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运动调控海马神经元结构可塑性和神经发生改善高脂饮食诱导的肥胖小鼠记忆损害*

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摘要 目的 本研究以高脂饮食诱导的肥胖小鼠为研究对象，以海马神经元结构可塑性和神经发生为切入点，探讨8周运动干预对肥胖小鼠记忆功能的影响及其可能的神经机制。**方法** 6周龄雄性C57BL/6小鼠（20~30 g，60只）随机分为对照组（CON）、高脂饮食组（HFD）和高脂饮食运动组（HFD-Ex）。运动干预前，高脂饮食组和高脂饮食运动组进行20周高脂饮食。运动组小鼠进行8周跑台运动。运动方案为前10 min运动负荷8 m/min，后50 min运动负荷12 m/min，1 h/d，5 d/周，跑台坡度0°。利用Y迷宫和新物体识别测试评估小鼠的记忆水平，并应用免疫荧光染色、蛋白质印迹法（Western blot）、高尔基体染色和酶联免疫吸附分析（ELISA）探究神经元轴突、树突、树突棘、c-fos、双皮质素（DCX）、突触后致密物95（PSD95）、突触素（Syn）、炎症因子IL-1β和主要组织相容性复合体II（MHC-II）阳性小胶质细胞水平。**结果** 肥胖小鼠呈现记忆损害，而运动干预有效改善肥胖小鼠海马依赖性记忆损害。运动通过提高肥胖小鼠海马神经元轴突长度、树突复杂性、树突棘数量、DCX和PSD95表达以增强神经发生和神经元结构可塑性。同时，运动降低肥胖小鼠海马MHC-II阳性小胶质细胞数和IL-1β水平。**结论** 8周有氧运动有效提高肥胖小鼠海马神经发生和神经元结构可塑性，并降低小胶质细胞活化和神经炎症，这可能是运动改善高脂饮食诱导的肥胖小鼠海马依赖性记忆损害的机制之一。

关键词 运动，记忆，海马，神经可塑性，神经发生，肥胖
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中国疾病预防控制中心在《中国居民营养与慢性病状况报告（2020年）》中指出，中国成年居民超重（16.4%）和肥胖（34.4%）的比率超过50%，其中肥胖人数约为8 500万。此外，45~54岁中年人的超重和肥胖比率是18~24岁年轻人的3.83倍。超重和肥胖已经成为当今社会亟待解决的公共健康问题之一。超重和肥胖是心血管疾病、2型糖尿病、多种癌症、一系列骨骼和肌肉疾病的重要风险因素，并可能进一步增加相关疾病的发病率和死亡率。基于人群的研究表明，超重会使痴呆症的发病风险增加35%，而肥胖人群患痴呆症的风险比体重正常者高74%[1]。同时，高脂饮食（high fat diet，HFD）引起的肥胖已被确定为轻度认知障碍、老年性认知功能衰退、阿尔茨海默病（Alzheimer's disease，AD）最重要的风险因素之一[2]。HFD诱导的肥胖小鼠模型可以复制人类因高脂肪或高糖等饮食模式而导致的超重和肥胖，已被广泛用于研究肥胖及相关疾病[3]。基于Y迷宫、Morris水迷宫和新物体识别测试进行的研究表明，HFD会诱发不同年龄C57BL/6小鼠和AD（3×TgAD）模型小鼠海马依赖性记忆损伤[4-5]。有研究甚至发现，6周龄雄性瑞士小鼠进行3 d 60% HFD即可导致小鼠呈现出认知障碍[6]。综上，肥胖与记忆损害密切相关。

海马是哺乳动物大脑中形成记忆的关键脑区，具有明显的神经可塑性和神经发生能力[7-8]。海马神经可塑性和神经发生是各种海马依赖性学习和记

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忆形成的基础。HFD会损害海马神经可塑性，抑制神经发生，并导致海马结构和功能受损[9]。在雌性小鼠中，18周的HFD会显著降低海马背侧未成熟神经元的数量[10]。此外，使用透射电子显微镜进行的研究发现，HFD诱导的肥胖小鼠海马脑区突触密度和突触后密度厚度降低，突触间隙增宽[6, 11]。突触素（synaptophysin，Syn）和突触后致密物95（postsynaptic density-95，PSD95）分别是突触前和突触后的特异性标记物。在HFD诱导的肥胖小鼠海马脑区中，Syn和PSD95的蛋白质表达水平也明显下降[11]。这些结果有力地证明，HFD会损害肥胖小鼠海马神经元结构可塑性和神经发生，并可诱发认知损害。小胶质细胞是大脑驻留的免疫细胞，可介导突触修剪以维持大脑发育过程中的正常突触传递[12]。此外，小胶质细胞还能清除受损细胞和功能障碍的突触（突触剥离）[13]。然而，HFD会诱导小胶质细胞活化和神经炎症，并促使小胶质细胞对海马脑区的突触进行特异性剥离[14]。因此，小胶质细胞的突触剥离可能是饮食诱导肥胖过程中海马功能受损的诱因之一。

中等强度有氧运动能够有效改善心血管功能和肌肉力量，并降低超重、肥胖、心脏病、2型糖尿病和痴呆症的风险[15-16]。本实验室及其他课题组的研究表明，8周规律的有氧运动可提高3×TgAD小鼠、衰老大鼠、血管性痴呆大鼠模型和大脑中动脉闭塞/再灌注（middle cerebral artery occlusion/reperfusion，MCAO/R）模型小鼠海马和皮层神经元突触可塑性，并改善小鼠的学习和记忆缺陷[17-20]。同时，12周跑台运动可通过增强HFD诱导的肥胖小鼠海马脑区内胰岛素信号以改善小鼠在Morris水迷宫测试中的认知功能[21]。Morris水迷宫测试用于评估实验动物的空间学习和工作记忆水平[22]。然而，目前还不清楚跑台运动是否以及如何影响HFD诱导的肥胖小鼠海马依赖性记忆损伤。本研究验证8周跑台运动是否可以改善HFD诱导肥胖小鼠的海马依赖性记忆损伤，并探讨其中的潜在机制，为有氧运动促进肥胖人群神经功能的恢复提供一定的理论依据。

