· 基础研究 ·

APP/PS1 小鼠不同时期灰质体积变化与 A β 沉积和学习记忆功能的相关性分析

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摘要 目的:分析阿尔兹海默病(AD)双转基因 APP/PS1 模型小鼠大脑灰质体积变化与其学习记忆能力和相关脑区 β -淀粉样蛋白(A β)沉积的相关性, 以期为阐明 AD 动物模型的病理特征提供可靠的行为学和影像学依据。**方法:**共纳入野生组(WT)和 APP/PS1 组 2 组小鼠, 每组 36 只。WT 组小鼠选用 C57-BL/6 小鼠, APP/PS1 组选用 APP/PS1 双转基因小鼠, 2 组均分别由 2、6、12 不同月龄小鼠组成, 每组每个月龄小鼠 12 只。通过 Morris 水迷宫测试(定位航行实验和空间探索实验)检测 2 组小鼠空间记忆学习能力和空间记忆提取能力; 采用磁共振 T2 加权成像(MRI T2WI)扫描检测小鼠大脑双侧内嗅皮质和海马的灰质体积变化情况; 分离和制备 2 组不同月龄小鼠大脑石蜡切片, 采用 Thioflavine-s(Th-s)荧光染色检测 2 组小鼠大脑双侧内嗅皮质和海马的 A β 沉积情况; 采用 Pearson 相关分析法分析 2 组小鼠不同时期的灰质体积变化与其学习记忆能力和对应脑区 A β 沉积的相关性。**结果:**① Morris 水迷宫测试结果: 与 WT 组比较, APP/PS1 组 2 月龄小鼠行为学结果无明显区别, 差异无统计学意义($P>0.05$); APP/PS1 组 6、12 月龄小鼠逃避潜伏期明显增加、穿越平台次数明显减少, 差异具有统计学意义($P<0.05$)。② MRI T2WI 扫描结果: 与 WT 组比较, APP/PS1 组 2 月龄小鼠灰质体积无明显变化, 差异无统计学意义($P>0.05$); APP/PS1 组 6 月龄小鼠内嗅皮质体积明显缩小, APP/PS1 组 12 月龄小鼠内嗅皮质和海马体积均明显缩小, 差异具有统计学意义($P<0.05$)。③ Th-s 荧光染色检测结果: WT 组、APP/PS1 组 2 月龄小鼠均未检测出 A β 沉积; 与 WT 组比较, APP/PS1 组小鼠 6 月龄内嗅皮质和海马可检测到少量 A β 沉积, 而 12 月龄时内嗅皮质和海马则可检测出大量致密 A β 斑块, 差异具有统计学意义($P<0.05$)。④ 相关性分析结果: APP/PS1 组小鼠内嗅皮质和海马体积变化与逃避潜伏期呈明显负相关关系($r=-0.729, P<0.001; r=-0.643, P<0.001$); APP/PS1 组小鼠内嗅皮质和海马体积变化与穿越平台次数呈明显正相关关系($r=0.705, P<0.001; r=0.719, P<0.001$); APP/PS1 组小鼠内嗅皮质和海马体积变化与 A β 沉积呈明显负相关关系($r=-0.865, P<0.001; r=-0.885, P<0.001$)。**结论:** APP/PS1 转基因小鼠可能于 6 月龄时就发生学习记忆功能障碍, 且随时间渐进性加重; 这种学习记忆功能障碍可能与大脑内嗅皮质和海马体积萎缩有关; 而 A β 过度沉积可能是导致大脑灰质体积萎缩的因素之一。

关键词 阿尔茨海默病; 学习记忆; 功能障碍; 内嗅皮质; 海马; 灰质萎缩; β -淀粉样蛋白

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阿尔茨海默病(Alzheimer's disease, AD)作为痴呆类疾病中发病率最高的疾病^[1-2],其临床表现主要为学习记忆功能逐渐受损以及一般认知损伤症状,病理变化特征包括大脑体积萎缩、 β -淀粉样蛋白($\text{amyloid } \beta\text{-protein, A}\beta$)沉积、神经纤维缠结以及神经元凋亡等^[3]。据统计,2015年全球痴呆症患者约4 680万,每年新增1 000万病例,2017年仅美国就有550万成年人患有AD^[4]。因此,探究AD的发病机制,将有可能为临床防治AD提供可靠的理论依据。

