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Neuron Activity in the Retrosplenial Cortex of the Rat at the Early and Late Stages of Memory Consolidation

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Activity was recorded from single neurons in the retrosplenial cortex during performance of an operant food-procuring behavior in two groups of rats; in the first six days after training to this behavior (group 1) and one week later, during which the animal did not perform the learned skill (group 2). At the same time, these groups showed no significant difference in the percentages of neurons specialized with respect to the learned behavior; in group 1, 40% of the cells of this category showed activation occurring in 80–90% of performances of the specific act, and not in all (100%), which was significantly different from the proportion of such cells (4%) in animals of group 2. All neurons with less than 100% activation at the early post-training stage were specialized with respect to the most recent act in the training history: approach to and pressing of the pedal. It is suggested that at the first stages of consolidation of the operant skill, its realization may occur by means of a variable set of cells in the retrosplenial cortex specialized with relative to the system of new behavioral acts.

Keywords: consolidation, rats, retrosplenial cortex, training, operant behavior, activity of specialized neurons, activation probability.

Many studies of memory consolidation have shown that the characteristics of the activity of various brain structures and the properties of the execution of learned behavior depend on the period of time between its formation and the beginning of reproduction [Dudai, 2004; Sozinov et al., 2013]. Recording of the activity of individual nerve cells during prolonged training to a new skill has demonstrated the dynamics of the frequency and quantity indicators of the involvement of cells in different areas in different stages of learning and repetition [Jog et al., 2007; Smith et al., 2012; Weible et al., 2009]. At the same time, the specific activity of neurons and the set of cells activated in repeat performances are generally stable in definitive food-procuring behavior [Shvyrkov, 2006; Gorkin and Shevchenko, 1990; Gavrilov et al., 1998; Thompson and Best, 1990; Greenberg and Wilson, 2004; and others]. As consolidation is one aspect of the process of the systemic differentiation of individual experience [Aleksandrov et al., 2014], which includes description of the process of neuron specialization in relation to the new system and reorganization of the system formed by previous experience, involved in behavior [Svarnik et al., 2013; Aleksandrov et al., 2014], the presence or absence of repeated actualization of the acquired skill after training can be reflected in these processes in different ways. This suggestion was tested by recording the activity of individual neurons in the retrosplenial cortex (RC) in rats during the first 6–7 days after training and one week (7–15 days after training), which correspond to the standard periods at which data on the stability of the activation of neurons of different behavioral specializations are obtained [Gorkin and Shevchenko, 1990; Gavrilov et al., 1998].

Methods

Hooded Long-Evans rats aged 8–12 months and weighing 250–350 g were used in the present studies. During training and the experiment, the animals were housed in individual cages and kept in conditions of partial food deprivation. Weight loss over the whole of the study period was by no more than 10–15%.

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TABLE 1. Classification of the Activity of Neurons in the Retrosplenial Cortex of Rats during Food-Procuring Behavior at the Early and Late Stages of Consolidation of the Skill

Group of neurons		Group of animals					
		1–6 days after training, $n = 6$			7–15 days after training, $n = 5$		
N neurons	specialization	pedal	feeder	total	pedal	feeder	total
Probability of activation	100%	11	4	15	13	9	24
	Less than 100%	10	0	10	0	1	1
D neurons			25			28	
NS			150			126	
Total			200			179	

All animals were trained stepwise to press a pedal to obtain cheese from a feeder. The training steps were: approach to the feeder, departure from the feeder, approach to the pedal, pressing the pedal. The duration of each session (step) was 20–30 min per day. The training sequence and construction of the experimental cage have been described in detail elsewhere [Kuzina, 2013]. Neuron activity was recorded in animals of group 1 ($n = 5$) during the first six days immediately following training to press the pedal, while recording in animals of group 2 ($n = 7$) was in the following week, during which the animals did not perform the acquired skill.

