THE EFFECT of PINEALOCTOMY on the DEVELOPING SUPERIOR Cervical GANGLION of the RAT

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Abstract

Back ground: In the present study Pinealoctomy was used to study the sympathetic innervations of the pineal gland by the superior cervical ganglion (SCG) of the albino rat.

Objective: Following Pinealoctomy, it is expected to observe the Chromatolysis reaction in some neurons of the SCG if they were to innervate the pineal gland (i.e. retrograde Chromatolysis changes).

Methods: Fifty albino rats were used in this study, Pinealoctomy was done, then after a different time interval ganglionectomy was done, in order to study the Chromatolysis in their cell body.

Result: The present study has demonstrated that the most obvious Chromatolysis reaction in the neurons which innervate the pineal gland appeared one day following Pinealoctomy in the young and adult rats. In the SCG of animal, one week following Pinealoctomy,
the number of the Chromatolysis neurons was much less than these seen in the previously mentioned one day animals. In (2 – 6) weeks following Pinealoctomy, the number of the Chromatolysis neurons kept a constant decline.

Conclusion: The work presented in this study showed that the method of tracing the innervations of the pineal gland by removing the target tissue (the pineal gland in this study) appeared to be justified and conclusive.
It has also shown that younger animals, subjected to Pinealoctomy, would react more vigorously with respect to Chromatolysis than adult animals. The present study has also demonstrate that the distribution of these Chromatolysis neurons and for all age groups all over the ganglion being inspected. However they were more abundant in the rostral 2/3 of these ganglions.

Key words: Pineal gland **,** Pinealoctomy , Superior cervical ganglion (S.C.G.), Pinealoctomy, Ganglionectomy Chromatolysis

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Introduction

1- The aim of this study:

The retrograde cell reaction (Chromatolysis reaction) is a well established method for studying neuronal projections. However this method has not been employed in the study of the innervations of the pineal gland. It would be suspected that following Pinealoctomy some neurons with in the SCG which are involved in the innervations of the pineal gland would shown some form of a Chromatolysis reaction.

Therefore a set of experiments were designed to elucidate the following:-

1. To confirm the sympathetic innervations of the pineal gland following Pinealoctomy.
2. To localize and determine the number of the Chromatolysis neurons with in the SCG which have affected by Pinealoctomy.
3. To study the effect of Pinealoctomy on the developing SCG:
   a) At different post – operative intervals with in the same age group.
   b) At different age groups following the same post operative intervals.

Methods

2.1. Grouping of the experimental animals: -

Fifty albino rats (Rattus norrvegicus), both sexes, were used in this study.

The animals were grouped into two major groups: -

A) The young animals group (35 animals): -

In this group, the pineal glands were removed from these animals at the age of 2 weeks.

The animals were kept a live and scarified after different time intervals (i.e. 1 day , I weeks , 2 weeks , 3 weeks , 4 weeks , 5 weeks and 6 weeks , . .), for the removal of the SCG in order to study the Chromatolysis effect on their neurons.

B) The adult animals group (12 animals): -

In this group, the pineal gland was removed from these animals at the age of 10 weeks. The animals were kept a live and scarified after different time intervals (i.e. 1 day, 1 week, 3weeks and 6 weeks) for the removal of the SCG in order to study the Chromatolysis effect on their neurons.

2.2. The procedure of Pinealoctomy.

2.3 The procedure of ganglionectomy.

2 .4 Sham operations : two animals were used.

2. 5 the control animal: one animal was used.

2.6 Processing for light microscope :-

A) Fixation). B) Dehydration. C) Clearing. D) Embedding. E) Sectioning. F) Staining: - cresyl fast violet was used to stain the tissues.

2. 7 Quantization and localization of the Chromatolysis neurons in the SCG.

Results

The results will include the following observations: -

1. The effect of Pinealoctomy on the neurons of the SCG of albino rats.
2. Observation on the SCG one day following Pinealoctomy.
3. Observation on the SCG one week following Pinealoctomy.
4. Observation on the SCG (2 – 6) weeks following Pinealoctomy.

