GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Increase in Slow Afterhyperpolarization Led to Learning Delay in DBA mice

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We showed that differences in learning capacity between DBA and C57Bl/6 mice correlates with differences in slow afterhyperpolarization amplitude in hippocampal CA1 pyramid neurons. In DBA mice learning capacity is lower, but the amplitude of slow afterhyperpolarizations higher than in C57Bl/6 mice.

Key Words: *DBA and C57Bl/6 mice; slow neuronal afterhyperpolarization; long-term potentiation of synaptic transmission*

According to modern views, two major cellular mechanisms underlie learning and memory function: synaptic plasticity (long-term potentiation, LTP) [2,10,15] and the phenomenon of plasticity of neuronal excitability regulated by slow afterhyperpolarization (sAHP) [4-6,13]. Learning and memory deficits observed during aging are associated with impairment of these two mechanisms [1,3,11,14].

Behavioral experiments on mice showed that different mouse strains exhibited different learning and memory abilities. For example, DBA mice are characterized by poor learning in Morris water maze compared to C57Bl/6 mice [7,8], but demonstrate much better performance in some other behavioral paradigms [14]. In the present study we tested young (3-month-old) DBA and C57Bl/6 mice in Morris water maze task and examined synaptic plasticity and slow afterhyperpolarization in hippocampal CA1 neurons.

MATERIALS AND METHODS

First, we confirmed that DBA mice have specific impairment in learning the water maze task. Second, we found that short-term LTP was increased in DBA mice, compared to C57Bl/6 , while the long-term LTP was the same in both strains. Third, sAHP amplitude was significantly increased in DBA mice. These data suggests that the increase in sAHP accompanied by decrease in neuronal excitability can underlie the delay in learning of DBA mice.

The mice were trained to find an invisible platform in Morris water maze (1.5-m round pool with round 14.5-cm escape platform located in the center of one of pool quadrants 2 cm below the water surface). The animals were given 21 trials over 7 consecutive days (three trials with 20 min intervals; 120 sec maximum trial duration). During the last trial the platform was pneumatically lowered out of reach of the mice for 1 min and then returned to its original position. The number of crossings of the exact position of the platform was used to assess test performance. Latencies of finding hidden platform during learning and the number of crossings of the platform location during testing were recorded and analyzed using a computer-based tracking system (San Diego Instruments, San Diego, CA).

RESULTS

During first three days DBA mice learned to find the platform much slower than C57Bl/6 (day 2: 16.8±1.9 and 35.1±4.9 sec, for C57Bl/6 and DBA, respectively,

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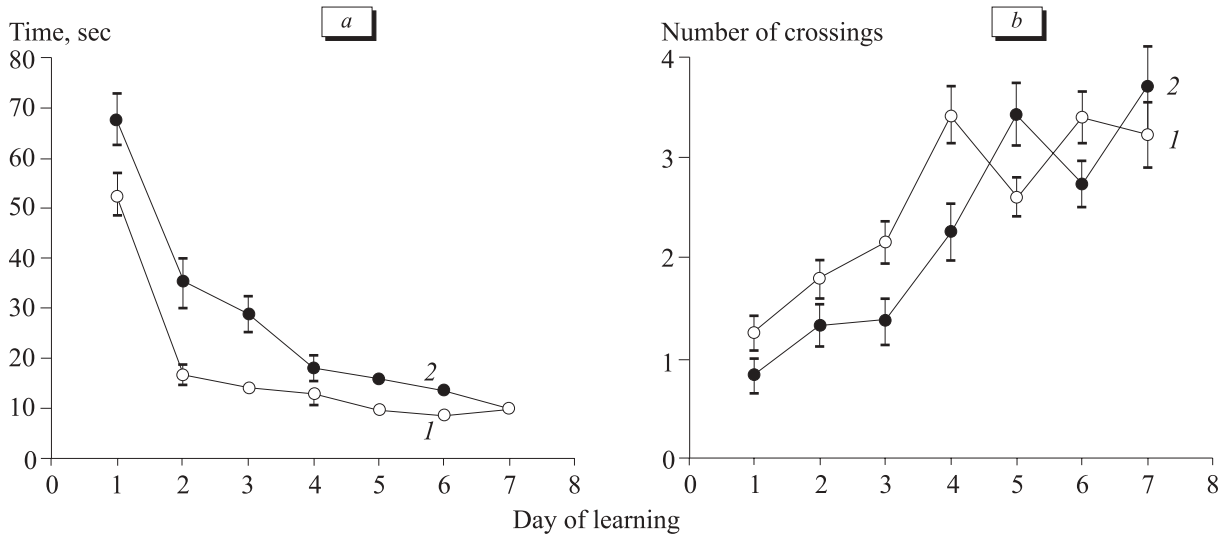

