

国人胎儿三叉神经节细胞分化的透射电镜观察 *

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摘要 目的 观察国人胚胎三叉神经节细胞分化及发育过程。方法 取水囊引产 18-36 周国人胎儿三叉神经节 ,HE 染色及透射电镜观察。结果 :18-20 周胎儿三叉神经节神经元排列紧密 ,胞质少 ,可见到数量不多的线粒体 ,且其内几乎看不到嵴 ,其它细胞器少。25 周时 ,线粒体嵴变长 ,粗面内质网雏形出现 ,有纵形小管出现 ,27 周时可观察到成熟的高尔基复合体 ,32 周后 ,线粒体、粗面内质网等细胞器发育趋于成熟。到 33 周电镜下可见溶酶体 ,36 周时细胞内各种细胞器结构和功能基本完善。结论 :人胚胎三叉神经节细胞发育过程中随胎龄增加 ,其结构和功能逐步完善 ,32~36 周(8~9 月)是细胞的分化发育重要时期。

关键词 三叉神经节细胞 电镜 人胚胎

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Electron Microscopic Observations of Trigeminal Ganglion Cells
Differentiation in Chinese Fetus*LIAO Li-bin¹, Tuerxunjiang·Dadihan², Gulnisa·Kerem^{2△}, GUO Lian-ping³, WANG Shui-quan²

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ABSTRACT Objective: To investigate the development and differentiation of human fetus trigeminal ganglion cells with the age increased. **Methods:** Chinese human trigeminal ganglia were obtained from 18-36 weeks old abortion fetuses, and the ultrastructural changes of cells were detected by using optical and transmission electron microscope. **Results:** After 18 to 20 weeks' development, the neurons in the trigeminal ganglion cells tightly packed, with little cytoplasm and can observe a small number of mitochondria which is barely seen the cristae, there were few other organelles, 25 weeks, mitochondria cristae became longer, rough endoplasmic reticulum shape appeared, some showed a longitudinal tubules, the mature Golgi complex appeared at 27 weeks old fetus. After 32 weeks, the development of mitochondria, rough endoplasmic reticulum and other organelles approached maturity; the lysosome can be seen under electron microscope until 33 weeks; At 36 weeks, the structure and function of all kinds of intracellular organelles basically have completely developed. **Conclusion:** The structure and function gradually improved with gestational age increased during human fetal trigeminal ganglion cells development, 32 to 36 weeks (8 ~ 9 months) was an important period of cells differentiation and development.

Key words: Trigeminal ganglion cells; Electron microscopic; Human fetus

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前言

三叉神经痛是累及面部三叉神经一支或几支感觉分布区反复发作的阵发性剧烈疼痛,其病因及发病机制到目前为止尚不明确。从上世纪 60 年代开始国外对三叉神经形态学研究报道^[3-15]较多,治疗手段也在不断改善^[1-3],到八九十年代国内对动物三叉神经节细胞、根及节进行了大量的形态学观察,新近^[16-17]针对人胚胎三叉神经节细胞定位进行研究。但大多都限于动物或者只研究某一时期的发育情况。本研究主要应用 HE 染色和超薄切片技术,对 18-36 周胎儿三叉神经节细胞发育及分化进行观察,试图为三叉神经痛的发病机制提供更详尽的形态学

上的依据,同时给国人胚胎学神经系统的发育添补新的内容。

1 材料与方法

1.1 材料

水囊引产妊娠 18-36 周正常胎儿,共 16 例,性别不限。其中 18、20、23、25、26、28、33 周各 1 例,27、34、36 周各 2 例,32 周 3 例。胎龄根据孕妇末次月经、B 超及标本体检^[18](测量胎儿坐高及称量体重)综合确定。标本均由临床科室提供,通过伦理学会审核,由孕妇自愿捐献,平均死亡时间 2.5h。

1.2 方法

1.2.1 标本固定及取材 从医院获取标本后,生理盐水心脏冲

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洗,再以 10%福尔马林溶液 500-1000 ml 心脏灌注固定,然后将标本浸泡在同浓度福尔马林溶液进行后固定,常温避光保存。保存一段时间后,取胎头开颅,移去端脑和间脑,充分暴露三叉神经节,在外科显微镜下找到其三大分支,切断。从内侧三叉神经根处连同硬脑膜剥离下来,左侧置于新鲜配制的 10%福尔马林溶液中,进行光镜观察。右侧置于 2.5%戊二醛溶液,进行透射电镜观察。

