

·基础研究·

基于BDNF/TrkB信号通路探讨高频重复经颅磁刺激对老年小鼠术后认知功能障碍的影响

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摘要 目的 探讨高频重复经颅磁刺激(rTMS)对老年小鼠术后认知功能障碍(POCD)的影响,并分析其作用机制。**方法** 选择24只18月龄C57BL/6J雄性老年小鼠,采用随机数字表法分为对照组、手术组和rTMS组,每组8只。rTMS组术前连续10 d进行5 Hz rTMS真刺激,随后进行实验性剖腹手术,术后继续4 d rTMS真刺激,1次/d,磁刺激强度0.84 T,频率5 Hz,每个序列100脉冲,间歇时间30 s,重复10个序列,共1 000个脉冲。对照组和手术组术前连续10 d进行伪刺激,对照组不手术,手术组进行实验性剖腹手术,术后对照组和手术组接受4 d伪刺激。术后第3天采用旷场实验评估小鼠的运动能力(中心区域停留时间百分比、运动总距离和平均运动速度);术后第4天采用Y迷宫测试评估小鼠认知功能(进入新异臂次数百分比、新异臂停留时间百分比和新异臂移动总距离);采用Western blot法检测小鼠海马组织脑源性神经营养因子(BDNF)、原肌球蛋白相关激酶B(TrkB)和神经生长因子(NGF)蛋白表达水平。**结果** ①运动功能:3组旷场中心区域停留时间百分比、运动总距离和平均运动速度比较,差异均无统计学意义($P>0.05$)。②认知功能:3组小鼠探索新异臂次数百分比差异无统计学意义($P>0.05$);与对照组比较,手术组新异臂停留时间百分比、新异臂移动总距离均明显降低,差异均具有统计学意义($P<0.05$);与手术组比较,rTMS组新异臂停留时间百分比、新异臂移动总距离均明显升高,差异均具有统计学意义($P<0.05$)。③海马组织蛋白表达水平:与对照组比较,手术组海马组织中BDNF、TrkB和NGF蛋白表达水平均明显降低,差异均具有统计学意义($P<0.05$);与手术组比较,rTMS组海马组织中BDNF、TrkB和NGF蛋白表达水平均明显升高,差异均具有统计学意义($P<0.05$)。**结论** 高频rTMS能改善老年小鼠POCD,其作用机制可能与rTMS激活BDNF/TrkB信号通路和增加NGF表达有关。

关键词 术后认知功能障碍;重复经颅磁刺激;脑源性神经营养因子;原肌球蛋白相关激酶B;神经生长因子

术后认知功能障碍(postoperative cognitive dysfunction, POCD)是指在麻醉/手术后出现的记忆力、注意力、理解能力及社交能力等方面的功能障碍,是手术后常见的中枢神经系统并发症^[1-2]。POCD的

发生率随着年龄增长而升高,青、中年手术患者POCD发生率为30%~36%^[3],而老年手术患者发生率高达51%^[4]。POCD的发病机制复杂,目前认为与麻醉/手术等因素降低脑源性神经营养因子(brain

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derived neurotrophic factor, BDNF)和原肌球蛋白相关激酶B(tropomyosin related kinase B, TrkB)蛋白表达,干扰二者结合从而导致神经功能受损有关^[5-6]。POCD不仅增加医疗时间和成本,降低患者生活质量,还会导致病死率增高。POCD是麻醉与围手术期医学、外科学、神经科学和康复医学等多学科领域交叉的共性难题,至今仍缺乏明确有效的防治措施。

经颅磁刺激(transcranial magnetic stimulation, TMS)是一种无痛、非侵入性的物理治疗技术,重复经颅磁刺激(repetitive transcranial magnetic stimulation, rTMS)是目前广泛使用的磁刺激脉冲,指在大脑皮质的特定部位给予重复、连续的磁刺激,以达到兴奋或抑制神经细胞的作用^[7]。研究表明,高频rTMS能调节BDNF和TrkB蛋白表达水平促进大脑可塑性,提高学习能力和记忆能力^[8]。团队前期研究结果也证实,5 Hz高频rTMS可改善老年小鼠剖腹手术后出现的空间学习能力降低和记忆缺陷^[9],但其机制仍不明确。鉴于老年群体有更高的POCD发生率,本研究拟对老年小鼠进行实验性剖腹手术以构建POCD模型,在围手术期使用高频rTMS进行预防性干预,探讨高频rTMS能否改善老年小鼠POCD,并基于BDNF/TrkB信号通路探讨高频rTMS改善POCD的作用机制。

