Articles

Gradual increase in neuronal density of rats' lateral geniculate nucleus from anterior to posterior

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ABSTRACT

الأهداف: توضيح المركز الرئيسي للنظام البصري LGN في للفئران.

الطريقة: استخدمنا في هذه الدراسة 50 من فئران البينو، والتي تبلغ من العمر شهرين. تم بذل الكثير من الجهد للتخفيف من معاناتها. وكانت الفئران تحت التخدير العميق، بدايتاً محلول ملحي، تلاه مثبت حل يحتوي على 4% بارافورمالديهايد، و0.2% جلوترالدهيد، في 0.1M فوسفات عازلة الهيدروجيني pH 7.2. تم استخراج المخ وتجهيزه، وبعد ذلك 50µm قسم إكليلي للنظام البصري LGN، تم تقطيعها وتقسيمها إلى ثلاثة مجموعات: أمامية، وسطى وخلفية. تمت دراسة Cresyl البنفسجي الملطخة باستخدام اضائة مجهرية LM، تم حساب عدد الخلايا العصبية ونفذت باستخدام Olysiabioreports، وتم تطبيقها في الأقسام المتبقية. أجريت هذه الدراسة خلال عام 2007م، في جامعة إيران للعلوم الطبية (IUMS).

النتائج: تبين لنا وجود اختلاف إحصائي في كثافة الخلايا العصبية في المجموعات الثلاث: الأمامية، الوسطى والخلفية.

خاتمة: يمكننا أن نستخلص، بأن تركيز الخلايا العصبية والوصلات الطرفية للخلايا العصبية، قد تسبب تغييرات في كثافة الخلايا العصبية.

Objectives: To clarify the organization of the rat lateral geniculate nucleus (LGN).

Methods: A total of 50 male Sprague-Dawley albino rats of 2 months of age were used in this study carried out in the Iran University of Medical Sciences, Tehran, Iran in Spring-Fall 2007. The rats were cardially perfused under deep ether anesthesia, first with a small amount of saline then with a fixative solution containing 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1M phosphate buffer pH 7.2. The brains were removed, processed, and then 50 µm coronal sections of the LGN were cut and divided into 3 groups: anterior, middle, and posterior third. Cresyl violet stained sections were studied by light microscopy and counts of neurons were carried out with Olysiabio report software of Olympus Microscope in every other section.

Results: We observed that the neuronal density in the anterior, middle, and posterior thirds were statistically different.

Conclusion: The concentration of neuronal terminals and neuronal connections causes changes in neuronal density.

The dorsal lateral geniculate nucleus (dLGN) is the primary structure that integrates visual information from the retina to the visual cortex in mammals.[1] Light, which is converted into a chemical signal, is transmitted to the LGN,[2] and then projected to the visual cortex by relay neurons in the LGN,[3] which also receives cortical and subcortical afferents.[4] Numerous studies have been conducted on the LGN of rats without a precise view of its structure and neuronal organization. In addition, available references on the structure of the LGN are not up-to-date. Anatomical studies show that the layer and cellular organizations of LGN in rat are different from those in cats and primates.[5] The LGN in primates and humans consists of 6 distinct layers in which each retinal axon terminal ends in a certain layer, and in cats, the LGN has 5 layers: A1, A2, C, C1, and C2.[6] The organization of the LGN in rats is based on neither layer nor cellular array, but on the morphology of synaptic vesicles and electron microscope analyses.[6] In this study, our aim is to clarify the organization of the LGN, and to provide a criterion and a review of the rat LGN for future studies in this area.

Methods. A total of 50 Sprague-Dawley albino rats of 2 months of age were used in the study carried out in the Iran University of Medical Sciences, Tehran, Iran in Spring-Fall 2007. Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 for the care and use of laboratory animals, and we received study approval from the Animal Experiments and Ethics Committee of Iran University of Medical Sciences. All efforts were made to reduce animal suffering. The rats were cardially perfused under deep ether anesthesia first with a small amount of saline then with a fixative solution containing 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2. The brains were subsequently removed from the skulls, postfixed for 18-24 hours, and processed for light microscopy. The anatomical location of the LGN was determined, and the boundaries of the LGN according to Paxinos rat brain atlas[7] are: laterally, the third ventricle, medially, the interthalamic tract, and rostrocaudally from interaural 5.70 mm to interaural 3.70 mm. In accordance with Paxinos rat brain atlas, 50 µm coronal sections of LGN were cut and divided into 3 groups: anterior, middle, and posterior third. Cresyl violet stained sections were studied by light microscopy and detailed quantitative analysis, specifically, counts of the number of neurons were carried out with Olysiabio reports. All viable neurons were counted in every other section, and the length of single LGN neurons (45-35 µm from the anterior third to the posterior third) was determined by the Olysiabioreports. The neural density was measured according to the formula: Multiplication of total area of LGN section into the thickness of the section results in the volume of each LGN section where N_n: total number of neurons in each LGN section, V: total volume of each LGN section, A: total area of each section, T_t: tissue thickness, N_s: total number of sections in each third of LGN, V= A*T_t, neuronal density of each LGN section = (N_n/V), neuronal density of each third of LGN= $\sum$ (N_n/V) / N_s.

