Distribution of Behaviorally Specialized Neurons and Expression of Transcription Factor c-Fos in the Rat Cerebral Cortex during Learning

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Studies of learning are now performed at very different levels, including the neuronal and molecular biological levels. Integration of data obtained at these two levels can facilitate the development of overall concepts of processes occurring in the brain during learning.

Data obtained using neurophysiological methods provide evidence that learning is mediated by a process of "behavioral specialization" of silent reserve neurons [1, 3, 5, 7]. According to the systems-selection theory of the formation of a new behavioral act, learning involves the formation of a new functional system, i.e., systemogenesis; at the neuronal level, this corresponds to the formation of neuron specializations for this system [7]. Neuron specialization consists of the appearance of activation of previously "silent" neurons every time the relevant formed behavioral act takes place.

It has repeatedly been demonstrated that different brain structures are characterized by different patterns of behavioral neuron specializations [1]. Thus, the motor cortex is dominated by neurons specialized with regard to systems formed at the early stages of individual development: so-called old system neurons, for example, "movement" neurons or "food taking" neurons. The cingulate cortex is dominated by neurons specialized with regard to new systems formed when animals learn in an experimental cage, for example, "pedal-pressing" neurons [4]. Comparison of the patterns of specialization in the retrosplenial area of the cingulate cortex and in the anterolateral area of the motor cortex in rabbits shows that the number of "new" neurons in

the former is an order of magnitude greater than in the latter. It has also been demonstrated that the patterns of specialization in the cingulate cortex are similar in rabbits and rats: "new" neurons predominate in both species [3]. Further studies reported by Gavrilov et al. have demonstrated a similarity between the patterns of specialization in the motor cortex of rats and rabbits. Thus, the ratio of the patterns of neuron specializations in rats is the same as that in rabbits.

The processes underlying the formation of neuron specialization are evidently based on long-term changes in cell functions and cell connections, which must require activation of gene expression. Learning has been shown to induce a cascade of molecular rearrangements in neurons, and expression of early genes has been shown to be one of the critical elements of these modifications [2]. Induction of the *c-fos* gene during formation of a new behavior, this being one of the main members of the immediate early gene family, varies in different brain structures. Zhu et al. [9] have established that the set of structures activated in the rat brain, demonstrated by immunohistochemical mapping, coincides with the set of structures identified as activated by recording of neuron activity during presentation of familiar and unfamiliar objects. These points suggest that differences in the levels of gene expression between structures may be associated with the different contributions of these brain structures to the process of neuron specialization during learning. Confirmation of this suggestion and improvement of our understanding of the molecular biological bases of the formation of neuron specialization require comparisons of the numbers of neurons expressing early genes in a given brain structure during learning with the level of involvement of that structure in the formation of new spe-

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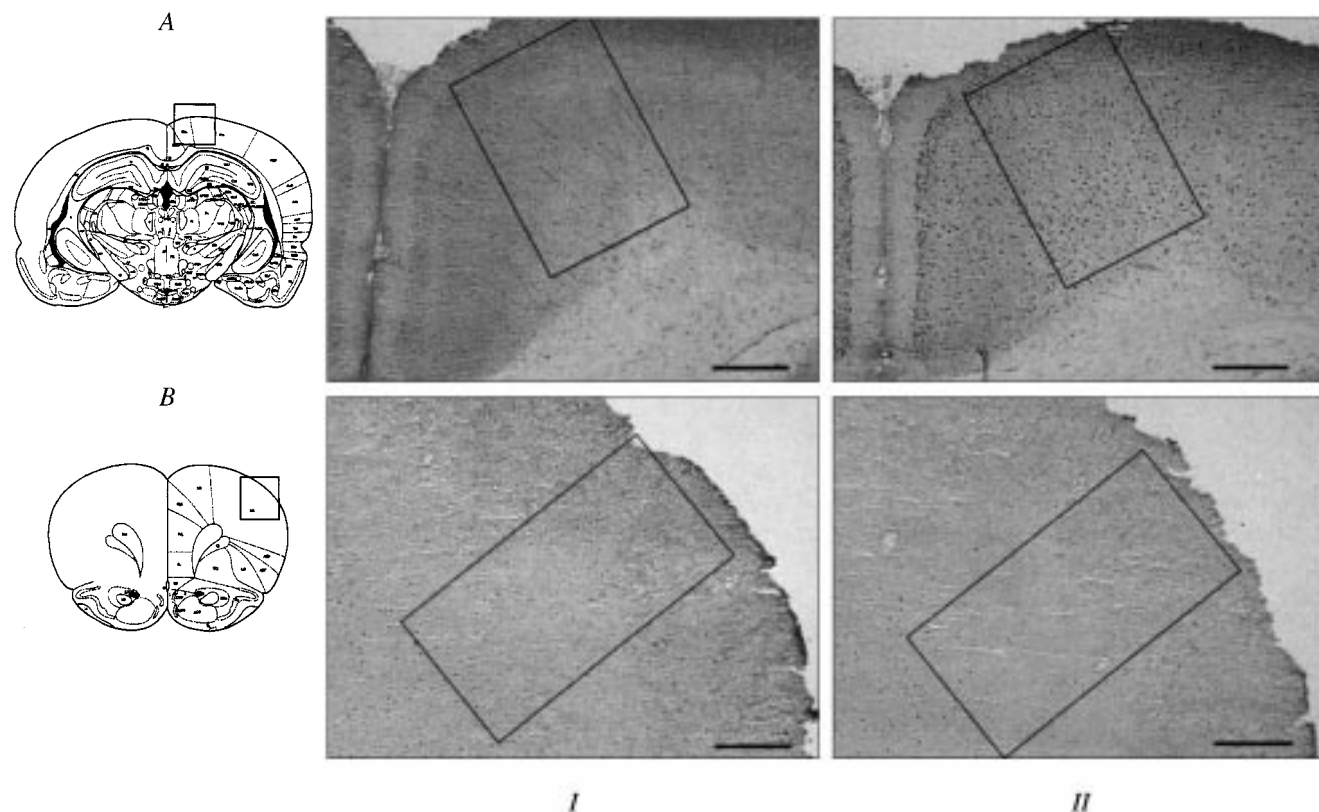
Fig. 1. Diagrams of frontal sections identifying the study areas and photomicrographs of frontal sections showing Fos-stained neuron nuclei in the cingulate (*A*) and anterolateral (*B*) areas of the rat cortex in the passive control group (*I*) and the group which had formed a new behavior (*II*). Calibration bar: 500 μm.

