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针刺百会穴对产后抑郁小鼠行为学改变和海马区 NMDAR 相关蛋白表达的影响 *

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摘要 目的:研究针刺百会穴对产后抑郁小鼠行为学改变和海马区 N-甲基-D-天冬氨酸受体(NMDAR)相关蛋白表达的影响。**方法:**30只 C57BL/6 母鼠被随机分为对照组、模型组和治疗组,每组 30 只。模型组和治疗组小鼠在妊娠期间通过皮下注射地塞米松磷酸钠建立产后抑郁小鼠模型,对照组小鼠皮下注射等量的生理盐水作为对照。治疗组小鼠分娩后通过针刺百会穴治疗 14 天,模型组和对照组小鼠不进行治疗。比较三组小鼠 24 h 食物消耗量和体重,黑白箱实验中白箱停留时间和黑白箱穿梭次数,以及强迫游泳实验不动状态时间和悬尾实验中悬尾不动时间。同时,通过免疫印记法检测三组小鼠海马去 NMDA 受体(NR2A 和 NR2B)、cAMP 结合蛋白(CREB)和钙调蛋白激酶 II(CaMKII)蛋白表达水平。**结果:**治疗前,产后抑郁小鼠 24 h 食物消耗量、体重、白箱停留时间、黑白箱穿梭次数以及海马区 CREB 蛋白表达水平均显著低于对照组小鼠($P<0.05$),而游泳不同状态时间、悬尾不动时间和海马区 NR2A、NR2B、cAMP 蛋白表达水平均显著高于对照组小鼠($P<0.05$)。治疗后,针刺百会穴治疗组小鼠 24 h 食物消耗量、体重、白箱停留时间和黑白箱穿梭次数以及海马区 CREB 蛋白表达水平均显著高于模型组小鼠($P<0.05$),而游泳不同状态时间、悬尾不动时间和海马区 NR2A、NR2B、cAMP 蛋白表达水平均显著低于模型组小鼠($P<0.05$)。**结论:**针刺百会穴可以显著改善产后抑郁小鼠行为学情况,提高其运动能力,其可能与影响产后抑郁小鼠 NMDAR 相关蛋白表达有关。

关键词:百会穴;针刺;产后抑郁;NMDAR 相关蛋白

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Effects of Acupuncture at Baihui Point on Behavioral Changes and Expression of NMDAR-related Proteins in Hippocampus of Mice with Postpartum Depression*

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ABSTRACT Objective: To study the effect of acupuncture at Baihui point on the behavioral changes and the expression of N-methyl-D-aspartate receptor (NMDAR)-related proteins in the hippocampus of mice with postpartum depression. **Methods:** Thirty C57BL/6 female mice were randomly divided into control group, model group and treatment group, 30 mice in each group. The mice in the model group and the treatment group were subcutaneously injected with dexamethasone sodium phosphate to establish a postpartum depression mouse model during pregnancy, and the mice in the control group were subcutaneously injected with the same amount of normal saline as a control. The mice in the treatment group were treated with acupuncture at Baihui point for 28 days after delivery, while the mice in the model group and control group were not treated. The 24-hour food consumption and body weight of the three groups of mice, the time spent in the white box and the number of shuttles in the black and white box in the black and white box experiment, and the immobility time in the forced swimming experiment and the immobility time in the tail suspension experiment were compared. At the same time, the expression levels of NMDA receptors (NR2A and NR2B), cAMP-binding protein (CREB) and calmodulin kinase II (CaMKII) protein in the hippocampus of the three groups of mice were detected by immunoblotting. **Results:** Before treatment, the 24-hour food consumption, body weight, white box residence time, black and white box shuttle times and CREB protein expression in hippocampus of postpartum depression mice were significantly lower than those of control mice ($P<0.05$). The time of swimming in different states, the time of tail suspension and the protein expression levels of NR2A, NR2B and cAMP in the hippocampus were significantly higher than those in the control group ($P<0.05$). After treatment, the 24-hour food consumption, body weight, white box residence

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time, number of black and white box shuttles, and CREB protein expression in hippocampus of the acupuncture Baihui point treatment group were significantly higher than those of the model group ($P<0.05$). However, the swimming time in different states, tail suspension immobility time and the expression levels of NR2A, NR2B and cAMP proteins in the hippocampus were significantly lower than those in the model group ($P<0.05$). **Conclusion:** Acupuncture at Baihui point can significantly improve the behavior of mice with postpartum depression and improve their exercise ability, which may be related to the expression of NMDAR-related proteins in mice with postpartum depression.