The SPSS was used for statistical analysis. We used one-way ANOVA to test the equality of means and then we compared the mean differences of different thirds with paired sample t-test.

Results. Light microscopic observation showed that the neuronal density in the anterior third of the LGN is lower than the middle and posterior third and the difference is statistically significant. The results of quantitative analysis of the neuronal density in the LGN are summarized in **Tables 1 & 2**, and also in **Figures 1 & 2**. As shown in these tables and figures, the density of the middle third of the LGN is significantly higher than the anterior third, and lower than the posterior third.

Table 1 - Comparing mean squares between and within the 3 thirds of the lateral geniculate nucleus.

Location	ANOVA	Mean squares	F	*P*-value
Anterior third	Between Groups Within Groups	132 20	6.354	0.004
Middle third	Between Groups Within Groups	104 34	3.071	0.056
Posterior third	Between Groups Within Groups	154 53	2.858	0.068

Table 2 - Correlation of mean squares.

Paired samples test	Correlation	Standard deviation	*P*-value
Anterior third Middle third	0.909	6.140 6.680	0.000
Anterior third Posterior third	0.856	6.140 8.308	0.000
Middle third Posterior third	0.961	6.680 8.308	0.000
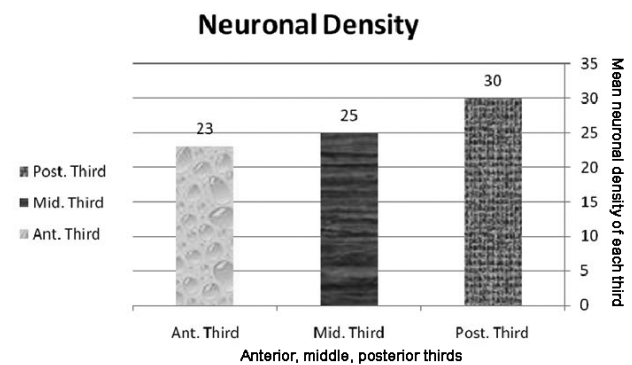
Neuronal Density

Figure 1 - Showing means of neuronal density in anterior (Ant), middle (Mid) and posterior (Post) thirds of the lateral geniculate nucleus.

Discussion. Although there were technical difficulties in measuring the size and volume of LGN neurons without using an electron microscope, the present light microscopic analysis shows that the neuronal density in the rat LGN gradually increases from the anterior to the posterior part of the LGN. The emerging pictures of LGN organization suggest that in rats, it contains a neuronal density gradient that integrates visual information and processes extra-visual information. The subsequent account is based largely on work in the primate and the cat, for which a larger body of evidence is available. Considering the strong concordance across species in LGN neuronal architecture, it is appropriate to use this as a device to propose neuronal organization and to summarize species differences.
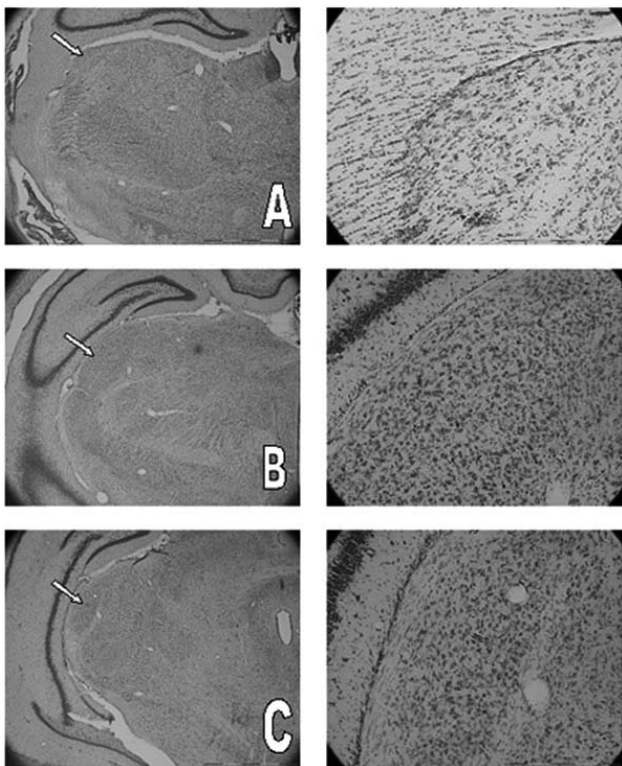
Figure 2 - Coronal sections of the lateral geniculate nucleus (LGN) showing: **A)** anterior third, **B)** middle third and, **C)** posterior third, magnification x 4. The arrows indicate the location of the LGN. The figures on the right are x 20 magnification of the adjacent figures on the left.