1.2.2 HE 染色与超薄切片 不同胎龄三叉神经节取材后,左侧常规石蜡包埋,切片厚度 $6\text{ }\mu\text{m}$,常规 HE 染色,光镜观察。右侧三叉神经节 2.5%戊二醛溶液中固定 72 h 后,移入 1/15M 磷酸缓冲液(pH7.4)中漂洗 3 次,每次各 15 min,漂洗后组织 1% 饿酸固定液中后固定 1 小时后,相同方法漂洗,丙酮梯度脱水,环氧树脂包埋。用 LKB-2188 超薄切片机切片,选其中 1-2 张切片,甲苯胺蓝染色后在光学显微镜下观察,根据甲苯胺蓝染色结果提供的依据,选择神经元较多的部位再进行超薄切片,定位后选用醋酸双氧铀-枸橼酸铅双染色方法对切片进行染色,切片置于透射电镜观察。

2 结果

2.1 18-20 周胎儿三叉神经节细胞

电镜发现 20 周时,神经元排列紧密,胞质少,突起亦少。细胞的核周质中核糖体丰富,可见到数量不多的线粒体,且其内几乎看不到嵴。高尔基体,内质网,以及神经微管和微丝的结构少见,细胞核较圆,核质较密(图 1)。

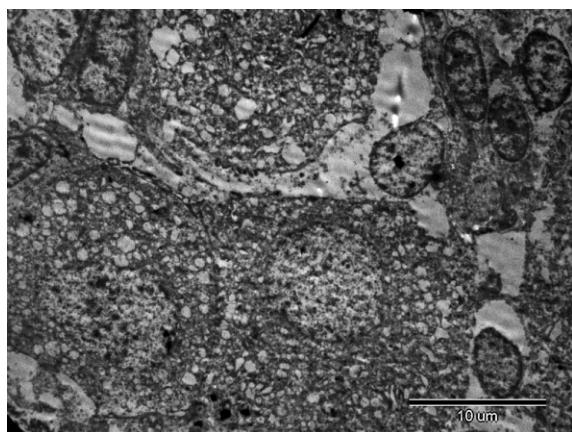


图 1 20 周时三叉神经节细胞透射电镜观察(各细胞器在幼稚形态,结构不完善,TEM $\times 3000$)

Fig.1 Transmission electron microscope observation of trigeminal nerve ganglion cells with immature organelles and imperfect structure of a 20-week-old-fetus

2.2 21-25 周胎儿三叉神经节细胞

光镜可见神经节被结缔组织包被的实质内有许多神经元,由成束的神经纤维分割成大小不等的群状或索状,神经元多呈卵圆形,大小不一,胞质淡蓝紫色。卫星细胞呈扁平形,细胞浆除有核部位较多外,其余部分较少,位于神经元胞体表面。而神经胶质细胞与胞体间隙较大,神经元和神经纤维间有少量间质成份及血管断面,神经纤维颜色浅淡,内含淡红色的轴索和蛋白质网,网眼中有空泡(图 2)。

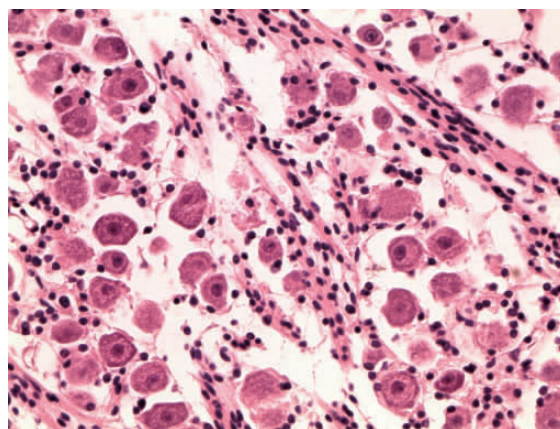


图 2 25 周三叉神经节细胞光镜观察(节细胞发出纤维颜色浅淡,内含淡红色轴索和蛋白质网,网眼中有空泡 HE $\times 400$)

Fig.2 Microscope observation of trigeminal nerve ganglion cells of a 25-week-old-fetus,showing Ganglion cells send light colored fibers with pink axonal and protein network mesh in vacuoles (HE $\times 400$)

电镜下可见粗面内质网雏形,均匀的分布在胞质内,呈小管状,有少许纵形小管,尼氏体也较少。线粒体数量不多,个体不大,基质多,嵴少,主要分布在内膜下,在腔内呈放射状排列,未见高尔基体(图 3)。

