Involvement of Dysfunctional Mastication in Cognitive System Deficits in the Mouse

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Summary

A systemic effect of dysfunctional mastication has been suggested as a possible epidemiological risk factor for senile dementia. In recent years, we have evaluated the effects on cognitive function deficits in SAMP8 mice. Aged mice with dysfunctional mastication showed significantly reduced learning ability in a water maze test compared with age-matched control mice, whereas there was no difference between control and molarless young adult mice. Immunohistochemical analysis revealed that in the CA1 region of the hippocampus the molarless condition not only enhanced the age-dependent increase in the density and hypertrophy of GFAP-labeled astrocytes, it decreased the density of Fos-positive neurons and Nissl-stained neurons, or the amount of acetylcholine (ACh) release in the hippocampus, in the same manner. There was a similar age-dependent decrease in choline acetyltransferase in the medial septal nucleus. Furthermore, dysfunctional mastication induced an increase in plasma corticosterone levels. The findings suggest that dysfunctional mastication in aged SAMP8 mice causes abnormalities in the hippocampus through stress, leading to deficits in learning and memory.

Key words Mastication, Hippocampus, Memory, Neuronal degeneration, Stress

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Introduction

A reduced ability to masticate as a result of root caries, a large number of missing teeth, and oral dyskinesia have been suggested to be associated with senile dementia. For example, a systemic effect of tooth loss has been suggested as a possible epidemiological risk factor for Alzheimer's dementia [1]. In experimental studies, it has also been shown that a soft diet from the weanling period onward causes later impairment of avoidance performance in mice [2], and that differences in neuronal density between the right and left cerebral hemispheres are seen in rats with unilateral mastication [3]. Recently, many investigators have extensively studied the mechanism(s) connecting dysfunctional mastication with senile deficits in cognition.

In this chapter, we describe our recent findings obtained using behavioral, immunohistochemical, and biochemical methods in the hippocampus of mice with accelerated senescence (SAMP8 mice).

Impaired Spatial Cognition in the Molarless Condition

To achieve an animal model with dysfunctional mastication, we extracted or cut off the maxillary molar teeth (molarless condition) of SAMP8 mice. Control animals underwent the same surgical procedure except for tooth removal or extraction. Ten days after operating, animals were tested in the spatial memory version of the Morris water maze [4]. In this behavioral experiment, the mice were placed in a pool of nontranslucent water and given spatial cues to help them find a hidden platform [5]. Figure 1 shows the time required for the mice to arrive at the platform (escape latency), revealing that the escape latency of the aged group was longer than that of the young group. In control mice, significant overall differences were seen between the three age groups in terms of the time taken to reach the platform

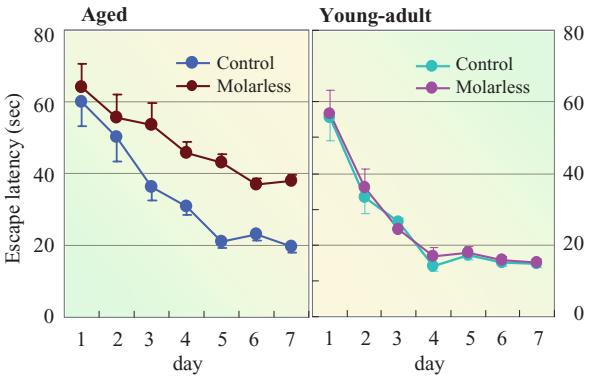

Fig. 1. Spatial learning in the water maze test. Ten days after surgery, learning tests were started. The results are expressed as the mean score of four trials per day. Note that molarless mice take a significant longer time to reach the platform than do controls

[5], indicating age-related impairment of spatial learning. Although young molarless mice performed as well as their age-matched controls, middle-aged and aged molarless mice performed significantly less well than their age-matched controls. When a visible probe test was performed on the same mice at the end of the test, no significant difference was seen between the control and molarless groups [5–8]. In addition, the swimming speed of each mouse, estimated from the latency and swim paths recorded in the computer system, showed no difference between the age-matched control and molarless groups.

