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Activity Patterns in Neurons in the Retrosplenial Area of the Cortex in Operant Food-Procuring Behavior in Rats of Different Ages

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Neuron spike activity was recorded in the retrosplenial area of the cortex during execution of acquired cyclic operant food-procuring behavior (COFPB) in adult (8–12 months) and elderly (20–27 months) Long-Evans rats. As compared with adult rats, elderly animals showed a significant decrease in the proportion of neurons specialized for COFPB. The normalized discharge frequency of all neurons in elderly animals during execution of basic food-procuring acts was significantly greater than that in adults. Elderly rats showed significantly fewer pairs of acts with significant differences in discharge frequency than adults, indicating that neuron activity on execution of COFPB was more uniform. These data indicate that in old age, learning involves less "extension" of existing experience due to formation of new neuronal specializations than in earlier stages of an individual's life and that the internal system structure of the newly formed behavior is more "homogeneous."

Keywords: aging, rats, individual neuron activity, neuron specialization, behavioral act, system, retrosplenial cortex.

Aging in humans and animals is accompanied by changes in brain activity both at rest and on execution of a wide range of tasks [Sala-Llonch et al., 2015]. Thus, hippocampal "place" cells have been seen to be less stably activated in old animals in their spatial "field" than in adults (instability) [Barnes et al., 1997; Wilson et al., 2004] or to maintain local activation when the external context changes (rigidity) [Wilson et al., 2004]. Aging can involve impairment to the formation of the olfactory selectivity of neurons in the orbitofrontal area of the cortex on learning and its loss on retraining [Schoenbaum et al., 2006].

One of the areas in which age-related morphofunctional changes can be seen even before behavioral and cognitive impairments are detectable is the retrosplenial cortex (RC) [Huijbers et al., 2012], which is also active on reproduction of long-term memories in humans [Huijbers et al., 2012] and animals [Miller et al., 2014]. Experiments with recording of RC neuron activity in adult rabbits and rats showed that from 45% to 55% of all cells have specific activation patterns (in all performances of specific behavioral acts) [Alexandrov et al., 1999; Gorkin and Shevchenko, 1991; Gavrilov et al., 1998]. Among them, up to 30% of neurons (depending on the number of acts learned) were specialized only for relatively new acts formed in the experimental cage on training to this behavior [Gorkin and Shevchenko, 1991; Gavrilov et al., 1998; Kuzina et al., 2015], while others were activated on execution of defined movements or their sequences (independently of the act in which the movement is carried out) [Alexandrov et al., 1990; Tabuchi et al., 2005]. As adult individuals demonstrated a significant role for the RC in forming new behavior (see above) and views on the characteristics of its activation on solution of novel tasks in old age are limited to data obtained using noninva-

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sive methods, identification of the characteristics of the neuronal support for cyclic operant food-procuring behavior (COFPB) in old age was sought by recording spike activity from individual RC neurons in elderly and adult rats during execution of learned behavior.

