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ENVIRONMENTAL DOXYCYCLINE ON ANXIOUS BEHAVIOR, COGNITIVE MEMORY ABILITY AND INTESTINAL MICROFLORA OF ZEBRAFISH

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环境浓度多西环素对斑马鱼焦虑行为、认知记忆能力的影响与肠道菌群变化的关联

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摘要: 为探讨环境浓度多西环素长期暴露对斑马鱼焦虑行为、学习记忆、认知灵活性及肠道菌群的影响, 采用不同环境浓度多西环素(0、0.01、0.1和1 μg/L)对斑马鱼成鱼进行水体暴露染毒21d后, 采用新缸实验、Y迷宫、明暗实验和斑马鱼行为学高通量监测系统方法检测环境浓度多西环素对斑马鱼行为学和肠道菌群的影响。结果表明: 在新缸实验中, 与对照组相比, 0.01、0.1和1 μg/L浓度组斑马鱼平均游泳速度显著降低; 随着多西环素浓度升高, 斑马鱼最大游泳速度和总游动距离与对照组相比呈现下降趋势, 其中1 μg/L浓度组同对照组相比差异显著; 在底部区域停留时间上, 与对照组相比, 0.01、0.1和1 μg/L组均有升高, 0.01 μg/L差异显著。在Y迷宫实验中, 0.01 μg/L浓度组斑马鱼在探索臂停留时间与对照组相比显著增加, 0.1和1 μg/L浓度组显著降低; 0.01 μg/L浓度组斑马鱼在探索臂的转弯角度与对照组相比增加, 而0.1和1 μg/L浓度组显著降低。在明暗实验中, 斑马鱼在光区停留时间随着多西环素浓度增加具有上升的趋势。在斑马鱼行为学高通量监测系统实验中, 斑马鱼总游动距离随着多西环素浓度的增加呈现下降趋势, 其中1 μg/L浓度组同对照组相比差异显著。基于16S rRNA测序技术对肠道菌群进行分析, 12份肠道样本共获得1454814条原始有效序列, 筛选后获得1314404条优质序列供后续分析。同时利用DADA2方法降噪, 以100%相似度聚类, 发现多西环素各浓度组减少了斑马鱼肠道菌群的丰度, 博斯氏菌属、副球菌属、不动杆菌等菌属丰度降低, 棒状杆菌属和漫游球菌等菌属丰度升高。香农指数、辛普森指数和chao1指数染毒组同对照组相比显著降低, 此外, 主坐标分析中多西环素组和对照组之间的群落不同。综上, 环境浓度多西环素长时间暴露能够引起斑马鱼行为改变, 产生焦虑样行为, 0.01 μg/L浓度组能够提高斑马鱼短期学习记忆能力和认知灵活性; 多西环素长期暴露引起斑马鱼肠道菌群丰度和多样性改变。

关键词: 环境浓度; 多西环素; 焦虑行为; 认知记忆; 肠道菌群; 斑马鱼

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多西环素(Doxycycline)又称脱氧土霉素, 广泛用于革兰氏阳性菌和革兰氏阴性杆菌的治疗。近年来抗生素的滥用造成了养殖废水和自然水中多西环素的残留[1, 2]。Wei等[1]于江苏省某大型畜牧场的废水中检测到多西环素残留最大浓度为39.5 μg/L。Wang等[2]对长江饮用水源的旱季和常规季多西环素的残留水平进行测定, 旱季时最大残留浓度为56.09 ng/L, 常规季最大残留浓度为4.96 ng/L。Yan等[3]在长江口沿线的7个地点采集了地表水样本, 其中监测到残留的多西环素浓度最大为5.6 ng/L。Jiang等[4]在黄浦江水样检测到地表水多西环素浓度最大为46.93 ng/L。多西环素的残留对机体健康造成潜在威胁。研究表明, 多西环素能够对人类的肝脏造成不良影响并诱发病症[5, 6]。此外, 抗生素残留还