At 6 months of age, SAMP8 mice exhibit clear deficits in learning and memory in various learning tests (e.g., passive avoidance test [9, 10] and one-way [9], T maze [11], and Sidman active avoidance tests [12]), suggesting that the molarless condition enhances the age-dependent decrease in cognitive function. Kubota et al. [13] demonstrated that sensory input from the sensory receptor on the tooth root decreases as a result of long-term dysfunction of mastication following extraction of teeth. Kato et al. [14] also showed impaired performance in a radial arm maze task in aged rats lacking their molar teeth, implying that the molarless condition is linked to age-related memory deficits. Several studies have also shown that mastication increases muscular activity, regardless of caloric intake, and that brain neuronal activity and cerebral blood flow increase simultaneously [15, 16]. Thus, it is likely that there may be an important link between mastication-induced stimulation and hippocampal function.

Attenuation of Hippocampal Fos Induction by the Molarless Condition

In our study using the combined technique of behavior and immunohistochemistry in aged SAMP8 mice, the molarless condition decreased Fos induction in the hippocampus, which is related to impaired development of learning ability in a water maze [6]; this suggests that functional molar teeth may be one of the factors responsible for maintaining spatial learning and memory during old age [16]. This conclusion derives from the following findings: (1) in molarless mice, the reduction in Fos-positive cell numbers in the hippocampal CA1 region paralleled the reduction in learning ability in a water maze [6, 8]; and (2) the extent of the decrease in the number of these cells and the reduced learning ability [5, 6, 17] both depended on the duration of the molarless condition. Furthermore, as shown in Fig. 2, it was found that the suppressive effects induced by the molarless condition were considerably reduced by restoring the lost molar with an artificial crown [6].

The mechanism by which dysfunctional molar teeth and the consequent reduction in masticatory ability accelerate senile impairment of spatial learning has not yet been defined. However, one possibility is that in the molarless condition input activity to the somatic sensory cortex (in which some neurons are indirectly linked with hippocampal neurons) from receptors coupled to mastication and mastication-associated face and jaw movements may decrease. In addition, given the fact that

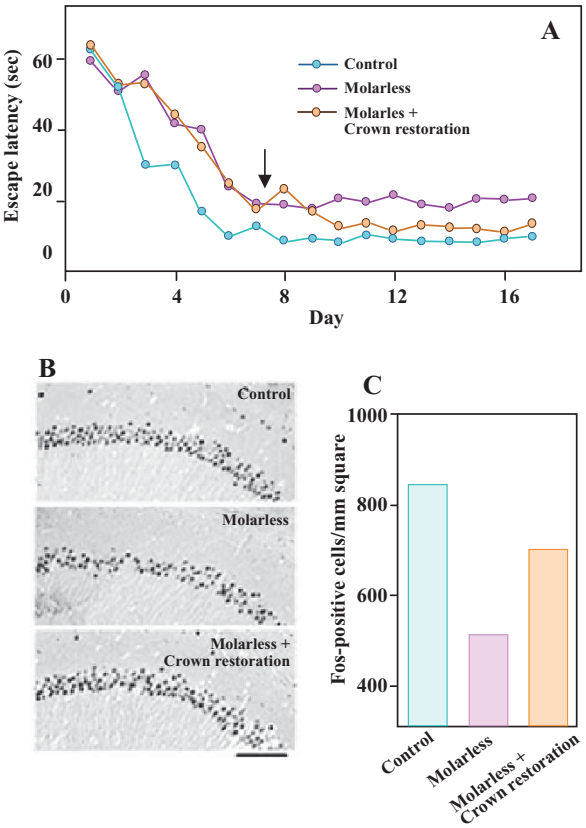

Fig. 2. Effect of placing an artificial crown on spatial learning and the number of Fos-positive cells in the CA1 region in aged molarless mice. **A** On postoperative day 10, the control group and the two molarless groups were subjected to the water maze learning test; one group of molarless mice then received an artificial crown between the tests on test days 7 and 8 (*arrow*). The results are expressed as the mean score. **B** Fos immunohistochemistry (IHC) in the CA1. IHC analysis was carried out following the learning task on the final test day (day 17). *Bar* 100 µm. **C** Quantitative results for Fos-positive cells in the CA1. Each *column* indicates the mean. (From Watanabe et al. [6], with permission)

the expression of c-fos and Fos is a useful marker for elevated levels of neuronal activity generated in the brain following various stimuli [18, 19] and the finding that trigeminal input has a facilitatory effect on synaptic transmission in various regions of the cerebral cortex [20, 50], it is assumed that deficits in spatial learning in aged molarless mice are largely due to reduced afferent input from masticatory work and mastication-associated face and jaw movements to the cerebral cortex. It has been shown that chewing increases neuronal activity and blood flow rate in various cortical regions, including the primary sensory cortex [21], and that the suppressed behavioral and hippocampal responses in appetitive trace classic conditioning are paralleled by a reduced frequency of rhythmic jaw movement [22].