大脑皮层灰质是人类思维活动的物质基础,是大脑神经元聚集部位,调控机体各种活动的最高中枢。有研究指出,内嗅皮质(entorhinal cortex, EC)和海马(hippocampus, HP)是大脑参与学习记忆的关键结构^[5-6]。AD患者大脑核磁共振成像结果显示,其灰质体积只有正常受试者的70%~80%,萎缩明显^[7]。AD患者大脑内A β 的产生会导致老年斑的形成、神经纤维的缠绕及神经元的死亡,并随着时间的推移导致痴呆^[8]。APP/PS1转基因小鼠动物模型是探究AD病变机制常用的双转基因模型之一,目前关于APP/PS1灰质体积变化与学习记忆功能的下降以及其A β 沉积的关系仍缺少较为完善的证据,因此本研究通过小动物磁共振扫描观察APP/PS1小鼠灰质体积变化特征,并采用Th-s荧光染色检测其A β 沉积,探讨APP/PS1小鼠灰质体积变化与学习记忆功能和A β 沉积之间的相关性,为该AD动物模型的病理特征及干预性实验研究提供可靠的行为学和影像学依据。

1 材料与方法

1.1 实验动物

本实验共纳入野生组(wild type, WT)和APP/PS1组2组小鼠,每组36只。WT组小鼠选用C57-BL/6小鼠,APP/PS1组选用APP/PS1双转基因小鼠,2组均分别由2、6、12不同月龄小鼠组成,每组每个月龄小鼠12只。分批购买于南京大学南京生物医药研究院[批号:SCXK(苏)2015-0001]。实验前,通过聚合酶链式反应(polymerase chain reaction, PCR)鉴定每组不同月龄小鼠DNA基因型,然后在福建中医药大学实验动物中心SPF级实验室饲养。所有动物实验程序均按国家标准进行并经学校动物实验伦理委员会批准。

1.2 主要实验试剂和设备

Th-s抗荧光衰减封片剂(中国索莱宝有限公司,

S2110);免疫荧光检测试剂盒(上海一基实业有限公司, YJ1012576);异氟烷(中国RWD生命科技有限公司, 1602801);多聚甲醛(中国Biosharp生物科技有限公司, BL539A);Morris水迷宫(中国上海市欣软信息科技有限公司, XR-XM101);7.0T小动物磁共振仪(德国布鲁克科技有限公司, 70/20 USR);徕卡荧光显微镜(德国LEICA公司, DFC425C)。

1.3 实验方法

1.3.1 Morris水迷宫测试 Morris水迷宫为直径120 cm的圆形水池,共分为4个象限,其中东南角为象限1,顺时针以此类推。迷宫水深30 cm左右,逃生平台放置在3象限,为一直径5 cm的透明原型平台,保持其在水面下2 cm左右。水迷宫上方正中配置1个摄像头,记录小鼠游泳轨迹,为方便镜头精确捕捉小鼠影像,在迷宫水面铺洒1层白色塑料颗粒使水面与实验灰黑色小鼠形成颜色对比。实验开始后,拉上水迷宫四周帘幕,以免外界光线干扰摄像机记录小鼠游泳画面。Morris水迷宫分别通过前4 d的定位航行实验和第5天的空间探索实验检测2组小鼠以空间为参考的学习记忆功能。

Morris水迷宫定位航行实验共进行4 d,通过小鼠寻找到逃生平台的时间,检测2组小鼠的空间记忆学习能力。第1天,将小鼠从水迷宫第1象限内侧壁放入水中,测试90 s,其找到逃生平台的时间即为此次逃避潜伏期,若90 s内仍未找到,则逃避潜伏期记为90 s,并引导其找到逃生平台,休息,适应学习平台所在位置15 s,然后从第2象限放入,4个象限依次进行定位航行测试。第2天从第2象限放入,以此类推共进行4 d。第5天进行空间探索实验测试,实验开始前,去掉第3象限的逃生平台。每只小鼠均从第1象限放入,测试90 s,通过记录比较其穿越原逃生平台所在位置的次数,检测其空间学习记忆的提取能力。

1.3.2 磁共振T2加权成像扫描 采用7.0T磁共振(magnetic resonance imaging, MRI)进行T2加权成像(T2-weighted imaging)扫描。扫描前,用1.5%的异氟烷对小鼠进行麻醉,同时监测呼吸情况,体感线圈为激发线圈,表面线圈为接收线圈。检测结束,用ITK-SNAP软件分析对应灰质体积。

