RNA SYNTHESIS IN THE RAT BRAIN DURING CHANGES IN CNS FUNCTION

V. V. Dergachev and O. A. Tyutyaeva

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RNA synthesis in the phenolic fractions of nuclei and cytoplasm from higher structures of the rat brain under normal conditions, during training in defensive movements in a maze, and in an active control (irregular presentation of stimuli evoking defensive movements) was investigated by electrophoresis in polyacrylamide gel. Behavioral stimulation and irregular presentation of the same stimuli to the animals as during training, but not leading to the formation of defensive skills, activates synthesis of 18S, 28S, and higher forms of RNA in the phenolic extracts of the nuclei. An increase in the incorporation of labeled precursor in the 18S region was found for cytoplasmic RNA.

KEY WORDS: RNA synthesis in nuclei and cytoplasm; rat brain; mechanism of memory; electrophoresis of RNA.

The storage, recognition, and recall of information are important brain functions. During the encoding of information in the CNS, changes have been found in the synthesis of macromolecules, especially of RNA [2, 3, 10, 11, 13, 15]. However, there is as yet no general agreement on the kinetics of RNA synthesis in nerve tissue. Some workers have not found a peak of fast-labeled high-molecular-weight RNA's in the brain [8, 9, 16]. The presence of a 45S-ribosomal precursor and of its subsequent degradation in brain cell nuclei have recently been reported [12, 18, 19]. In addition, despite frequent confirmation that RNA synthesis is activated during learning, it is not yet clear on account of what forms of RNA this activation takes place, although this could be a key step toward the elucidation of the molecular mechaisms of memory.

It was accordingly decided to use the method of RNA fractionation by electrophoresis in polyacrylamide gel to study the sedimentation profile of RNA in the higher structures of the rat brain under normal conditions, during training of the animals in defensive movements, and during irregular presentation of stimuli evoking defensive movements to the animals.

EXPERIMENTAL METHOD

Two series of experiments were carried out on male albino rats weighing about 200 g. The animals of series I were divided into three groups. The rats of group I were controls. The rats of group 2 were trained in defensive movements in a maze for 3 days [2]. The rats of group 3 (active control) were placed in the maze and subjected to the same stimulation as the trained animals, but the stimuli were applied irregularly and the rats had no opportunity of escaping the shock. One day before the experiment began, a burr-hole was drilled in the rats' skull under local anesthesia under the control of a stereotaxic apparatus in the regions of the right and left lateral ventricles. Immediately before the animals were placed in the maze on the third day of the experiment, 30 μCi uridine-C^{14} in 0.15 ml physiological saline was injected by means of a microinjector into each ventricle of the rats. The animals were decapitated 10 min after removal from the maze. Uridine was injected into the control animals in the same dose and at the same time before sacrifice as in the experimental animals.

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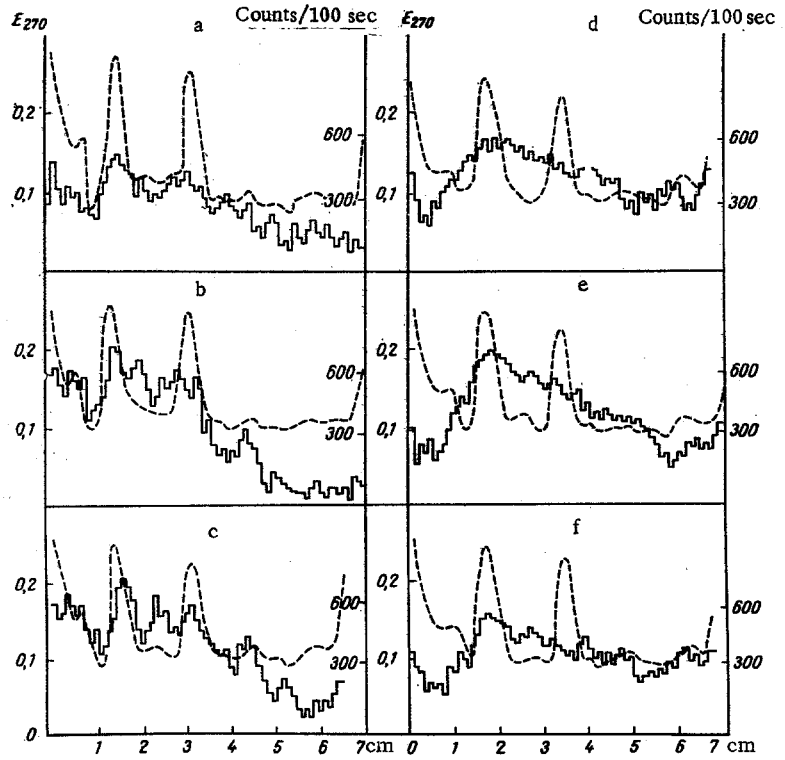