Astroglial Responsiveness Under the Molarless Condition

In the hippocampus, aging has been shown to cause an increase in astrocyte numbers [23, 24]; and glial fibrillary acidic protein (GFAP), produced as an intermediate filament protein [25], has been widely used to monitor astrocyte changes in response to neuronal degeneration and aging [26] and glial reactivity during aging [27]. Thus, it seems likely that reduced mastication may be involved in astroglial responsiveness in the hippocampus.

To evaluate the mechanism(s) responsible for senile impairment of cognitive function as a result of reduced mastication, we immunohistochemically examined the effects of the molarless condition on the hippocampal expression of GFAP and on spatial memory in young adult and aged SAMP8 mice [8]. Figure 3 shows the immunohistochemical results, which indicate that the molarless condition enhanced the age-dependent increase in the density and hypertrophy of GFAP-labeled

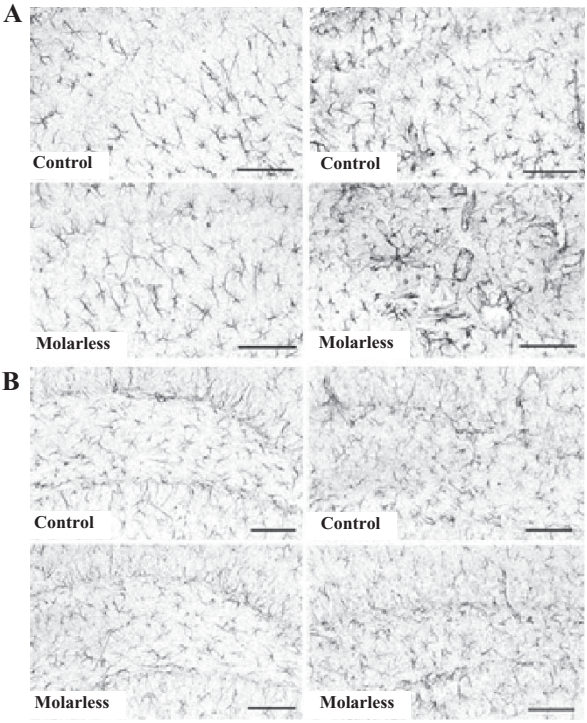

Fig. 3. Glial fibrillary acidic protein (GFAP) IHC in the CA1 region (**A**) and dentate gyrus (**B**) of control and molarless young adult (*left*) and aged (*right*) mice. Ten days after surgery, the mice began 7 days of testing in the water maze after which IHC was performed. *Bars* 150 μm. (From Onozuka et al. [8], with permission)

astrocytes in the CA1 region of the hippocampus. These effects increased as the molarless condition persisted [8]. When the extracellular K^+ concentration ($[K^+]_o$) was increased from 4 mM to 40 mM for hippocampal slices in vitro, the mean increase in the membrane potential was about 57 mV for fine, delicate astrocytes, the most frequently observed type of GFAP-positive cell in the young adult mice, and about 44 mV for the hypertrophic astrocytes of aged mice. However, there was no significant difference in resting membrane potential among these cell types. These data suggest that changes in astroglial responsiveness occur under the molarless condition in aged SAMP8 mice.

It has been suggested that the increased GFAP expression seen following deafferentation may reflect, in part, astrocyte responsiveness to changes in neuronal electrical activity [28]. For instance, Canady and Rubel demonstrated proliferation of GFAP-immunoreactive astrocytic processes in the chick cochlear nucleus following action potential blockade in the afferent nerve, indicating that neuronal activity may regulate the structure of astrocytic processes [29]. Furthermore, Rubel and MacDonald [30] have shown that an increase in GFAP-immunopositive and silver-impregnated glial processes in the chick nucleus magnocellularis occurs following cochlea removal; and they speculated that modulation of glial processes as a function of afferent activity may influence synaptic efficacy. Thus, the increase in GFAP-immunoreactive astrocytes in the CA1 region of molarless aged mice suggests a link between reduced sensory input and astroglial changes in the hippocampus.

