Age-Specific Peculiarities of Formation of Long-Term Posttetanic Potentiation in OXYS Rats

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OXYS rats with hereditary hyperproduction of active oxidative radicals and early disorders in the mitochondrial structure and functions are an interesting model for studies of age-specific features of synaptic plasticity. The formation of long-term posttetanic potentiation in the mossy fibers–CA3 pyramidal neuron system were studied in hippocampal slices from Wistar and OXYS rats aged 3 and 4.5 months (young), 11 (middle-aged), and 18 months (old). No appreciable age-related differences were detected in the amplitudes and latencies of stimulatory postsynaptic summary potentials of the mossy synapses evoked by test stimuli in Wistar and OXYS rat groups of different age and between the two strains. The capacity to induction and formation of long-term posttetanic potentiation and its value decreased in 18-month-old Wistar rats, which attested to disorders in synaptic plasticity of old animals. The capacity to induction and formation of long-term posttetanic potentiation and its value in OXYS were lower than Wistar rats of the same age in all the studied groups.

Key Words: *OXYS rats; hippocampus; long-term posttetanic potentiation*

Age-specific changes in synaptic plasticity underlie disorders in cognitive function during aging. Changes in neurons and neuronal network associated with aging are not simultaneous in different brain structures [14]. The main processes determining them are loss of mitochondrial functional activity, accumulation of active oxidative radicals, and disorders in intracellular calcium homeostasis [13].

Hyperproduction of free radicals and progressive disorders in mitochondrial structure and functions are characteristic of OXYS rats [7]. A significant reduction of the research and exploratory activity, increase of anxiety, disorders of learning capacity are detected in these animals starting from the age of 3-4 months [7]. Animals of this strain seem to be an interesting object for studies of age-specific changes in synaptic plasticity.

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Long-term posttetanic potentiation (LTP) in the hippocampus (structure directly involved in learning and memory processes) is the main model for studies of cellular molecular mechanisms of plasticity in health, during natural aging, and various diseases [9].

We studied the formation of LTP in hippocampal mossy fibers in Wistar and OXYS rats of different age.

MATERIALS AND METHODS

Experiments were carried out on male Wistar and OXYS rats (Laboratory of Experimental Animals, Institute of Cytology and Genetics, Novosibirsk) of three age groups: young (3 and 4.5 months: 12 and 9 OXYS, 11 and 9 Wistar rats), middle-aged (11 months: 12 OXYS and 10 Wistar rats), and old animals (18 months: 10 OXYS and 10 Wistar rats). The rats were kept 2 per cage at 12:12 day:night regimen on standard combined ration with free access to water. All experimental procedures were carried out in accordance with Regulations on Studies on Experimental Animals.

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Transverse hippocampal slices (400 μ) were incubated in a thermcontrolled (32-35°C) flow chamber with medium of the following composition (in mM): 124 NaCl, 4.9 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 25.6 $NaHCO_3$, and 10 D-glucose (pH 7.5) aerated with carbogen (95% O_2, 5% CO_2) for at least 40 min before the experiment for adaptation to *in vitro* conditions.

The summary excitatory postsynaptic potentials (EPSP) of mossy fibers were recorded and tetanization procedure was carried out as described previously [1]. Changes in the amplitude of the summary EPSP after tetanization were calculated by the formula $(A_t-A_0)\times100\%$, where A_0 and A_t were the response amplitudes to the test stimulus before and after tetanization, respectively.

Statistical processing of the results was carried out and the mean value of the studied parameter and the standard error in the mean (*M*±*SEM*) were estimated. The significance of differences in the means was evaluated by Student's *t* test and Mann–Whitney's test using OriginPro 8.1 and Statistica 9 software. The differences between the groups were considered significant at $p\leq0.05$.

RESULTS

The age groups were formed with consideration for the time of manifestation of behavioral and learning disorders and for the shorter life span of OXYS rats (36.8±5.0 months in Wistar and 24.6±6.0 months in OXYS rats).

The amplitude and latency of mossy synapse responses evoked by the test stimuli virtually did not differ in OXYS and Wistar rats and did not change with age in each group of animals.

The probability of potentiation induction in OXYS was lower that in Wistar rats in all the studied age groups. In Wistar rats, the induction capacity decreased only at the age of 18 months (Table 1). Induction caused the formation of only short-term potentiation and did not lead to the development of LTP in a greater number of OXYS *vs.* Wistar rats (Table 2).

The mean amplitudes of mossy synapse summary EPSP 60 min after tetanization virtually did not differ in young and middle-aged Wistar rats, though a trend to an increase of the LTP amplitude with age was noted (Fig. 1). In the oldest age group, the amplitude of the evoked response decreased significantly ($p=0.02$) in comparison with 11-month-old animals.

The mean amplitudes of summary EPSP were lower in the hippocampal slices from OXYS rats after tetanization in all age groups in comparison with those in Wistar rats of similar age (Fig. 1), but the differences were significant only at the age of 4.5 and 11 months ($p=0.09$ at 3 and 18 months).