1 材料与方法

1.1 实验动物与造模

6周龄雄性C57BL/6小鼠（20~30 g，60只）购买于广东省医学实验动物中心。将小鼠分为普通饲料（脂肪含量10%）喂养组（对照组，CON，n=20）和高脂（脂肪含量60%）饮食组（HFD，n=40）。喂养20周后，称量小鼠的体重。当HFD组小鼠的体重高于CON组小鼠的20%，则表明HFD诱导的肥胖小鼠造模成功。HFD组小鼠继续进行HFD并随机选取20只作为高脂饮食运动组（HFD-Ex），进行8周规律的跑台运动，而其他小鼠不进行任何形式的运动干预。在整个实验周期中，各组小鼠可自由进食和饮水。小鼠饲养环境温度(23±1)°C、湿度40%~60%、光暗周期为12 h。本研究严格按照广州体育学院伦理委员会批准的实验方案进行小鼠饲养和处理（批准号：2024-DWLL-46）。

1.2 运动干预方案

HFD-Ex组小鼠进行1 h/d、5 d/周，连续8周的跑台运动。1 h/d的运动方案为跑台坡度0°，前10 min运动负荷8 m/min，后50 min运动负荷12 m/min。此外，CON组和HFD组小鼠在同一时间放置于跑台，但不进行运动干预。运动干预后，用Y迷宫和新物体识别测试检测小鼠（34周龄）的记忆水平，并用高尔基体染色、蛋白质印迹法（Western blot）、免疫荧光染色和酶联免疫吸附分析（ELISA）检测各组小鼠的组织学差异。

1.3 行为学测试

Y迷宫由3个开放臂（长×宽×高，21 cm×7 cm×15.5 cm）组成，且彼此成120°角。将小鼠放入一个开放臂远端并允许其自由探索8 min，测试过程中无奖励、惩罚或训练。小鼠的入臂顺序由仪器上方的摄像头记录。当小鼠的4只爪子都进入迷宫的一个臂，则记录为一次进入。分析小鼠的入臂总次数和交替次数（连续进入所有3个臂，即ABC、BCA或CAB，但不包括ABA）。交替率用于衡量小鼠的短期空间记忆。交替率（%）的计算公式如下：交替率=(交替次数/(入臂总次数−2))×100%。

旷场（40 cm×40 cm×40 cm）用于小鼠的新物体识别测试。测试过程分为适应阶段和测试阶段。适应阶段（2 d）：小鼠放入旷场中自由探索10 min。训练阶段：旷场内两个对角的位置各放置1个大小、形状、颜色相同的物体。将小鼠放入旷场中并允许其自由探索10 min。运用VisuTrack动

物行为分析系统进行视频采集和数据分析。测试阶段：0.5 h和2 h后，将旷场中一个物体替换为形状、颜色不同的新物块，并让小鼠自由探索10 min。记录小鼠对两个物体的探索时间。计算小鼠的总探索时间和分辨指数（DI）。分辨指数=新物体探索时间/总探索时间×100%。

1.4　高尔基染色

小鼠用异氟烷麻醉后，取全脑并分离左右半球。将脑组织浸泡于20 ml Golgi-Cox溶液并避光放置17 d。用冰冻切片机将脑组织切为60 μm的脑切片。切片经过染色液孵育10 min、梯度酒精（50%、70%、95%和100%）脱水5~10 min、二甲苯清洗5~10 min。将脑片用Permount封片液封片。在200×和1 000×显微镜下镜检海马脑区的锥体神经元。神经元应选择完全染色且未被血管、神经胶质细胞或其他神经元遮挡。选取200×镜检神经元，并通过手动测量神经元边界到轴突顶端的距离以定量轴突长度。同时，以神经元的胞体为圆心作间距为10 μm的同心圆以计数同心圆与树突的交点数之和。同心圆与树突的交点数之和（number of dendritic branch points）反映神经元树突的整体分枝密度，即树突复杂程度（其交点数之和越多代表树突复杂程度越高）。选取1 000×镜检神经元，以神经元胞体发出树突的第一次分枝（>10 μm）为观察目标，统计树突棘的总数。

1.5　蛋白质印迹法（Western blot）

将海马脑组织放置于1 ml含有蛋白酶抑制剂和磷酸酶抑制剂（Roche，Indianapolis，IN，USA）的RIPA裂解缓冲液（Thermo Scientific Pierce，Waltham，MA，USA）中匀浆。将匀浆液在4ºC下以13 000*g*离心30 min，并提取上清液。用BCA法测定蛋白质浓度。将等量蛋白质（20 μg/孔）在10% SDS聚丙烯酰胺凝胶电泳（SDS-PAGE）上电泳分离，并转移到PVDF膜。室温下，5% BSA缓冲液孵育膜1 h，并用一抗（c-fos，1∶5 000；DCX，1∶8 000；Syn，1∶5 000；PSD95，1∶5 000；GAPDH，1∶10 000）孵育过夜。TBST冲洗3次后，用HRP结合的山羊抗兔或HRP结合的山羊抗鼠二抗（1∶15 000，Proteintech Group，Rosemont，IL，USA）孵育1 h。ECL发光液显影，并用Image J软件进行半定量分析。用内参蛋白GAPDH进行校准以获得实验数据。

1.6　免疫荧光染色

小鼠经异氟烷麻醉后，取脑组织并将右侧大脑放置于4℃的4%多聚甲醛溶液中浸泡24 h。将脑组织依次经过20%和30%蔗糖溶液脱水。用最佳切割温度复合物（optimal cutting temperature compound，OCT）包埋脑组织，并于冰冻切片机（CM1850，Leica，Germany）中进行冠状切片，厚度30 μm。用PBS冲洗海马切片，并于室温下用PBS封闭液（5%山羊血清+0.3% Triton X-100）孵育2 h。海马切片在室温避光条件下孵育二抗（ab150080，1∶15 000，Cambridge，UK）溶液4 h。切片经含DAPI的封片液封片。用尼康Eclipse TE-2000U显微镜成像。在每只小鼠海马的5个代表性切片（约0.1 mm^2）定量主要组织相容性复合体II（major histocompatibility complex II，MHC-II）阳性小胶质细胞的数量。