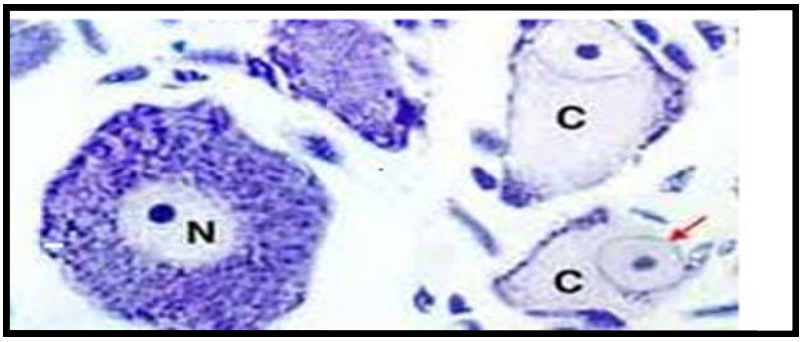
1. The effect of Pinealoctomy on the neurons of the SCG of rats: -

Following different time intervals after removal of the pineal gland, certain characteristic changes (i.e. retrograde changes) appeared in the perikaryon of the SCG which innervate the pineal gland. These changes, as seen by light microscope included: -

A) Changes in the size & shape of the perikaryon: - An early increase in the perikaryon volume of these neurons was a consistent feature after Pinealoctomy. The neurons appeared rather round in shape probably because of the swelling of their cell bodies.

B) Eccentricity of the nucleus: - The nucleus was displaced to the periphery of the cell body. However it was difficult by light microscope to determine whether this displacement was toward the opposite pole of the axon hillock or not.

C) Central Chromatolysis: - Chromatolysis was seen as a fragmentation of the basophilic Nissl substance, a redistribution of basophilic material toward the cell periphery & an apparent reduction in cytoplasmic basophile (with Nissl stains, cytoplasm basophile is due almost entirely to dye – binding by cytoplasm RNA in the form of ribosomes). Picture (1).



Picture 1:- Changes in the shape & size of the perikaryon: normal neuron (N), Chromatolysis (C).

2. Observation on the SCG one day following Pinealoctomy:

In this set of experiments a number of neurons within the SCG demonstrated clear cut Chromatolysis reaction which is recognized by the swelling of these neurons, the eccentricity of their nuclei and their central Chromatolysis. (Picture 1).

In the young age group (2 weeks of age at Pinealoctomy), the average number of the Chromatolysis neurons one day post Pinealoctomy was about (250). (Table 1). The percentages in the three parts of the ganglia were

(42%, 45%, and 13%) (rostral to caudal). (Table3).

The Chromatolysis neurons where distributed all over the SCG. However they were more abundant in the rostral 2/ 3.

In the adult age group (10 weeks of age at Pinealoctomy) the average number of the Chromatolysis neurons was about (55)(Table 2) .The percentages in the three parts of the ganglia were about (37%,50%,13%) (Table 4). (rostral to caudal respectively) . The Chromatolysis neurons were again distributed all over the SCG and mainly in the rostral 2/3. In the sham operated animals, no Chromatolysis neurons were found. Similarly no sign of Chromatolysis were found in the neurons of SCG of the control animal.

3. Observation of the SCG one week following Pinealoctomy:

In the young age group :One week post Pinealoctomy ; the number of Chromatolysis neurons within the SCG was considerably less than in the one day post Pinealoctomy experiments. The average number of these neurons was (120) (Table 1). The distribution of these neurons in the three parts of the ganglion was (33%, 53%, and 14%) (Table 3). (rostal to caudal) respectively . The distribution of the neurons was similar to the previous interval (i . e they were distributed all over the SCG and they were more abundant in the rostal 2/3) .