All animals underwent surgery under anesthesia with a mixture of Zoletil (Virbac Santé Animal, France, 25 mg/kg) and Rometar 2% (Spofa, Czech Republic, 10 mg/kg) given i.m. An opening about 2 mm in diameter was drilled in the skull over the retrosplenial agranular cortex (P = 4.5–5, L = = 1.1–1.2), and a platform for a detachable micromanipulator was placed and fixed over this with dental cement. Activity was recorded from individual neurons using glass microelectrodes filled with isotonic NaCl solution with impedance 3–7 MΩ at a frequency of 1 kHz. Video recordings were made in parallel with neuron activity traces, along with event markers. Morphological reconstructions of the recording site were made when experiments were complete. All experiments were performed in compliance with European Union Directive No. 86/609 EC regarding the humane treatment of experimental animals.

Neuron activity and behavioral characteristics were processed in DMain (Yu. Raigorodskii). Analyses included only those traces in which the animal performed at least 10 successful behavioral cycles including pedal pressing and running to the feeder. Mean activity frequencies throughout the whole recording period were determined for each neuron. Activation in one or more acts was taken as an increase in the activity frequency in these acts to at least 1.5 times the mean throughout the recording period. For the purposes of this study, cells were regarded as "specialized" in relation to particular systems for these and other acts in the repertoire when the probability of activation during these acts was not only unity, as used in our previous studies of definitive behavior [Gorkin and Shevchenko, 1990; Gavrilov et al., 1998; Aleksandrov et al., 2014], but also 0.75–0.9, given that recordings in the present study were made at the early stages of formation of behavior. The distributions of neuron activity in the two groups of rats were compared with all cells being assigned to one of three categories: 1) neurons specialized in relation to acts of behavior learned in the experimental cage ("new," N); 2) neurons with activation both during acts of learned behavior and outside such acts, apparent as a link with particular movements (left-right, up-down, etc.) in whichever behavior they might be involved ("old," D), and 3) cells not showing consistent activation in cyclic behavior (neurons with unidentified specialization, NS) [Aleksandrov, 2012; Gorkin and Shevchenko, 1990; Gavrilov et al., 1998]. Statistically significant differences in the proportions of neurons with different specializations in the two groups of animals were identified using Fisher's exact test. Neuron activity frequency parameters and behavioral characteristics were compared using the nonparametric Mann–Whitney test. Differences were regarded as significant at $p < 0.05$ (one-tailed test for deviation from the null hypothesis). All calculations were run in Statistica 6.0.

Results

Neuron sets. Table 1 shows data on the classification of all the neurons recorded ($n = 379$) in the two groups of animals. The two groups were characterized by identical proportions of NS neurons (Fisher's exact test, $p = 0.18$), and there was also no significant difference in terms of the proportions of D neurons (Fisher's exact test, $p = 0.23$). However, comparison of the proportions of N neurons with stable (100%) activation in the learned behavior showed that there were fewer such cells in the first days after training than one week after completion of training (Fisher's exact test, $p = 0.042$). The mean frequency throughout the recording period, the frequency during the specific act, and the ratio of the frequency during activation to the mean frequency were also calculated for this category of neurons. The ratio of the frequencies in specific and other acts in the behavioral cycle was significantly greater in specialized neurons in the