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会导致耐药菌的出现, 影响抗生素发挥作用, 增加患者的死亡率[7], 多西环素能够影响肠道菌群的平衡[8]。故抗生素不合理的排放和使用对人类和自然界微生物会产生强大的选择压力, 造成巨大影响[9, 10]。

目前, 斑马鱼是毒理学研究的重要模式动物[11], 同时也是研究神经生物学行为现象的一种模式动物[12]。Qiu等[13]用恩诺沙星暴露于斑马鱼, 引起了肠道菌群丰度的降低。Maselli等[14]将广谱抗生素暴露于斑马鱼, 同样对肠道菌群水平造成了影响。目前研究集中于肠道菌群同炎症和免疫方面较多, 同斑马鱼行为关联的研究较少。研究表明, 肠道菌群的变化同行为的改变关联密切[15, 16]。目前, 关于多西环素长期暴露对斑马鱼行为的影响研究比较少, 尤其是行为学变化与菌群变化的关联还有待深入探讨。本实验以斑马鱼作为一种环境危害评估模型动物, 采用环境浓度多西环素对斑马鱼进行长期暴露, 研究多西环素对斑马鱼焦虑行为、记忆、认知灵活性和肠道菌群的影响, 为探讨多西环素对斑马鱼行为的影响及其作用机制研究提供一定的参考。

1　材料与方法

1.1　实验动物

选择体重变化较小的野生斑马鱼雄鱼(4—5月龄)作为实验动物, 斑马鱼购自中国沈阳某市场, 随机平均分配在两个饲养缸(34 cm×22.5 cm×19.5 cm)中(n=100), 于内蒙古毒物监测与毒理学重点实验室暂养15d, 水温22℃, pH 7.8—8.0, 14L∶10D明暗循环。每天2次适量饲喂斑马鱼饲料(日本日清丸红饲料株式会社公司, 粗蛋白大于等于≥50%, 粗脂肪≥10%), 实验用斑马鱼遵循内蒙古民族大学动物伦理规范。

1.2　实验药品

盐酸多西环素标准品(97%)购自Solarbio公司。CAS号为24390-14-5, 分子式为$C_{22}H_{24}N_2O_8\cdot HCl\cdot 0.5H_2O\cdot 0.5C_2H_6O$, 分子量512.94。

1.3　多西环素暴露方法

将盐酸多西环素标准品粉末溶于蒸馏水中, 配制0 (对照组, CO)、0.01、0.1和1 μg/L多西环素环境浓度。选取透明亚克力鱼缸(26.5 cm×12.5 cm×13 cm)12个, 每个缸中加入2 L斑马鱼系统循环水[水温(22±1)℃, pH 7.8—8.0)。每个缸中随机放入8条斑马鱼, 每个浓度组设置3个平行。暴露时间为21d, 每天换1/2水。14L∶10D明暗循环, 饲喂方法同上。

1.4　行为学检测方法

新缸实验　新缸实验是观测斑马鱼焦虑行为实验方式之一[17], 具体为将透明矩形水缸(40 cm×18 cm×20 .5 cm)水体填充至15 cm的高度, 水体高度被平均分成上、中、下三部分。实验前将斑马鱼放入缸中适应5—10min, 然后拍摄6min的视频。利用Any-maze视频跟踪技术分析斑马鱼的最大游泳速度和平均游泳速度、总游动距离及底部区域停留时间。

Y迷宫实验　Y迷宫实验用于评估斑马鱼的学习记忆和认知灵活性[18]。Y迷宫像字母“Y”般, 分为三个臂, 分别为起始臂、开放臂和探索臂, 每两个臂的夹角为60°, 每个臂的参数为30 cm×6 cm×15 cm。实验前关闭探索臂, 斑马鱼在其他两个臂自由游动5min。在实验开始后, 令其强制回到起始臂, 并开放探索臂, 录制5min视频后, 利用Any-maze软件分析录制好的视频, 检测其在探索臂停留的时间、探索臂的转弯角度及进入探索臂的次数[19]。