On the other hand, the dynamics of age-specific changes in the mossy synapse LTP was different in OXYS and Wistar rats. The probability of induction and LTP formation in general and its value decreased at the age of 18 months in Wistar and OXYS rats. That could be referred to age-associated disorders in synaptic plasticity in the studied system of synaptic contacts. On the other hand, certain improvement of the parameters of LTP formation was observed in middle-aged OXYS rats in comparison with animals aged 4.5 months, which could indicate the involvement of compensatory mechanisms supporting synaptic plasticity.

Age-associated changes in plasticity in different synaptic systems of the hippocampus differed by time of development and by the mechanisms. The age-associated changes in synapses of the medial perforant pathway on CA3 pyramidal neurons did not involve induction of NMDA-dependent potentiation, but shortened the late LTP phase [4], while in Schaffer collaterals of old rats the LTP induction was disordered [5]. A possible mechanism of age-associated changes has been described for the best studied synaptic relationship system (Schaffer collaterals–CA1 pyramidal neurons) [5]. The contribution of NMDA glutamate receptors to the formation of LTP was reduced in old animals, Ca entry through the voltage-dependent

TABLE 1. Disorders in Mossy Fiber LTP Induction in Rat Hippocampal Sections (% of Examined Slices)

Animal age, months	OXYS	Wistar
3	22.7 (*n*=22)	11.7 (*n*=17)
4.5	22.2 (*n*=18)	12.3 (*n*=16)
11	21.7 (*n*=20)	10.0 (*n*=20)
18	25.0 (*n*=20)	18.8 (*n*=19)

Note. Here and in Table 2: *n*: number of examined slices.

TABLE 2. Formation of Mossy Fiber LTP in the Rat Hippocampal Slices

Rat age, months	OXYS	Wistar
3	59.6 (*n*=22)	81.6 (*n*=17)
4.5	58.4 (*n*=18)	79.1 (*n*=16)
11	65.8 (*n*=20)	83.4 (*n*=20)
18	37.5 (*n*=20)	68.7 (*n*=19)

Note. The LTP was assumed to have formed if 60-80 min after the second tetanization the amplitude of summary EPSP in response to the test stimulus surpassed the response before tetanization by 50% and more. The data are presented as % of slices with LTP.

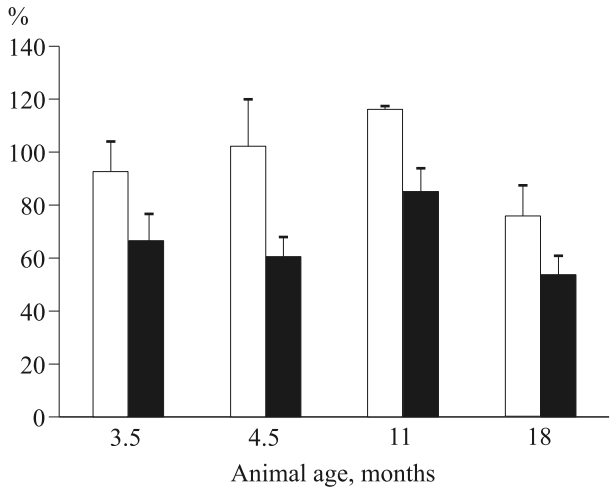

Fig. 1. Changes in EPSP amplitude 60 min after tetanization in Wistar (light bars) and OXYS (dark bars) rats. Significant ($p \leq 0.05$) differences between OXYS and Wistar for age groups of 4.5 and 11 months; between OXYS rats aged 4.5 and 11 months, 11 and 18 months; between Wistar rats aged 11 and 18 months.

L-type Ca channels and from intracellular depots increased, which led to an increase in the amplitude and duration of trace hyperpolarization and to disorders in LTP induction. L-type Ca channel blockers improved learning in old animals [10]. On the other hand, study of LTP in old rats with disordered or retained learning ability showed that though the NMDA-dependent potentiation was reduced in both groups, LTP mediated through L-type Ca channels was significantly higher in group 2 [3]. Hence, stimulation of Ca channels can play an adaptive role in age-specific modification of plasticity; importantly that the enhanced Ca entry through these channels and increased intracellular Ca content were detected already in middle-aged (12-15 months) rats [11].

Disorders in induction and values of mossy synapse LTP were detected in 18-month-old animals in our study. Age-specific changes in synaptic plasticity in this system of relationships have never been described. Mossy fiber LTP are largely supported by the presynaptic mechanisms. Glutamate release by mossy synapses in old is reduced compared to that in young and middle-aged animals. Proximal dendrites of pyramidal CA3 neurons are characterized by high density of voltage-dependent L-type Ca channels, which suggests their involvement in age-specific modification of synaptic plasticity; this hypothesis should be verified in special studies.

Active oxygen radicals are an obligatory component of normal LTP, but they act as toxic agents in age-associated disorders [12]. Increased level of active radicals in hippocampal neurons stimulates calcium release from depots [6], increases its entry through voltage-dependent L-type Ca channels [2], and reduces activities of ionic channels coupled with NMDA receptors [8]. Impaired induction and formation of LTP and its reduced value were found in OXYS rats, characterized by hereditary hyperproduction of active radicals. These data are in good agreement with the concepts on the role of active radicals in age-specific changes in synaptic plasticity. On the other hand, the dynamics of changes in LTP characteristics in this rat strain attests to possibility of compensation; the mechanisms of this compensation are particularly interesting for the search for methods correcting age-specific disorders.

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