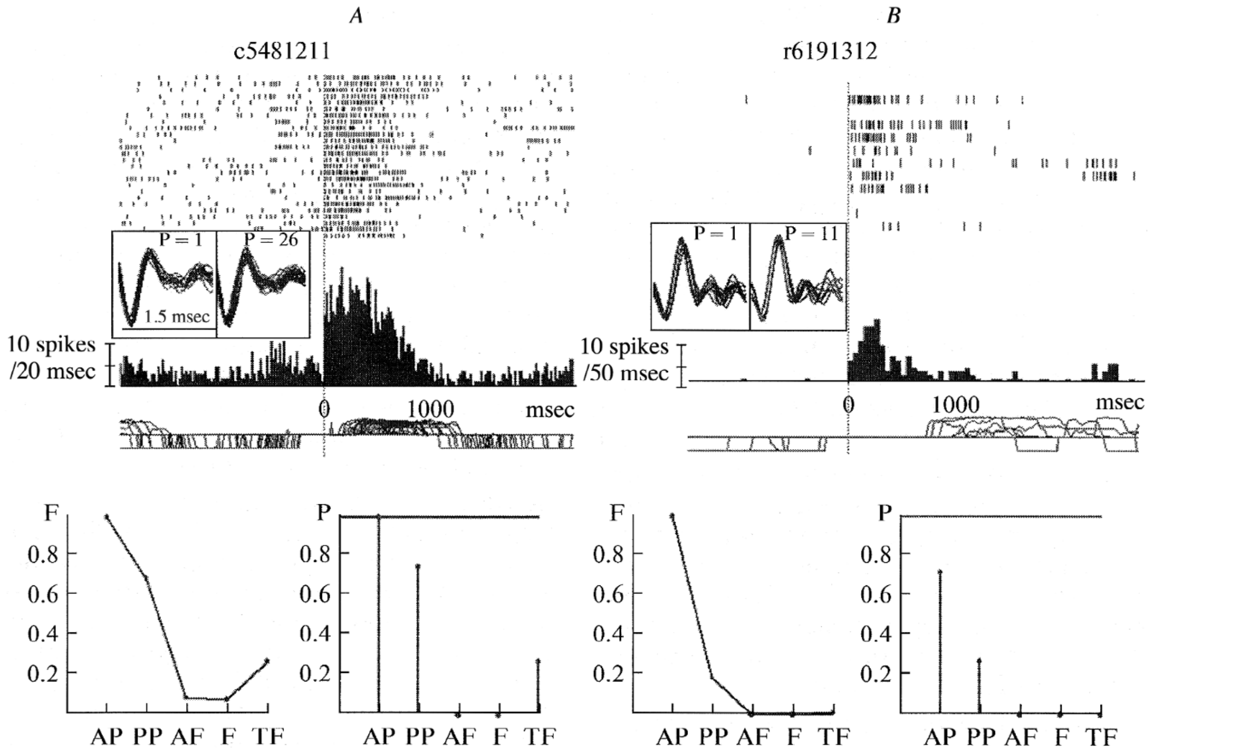

Fig. 1. *Above*: Raster plots and histograms showing neuron activity: *A*) A neuron in which activation on approaching the pedal (c5481211, mean background frequency 9.35 Hz, frequency during specific act 33 Hz) appeared with a probability of 100%. *B*) A neuron in which activation on approaching the pedal appeared in only 75% of acts (r6191312, mean background frequency 1.9 Hz, frequency during specific activity 9.45 Hz). Averaged prestimulus histograms of neuron activity. Abscissas show time, in msec; "0" corresponds to the beginning of the approach to the pedal. The ordinates show numbers of spikes in histogram channels. Activity raster plots are shown above histograms: rows correspond to individual realizations; points correspond to neuron spikes. Frames in the lower left corner of each raster show examples of spike shapes for the corresponding neurons in the first and last realizations (P) of acts consisting of approaching the pedal. Actograms of behavior are shown beneath histograms (superimpositions); deviations upward show pedal presses; deviations downwards show lowering the snout into the feeder. Histogram channel width for neuron r6191312 is 50 msec, while that for neuron c5481211 is 20 msec. *Below*: Ordinates show: F – normalized mean frequency; P – probability of activation of neurons in cyclic behavior acts. Abscissas show acts: AP = approach to pedal; PP – pedal press; AF – approach to feeder; F – taking food from feeder; TF – turning away from feeder.

first days of training (by a factor of 3.5) than at the later periods (by a factor of 2.5) of recording (M – W(U) = 2.22, p = = 0.026). We have previously observed that a group of cells specifically active during not all, but only 75–90% of acts in the learned behavior, could be seen during the first post-training days [Kuzina, 2013]; Fig. 1 shows examples of neurons with 100% and non-100% activation probabilities. The "one week after training" group contained significantly fewer (n = 1) new neurons with non-100% activation probabilities, as compared with the "first post-training days" group (n = 10) (Fisher's exact test, p = 0.01). All neurons with new specializations with non-100% activation in the first post-training days had increased activity frequencies during the approach and pedal-pressing acts (n = 10), while all cells (n = 4) specialized in relation to "feeder" acts had 100% activation (Fisher's exact test, p = 0.003). Combination of the N group with different activation probabilities in the first post-training days had the result that the proportion of N neurons became identical for the two time stages (13 and 13.96%). Nonetheless, the ratio of "pedal" and "feeder" neurons changed significantly: during the first days, there were significantly more neurons for "pedal" acts than for "feeder" acts (Fisher's exact test, p = 0.0003), while after a week the proportions were equal (Fisher's exact test, p = 0.28). In addition, although no significant differences were seen in the frequencies of all N neurons in the lower and upper cortical layers (Fisher's exact test, p = 0.233 (group 1); p = 0.5 (group 2)), mean activity frequencies were higher in cells in the upper layers in the first post-training days (F_{mean} = 3.8 ± 1.9 Hz) than in the lower layers, F_{mean} = 1.78 ± 1 Hz (M – W = 2, p = 0.045) and the ratio of specific to background activity was greater in neurons in the lower layers (M – W = –2, p = = 0.045). Analysis of the proportion of active neurons in the track (the ratio of the number of neurons found per insertion of the electrode to the track length) showed that the number of active neurons in tracks with ≥ 1 active neuron was greater in rats one week after training (1.5%) than in the first days after training (1.1%) (M – W(U) = 02.22, $p = 0.026$).