Neuronal Degeneration in the Hippocampus Induced by the Molarless Condition

When dendritic spines in the hippocampus CA1 region of the SAMP8 mice were assessed using Golgi-Cox staining, pyramidal cells with apical and basal dendrites were seen in all mice, but the spine number was significantly decreased in aged molarless mice compared age-matched control mice (Fig. 4) [17]; this finding suggests the involvement of the molarless condition in an attenuation of input activities in hippocampal synapses. If this hypothesis is true, age-dependent neuronal cell death must be enhanced in the molarless condition. As expected, the age-dependent decrease in CA1 pyramidal neurons was enhanced by the molarless condition (Fig. 5) [8]. Quantitative analysis revealed that the percents of these neurons in the CA1 subfield of young, middle-aged, and aged molarless mice were 96.7%, 85.1%, and 77.8%, respectively, of that in age-matched control mice, indicating that a reduction in neuron numbers parallels the reduction in learning ability in a water maze test [5]. This implies that the reduction in hippocampal neuron numbers may be related to the impaired spatial memory seen in middle-aged and aged molarless mice. However, no significant difference in neuronal number was seen in either the CA3 subfield or the dentate gyrus between any of the groups.

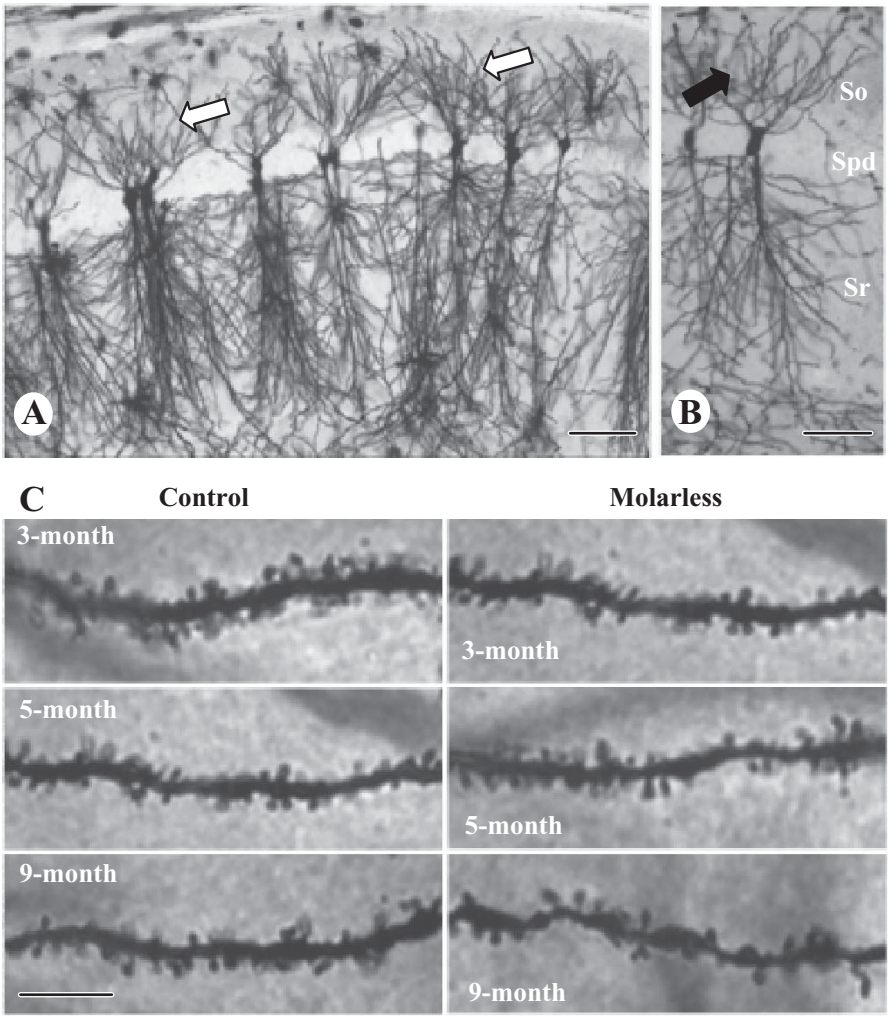

Fig. 4. Photomicrographs showing hippocampal CA1 pyramidal cells (**A**), a pyramidal cell (**B**), and dendritic spines in CA1 basal dendrites of control and molarless mice (**C**). *So*, stratum oriens; *Spd*, stratum pyramidale; *Sr*, stratum radiatum; *arrows*, basal dendrites. *Bars* 100 µm (**A**, **B**) and 10 µm (**C**). (From Kubo et al. [17], with permission)