Fig. 1. Profile of incorporation of uridine-C^{14} into RNA of "phenol" nuclei of rat brain. RNA isolated 40 min (a, b, c) and 4 h (d, e, f) after injection of uridine-C^{14}. a, d) Control; b, e) training; c, f) nonspecific stimulation. Broken line represents optical density at 270 nm, continuous line shows radioactivity (counts/100 sec). Abscissa, height of gel.

The experiments of series II differed from those of series I in that the uridine-C^{14} was injected into the animals of the corresponding three groups 4 h before decapitation.

RNA was isolated from all the higher (above the pons) brain structures. The brain tissue was homogenized at 0–4° C in 0.01 M sodium acetate buffer (1 : 10), pH 5.1, containing 20 mg/liter polyvinyl sulfate. The homogenate was treated with an equal volume of cold 80% phenol, pH 5.8. The mixture was shaken for 15 min and centrifuged for 30 min at 2000 g. The aqueous phase was collected and used to obtain cytoplasmic RNA by cold phenolic extraction. Cell nuclei ("phenol" nuclei), containing RNA of the chromosomes and nucleoli [1], collected in the intermediate layer that formed at the boundary between the aqueous and phenolic phases. The intermediate layer was used to obtain RNA from the "phenol" nuclei by hot phenolic extraction. The purified RNA preparation was dissolved in the minimal volume of acetate buffer. The RNA content was determined spectrophotometrically by Spirin's method [5]. To separate the RNA (200 μg) into fractions, gel containing 2.5% acrylamide and 0.15% ethylene-bis-acrylamide was used [14]. Electrophoresis was carried out in the Reanal (Hungary) apparatus in calibrated quartz tubes measuring 0.6 × 8 cm. The height of the gel was 7 cm. RNA extracted from E. coli was used as the marker. After electrophoresis (2.5 h, pH 7.8) the gels in the quartz tubes were scanned at 270 nm using a special attachment to the SF-4A spectrophotometer [4]. The gels were removed from the tubes and cut into disks 1 mm thick. The disks of gel were placed with a drop of 20% Triton X-350 on a foil target, dried, and counted on the NAG-βM gas-flow counter.

EXPERIMENTAL RESULTS AND DISCUSSION

The distribution of labeled precursor in the phenolic fraction of nuclei is illustrated in Fig. 1. After incorporation for 40 min, labeled uridine formed four principal peaks (Fig. 1a, b, c). Two of these peaks coincided in optical density with 28S- and 18S-RNA peaks. The peak of activity of RNA with a sedimentation constant above 28S was most marked in nuclei extracted from the brain of animals trained in defensive movements or exposed to irregular stimulation. The small peak between the 28S- and 18S-RNA's, accord-