明暗实验　用矩形透明缸作为实验鱼缸(40 cm×18 cm×20.5 cm), 缸内水体高度15 cm。将矩形缸体按照其最长的长度平均分为左、右两个部分, 为黑暗区和光明区, 中间用隔板隔开, 黑暗区用不透光黑色薄膜包裹。实验前将斑马鱼放入缸内适应5—10min, 随后将其放入光明区, 拍摄5min视频。将视频内容放入Any-maze软件中分析斑马鱼在光明区所停留的时间。

斑马鱼行为学系统检测　斑马鱼行为学系统为鱼类高通量监测系统(法国Viewpoint Life Sciences公司), 将每条斑马鱼单独放在透明矩形方盒中(11.5 cm×6.5 cm×6.5 cm), 水体占整个高度2/3, 斑马鱼在其中适应5—10min后, 放入鱼类高通量监测系统, 系统设置为光暗交替, 每5min变换1次, 总时间为40min。检测斑马鱼的总游动距离及游动轨迹图。

1.5　肠道菌群检测

斑马鱼在多西环素中暴露21d后, 取出斑马鱼放入冰块中麻醉后解剖, 取其肠道内容物放入冻存管内。每个鱼缸取3条鱼的肠道内容物放入1个冻存管中, 每个浓度组包含3个样本。样本经干冰保存后送检至诺禾致源公司进行16S V4区域微生物群落扩增子测序分析, 内容包括肠道菌群的特征分析、α和β多样性分析及相关性分析和通路功能预测。

1.6　统计分析

利用GraphPad Prism 8.0软件绘制图表, 采用单因素方差分析(One-way analysis of variance, ANOVA)确定统计学差异, P<0.05表示差异显著。数据以均值±标准差(SD)表示。

2 结果

2.1 多西环素对斑马鱼焦虑行为的影响

新缸实验 斑马鱼新缸实验热图见图 1, 随着多西环素浓度的升高, 斑马鱼在底部区域的停留时间延长, 0.01 μg/L浓度组同对照组相比差异显著($P<0.05$; 图 2B)。斑马鱼在多西环素的暴露组中平均速度均低于对照组, 差异显著(图 2A)。随着多西环素浓度增加, 斑马鱼总的游泳距离(图 2C)和最大游泳速度(图 2D)呈现下降趋势, 1 μg/L浓度组与对照组相比差异显著($P<0.05$)。

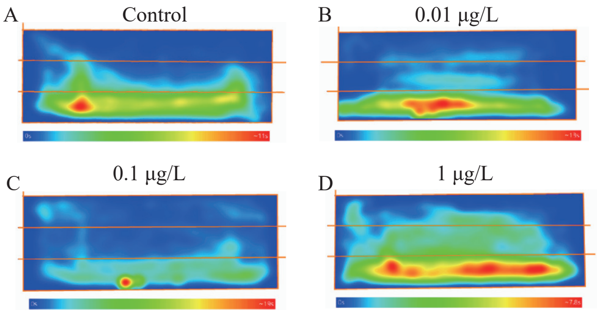

图 1 斑马鱼新缸实验停留时间热图

Fig. 1 Heat map of experimental residence time of zebrafish in new tank

颜色由绿色至红色表示斑马鱼停留时间逐渐增加

The color from green to red indicates a gradual increase in the residence time of zebrafish

明暗实验 明暗实验结果(图 3)显示斑马鱼在光区所停留的时间随着多西环素浓度的增加而上升, 但0.01、0.1和1 μg/L浓度组同对照组相比差异不显著(图 2H; $P>0.05$)。

斑马鱼行为学高通量监测系统 斑马鱼总游动距离随着多西环素浓度的增加, 产生了下降的趋势, 1 μg/L组同对照组相比显著减少(图 2I; $P<0.05$)。

2.2 Y迷宫

斑马鱼在探索臂停留的时间见图 2E, 同对照组相比, 0.01、0.1和1 μg/L浓度组差异显著($P<0.05$), 0.01 μg/L浓度组差异极显著($P<0.01$)。在探索臂的转弯角度(图 4和图 5) 0.1和1 μg/L浓度组同对照组相比差异显著。各浓度组斑马鱼进入探索臂的次数均不显著(图 2F; $P>0.05$)。

