



运动通过肠道菌群对孤独症样大鼠行为及下丘脑-垂体-肾上腺轴的影响*

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摘要 **目的** 探究自主跑轮运动通过肠道菌群对孤独症谱系障碍 (autism spectrum disorder, ASD) 样大鼠行为异常与下丘脑-垂体-肾上腺 (hypothalamic-pituitary-adrenal, HPA) 轴激活状态的影响。**方法** 选取SD母鼠, 于孕期第12.5天对孕鼠腹腔注射400 mg/kg丙戊酸盐 (valproic acid, VPA) 溶液或等量生理盐水, 所产仔鼠分别为ASD模型组 (PASD, $n=35$) 和正常对照组 (PCON, $n=16$)。在仔鼠出生后第23天, 采用三室社交、旷场、Morris水迷宫试验进行干预前行为学测试。行为学测试结束后, 从两组中各随机选取8只大鼠 (PCON, PASD), 采用酶联免疫吸附分析 (ELISA) 检测血清促肾上腺皮质激素释放激素 (corticotrophin-releasing hormone, CRH)、促肾上腺皮质激素 (adrenocorticotrophic hormone, ACTH) 及皮质醇 (corticosterone, CORT) 浓度, 以评估HPA轴功能状态。在仔鼠出生后第28天, 将PCON组剩余8只大鼠作为CON对照组 (CON, $n=8$), PASD组剩余27只大鼠随机分为4组: ASD无干预组 (ASD, $n=6$)、ASD运动组 (ASDE, $n=8$)、ASD移植组 (FMT, $n=8$) 和ASD假移植组 (sFMT, $n=5$)。其中, ASD组和CON组常规饲养, ASDE组从出生后第28天起, 进行6周自主跑轮运动干预, FMT组从出生后第42天起, 每日灌胃运动2周后的ASDE组大鼠新鲜粪便悬液 (1 ml/100g), 5 d/周, 持续4周, sFMT组则灌胃等量生理盐水。干预结束后, 检测各组行为学和HPA轴指标。**结果** 干预前, 与正常对照组相比, ASD模型组的社交能力和社交新颖性偏好、自发活动和探索兴趣, 以及空间学习、记忆和导航能力均显著下降 ($P<0.05$), PASD组血清中CRH、ACTH和CORT浓度显著高于PCON组 ($P<0.05$)。经6周自主跑轮运动干预后, 与ASD组相比, ASDE组大鼠的社交能力和社交新颖性偏好、自发活动和探索兴趣, 以及空间学习、记忆和导航能力均显著改善 ($P<0.05$), 血清CORT浓度显著降低 ($P<0.05$), CRH及ACTH浓度呈下降趋势。在接受4周运动大鼠的粪菌移植干预后, 与ASD组和sFMT组相比, FMT组大鼠的社交能力和社交新颖性偏好、自发活动和探索兴趣、空间学习、记忆和导航能力均显著改善 ($P<0.05$), 血清ACTH、CORT浓度显著降低 ($P<0.05$), CRH浓度呈下降趋势。**结论** 运动可能通过抑制HPA轴激活改善ASD相关行为, 肠道菌群可能在这一过程中发挥关键作用。

关键词 孤独症谱系障碍, 肠道菌群, 下丘脑-垂体-肾上腺轴, 运动, 粪菌移植

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孤独症谱系障碍 (autism spectrum disorder, ASD) 是一种异质性神经发育障碍性疾病, 其核心症状包括社交障碍、兴趣和活动受限, 以及重复刻板行为^[1]。ASD常伴有多种并发症, 如胃肠道紊乱、运动缺陷、睡眠问题和智力障碍^[2]。流行病学显示, ASD全球发病率为1%~2%^[3-4]。其中美国8岁儿童的ASD患病率已达1/36^[5], 而中国ASD患病率已上升至0.7%^[6]。ASD病因极其复杂, 涉及基因突变、母体免疫激活和环境因素等^[7], 目前尚无特异性治疗手段, 给患者、家庭及社会带来沉重负担^[8]。因此, 深入研究ASD的

病因机制并制定针对性治疗策略, 仍是亟待解决的重要问题。

研究显示, 下丘脑-垂体-肾上腺 (hypothalamic-pituitary-adrenal axis, HPA) 轴在ASD发病机制中发挥关键作用^[9]。临床研究发现, ASD儿童皮质醇水平升高, HPA轴呈现过度激活

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状态^[10]。据报道,HPA轴的长期激活与慢性炎症状态相关,炎症因子可以参与调控大脑功能,包括情绪和行为调节^[11-12]。在ASD患者中,体内炎症因子水平的升高会刺激下丘脑分泌更多的促肾上腺皮质激素释放激素(corticotrophin-releasing hormone, CRH),进而促使垂体释放更多促肾上腺皮质激素(adrenocorticotrophic hormone, ACTH),导致肾上腺皮质酮(corticosterone, CORT)的合成和分泌增多^[13]。研究发现,由肠道菌群介导的HPA轴功能障碍与多种精神疾病的病理生理学有关^[14]。例如,针对无菌小鼠的研究表明,肠道菌群缺失可导致HPA轴对应激刺激的反应性显著增强,引发焦虑和社交行为受损,进而介导ASD的发病进程^[15]。此外,研究显示,ASD模型动物普遍存在肠道菌群失调,其可能破坏肠道屏障,导致炎症加剧、HPA轴启动、神经递质和细菌代谢物水平改变,最终导致ASD样行为异常^[16-17]。值得注意的是,HPA轴的功能与社交行为密切相关,抑制其活性可改善肠道菌群紊乱小鼠的异常社交行为,激活则使正常小鼠出现社交缺陷^[11]。这提示HPA轴可能是影响ASD行为异常的关键调控点。

近年来,运动被证实能够改善ASD儿童的核心症状及认知功能,同时调节肠道菌群的组成和多样性^[18]。本课题组前期研究发现,6周的有氧运动可显著改善ASD患儿核心症状和胃肠道问题,与其对肠道菌群的调节有关^[19]。此外,动物实验表明,自主跑轮运动能改善ASD大鼠的社交行为及自主活动,且通过粪菌移植(fecal microbiota transplantation, FMT)进一步证实,运动通过肠道菌群影响中枢神经递质系统,从而改善ASD大鼠的行为^[20]。其中,FMT作为一种调整肠道菌群的治疗手段,已被证实对ASD儿童的肠道及行为症状有长期改善的作用^[21-22]。然而,运动是否通过调节肠道菌群抑制HPA轴的过度激活进而改善ASD行为症状,其具体机制尚不清楚。因此,本研究基于前期成果,采用孤独症样大鼠模型,探究自主跑轮运动对ASD行为及HPA轴的影响及肠道菌群在其中的作用,旨在探讨运动改善ASD症状的神经生物学机制,为ASD治疗提供新策略和科学依据。

1 材料与方法

1.1 主要仪器和试剂

自主跑轮系统(XR-PL107,上海欣软信息科

技有限公司);行为学检测系统(上海欣软信息科技有限公司);离心机(5424R, Eppendorf);酶标仪(SYNERGY LX, BioTek);丙戊酸盐(货号P4543, Sigma);三箱社交实验箱(RD1150-SIT, 上海移数信息科技有限公司);旷场实验箱(RD1112-OF-G, 上海移数信息科技有限公司);水迷宫游泳池(RD1101-MWM-G, 上海移数信息科技有限公司)。皮质醇酶联免疫吸附分析(enzyme-linked immunosorbent assay, ELISA)试剂盒(货号:CSB-E07014r)、促肾上腺皮质激素ELISA试剂盒(货号:CSB-E06875r)和促肾上腺皮质激素释放激素ELISA试剂盒(货号:CSB-E08038r)均购自武汉华美生物工程有限公司。

1.2 实验方法

1.2.1 实验动物

选取8~9周龄SPF级Sprague-Dawley (SD)大鼠(购自南方医科大学实验动物中心,许可证号:SCXK(粤)2016-0041)雌、雄各数只,饲养于同一SPF级动物房,环境温度(22±2)℃,相对湿度为35%~75%,光照周期为12/12 h,自由饮水和普通饲料饮食。大鼠被适应性喂养2周后,雌、雄大鼠按照1:2的比例合笼,次日观察到雌鼠阴栓并经阴道涂片确认有精子即表明交配成功,记为孕第1天(embryoic 1, E1),受孕大鼠进行单笼喂养。所有动物处理严格遵守《关于善待实验动物的指导性意见》,且本实验已得获广州体育学院实验动物委员会批准(审批编号:2021DWLL-07)。

1.2.2 模型制备及分组

雌性SD大鼠于孕期第12.5天腹腔注射400 mg/kg丙戊酸盐(valproic acid, VPA, 溶于0.9%生理盐水配成250 g/L溶液),其子代作为ASD模型大鼠(PASD, $n=35$)。同期选取SD孕鼠在相同孕期腹腔注射等量生理盐水,其子代作为正常对照组(PCON, $n=16$)。在仔鼠出生后第23天进行干预前行为学测试后,从两组中各随机选取8只大鼠(PCON, PASD),采用ELISA检测HPA轴相关指标。在仔鼠出生后第28天,将PCON组剩余8只大鼠作为CON对照组(CON, $n=8$);将PASD组剩余27只大鼠随机分为4组:ASD无干预组(ASD, $n=6$)、ASD运动组(ASDE, $n=8$)、ASD移植组(FMT, $n=8$)和ASD假移植组(sFMT, $n=5$)。其中,ASD组和CON组常规饲养,ASDE组从出生后第28天起,进行6周自主跑轮运动干预,FMT组从出生后第42天起,每日以运动2周后的ASDE

组大鼠新鲜粪便悬液灌胃(每100 g体重1 ml), 5 d/周, 持续4周, sFMT组则灌胃等量生理盐水。干预结束后, 检测各组行为学和HPA轴指标。

1.2.3 运动干预方案

本实验采用自主跑轮运动干预模式, 运动开始于行为学测试后第2天(即大鼠出生后第28天)。运动组大鼠饲养笼内放置跑轮装置(直径36 cm, 跑道宽度10 cm, 整体尺寸42 cm×26 cm×19 cm), 24 h供其自由活动。正式运动干预前, 所有大鼠均进行为期3 d的适应性训练, 随后进行为期6周的自主跑轮运动。运动参数, 即运动量(跑步距离)与运动时间的监测, 由同一实验员每日18:00通过电子计数系统进行记录。最终选择平均运动量大于2 km/d的大鼠进行分析^[23]。

1.2.4 粪菌移植方案

为了探索运动改善孤独症的行为是否来自运动对肠道菌群的修饰效应, 进一步采用整体肠道菌群移植方案。每天固定时间收集运动量大于2 km/d的运动组大鼠作为供体^[24], 常规饲养的ASD大鼠为受体组(即为移植组FMT), 假移植组(sFMT)以生理盐水灌胃作为对照。粪菌移植从运动组进行2周运动后, 收集运动组大鼠的直肠粪便。粪便经处理后以灌胃方式移植至常规饲养的ASD大鼠体内, 持续4周(5 d/周, 共20 d)。灌胃操作由同一实验员在相同时间和条件下执行。具体步骤如下^[25]: a. 供体粪便收集, 每日8:00~9:00采集ASD运动组大鼠肛门的新鲜粪便, 保存于无菌冻存管; b. 稀释, 称取粪便, 按1:5比例加入生理盐水, 搅拌成混悬液; c. 过滤, 采用医用纱布过滤掉粪便中的食物纤维, 获得二级混悬液; d. 将二级混悬液于4℃离心机中以6 000 r/min、15 min进行离心, 弃掉上清液, 得到沉淀物; e. 将与b步骤等量的无菌生理盐水加入沉淀物中, 并混合成悬液即粪菌滤液; f. 将所收集的滤液混合后, 按每100 g体重1 ml进行灌胃。