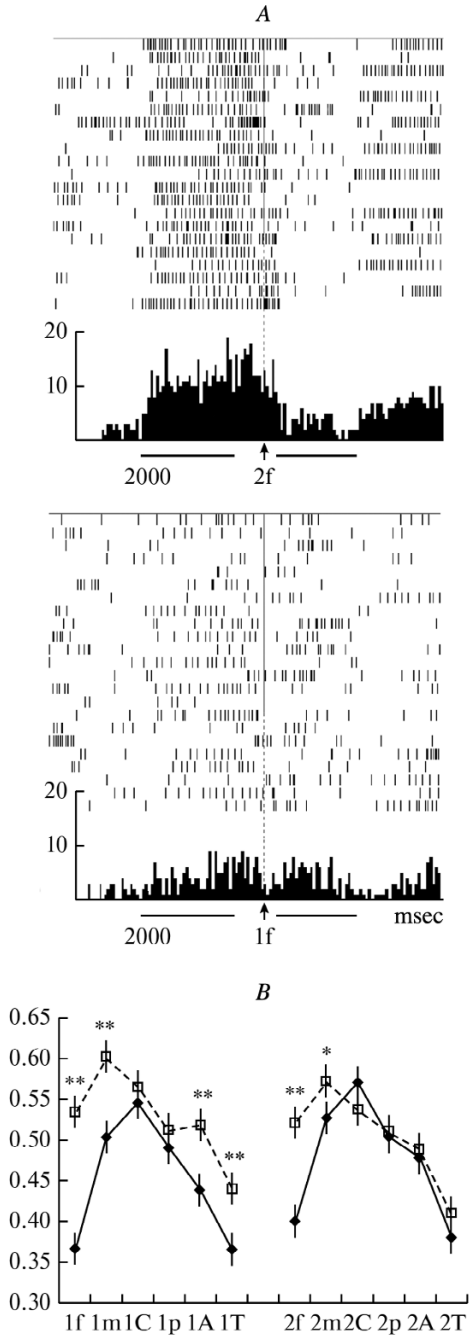
Methods. All experiments were performed in compliance with European Community Directive No. 86/609 EEC of November 24, 1986 on the Humanitarian Treatment of Experimental Animals. Studies used Long-Evans rats weighing 230–280 g at age 8–12 months ("adult" group, $n = 4$) and 20–27 months ("old" group, $n = 4$). During training and experiments, animals were placed in individual cages and were kept in conditions of partial food deprivation. Weight loss throughout the study period was by no more than 15%. In the experimental cage, different corners of which were fitted with two feeders and two pedals, the animals underwent stepwise training to press the pedal to obtain cheese from the feeder. After forming behavior on the first side of the cell, animals were trained to the same behavior on the other side for 1–2 sessions. Groups of adult and old animals were balanced in terms of the initial training side. Neuron activity was recorded in animals of both groups with the acquired food-procuring behavior on both sides of the cage. Surgery to attach a micromanipulator platform and to implant ground and reference electrodes was performed as described previously [Kuzina et al., 2015]. The activity of individual neurons was recorded using microelectrodes with impedance of 2–7 MΩ at a frequency of 1 kHz. In parallel with recording of neuron activity in the RC, video recordings were made, with behavioral markers for visits to the feeders and pressing the pedals. After experiments were complete, recording sites were reconstructed morphologically. Neuron activity and behavioral characteristics were recorded using DMain (written by D. Raigorodskii) and Neuru by A. Krylov) software.

Five types of act were identified in the behavior on each side of the cage, in accordance with the stages of learning, which together made up a single food-procuring cycle: placing of the animal's snout on the food-containing feeder (f), elevation of the head from the feeder and turning to the middle of the side of the cage (m), approach to the pedal corner (C), pressing the pedal (p), and returning from the pedal to the feeder (A). The act of placing the animal's snout in an empty feeder (T) was also identified. Analysis of behavior on each side of the cage assessed the mean duration of cycle execution and the ratio of the number of check visits to the feeders to the number of productive visits in each neuron spike activity recording session in which empty feeders were checked.

For each neuron, the mean activity frequency throughout the recording period was determined. Activation of one or several acts was identified in terms of the activity frequency during these acts being at least 1.5 times the mean in the other behavioral acts. Cells which were "specialized" in relation to a particular system for one or another act in the repertoire were identified in the present study as those showing activation during all performances of the act or group of acts [Gorkin and Shevchenko, 1991; Gavrilov et al., 1998; Aleksandrov et al., 2014]. Activity patterns (mean spike frequency distributions) in the acts of the behavioral cycle were constructed for all the neurons recorded, and mean activity frequencies were determined throughout the recording period. Thus, activity patterns were described by the distribution of 12 mean activity frequencies in individual acts normalized with respect to the highest of their frequencies. Averaged activity patterns for all the neurons recorded were determined for both groups of animals. The set of all neurons was also used for comparing patterns in adult and old rats for individual analogous acts, such as the approach and pedal pressing in the first and second food-procuring cycles or the approach and climbing into the first or second feeder. Pairwise comparisons of normalized activity frequencies in all combinations of pairs of acts of the food-procuring cycle (apart from empty feeder checking acts) on both sides were made within groups to determine the level of differentiation of the overall activity pattern. Relative numbers of specialized neurons in the sets in old and adult animals were also compared. All statistical computations were run in SPSS 15.0 (SPSS Inc., USA).