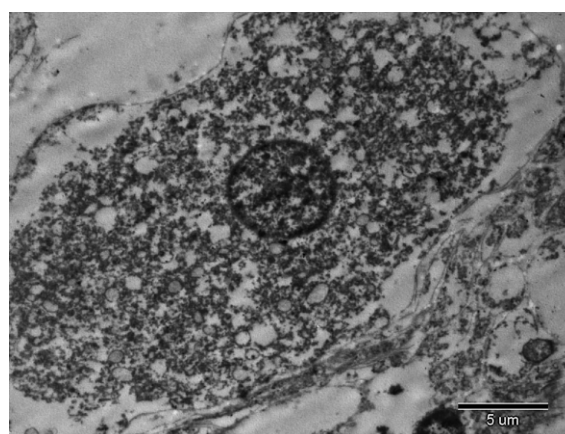


图 3 25 周三叉神经节细胞透射电镜观察(粗面内质网雏形初现,TEM $\times 4000$)

Fig. 3 Rough endoplasmic reticulum beginning to appear in 25-week-old-fetus under transmission electron microscope TEM $\times 4000$

2.3 26-32 周胎儿三叉神经节细胞

光镜下发现(图 4)此时期神经胞体明显比 25 周龄大,细胞间质明显增多,神经胶质细胞与胞体间隙变窄,神经纤维上施旺细胞增多,包绕神经纤维,纤维内空泡减少,纤维的颜色比较深。

电镜可见 28 周(7 月)有较成熟的高尔基体,旁边可见大量分泌泡(图 5),高尔基体是合成突触小泡必不可少的细胞器之一,因此它的成熟对突触的形成有重要的作用。32 周时(图 6)可见细胞内有大量的膨大形线粒体及小型线粒体散布胞质中,前者呈球形或者椭圆形,嵴较疏、有的很短,个别明显贯穿整个线粒体腔,基质电子密度低。数目明显比前面胎龄的要多。粗面内质网和核蛋白体少,均匀分布,有明显的纵形管状粗

面内质网出现。

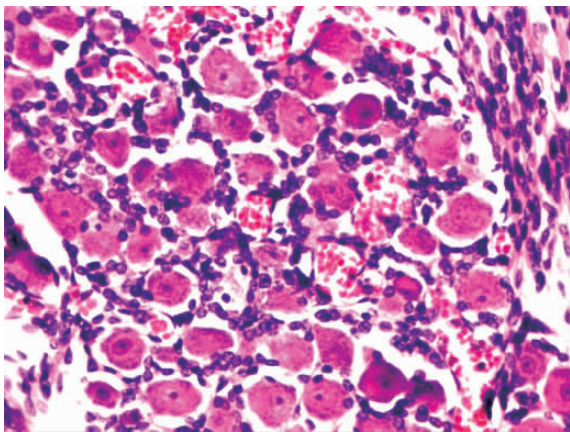


图 4 27 周三叉神经节细胞光镜观察(神经纤维上施旺细胞增多,纤维内空泡减少,纤维颜色比较深,HE× 400)

Fig.4 Microscope observation of trigeminal nerve ganglion cells of a 27-week-old-fetus, showing Schwann cells increased on nerve fibers and the vacuoles decreased in fiber, the color fiber became darker (HE× 400)

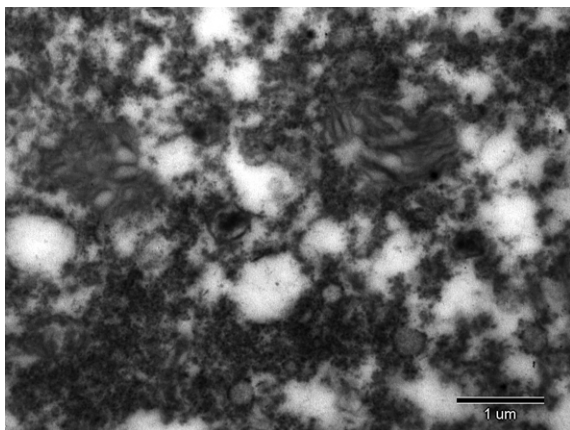


图 5 28 周三叉神经节细胞透射电镜观察(较成熟的高尔基体 TEM× 20000)

Fig. 5 Mature Golgi complex under transmission electron microscope of 28-week-old-fetus.