1.3.3 Th-s荧光染色检测 使用3.0%异氟烷混合纯氧将小鼠麻醉,从腹部向上打开胸腔,然后用止血钳轻微夹住心脏,灌注针刺破左心室,依次灌注生理盐水和4%多聚甲醛,待小鼠全身抽搐、僵硬后断头取出完整脑组织放入多聚甲醛中,24 h后进行

石蜡包埋切片。用 0.125% 的 Thioflavine-s 溶液避光浸泡 8 min。磷酸缓冲盐溶液(phosphate buffer saline, PBS)洗 3 次,5 min/次,然后用封片剂封片,最后在显微镜下拍摄。分析 2 组不同月龄小鼠大脑双侧内嗅皮质和海马内的 Aβ 沉积情况。

1.4 统计学方法

使用 SPSS 21.0 统计软件分析。计量资料采用 ($\bar{x} \pm s$) 表示。水迷宫各组小鼠的逃避潜伏期符合正态分布且方差齐采用重复测量方差分析,两两比较采用 LSD 法;各组小鼠穿越平台次数、Aβ 沉积以及灰

质体积数据符合正态性分布且方差齐,组间比较采用单因素方差分析,两两比较采用 LSD 法;2 组数据间相关性分析则选择 Pearson 相关分析。 $P < 0.05$ 为差异具有统计学意义。

2 结果

2.1 2 组小鼠学习记忆功能 Morris 水迷宫检测结果比较

见图 1、表 1 和图 2、表 2。

图 1 2 组小鼠逃避潜伏期
Figure 1 Escape latency periods between two groups

表 1 2 组小鼠逃避潜伏期比较 ($\bar{x} \pm s$)
Table 1 Comparison of escape latency between two groups ($\bar{x} \pm s$)

组 别	月龄	n	第 1 天	第 2 天	第 3 天	第 4 天
WT 组	2	12	48.41±15.13	41.93±12.73	33.89±13.29	27.87±7.55
	6	12	59.76±13.81	51.41±16.03	35.13±10.16	26.87±11.38
	12	12	63.70±20.78	48.68±19.07	40.29±19.54	28.71±11.19
APP/PS1 组	2	12	51.52±11.86	46.35±20.21	39.48±11.04	31.19±10.99
	6	12	65.41±12.63	55.42±17.12	50.38±18.20 ¹⁾	41.25±11.97 ¹⁾
	12	12	78.36±11.01	66.70±16.71 ¹⁾	68.10±15.38 ¹⁾	53.12±10.77 ¹⁾

注:与 WT 组同月龄小鼠比较,1) $P < 0.05$ 。
Note: Compared with WT group at the same month, 1) $P < 0.05$.

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Note: Compared with WT group, 1) $P < 0.05$.

图 2 2 组穿越平台次数
Figure 2 Times of crossing the platform between two groups

表 2 2 组穿越平台次数比较 ($\bar{x} \pm s$)
Table 2 Comparison of times of crossing the platform between two groups ($\bar{x} \pm s$)

组 别	月龄	n	穿越平台次数
WT 组	2	12	2.81±0.51
	6	12	2.43±0.67
	12	12	2.45±0.60
APP/PS1 组	2	12	2.43±0.88
	6	12	1.53±0.87 ¹⁾
	12	12	0.61±0.28 ¹⁾

注:与 WT 组同月龄小鼠比较,1) $P < 0.05$ 。
Note: Compared with WT group at the same month, 1) $P < 0.05$.

2.2 2 组小鼠灰质体积 T2W1 扫描检测结果比较
见表 3。

表 3 2 组小鼠灰质体积比较 ($\bar{x}\pm s$)
Table 3 Comparison of gray matter volume
between two groups ($\bar{x}\pm s$) mm^3

组 别	月 龄	n	内嗅皮质	海马
WT 组	2	12	3.48 ± 0.14	25.08 ± 0.84
	6	12	3.53 ± 0.19	24.76 ± 0.83
	12	12	3.64 ± 0.34	25.45 ± 1.32
APP/PS1 组	2	12	3.45 ± 0.15	25.17 ± 0.64
	6	12	$3.09\pm 0.17^{1)}$	24.30 ± 0.81
	12	12	$2.60\pm 0.13^{1)}$	$20.34\pm 1.07^{1)}$

注:与 WT 组同月龄小鼠比较,1) $P<0.05$ 。
Note: Compared with WT group at the same month, 1) $P<0.05$.