1.2.5 行为学测试

所有行为学试验均在标准化的行为学实验室内完成, 该实验室具备隔音条件, 能保持一致的适宜光照强度、温度和湿度。所有测试均由经过统一培训的实验人员在固定时间段(9:00~16:00)完成, 测试前后均用75%乙醇清洁装置, 全程采用视频记录系统采集数据。

a. 三室社交试验。用于评估大鼠的社交能力和

社交新颖性偏好。实验设备为150 cm×50 cm×40 cm的三室偏好箱, 包含左、中、右3个箱体。实验开始前, 让受试大鼠在中央箱自由探索5 min。随后将一只同龄同性别的陌生大鼠(stranger1)放入左箱铁笼中, 记录受试大鼠10 min内在三箱的活动情况。清洁笼具后, 将另一只陌生大鼠(stranger2)放入右箱铁笼, 左箱的stranger1位置不变, 再次观察10 min。记录受试大鼠在各箱停留时间、进入次数以及接近陌生大鼠的次数和时长。

b. 旷场试验。用于评估大鼠在新环境中的探索行为、自发活动及焦虑反应。将大鼠放入100 cm×100 cm的开放式试验箱中央, 记录大鼠10 min内的运动距离、速度、中央区域活动时间及持续时间等。

c. Morris水迷宫试验。用于评估大鼠空间学习、记忆及导航能力, 主要包括定位航行实验和空间探索实验。实验在直径120 cm的圆形水池中进行, 池内设有直径10 cm, 位于水面下约1 cm处的逃生平台, 水温保持24℃。正式实验前1天, 让受试大鼠进行120 s适应性游泳训练。随后进行3 d(4次/d)的定位航行实验, 将大鼠从随机象限放入水池, 计时120 s。大鼠找到平台后停留30 s, 若未找到, 引导至平台并停留30 s, 记录逃避潜伏期(即寻找平台的时间)。第4天进行空间探索实验, 撤去平台, 将大鼠从固定象限放入水池, 记录60 s内其在原平台象限游泳的路程、时间、平均速度及穿越次数等。

1.2.6 酶联免疫吸附分析(ELISA)

行为学检测结束后, 对各组大鼠腹腔注射1%戊巴比妥钠溶液(1 ml/kg)进行麻醉, 经腹主动脉采血至预冷的1.5 ml离心管中。样本于4℃、3 000 r/min条件下离心10 min, 收集上清液(血清)转移至新管, 保存于-80℃冰箱待测。采用ELISA试剂盒检测血清中HPA轴相关激素浓度。具体步骤如下: 将血清样本及标准品按试剂盒说明书稀释后加入酶标板, 室温孵育2 h; 弃去孔内液体, 用洗涤液洗板5次, 静置30 s/次; 加入生物素化抗体工作液, 室温孵育1 h, 重复洗板; 随后加入辣根过氧化物酶(horseradish peroxidase, HRP)标记的亲合素工作液, 室温避光孵育30 min, 再次洗板; 最后加入显色底物3,3',5,5'-四甲基联苯胺(3,3',5,5'-Tetramethylbenzidine, TMB), 37℃避光反应15 min, 加入终止液终止反应。使用酶标仪在

450 nm 波长处测定各孔吸光度值 (A), 根据标准曲线计算 CRH、ACTH 及 CORT 的浓度 (单位: $\mu\text{g/L}$)。

1.2.7 统计学处理

采用 GraphPad Prism 10.0 进行统计学分析。所有计量数据均以均数 $\pm$ 标准误 (mean $\pm$ SEM) 表示。干预前两组间比较采用独立样本 t 检验, 干预后多组间比较采用单因素方差 (one-way analysis of variance, ANOVA) 分析。若数据不符合正态性和方差齐性时, 均采用非参数检验: 两组间比较采用 Mann-Whitney test, 多组间比较采用 Kruskal-Wallis test, 以 $P<0.05$ 为差异有统计学意义。

2 结 果

2.1 孕期 VPA 暴露对子代大鼠行为、HPA 轴的影响

2.1.1 孕期 VPA 暴露对子代大鼠行为的影响

三室社交试验结果显示, 在干预前, PASD 大鼠与 stranger1 的头部接触时间、所在空间停留时间及接触次数较 PCON 组均显著减少 ($P<0.05$), 在右箱放入 stranger2 后, PASD 组与 stranger2 的头部接触时间、所在空间停留时间及接触次数较 PCON 组也均显著减少 ($P<0.05$, 图 1)。表明 ASD 大鼠存在社交能力下降及社交新颖性偏好能力缺陷。

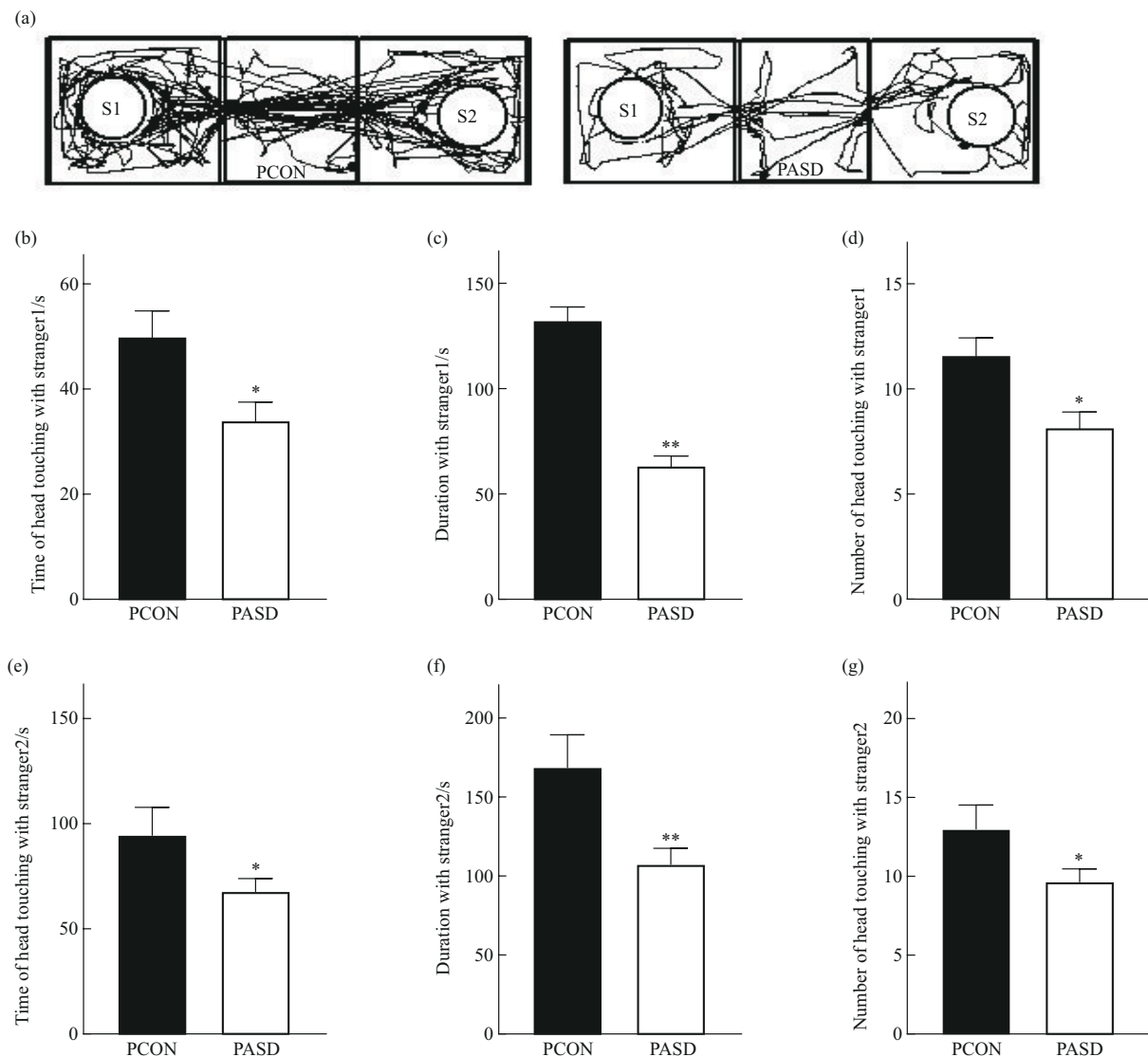


Fig. 1 Influence of VPA exposure on the social behavior of rats

(a) Representative trajectories of rats in each group in the three-compartment social behavior test, S1 and S2 represent stranger 1 and stranger 2 respectively in the trajectories. (b, e) The head contact time of each group of rats with stranger 1 and stranger 2 respectively. (c, f) The time each group of rats stayed in the space where the stranger 1 and stranger 2 respectively. (d, g) The number of times each group of rats had head contact with stranger 1 and stranger 2 respectively. * $P<0.05$, ** $P<0.01$ vs PCON group.

