

## Research Article

### Does Puberty Affect the Ultrastructure of the Superior Colliculus in Male Albino Rats?

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#### Abstract

**Background:** Puberty is the changes in the period of adolescence. The Superior Colliculus (SC) is located in dorsal midbrain. In mammals, the SC formed of seven layers. These layers are classified into superficial and deep parts. g-ratio is the ratio between the axon diameter and the fiber Identification of the g-ratio is valuable for the functional brain studies. The change in the g-ratio may indicate change in the myelination.

**Aim of the work:** The purpose of this study is to investigate the effect of puberty on the histological structure and ultrastructure of the superior colliculus, and the possible changes in the g-ratio, if present, between prepubertal and post pubertal male rats.

**Materials and Methods:** Twenty male albino rats were divided into pre pubertal and post pubertal rats. Rats of each group were anaesthetized with Ketamine, the superior colliculi were dissected; one used for paraffin sections, the other used for semi thin and ultrathin sections. The diameter of the axons and thickness of myelin, then g-ratio, and synaptic density were measured.

**Results:** The ultrastructural study showed that in post pubertal group there is significant reduction in axon diameter and increased myelin thickness which disturbed the g-ratio. The synapses showed significant reduction in the post-pubertal group.

**Conclusion:** The superior colliculus exhibited post-pubertal changes in the axon diameter, myelin thickness and g-ratio which might affect the nerve conduction velocity, and in turn, may affect its function in the visual pathway, and other cognitive functions.

**Keywords:** G-Ratio; Puberty; Superior Colliculus

#### Introduction

Puberty is the changes in the period of adolescence. It is due to action of the sex hormones which affect physical and sexual growth. The changes in the brain while transforming from childhood to adulthood stages are mostly studied in animal, as the behavior may be the same in all mammals [1]. The study of adolescence on the cellular and molecular levels may confirm the effect of hormones on the brain [2]. The most studied brain regions are the mygdala and the prefrontal cortex (PFC) [3,4]. It has been postulated that the PFC and amygdala may be major targets for the hormones during the puberty [5]. During neuronal growth, the myelin is formed around the axon, secreted by glial cells. Myelin acts as an insulator and helps to increase the speed of impulse transmission between neurons. Although the most of brain myelination

is in the first years of life, axons of the frontal cortex complete their myelination in adolescence. So, the neural transmission in the frontal cortex increase from childhood to adolescence [6]. The synaptic density in the brain change with age. In early postnatal period, there are new synapses, so the synaptic density is more than that in adult [7].

The Superior Colliculus (SC) is located in dorsal midbrain [8]. In mammals, the SC formed of seven layers. These layers are classified into superficial and deep parts. The superficial layers (strata; zonale, griseum superficial and opticum) receive inputs concerning with vision from contralateral field [9]. The output of the superficial part of superior colliculus reaches the thalamus and its pulvinar. The deep layers (strata; griesum intermedium, album intermedium, griseum profundum and album profundum) receive sensory inputs [10]. Those layers were reported to play a major role in saccadic eye movement [11].

G-ratio is the ratio between the axon diameter (d) and the fiber diameter (D);  $g=d/D$ . The fiber diameter refers to the sum of the axon caliber and the myelin thickness [12]. Identification of the g-ratio is valuable for the functional brain studies [13]. The change in the g-ratio may indicate change in the myelination [14]. The increase in the g-ratio is supposed to reflect a reduction in myelination, while the decrease in the g-ratio indicates increased myelination [15]. In the same time, increased fibers' diameter will increase in the g-ratio index [16]. This study was designed to investigate the effect of puberty on the histological structure and ultrastructure of the superior colliculus, and the possible changes in the g-ratio, if present, between prepubertal and post pubertal rats.

## Material and Methods

### Experimental animals

Twenty male albino rats (150-250 gm weight) were used. The use of experimental animals was approved by the Institutional Research Board at Mansoura University, Faculty of Medicine. The rats were housed in Animal Care Centre of Mansoura Faculty of Pharmacy. The animals were housed two rats in a cage, temperature 18 °Cm, the humidity 45%. Fresh food and water was applied to the rats; they were observed for sign of infection.

### Experimental design

Animals were divided into two groups (10 rats each). The first group is pre-pubertal rats (age: 3 weeks to 1 month). The second group is post-pubertal rats, (2 months in average). Puberty in rats was confirmed by the preputial separation which is separation of the penile skin from the glans penis, this occurs from 40 to 50 postnatal days [17].