Fig. 1. Performance of young C57Bl/6 (*1*, *n*=43) and DBA (*2*, *n*=30) mice in Morris water task. *a*) latency of finding the platform; *b*) number of crossings of the platform location.

$p<0.001$; day 3: 14.0±1.6 and 29.1±3.6 sec, $p<0.001$; Fig.1). Starting from day 4 the time of finding the platform was similar in C57Bl/6 and DBA mice (12.9±2.0 and 18.0±2.5 sec, $p=0.11$, *t* test). C57Bl/6 and DBA mice also demonstrated initially different number of crossings of the zone around the platform center. For C57Bl/6 mice the number of crossing gradually increased during the first three training days and reached a plateau on day 4 (3.4±0.3 crossings). In DBA mice this parameter reached a plateau on day 5 (3.4±0.3 crossings). On day 4 of training in number of crossings significantly differed in C57Bl/6 and DBA mice (3.4±0.3 and 2.3±0.3 sec, respectively, $p<0.001$). These findings suggest that impaired learning in DBA is determined by deficiency in searching strategy leading to a delay in spatial learning.

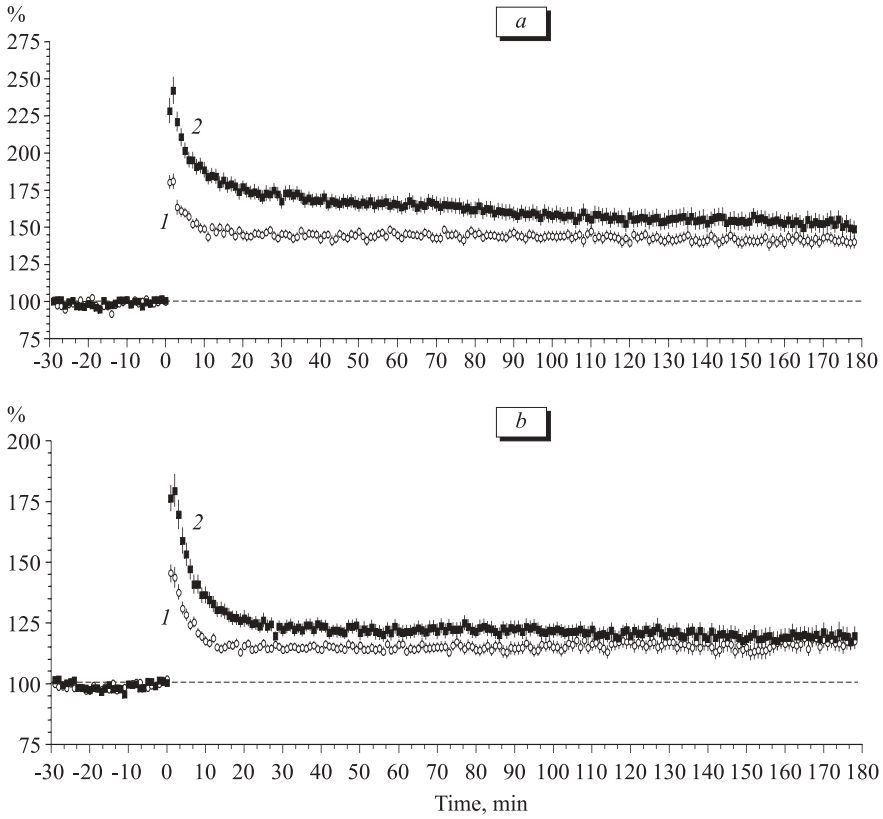

Fig. 2. Increase in synaptic transmission after tetanic stimulation in C57Bl/6 (*1*, *n*=31) and DBA (*2*, *n*=27) mice. *a*) 10 theta bursts (4 stimuli with 100 Hz frequency, 10 bursts with 200 msec interval) tetanic stimulation; *b*) 2 theta bursts (4 stimuli with 100 Hz frequency, 2 bursts with 200 msec interval) tetanic stimulation.