旷场试验结果显示, PASD组大鼠自由活动的总路程、平均速度、中心区域路程、中心平均速度及穿过中心次数均显著低于PCON组 ($P<0.05$,

图2)。表明ASD大鼠自发活动水平降低, 且对新环境的探索兴趣下降。

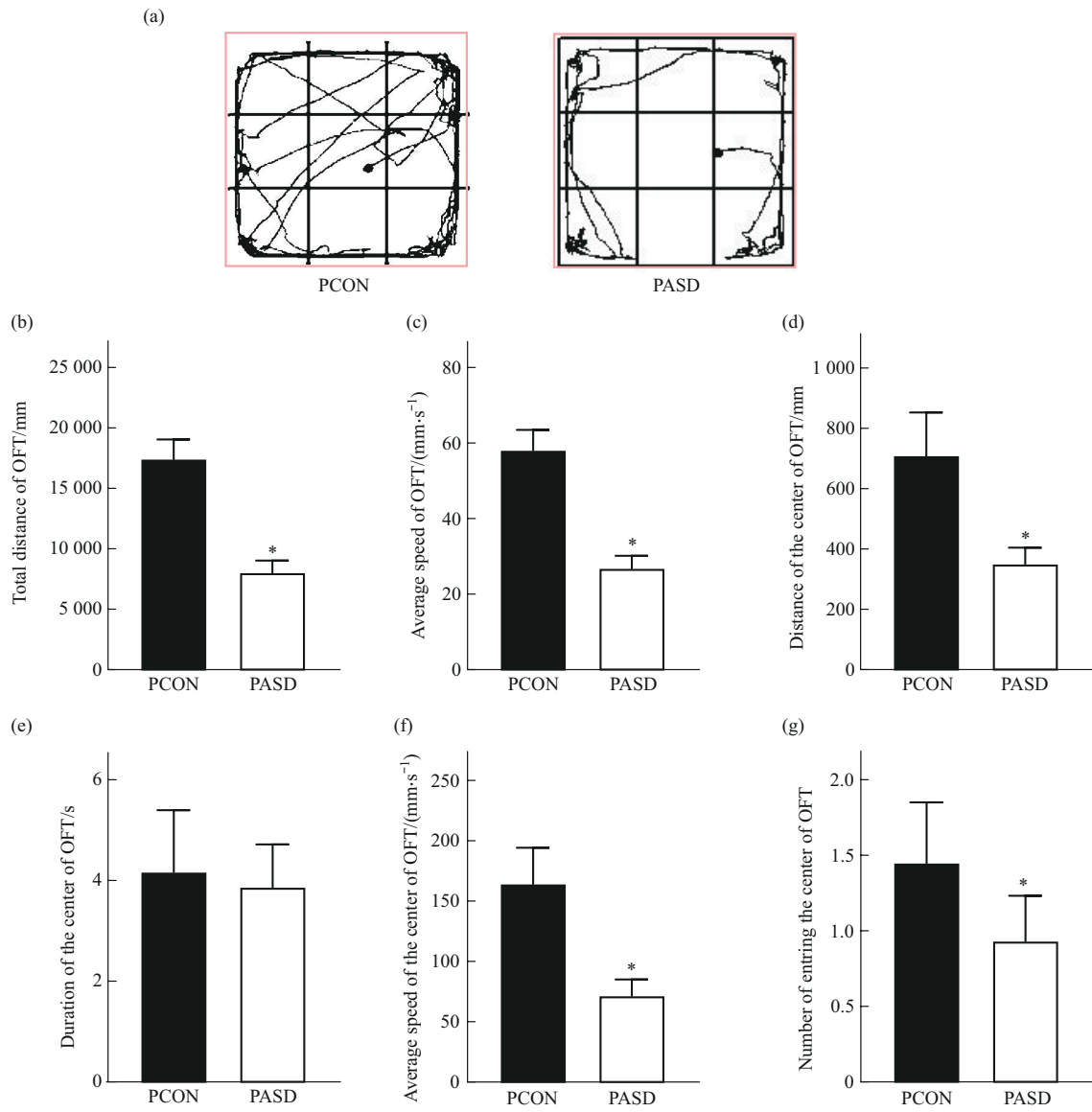


Fig. 2 Influence of VPA exposure on the spontaneous activity of rats

(a) Representative trajectories of rats in each group in the open field test (OFT). (b) Total distance traveled in the open field. (c) Average speed in the open field. (d) Distance traveled in the center area of the open field. (e) Duration of staying in the center area of the open field. (f) Average speed in the center area of the open field. (g) Number of times crossing the center area of the open field. * $P<0.05$ vs PCON group.

水迷宫试验结果显示, PASD组大鼠在平台象限区域的游泳路程、游泳时间及穿越次数显著低于PCON组 ($P<0.05$), 且进入平台象限的潜伏期明显长于PCON组 ($P<0.05$, 图3)。表明ASD大鼠存在空间学习、记忆及导航能力的下降。

2.1.2 孕期VPA暴露对子代大鼠HPA轴的影响

血清检测结果显示, 与PCON组相比, PASD

组大鼠血清中CRH、ACTH及CORT的浓度均显著升高 ($P<0.05$, 图4)。表明ASD大鼠的HPA轴呈现过度激活状态。

2.2 运动对ASD大鼠行为、HPA轴的影响

2.2.1 运动对ASD大鼠行为的影响

三室社交试验结果显示, 干预后, 与CON组相比, ASD组大鼠与stranger1的头部接触时间显

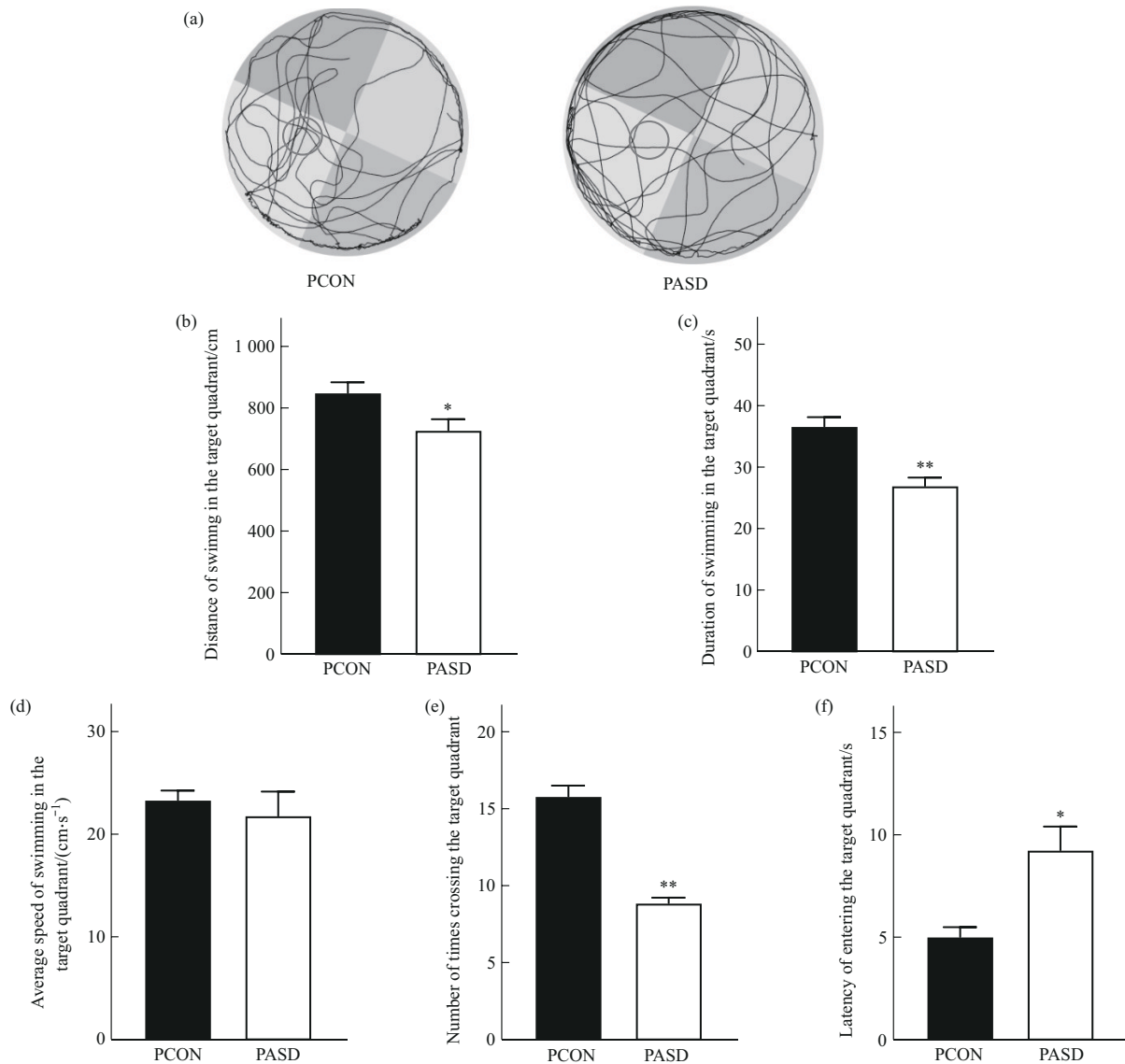


Fig. 3 Influence of VPA exposure on the learning and memory abilities of rats

(a) Representative trajectories of rats in each group in the Morris water maze test. (b) Distance swum in the platform quadrant area. (c) Residence time in the platform quadrant area. (d) The average swimming speed in the platform quadrant area. (e) Number of times of crossing the platform quadrant area. (f) Latency of entering the platform quadrant area. * $P<0.05$, ** $P<0.01$ vs PCON group.

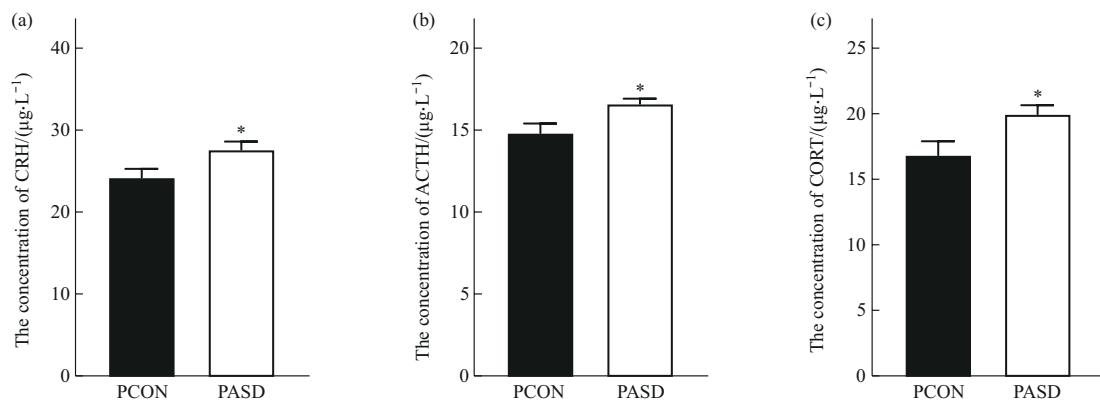


Fig. 4 Influence of VPA exposure on the HPA axis of rats

(a) The concentration of corticotropin-releasing hormone (CRH) of rats in each group. (b) The concentration of adrenocorticotrophic hormone (ACTH). (c) The concentration of corticosterone (CORT). * $P<0.05$ vs PCON group.

著缩短 ($P<0.05$), 与 stranger2 的头部接触时间、所在空间停留时间及接触次数均显著减少 ($P<0.05$), 与 ASD 组相比, ASDE 组大鼠与 stranger1 和 stranger2 的头部接触时间、所在空间停留时间及接触次数均显著增加 ($P<0.05$, 图 5)。表明 6 周自主跑轮运动干预可显著改善 ASD 大鼠的社交能力及社交新颖性偏好能力。