2.3 多西环素对斑马鱼肠道菌群的影响

采用16S rRNA测序技术, 12份肠道样本共获得

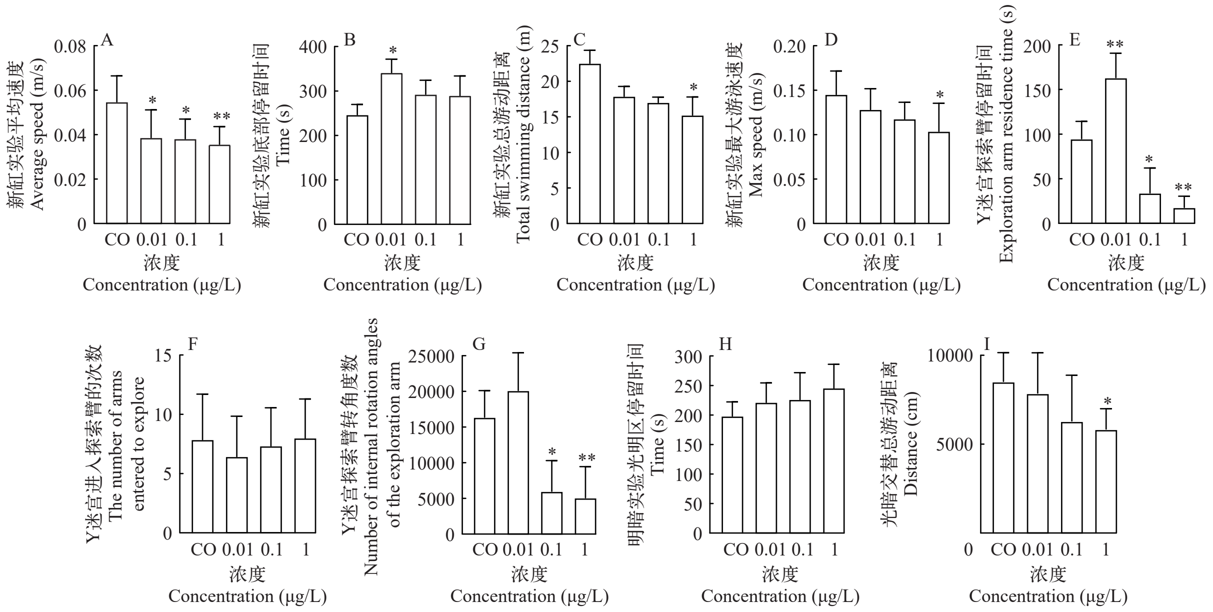

图 2 多西环素对斑马鱼焦虑行为影响

Fig. 2 Effect of doxycycline on anxious behavior of zebrafish

A为新缸实验多西环素对斑马鱼平均游泳速度影响; B为新缸实验底部停留时间; C为新缸实验总游动距离; D为新缸实验最大游泳速度; E为Y迷宫探索臂停留时间; F为Y迷宫进入探索臂次数; G为Y迷宫探索臂转角度数; H为明暗实验斑马鱼光区停留时间; I为高通量斑马鱼光暗交替总游动距离(*表示差异显著, 即$P<0.05$; **表示差异极显著$P<0.01$)

A. Effect of doxycycline on average swimming speed of zebrafish in new tank experiment; B. New tank test bottom residence time; C. the total swimming distance of the new tank experiment; D. New tank test maximum swimming speed; E. the residence time of Y maze exploration arm; F. the number of times that Y maze exploration arm enters the exploration arm; G. the number of degrees of Y maze exploration arm; H. the light area residence time of zebrafish in the light and dark experiment; I. the total swimming distance of high-flux zebrafish alternating light and dark (* indicates significant difference, $P<0.05$; ** means extreme significant difference $P<0.01$)

