

Quantitative Analysis of RNA Content in the Brain of Five Different Vertebrate Species – Pisces, Amphibian, Reptile, Avian and Mammalian Families

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Abstract: The increase in brain size during development of an organism is due to an increase in the number and the size of the cells and therefore DNA and RNA content. RNA amount is considered as a reflection of basic functional activity. The prime goal of this study is to compare the accumulated data about various species by defining the differences and the similarities of RNA content in the cerebrum and cerebellum. Differences in the whole body weight, cerebrum and cerebellum weight between in relation to total brain weight of all the five vertebrate species have been compared. Total RNA in cerebrum as a ratio of five vertebrate species. In the case of murrel and gecko the values of male and female significantly different. In the case of toads, avian and rat was not significant. The ratio of total RNA was lowest in murrel and rat shows that highest ratio. Though a defined heirachal patterns in RNA were found in this study, further detailed RNA studies are required in future.

Keywords: RNA; Physiology; Invertebrates; Vertebrates; Cerebrum; Cerebellum

I. Introduction

The increase in brain size during development of an organism is due to an increase in the number and the size of the cells and therefore DNA and RNA content. While DNA is taken as an index of cellular richness, RNA amount is considered as a reflection of basic functional activity. Thus the comparisons of the RNA concentration are an indication of average RNA per cell. Moreover incorporation of the labeled precursors into RNA may give interesting information concerning the kinetics of brain development [1, 2]. This RNA information is heritable and can be passed through successive generations via reverse transcription of genome [3,4]. Over time, RNA information can produce viable phenotypes within a population, contributing to evolution. Studies have shown that RNA processing events have been critical in rapid fast phenotypic evolution of vertebrates [5]. On the whole, RNA processing expands the range of phenotypes for a given genotype and thus contributes to the evolution and biodiversity.

The prime goal of this study is to compare the accumulated data about various species by defining the differences and the similarities of RNA content in the cerebrum and cerebellum. It is possible that variations in the distribution and concentrations of RNA may provide a clue to some aspect of physiological evolution.

II. Material And Methods

The study was conducted at Nizam's Institute of Pharmacy, Medici Institute of Medical Sciences, (Hyderabad, India) Genetics labortiy. This study was approved by the Nizam's Institute of Pharmacy, (Reg.No. 1330/ac/10/CPCSEA dated 30 June 2014). In this study five different male and female vertebrates *Channa striata* (weighing 150-180 gms), *Duttaphrynus melanostictus* (weighing 75-150 gms), *Hemidactylus frenatus* (weighing 60-110 gms), *Gallus gallus domesticus* (weighing 75-150 gms) were purchased from local suppliers and *Rattus norvegicus* (weighing 150-180 gms) were obtained from Nizam Institute of Pharmacy Hyderabad, India, (N=6 animals per each group). During the acclimatisation period, the species were fed daily (Safe feed 7711, Charoen Pokphand Foods PCL, Thailand) about 1% of the body weight. They were fasted for 24 hours before the experiment. They were sacrificed and the brain was rapidly removed, weighed, and dissected out for quantitative analysis of RNA.

2.1 Isolation of RNA from vertebrate's brain

Total RNA was extracted from the cerebrum and cerebellum of all five vertebrate species using RNeasy Mini Kit (QIAGEN GmbH, Germany). The RNeasy Mini Kit allows efficient purification of total RNA

from small amounts of starting material. RNeasy technology simplifies total RNA isolation by combining the stringency of guanidine-isothiocyanatolysis with the speed and purity of silica-membrane purification. RNeasy Kits provide the highest-quality RNA with minimum purification of DNA. With the RNeasy Mini Kit, total RNA is easily purified from 10 to 1×10^7 animal or human cells, 0.5–30 mg animal or human tissues, and $<5 \times 10^7$ yeast cells. Samples were first lysed and then homogenised. Ethanol is added to the lysate to provide ideal binding conditions. The lysate was then loaded onto the RNeasy silica membrane (see figure RNeasy Mini spin column). RNA binds (up to 100 μg capacity), and all contaminants are efficiently washed away. For certain RNA applications that are sensitive to very small amounts of DNA, the residual amount of DNA remaining was removed using a convenient on-column DNase treatment. Pure, concentrated RNA was eluted in 30–100 μl water (see figure RNeasy Mini procedure). RNA was analyzed in 1% Agarose gel, containing ethidiumbromide and visualized with UV light. The 1 Kb DNA ladder plus and 100 bp DNA ladder plus (Fermentas, USA) was used as molecular marker.