1.7　酶联免疫吸附分析（ELISA）

根据ELISA试剂盒说明书检测各组小鼠海马IL-1β水平。海马样本在含有蛋白酶抑制剂的RIPA裂解缓冲液（100 g/L；Thermo Scientific Pierce，Waltham，MA，USA）中匀浆，并以13 000*g*离心30 min以提取上清液。将100 μl上清液样品加入微孔板，在4℃孵育过夜。第二天，用洗涤液清洗微孔板，并与生物素化IL-1β抗体孵育60 min。洗涤后，用HRP-链霉亲和素溶液孵育微孔板45 min。然后用TMB底物试剂孵育30 min。加入50 μl终止液，并立即用酶标仪读取450 nm波长下各孔的光密度值（*A*）。用Gen 5软件进行数据分析，并将数值归一化为每个样品的海马上清液体积（100 g/L）。

1.8　数据处理与分析

本研究中数据均以平均数±标准误（mean±SEM）表示。数据的统计分析使用SPSS 23.0软件包以单因素方差分析或*t*检验（c-fos）进行组间统计。$P<0.05$为具有显著性差异水平，具有统计学意义。

2　结　　果

2.1　运动干预降低HDF诱导的肥胖小鼠体重、内脏脂肪和皮下脂肪质量

行为学测试后，称量小鼠体重并通过解剖肾周脂肪和附睾脂肪来分别评估小鼠的内脏脂肪和皮下脂肪水平（图1a）。HFD组小鼠的体重明显高于CON组小鼠（P<0.001，图1b）。8周有氧运动干预明显降低HFD-Ex组小鼠的体重（P<0.001，图1b）。HFD组小鼠的肾周脂肪（P<0.001，图1c）和附睾脂肪（P<0.001，图1d）的质量显著高于CON组小鼠。运动干预明显降低HFD-Ex组小鼠的肾周脂肪（P=0.003，图1c）和附睾脂肪（P=0.001，图1d）水平。

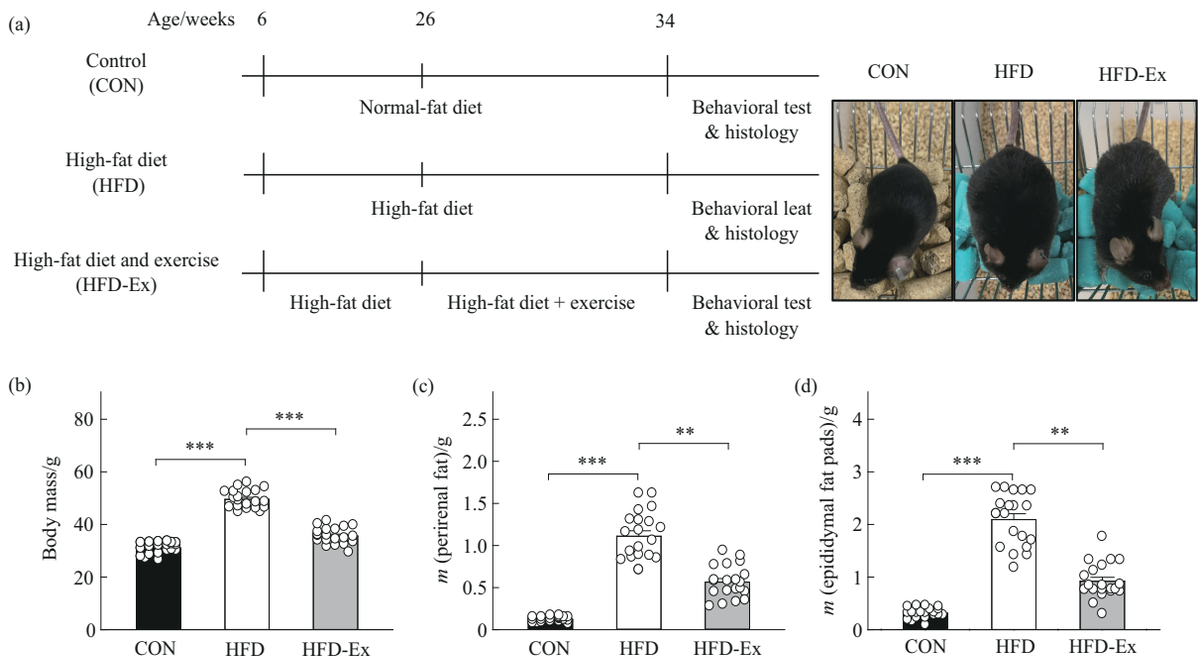

Fig. 1　Effect of high−fat feeding and treadmill exercise on body mass, perirenal fat and epididymal fat pads

(a) Timeline of the experiment. (b−d) The body mass (b, ***P<0.001, n=20), mass of perirenal fat (c, ***P<0.001, n=20), mass of epididymal fat pads (d, ***P<0.001, n=20) of animals in the high-fat diet (HFD) group was significantly higher than that of the animals in the normal-fat diet (CON). However, the body mass (b, ***P<0.001, n=20), mass of perirenal fat (c, **P<0.01, n=20) and mass of epididymal fat pads (d, **P<0.01, n=20) of animals in the high-fat diet and exercise (HFD-Ex) group was significantly lower than that of the animals in the HFD group.

2.2　运动干预改善HFD诱导的肥胖小鼠记忆损伤

在Y迷宫测试中（图2a），HFD组小鼠的交替率显著低于CON组小鼠（P<0.001，图2c），但总入臂次数无显著性差异（P=0.986，图2d）。运动干预明显提高HFD-Ex组小鼠在Y迷宫测试中的交替率（P=0.001，图2c）和总入臂次数（P<0.001，图2d）。在新物体识别测试中（图2b），HFD组小鼠在训练后的0.5 h（P<0.001，图2e）和2 h（P<0.001，图2f）的分辨指数显著低于CON组小鼠。运动干预明显提高HFD-Ex组小鼠在新物体识别测试中的训练后的0.5 h（P<0.001，图2e）和2 h（P=0.015，图2f）的分辨指数。但是，3组小鼠在训练后的0.5 h（P=0.800，图2g）和2 h（P=0.750，图2h）的总探索时间无显著性差异。