1454814条原始有效序列, 筛选后获得1314404条优质序列供后续分析。同时利用DADA2方法降噪, 以100%相似度聚类。

在生物各层级数目(图 6)统计中, 从总体上看, 随着多西环素浓度的增加, 斑马鱼肠道菌群丰度逐渐减少(图 7), 同对照组相比, 0.01、0.1和1 μg/L组别分别下降了0.072倍、0.065倍和0.221倍。种和属所占比例位居前两位, 所占比例分别为31.5%、48.2%、19.9%、29.9%和58.5%、48.3%、74.4%、60.2%, 具有一定代表性。

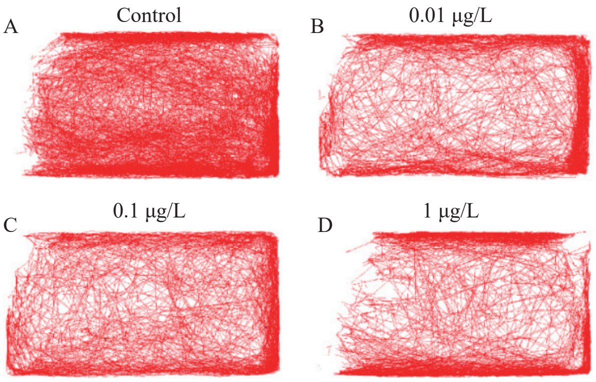

图 3　斑马鱼高通量光暗交替环境下行为检测轨迹图

Fig. 3　Behavior detection trajectory of zebrafish in high-flux lightdark alternating environment

线条为斑马鱼的游动轨迹

The line is the swimming track of zebrafish

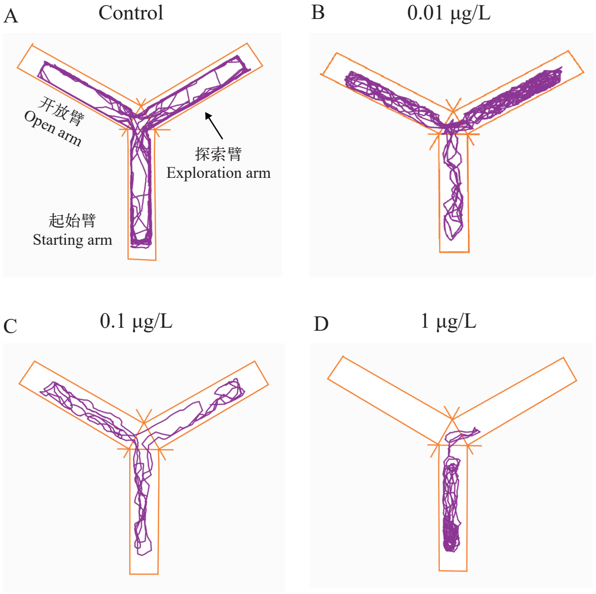

图 4　斑马鱼Y迷宫轨迹图

Fig. 4　Labyrinth track of zebrafish Y

线条为斑马鱼的游动轨迹, Open arm为开放臂, Starting arm为起始臂, Exploration arm为探索臂

The line is the swimming track of zebrafish

香农指数、辛普森指数, 物种丰富度指数和chao1指数多西环素组同对照组相比整体上均有所下降, Goods_coverage指数和优势度指数多西环素组同对照组相比整体上有所增加(图 8)。

在KEGG功能预测聚类热图中, 代谢通路数据库(图 9)中细胞过程通路水平同对照组相比提高显著, 新陈代谢通路水平下降显著。基因数据库中(图 10), 信号转导通路及细胞运动性通路和聚糖的生物合成和代谢通路功能同对照组相比提高, 氨基