In the adult age group (10 weeks): One week post Pinealoctomy, the average number of the Chromatolysis neurons was about (51) (Table 2). This number is close to that of the previous interval (i.e. one day post Pinealoctomy).

The distribution of these neurons in the three parts of the ganglia was (18%, 69% & 13%)(Table 4). (rostral to caudal). This distribution was similar to the previous interval (i.e. they were distributed all over the SCG & they were more abundant in the rostal 2/3) .In the sham operated animals and in the control animals no sign of Chromatolysis were seen in the neurons of the SCG.

4. Observation of SCG (2 – 6) weeks following Pinealoctomy:

In these set of experiments, the average number of the Chromatolysis neurons with in the SCG declined gradually. The longer the post operative interval, the fewer Chromatolysis neurons were seen.

For example, in the young age group (i. e 2 weeks) the average number of the Chromatolysis neurons, 2 week after Pinealoctomy was (100) neurons. After 3 weeks the number remained (100) neurons, after 4 weeks the number declined to(68) neurons, after 5 weeks to(55) and after 6 weeks to (53). (Figure A).

However the distribution of the Chromatolysis neurons in all these experiments was all over the ganglion, and more abundant in the rostral 2/3 of the ganglion. (Figure C),

In the adult age group (i. e. 10 weeks); the average number of Chromatolysis neurons three weeks after Pinealoctomy was (47) neurons. This number declined to (40) neurons six weeks after Pinealoctomy. (Figure B).

With regard to the distribution of the Chromatolysis neurons within the SCG; it was found that their percentages three weeks after Pinealoctomy, were(40%) in the rostral part,(51%) in the middle part and(9%) in the caudal part; while their percentages, six weeks after Pinealoctomy were (37.5%) in the rostral part, 52.5% in the middle part & 10% in the coudal part (Figure D).

Table 1: The total number of the Chromatolysis neurons in each post Pinealoctomy interval compared to the total number of the neuronal population in the SCG in the young age group (2 weeks of age at Pinealoctomy).

Post Pinealoctomy Interval	total number of neuron	average number of Chromatolysis neurons	%of the chromtoytic Neurons to the total no.
1 day	14500	250	1.72
1 week	15765	120	0.76
2 week	19605	100	0.51
3 week	19590	100	0.51
4 week	16005	68	0.48
6 week	18420	53	0.24

Table 2: The total number of the Chromatolysis neurons in each post Pinealoctomy interval compared to the total number of the neuronal population in the SCG in the adult age group (10 weeks of age at Pinealoctomy).

post Pinealoctomy interval	total number of neurons	average number of Chromatolysis neurons	% of the Chromatolysis neurons to the total no.
1 day	18020	55	0.30
1 week	16120	51	0.31
2 weeks	12230	47	0.38
6 weeks	11955	40	0.33

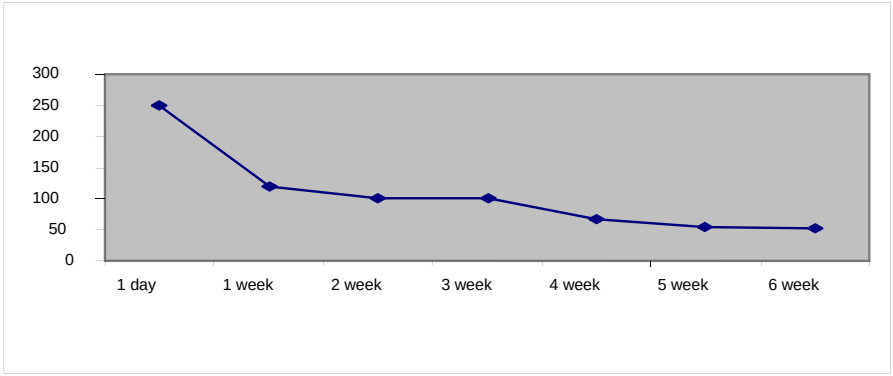


Figure A: The average number of the Chromatolysis neurons in the rat SCG at different post – Pinealoctomy intervals of the young age group.