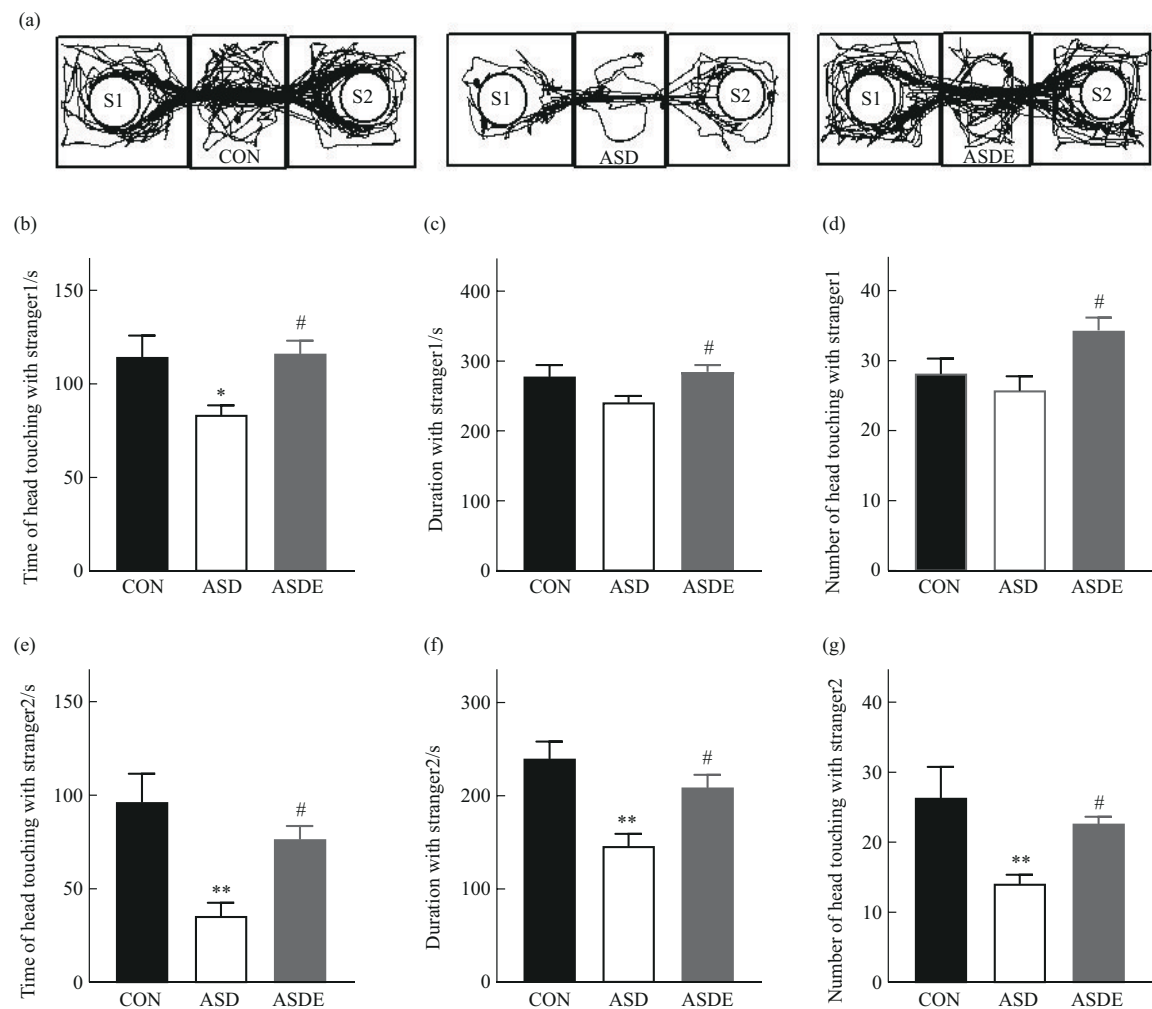


Fig. 5 Effect of exercise training on the social behavior of ASD rats

(a) Representative trajectories of rats in each group in the three-compartment social behavior test, S1 and S2 represent stranger 1 and stranger 2 respectively in the trajectories. (b, e) The head contact time of each group of rats with the stranger 1 and stranger 2 respectively. (c, f) The time each group of rats stayed in the space where the stranger 1 and stranger 2 respectively. (d, g) The number of times each group of rats had head contact with stranger 1 and stranger 2 respectively. * $P<0.05$, ** $P<0.01$ vs CON group; # $P<0.05$ vs ASD group.

旷场试验结果显示, 与 CON 组相比, ASD 组大鼠自由活动的总路程、平均速度、中心区域路程、时间及穿过中心次数均显著降低 ($P<0.05$), 与 ASD 组相比, ASDE 组大鼠自由活动的总路程、平均速度、中心区域路程、时间及穿过中心次数均显著增加 ($P<0.05$, 图 6)。表明 6 周自主跑轮运动可显著提升 ASD 大鼠的自发活动水平, 并增强其对新环境的探索兴趣。

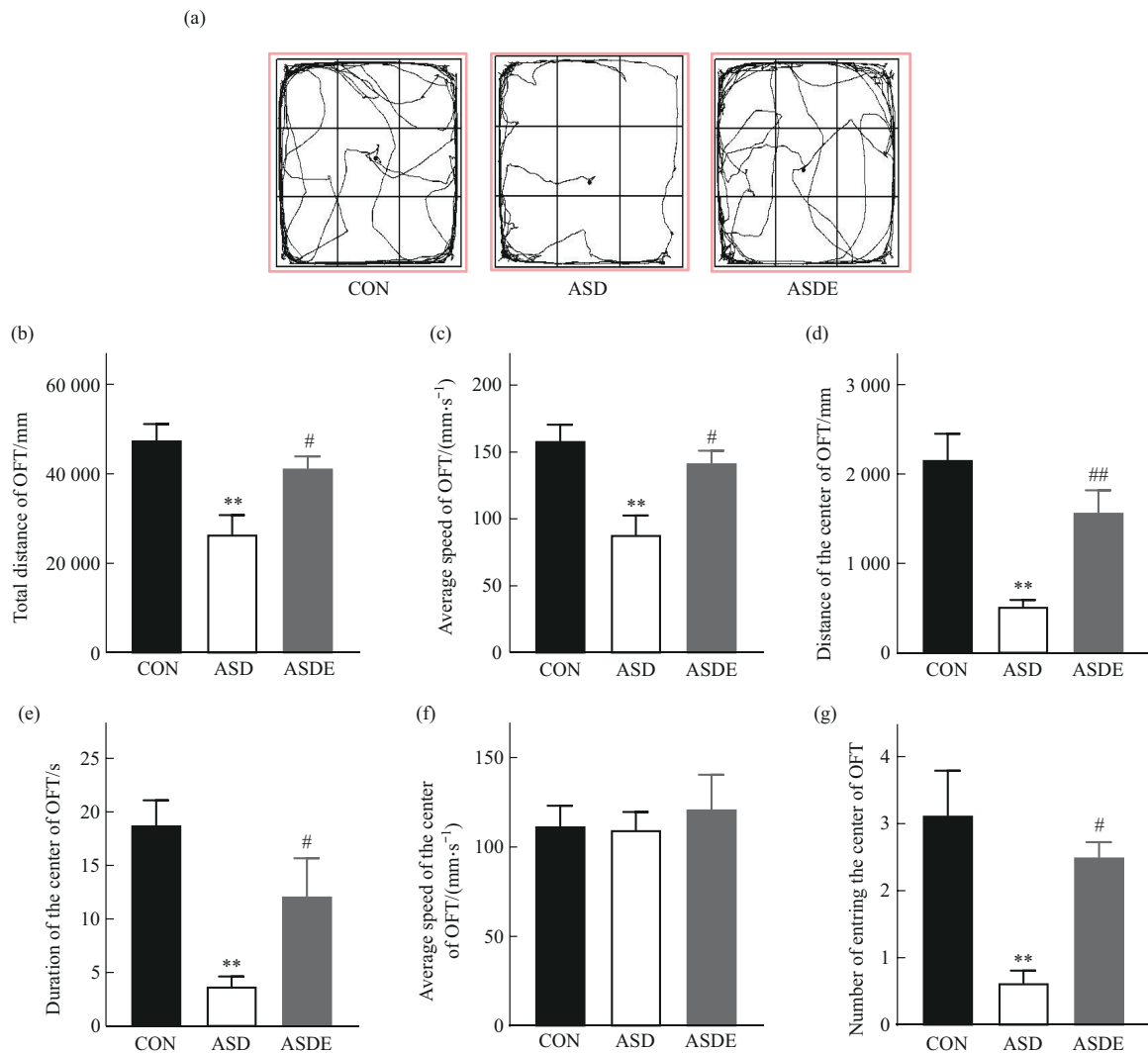


Fig. 6 Effect of exercise training on the spontaneous activity of ASD rats

(a) Representative trajectories of rats in each group in the open field test (OFT). (b) Total distance traveled in the open field. (c) Average speed in the open field. (d) Distance traveled in the center area of the open field. (e) Duration of staying in the center area of the open field. (f) Average speed in the center area of the open field. (g) Number of times crossing the center area of the open field. ** $P < 0.01$ vs CON group; # $P < 0.05$, ## $P < 0.01$ vs ASD group.

水迷宫试验结果显示, 与CON组相比, ASD组大鼠在平台象限区域的游泳路程、游泳时间及穿越次数均显著减少 ($P < 0.05$), 且进入平台象限的潜伏期显著延长 ($P < 0.05$), 与ASD组比较, ASDE组大鼠在平台象限区域的游泳路程、游泳时间及穿越次数均显著增加 ($P < 0.05$), 且进入平台象限的潜伏期显著缩短 ($P < 0.05$, 图7)。表明6周自主跑轮运动可显著改善ASD大鼠的空间学习、

记忆及导航能力。

2.2.2 运动对ASD大鼠HPA轴的影响

血清检测结果显示, 与CON组相比, ASD组大鼠血清中CRH、ACTH及CORT浓度均显著升高 ($P < 0.05$), 与ASD组相比, ASDE组大鼠血清中CORT浓度显著降低 ($P < 0.05$, 图8), ACTH及CRH浓度呈下降趋势。表明6周自主跑轮运动可降低ASD大鼠HPA轴的激活。

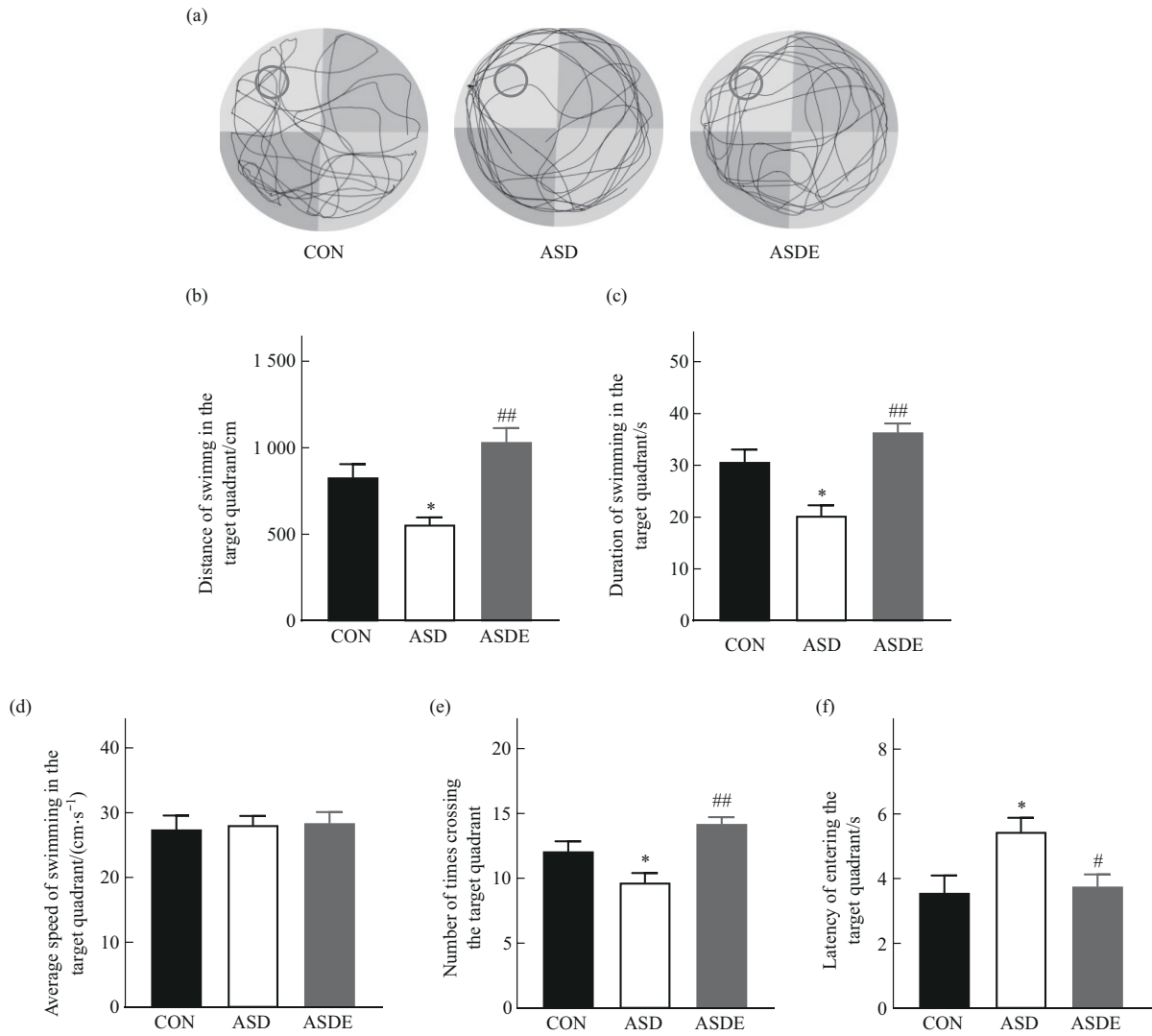


Fig. 7 Effect of exercise training on the learning and memory abilities of ASD rats

(a) Representative trajectories of rats in each group in the Morris water maze test. (b) Distance swum in the platform quadrant area. (c) Residence time in the platform quadrant area. (d) The average swimming speed in the platform quadrant area. (e) Number of times of crossing the platform quadrant area. (f) Latency of entering the platform quadrant area. * $P<0.05$ vs CON group; # $P<0.05$, ## $P<0.01$ vs ASD group.