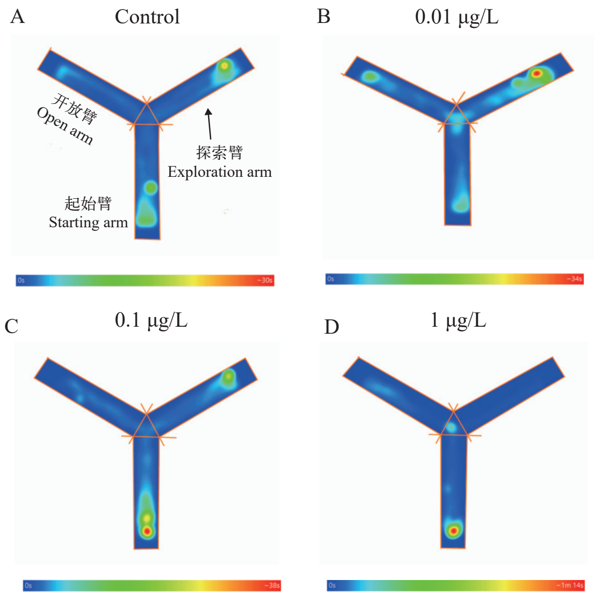

图 5　斑马鱼Y迷宫停留时间热图

Fig. 5　Residence time heat map of zebrafish Y maze

颜色由绿至红表示斑马鱼停留时间逐渐增加

The color from green to red indicates that the residence time of zebrafish increases gradually

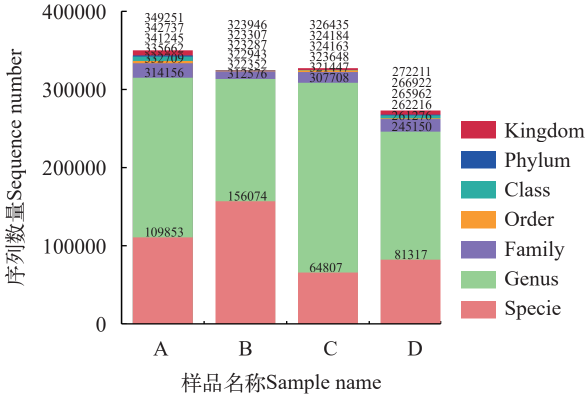

图 6　斑马鱼肠道菌群各个层级数目柱状图

Fig. 6　Histogram of the number of intestinal flora at each level of zebrafish

A代表对照组; B代表 0.01 μg/L; C代表 0.1 μg/L; D代表1 μg/L 界Kingdom; 门Phylum; 纲Class; 目Order; 科Family; 属Genus; 种Species

酸代谢通路、免疫系统通路、辅助因子和维生素的代谢通路功能下降。配体数据库中(图 11)双组分信号系统和细菌运动蛋白通路功能增加, 线粒体生物合成和维生素的代谢、疾病内分泌代谢和甘氨酸、丝氨酸和苏氨酸的代谢水平下降较为显著。

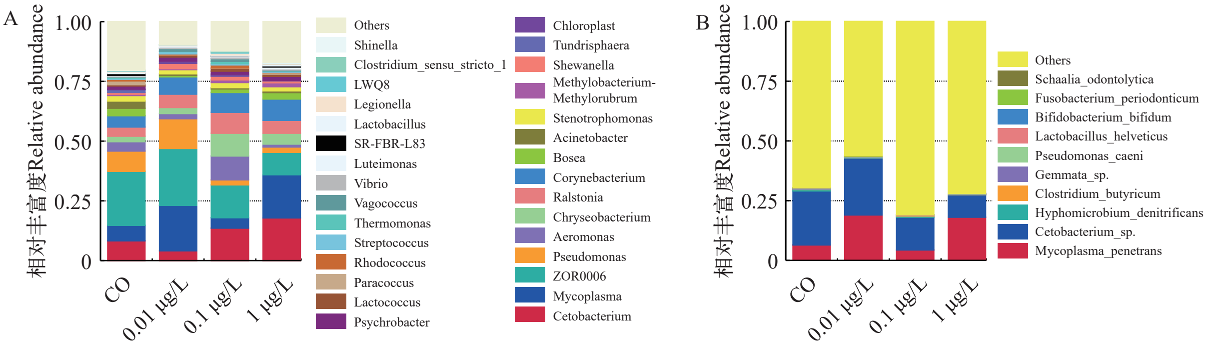

图 7 各组别变化显著菌属和菌种

Fig. 7 There were significant changes in bacteria genera and species in each group