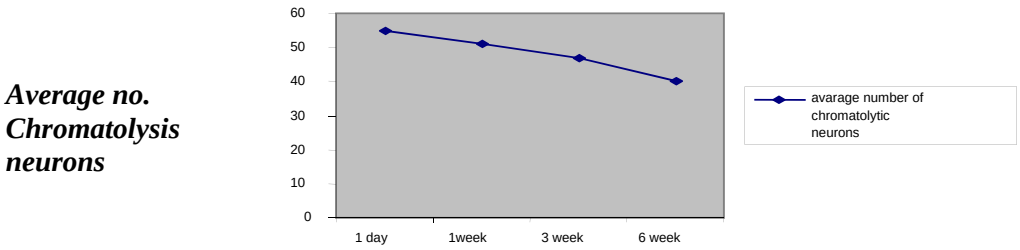


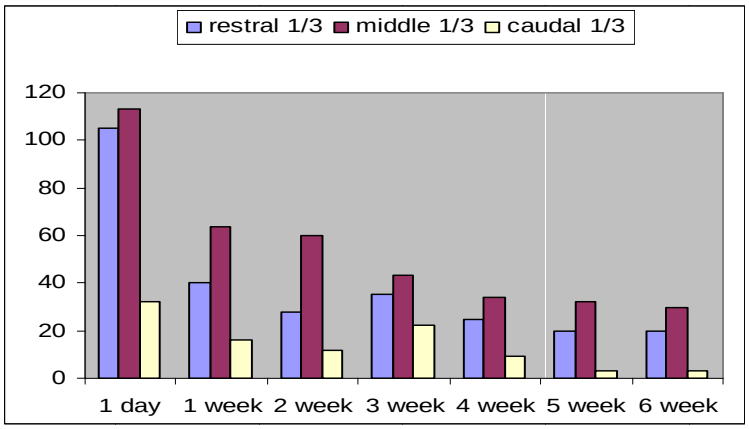
Figure B: The average number of the Chromatolysis neurons in the rat SCG at different post-Pinealoctomy intervals of the adult age group.

Table 3:The total number of Chromatolysis neurons, their number and the percentages of their distributions in the three parts of the SCG in the young age group (i. e 2 weeks of age at Pinealoctomy).

post Pinealoctomy interval	average No. of Chromatolysis neurons	No. &% of Chromatolysis neurons in rostral 1/ 3 No %	No. &% of Chromatolysis neurons in middle 1 / 3 No %	No. &%of Chromatolysis neurons in caudal 1 / 3 No %
1 day	250	105 42%	113 45%	32 13%
1 week	120	40 33%	64 53%	16 14%
2 weeks	100	28 28%	60 60%	12 12%
3 weeks	100	35 35%	43 43%	22 22%
4 weeks	68	25 36%	34 50%	9 14%
5 weeks	55	20 36%	32 58%	3 5%
6 weeks	53	20 37%	30 56%	3 7%
Average percentage	115	33.5%	54%	12 .5%

Table 4:The total number of Chromatolysis neurons, their number and the percentages of their distributions in the three parts of the SCG in the adult age group (10 weeks of age at Pinealoctomy).