Key words: Baihui point; Acupuncture; Postpartum depression; NMDAR-related protein

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前言

产后抑郁症是产妇产褥期出现典型抑郁的发作,属产褥期精神综合征之一^[1,2]。流行病学统计数据显示,在全球范围内产后抑郁患病率约为 10~15%,我国研究报道的产后抑郁发病率约为 1.1~52.1%,并且初胎产后抑郁患者再次分娩产后抑郁的发生率高达 50%。尽管大部分产后抑郁患者在发病 3~6 月内可自行恢复,但严重的产后抑郁患者其临床症状可持续 1~2 年^[3~6]。产后抑郁症患者常常感觉身体不适,虽然这是一种精神类疾病,不过大多人都会有躯体症状,比如便秘,食欲减退,消化不良,胸闷气短等,并且患者也常常会有自杀倾向^[7,8]。此外,产后抑郁不仅会引起产妇身体不适,而且会影响新生儿的语言能力、智力发育以及身高体重^[7,8]。因此,对产后抑郁患者因进行及时的干预治疗。近年来,多项研究表明^[9~12],针灸可有效改善抑郁患者临床症状。百会穴位于头顶正中线与两耳尖连线的交叉处,穴居颠顶,联系脑部,是调节大脑功能的要穴,针灸百会穴对头痛、目眩、中风、失语、脱肛、阴挺、久泻久痢等临床症状具有较好的改善作用^[13,14]。然而,目前关于针灸百会穴对产后抑郁影响的报道较少。本研究通过皮下注射地塞米松磷酸钠建立产后抑郁小鼠模型以研究针灸百会穴对产后抑郁的影响,为针灸百会穴应用于临床治疗提供参考依据。

1 材料与方法

1.1 实验动物与分组

30 只 C57BL/6 雌性小鼠(6~8 周龄,SPF 级,23~25 g)由齐齐哈尔医学院提供。适应性喂养一周后,随机分为对照组、模型组和治疗组,每组 10 只。本研究过程中动物实验均严格按照国际动物伦理文件相关内容展开,正式实验前动物均适应性饲养一周。

1.2 产后抑郁小鼠模型的建立

所有母鼠均与雄鼠配对饲养,24 h 后通过阴道涂片确认雌鼠怀孕后将其单独饲养。所有妊娠雌鼠在妊娠第 1~14 天皮下注射生理盐水以排除注射影响,妊娠第 15~23 天模型组和治疗组小鼠皮下注射 0.2 mg/kg/d 地塞米松磷酸钠以诱导产后抑郁,对照组小鼠注射等量的生理盐水。

1.3 治疗方案

治疗组小鼠在分娩后每日针刺小鼠百会穴,具体方案如下:针刺小鼠百会穴向前平刺 1~2 mm,平转/平捻 60 次/min,针留 10 min,每日一次,连续 14 天。对照组和模型组小鼠不进行任何治疗。

1.4 观察指标

1.4.1 24 h 食物消耗量 在所有小鼠分娩后和最后一次针刺治疗后,每组小鼠给予准确称重的足量的水和饲料,24 h 后称量饲料重量,计算 24 h 期间饲料重量减少数值,即为 24 h 食物消耗量。

1.4.2 黑白箱行为实验 黑白箱是一个由有盖的不透明黑箱(30 cm×20 cm×30 cm)和无盖的透明白箱(30 cm×20 cm×30 cm)组成,黑白箱之间连壁开一个 5 cm×7 cm 的门洞,记录小鼠在 5 min 内白箱的停留时间和在黑白箱之间穿梭次数。

1.4.3 强迫游泳实验 将小鼠放入一个直径 15 cm,水深 10 cm 的烧杯中(水温 25~27 摄氏度),待小鼠被放入烧杯 2 min 后,记录接下来 4 min 小鼠在水中保持不动状态时间。

1.4.4 悬尾实验 在距小鼠尾尖 2 cm 处,使用胶带粘贴尾巴,并将其固定在距离地面 50 cm 的支架上,首先观察 2 min,然后记录接下来 4 min 小鼠静止不动的时间。

1.4.5 NMDAR 相关蛋白 待治疗组小鼠完成针刺百会穴治疗 and 所有测试后,将所有小鼠通过颈椎脱臼安乐死,分离海马区脑组织,通过组织匀浆器进行组织匀浆,离心以收集组织匀浆液上清,通过免疫印记法检测 NMDA 受体(NR2A 和 NR2B)、cAMP 结合蛋白(cAMP response element binding protein, CREB)和钙调蛋白激酶 II(calmodulin kinase II, CaMKII)蛋白表达水平。

1.5 统计学分析方法

研究数据通过 SPSS20.0 进行统计,以(均值±标准差)计量资料,t 检验比较两组间差异,单因素方差分析比较三组间差异。 $P<0.05$ 表示差异显著具有统计学意义。