1 材料与方法

1.1 实验动物

选择18月龄C57BL/6J雄性小鼠24只,体质量(32±2)g,由上海市计划生育研究所实验动物经营部[实验动物生产许可证号:SCXK(沪)2023-0009]提供,饲养于同济大学附属上海市第四人民医院实验动物中心[实验动物使用许可证号:SYXK(沪)2023-0040]。本研究已通过同济大学动物伦理委员会审批(审批号:TJHBLAC2020-68)。

1.2 主要实验仪器和试剂

1.2.1 主要实验仪器 经颅磁刺激治疗仪(武汉依瑞德医疗设备新技术有限公司,型号:CCY-II);旷场装置(型号:63008)、Y迷宫(型号:63010)均购自深圳市瑞沃德生命科技有限公司;行为学跟踪系统(美国Harvard Apparatus公司,型号:Smart 3.0);垂直电泳槽、电泳仪及转膜、凝胶扫描成像仪和Image Lab凝胶自动分析系统均购自美国Bio-Rad公司。

1.2.2 主要实验试剂 BCA蛋白浓度测定试剂盒、RIPA组织细胞快速裂解液均购自上海碧云天生物

技术研究所;蛋白酶抑制剂、磷酸酶抑制剂、发光液均购自上海雅酶生物医药科技有限公司;BDNF抗体(批号:ab108319)、神经生长因子(nerve growth factor, NGF)抗体(批号:ab52987)、 β -actin抗体(批号:ab8226)均购自英国Abcam公司;TrkB抗体(德国Merck Millipore公司,批号:SAB4300702)。

1.3 实验方法

1.3.1 实验动物分组 小鼠适应性饲养1周后,按随机数字表法分对照组、手术组和rTMS组,每组8只。

1.3.2 干预方法

1.3.2.1 rTMS组 术前连续10 d进行5 Hz rTMS真刺激后,随后进行实验性剖腹手术,术后继续4 d rTMS真刺激。将小鼠放置在特制塑料固定器中,该固定器为圆柱形,能暂时限制小鼠活动并暴露小鼠的头顶而不会造成损伤。圆形动物线圈(直径:6.5 cm)连接到经颅磁刺激治疗仪上,使用固定支架将动物线圈固定于小鼠颅顶正中心并轻轻接触颅顶进行治疗。rTMS在每天9:00—11:00进行,1次/d,磁刺激强度0.84 T,频率为5 Hz,每个序列100脉冲,间歇时间30 s,重复10个序列,共1 000个脉冲^[10]。

1.3.2.2 对照组 以手术组和rTMS组手术时间为节点,术前连续10 d rTMS伪刺激,术后继续4 d伪刺激。

1.3.2.3 手术组 术前连续10 d rTMS伪刺激后进行实验性剖腹手术,术后继续4 d伪刺激。

将对照组和手术组接受伪刺激干预的小鼠放置于与rTMS组相同的塑料固定器中,但仅暴露于经颅磁治疗的噪声中而不放置在线圈下进行伪刺激。

1.3.3 动物模型制备 手术组和rTMS组小鼠采用实验性剖腹手术制作POCD模型^[11]。术前所有小鼠禁食禁水6 h,异氟醚麻醉下对小鼠进行实验性剖腹手术。通过面罩输送异氟醚(1.5%~2.0%异氟醚,0.5 L/min氧气)麻醉小鼠后,将小鼠放在恒温垫上(36.5~37.0 °C),刮除腹毛后用75%酒精对皮肤进行消毒,于小鼠腹部中线纵向切开1 cm切口,向两侧分开腹部皮肤和肌肉并轻柔固定,将小肠轻轻从腹腔向外拉出约5 cm,用湿纱布覆盖并轻轻擦拭10 min,然后回纳小肠,逐层缝合腹肌和皮肤,并用0.3 mL 0.2%罗哌卡因涂抹伤口镇痛。术后允许小鼠在35 °C的培养箱中恢复30 min,然后返回其笼中。对照组放置于同一手术室,但不进行手术。

1.4 观察指标

1.4.1 运动功能 术后第3天,采用旷场测试评估

3组小鼠的运动功能^[12]。在旷场测试之前,将小鼠转移到测试室适应30 min。旷场为40 cm×40 cm×20 cm的透明塑料室,底部均分为25个正方形小格,内部9格为中心区域,外部16格为外圈,将小鼠置于旷场中央,让其在旷场内自由活动7 min,采用Smart 3.0行为学系统全程记录并选取后5 min数据进行分析,计算小鼠中心区域停留时间百分比、运动总距离和平均运动速度。不同小鼠测试间隔期间用70%乙醇清洁旷场内部以清除气味。