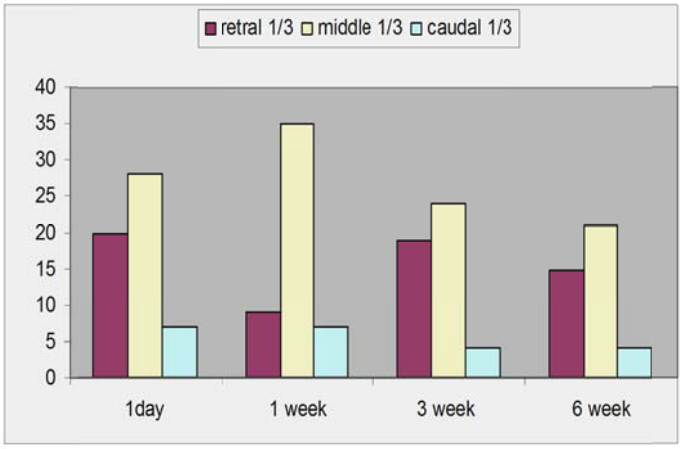
post Pinealoctomy interval	average No. of Chromatolysis neurons	No. &% of Chromatolysis neurons in rostral 1 / 3 No. %	No. &% of Chromatolysis neurons in middle 1 / 3 No. %	No. &% of Chromatolysis neurons in caudal 1 / 3 No. %
1 day	55	20 37%	28 50%	7 13%
1 week	51	9 18%	35 69%	7 13%
3 weeks	47	19 40%	24 51%	4 9%
6 weeks	40	15 37.5%	21 52.5%	4 10%
Average percentage	48.25	33%	56%	10.5%



The number of Chromatolysis Neurons

Post Op. Interval

Figure C: The number of the Chromatolysis neurons in the three parts of the rat SCG in the young age group.



The number of Chromatolysis Neurons

Post Op. Interval

Figure D: The number of the Chromatolysis neurons in the three parts of the rat SCG of the adult age group.

Discussion

1- The sympathetic innervations of the pineal gland using the procedure of Pinealoctomy.
2- The effect of Pinealoctomy on the developing SCG.
A – The post Pinealoctomy time interval.
B – The age of animals at Pinealoctomy.
3- The localization of the Chromatolysis neurons within the SCG following Pinealoctomy.

1-The sympathetic innervations of the pineal gland using the procedure of Pinealoctomy:

The work presented in this study showed that the method of tracing the innervations of the pineal gland by removing the target tissue (the pineal gland in this study) appeared to be justified and conclusive. The importance of this work lies in that it confirms the previous work of kapper (1960) , in that the SCG is the source of the sympathetic supply to the pineal gland , the methods adopted to prove this fact used the retrograde transport of macromolecules such as HRP (Bowers & zigmoid , 1979 , 1984 (1) ; patrickson , 1987 (2)) and the use of

fluorescence microscopy to demonstrate sympathetic nerve ending within the pineal gland (Bartsch H 2000)(3) . Retrograde cell reaction (Chromatolysis) has not been tried in this system.

Until a few years ago the method of axonal injury was the only one that could be used for experimental studies of the connections of the nervous system. This was based on study of the degenerative changes that occur in the neurons when they were damaged. Even though the recent method based on the retrograde transport of macromolecules have proved to be superior in many respects , the older method deserves attention , since it forms the basis for much of our fundamental knowledge of the organization of the nervous system .

Beside that, tracer substance such as HRP could not be used in our study of the role of the developing SCG in the innervations of the pineal gland; the reason being that the tracer would not be available in the neurons of the SCG for periods more than (24 – 36) hours following its injection into the pineal gland (Conti A 2001)(4) . On the other hand- Pinealoctomy would provide us with the tool by which this matter could be investigated.

2- The effect of Pinealoctomy on the developing SCG:-

A) The post Pinealoctomy time interval:

In the present study, the length of the post operative period following Pinealoctomy appeared to be an important factor in determining the number of the Chromatolysis neurons seen in the SCG. The earlier the post operative period, the more chromalolytic neurons were found.

It is known from studies on vertebrate's embryos that survival of neurons in the central nervous system and spinal ganglia depends to some extent on their appropriate connections with their target tissues (Parwani AV 2005) (5). Death of neurons take place in some centers normally when their axons do not make proper connections with their targets. For the various groups of neurons there a critical time period during which the proper connection and activation with the target tissue is established and it's the same critical time or slightly longer , that the normal neuronal death occurs in the neuronal group that innervates this tissue (Hopkins & Brown 1984) (6) .