cializations. As far as we know, no such comparisons have yet been made. The aim of the present work was to compare the patterns of behavioral specializations of neurons in the anterolateral and cingulate areas of the cortex with induction of the expression of transcription factor c-Fos in these brain areas during learning.

Capuchin rats (Long Evans, males weighing 250 g, $n = 8$) were trained to an operant food-procuring behavior using a stepwise method in an experimental cage with one feeder and one pedal. This behavior was analogous to the animals' behavior during recording of neuron activity in experiments performed to identify neuron specialization [1, 3]. Training was conducted over a period of 13 days in a number of stages. At the first stage, rats were trained to take food from the feeder; at the second stage they were trained to turn away from the feeder to the middle of the cage; at the third stage, animals were trained to move away from the feeder and approach the middle of the cage wall; at the fourth stage, rats were trained to approach the pedal; at the fifth stage, animals were trained to press the pedal. Each step occupied two 30-min training sessions; one session was performed each day. Animals were trained to press the pedal during the last 30-min training session. Rats were then placed in their home cages for 1 h

15 min, and were then subjected to inhalational anesthesia and sacrificed by decapitation. Brains were immediately removed and frozen in liquid nitrogen. Animals of the passive control group (Long Evans males weighing 250 g, $n = 3$) were taken from the home cage immediately before decapitation. Frontal cryostat sections of thickness 20 μm were prepared from brains. The brain of each animal yielded ten pairs of sequential sections of the anterolateral cortex (+2.5 ... +3.5 mm from the bregma) and ten pairs of sequential sections of the cingulate cortex (–4.0 ... –5.0 mm from the bregma). These coordinates correspond to the coordinates used for recording spike activity from rat brain neurons in experiments performed to identify behavioral specialization of neurons [3]. The first section of each pair was used for immunohistochemical staining using a Vector Laboratories (USA) streptavidin-biotin-peroxidase kit. Reactions used polyclonal rabbit antibodies against Fos protein (Calbiochem, Ab-5, Cat. No. PC38, USA) at a dilution of 1:2000. The second section of each pair was stained by the Nissl method. Images of the right hemisphere derived from micropreparations of brain slices were obtained using an Olympus BX-50 microscope (Japan) and a Panasonic WV-CP230 video camera (Japan), with analysis using the computer program Image Pro
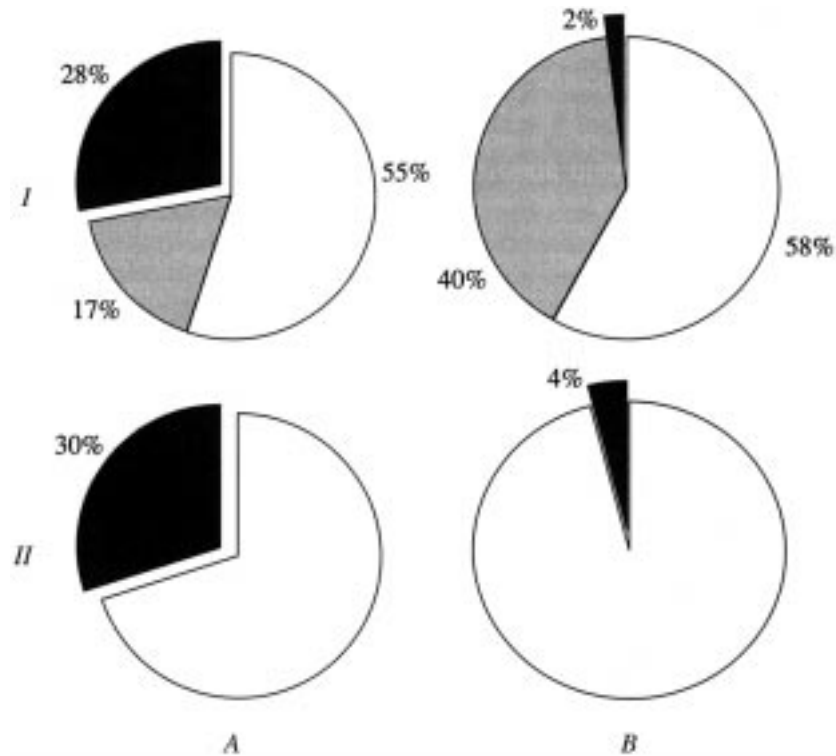
Fig. 2. Comparison of the patterns of behavioral specializations of neurons (*I*) in the cingulate (*A*) and anterolateral (*B*) areas of cortex [1, 3] with the relative proportions of Fos-positive neurons (*II*) in these areas. *I*) The dark sector shows the proportion of neurons belonging to new systems (%); the shaded sector shows those belonging to old systems; the white sector shows those not involved in the behavior of interest. *II*) The dark sector shows the proportion of neurons expressing c-Fos (%); the light sector shows the proportion not expressing c-Fos.