Behavior. During the first post-training days, pedal pressing in rats occurred with a decrease in the duration (t) and variability (v) of performing the behavioral cycle on daily repetition of the skill (from 5 to 10 days in different animals), in contrast to the "one week after training" group (comparison of mean durations and standard deviations of the cycle time in the first half of the recording period (1–5 days) and the second half of recording period (6–10 days): "first days" group, M – W(t, v) = 2.61, $p = 0.009$; "one week" group, M – W(t) = 0.57, $p = 0.56$; M – W(v) = 0.28, $p = 0.77$). In the group of rats one week after initiation of training, conversely, there was an increase in the duration and variability of realizations by day 3 of repetitions (the smallest value of the test on pairwise comparisons: M – W(t) = –3.28, $p = 0.0011$; M – W(v) = –2.37, $p = 0.017$) and by day 7 (M – W(t) = 3.93, $p < 0.0001$; M – W(v) = 2.08, $p = 0.036$) – a decrease.

Discussion

Comparison of the proportion of RC neurons with different behavioral specializations in animals in the first day and a week after formation of the cyclic food-procuring pedal-pressing skill showed that in the first post-training days significantly more cells (40% in the first days and 4% after a week) were specialized in relation to approaching and pressing the pedal had unstable (non-100%) activation in their specific acts. As at one week and in the first post-training days there were no significant differences in the frequency characteristics or distributions in the lower and upper layers of the cortex *between* the sets being compared, instability of specific activation of some of these cells in definitive behavior may reflect both the processes of reorganization of the systems for the preceding training [Svarnik et al., 2014] and the sequence in the behavioral continuum of the act (approach to the pedal) [Aleksandrovich et al., 2014] on the one hand and, on the other, the characteristics of the formation of the new system [e.g., Smith et al., 2012; Weible et al., 2012], including the processes forming intersystem connections between it and other elements of experience, i.e., the processes "writing" the system into the integral structure of experience, leading to further differentiation of the structure. In animals which were kept in their home cages for a week after training, the time and variability of performing the cyclic behavior in the first days of testing were no different from those in the group of animals immediately after training; while rats of the first group showed a smooth decrease in the time and variability of the cycle with weekly repetition, the second group, conversely, showed an increase in duration. Along with a significant increase in the proportion of active neurons in the track, as compared with the set at the early post-training stage, these differences show that on actualization of experience at least a week after its formation, the processes of accommodative reconsolidation could be more minor than in the first days after training.

Conclusions

No significant differences were seen in the patterns of specialization of neurons in the retrosplenial cortex in the early and late stages of consolidation of the operant skill. However, the characteristics of the activity within the groups of neurons specialized in relation to the learned behavior were different. At the early post-training stages, 40% of the neurons specialized in relation to the systems for the behavioral acts were involved in the new behavior, but not in 100% of realizations of the behavior. All these cells had increased activity frequency in acts consisting of approaching and pressing the pedal, which the animals had learned immediately before the experiment. At the late stages, the proportion of such cells decreased significantly (to 4%). It is suggested that these observations, along with the significant differences in the rates of the learned behavior and the proportions of active neurons along tracks at comparable stages may be evidence that systemic differentiation accompanying consolidation-related modifications of the neuronal support of behavior may occur differently in the retrosplenial area of the cortex, depending on the period of time between training and the beginning of the realization of the learned behavior.

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