The critical time for the developing SCG is not defined. The very marked Chromatolysis changes in the SCG of young animals, one day following Pinealoctomy could be explained in the basis that Pinealoctomy was done during the critical period.

On the other hand the decline in the number of the Chromatolysis neurons seen in the SCG after longer post operative intervals may reflect the results of recovery or complete degeneration of Chromatolysis neurons.

Regeneration of the Nissels substance following axontomy was reported by many workers. Moreover, liebman PM (2001) (7) suggested that even neurons prevented from reestablishing peripheral contacts may show sign of recovery from the early Chromatolysis. In spite of this, degeneration and complete disintegration of the axotomized neurons were reported by many workers (Ishizawa K 2008) (8).

B. The age of the animal at Pinealoctomy: -

It is well known that most types of neurons are vulnerable to axonal injury in fetal, newborn & Young animals. Peripheral neurons and many classes of intrinsic CNS neurons degenerate in much larger number in young animals than in the adult, even after mild crush or simple transaction lesion.

In the present study, the number of the Chromatolysis neurons in the rat SCG of young animals was greater and more obvious than those seen in adult animals, following the same post Pinealoctomy time interval.

In other words the retrograde charges are always more severe in young animals, (Table 5 & Fig.E).

In considering why immature neurons behave as they do after axotomy;(Kanishi Y 2000)(9) suggested that cellular machinery involved in the normal metabolic system, is not fully developed in the immature neurons & cannot sustain the anabolic response following axonal injury and furthermore the development of a normal content and distribution of Nissel substance is commonly taken as one of the important cytological index of normal maturation .

Kumar (2006) (10) considered that in vulnerable neurons the characteristic adult pattern of Nissl substance has not yet been established it's adult configuration . Liebman (2001) has postulated that immature neurons may have a different metabolic program than do adult neurons & that theory may lack a number of basic homeostatic mechanisms that appear with maturation. Possibly relevant to this issue is the fact found by Parwani (2005) that when adult neurons are axotomized, they shift to a pattern of protein metabolism more nearly resembling that in the normal young animal .

Table 5: The effect of the age of the animal at Pinealoctomy in the number of the Chromatolysis neurons seen in both young and adult rat SCG at the same post operative interval.

	1 day Post-Pinealoctomy	1 week post-Pinealoctomy	3 weeks post-Pinealoctomy	6 weeks post-Pinealoctomy
Young animal	250	120	100	53
Adult animal	55	51	47	40

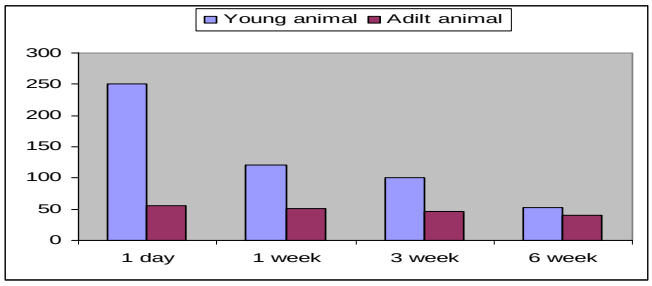


Figure E: The effect of the age of the animals at Pinealoctomy in the number of the Chromatolysis neurons in both young and adult rat at the same post operative intervals.

3-The localization of the Chromatolysis neurons within the SCG following Pinealoctomy:

With regard to the localization and total number of the neurons which project into the rat pineal gland; Bower & Zigmond (1984) have found that the injection of HRP into the rat pineal gland resulted in the labeling of about 250 neurons within the SCG. These neurons were distributed throughout the ganglia , but the majority is found in the rostral part of the ganglia . No labeled neurons were found in the middle or inferior cervical ganglia. Patrickson & smith (1987) using HRP as tracer substance have found a similar distribution of labeled neurons projecting in to the pineal gland from the rat SCG. In the present study, the average numbers of neurons which project in to the pineal gland, seen in the SCG of young age group animal, on day after Pinealoctomy were around (250 + 5). (Table 1) The distribution of these neurons was 105 neurons in the rostral part, 113 neuron in the middle part, and 32 neurons in the caudal part. So the percentage of these neurons was, (42%, 45% & 13%) (rostral to caudal). (Table 3).