2.3 2 组小鼠 A β 沉积结果比较
对 2 组小鼠大脑切片进行 Th-s 荧光染色后, A β 沉积呈白色高密度荧光斑块,见图 3。其中,2 组小鼠 2 月龄时脑内均未检测出 A β 沉积;APP/PS1 组小鼠 6 月龄内嗅皮质和海马即可检测到少量 A β 沉积,而到 12 月龄则可检测出大量致密 A β 斑块。此病理性斑块仅集中于海马和内嗅皮质,其他脑区分布较少。统计分析斑块数量,结果见表 4、图 4。

2.4 APP/PS1 小鼠灰质体积变化与学习记忆能力的相关性分析结果

相关性分析结果显示,APP/PS1 小鼠的灰质体积萎缩与其对应的逃避潜伏期和穿越平台次数分别呈负相关和正相关关系,见图 5、图 6。

图 3 2 组小鼠脑区 A β 沉积情况
Figure 3 A β deposition in brain area of mice between two groups

表 4 APP/PS1 小鼠 6 月龄和 12 月龄 A β 斑块
结果比较 ($\bar{x}\pm s$)

Table 4 Comparison of A β plaque of APP/PS1
group at 6 and 12-month-old ($\bar{x}\pm s$)

月龄	n	内嗅皮质区	海马区
6 月龄	12	13.25 $\pm$ 2.01	12.42 $\pm$ 1.96
12 月龄	12	33.86 $\pm$ 3.61 ¹⁾	28.26 $\pm$ 3.64 ¹⁾

注:与 6 月龄 APP/PS1 组小鼠比较,1) $P<0.05$ 。
Note: Compared with APP/PS1 group at the 6th month,
1) $P<0.05$.

注:与 6 月龄 APP/PS1 组小鼠比较,1) $P<0.05$ 。
Note: Compared with APP/PS1 group at the 6th month,
1) $P<0.05$.

图 4 APP/PS1 小鼠 6、12 月龄 A β 斑块结果比较
Figure 4 Comparison of A β plaque of APP/PS1
group at 6 and 12-month-old

注:图 A 和图 B 分别是 APP/PS1 小鼠内嗅皮质和海马体积与逃避潜伏期的相关性分析。
Note: Figures A and B are the correlation analysis between the volume of the entorhinal cortex, hippocampus and the
escape latency in APP/PS1 group.

图 5 APP/PS1 小鼠灰质体积变化与逃避潜伏期的相关性分析

Figure 5 Correlation analysis between changes in gray matter volume and escape latency in APP/PS1 group

注:图 A 和图 B 分别是 APP/PS1 小鼠内嗅皮质和海马体积与穿越平台次数的相关性分析。
Note: Figures A and B are the correlation analysis between the entorhinal cortex and hippocampus volume and the number of crossing
platforms in APP/PS1 group.

图 6 APP/PS1 小鼠灰质体积变化与穿越平台次数的相关性分析

Figure 6 Correlation analysis between changes in gray matter volume and the number of crossing platforms in APP/PS1 group

2.5 APP/PS1 小鼠灰质体积变化与 A β 沉积相关性分析

见图 7。

注:图 A 和图 B 分别是 APP/PS1 小鼠内嗅皮质和海马体积与 A β 沉积的相关性分析结果。

Note: Figures A and B are the correlation analysis between the entorhinal cortex and hippocampus volume of the APP/PS1 group and A β deposition.

图 7 APP/PS1 小鼠内嗅皮质、海马体积与其 A β 沉积的相关性分析

Figure 7 Correlation analysis between the entorhinal cortex and hippocampus volume and A β deposition in APP/PS1 group

3 讨论

本研究采用 APP/PS1 双转基因模型小鼠作为 AD 模型组,APP/PS1 双转基因小鼠携带与早发 AD 相关的人类淀粉样前体蛋白瑞典型突变基因 (APPSW) 和外显子 9 缺失的 PS1 基因 (PS1 dE9), 同 WT 组实验小鼠一致,均具有 C57-BL/6 背景。C57BL/6 小鼠的生命周期大致可分为幼年期、成年期、中年期和老年期,分别对应 1~3 月龄、3~6 月龄、10~15 月龄和 18~24 月龄^[9-10]。多数研究证实,APP/PS1 小鼠在 6 月龄即会表现出学习记忆功能障碍,并出现神经退行性改变^[11-12],为探究 APP/PS1 小鼠发病特征及其早期病变表现,本实验分别选取 2 月龄、6 月龄和 12 月龄即小鼠幼年期、成年期与中年期进行实验。