1.4.2 认知功能 术后第4天,采用Y迷宫测试评估3组小鼠海马相关的辨别性工作记忆,以判断小鼠认知功能^[13]。Y迷宫由3个臂(46 cm×11 cm×25 cm)组成,彼此夹角为120°。在测试之前,让小鼠在测试室中适应30 min。在训练阶段,迷宫的1个臂(新异臂,N)用挡板阻断,将小鼠放置在3条手臂的交叉处,让其在其他2条臂(其他臂,O)中自由探索5 min。2 h后进入测试阶段,打开新异臂的挡板,让小鼠在3条臂中再次探索5 min。测试阶段全程采用Smart 3.0行为学系统记录小鼠进入新异臂次数百分比、新异臂停留时间百分比和新异臂移动总距离,分析小鼠海马相关的辨别性工作记忆。

1.4.3 海马组织蛋白表达水平 采用Western blot法检测小鼠海马组织中BDNF、TrkB、NGF蛋白表达水平。完成所有行为学测试后,每组随机选择4只

小鼠安乐死并立即剥离大脑,分离海马体投入干冰中,移入-80℃冰箱保存使用。海马组织在RIPA缓冲液中裂解匀浆,4℃下1 500 r/min离心10 min,取上清液用BCA法测定总蛋白浓度。根据BCA定量浓度加入适量缓冲液,煮沸5 min后通过8%和15%聚丙烯酰胺凝胶电泳分离蛋白质。将分离的蛋白质转移到PVDF膜上,室温下5%BSA在含有0.1%TBST的TBS缓冲液中封闭膜2 h。去除封闭液后TBST洗膜,加入BDNF(1:1 000)、NGF(1:1 000)、TrkB(1:100)和 β -actin(1:5 000)配置液于4℃摇床孵育过夜,TBST清洗后加入二抗,室温下孵育2 h,再次TBST洗涤后加入化学发光试剂。使用凝胶图像分析成像软件对获得的目标蛋白进行分析和处理。

1.5 统计学方法

采用SPSS 27.0统计软件进行数据分析。计量资料服从正态分布以 $(\bar{x}\pm s)$ 表示,多组比较采用单因素方差分析,组间比较采用Dunnett's T3检验。 $P<0.05$ 表示差异具有统计学意义。

2 结果

2.1 3组旷场测试比较

3组旷场中心区域停留时间百分比、运动总距离和平均运动速度比较,差异均无统计学意义($P>0.05$)。见表1。

表1 3组旷场测试比较 $(\bar{x}\pm s)$

Table 1 Comparison of open field test in three groups $(\bar{x}\pm s)$

组别	n	中心区域停留时间百分比/%	运动总距离/cm	平均运动速度/(cm/s)
对照组	8	14.82±9.11	1 209.02±237.78	4.03±0.79
手术组	8	13.85±10.29	1 131.31±489.22	3.77±1.63
rTMS组	8	12.13±7.31	1 264.56±265.27	4.21±0.88

2.2 3组Y迷宫测试比较

3组探索新异臂次数百分比差异无统计学意义($P>0.05$);与对照组比较,手术组新异臂停留时间

百分比、新异臂移动总距离均明显降低($P<0.05$);与手术组比较,rTMS组新异臂停留时间百分比、新异臂移动总距离均明显升高($P<0.05$)。见表2。

表2 3组Y迷宫测试比较 $(\bar{x}\pm s)$

Table 2 Comparison of Y-maze test in three groups $(\bar{x}\pm s)$

组别	n	进入新异臂次数百分比/%	新异臂停留时间百分比/%	新异臂移动总距离/cm
对照组	8	42.50±13.02	50.18±13.25	320.41±85.61
手术组	8	29.88±6.33	28.72±5.95 ¹⁾	207.68±56.11 ¹⁾
rTMS组	8	36.50±8.88	45.63±16.75 ²⁾	297.16±76.38 ²⁾

注:与对照组比较,1) $P<0.05$;与手术组比较,2) $P<0.05$ 。

Note: Compared with the control group, 1) $P<0.05$; compared with the operation group, 2) $P<0.05$.