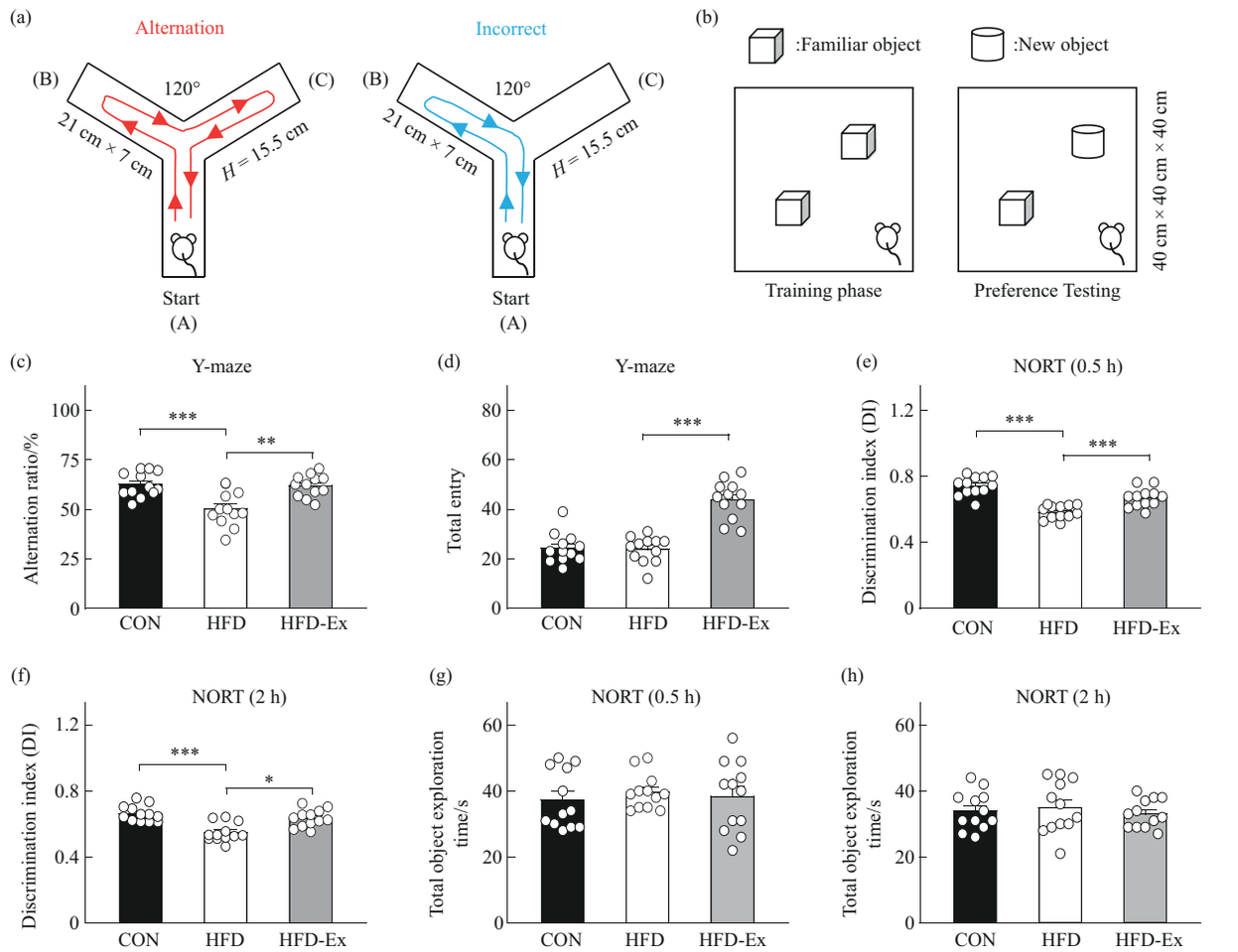

Fig. 2　Treadmill exercise prevents decline in spatial learning and memory in high−fat diet−induced obese mice

(a, b) Schematic drawings of the Y-maze (a) and novel object recognition (b) tests performed in our experiment. (c−f) The alternation ratio (c, ***P< 0.001, n=12) and discrimination index (DI) (e, f, ***P<0.001, n=12) were significantly decreased in HFD mice compared to the CON mice. Treadmill exercise reversed the decrease in the alternation ratio (c, **P<0.01, n=12) and DI (e, ***P<0.001, n=12; f, *P<0.05, n=12) in the HFD-induced obese mice. The total arm entry (d) was significantly increased in the HFD-Ex mice compared to the HFD mice (***P<0.001, n=12). (g, h) The total object exploration time did not differ among all 3 experimental groups at 0.5 h (g) and 2 h (h) post-training intervals (P=0.8, n=12). NORT: novel object recognition test.

2.3　运动干预提高小鼠海马神经元活动和神经发生

由于神经元受到刺激时可短暂表达c-fos蛋白，其可作为神经元活动的标志。本研究检测了运动干预对小鼠海马脑区c-fos蛋白表达以评估海马神经元的活动。运动干预明显提高HFD-Ex组小鼠海马脑区c-fos蛋白表达水平（P<0.001，图3a，c）。此外，我们检测了神经前体细胞和新生神经元特异性标志蛋白双皮质素（doublecortin，DCX）水平。HFD组小鼠海马脑区DCX蛋白表达水平显著低于CON组小鼠（P=0.005，图3b，d）。运动干预明显提高HFD-Ex组小鼠海马脑区DCX蛋白表达水平（P=0.037，图3d）。