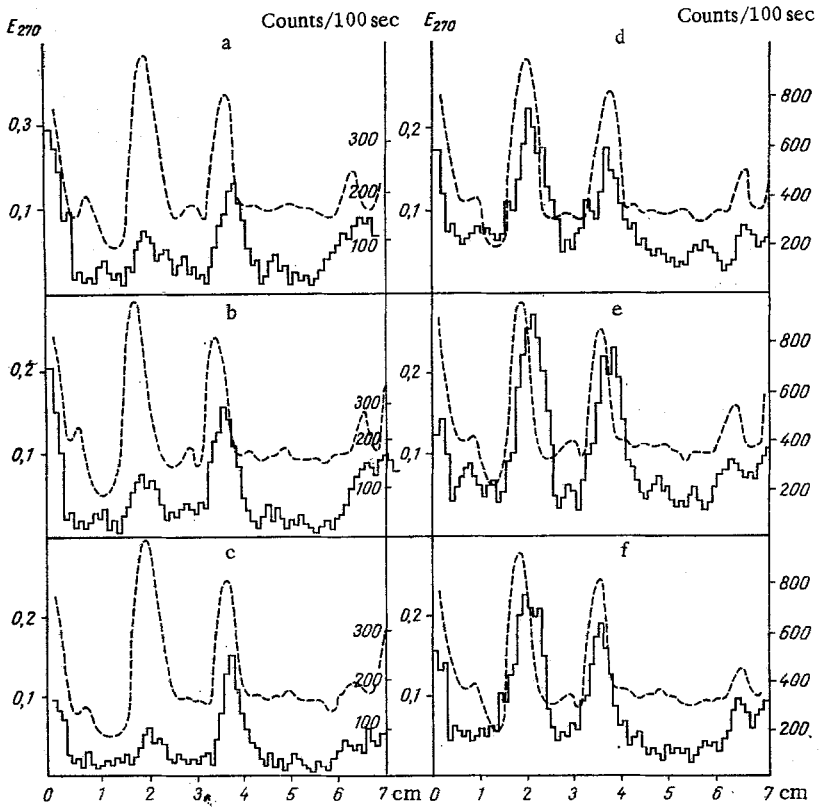

Fig. 2. Profile of uridine-C^{14} incorporation into cytoplasmic RNA of rat brain cells. Legend as in Fig. 1.

ing to Takahashi et al. [18], consisted of minor brain RNA's, and this also was most marked in the two experimental groups of animals.

After incorporation of uridine-C^{14} for 4 h (Fig. 1d, e, f), the radioactivity peaks of RNA with sedimentation constant of 28S-18S were ill-defined and no peak was found in the high-molecular-weight region. However, in both groups of experimental rats a higher level of incorporation of labeled precursor was found in all RNA fractions than in the intact animals.

Fractionation of the cytoplasmic RNA after incorporation of uridine-C^{14} for 40 min showed that most radioactivity corresponded to RNA with a sedimentation constant of 18S (Fig. 2a, b, c). The discovery of radioactivity for this peak of cytoplasmic RNA after a short incorporation time of the precursor was evidently the result of liberation of fast-labeled RNA from nucleus into cytoplasm [17]. There was a small increase in the level of incorporation of labeled precursor into 18S RNA extracted from the cytoplasm of the brain cells of the experimental animals compared with the controls.

When uridine-C^{14} was injected 4 h before the animals were killed, two well-defined peaks were found in the cytoplasmic RNA: in the 28S and 18S regions (Fig. 2d, e, f); there was no difference in the distribution of labeled precursor between the two groups studied.

The electrophoretic profile of distribution of the label discovered for brain RNA of the control animals agreed with that described in the literature [12, 18, 19]. Teaching rats behavioral skills and irregular presentation to the rats of the same series of stimuli as required to produce the skill, but not leading to its formation, were found to have a marked effect on the synthesis of fast-labeled high-molecular-weight brain RNA's. Investigations [11, 15] have shown that during the formation of behavioral skills in animals new types of RNA specific for a particular skill appear but are not found in the active control. No difference was found in the present investigation between RNA synthesis in the animals in which skilled movements were developed and in animals exposed to irregular stimulation. The reason could be that if such types of RNA existed, they probably differed very little in their sedimentation properties from the bulk of the RNA that was found. According to Becker [6], this RNA, embodying the specific features of learning, accounts for 3% of the total quantity of newly synthesized mRNA. It is obvious, however, that exposure of the CNS to an extraordinary situation, as during training in behavioral skills or in nonspecific stimulation in a maze, can act

as equally strong stimulators of the synthesis of high-molecular-weight RNA. This could perhaps be one cause of the differences of opinion regarding the synthesis of specific RNA molecules during learning [7, 15, 20].

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