellation of long-term alterations in cellular mechanisms that, together with specific mechanisms whose impairment is further exacerbated by acute cool incubation, may underlie the blunted LTP in senescent rats. Ion channels permeable to Ca²⁺ are clearly altered in aging rats [15]. While mild cooling is generally considered neuroprotective [29], recovery mechanisms in cold-exposed slices from senescent rats may be further strained, resulting, after a latent period, in an additional loss of Ca²⁺ homeostasis. Nicholls and Budd [23] have reviewed the key role played by reactive oxygen species and mitochondria in neuronal survival. Other long term factors potentially contributing to the attenuated LTP in cold-exposed slices from senescent vs. presenescent rats include exacerbated synaptic sparseness [1], altered GABA-mediated inhibition [1,25,26] and reduced presynaptic release of glutamate [13]. Additional acute effects of cool incubation include an increase in PKC in homogenized hippocampal slices and a reduction in GABAergic IPSPs that are blocked by chelerythrine, a selective inhibitor of PKC [30]. If PKC is also increased in pyramidal cells, this increase together with a reduction in GABAergic

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Fig. 6. Flow diagram for the induction and establishment of LTP showing age-dependent steps. Initial events, LTP induction, are shown in the top half of the figure. The release of glutamate from Schaffer/commissural fibers opens AMPA channels in CA1 pyramidal cells to depolarize the cell. NMDA receptor channels are then opened by coincident cell depolarization (signal A) and glutamate binding (signal B), and Ca²⁺ enters the cell through NMDA channels. Events triggered by Ca²⁺ influx and culminating in LTP expression are indicated in the bottom half of the figure.
inputs would contribute to the altered state of hippocampal neurons in senescent animals.

4.3. Blunted LTP absence is a property of the senescent state, not weight loss

While body weight measurements of old rats (Fig. 1A) provide a simple method for identifying animals that have entered senescence [3,4,11], weight loss is only one of several characteristics of this state [20,21]. Even though rats live only a few weeks after entry into senescence, they display no common pathology [3]. In many respects the senescent state in rats is analogous to a syndrome seen in elderly humans near the end of their life, a syndrome termed geriatric “failure to thrive”.

To determine if the weight loss per se in old rats was sufficient to dampen LTP, old presenescence (weight stable) rats were food restricted until they had reached the average weight loss of the senescent animals. In marked contrast to our findings on senescent rats, food-restricted old rats still showed robust LTP (Fig. 5) in cold-exposed slices. This preservation of LTP with age in presenescence rats is consistent with observations by Hori et al. [12] on a group of old animals (most of whom were most likely presenecent. Thus, the loss of plasticity in senescent rats is a consequence of the senescent state rather than the weight loss associated with this state.

In summary, the blunted enhancement of PS amplitudes and iEPSP slopes in cold-exposed slices appears to be characteristic of senescence.

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References

[22] C.I. Moore, M.D. Browning, G.M. Rose, Hippocampal plasticity induced by primed burst, but not long-term potentiation, stimulation is impaired in area CA1 of aged Fischer 344 rats, Hippocampus 3 (1993) 57–66.
[27] A.V. Raghavan, J.M. Horowitz, C.A. Fuller, Diurnal modulation of...
long-term potentiation in the hamster hippocampal slice, Brain Res. 833 (1999) 311–314.