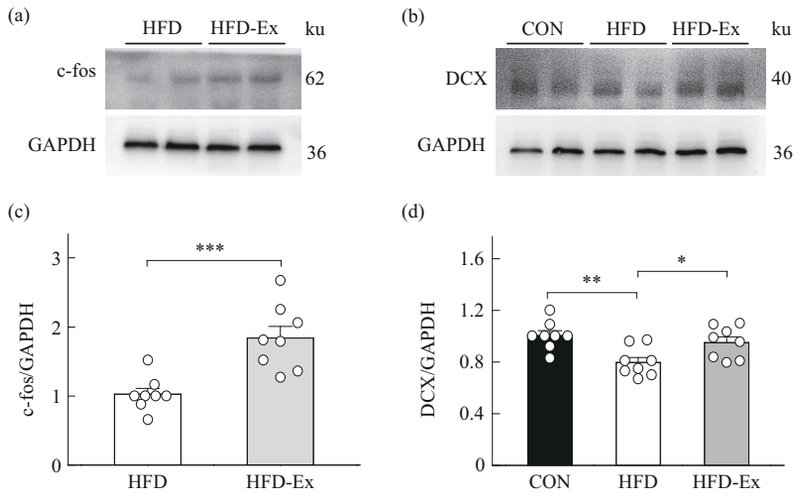

Fig. 3 Effects of treadmill exercise on the expression of c−fos and DCX in the hippocampus of high−fat diet−induced obese mice

(a, b) Representative Western blots for c-fos, Dcx, and GAPDH in the hippocampus of mice in each group. (c) Summarized data showed a significant increase in c-fos expression in the hippocampus of the HFD-Ex group compared to the HFD group (***P<0.001, n=8). (d) Summarized data showed a significant decrease in DCX expression in the hippocampus of the HFD group compared to the CON group (**P<0.01, n=8), which was blocked by treadmill exercise (*P< 0.05, n=8).

2.4 运动干预增强HFD诱导的肥胖小鼠海马神经元结构可塑性

本研究通过高尔基染色量化各组小鼠海马脑区神经元轴突长度、树突复杂程度和树突棘数量（图4a，b）。HFD组小鼠海马脑区神经元轴突长度（P<0.001，图4c）、树突复杂程度（P<0.001，图4d）和树突棘数量（P<0.001，图4e）均显著低于CON组。运动干预明显提高HFD-Ex组小鼠海马脑区神经元轴突长度（P=0.002，图4d）、树突复杂程度（P<0.001，图4d）和树突棘数量（P<0.001，图4e）。Syn和PSD95分别是突触前和突触后的特异性标记物。本研究进一步检测各组小鼠海马脑区Syn和PSD95蛋白表达水平（图5a，b），并发现HFD组小鼠海马脑区PSD95表达水平（P<0.001，图5c）显著低于CON组小鼠。运动干预明显提高HFD-Ex组小鼠海马脑区PSD95表达水平（P=0.001，图5c）。然而，CON、HFD和HFD-Ex组小鼠海马脑区Syn蛋白表达水平无显著性差异（P=0.396，图5d）。

2.5 运动干预降低HFD诱导的肥胖小鼠海马脑区小胶质细胞激活和炎症因子IL−1β水平

MHC-II是小胶质细胞活化的标志性蛋白。免疫荧光染色结果表明，HFD组小鼠海马脑区MHC-II阳性小胶质细胞数量显著性高于CON组小鼠（P<0.001，图6a，b）。运动干预明显降低HFD-Ex组小鼠海马脑区MHC-II阳性小胶质细胞数量（P<0.001，图6b）。同时，HFD组小鼠海马脑区炎症因子IL-1β水平显著高于CON组小鼠（P<0.001，图6c）。运动干预明显降低HFD-Ex组小鼠海马脑区IL-1β水平（P<0.001，图6c）。

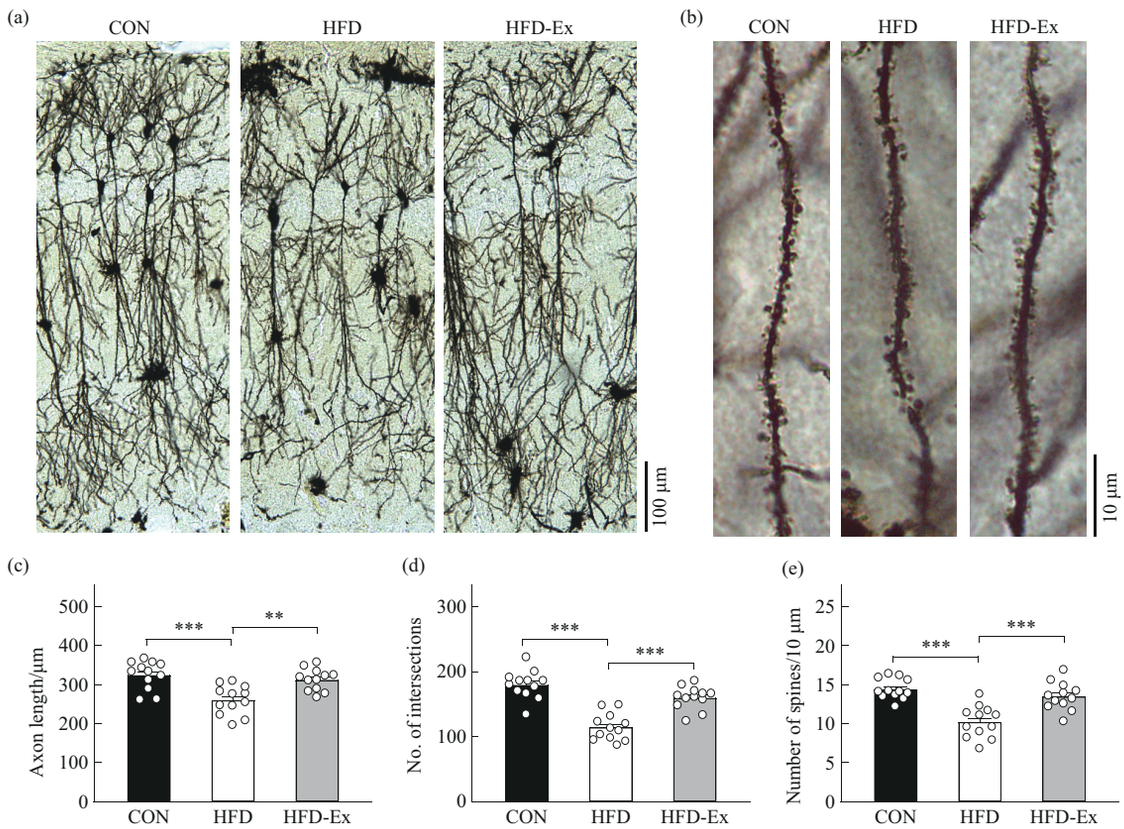

Fig. 4　Treadmill exercise increases the axon length, dendritic complexity, and the dendritic spines numbers in the hippocampus of high−fat diet−induced obese mice

(a, b) Representative Golgi staining images of the hippocampus neurons (CA1) (a) and dendritic spines (2nd-oder dendrites) (b) in the CON, HFD, and HFD-Ex groups. (c−e) The axon length (c) (***P<0.001, n=12), dendritic complexity (d) (***P<0.001, n=12), and the number of spines (e) (***P<0.001, n=12) of the hippocampus were significantly decreased in the HFD-induced obese mice compared to the control group. Treadmill exercise increased the axon length (c) (**P<0.01, n=12), dendritic complexity (d) (***P<0.001, n=12), and the number of spines (e) (***P<0.001, n=12) of the hippocampus in the HFD-induced obese mice.