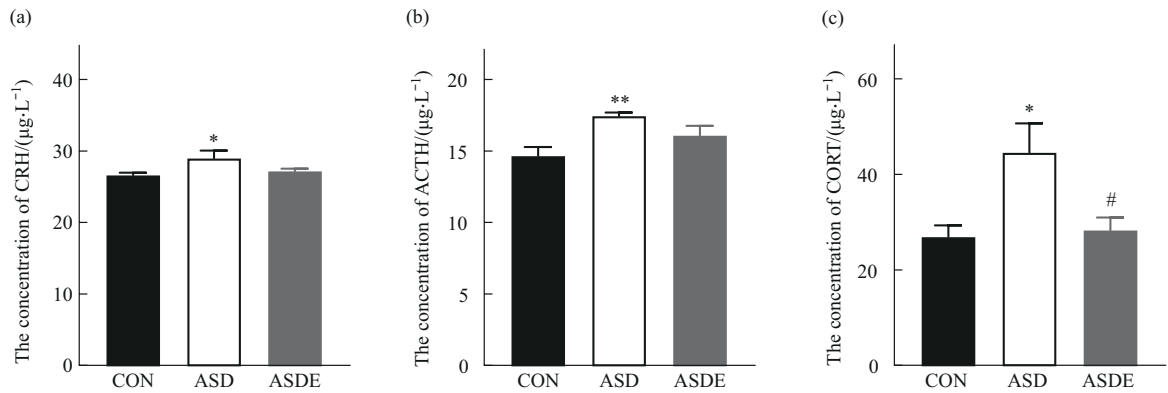


Fig. 8 Effect of exercise training on the HPA axis of ASD rats

(a) The concentration of corticotropin-releasing hormone (CRH) of rats in each group. (b) The concentration of adrenocorticotrophic hormone (ACTH). (c) The concentration of corticosterone (CORT). * $P<0.05$, ** $P<0.01$ vs CON group; # $P<0.05$ vs ASD group.

2.3 运动大鼠的粪菌移植对ASD大鼠行为、HPA轴的影响

2.3.1 运动大鼠的粪菌移植对ASD大鼠行为的影响

三室社交试验结果显示,与ASD组相比,FMT组大鼠与stranger1的所在空间停留时间显著延长 ($P<0.05$),与stranger2的头部接触时间、所在空间停留时间及接触次数均显著增加 ($P<0.05$),与sFMT组相比,FMT组大鼠与stranger1及

stranger2的头部接触时间、所在空间停留时间及接触次数均显著增加 ($P<0.05$),与sFMT组相比,ASDE组大鼠与stranger1的接触次数,与stranger2的头部接触时间、所在空间停留时间及接触次数均显著增加 ($P<0.05$,图9)。表明移植运动大鼠的粪菌可显著提高ASD大鼠的社交能力及社交新颖性偏好。

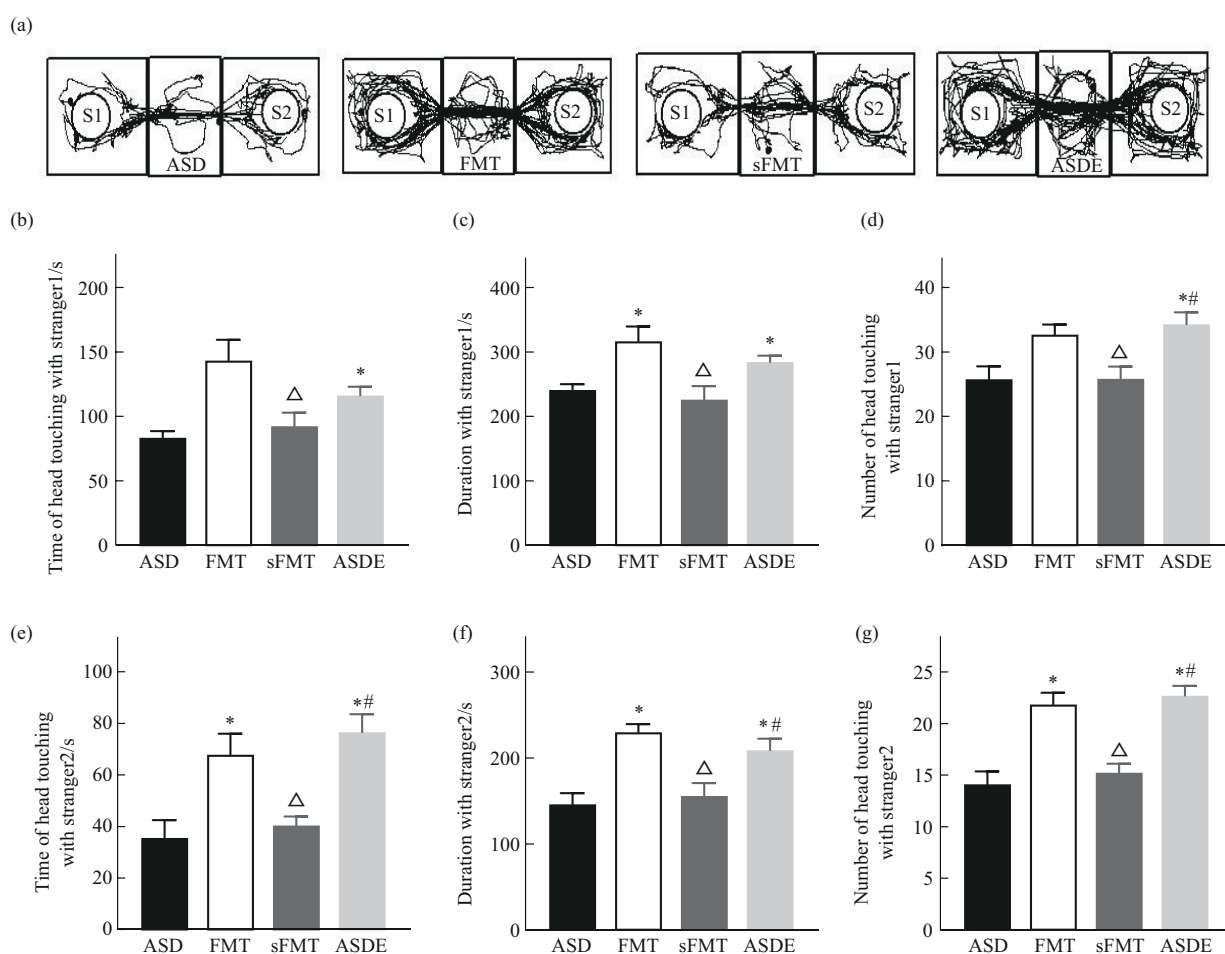


Fig. 9 Effect of fecal microbiota transplantation on the social behavior of ASD rats

(a) Representative trajectories of rats in each group in the three-compartment social behavior test. (b, e) The head contact time of each group of rats with the stranger 1 and stranger 2 respectively. (c, f) The time each group of rats stayed in the space where the stranger 1 and stranger 2 respectively. (d, g) The number of times each group of rats had head contact with the stranger 1 and stranger 2 respectively. * $P<0.05$ vs ASD group; $\Delta P<0.05$ vs FMT group; # $P<0.05$ vs sFMT group.

旷场试验结果显示,与ASD组和sFMT组相比,FMT组大鼠自由活动的总路程、平均速度、中心持续时间及穿过中心次数均显著增加 ($P<0.05$),与sFMT组相比,ASDE组大鼠自由活动的

总路程、平均速度、中心路程、中心持续时间及穿过中心次数均显著增加 ($P<0.05$,图10)。表明移植运动大鼠的粪菌可显著提升ASD大鼠的自发活动水平,并增强其对新环境的探索兴趣。

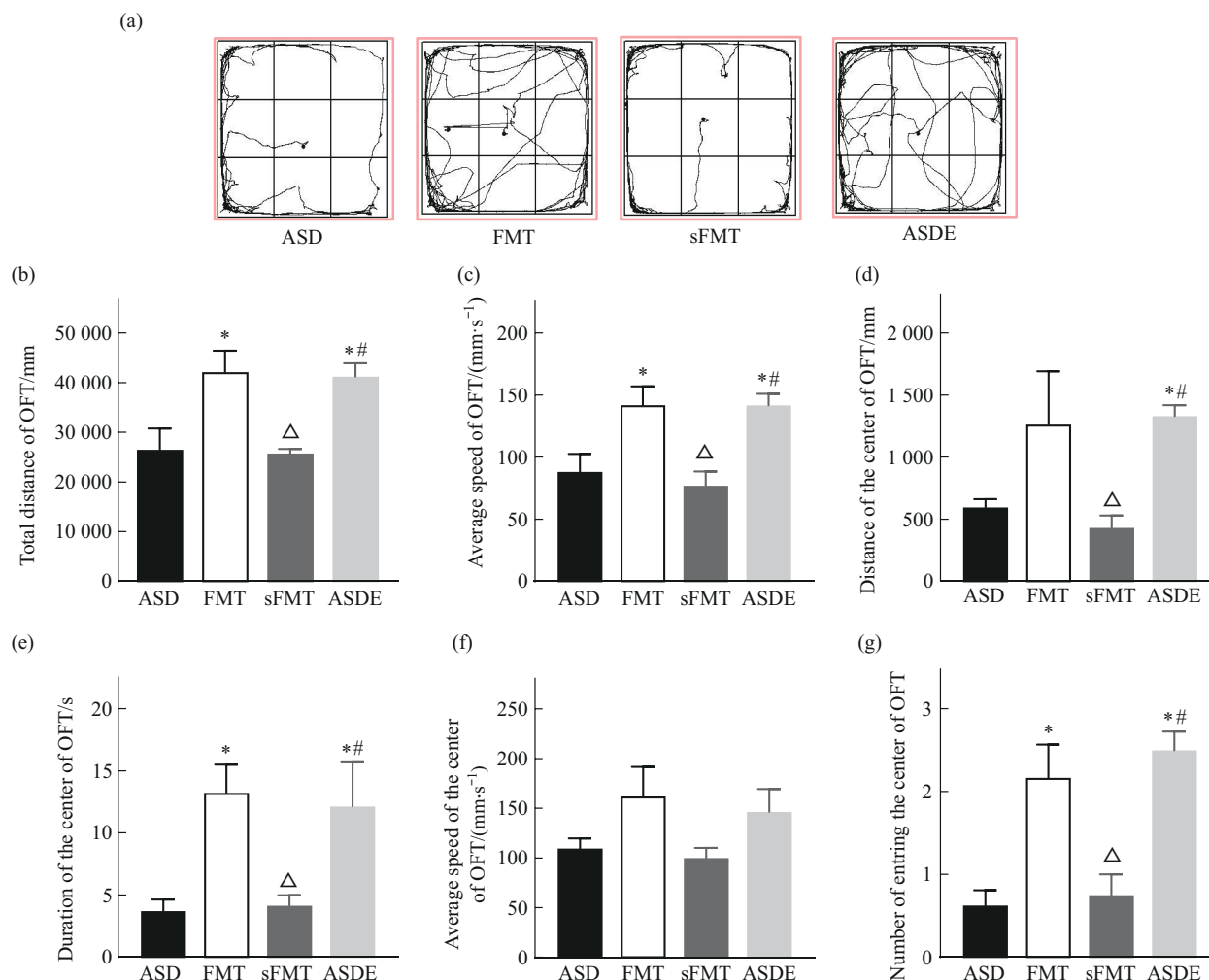


Fig. 10 Effect of fecal microbiota transplantation on the spontaneous activity of ASD rats