Results and Discussion. ***Behavior.*** There were no significant differences between old and adult rats in the mean duration of performance of the food-procuring cycle on the first and second sides of the experimental cage in the training sequence (mean cycle time in old rats was 4.64 ± 1 sec on the first side, 5.17 ± 1.37 sec on the second; values in adults were 4.54 ± 1.59 sec on the first side and 5.14 ± 2 sec on the second, Mann–Whitney test (M–W), Z = 1.05–0.92, $p = 0.29$–0.35). However, old rats checked empty feeders significantly more often than adult rats (the mean ratios of check visits to productive visits in old rats were 40.7% for the first feeder and 33.3% for the second; in adults, values were 25% and 29.8%, respectively, M–W, Z = –8.379, $p < 0.001$ for the first side, Z = –4.179, $p < 0.001$ for the second). Similar results were obtained in two comparable studies, which showed that old rats spent longer at feeders than young rats [Caetano et al., 2012; Samson et al., 2014]. This feature evidently helped old animals to rearrange their behavior more quickly when its productivity was lost [Samson et al., 2014].

Spike activity of RC neurons during behavior. We recorded the activity of 241 neurons in four adult rats and 268 neurons in four elderly rats. Comparison of mean neuron spike frequencies during food-procuring behavior identified a significantly higher level of activity in RC neurons in adult animals (F_{mean} in adults = 5.01 Hz; F_{mean} in old animals = 2.71; M–W, Z = –5.506, $p < 0.001$). These data are consistent with results from other studies, which also demonstrated age-related decreases in mean neuron activity frequency in the prefrontal cortex [Caetano et al., 2012] and a decrease in the proportion of high-activity (frequencies of



6–30 Hz) neurons in the hippocampus and sensorimotor area of the cortex [Kopytova et al., 2003; Kopytova et al., 1992].

The criterion for specialization for new systems of behavioral acts formed during training in the experimental chamber (activity frequency 1.5 times the mean in all, without exception, performances of the act or group of acts) was met by the activity of eight neurons of the 268 recorded in old animals and 21 neurons of the 241 recorded in adult animals. An example of the activity of a neuron specialized in relation to the acts of approaching and climbing into the first or second feeder in the training sequence is shown in Fig. 1, *A*. Statistical comparison of the proportions of these neurons, χ^2 test, revealed a significantly greater number in adult animals than elderly ($\chi^2 = 6.687$, $p < 0.01$). Other authors have used only statistically significant differences in discharge frequency as the criterion for linkage between activity and behavior, leading to greater proportions of behavior-linked neurons being identified (see, for example, Tabuchi et al. [2005]). However, these authors did not indicate how stably these activation events occurred, so we cannot know which of these neurons was specialized for the new systems of behavioral acts formed on training in the experimental cage. The decrease in the proportion of specialized RC neurons seen here on aging, along with data obtained on recording of spike activity in other brain structures [Barnes et al., 1997; Burke et al., 2012; Caetano et al., 2012; Schoenbaum et al., 2006; Wilson et al., 2004] indicates that aging involves a reduction in the specificity of the link between neuron activity and newly formed behavior.

Comparison of the averaged activity patterns of all the neurons recorded in adult rats and all neurons in elderly rats in the 12 acts identified in the cyclic operant behavior revealed significant differences in the normalized mean activity frequencies in six acts. This result is presented in Fig. 1, *B*. The normalized neuron discharge frequency in elderly animals was significantly greater than that in adults in acts consisting of approach to the first feeder in the training sequence (act 1A) (M–W, Z = –3.344, $p < 0.01$), on tilting and grasping the food within it (1f) (M–W, Z = –6.682, $p < 0.001$), on checking it without pressing the pedal (1T) (M–W, Z = –2.875, $p < 0.01$), taking the head out of the feeder and turning to the middle of the wall (1m) (M–W, Z = –3.869, $p < 0.01$), tilting towards the second feeder