2.2 Estimation of RNA

Purity of the RNA was checked before subjecting to molecular analysis. Nucleic acid has maximum absorbance at 280nm. The ratio between the readings at 260nm and 280nm (OD 260/280) provides an estimate of the purity of nucleic acid. Pure preparations of RNA have a ratio approximately 1.6 to 1.9. The estimation of RNA was done with the help of Nanodrop 1000 (Thermo scientific).

2.3 Qualitative analysis by Denaturing Agarose Gel electrophoresis

The samples were electrophoresed on 2% Agarose gel using buffer 1X TAE. For analysis 5 μl of RNA sample was taken and 6 μl of 6x Gel loading dye was added and loaded to the Agarose gel. (Figs 1 to 4).

2.4 Statistical analysis

Sigmaplot version 12.0 for windows (Systatsoft ware, USA) was employed. Descriptive analysis was done Student t-test was performed for means and standard deviation for determining RNA quantity .P value of < 0.05 was considered as statistically significant.

III. Results

Differences in the whole body weight, cerebrum and cerebellum weight between in relation to total brain weight of all the five vertebrate species have been compared. Fig. 5 shows the total brain weight has a ratio of body weight of five vertebrate species. In the case of murrel and rat, the values male and female are significantly different. In the case of toads, gecko and avian, it was not significant. The ratio of brain to body weight was lowest in male murrel, avian and rat showed that highest ratio. Toads and gecko, avian in between. The ratio of brain to body weight was lowest in female murrel, avian and rat showed that highest ratio. Toads and gecko in between.

Fig. 6 shows the cerebrum weight as a ratio of brain weight of five vertebrate species. In the case of toads and gecko the values of male and female significantly different. In the case of murrel, avian and rat was not significant. The ratio cerebrum weight to total brain weight was lowest in murrel and rat shows that highest ratio. Toads, gecko and avian in between. The same results were observed in male as well as female.

Fig.7 shows the cerebellum weight as a ratio of brain weight of five vertebrate species. In the case of toads the values of male and female significantly different. In the case of murrel, gecko, avian and rat was not significant. The ratio cerebellum weight to total brain weight was lowest in murrel and rat shows that highest ratio. Toads, gecko and avian in between. The same results were observed in male as well as female.

Fig.8 shows the cerebrum weight as a ratio of total cerebrum of five vertebrate species. In the case of murrel the values of male and female significantly different. In the case of toads, gecko avian and rat was not significant. The ratio cerebrum weight to total cerebrum weight was lowest in murrel and rat shows that highest ratio. Toads, gecko and avian in between. The same results were observed in male as well as female.

Fig.9 shows the cerebellum weight as a ratio of total cerebellum of five vertebrate species. In the case of murrel and toads the values of male and female significantly different. In the case of gecko, avian and rat was not significant. The ratio cerebellum weight to total cerebellum weight was lowest in murrel and rat shows that highest ratio. Toads, gecko and avian in between. The same results were observed in male as well as female.

Fig. 10 shows the total RNA in cerebrum as a ratio of five vertebrate species. In the case of murrel and gecko the values of male and female significantly different. In the case of toads, avian and rat was not significant. The ratio of total RNA was lowest in murrel and rat shows that highest ratio. Toads, gecko and avian in between. The same results were observed in male as well as female.

Fig.11 shows the total RNA in cerebellum as a ratio of five vertebrate species. In the case of murrel, toads, gecko and rat the values of male and female significantly different. In the case of avian was not

significant. The ratio of total RNA in cerebellum was lowest in murrel and rat shows that highest ratio. Toads, gecko and avian in between. The same results were observed in male as well as female.