### Histological Analysis

After two weeks of acclimatization, rats of each group were anaesthetized with Ketamine (60 mg/kg i.p.), the brains were dissected [18] From the dorsal aspect of the midbrain, the superior colliculi were dissected. One colliculus was immersed in Bouin's solution for one day, used for paraffin sections and H & E stain [19].

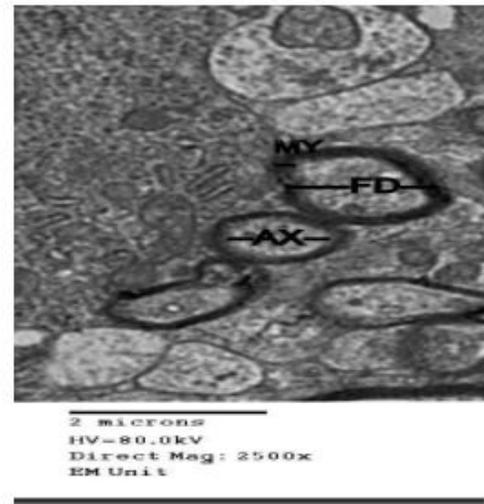
### Tissue preparation for ultrastructural study

The other colliculus was cut into 1mm<sup>3</sup> pieces, fixed in 3.5% glutaraldehyde in 0.1 M phosphate buffer. Then, fixed with 1% osmium tetroxide, and embedded in epon. Semi thin toluidine blue sections (1µm) were prepared. Ultrathin sections (50-70 nm thick) were stained with uranyl acetate and lead citrate [20]. Those

sections examined by JEOL-100SX transmission electron microscope, Tanta University, Faculty of Medicine.

### Quantitative & statistical analysis

The diameter of the axons and thickness of myelin were measured in TEM photos, then g-ratio was calculated (Figure 1). The synaptic density was measured through TEM photos. All synapses have been measured (symmetrical, unsymmetrical and uncharacterized), the synaptic density (NV) was calculated via size-frequency method;  $NV = NA/d$ , NA is the number of synapses per unit area, d is the synaptic junction cross-sectional diameter [21]. All measurements were through Image J analysis software. Statistical analysis was carried out by SPSS program (statistical package for social science) version 10. The Kruskal-Wallis H test (one-way ANOVA) was used for the comparison of the data (g-ratios).  $P<0.05$  was accepted as a significant difference .



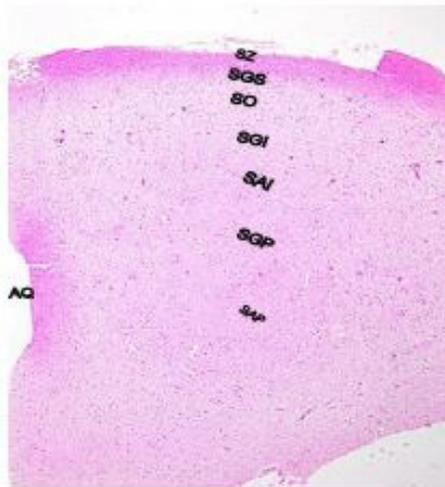
**Figure 1:** Measurements used for calculation of g-ratio; Axon Diameter (AX), Fiber Diameter (FD) and Myelin Thickness (MY).

## Results

### Histological & Ultrastructural results

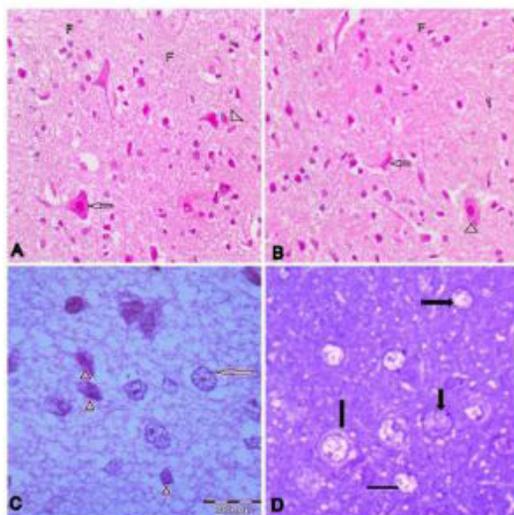
#### Pre-pubertal rats

Light microscopic examination of the Superior Colliculus (SC) in the prepubertal rats showed that it was formed of subsequent layers. From superficial to deep, strata; zonale, griseum superficial, opticum, griseum intermediale, album intermediale, griseum profundum and album profundum (Figure 2).