Decrease in Septohippocampal Cholinergic Activities by the Molarless Condition

The cholinergic neuronal system in the hippocampus plays an important role in spatial cognition and undergoes a variety of age-dependent changes (reviewed in [31]). In rodents, hippocampal acetylcholine (ACh) release declines with age [17], and there is an age-dependent decline in memory function [32]. Furthermore,

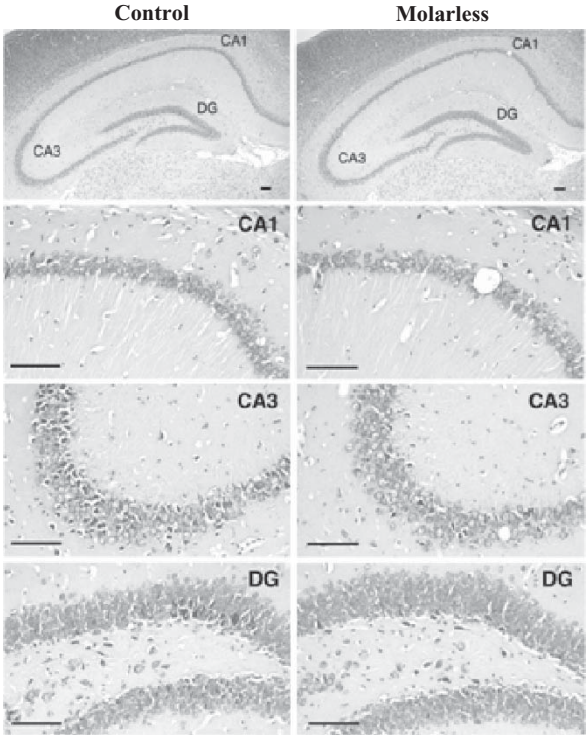

Fig. 5. Nissl staining of the hippocampal formation of a control (*left*) or molarless (*right*) SAMP8 mouse. *DG*, dentate gyrus. *Bars* 200 μm. (From Onozuka et al. [36], with permission)

the ACh-synthesizing enzyme choline acetyltransferase (ChAT) levels in the hippocampus decrease with age [33], and cholinergic neurons in the medial septal nucleus, projecting to the hippocampal formation [34], degenerate during aging [35].

In the experiments using microdialysis, biochemical, and immunohistochemical approaches in young adult and aged SAMP8 mice, we found that the molarless condition enhances a normal age-related decrease in the functioning of the septo-hippocampal cholinergic system, which may be linked to impaired learning ability measured in a water maze [8, 36]. In addition, KCl-evoked ACh release in the hippocampus of the aged molarless group was significantly less than that in age-matched molar-intact controls (Fig. 6) [7], implying that the molarless condition suppresses hippocampal ACh release in aged SAMP8 mice. In agreement with our results, it has been reported that hippocampal ACh release in response to depolarizing stimulation using high potassium concentrations is decreased in aged rats [37], suggesting that the molarless condition in aged SAMP8 mice may be involved in the development of age-related functional impairment of the cholinergic system in the hippocampus.

In our study, aged SAMP8 mice lacking molar teeth showed a significant reduction in the number of ChAT-positive neurons in the septal nucleus compared to age-matched molar-intact SAMP8 mice (Fig. 7) [7]. In contrast, the molarless