IV. Discussion

Difference in RNA content in cerebrum and cerebellum in five species of vertebrates male and female were evaluated. The RNA content in cerebrum of lizard female is surprisingly significantly high in comparison to male and female species and also found the lowest content of RNA in cerebrum of Murrel female. But, compared male species only the RNA content in cerebrum of Toads are highest. The data for rest of species are very close.

In case of cerebellum the highest content of RNA was found in female lizard following toads male. It shows that overall content of RNA in both cerebrum and cerebellum was highest in lizard followed by toad. Relative sizes of different brain regions have changed as vertebrates evolved in same line the content of RNA was also changed.

There is considerable evidence in vertebrates that intensive electrical activity augments neuronal RNA synthesis [6]. and may do so relatively specifically to functional systems known to be involved, as examined by histochemical microspectrophotometry[7], by single-cell dissection and microanalysis[8], and by microradiography[9]. Experimental designs in this field must therefore be capable of picking out the RNA changes, if any, which are correlated specifically with retention rather than with sensory, motor, or motivational activation or with the integrating processes reading into or out of long-term memory.

Differences in RNA between trained animals and adequate primary controls were first reported for an experiment in which rats were forced to reach for food with the nonpreferred front paw [10]. From each cerebral hemisphere of five such rats, 10 single neurons were dissected out of the layer of large pyramidal cells in the area of cortex known to be necessary for transfer of handedness under forced reaching. Extracted RNA preparations were assayed by microchemical techniques shown to be reliable for RNA from other sources. RNA from cortex contralateral to the trained paw occurred in greater quantities per neuron than in ipsilateral cortex, and with a different base composition. The control ipsilateral cortex (not necessary to transfer of handedness) had neuronal RNA in amounts and composition not distinguishable from that in either cortex of untrained animals. It was stated without quoting data that three rats given training with their preferred paws showed slight RNA increase in contralateral cortex but no apparent base ratio changes.

These findings indicated that something was happening with training of the no preferred paw which did not occur with practice by the preferred path and as the base composition changes moved the RNA nearer to the gross base composition complementary to DNA, synthesis of novel messenger RNA by gene activation was suggested. The above discussed experiment shows that during development or training the RNA content of brain increased significantly and also increases memory.

In this study, we found that in avian female cerebellum there is 886.2 ± 3.04 RNA content which was highest among all vertebrate species and if we take male alone, we found that Toads male cerebellum contain maximum 875.8 ± 2.11 RNA content. RNA functions are so varied that neither the necessity nor the impossibility of specific functions of RNA in memory can be prejudged, nor can the ways in which it might be involved. Based on above fact, future investigation in both of basic nucleic acid biochemistry, especially in brain tissue, and of the special problems of biochemistry of memory is warranted.

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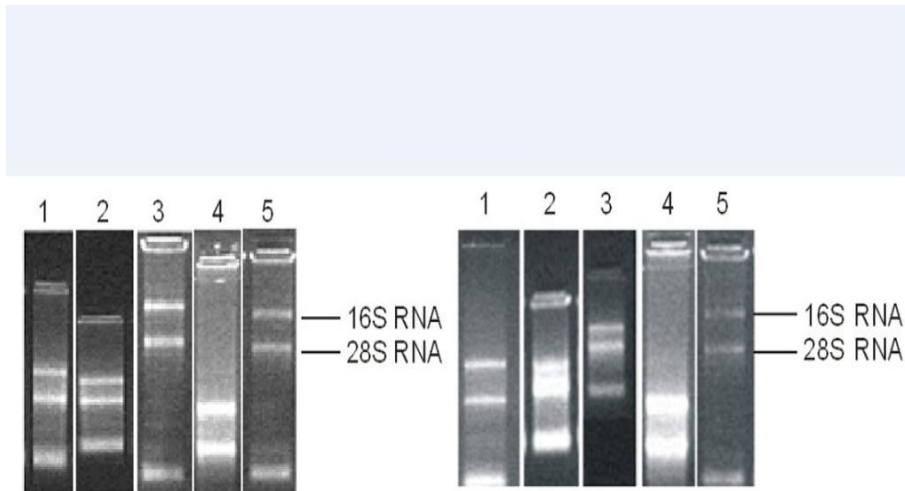