(a) Representative trajectories of rats in each group in the open field test (OFT). (b) Total distance traveled in the open field. (c) Average speed in the open field. (d) Distance traveled in the center area of the open field. (e) Duration of staying in the center area of the open field. (f) Average speed in the center area of the open field. (g) Number of times crossing the center area of the open field. * $P < 0.05$ vs ASD group; $\Delta P < 0.05$ vs FMT group; # $P < 0.05$ vs sFMT group.

水迷宫试验结果显示, 与 ASD 组相比, FMT 组大鼠在平台象限区域的游泳路程、游泳时间及穿越次数均显著增加 ($P < 0.05$), 与 sFMT 组相比, FMT 组大鼠与 ASDE 组大鼠在平台象限区域的游泳路程、游泳时间及穿越次数均显著增加 ($P < 0.05$), 进入平台象限的潜伏期均显著缩短 ($P < 0.05$, 图 11)。表明移植运动大鼠的粪菌可显著改善 ASD 大鼠的空间学习、记忆及导航能力。

2.3.2 运动大鼠的粪菌移植对 ASD 大鼠 HPA 轴的影响

血清检测结果显示, 与 ASD 组相比, FMT 组

大鼠血清中 ACTH 和 CORT 浓度均显著降低 ($P < 0.05$), CRH 浓度呈下降趋势, 与 sFMT 组相比, FMT 组和 ASDE 组大鼠血清中 ACTH 和 CORT 浓度均显著降低 ($P < 0.05$, 图 12), CRH 浓度也均呈下降趋势。可见移植组与运动组大鼠的 HPA 轴激活状态呈现相同活性降低的趋势。表明移植运动大鼠的粪菌可以降低 ASD 大鼠 HPA 轴的激活, 尤其对 ACTH 和 CORT 浓度的降低作用更为显著。

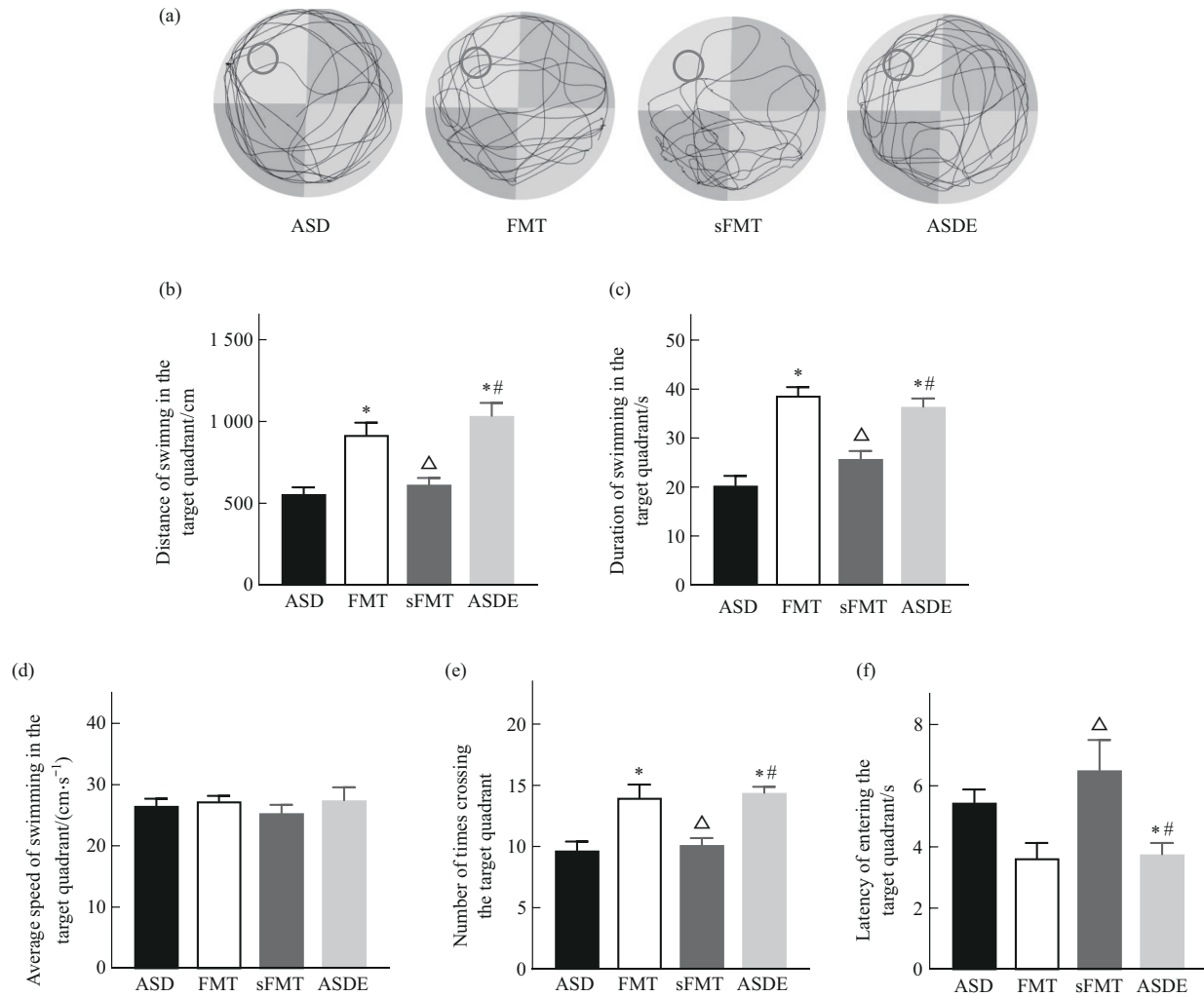


Fig. 11 Effect of fecal microbiota transplantation on the learning and memory abilities of ASD rats

(a) Representative trajectories of rats in each group in the Morris water maze test. (b) Distance swum in the platform quadrant area. (c) Residence time in the platform quadrant area. (d) The average swimming speed in the platform quadrant area. (e) Number of times of crossing the platform quadrant area. (f) Latency of entering the platform quadrant area. * $P < 0.05$ vs ASD group; Δ $P < 0.05$ vs FMT group; $\#$ $P < 0.05$ vs sFMT group.

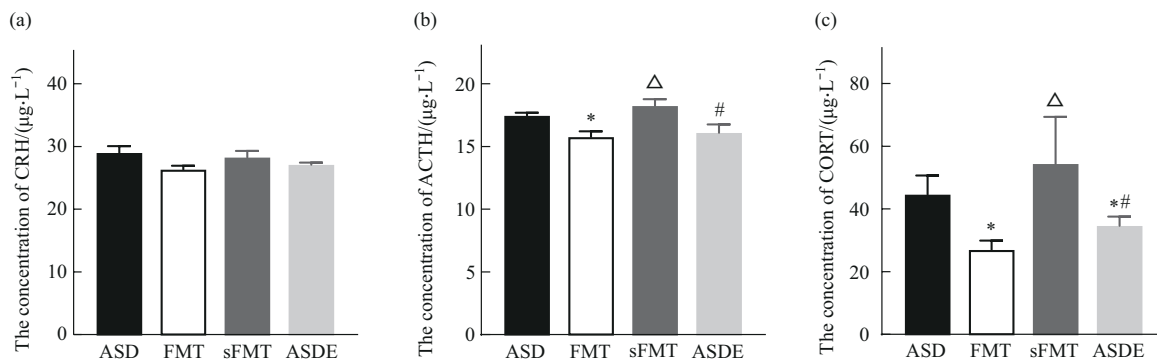


Fig. 12 Effect of fecal microbiota transplantation on the HPA axis of ASD rats

(a) The concentration of corticotropin-releasing hormone (CRH) of rats in each group. (b) The concentration of adrenocorticotrophic hormone (ACTH). (c) The concentration of corticosterone (CORT). * $P < 0.05$ vs ASD group; Δ $P < 0.05$ vs FMT group; $\#$ $P < 0.05$ vs sFMT group.

3 讨 论

妊娠期和围产期是神经系统发育易受影响的的关键时期^[26-27]。研究发现, 孕鼠第12.5天腹腔注射VPA可显著影响胎鼠脑部发育, 导致脑组织异常, 其与孤独症患者临床病因、病理以及行为学症状等具有明显的相似性^[28], 是目前比较经典成熟的ASD动物模型^[29-30]。一项研究发现, 孕鼠腹腔注射VPA后, 其子代在三箱社交检测等行为学实验中, 表现出社交行为减少和社交行为潜伏期增加, 埋珠、理毛等重复刻板动作增加^[31]。与前人结果一致, 本研究造模后三室社交、旷场和水迷宫行为学试验结果显示, ASD大鼠社交能力和社交新颖性偏好下降、自发活动和探索兴趣减少, 以及空间学习, 记忆和导航能力减弱, 表明ASD仔鼠模型构建成功, 后续实验具有可行性。

动物试验显示, 产前应激刺激会诱导高反应性HPA轴应激反应^[32]。例如, 孕期脂多糖(lipopolysaccharide, LPS)干预可激活子代大鼠免疫系统并引发相应炎症, 而这些炎症反应不仅会影响子代大鼠的大脑发育, 还会作为重要的压力信号分子直接激活HPA轴^[33]。本研究同样发现, 孕鼠VPA暴露诱导的子代ASD大鼠血清中CRH、ACTH和CORT含量较对照组均显著增加, 提示其HPA轴功能亢进。其中皮质醇是主要的HPA轴激素, 研究发现过多的皮质醇释放会激活小胶质细胞, 影响中枢神经系统的兴奋与抑制失衡, 这可能导致ASD的病理发展^[34]。本研究发现, ASD大鼠血清中皮质醇浓度显著高于对照组, 这可能与皮质醇参与应激反应的调控有关。研究显示, 皮质醇在反复约束期间下降, 但在不可预测的压力下保持高水平^[12]。此外, 当机体受到压力应激时下丘脑会释放CRH, 进而触发ACTH和CORT的合成和分泌增加。值得注意的是, CRH的调节紊乱与多种疾病密切相关, 如抑郁症、焦虑症和炎症性肠病等^[35]。本研究结果显示, CRH异常可能参与ASD的发病机制, Tsilioni等^[36]针对ASD儿童的研究也进一步验证了这一结论。可以推测, HPA轴的激活在ASD的病理机制中可能发挥着重要作用, 但其具体机制尚不清楚。