2.3 3组BDNF、TrkB和NGF蛋白表达水平比较

与对照组比较,手术组海马组织中BDNF、TrkB和NGF蛋白表达水平均明显降低,差异均具有统计学意义($P<0.05$);与手术组比较,rTMS组海马组织中BDNF、TrkB和NGF蛋白表达水平均明显升高,差异均具有统计学意义($P<0.05$)。见表3、图1。

表3 3组BDNF、TrkB、NGF蛋白表达水平比较($\bar{x}\pm s$)

Table 3 Comparison of expression levels of BDNF, TrkB, and NGF proteins in three groups ($\bar{x}\pm s$)

组别	n	BDNF	TrkB	NGF
对照组	4	1.52±0.01	1.59±0.06	1.90±0.23
手术组	4	1.29±0.03 ¹⁾	1.35±0.14 ¹⁾	1.20±0.19 ¹⁾
rTMS组	4	1.58±0.18 ²⁾	1.70±0.12 ²⁾	1.68±0.28 ²⁾

注:与对照组比较,1) $P<0.05$;与手术组比较,2) $P<0.05$ 。

Note: Compared with the control group, 1) $P<0.05$; compared with the operation group, 2) $P<0.05$.

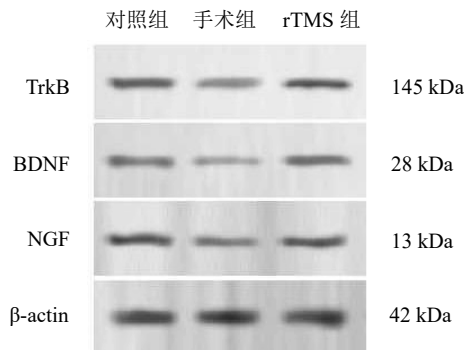


图1 3组海马组织BDNF、TrkB、NGF蛋白条带图

Figure 1 Band diagrams of BDNF, TrkB and NGF proteins of hippocampal tissue in three groups

3 讨论

3.1 高频rTMS可改善老年小鼠术后认知功能障碍

POCD是手术患者,尤其是老年患者术后康复的主要健康问题之一,其发病机制可能与麻醉/手术和衰老等多种危险因素导致的神经功能受损有关^[14]。本研究结果显示,对照组、手术组和rTMS组旷场中心区域停留时间百分比、运动总距离和平均运动速度差异均无统计学意义,提示麻醉/手术因素和经颅磁干预均不会影响小鼠的运动功能。与对照组比较,手术组新异臂停留时间百分比、新异臂移动总距离均明显降低,提示麻醉/手术会导致老年小鼠术后出现认知功能障碍。与手术组比较,rTMS组新异臂中停留时间百分比和移动总距离均

明显升高,提示高频rTMS可改善老年小鼠术后认知功能障碍。这可能与以下因素有关:① 诱导突触可塑性。突触可塑性是学习和记忆的神经基础,麻醉/手术会造成海马神经元的突触可塑性降低,导致POCD发生^[15]。高频rTMS可以调节海马突触可塑性,改善认知功能障碍^[10]。② 提高神经营养因子水平。神经营养因子主要包括BDNF和NGF,对维持神经元的存活、生长、分化及神经损伤后的修复、再生具有重要作用。手术应激和麻醉药物的使用导致大脑和外周血清中BDNF、NGF降低是POCD发生的重要原因^[5,16]。高频rTMS能够增加皮质及海马-皮质环路中BDNF和NGF表达水平,改善认知功能^[17]。③ 降低中枢炎症。麻醉/手术引起炎症因子过度释放导致血脑屏障破坏,继而引起迟发性中枢神经炎症导致神经元损伤,最终表现为各种认知功能受损^[18]。高频rTMS能抑制海马体中促炎因子白细胞介素-1 β (interleukin-1 β , IL-1 β)、白细胞介素-6(interleukin-6, IL-6)和肿瘤坏死因子- α (tumor necrosis factor- α , TNF- α)的释放,改善认知功能障碍^[19]。

3.2 高频rTMS改善老年小鼠POCD可能与激活海马区BDNF/TrkB信号通路有关

本研究结果显示,与对照组比较,手术组海马组织中BDNF、TrkB和NGF蛋白表达水平均明显降低;与手术组比较,rTMS组海马组织中BDNF、TrkB和NGF蛋白表达水平均明显升高,提示高频rTMS可能激活BDNF/TrkB信号通路,改善老年小鼠POCD。可能与以下因素有关:① 提高BDNF和TrkB表达水平。BDNF通过与TrkB结合激活相关下游信号通路发挥神经调控作用,两者表达水平下降或结合不足会导致认知功能异常^[20-21]。麻醉/手术会抑制BDNF/TrkB信号通路而引起学习障碍,激活BDNF途径能有效预防老年小鼠POCD发生^[22-23]。高频rTMS可增加BDNF和TrkB表达,激活BDNF/TrkB通路信号,增强突触可塑性和认知功能,从而改善老年小鼠POCD。这与SHANG等^[24]和ZHAI^[25]等研究结果类似。② 驱动N-甲基-D-天冬氨酸(N-methyl-D-aspartic acid, NMDA)受体激活。NMDA受体与谷氨酸结合可加强突触前和突触后神经元的功能联系,诱导海马的长时程增强(long-term potentiation, LTP)效应,促进突触可塑性,这与学习和记忆密切相关^[26]。谷氨酸能NMDA受体的激活受BDNF/TrkB信号通路的驱动,高频rTMS可通过BDNF/TrkB信号通路增强下游谷氨酸与NMDA受体的亲和力,增