Fig. 1: Agarose Gel electrophoretogram profile for qualitative RNA assay in male cerebrum (left panel) and male cerebellum (right panel) of 5 species

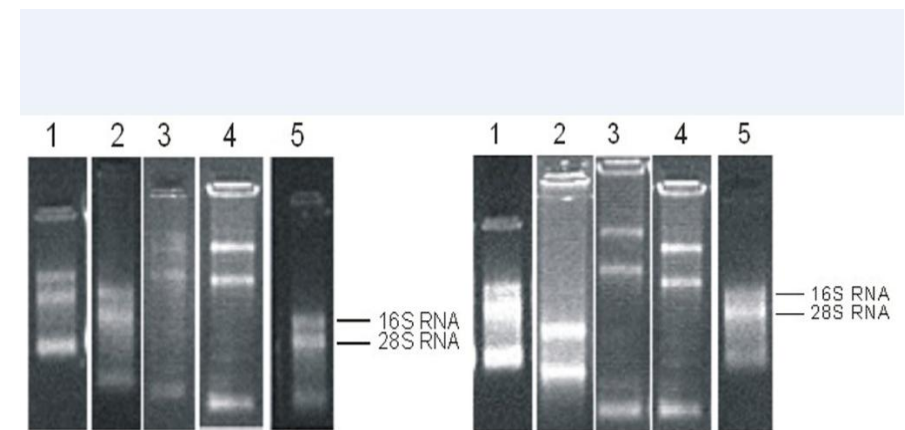


Fig. 2: Agarose Gel electrophoretogram profile for qualitative RNA assay in female cerebrum (left panel) and cerebellum (right panel) of five species

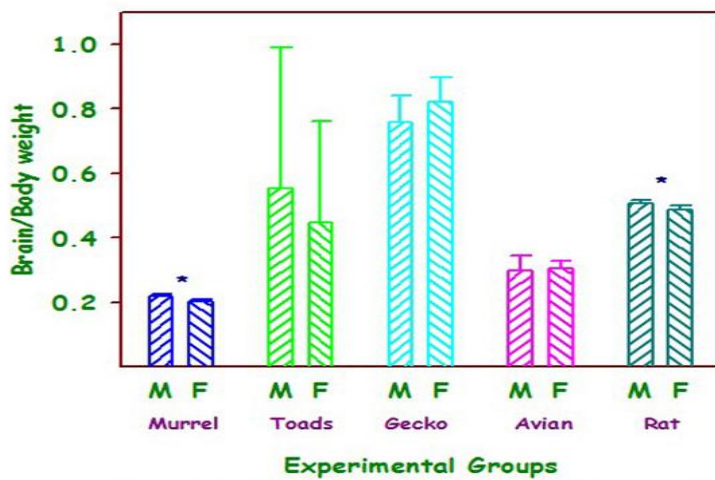


Fig. 3: Bar diagram showing the total brain/body weight of five vertebrate species