**Figure 2:** Photomicrograph of section in the superior colliculus, shows superficial layers, Strata Zonale (SZ), griseum superficiale (SGS), Opticum (SO). The deep layers, strata; Griseum Intermediale (SGI), Album Intermediale (SAI), Griseum Profundum (SGP) and Album Profundum (SAP). Aqueduct of Sylvius is noticed (AQ) (H&EX 100).

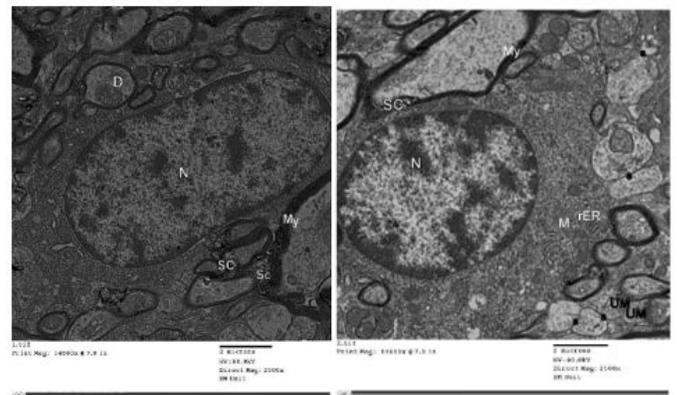
The superior colliculus (superficial and deep layers) showed many small and medium sized neurons with round vesicular nuclei. There were rounded, bipolar and multipolar cells. The neuropil space between the cells was occupied by bundles of transverse nerve fibers. The neuroglial cells appeared small rounded with small nuclei (Figure 3A, 3C).



**Figure 3:** A. Photomicrograph of section in the prepubertal superior colliculus shows many small and medium sized neurons with round vesicular nuclei, bipolar (arrow head) and multipolar cells (arrow). The neuropil

space between the cells is occupied by bundles of transverse nerve fibers (F), the neuroglia cells (G). B. Photomicrograph of section in the postpubertal superior colliculus shows many small and medium sized neurons with round vesicular nuclei, bipolar (arrow head) and multipolar cells (arrow). The neuropil space between the cells is occupied by bundles of transverse nerve fibers (F), the neuroglia cells (G). (A, B: H&E, X400, scale bar=50 µm) C. Photomicrograph of semi thin section in prepubertal superior colliculus shows round neurons with vesicular nuclei (arrow), glial cells (arrow heads) D. photomicrograph of semi thin section in postpubertal superior colliculus shows round neurons with vesicular nuclei (arrows) (C, D: Toulidine blue X 1000, scale bar=200 µm).

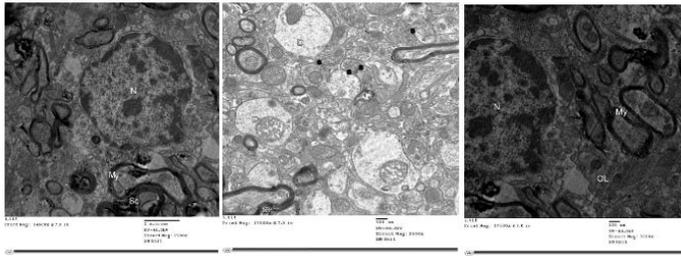
The ultrastructural study showed that neuronal cells had oval or rounded euchromatic nucleus with regular nuclear membrane, rough endoplasmic reticulum, and mitochondria were scattered in the cytoplasm. The nerve fibers and dendrites were variable in shape and diameter. Most of fibers had regular thick myelin. However, there were some unmyelinated fibers. Schwann cells were observed in close relation to the nerve fibers. Synapses were noticed (Figure 4,5).



**Figure 4,5:** Electron micrograph of ultrathin section in pre-pubertal superior colliculus shows that neuronal cells with oval or rounded euchromatic Nucleus (N), Rough Endoplasmic Reticulum (rER), and Mitochondria (M) The nerve fibers with Myelin sheath (My), Schwann Cell (Sc), Unmyelinated Fibers (UM), Dendrites (D), Synapses (s).