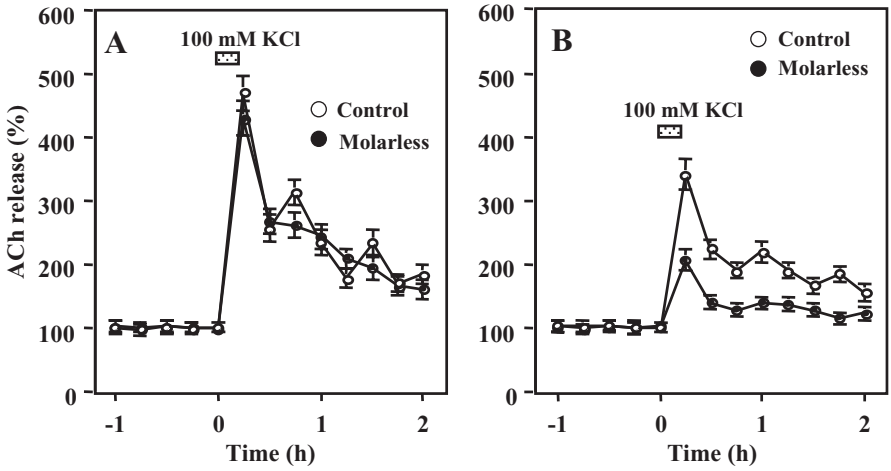

Fig. 6. Effect of the molarless condition on hippocampal acetylcholine (*ACh*) release. **A** Young adult. **B** Aged mice. Values are the mean (percent of the basal level) ± SE ($n = 5$ for each group). Basal levels were defined as the average value for the four samples taken before KCl perfusion. (From Onozuka et al. [7], with permission)

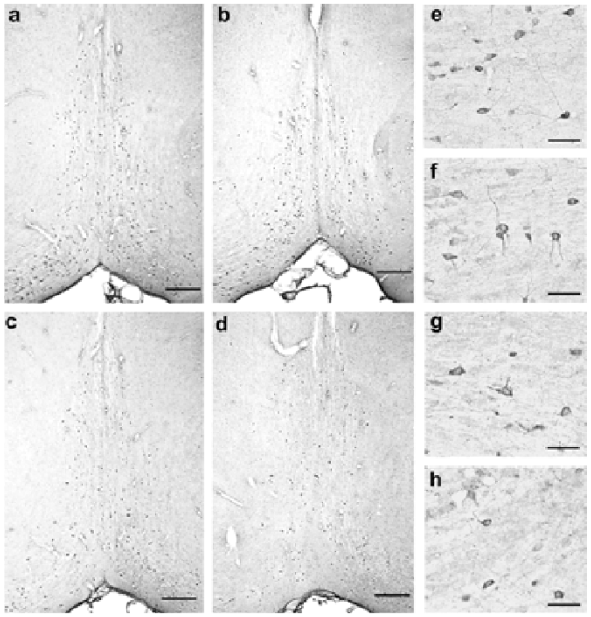

Fig. 7. Choline acetyltransferase (ChAT)-positive cells in the basal forebrain. **a**, **e** Young adult control. **b**, **f** Young adult molarless mouse. **c**, **g** Aged control. **d**, **h** Aged molarless mouse. *Bars* 200 μm (**a**–**d**) and 50 μm (**e**–**h**). (From Onozuka et al. [7], with permission)

condition had no effect on the number of ChAT-positive neurons in the vertical limb of the diagonal band of Broca (vDBB). During aging, various degenerative changes in basal forebrain cholinergic neurons have been shown in experimental animals [38, 39]. Lee et al. [40] reported a significant decrease in the number of septohippocampal cholinergic neurons in aged animals. The hippocampus is supplied by cholinergic fibers arising from the medial septal nucleus and ending on pyramidal neurons of the hippocampus and granule neurons of the dentate gyrus [41]. Taken together with the fact that an age-related impairment of cholinergic neurons in the medial septal nucleus is associated with cognitive impairment [41], it is likely that, in molarless aged SAMP8 mice, the reduced number of cholinergic neurons in the medial septal nucleus is related to impaired spatial memory [7].

Glucocorticoid Response to the Molarless Condition

Previous studies have shown that basal plasma corticosterone levels in aged rats correlate significantly with hippocampal degeneration and spatial learning deficits [23, 42]. Elevated plasma corticosterone levels are found only in aged rats with spatial memory deficits and not in those with normal spatial memory [43]. Cumulative exposure to high glucocorticoid levels throughout life disrupts electrophysiological function, leading to atrophy and ultimately the death of hippocampal neurons, all of which can cause severe cognitive deficits in hippocampus-dependent learning and memory [44]. Combined with the fact that hippocampal neuronal loss occurs in both patients with symptoms of senile dementia [45] and animals with senile deficits of cognitive function [44, 45] , it is conceivable that the molarless condition-induced deficits in spatial learning and hippocampal neurons seen in aged SAMP8 may be due to the damaging effects of glucocorticoid.