These finding indicate that the Chromatolysis neurons were found all over the SCG and occupying mainly the rostral 2 / 3 of the ganglia. (Figure C).

In the SCG of the young animal, one week following Pinealoctomy to the average total number of the Chromatolysis neurons was (120) neurons. Of these neurons, (40) neurons found in the rostral part, (64) neurons found in the middle part and (16) neurons in the caudal part. The percentage of these neurons was (33%, 52% & 14%) (rostral to caudal) (Table 3). This distribution indicates that Chromatolysis neurons are found all over the ganglion however they were abundant in the rostral 2/3. (Figure C), In the SCG from 2 – 6 weeks after Pinealoctomy, for all age groups, the distribution of the Chromatolysis neurons remain similar to the one day & one week post operative interval. Therefore , in the present study , the localization & distribution of the neurons that project in to the pineal gland are similar to these obtained by

Bower & Zigmond (1984) and patrickson & smith; 1987) who have used HRP tracer substance , to retrograde label neuronal perikaryon within the SCG that project into the pineal gland of rat .

Conclusion

The work presented in this study showed that the method of tracing the innervations of the pineal gland by removing the target tissue (the pineal gland in this study) appeared to be justified and conclusive.

It has also shown that younger animals, subjected to Pinealoctomy, would react more vigorously with respect to Chromatolysis than adult animals. The present study has also demonstrate that the distribution of these Chromatolysis neurons and for all age groups all over the ganglion being inspected. However they were more abundant in the rostral 2/3 of these ganglions.

References

1. Bowers, C.W Dalm, L.M. and Zigmoid, R.E. (1984). The number and distribution of sympathetic neurons that innervate the rat pineal gland. Neuroscience. B, 87-96.
2. Patrickson, J.W. and smith, T.E. (1987). Innervations of the pineal gland in the rat. An HRP. Study. Exp. Neurology. 95, 207-215.
3. Bartsch C, Bartsch H, Blask DE. Eds. The Pineal Gland and Cancer: Neuro-immuno-endocrine Mechanisms in Malignancy. Berlin: Springer. 2001.
4. Conti A, Maestroni GJM. Melatonin rhythms in mice: role in autoimmune and lymphoproliferative diseases In: Bartsch C, Bartsch H, Blask DE et al, Eds. The Pineal Gland and Cancer: Neuroimmunoendocrine Mechanisms in Malignancy Berlin: Springer, 2001. 395–407. .
5. Parwani AV, Baisden BL, Erozan YS, Burger PC, Ali SZ. Pineal gland lesions: A cytopathologic study of 20 specimens. *Cancer cytopathology*. 2005;25:105. 80-86.
6. Hopkins, W.C and Brown, M.C. (1984). Development of nerve cells and their connections. Cambridge university press. 4, 20-22,
7. Liebman PM, Wolfler A, Schauenstein K. Melatonin and immune functions In: Bartsch C, Bartsch H, Blask DE et al, eds. The Pineal Gland and Cancer: Neuroimmunoendocrine Mechanisms in Malignancy Berlin: Springer,2001. 371–383.
8. Ishizawa K, Komori T, Shimada S, Hirose T. Olig2 and CD99 are useful negative markers for the diagnosis of brain tumors. *Clin Neuropathology*. 2008;27(3):118–28.
9. Kanishi Y, Kobayashi Y, Noda S. et al. Differential growth inhibitory effect of melatonin on two endometrial cancer cell lines. J Pineal Res. 2000; 28: 227–233.
10. Kumar P, Tatke M, Sharma A, Singh D. Histological analysis of lesions of the pineal region: A retrospective study of 12 years. *Pathology - Research and Practice 202.* 2006. pp. 85–92.

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