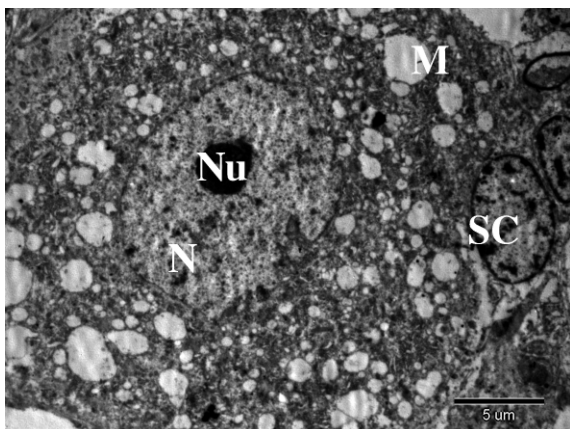


图 6 32 周三叉神经节细胞透射电镜观察(成熟线粒体,有明显的纵行管形状粗面内质网 TEM× 4000)

Fig.6 Transmission electron microscope observation of trigeminal nerve ganglion cells of 32-week-old-fetus, showing mature mitochondria, clear vertical-tube shape of rough endoplasmic

2.4 33-36 周胎儿三叉神经节细胞

33 周胎儿三叉神经节细胞电镜下可看到线粒较大,嵴,完全贯通或半贯通线粒体腔(图 7),贯通所占的比例上比 32 周多,个体普遍比 32 周大。粗面内质网丰富,有很多游离核糖体,在胞质中还可见到散在分布的溶酶体(图 8)。36 周时电镜下(图 9)可见线粒体几乎遍布整个胞质,密度大,基质内充满致密颗粒较多与 33 周比较。光镜下神经胞体排列散在分布于纤维束之间,胞体大小无明显变化,纤维上施旺细胞增多。

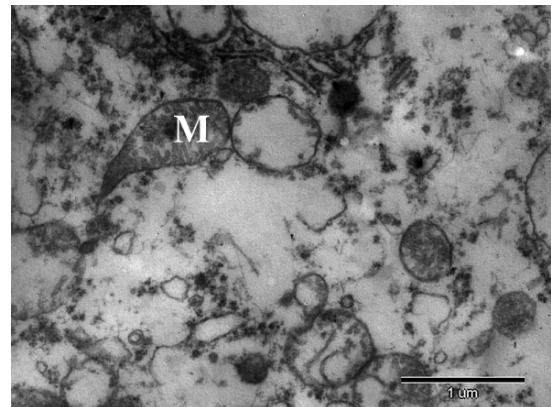


图 7 33 周三叉神经节细胞透射电镜观察(成熟线粒体 TEM× 20000)

Fig. 7 Transmission electron microscope observation of trigeminal nerve ganglion cells of 33-week-old-fetus, showing mature mitochondria TEM× 20000

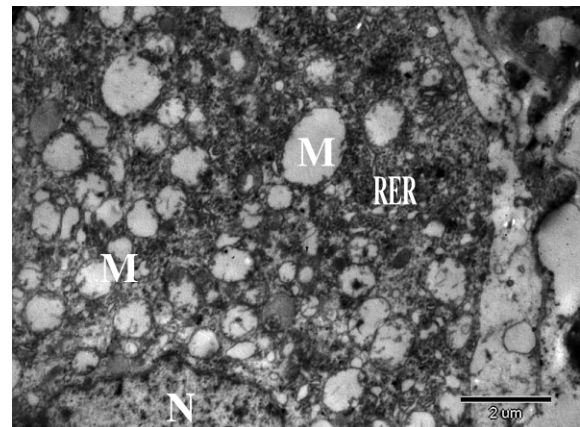


图 8 33 周三叉神经节细胞透射电镜观察(溶酶体 TEM× 10000)

Fig.8 Transmission electron microscope observation of trigeminal nerve ganglion cells of 33-week-old-fetus, showing a lot of lysosomal TEM× 10000

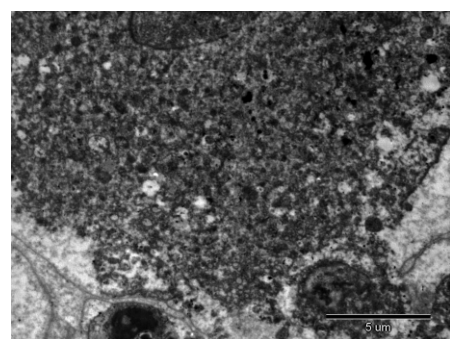


图 9 36 周人胚胎三叉神经节细胞透射电镜观察 TEM× 10000

Fig. 9 Trigeminal nerve ganglion cells of 36-week-old-fetus under transmission electron microscope TEM× 10000