2 结果

2.1 各组小鼠 24 h 食物消耗量和体重比较

治疗前,与对照组小鼠相比,模型组和治疗组产后抑郁小鼠 24 h 食物消耗量和体重均显著降低($P<0.05$);治疗后,治疗组产后抑郁小鼠 24 h 食物消耗量与对照组小鼠相比差异无统计学意义($P>0.05$),但显著高于模型组产后抑郁小鼠($P<0.05$);治疗后,治疗组产后抑郁小鼠体重显著高于模型组产后抑郁小鼠($P<0.05$),而显著低于对照组小鼠体重($P<0.05$)。具体如表 1 所示。

2.2 各组小鼠黑白箱实验行为评估结果比较

治疗前,模型组和治疗组产后抑郁小鼠在黑白箱白箱停留时间和黑白箱穿梭次数均显著低于对照组小鼠($P<0.05$);治疗后,治疗组产后抑郁小鼠白箱停留时间和黑白箱穿梭次数均显著高于模型组产后抑郁小鼠($P<0.05$),而显著低于对照组小鼠($P<0.05$)。具体如表 2 所示。

表 1 三组小鼠治疗前后 24 h 食物消耗量和体重比较

Table 1 Comparison of 24-hour food consumption and body weight of three groups of mice before and after treatment

Groups	n	24 hour food consumption (g)		Weight (g)	
		Before treat	After treat	Before treat	After treat
Control group	10	0.21± 0.02	0.24± 0.03	24.15± 2.35	29.72± 3.23
Model group	10	0.15± 0.02*	0.16± 0.05*	21.13± 2.45*	20.19± 3.16*
Treat group	10	0.15± 0.03*	0.23± 0.04* [#]	21.35± 2.71*	26.69± 3.02* [#]
F		7.321	5.317	1.031	4.325
P		<0.001	0.038	0.191	0.047

Note: Compared with Control group, * $P<0.05$; Compared with Model group, [#] $P<0.05$. The same below.

表 2 三组小鼠治疗前后黑白箱实验白箱停留时间和黑白箱穿梭次数比较

Table 2 Comparison of white box residence time and black and white box shuttle times before and after treatment in three groups of mice

Groups	n	White box dwell time (s)		Black and white box shuttle times (n)	
		Before treat	After treat	Before treat	After treat
Control group	10	105.32± 36.31	106.37± 35.42	11.0± 1.35	11.00± 1.71
Model group	10	78.23± 27.15*	76.32± 29.02*	7.00± 1.82*	5.00± 2.05*
Treat group	10	74.39± 26.42*	93.26± 30.18* [#]	7.00± 1.65*	10.00± 2.05* [#]
F		6.328	8.131	10.105	13.205
P		<0.001	<0.001	<0.001	<0.001

2.3 各组小鼠治疗前后强迫游泳和悬尾实验结果比较

治疗前,模型组和治疗组产后抑郁小鼠在强迫游泳实验中不动状态时间和悬尾不动时间均显著低于对照组小鼠($P<0.05$);

治疗后,治疗组产后抑郁小鼠不动状态时间和悬尾不动时间均显著高于模型组产后抑郁小鼠($P<0.05$),而显著低于对照组小鼠($P<0.05$)。具体如表 3 所示。

表 3 三组小鼠治疗前后黑强迫游泳不动状态时间和悬尾不动时间比较

Table 3 Comparison of black forced swimming immobility time and tail suspension immobility time before and After treatment in three groups of mice

Groups	n	Pool not moving time (s)		Tail suspension time (s)	
		Before treat	After treat	Before treat	After treat
Control group	10	110.35± 37.12	112.24± 36.37	23.25± 3.12	24.05± 3.25
Model group	10	168.72± 40.15*	170.65± 41.23*	135.68± 10.28*	145.38± 12.49*
Treat group	10	171.26± 45.62* [#]	120.18± 40.35* [#]	138.65± 11.17* [#]	48.65± 7.74* [#]
F		11.035	10.224	8.713	9.871
P		<0.001	<0.001	<0.001	<0.001

2.4 各组小鼠治疗前海马区 NMDAR 相关蛋白表达

治疗后,模型组和治疗组产后抑郁小鼠海马区 NR2A、NR2B 和 CaMK II 蛋白表达水平均显著高于对照组小鼠,CREB 蛋白表达水平则显著低于对照组($P<0.05$);治疗后,治

疗组产后抑郁小鼠海马区 CREB 蛋白表达水平显著高于模型组小鼠,CREB 蛋白表达水平则显著高于模型组($P<0.05$)。具体如表 4 所示。

表 4 三组小鼠治疗前海马区 NR2A、NR2B、CaMK II 和 CREB 蛋白表达比较

Table 4 Comparison of NR2A, NR2B, CaMK II and CREB protein expression in hippocampus of three groups of mice after treatment