### Post-pubertal Rats

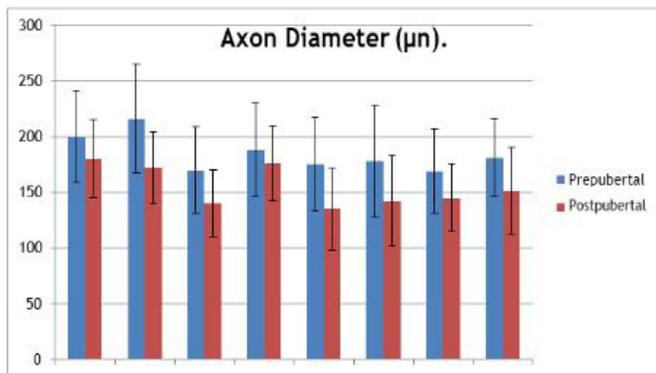
The superior colliculus showed also mixture of rounded, bipolar and multipolar neurons. The neuropil space between the cells was occupied by bundles of nerve fibers. The neuroglia cells appeared small rounded with small nuclei (Figure 3B, 3D). Ultrastructural examination revealed regular, well defined thick myelin sheath around nerve fibers and adjacent oligodendrocyte. The neural cells showed normal cytoplasmic organelles and chromatic nuclei with regular nuclear membrane. More dendrites and synapses were noticed (Figure 6-8).



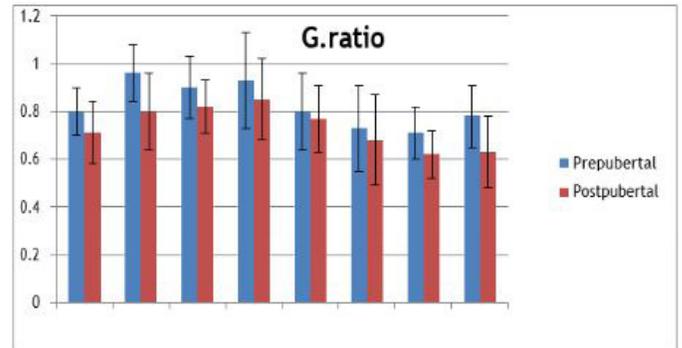
**Figure 6-8:** Electron micrograph of ultrathin section shows that neuronal cells with oval or rounded euochromatic nucleus (N), The nerve fibers with Myelin sheath (My), Schwann cell (Sc), Oligodendrocyte (OL) Dendrites (D), Synapses (s), dendrites (D).

### Morphometric results

1. **Axon diameter:** The post puberal group showed significant reduction in axon diameter when compared to the pre-pubertal group (Histogram 1)
2. **G-ratio:** the myelin thickness increased in post-pubertal group in comparison with the prepubertal group. The decreased axon diameter and increased thickness of myelin sheath in the post pubertal rats disturbed the g-ratio (Histogram 2), it remained in the range (.6) not less but could be more.
3. **Synapses:** Based on size-frequency formula, the synapses showed significant reduction in the post pubertal group ( $2.72 \pm 0.4$  in prepubertal rats,  $1.85 \pm 0.25$  in post pubertal).



**Histogram 1:** The axon diameter changes in pre and post-pubertal superior colliculus.



**Histogram 2:** The g-ratio changes in pre and post-pubertal superior colliculus.

### Discussion

This is the first report, to my knowledge, that refers to effect of puberty on the ultrastructure of superior colliculus in experimental animals. Light microscopic examination of the superior colliculus in the prepubertal rats showed that it was formed of subsequent layers. From superficial to deep, strata; zonale, griseum superficiale, opticum, griseum intermediale, album intermediale, griseum profundum and album profundum. Similar identification of layers in the superior colliculus was reported [22]. Our results in prepubertal superior colliculus (superficial and deep layers) showed many small and medium sized neurons with round vesicular nuclei. There were rounded, bipolar and multipolar cells. The neuropil space between the cells was occupied by bundles of transverse nerve fibers. The neuroglial cells appeared small rounded with small nuclei. The ultrastructural study showed that neuronal cells had oval or rounded euchromatic nucleus with regular nuclear membrane, rough endoplasmic reticulum, and mitochondria were scattered in the cytoplasm. The nerve fibers and dendrites were variable in shape and diameter. Schwann cells were observed in close relation to the nerve fibers. Synapses were noticed. Most of fibers had regular thick myelin. However, there were some unmyelinated fibers. Our finding in superior colliculus comes in agreement with the previous finding in the spinal cord, it was reported that the posterior funiculus myelination in the rat was incomplete at two weeks postnatal, some unmyelinated fibers were observed [23].