Species significance: Male: Murrel < Toads = Gecko < Avian = Rat

Female: Murrel < Toads < Gecko < Avian = Rat

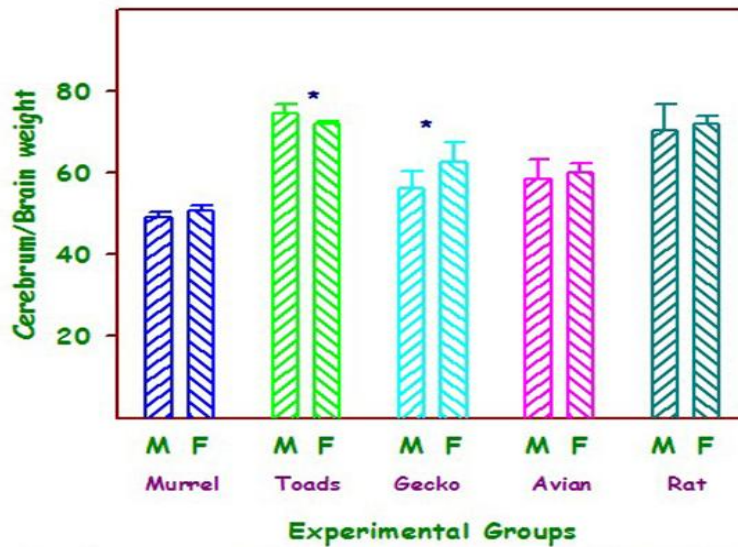


Fig. 4: Bar diagram showing cerebrium weight/brain weight of five vertebrate species

Species significance: Male: Murrel < Toads = Gecko < Avian = Rat

Female: Murrel < Toads < Gecko < Avian = Rat

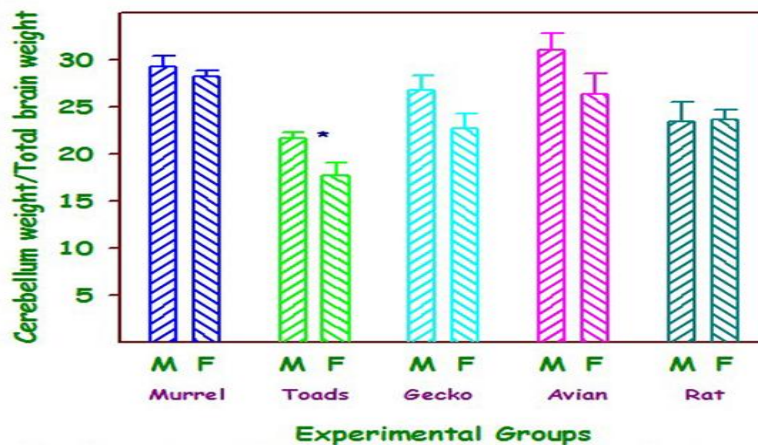


Fig. 5: Bar diagram showing cerebellum weight/brain weight of five vertebrate species

Species significance: Male: Murrel < Toads = Gecko < Avian = Rat

Female: Murrel < Toads < Gecko < Avian = Rat

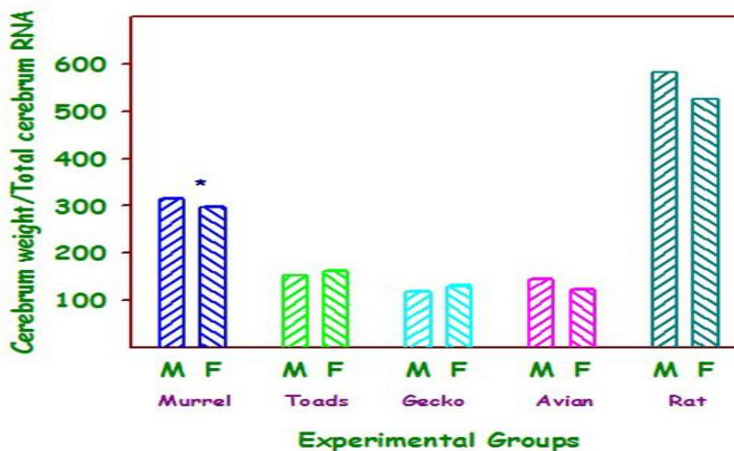


Fig. 6: Bar diagram showing cerebrium weight/total cerebral RNA of five vertebrate species