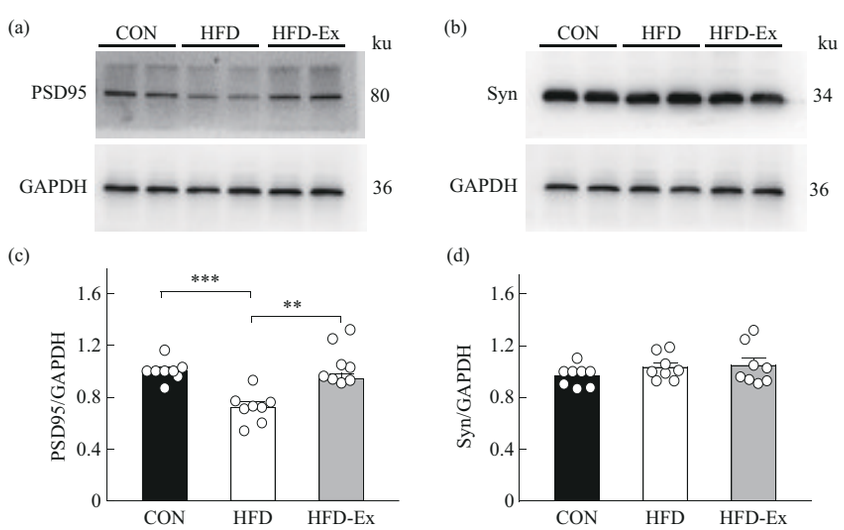

Fig. 5　Effects of treadmill exercise on the expression of PSD95 and Syn in the hippocampus of high−fat diet−induced obese mice

(a, b) Representative Western blots for PSD95, Syn, and GAPDH in the hippocampus of mice in each group. (c) Summarized data showed a significant decrease in PSD95 expression in the hippocampus of the HFD group compared to the CON group (***P<0.001, n=8), which was blocked by treadmill exercise (**P<0.01, n=8). (d) There were no significant difference on Syn expression in the hippocampus among the CON, HFD, and HFD-Ex groups (P=0.396. n=8).

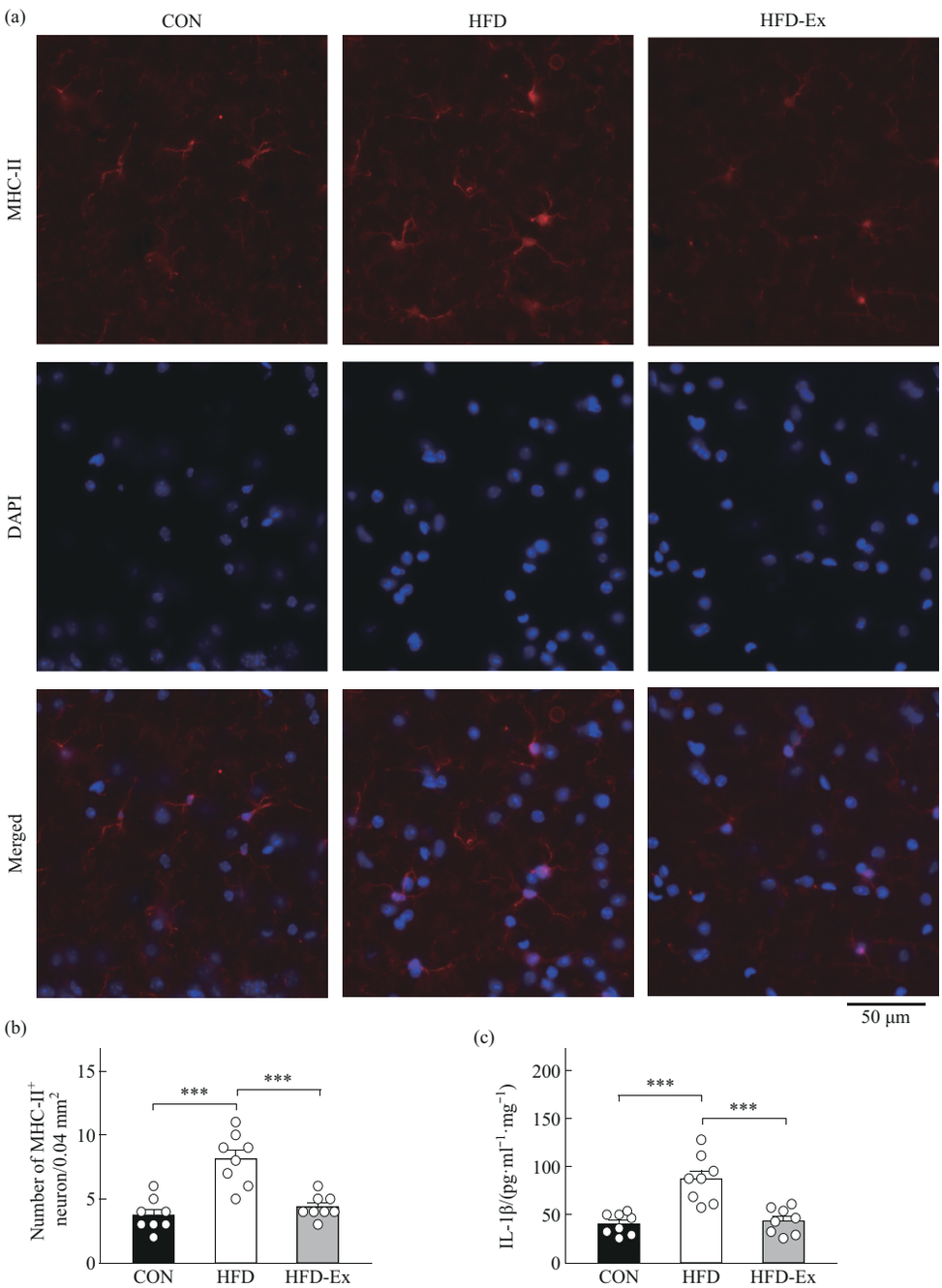

Fig. 6　Effects of treadmill exercise on microglia (MHC−II) and IL−1β in the hippocampus