加海马突触可塑性,提高学习和记忆能力,从而改善认知功能^[27]。

4 小 结

高频rTMS能改善老年小鼠POCD,其作用机制可能与激活海马区BDNF/TrkB信号通路、增加NGF表达有关。虽然氯胺酮、地塞米托米丁、类固醇等药物可在一定程度上改善POCD,但是这些药物多数在POCD发生后使用,且长期使用具有一定毒副作用^[28]。早期防治是改善POCD的关键策略。本研究在术前10 d开始采用高频rTMS对老年小鼠进行干预,结果显示高频rTMS可改善老年小鼠术后POCD,这为POCD的预防策略提供了新思路,是对传统POCD预防性策略的补充^[29]。但本研究仍存在一些不足之处,如未对术后海马组织细微结构和神经功能进行观察。下一步研究可采用免疫荧光、功能磁共振等技术观察术后海马区结构和功能改变情况,为rTMS防治POCD提供进一步证据。

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Effect of High Frequency Repetitive Transcranial Magnetic Stimulation on Aged Mice with Postoperative Cognitive Dysfunction Based on BDNF/TrkB Signaling Pathway

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ABSTRACT Objective To explore the effect and its mechanism of high frequency repetitive transcranial magnetic stimulation (rTMS) on aged mice with postoperative cognitive dysfunction (POCD). **Methods** A total of twenty-four 18-month-old C57BL/6J male aged mice were randomly divided into control group, operation group and rTMS group, with eight cases in each group. The rTMS group received 5 Hz rTMS real stimulation for 10 consecutive days before operation, then underwent an experimental laparotomy, and continued with another four days of rTMS real stimulation after operation, once a day, with a magnetic stimulation intensity of 0.84 T, frequency of 5 Hz, 100 pulses per sequence, a 30-second interval time, 10 repetitions for a total of 1 000 pulses. The control group and the operation group received sham stimulation for 10 days before operation, the control group did not undergo the operation, while the operation group received experimental laparotomy, and then the control group and the operation group received sham stimulation for four days. Open field test was used to evaluate motor function (percentage of time spend in the central area, total distance traveled, and average speed of movement) at the 3rd day after operation; Y-maze test was used to evaluate cognitive function (the percentage of entries into the novel arm, the percentage of time to stay in the novel arm and the total distance moved in the novel arm) at the 4th day after operation; Western blot was used to detect the protein expression levels of brain-derived neurotrophic factor (BDNF), tropomyosin related kinase B (TrkB) and nerve growth factor (NGF) in the hippocampal tissue of the mice. **Results** (1) Motor function: there were no significant differences in the percentage of time spent in the central area of the open field, total distance traveled and average speed of movement among the three groups ($P>0.05$). (2) Cognitive function: there was no significant difference in the percentage of entries into the novel arm among the three groups ($P>0.05$); compared with the control group, the percentage of time spent in the novel arm and the total distance moved in the novel arm in the operation group decreased significantly, and the differences were statistically significant ($P<0.05$). Compared with the operation group, the percentage of time spent in the novel arm and the total distance moved in the novel arm in the rTMS group increased significantly, and the differences were statistically significant ($P<0.05$). (3) Protein expression level of hippocampal tissue: compared with the control group, the protein expression levels of BDNF, TrkB and NGF in the hippocampal tissue in the operation group decreased significantly, and the differences were statistically significant ($P<0.05$). Compared with the operation group, the protein expression levels of BDNF, TrkB and NGF in the hippocampus in the rTMS group increased significantly, and the differences were statistically significant ($P<0.05$). **Conclusion** High frequency rTMS can improve POCD in aged mice, and its mechanism may be related to rTMS activating BDNF/TrkB signaling pathway and increasing NGF expression.

KEY WORDS postoperative cognitive dysfunction; repetitive transcranial magnetic stimulation; brain derived neurotrophic factor; tropomyosin receptor kinase B; nerve growth factor

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