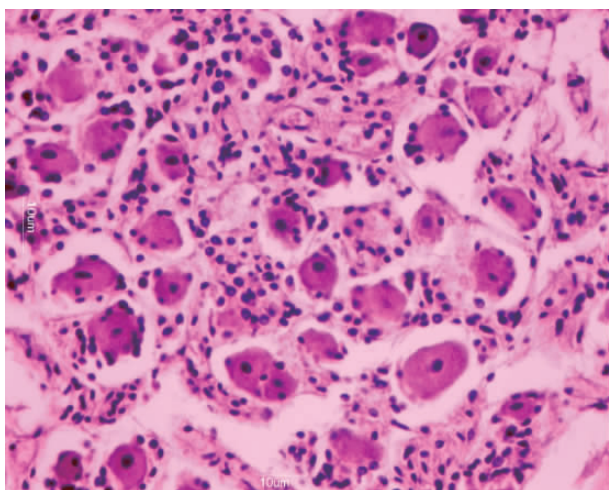


图 10 36 周三叉神经节细胞光镜观察 (HE× 400)

Fig.10 Trigeminal nerve ganglion cells of 36-week-old-fetus under microscope (HE× 400)

注 N 细胞核 ;Nu 核仁 SC 卫星细胞 M 线粒体 ;RER 粗面内质网 ;G 高尔基复合体。

Note: N: nucleus; SC : satellite cell; M: mitochondria RER: rough endoplasmic reticulum G: Golgi complex.

3 讨论

分化指胚胎细胞或幼稚细胞发育为具有特殊形态和功能的专一化细胞的过程。神经细胞分化是解剖学研究领域的热点之一,许多问题尚未完全明了,目前为人们所接受的观点是神经干细胞(未分化细胞学说)。此学说认为,胚胎早期的神经上皮细胞及其向室管膜下层和中间层迁徙的细胞属多能干细胞,这些多能干细胞在一定条件下可以向神经细胞与神经胶质细胞分化。人神经系统的发育始于胚胎第 18 天,神经细胞的分化与成熟是不同步的,因而不同部位不同类型的细胞也是不同时期产生的,一般因为有以下几方面的规律^[18]:大细胞比小细胞发育早,运动神经元比感觉神经元分化早,中间神经元分化较迟,胶质细胞在神经元之后分化。本研究中各时期的三叉神经节细胞随胎龄增长,细胞内个细胞器逐步成熟,线粒体从 20 周幼稚,无嵴,到 33 周时可以清楚看到嵴贯穿整个腔,在数量和结构上也日趋成熟。线粒体含有三羧酸循环、氧化磷酸化及呼吸链等一系列重要酶系,与细胞生命活动有极为密切的关系。同时,它含有自身基因物质即线粒体 DNA、DNA 聚合酶以及合成蛋白质所需的全套物质,因而能够实现自身分裂、繁殖和生长。由于线粒体的高度自主性,在细胞分化过程中,其线粒体的形状、大小及数量可发生改变,成为反应细胞分化、代谢最敏感的细胞器之一。粗面内质网在 25 周出现雏形至 32 周时已经基本成熟,28 周时高尔基体结构清晰可见,高尔基体是合成突触小泡不可少的细胞器之一,它的成熟对突触的形成有重要作用,突触数量和结构发育也在此时期,这与钱雪松^[19]研究的结果相符。神经纤维是神经细胞的突起形成的,周围神经从中枢或神经节出发,刚开始并无髓鞘,被施旺细胞包裹后才产生髓鞘,25 周胎龄光镜下节细胞间有髓神经纤维多,施万细胞的数量少,颜色浅淡,32 周时节细胞周围纤维束紧密,纤维中有

髓神经纤维较少,而无髓的神经纤维较多,施旺细胞多,纤维内空泡少,即髓鞘量少,颜色比较深,三叉神经节含有髓神经纤维和无髓神经纤维,随胎龄增加有髓神经纤维逐渐增多。说明运动纤维的发育比感觉纤维早,即运动神经元比感觉神经元分化早,与神经细胞分化规律相符。

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期不断变换。

本例患者食道电生理检查时出现两个心动过速频率(186次/分 215次/分)提示存在多径路可能,为此在第一次右延伸消融慢径成功后终止一种200次/分的心动过速后,我们观察了40分钟,并多次行完整心内电生理检查,最后终于发现另一频率为158次/分的室上速,在右延伸处不能再成功消融,后于左延伸处消融慢径成功,并最终完全终止室上速的发生。

本例患者提示在食道电生理检查如果发现存在多径路特征时,一定要做出提示,这样心内电生理检查时才会更加注意,必要时会给予延长观察时间、药物刺激等方法加以检验,以争取一次手术解决多种心动过速,减少复发和重复手术,减轻患者痛苦^[14,15]。

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