近年来, 运动对改善孤独症的效果已得到广泛证实^[37-38]。现有研究表明, 适宜的运动可通过刺激迷走神经调节HPA轴的神经内分泌活动, 进而改善肠道菌群结构及肠道功能^[39]。同时, 运动能

够刺激下丘脑释放内啡肽等神经递质, 抑制CRH释放, 降低ACTH及CORT水平, 减轻HPA轴亢进^[40]。本研究发现, 经自主跑轮运动干预后, ASD大鼠的异常行为得到显著改善; CORT浓度显著下降, CRH及ACTH浓度也呈现下降趋势。动物研究表明, 环境因素引起的HPA轴功能障碍可诱导孤独症样行为, 而调节HPA轴可缓解孤独症样行为^[41]。因此, 本研究结果提示, 运动改善ASD大鼠异常行为的作用机制可能与HPA轴功能调节密切相关。

在微生物群调控领域, 运动的作用机制正被深入挖掘。研究表明, 运动可通过重塑肠道微生物群的组成与功能, 发挥减少炎症反应、调节肠道神经系统免疫及内分泌功能的效应^[42]。小鼠模型及人体研究均发现, 单纯依靠运动就可以改变肠道微生物的组成^[43], 其积极作用主要与转基因多样性的改变以及有益菌与致病菌群落之间的平衡关系有关^[44]。一项动物实验进一步验证了运动调节菌群的健康效应: 将运动小鼠和久坐小鼠的菌群移植至无菌小鼠后, 运动小鼠表现出显著的健康改善^[45]。然而, 目前运动通过调节肠道菌群改善ASD核心症状的研究仍较少^[46]。研究显示, 许多ASD患者肠道中异常菌群(如类杆菌)的相对丰度显著升高, 而运动干预可降低其相对丰度^[45, 47]。结合前期课题组发现, 经6周自主跑轮运动干预后, ASD大鼠的乳杆菌属(*Lactobacillus* spp.)和黏乳杆菌属(*Limosilactobacillus* spp.)丰度显著升高, 而异杆菌属(*Allobaculum* spp.)、普雷沃菌属(*unidentified_Prevotellaceae* spp.)和布劳特氏菌属(*Blautia* spp.)的丰度则显著降低^[20]。近期指出, 运动能够通过调节链球菌、双歧杆菌、梭菌、乳杆菌属和拟杆菌属等菌群的丰度, 影响肠-脑轴改善ASD症状^[48]。一项研究发现, 补充植物性的乳杆菌4周可以改善ASD儿童的相关症状^[42]。本研究将运动干预2周后的ASD大鼠肠道菌群移植至未运动的ASD大鼠体内, 持续4周后, 移植组大鼠表现出与运动组相似的行为学改善趋势。与此同时, Li等^[49]研究证实, 4周的FMT治疗能够有效促进ASD儿童肠道中供体微生物的定植。因此, 推测运动组大鼠的肠道菌群在移植后, 可能通过重塑受体大鼠的肠道菌群, 进而发挥调节行为异常的作用。

研究表明, 肠道菌群可以通过多种途径影响HPA轴的功能, 在ASD的病理机制中发挥重要作用。

用^[15]。许多研究发现,肠道菌群及其衍生的益生菌可恢复HPA轴过度激活,并改善认知、焦虑及社交行为^[39]。微生物可促进一系列神经递质及细胞因子的分泌来调控HPA轴,反过来HPA轴的激活也能作用于肠道活动性、通透性、屏障功能、免疫功能和黏液分泌等维持肠道微生物的稳态^[50]。此外,肠道菌群失调会导致HPA轴的过度激活^[51-52]。一项研究发现,将来自SPF供体的肠道细菌移植到无菌小鼠中,降低了其皮质酮并纠正了社会活动^[11]。本研究选择运动后大鼠的肠道菌群作为供体,移植至ASD大鼠后,发现移植后的ASD大鼠HPA轴激活较对照组下降。此外,运动组和移植组的HPA轴指标无显著差异,推测肠道菌群可能在其中发挥关键作用。同时值得注意的是,移植组与运动组的HPA轴抑制结果趋势并不完全一致,运动大鼠的粪菌移植显著降低了ASD大鼠ACTH和CORT浓度,提示可能是运动调节了大鼠体内肠道菌群中有益菌属的结构,肠道菌群可能通过肠-脑轴直接或间接调节垂体功能。同时课题组前期发现,接受了运动大鼠的肠道菌群移植后,ASD组大鼠肠道中*Lactobacillus* spp.、*Romboutsia* spp.的相对丰度增加^[20]。有学者发现增加*Lactobacillus* spp.、双歧杆菌(*Bifidobacterium* spp.)等有益菌属的摄入可以减少应激诱导的HPA反应,进而改善记忆和学习缺陷以及焦虑和抑郁相关行为^[53]。本研究在干预后行为学检测结果也发现,移植后ASD大鼠的社交能力和社交新颖性偏好、自发活动和探索兴趣,以及空间学习,记忆和导航能力均显著改善。因此推测,运动可能通过影响肠道菌群抑制HPA的激活,进而发挥改善ASD大鼠行为能力的作用。

4 结 论

VPA诱导的孤独症样大鼠存在社交能力、空间学习记忆等行为障碍及HPA轴异常激活。运动可能通过抑制HPA轴激活改善其孤独症样行为,且肠道菌群可能在这一过程中发挥关键作用。然而,值得注意的是,运动如何改变肠道菌群进而改善孤独症的作用机制,以及如何有效利用运动充分发挥肠-脑互动的作用仍将是今后可研究的重点。

参 考 文 献

- [1] First M B. Diagnostic and statistical manual of mental disorders, 5th edition, and clinical utility. J Nerv Ment Dis, 2013, **201**(9): 727-729
- [2] Rylaarsdam L, Guemez-Gamboa A. Genetic causes and modifiers of autism spectrum disorder. Front Cell Neurosci, 2019, **13**: 385
- [3] Lord C, Charman T, Havdahl A, *et al.* The Lancet Commission on the future of care and clinical research in autism. Lancet, 2022, **399**(10321): 271-334
- [4] Zeidan J, Fombonne E, Scora J, *et al.* Global prevalence of autism: a systematic review update. Autism Res, 2022, **15**(5): 778-790
- [5] Maenner M J, Warren Z, Williams A R, *et al.* Prevalence and characteristics of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2020. MMWR Surveill Summ, 2023, **72**(2): 1-14
- [6] Jiang X, Chen X, Su J, *et al.* Prevalence of autism spectrum disorder in mainland China over the past 6 years: a systematic review and meta-analysis. BMC Psychiatry, 2024, **24**(1): 404
- [7] Wang L, Wang B, Wu C, *et al.* Autism spectrum disorder: neurodevelopmental risk factors, biological mechanism, and precision therapy. Int J Mol Sci, 2023, **24**(3): 1819
- [8] Cakir J, Frye R E, Walker S J. The lifetime social cost of autism: 1990 - 2029. Res Autism Spectr Disord, 2020, **72**: 101502
- [9] Gao J, Zou J, Yang L, *et al.* Alteration of peripheral cortisol and autism spectrum disorder: a meta-analysis. Front Psychiatry, 2022, **13**: 928188
- [10] Hubstenberger A, Courel M, Bénard M, *et al.* P-body purification reveals the condensation of repressed mRNA regulons. Mol Cell, 2017, **68**(1): 144-157.e5
- [11] Wu W L, Adame M D, Liou C W, *et al.* Microbiota regulate social behaviour via stress response neurons in the brain. Nature, 2021, **595**(7867): 409-414
- [12] Knezevic E, Nenic K, Milanovic V, *et al.* The role of cortisol in chronic stress, neurodegenerative diseases, and psychological disorders. Cells, 2023, **12**(23): 2726
- [13] Robinson-Agramonte M L A, Noris García E, Fraga Guerra J, *et al.* Immune dysregulation in autism spectrum disorder: what do we know about it?. Int J Mol Sci, 2022, **23**(6): 3033
- [14] Butler M I, Cryan J F, Dinan T G. Man and the microbiome: a new theory of everything. Annu Rev Clin Psychol, 2019, **15**: 371-398
- [15] De W C. Do bacteria shape our development? Crosstalk between intestinal microbiota and HPA axis. Neurosci Biobehav Rev, 2017, **83**: 458-471
- [16] Yousefi B, Kokhaei P, Mehranfar F, *et al.* The role of the host microbiome in autism and neurodegenerative disorders and effect of epigenetic procedures in the brain functions. Neurosci Biobehav Rev, 2022, **132**: 998-1009
- [17] Su Q, Wong O W H, Lu W, *et al.* Multikingdom and functional gut microbiota markers for autism spectrum disorder. Nat Microbiol, 2024, **9**(9): 2344-2355
- [18] Kim D, Kang H. Exercise training modifies gut microbiota with attenuated host responses to sepsis in wild-type mice. FASEB J, 2019, **33**(4): 5772-5781