Groups	n	NR2A	NR2B	CaMK II	CREB
Control group	10	0.23± 0.05	0.41± 0.12	0.38± 0.07	0.81± 0.24
Model group	10	0.48± 0.15*	0.89± 0.31*	0.75± 0.27*	0.35± 0.15*
Treat group	10	0.28± 0.11* [#]	0.55± 0.35* [#]	0.45± 0.15* [#]	0.74± 0.23* [#]
F		7.035	11.018	10.326	9.058
P		<0.001	<0.001	<0.001	<0.001

3 讨论

孕产妇在妊娠期间和产后出现心理紊乱和精神卫生问题的机会大于日常,尤其以抑郁和焦虑情绪障碍发病占有率最高^[15,16]。目前,产后抑郁的发病机制尚未被完全揭示,但研究证实产后抑郁发病受遗传因素、神经内分泌功能失调、脑电生理变化以及社会心理因素等因素的影响^[17,18]。不同于其他抑郁症患者,产后抑郁患者由于正处于哺乳期,在临床上一般不建议使用药物治疗,通常对其进行心理干预治疗^[20,21]。近年来,相关研究表明:中国传统的针灸疗法具有改善产后因内分泌、神经系统失调而导致的抑郁症状,对产妇康复有较大裨益^[9,13,14]。

本研究首先通过皮下注射地塞米松磷酸钠以建立产后抑郁小鼠模型,结果发现:皮下注射地塞米松磷酸钠的模型组和治疗组小鼠 24 h 食物消耗量、体质量以及黑白箱实验中白箱停留时间和黑白箱穿梭次数均显著低于对照组正常小鼠,表明本研究通过皮下注射地塞米松磷酸钠成功建立产后抑郁小鼠模型,这一结果与李腾等人^[22]的研究结果一致。李腾等人同样通过皮下注射地塞米松磷酸钠诱导建立产后抑郁模型,发现小鼠 24 h 食物消耗量、体质量以及黑白箱实验白箱停留时间和黑白箱穿梭次数显著低于正常小鼠表明产后抑郁小鼠模型建立成功。结合 Johnson S^[23]和 Atuhaire C^[24]等研究进一步分析可知:产后抑郁患者常常伴有焦虑紧张、失眠、食欲下降、体重减轻和精神病型症状,所以 24 h 食物消耗量和体质量是从宏观层面评估小鼠的抑郁症状。黑白箱实验主要是利用实验小鼠趋暗避光的本能和探索本能以评估小鼠行为学的一种测试,而抑郁小鼠由于感到精神疲乏、兴趣和愉快感丧失会不爱活动而降低趋暗避光的本能和探索本能^[25,26]。

本研究发现,经针灸百会穴治疗的治疗组产后抑郁小鼠 24 h 食物消耗量、体质量均显著升高,而强迫游泳实验不动状态时间和悬尾不动状态时间均显著降低,表明针灸百会穴可显著改善产后抑郁小鼠行为学状况和运动能力,这一结果与李腾等人^[22]的研究结果一致,该研究发现鼠李糖可显著增加产后抑郁小鼠 24 h 食物消耗量和体质量,而降低强迫游泳实验不动状态时间和悬尾不动状态时间,提示鼠李糖可显著改善产后抑郁小鼠行为学状况。进一步分析可知^[13,14]:百会穴是人体最高的一个穴位,又被称为长寿穴,首见于《针灸甲乙经》,归属督脉,别名“三阳五会”。《采艾编》云:“三阳五会,五之为言百也”,意为百脉于此交会。百脉之会,百病所主,故百会穴的治症颇多,为临床常用穴之一。该穴位的保健和锻炼可以提升人体的真气、调节心脑血管系统功能,对头痛、眩晕、低血压等都有很好的治疗效果。此外,本研究还发现,针灸百会穴可显著降低产后抑郁小鼠海马区 NR2A、NR2B 和 CaMK II 蛋白表达水平,而显著提高 CREB 蛋白的表达水平,提示针灸百会穴改善产后抑郁小鼠,与韩远山^[27]等研究结果一致,该研究发现,逍遥抗癌解郁方可通过显著降低海马区 NR2A、NR2B 和 CaMK II 蛋白表达,和提高 CREB 蛋白的表达水平而改善乳腺癌并发抑郁症小鼠抑郁焦虑症状。进一步分析可知^[28-30]:当机体受应激反应时会引起 NR 的过度激活,进而引起 Ca²⁺ 超载,最终引起海马区神经元细胞毒性,导致抑郁症的发生发展;CREB 和 CaMK II 蛋白是 NR 激活的正负反馈调节蛋白,对 NR 的激活发挥调控作用。

综上所述,针刺百会穴可以显著改善产后抑郁小鼠行为学状况,提高其运动能力,其可能与影响产后抑郁小鼠 NMDAR 相关蛋白表达有关,从而为产后抑郁的临床治疗提供动物实验理论基础。

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