3.1 APP/PS1 转基因小鼠学习记忆功能障碍可能发生于 6 月龄

Mirros 水迷宫实验结果显示,与 WT 组比较,APP/PS1 组 2 月龄小鼠的行为学检测结果无明显区别,提示 APP/PS1 小鼠早期无学习记忆功能障碍的表现。与 WT 组同月龄比较,APP/PS1 组小鼠 6 月龄时逃避潜伏期明显增高,穿越平台次数明显降低,且 APP/PS1 组 12 月龄时这种差异表现得更加显著,表明 APP/PS1 小鼠的空间学习记忆功能障碍可能发生于 6 月龄,且 12 月龄时出现加重的情况,这与 TASDEMIR 等^[13-14]研究结果一致。这提示,对于该 AD 模型的干预性实验研究可以选择在更早的时间采取干预措施以预防 AD 的进展。

3.2 大脑灰质进行性萎缩可能导致 AD 小鼠学习记忆功能障碍

本研究 T2WI 序列扫描结果显示,与 WT 组比较,APP/PS1 组 6 月龄小鼠内嗅皮质体积明显缩小,12 月龄小鼠内嗅皮质和海马体积也均明显萎缩,提示 APP/PS1 小鼠大脑灰质具有时间进行性萎缩的病理表现。这与 AD 患者早期阶段常出现的包括内嗅皮质和海马在内的脑实质萎缩,且内嗅皮质和海马的损伤程度常最为严重的研究结果一致^[15-16]。内嗅皮质和海马是保证空间学习记忆功能正常的 2 个重要脑区,内嗅皮质和海马体积萎缩,导致空间学习记忆的定位与导航功能失常,引起 AD 患者早期出现情景记忆损伤的情况^[17-21]。AD 患者脑区病理性变化最早出现的是内嗅皮质,而后逐渐累及海马和大脑新皮质^[22-24]。内嗅皮质、海马和海马旁回在内的内侧颞叶萎缩,被认为是 AD 患者灰质体积变化的主要特征^[25]。本研究结果显示,APP/PS1 组 6 月龄小鼠海马体积未发生明显变化,这与 REDWINE 等^[26]研究发现 PDAPP 小鼠在 3~4 月龄时即出现海马体积减少的结果不一致,这可能是由于核磁扫描序列参数、AD 模型小鼠的遗传背景以及海马体积的检测方法不同所导致的。

此外,APP/PS1 组小鼠大脑灰质体积萎缩与其相应月龄的学习记忆功能相关性分析结果显示:学习记忆功能与其灰质体积的进行性萎缩呈显著负相关关系,大脑灰质体积萎缩越厉害,AD 学习记忆功能障碍越严重,这提示,AD 学习记忆功能障碍可能是由大脑灰质萎缩所导致。这与有研究显示 AD

患者内嗅皮质^[27]、海马体积^[28]的萎缩程度与其认知功能具有一定相关性的研究结果一致。

3.3 A β 过度沉积可能导致 AD 大脑灰质萎缩

本研究通过免疫荧光检测 2 组不同月龄小鼠 A β 沉积情况,研究结果显示,与 WT 组比较,APP/PS1 组 6 月龄小鼠大脑内开始出现 A β 沉积,到 12 月龄时 AD 小鼠 A β 沉积进一步增加,这表明,随着月龄的增加 AD 小鼠脑组织中 A β 沉积逐渐增多。这提示,脑组织中 A β 沉积是 AD 患者的主要病理表现之一,它可能是导致 AD 患者出现进行性记忆功能障碍的最初诱因,这与 HAASS 等^[29-30]研究结果一致。

相关性分析结果显示,AD 小鼠灰质体积与其相应脑区的 A β 沉积程度呈负相关关系,即灰质体积越小,A β 沉积越多。这表明高水平的 A β 沉积和加剧的脑萎缩是 AD 小鼠神经退行性的主要表现,这提示 A β 沉积、灰质体积萎缩是认知功能障碍的主要原因之一。这与 JACK 等^[31-35]研究显示脑组织内出现 A β 沉积,其大海马和皮质的萎缩程度也更高的结果一致。有研究显示,AD 模型小鼠大脑内的 A β 沉积可能与神经元丢失和突触减少有关,其可能诱导大脑灰质体积萎缩性及营养不良性神经元^[36-37],但有关于其具体作用机制的研究还较少,还有待于包括大脑神经功能连接以及分子生物学水平等方面在内更深入的研究。