- [19] 马哲明. 有氧运动对自闭症儿童肠道菌群的影响[D]. 广州: 广州体育学院, 2021
- Ma Z M. Effects Aerobic Exercise on Gut Microbiota of Children With Autism[D]. Guangzhou: Guangzhou Sport University, 2021
- [20] 钟救根. 运动经肠道菌群调控孤独症大鼠中枢神经递质的研究[D]. 上海: 上海体育学院, 2024
- Zhong J G. Study of Exercise Regulating the Central Neurotransmitters of Autistic Rats Through Gut Microbiota[D]. Shanghai: Shanghai University of Sport, 2024
- [21] Tweedie-Cullen R Y, Leong K, Wilson B C, *et al.* Protocol for the gut bugs in autism trial: a double-blind randomised placebo-controlled trial of faecal microbiome transfer for the treatment of gastrointestinal symptoms in autistic adolescents and adults. *BMJ Open*, 2024, **14**(2): e074625
- [22] Wan L, Wang H, Liang Y, *et al.* Effect of oral faecal microbiota transplantation intervention for children with autism spectrum disorder: a randomised, double-blind, placebo-controlled trial. *Clin Transl Med*, 2024, **14**(9): e70006
- [23] Nokia M S, Lensu S, Ahtiaainen J P, *et al.* Physical exercise increases adult hippocampal neurogenesis in male rats provided it is aerobic and sustained. *J Physiol*, 2016, **594**(7): 1855-1873
- [24] Lambert J E, Myslicki J P, Bomhof M R, *et al.* Exercise training modifies gut microbiota in normal and diabetic mice. *Appl Physiol Nutr Metab*, 2015, **40**(7): 749-752
- [25] Sampson T R, Debelius J W, Thron T, *et al.* Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell*, 2016, **167**(6): 1469-1480.e12
- [26] Bilbo S D, Block C L, Bolton J L, *et al.* Beyond infection - maternal immune activation by environmental factors, microglial development, and relevance for autism spectrum disorders. *Exp Neurol*, 2018, **299**(Pt A): 241-251
- [27] Pelch K E, Bolden A L, Kwiatkowski C F. Environmental chemicals and autism: a scoping review of the human and animal research. *Environ Health Perspect*, 2019, **127**(4): 46001
- [28] Chaliha D, Albrecht M, Vaccarezza M, *et al.* A systematic review of the valproic-acid-induced rodent model of autism. *Dev Neurosci*, 2020, **42**(1): 12-48
- [29] Li W K, Zhang S Q, Peng W L, *et al.* Whole-brain *in vivo* base editing reverses behavioral changes in *Mef2c*-mutant mice. *Nat Neurosci*, 2024, **27**(1): 116-128
- [30] Christensen J, Grønborg T K, Sørensen M J, *et al.* Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. *JAMA*, 2013, **309**(16): 1696-1703
- [31] Fontes-Dutra M, Della-Flora Nunes G, Santos-Terra J, *et al.* Abnormal empathy-like pro-social behaviour in the valproic acid model of autism spectrum disorder. *Behav Brain Res*, 2019, **364**: 11-18
- [32] Sharan P, Vellapandian C. Hypothalamic-pituitary-adrenal (HPA) axis: unveiling the potential mechanisms involved in stress-induced Alzheimer's disease and depression. *Cureus*, 2024, **16**(8): e67595
- [33] Agirman G, Yu K B, Hsiao E Y. Signaling inflammation across the gut-brain axis. *Science*, 2021, **374**(6571): 1087-1092
- [34] Cornell J, Salinas S, Huang H Y, *et al.* Microglia regulation of synaptic plasticity and learning and memory. *Neural Regen Res*, 2022, **17**(4): 705-716
- [35] Laryea G, Arnett M G, Muglia L J. Behavioral studies and genetic alterations in corticotropin-releasing hormone (CRH) neurocircuitry: insights into human psychiatric disorders. *Behav Sci (Basel)*, 2012, **2**(2): 135-171
- [36] Tsilioni I, Dodman N, Petra A I, *et al.* Elevated serum neurotensin and CRH levels in children with autistic spectrum disorders and tail-chasing Bull Terriers with a phenotype similar to autism. *Transl Psychiatry*, 2014, **4**(10): e466
- [37] Arnell S, Jerlinder K, Lundqvist L O. Parents' perceptions and concerns about physical activity participation among adolescents with autism spectrum disorder. *Autism*, 2020, **24**(8): 2243-2255
- [38] Huang J, Du C, Liu J, *et al.* Meta-analysis on intervention effects of physical activities on children and adolescents with autism. *Int J Environ Res Public Health*, 2020, **17**(6): 1950
- [39] Monda V, Villano I, Messina A, *et al.* Exercise modifies the gut microbiota with positive health effects. *Oxid Med Cell Longev*, 2017, **2017**: 3831972
- [40] Allen J M, Mailing L J, Niemiro G M, *et al.* Exercise alters gut microbiota composition and function in lean and obese humans. *Med Sci Sports Exerc*, 2018, **50**(4): 747-757
- [41] Zhao X, Mohammed R, Tran H, *et al.* Poly (I: C)-induced maternal immune activation modifies ventral hippocampal regulation of stress reactivity: prevention by environmental enrichment. *Brain Behav Immun*, 2021, **95**: 203-215
- [42] Koutouratsas T, Philippou A, Kolios G, *et al.* Role of exercise in preventing and restoring gut dysbiosis in patients with inflammatory bowel diseases: a review. *World J Gastroenterol*, 2021, **27**(30): 5037-5046
- [43] Allen J M, Mailing L J, Cohrs J, *et al.* Exercise training-induced modification of the gut microbiota persists after microbiota colonization and attenuates the response to chemically-induced colitis in gnotobiotic mice. *Gut Microbes*, 2018, **9**(2): 115-130
- [44] Allen J M, Berg Miller M E, Pence B D, *et al.* Voluntary and forced exercise differentially alters the gut microbiome in C57BL/6J mice. *J Appl Physiol* (1985), 2015, **118**(8): 1059-1066
- [45] Zurita M F, Cárdenas P A, Sandoval M E, *et al.* Analysis of gut microbiome, nutrition and immune status in autism spectrum disorder: a case-control study in Ecuador. *Gut Microbes*, 2020, **11**(3): 453-464
- [46] Plaza-Diaz J, Radar A M, Baig A T, *et al.* Physical activity, gut microbiota, and genetic background for children and adolescents with autism spectrum disorder. *Children (Basel)*, 2022, **9**(12): 1834
- [47] Dan Z, Mao X, Liu Q, *et al.* Altered gut microbial profile is associated with abnormal metabolism activity of autism spectrum disorder. *Gut Microbes*, 2020, **11**(5): 1246-1267
- [48] Wang Y, Chen J, Ni Y, *et al.* Exercise-changed gut mycobiome as a potential contributor to metabolic benefits in diabetes prevention:

- an integrative multi-omics study. *Gut Microbes*, 2024, **16**(1): 2416928
- [49] Li N, Chen H, Cheng Y, *et al.* Fecal microbiota transplantation relieves gastrointestinal and autism symptoms by improving the gut microbiota in an open-label study. *Front Cell Infect Microbiol*, 2021, **11**: 759435
- [50] Lasheras I, Seral P, Latorre E, *et al.* Microbiota and gut-brain axis dysfunction in autism spectrum disorder: evidence for functional gastrointestinal disorders. *Asian J Psychiatr*, 2020, **47**: 101874
- [51] Sudo N, Chida Y, Aiba Y, *et al.* Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J Physiol*, 2004, **558**(Pt 1): 263-275
- [52] Palamarchuk I S, Slavich G M, Vaillancourt T, *et al.* Stress-related cellular pathophysiology as a crosstalk risk factor for neurocognitive and psychiatric disorders. *BMC Neurosci*, 2023, **24**(1): 65
- [53] Dinan T G, Stilling R M, Stanton C, *et al.* Collective unconscious: how gut microbes shape human behavior. *J Psychiatr Res*, 2015, **63**: 1-9

Effects of Exercise Training on The Behaviors and HPA Axis in Autism Spectrum Disorder Rats Through The Gut Microbiota *

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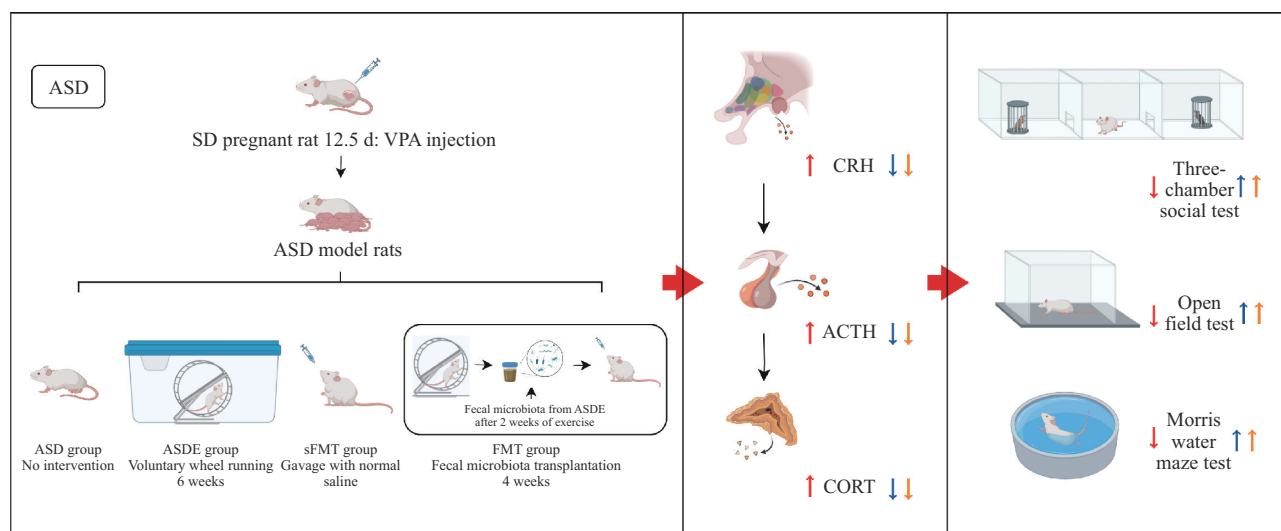
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Graphical abstract



Abstract Objective The study explores the influence of voluntary wheel running on the behavioral abnormalities and the activation state of the hypothalamic-pituitary-adrenal (HPA) axis in autism spectrum disorder (ASD) rats through gut microbiota. **Methods** SD female rats were selected and administered either 400 mg/kg of valproic acid (VPA) solution or an equivalent volume of saline *via* intraperitoneal injection on day 12.5 of pregnancy. The resulting offspring were divided into 2 groups: the ASD model group (PASD, $n=35$) and the normal control group (PCON, $n=16$). Behavioral assessments, including the three-chamber social test, open field test, and Morris water maze, were conducted on postnatal day 23. After behavioral testing, 8 rats from each group (PCON, PASD) were randomly selected for serum analysis using enzyme-linked immunosorbent assay (ELISA) to measure corticotropin-releasing hormone (CRH), adrenocorticotrophic hormone (ACTH), and corticosterone (CORT) concentration, to evaluate the functional state of the HPA axis in rats. On postnatal day 28,

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the remaining 8 rats in the PCON group were designated as the control group (CON, $n=8$), and the remaining 27 rats in the PASD group were randomly divided into 4 groups: ASD non-intervention group (ASD, $n=6$), ASD exercise group (ASDE, $n=8$), ASD fecal microbiota transplantation group (FMT, $n=8$), and ASD sham fecal microbiota transplantation group (sFMT, $n=5$). The rats in the ASD group and the CON group were kept under standard conditions, while the rats in the ASDE group performed 6 weeks of voluntary wheel running intervention starting on postnatal day 28. The rats in the FMT group were gavaged daily from postnatal day 42 with 1 ml/100 g fresh fecal suspension from ASDE rats which had undergone exercise for 2 weeks, 5 d per week, continuing for 4 weeks. The sFMT group received an equivalent volume of saline. After the interventions were completed, behavioral assessments and HPA axis markers were measured for all groups. **Results** Before the intervention, the ASD model group exhibited significantly reduced social ability, social novelty preference, spontaneous activity, and exploratory interest, as well as impaired spatial learning, memory, and navigation abilities compared to the normal control group ($P<0.05$). Serum concentration of corticotropin-releasing hormone (CRH), adrenocorticotrophic hormone (ACTH), and corticosterone (CORT) in the PASD group were significantly higher than those in the PCON group ($P<0.05$). Following 6 weeks of voluntary wheel running, the ASDE group showed significant improvements in social ability, social novelty preference, spontaneous activity, exploratory interest, spatial learning, memory, and navigation skills compared to the ASD group ($P<0.05$), with a significant decrease in serum CORT concentration ($P<0.05$), and a downward trend in CRH and ACTH concentration. After 4 weeks of fecal microbiota transplantation in the exercise group, the FMT group showed marked improvements in social ability, social novelty preference, spontaneous activity, exploratory interest, as well as spatial learning, memory, and navigation abilities compared to both the ASD and sFMT groups ($P<0.05$). In addition, serum ACTH and CORT concentration were significantly reduced ($P<0.05$), and CRH concentration also showed a decreasing trend. **Conclusion** Exercise may improve ASD-related behaviors by suppressing the activation of the HPA axis, with the gut microbiota likely playing a crucial role in this process.

Key words autism spectrum disorder, microbiota, hypothalamic-pituitary-adrenal axis, exercise, fecal microbiota transplantation

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