The superior colliculus in post pubertal rats (superficial and deep layers) showed also mixture of rounded, bipolar and multi-

polar neurons similar to those in the prepubertal group. The neuropil space between the cells was occupied by bundles of nerve fibers. Ultrastructural examination revealed regular, well defined thick myelin sheath around nerve fibers. The post puberal group showed significant reduction in axon diameter when compared to the pre-pubertal group, while the myelin thickness increased. These changes in axon diameter and myelin thickness resulted in change of g-ratio, it showed significant reduction in postnatal group, but remained in the range (.6) not less but could be more, this ratio was reported as an optimal g-ratio for maximum nerve conduction [24]. In parallel to our result, it was reported that the myelin sheaths continue to increase in thickness till 15 years of age but axons could reach their optimal thickness between 4-5 years of age in human sural nerve [25]. The reduced axon diameter may be a result of increased myelin thickness in adolescence [26]. According to previous reports, the myelin sheath thickness is more effective on conduction velocity than the axon diameter [24]. It is also known that the conduction velocity in any nerve fiber is proportional to its myelin thickness [27]. On the other hand, in contrast to our finding, it was reported that optic nerve diameter is constant with age due to increase in axonal diameter [28].

The reduced myelin g-ratio index in our result could be explained also by increased myelination due to oligodendrocytes over activity [14]. Sex steroid was reported to affect glial cells [29]. Testosterone, in particular, is known for its modulation role in myelination [30]. In the normal level, testosterone has a powerful demyelinating effect, which could be utilized in demyelinating diseases [31]. Based on animal and post-mortem human studies, it was reported that myelination is a continues process in adolescence and is more detected in males than females [32]. With aging, the genes of myelin formation are upregulated [33]. There is a proof that sex steroids have a major role in determination of structure of the brain white matter, like synapses, myelination, dendrites and its branching [34]. It was reported that the volume of the grey matter decreases in adolescence, while the white matter volume increases. This change in white matter volume may be a result of change in axon diameter [14], or myelination which may complete later in life [35]. It was documented that, in boys, the white matter in frontal, parietal and occipital lobes increase in density with puberty, while in corticospinal tracts, it decreases [36]. The brain new synapses tended to be formed in early postnatal life, synaptogenesis, then followed by synaptic pruning at puberty which caused reduction in synaptic density in frontal lobe during adolescence [37]. This comes in agreement with our finding that, based on size-frequency formula, the synaptic density showed significant reduction in post-pubertal superior colliculus when compared to the prepubertal group the synapses ( $2.72 \pm 0.4$  in prepubertal rats,  $1.85 \pm 0.25$  in post pubertal). It was reported that the synaptic density increases in rats (25-40 days of age) [38]. Also, the synaptic density showed significant reduction in visual cortex

and ventromedial prefrontal cortex in adolescent rat [39]. Pruning is mainly detected in asymmetric synapses in motor cortex [40], dentate gyrus molecular layer and dorsolateral prefrontal cortex [41]. This may be due to the fact that asymmetric synapses are excitatory while symmetric synapses are inhibitory. The GABA inhibitory neurons, which are abundant in symmetric synapses, are stable with age [42].

Our finding in this study in the post-pubertal superior colliculus may give a partial explanation for the changes and maturation of functions that the superior colliculus is involved in. Seassau et al. (2012) [43] reported that saccadic performance is age dependent. The horizontal saccades and latencies are well developed after 12 years in human. They suggested that neural circuits in brain regions responsible for saccades are well developed at 12 years old. Also, it was documented that the peak of saccadic velocity in human is in the age from 8-19 years [44]. The superior colliculus is also involved in many cognitive functions rather than its role in the visual pathway and saccadic movement. It was reported that it might have a role in spatial attention based on study of its superficial layer neurons in monkey [45]. In addition, it may be involved in complicated attentional functions and its convert shifts [46]. It may be involved in cognitive skills like planning skills, verbal fluency and motor tasks which were reported to be well developed in adolescence [47]. This may be achieved through projections from deep layers of superior colliculus to pulvinar in thalamus and prefrontal cortex [48]. The nerve conduction velocity, which depend mainly on myelin thickness, was tested in the visual pathway and other brain regions, it showed positive co-relation to intelligence level [49].

In conclusion, the superior colliculus is a unique structure in the central nervous system, it exhibited post-pubertal changes in the axon diameter, myelin thickness and g-ratio which might affect the nerve conduction velocity, and in turn, may affect its function in the visual pathway, saccadic movement and some cognitive functions like intelligence, attention and planning skills.

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