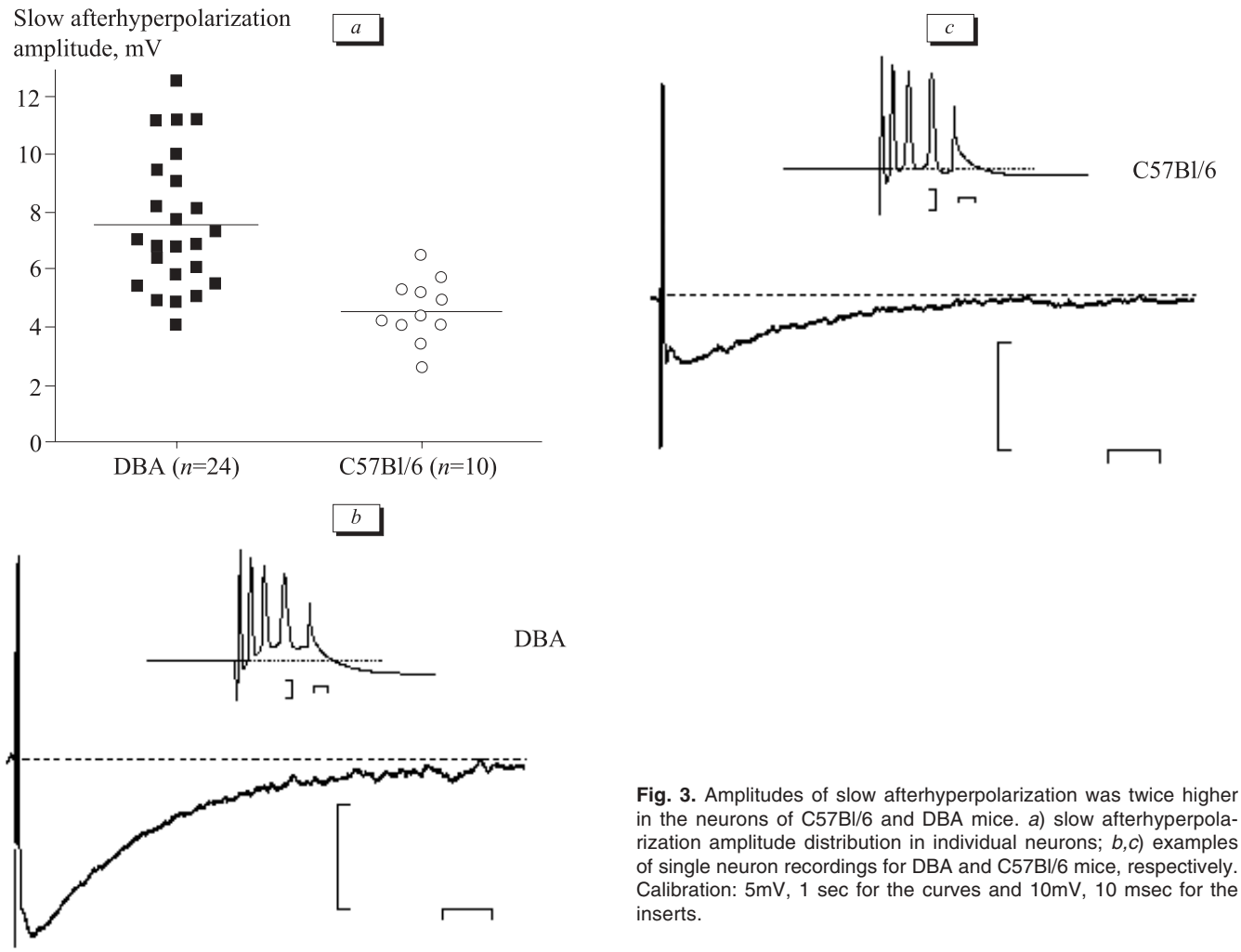

Fig. 3. Amplitudes of slow afterhyperpolarization was twice higher in the neurons of C57Bl/6 and DBA mice. *a*) slow afterhyperpolarization amplitude distribution in individual neurons; *b,c*) examples of single neuron recordings for DBA and C57Bl/6 mice, respectively. Calibration: 5mV, 1 sec for the curves and 10mV, 10 msec for the inserts.

In order to answer the question whether this deficiency results from impairment of synaptic plasticity, we recorded field potentials from CA1 hippocampal region and induced LTP by tetanic stimulation of afferent pathways (by two different protocols, Fig. 2). We showed that short-term (1 min and 1 hour) increase in the amplitude of field potentials after tetanization was higher in DBA mice (1 min after tetanus: 146±4 and 177±6% for C57Bl/6 and DBA mice, respectively, $p<0.001$, and 1 hour after tetanus 145±3 and 166±6% for C57Bl/6 and DBA mice, respectively, $p<0.01$), while long-term increase (3 h after tetanus) was almost the same for both strains (140±4 and 149±6% for C57Bl/6 and DBA mice, respectively, $p=0.24$). We also used less intensive tetanization paradigm (4+4 theta bursts) for LTP induction and found similar differences in potentiation between these mouse strains (Fig.2, *b*). Thus, we excluded the possibility that the decrease in synaptic plasticity underlies learning impairment in DBA mice.

The next step of our study was intracellular recording of slow afterhyperpolarization in hippocampal pyramidal CA1 neurons. We used standard blind neuron search technique. Glass electrodes were filled with the following solution: $KMeSO_3$ 100 mM; NaCl 20 mM. Electrode resistance was 9-11 MΩ. When the neuron was opened, membrane potential was about -58-61 mV and membrane resistance 100-120 MΩ. The amplitude of slow afterhyperpolariztion increased and stabilized within the first 3-5 min of recording. Measurements taken during this period showed a significant difference in slow afterhyperpolarization amplitude between mouse strains (Fig. 3). In DBA mice slow afterhyperpolarization was 5-12 mV (7.5±0.5 mV), while in C57Bl/6 it was only 3-5 mV (4.5±0.3 mV, $p<0.001$). There were no differences in membrane potentials or membrane resistance.

Our experiments showed that young DBA mice have specific learning impairment in Morris water maze during the first days of training. This impairment is determined by a deficiency in searching strategy, rather than in spatial learning. Additional days of learning allowed DBA mice to remember the platform position for the successful task performance.

This is consistent with another works in this field, suggesting that during first days of learning the mice rely more on the search strategy than on spatial cues [15]. The only difference we found in electrophysiological study was significant increase in slow afterhyperpolarization amplitude in DBA mice, which can result from decreased neuronal excitability. It is important to notice that the increase in slow afterhyperpolarization amplitude in DBA mice did not result in decrease of synaptic plasticity, caused both by strong (4×10 tetani) and weak (4+4) tetanic stimulation. A possible explanations of this discrepancy is different localization of both processes: soma of the neuron for the slow afterhyperpolarization and synapse for LTP.

These findings suggest that neuronal excitability regulated by slow afterhyperpolarization underlies the processes of collecting and retaining information and plays a distinct role in learning and memory. According to published data, the decrease in neuronal excitability due to increased slow afterhyperpolarization can be a cause of memory and learning deficiency in aged animals [3,11,14]. Thus DBA mice, shown to have increased sAHP amplitude, could serve as a possible model for investigating learning and memory deficiency, described in aged animals.

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