Plus 3.0. The distribution of Fos-positive cells in the areas of interest was analyzed as the base of a triangle, one side of which was determined by the depth of the cortex, the second being constant at 1 mm, which corresponded directly to the width of the area used for recording neuron spike activity.

The dynamics of the behavior of the animals of the experimental group during the last 30-min training session were as follows. The first third of the time spent in the experimental chamber consisted mainly of approaches to the pedal, which did not lead to presentation of food, along with orientational-investigative behavior and occasional pedal-pressing acts. There was then an increase in the number of pedal-pressings and a decrease in the manifestations of orientational-investigative behavior. Rats achieved the criterion of ten pressings in a row during the last third of the time spend in the cage, after which the behavioral act of pedal-pressing was regarded as formed.

Analysis of immunohistochemically stained brain sections from animals of the passive control group showed that transcription factor c-Fos was expressed at low levels in the study areas of these rats (Fig. 1, *I*). The density of c-Fos-positive cells in the cingulate cortex was 30 ± 9 neurons per mm^2, compared with 18 ± 8 neurons per mm^2 in the anterolateral cortex. Further analysis showed that the levels of c-Fos expression in these two areas were not significantly different: the percentages of Fos-positive neurons (of the total number of cells in sections) in each area were 3.7% and 3%. Analysis of histochemically stained brain sections from animals which had formed the new behavior revealed increases in the level of c-Fos expression in the cingulate cortex as compared with the level in control animals (Fig. 1, *II*). Expression was observed in both the cingulate and anterolateral areas of the cortex. The density of c-Fos-positive cells in the cingulate cortex was 258 ± 69 neurons per mm^2, compared with 29 ± 6 neurons per mm^2 in the anterolateral cortex. The percentage of c-Fos-positive neurons (of the total number of neurons on sections) in the cingulate area was 30.1%, which was significantly greater ($p < 0.05$, Wilcoxon T test) than in the anterolateral cortex, where the proportion of Fos-positive neurons was 3.6%.

Thus, comparison of the induction of transcription factor c-Fos expression during learning with the previously established pattern of behavioral neuron specializations showed that the *c-fos* gene is expressed mainly in the cingulate cortex, where the number of neurons with new spe-

cializations exceeded that in the anterolateral cortex by an order of magnitude (Fig. 2). The magnitude of the difference between the proportions of Fos-positive neurons in the cingulate and anterolateral areas of the cortex were analogous. These data provide evidence supporting the hypothesis that expression of transcription factor c-Fos is associated with the formation of new neuron specializations during learning.

The process of neuron specialization can currently be described only in terms of two hypotheses. Learning is known to associated with two phases of genetic processes: there is initial activation of early gene expression, which is followed by the expression of their target genes [2]. It is possible that this biphasic nature reflects stages in the process of neuron specialization with regard to the system for the behavioral act. It has been noted above that neuron specialization occurs because of silent or "reserve" neurons. Published data [4, 8] show that particular treatments altering the microenvironment of neurons (iontophoresis, electrical stimulation) lead to activation of previously silent cells. At the same time, changes in the microenvironment of a neuron, which occur in ischemia, thermal shock, administration of convulsants, and during learning, lead to activation of early gene expression [2, 6]. Thus, it can be suggested that changes in the microenvironment of a silent neuron during learning are, on the one hand, associated with activation of the cell and, on the other, with early gene expression, which is the first step in the cascade of processes leading to specialization.

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