Fig. 1. *A*) Raster plots and averaged prestimulus histograms of the activity of neurons specialized in relation to approaching and climbing into the right-hand feeder (second in the training sequence). Upper plot – pattern of activity on right side of experimental cage, averaged from removing the head from the right-hand feeder (2f). Lower plot – pattern of activity on the left-hand side, averaged from the moment of removing the head from the left-hand feeder (1f). The abscissa shows time, msec. The ordinate shows the number of spikes in the histogram channel. Beneath the histogram, black rectangles show the time distribution of acts of climbing into the feeder (left) and the start of turning towards the pedal (right). Neuron histogram channel width was 50 msec. *B*) Averaged patterns of normalized mean activity frequencies for all RC neurons recorded in adult and elderly rats. The abscissa shows behavioral acts on the first side in the training sequence (index 1) and the second side in the training sequence (index 2), respectively; f – positioning of the animal's snout in the food-containing feeder; m – elevation of the head from the feeder and turning towards the middle of the of the side wall of the cage; C – approach to the corner pedal; p – pressing the pedal; A – journey from pedal to feeder; T – positioning of the animal's snout into an empty feeder. The ordinate shows the normalized mean discharge frequency. Continuous lines and diamonds show values for adult rats; dotted lines and squares show values for elderly rats. Data are presented as mean ± standard error of measurements. *$p < 0.05$, **$p < 0.01$ – pairwise comparisons of normalized mean frequencies in behavioral acts in adult and elderly rats, Mann–Whitney test.

and grasping the food within it (2f) (M–W, Z = –4.815, $p < 0.001$), and also removing the head from the feeder and turning to the middle of the wall on the second side (2m) (M–W, Z = –1.974, $p < 0.05$). All these acts make up the "feeding" part of the behavioral cycle, which constitutes the initial baseline [Aleksandrov, 1989] for its operant food-procuring behavior in the experimental cage. The significance of the baseline acts for old individuals was demonstrated by Schoenbaum et al. [2006].

Pairwise comparison of analogous acts from two different behavioral cycles (for example, pressing the first and second pedals) did not identify any significant differences in the normalized mean activity frequencies, Mann–Whitney test, in either group of animals. At the same time, out of the possible 45 pairs of acts, we found significant differences in normalized frequencies for 18 pairs of acts in the group of old animals and 28 pairs in the group of adult animals (Wilcoxon test, $p < 0.05$). In terms of this parameter, the groups differed significantly (Fisher's exact test, $p = 0.029$, one way). As a result of these pairwise differences, each group of rats showed one act differing from all the others except the analogous act on the second side. For the group of adult animals, this was the act of visiting the first feeder in the training sequence and grasping food from it (act 1f, Fig. 1, *B*) with the lowest normalized frequency for the whole set of neurons, while for the group of old animals the act was the act of lifting the head from the first feeder and turning to the middle of the wall (1m, Fig. 1, *B*), where the normalized activity frequency was greater than in the other acts. The number of differences between individual acts in terms of normalized activity frequencies in the set of neurons recorded provided an indicator of the selectivity of the activity of the structure in behavior [Caetano et al., 2012], analogous to that used in studies with functional mapping of the "differentiatedness" criterion of activation of brain areas on execution of different tasks [Sara-Llonch et al., 2015]. In this case, the activity pattern in the RC during COFPB in elderly animals could be characterized by one strongly distinct component, corresponding to the "baseline" of feeding acts, and lower selectivity of activity in the other acts of the learned behavior. As studies recording cell activity in other brain structures in elderly animals have identified similar patterns, linked, inter alia, with the lower behavioral selectivity of sets of neurons [Caetano et al., 2012], the lower proportion of neurons specifically active at particular stages of behavior [Burke et al., 2014; Wilson et al., 2004], and the lower mean frequency activity of cells [Burke et al., 2014; Caetano et al., 2012; Kopytova et al., 1992], it can be suggested that in old age, the formation of a new behavior is associated with a smaller increase in the differentiation of the system structure of the individual's experience. One important factor in this decrease is the relatively large role of neurons specialized in relation to the past experience system in supporting this behavior.

Conclusions. Thus, these data suggest that learning in old age takes longer than at earlier stages in the individual's life, "tuning " of existing experience due to formation of new neuronal specializations occurs, and that internal systems structure of newly formed behavior ism ore "homogeneous." Keeping in view the criteria used, it can be suggested that the rate at which the differentiatedness of experience increases on learning in old age drops, while the relative contribution of groups of neurons specialized in relation to past experience to realizing the newly formed behavior, conversely, increases.

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