(a) The representative microscope imaging of MHC-II positive cells in the hippocampus of mice in each group. (b) Quantification of MHC-II positive microglia cell numbers in the hippocampal. The number of MHC-II positive microglia cells was significantly higher in HFD mice compared to the CON mice (***P<0.001, n=8), and this increase was blocked by treadmill exercise (***P<0.001, n=8). (c) ELISA analysis of IL-1β concentrations in the hippocampus of mice in each group. The concentration of IL-1β in the hippocampus were significantly increased in the HFD mice compared to the CON mice (***P<0.001, n=8). However, the concentration of IL-1β was decreased in the HFD-Ex mice compared to the HFD mice (***P<0.001, n=8).

3 讨　论

3.1 运动干预对记忆损伤的改善作用

肥胖被确定为认知功能障碍的重要风险因素之一。HFD诱导的肥胖小鼠模型可以复制人类因高脂肪或高糖饮食等饮食模式而导致的超重和肥胖，已被广泛用于研究肥胖及相关疾病[3]。本研究采用的HFD诱导的肥胖小鼠体重、内脏脂肪和皮下脂肪均明显高于正常饮食小鼠，而运动干预可有效降低肥胖小鼠的体重、内脏脂肪和皮下脂肪含量。结果提示，HFD可有效诱导小鼠肥胖，且运动干预能在一定程度上降低肥胖小鼠的体重并减少皮下和内脏脂肪堆积。运动增加肥胖小鼠的能量消耗并减少食物和能量的摄入量可能是其降低肥胖小鼠体重、皮下脂肪和内脏脂肪含量的关键原因[23-24]。动物研究表明，HFD会诱发不同年龄C57BL/6小鼠和AD模型（3×TgAD）小鼠海马依赖性记忆损伤[4-5]。Y型迷宫和新物体识别测试是研究海马依赖性短期记忆的常用行为学测试方法[25-26]。本研究应用Y型迷宫和新物体识别测试结果表明，HFD诱导的肥胖小鼠表现出短期记忆损害。同时，临床研究表明，4 d的高饱和脂肪和高糖饮食（西式饮食）会导致健康年轻人的海马依赖性学习和记忆能力下降[27]。本研究中，运动干预可有效提高HFD诱导的肥胖小鼠在Y迷宫和新物体识别测试中的交替率、总入臂次数和分辨指数。Y迷宫测试中，总入臂次数可以评估小鼠在测试期间的活动情况[28]。结果提示，运动可以有效改善HFD诱导的肥胖小鼠海马依赖性记忆损害，且提高肥胖小鼠的运动能力。

3.2 运动干预对海马神经发生和神经元结构可塑性的影响

海马和杏仁核等特定脑区神经元激活在记忆的形成中扮演者重要的作用[29]。即刻早期基因*c-fos*表达水平可以作为神经元活动的有效指标[30]。运动干预可明显提高HFD诱导的肥胖小鼠海马脑区中c-fos的表达水平，提示运动干预可以有效激活肥胖小鼠海马神经元[31]。运动诱导的海马神经元激活可能会促进海马神经可塑性和神经发生。海马神经可塑性和神经发生是各种海马依赖性学习和记忆形式的基础[32-33]。HFD会降低未成熟神经元的水平、突触密度以及Syn和PSD95的表达，从而损害海马神经可塑性和神经发生[6，9，11]。慢性有氧运动和阻力运动都能增加海马神经发生[34-35]。本研究通过定量海马神经元前体细胞标记物DCX蛋白水平发现，HFD诱导的肥胖小鼠海马脑区DCX蛋白表达水平显著降低，而运动干预则明显增加了肥胖小鼠海马脑区DCX蛋白表达水平。结果提示，运动干预可能通过激活海马神经元以增强神经发生。神经元轴突、树突和树突棘被认为是突触可塑性和记忆形成的基础[36]。在AD、帕金森病等多种疾病模型中，增强海马脑区神经可塑性可以改善动物的认知功能障碍[20，37-38]。运动干预可明显增加HFD诱导的肥胖小鼠海马脑区轴突长度、树突复杂程度和树突棘数量。Syn和PSD95分别是突触前和突触后的特异性标记物。事实上，Syn和PSD95蛋白表达减少与AD和自闭症等疾病的突触功能障碍高度相关[39-40]。进一步研究表明，HFD诱导的肥胖小鼠海马脑区Syn和PSD95蛋白表达水平明显降低，而运动干预有效提高肥胖小鼠海马Syn和PSD95表达水平，提示运动能有效改善HFD诱导的肥胖小鼠海马突触传递。综上所述，运动干预可能通过调控小鼠海马神经元结构可塑性和神经发生改善HFD诱导的肥胖小鼠记忆损害。

3.3 运动干预对海马脑区小胶质细胞激活和炎症因子水平的影响

学习和记忆需要在大脑中形成新的神经网络。兴奋性神经元之间的突触可在记忆事件发生后几秒钟内形成新的神经联系[41]。然而，活化的小胶质细胞可从神经元细胞体上移除突触[13]。HFD会诱导小鼠丘脑、杏仁核、海马脑区中小胶质细胞活化和炎症反应[14，42-43]。活化的小胶质细胞可以迁移到突触部位以扰乱小胶质细胞和突触之间的空间关系并介导突触剥离[14]。本研究表明，HFD诱导的肥胖小鼠海马脑区MHC-II阳性小胶质细胞数量显著增加，而运动干预可有效降低肥胖小鼠海马脑区中MHC-II阳性小胶质细胞的数量。结果提示，运动干预可能通过抑制肥胖小鼠海马脑区小胶质细胞的过度激活而减少神经元突触剥离。小胶质细胞是炎症反应的核心，也是IL-1β的主要来源[44]。IL-1β的水平与肥胖症患者的认知障碍直接相关[45]。海马内IL-1受体拮抗剂可预防突触功能障碍、炎症反应和认知障碍[45]。本研究结果同样发现，运动干预可以降低肥胖小鼠海马脑区炎症因子IL-1β水平。综上所述，运动干预能显著减少HFD诱导的肥胖小海马脑区小胶质细胞介导的突触剥离和炎