As shown in Fig. 8, the corticosterone levels showed significant circadian variation, peaking in both groups at the onset of the dark period (i.e., 8 p.m.). However,

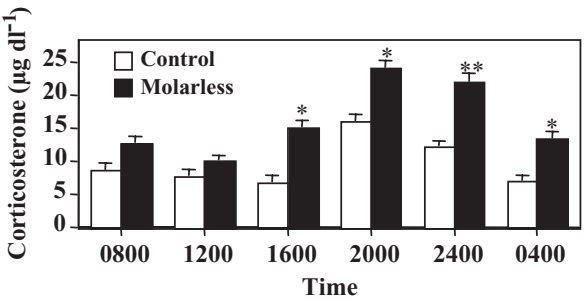

Fig. 8. Effects of the molarless condition on plasma corticosterone levels in aged SAMP8 mice. Mean ± SE plasma corticosterone levels in control and molarless mice ($n = 4$ for each column) at various times over a 24-h cycle. *$P < 0.05$ compared with controls; **$P < 0.01$ compared with controls. (From Onozuka et al. [47], with permission)

at all time points, the molarless group had significantly higher plasma corticosterone levels, indicating that the molarless condition causes increased plasma corticosterone levels. This finding was similar to that reported in a previous study [46] in showing a peak at the beginning of the dark period, when activity is generally greatest, and the lowest levels near the end of the dark period or the beginning of the light period, when rodents are least active. However, the molarless group had overall higher corticosterone levels than the control group, indicating that the molarless condition results in increased exposure to corticosterone and suggesting impaired hypothalamic-pituitary-adrenal negative feedback inhibition in molarless mice.

We next wanted to assess the effect of the corticosterone synthesis inhibitor metyrapone on the molarless condition-induced increase in corticosterone levels and reduction of CA1 neurons. Therefore, 1 day before the operation and every 2 days until the collection of blood, molarless mice received an injection of this inhibitor at a dosage known to inhibit the stress-induced rise in plasma corticosterone levels and hippocampal neuronal damage [48]. Ten days after the operation, we measured plasma corticosterone levels in the control (n = 10), molarless, and metyrapone-treated molarless groups. As shown in Fig. 9, it suppressed the molarless condition-induced increase in plasma corticosterone levels (a, in Fig. 9) but had no significant effect on plasma corticosterone levels in control mice [49]. Also, metyrapone prevented the molarless-induced reduction in neuronal number in this subfield (b and c, in Fig. 9), as no significant difference in the number was seen between vehicle-injected control mice and metyrapone-injected molarless mice. Furthermore, metyrapone prevented the increase in escape latency in the water maze test induced by the molarless condition, as a difference in latency was seen between metyrapone-injected molarless mice and vehicle-injected molarless mice but not between metyrapone-injected molarless mice and vehicle-injected control mice (d and e, in Fig. 9), implying that the molarless-induced deficits in spatial learning and hippocampal neuron numbers in aged SAMP8 mice may be related to exposure to increased corticosterone levels. Together with the observations that the adrenals of molarless aged SAMP8 mice are heavier than those of age-matched molar-intact control mice [8] and that increased mastication reduces the plasma corticosterone response during novelty exposure in mice [49], a prolonged stressful response to the molarless condition and the consequent increase in exposure to corticosterone could hasten hippocampal neuron damage in this species.

Conclusion

Based on the above findings, we propose that the reduced ability to masticate in aged SAMP8 mice induces deficits in spatial cognitive memory. The mechanism(s) underlying this phenomenon are as follows: (1) a decrease in input activities in the hippocampus; (2) degeneration of hippocampal neurons; (3) a decrease in the