Species significance: Male: Murrel < Toads = Gecko < Avian = Rat

Female: Murrel < Toads < Gecko < Avian = Rat

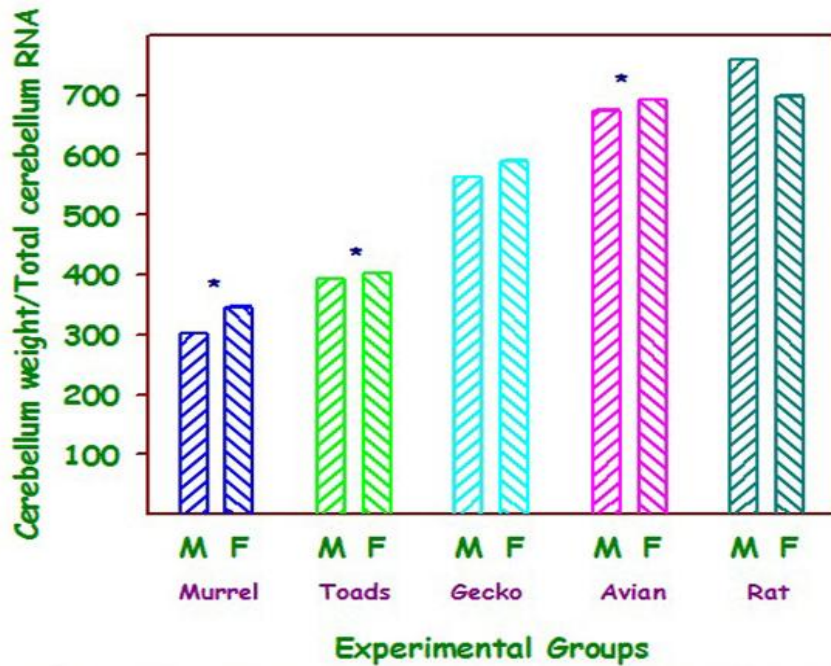


Fig. 6: Bar diagram showing cerebellum weight/total cerebellar RNA of five vertebrate species
 Species significance: Male: Murrel < Toads = Gecko < Avian = Rat
 Female: Murrel < Toads < Gecko < Avian = Rat

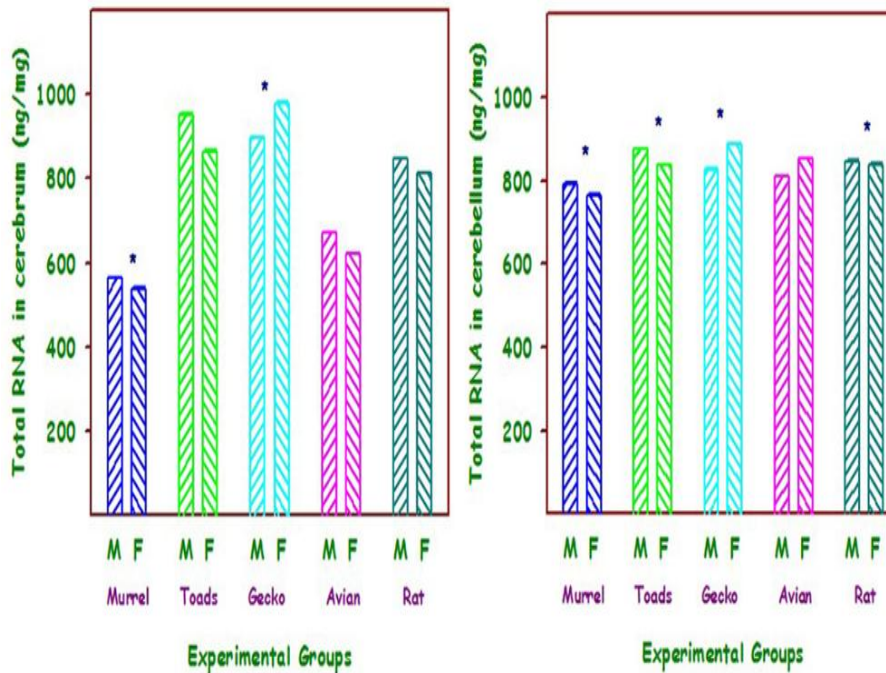


Fig. 8: Bar diagram showing total RNA in cerebrum (left panel) and cerebellum (right panel) of five vertebrate species. Mean \pm SD (n= 6 each)
 Species significance: Male: Murrel < Toads < Gecko < Avian < Rat.
 Female: Murrel < Toads < Gecko < Avian < Rat