症反应。

4 结　论

运动干预可有效提高HFD诱导的肥胖小鼠海马脑区神经元轴突长度、树突复杂程度、树突棘数量、PSD95蛋白表达水平，并降低MHC-II阳性小胶质细胞数和炎症因子IL-1β水平以减少突触剥离，促进海马脑区神经元结构可塑性和神经发生，可能是运动干预改善肥胖小鼠海马依赖性记忆力减退的机制之一。

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Exercise Regulates Structural Plasticity and Neurogenesis of Hippocampal Neurons and Improves Memory Impairment in High-fat Diet-induced Obese Mice*

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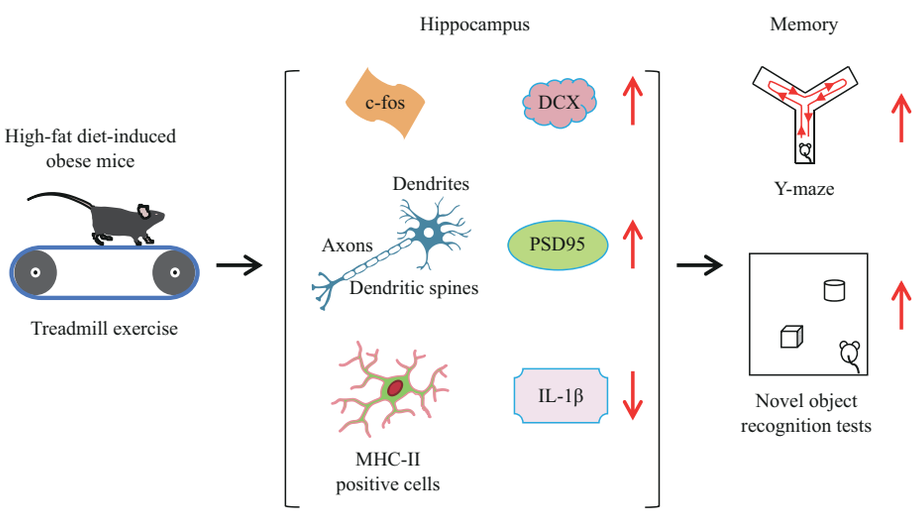
Graphical abstract



Abstract **Objective** Obesity has been identified as one of the most important risk factors for cognitive dysfunction. Physical exercise can ameliorate learning and memory deficits by reversing synaptic plasticity in the hippocampus and cortex in diseases such as Alzheimer's disease. In this study, we aimed to determine whether 8 weeks of treadmill exercise could alleviate hippocampus-dependent memory impairment in high-fat diet-induced obese mice and investigate the potential mechanisms involved. **Methods** A total of sixty 6-week-old male C57BL/6 mice, weighing between 20−30 g, were randomly assigned to 3 distinct groups, each consisting of 20 mice. The groups were designated as follows: control (CON), high-fat diet (HFD), and high-fat diet with exercise (HFD-Ex). Prior to the initiation of the treadmill exercise protocol, the HFD and HFD-Ex groups were fed a high-fat diet (60% fat by kcal) for 20 weeks. The mice in the HFD-Ex group underwent treadmill exercise at a speed of 8 m/min for the first 10 min, followed by 12 m/min for the subsequent 50 min, totally 60 min of exercise at a 0°

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slope, 5 d per week, for 8 weeks. We employed Y-maze and novel object recognition tests to assess hippocampus-dependent memory and utilized immunofluorescence, Western blot, Golgi staining, and ELISA to analyze axon length, dendritic complexity, number of spines, the expression of c-fos, doublecortin (DCX), postsynaptic density-95 (PSD95), synaptophysin (Syn), interleukin-1β (IL-1β), and the number of major histocompatibility complex II (MHC-II) positive cells. **Results** Mice with HFD-induced obesity exhibit hippocampus-dependent memory impairment, and treadmill exercise can prevent memory decline in these mice. The expression of DCX was significantly decreased in the HFD-induced obese mice compared to the control group (P<0.001). Treadmill exercise increased the expression of c-fos (P<0.001) and DCX (P=0.001) in the hippocampus of the HFD-induced obese mice. The axon length (P<0.001), dendritic complexity (P<0.001), the number of spines (P<0.001) and the expression of PSD95 (P<0.001) in the hippocampus were significantly decreased in the HFD-induced obese mice compared to the control group. Treadmill exercise increased the axon length (P=0.002), dendritic complexity (P<0.001), the number of spines (P<0.001) and the expression of PSD95 (P=0.001) of the hippocampus in the HFD-induced obese mice. Our study found a significant increase in MHC-II positive cells (P<0.001) and the concentration of IL-1β (P<0.001) in the hippocampus of HFD-induced obese mice compared to the control group. Treadmill exercise was found to reduce the number of MHC-II positive cells (P<0.001) and the concentration of IL-1β (P<0.001) in the hippocampus of obese mice induced by a HFD. **Conclusion** Treadmill exercise led to enhanced neurogenesis and neuroplasticity by increasing the axon length, dendritic complexity, dendritic spine numbers, and the expression of PSD95 and DCX, decreasing the number of MHC-II positive cells and neuroinflammation in HFD-induced obese mice. Therefore, we speculate that exercise may serve as a non-pharmacologic method that protects against HFD-induced hippocampus-dependent memory dysfunction by enhancing neuroplasticity and neurogenesis in the hippocampus of obese mice.

Key words exercise, memory, hippocampal, neuroplasticity, neurogenesis, obesity
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