4 小 结

本研究证实 APP/PS1 小鼠存在时间依赖性的学习记忆功能障碍,且随时间呈渐进性加重,这可能与大脑灰质进行性萎缩有关;AD 大脑灰质体积萎缩与 A β 沉积有明确的相关性,A β 过度沉积可能是 AD 大脑灰质体积萎缩的主要因素之一,但其具体机制尚未明确,今后还需进一步深入研究,探明具体作用机制为临床防治 AD 提供依据。

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Correlation of the Changes of Gray Matter Volume with A β Deposition and Learning and Memory Abilities in APP/PS1 Mice at Different Periods

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ABSTRACT Objective: To analyze the correlation in the changes of cerebral gray matter volume and the ability of memory learning, and the deposition of β -amyloid protein (A β) in related brain regions of Alzheimer's disease (AD) double-transgenic APP/PS1 model mice, which is expected to provide reliable behavioral and imaging basis for probing the pathological features of AD animal models. **Methods:** A total of two groups mice were included in this study, including the wild type (WT) group and the APP/PS1 group, with 36 mice in each group. The C57-BL/6 mice were selected in the WT group, and APP/PS1 double transgenic mice were selected in the APP/PS1 group. The two groups were composed of mice of different ages of 2, 6, and 12 months respectively, with 12 mice of each month in each group. The Morris water maze test (including location navigation test and spatial exploration test) was used to detect the spatial memory learning ability and spatial memory extraction ability of the two groups of mice, and the magnetic resonance T2-weighted imaging (MRI T2WI) scan was used to detect the changes of gray matter volume in the bilateral entorhinal cortex and hippocampus of mice. The brain paraffin sections of two groups of mice of different months were separated and prepared, and the A β deposition in bilateral entorhinal cortex and hippocampus of the two groups was detected by Thioflavine-s fluorescence staining, and the correlation between the changes of gray matter volume and memory learning ability and A β deposition in the corresponding brain area of the two groups was analyzed by Pearson correlation analysis. **Results:** ① The results of Morris water maze test: compared with the WT group, there was no significant difference in the behavioral results of the 2-month-old mice in the APP/PS1 group, and the difference was not statistically significant ($P>0.05$); the escape latency of mice of the APP/PS1 group at the 6 and 12 month-old increased significantly, the number of crossing platforms of the APP/PS1 group at the 6 and 12 month-old decreased significantly, and the difference was statistically significant ($P<0.05$). ② The results of MRI T2WI scan: compared with the WT group, there was no significant change in the gray matter volume of the APP/PS1 group at 2-month-old, and the difference was not statistically significant ($P>0.05$); the volume of the entorhinal cortex of the APP/PS1 group at 6-month-old decreased significantly, and the volume of the entorhinal cortex and hippocampus of the APP/PS1 group at 12-month-old mice decreased significantly, the difference was statistically significant ($P<0.05$). ③ The results of Th-s fluorescence staining test: A β deposition was not detected in WT group and APP/PS1 group at 2-month-old; compared with the WT group, a small amount of A β deposition could be detected in the entorhinal cortex and hippocampus of the APP/PS1 group at 6-month-old, while a large number of dense A β plaques can be detected in the entorhinal cortex and hippocampus of the APP/PS1 group at 12-month-old. ④ Correlation analysis results: APP/PS1 group mice's entorhinal cortex and hippocampus volume changes were significantly negatively correlated with escape latency ($r=-0.729$, $P<0.001$; $r=-0.643$, $P<0.001$); the volume changes of the entorhinal cortex and hippocampus in the APP/PS1 group showed a positive correlation with the number of crossing platforms ($r=0.705$, $P<0.001$; $r=0.719$, $P<0.001$); the volume changes of the entorhinal cortex and hippocampus in the APP/PS1 group were significantly negatively correlated with A β deposition ($r=-0.865$, $P<0.001$; $r=-0.885$, $P<0.001$). **Conclusion:** APP/PS1 transgenic mice may have learning and memory dysfunction at 6-month-old, and it gradually worsen with time; the learning and memory dysfunction may be related to the atrophy of the entorhinal cortex and hippocampus in the brain of mice, and excessive A β deposition may be the one of the factors that cause the brain gray matter volume to shrink.

KEY WORDS Alzheimer's disease; learning and memory; dysfunction; entorhinal cortex; hippocampus; gray matter atrophy; β -amyloid protein

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