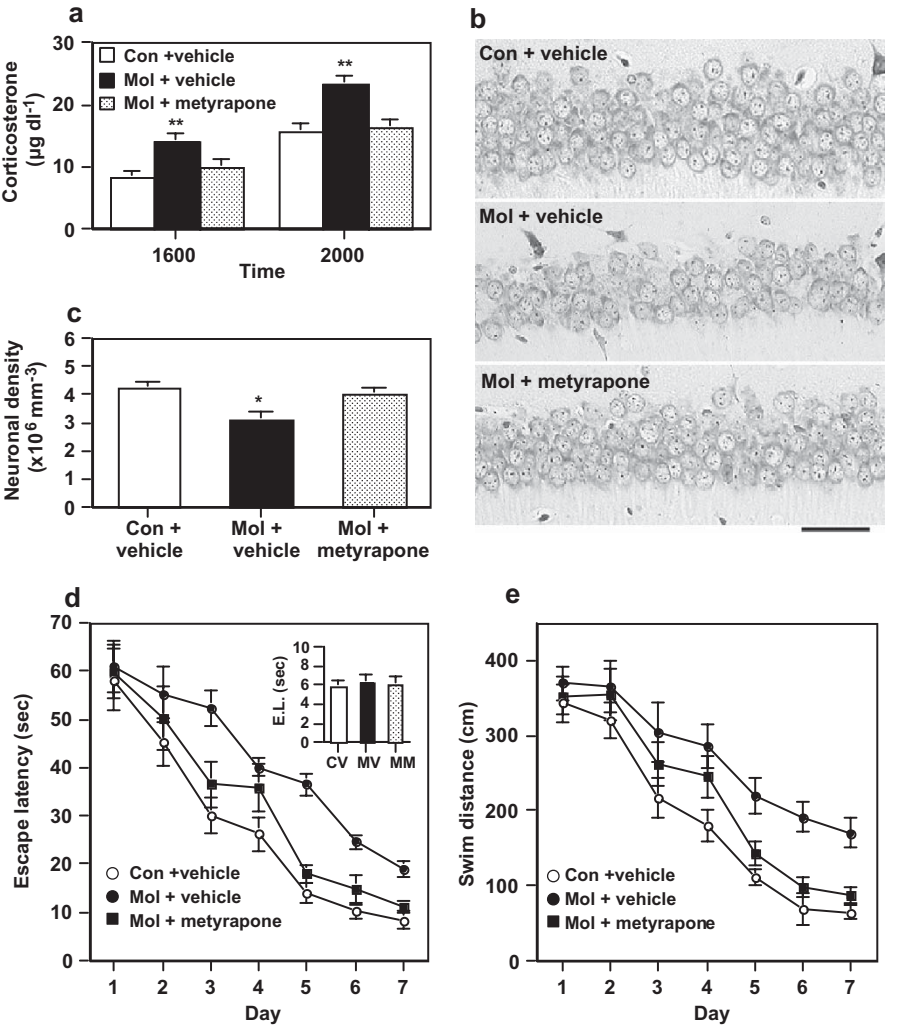

Fig. 9. Effects of metyrapone. **a** Mean ± SE (n = 10 for each column) plasma corticosterone levels in vehicle-injected control, vehicle-injected molarless, and metyrapone-injected molarless groups at 1600 and 2000 h (4 p.m. and 8 p.m.) $^{**}P < 0.01$ compared with vehicle-injected controls. **b** Representative photomicrographs of cresyl violet-stained sections showing the CA1 cell field in the control (*top*), molarless (*middle*) and metyrapone-treated molarless (*bottom*) groups. *Bar* 50 μm. **c** Mean ± SE (n = 7 for each column) neuron density in the CA1 pyramidal cell field in vehicle-injected control, vehicle-injected molarless, and metyrapone-injected molarless groups (n = 7 animals per group). $^{*}P < 0.05$. **d**, **e** Spatial learning in a water maze test. Ten days after surgery, the learning test was started. Mean ± SE (n = 10 for each group) latency (**d**) or swim distance (**e**) to locate a submerged platform in a Morris swim maze in vehicle-injected control, vehicle-injected molarless, and metyrapone-injected molarless groups. **Inset** Visible probe test. At the end of the maze test, animals performed a visible probe test. *Con*, molar-intact control group; *Mol*, molarless group; *E.L.*, escape latency; *CV*, vehicle-injected control group; *MV*, vehicle-injected molarless group; *MM*, metyrapone-injected molarless group. There was no significant difference between groups. (From Onozuka et al. [47], with permission)

septohippocampal cholinergic networks; and (4) involvement of the glucocorticoid response in the hippocampus. Therefore, we strongly suggest that dysfunctional mastication is one of the risk factors for senile dementia.

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