A为各组别变化显著的前30菌属; B为各组别变化显著的前10菌种。各组别变化显著的前30菌属包括申氏菌属(*Shinella*)、狭义梭菌属(*Clostridium_sensu_stricto_1*)、LWQ8 (*LWQ8*)菌属、军团菌属(*Legionella*)、乳酸杆菌SR-FBR-L83菌属(*LactobacillusSR-FBR-L83*)、藤黄色单胞菌属(*Luteimonas*) 、弧菌属(*Vibrio*)、阴道球菌属(*Vagococcus*)、嗜热单胞菌属(*Thermomonas*)、链球菌属(*Streptococcus*)、红球菌属(*Rhodococcus*)、副球菌属(*Paracoccus*)、乳球菌属(*Lactococcus*)、嗜冷杆菌属(*Psychrobacter*)、希瓦氏菌属(*Shewanella*)、寡养单胞菌属(*Stenotrophomonas*)、不动杆菌属(*Acinetobacter*)、博斯氏菌属(*Bosea*)、棒状杆菌菌属(*Corynebacterium*)、罗尔斯通氏菌属(*Ralstonia* sp.)、金黄杆菌属(*Chryseobacterium*)、气单胞菌属 (*Aeromonas*) 、假单胞菌属(*Pseudomonas*)、ZOR0006菌属(*ZOR 0006*)、鲸杆菌属(*Cetobacterium*)。各组别变化显著的前10菌种, 包括龋齿沙尔氏菌(*Schaalia odontolytica*)、牙周梭杆菌(*Fusobacterium periodonticum*)、双歧杆菌(*Bifidobacterium bifidum*)、瑞士乳杆菌(*Lactobacillus helveticus*) 、淤泥假单胞菌(*Pseudomonas caeni*)、出芽菌种(*Gemmata* sp.)、丁酸梭菌(*Clostridium butyricum*)、脱氮生丝微菌(*Hyphomicrobium denitrifican*)、醋酸杆菌(*Cetobacterium*_sp.)、渗透支原体(*Mycoplasma penetrans*)

A is the top 30 bacterial genera with significant changes in each group; B is the top 10 bacterial species with significant changes in each group