Comparison with other species. The organization of LGN neurons is well documented in the primate and the cat, though the anatomical data available in rodents are limited. Extending these findings to those in other species is problematic, since limited anatomical data are available for the rat visual thalamus. The LGN consists of similar structural elements in all mammals. One cell type has been identified in functional terms as a geniculocortical projection relay neuron, while a second type has been identified as an interneuron, whose direct influence remains strictly within the LGN, and these 2 cell types are morphologically distinct. These differences, fundamentally in cell size and characteristics of the dendritic appendages, have permitted investigators to classify the neurons of LGN in several studies of mammals.[8] Guillery[9] in the cat, Campos-Ortega et al[10] in the primate, and Kriebel[11,12] described 2 geniculocortical projection cell subtypes in LGN. Meyer and Albus[13] identified a wide variety of cell subtypes in the cat LGN. Their observation was further confirmed by Hitchcock and Hickey.[14]

Comparison with other studies. It is traditionally thought that retinal projections from each eye are conveyed to the visual cortex without being mixed binocularly. Therefore, the visual thalamus is monocularly laminated in most visual model animals such as cats and monkeys. In the traditional view, rodents have been less favored for visual investigations; despite comprehensive information on their CNS. Although the rodents' visual system has been less studied, their visual system is well developed and contains all the elements seen in cats and primates.[7,15] Retinal projections from each eye project to both superior colliculus and lateral geniculate nucleus.[16] The rats' LGN is not visibly laminated; but 90% of the retinal ganglion cell fibers cross at the optic chiasm to terminate in the contralateral LGN, while the remaining 10% course ipsilaterally to the ipsilateral LGN.[17] Therefore, neurons of this hidden lamination respond to either eye, monocularly.[18]

Anatomical studies of the LGN of the rat show that, although it appears relatively undifferentiated compared with that of the cat and primate, it has a number of features in common with these animals. There are 3 groups of synaptic patterns created by the ganglion cells terminals: complex encapsulated zones, simple encapsulated zones, and simple nonencapsulated zones. The complex encapsulated zones are seen only in the outer surface covering the LGN, while the simple zones can be seen throughout the LGN. The distribution of complex and simple zones in rats' LGN is similar in general pattern to that in the A and C laminae of the cat.[19] Light and electron microscopic observations made by Kriebel in 1975,[12] showed that neurons of the rat dLGN were classified into 3 categories based on location, dendritic patterns, and dendritic appendages. Type 1 and type 3 neurons were distributed throughout the dLGN, but type 2 neurons were located in the superficial zone. Dendritic appendages of type 1 and 2 neurons showed that these cells act as geniculocortical relay neurons, while type 3 neurons had lobulated dendritic appendages and an axon that terminated within the dLGN. So et al, in 1985,[20] reported that there were 2 types of neuropil in rodents' dLGN; glomerular neuropils partially enclosed by astrocyte processes, and simpler non-glomerular neuropils. Nonetheless, the organization of the LGN neurons is not well developed compared to the LGN of the primate and the cat.[12] In the dorsal division of the LGN, the pattern of organization is unique: there is no distinct layer in the dLGN, but a gradient array of neurons from anterior to posterior is observed in the present study. In addition, in another study, it has been shown that retinal projections play an important role in cellular organization of LGN.[21]

The rodents' dLGN is not cytoarchitectonically laminated. Input from the 2 eyes occupy separate regions of the dLGN.[22] Neuroanatomical tracing has revealed that the projections of superior colliculus to the dLGN

terminate in the posterior region of the nucleus.[23] The rodents' colliculogeniculate projection terminates in the dorsal part of the dLGN, which is also close to optic nerve terminations. The dorsal part of the dLGN in rodents, adjacent to the optic tract, contains the majority of neurons that receives the greatest density of colliculogeniculate input.[24] In this study, the increased neuronal density from the anterior to posterior third observed in the dLGN confirmed that termination of retinal input from the adjacent optic tract, and also termination of colliculogeniculate input causes the difference in neuronal density. As the optic tract reaches the posterior part of the dLGN, the majority of neurons have coagulated in this region to receive the majority of retinogeniculate inputs, in addition, the posterior region of the dLGN receives a considerable part of colliculogeniculate inputs. That is, normal increase in neuronal density from the anterior to posterior part of the dLGN is necessary to receive these large amounts of retinal and collicular inputs. It is really important to study neuronal organization of the LGN with labelling methods to see how this gradual increase in neuronal numbers of the LGN from anterior to posterior is related to neuronal connections with other visual areas.

We conclude that there is a direct relationship between neuronal connections, concentration of neuronal terminals, and neuronal density in the lateral geniculate nucleus of rats.

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