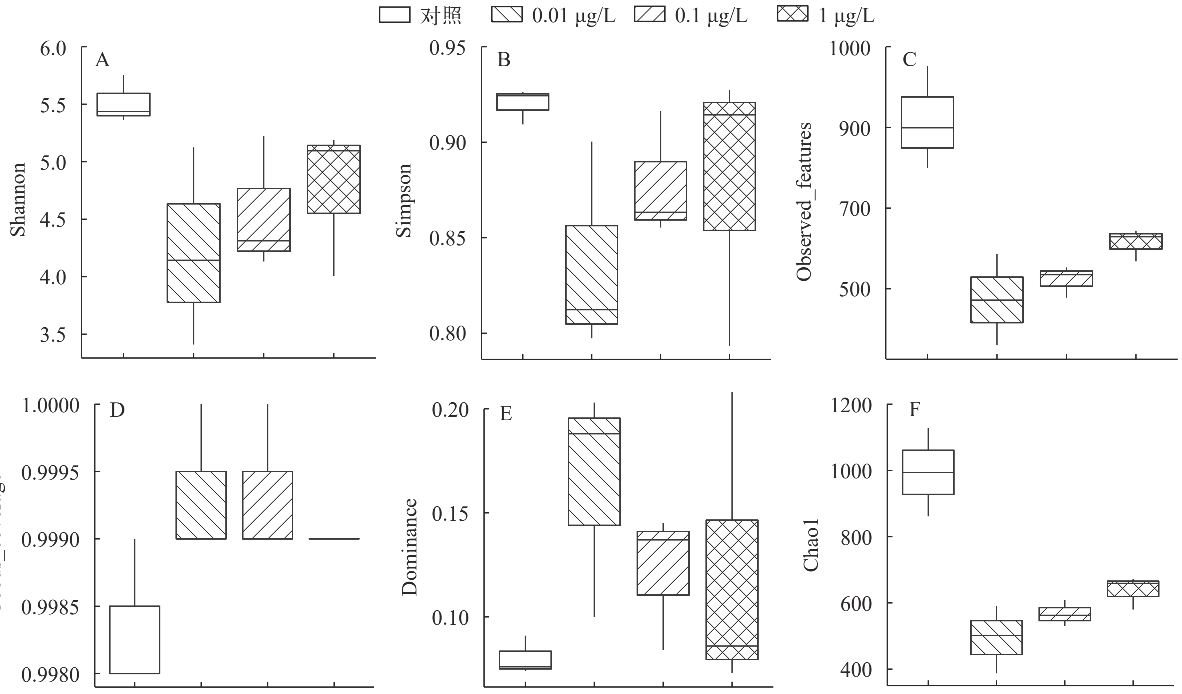

图 8 肠道菌群有关指数分析

Fig. 8 Analysis of relevant index of intestinal flora

A为香农指数; B为辛普森指数; C为物种丰富度指数; D为Goods_coverage指数; E为优势度指数; F为chao1指数

A is the Shannon index; B is the Simpson index; C is the species richness index; D is the Goods_coverage index; E is the dominance index; F is the chao1 index

3　讨论

3.1　环境浓度多西环素长期暴露引发斑马鱼行为变化

抗生素的长期暴露引起斑马鱼行为变化。在Wang等[20]的研究中, 斑马鱼长期暴露在氧氟沙星、环丙沙星、恩诺沙星、多西环素、金霉素和土霉素的混合物中, 在缸顶部的时间增加。ALMEIDA等[21]将斑马鱼成鱼暴露于0、0.1、10和10000 mg/L的土霉素中2个月后, 在新缸实验中发现暴露于土霉素组别的斑马鱼在底部的停留时间随土霉素浓度升高有上升的趋势。当斑马鱼花费较多时间停留于水底部, 减少在水体顶端的时间, 并表现出静止不动和运动的不稳定, 这些现象被认定为斑马鱼的一种焦虑表现[22—25]。在本实验中, 使用环境浓度的多西环素的长期暴露引起了斑马鱼行为变化, 并产生了焦虑行为。在鱼类高通量监测系统的分析结果中, 利用光暗交替的技术手段, 探究斑马鱼在此条件下的游动轨迹和距离情况。在不同的光照条件下, 运动被用来表征焦虑[26, 27]。在本实验中, 斑马鱼游动的总距离随着多西环素浓度增加而减少, 轨迹深度减弱, 基于以上相关数据, 环境浓度多西环素的长期暴露引起斑马鱼行为参数的改变。

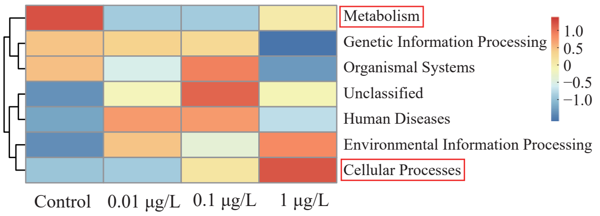

图 9　KEGG功能预测聚类热图(代谢通路数据库)

Fig. 9　KEGG functional prediction clustering heat map (Metabolic pathway database)

新陈代谢(Metabolism)、遗传信息处理(Genetic information processing)、环境信息处理(Environmental information processing)、细胞过程(Cellular processes)、生物体系统(Organismal systems)、人类疾病(Human diseasea)和其他(Unclassifiled)

3.2　环境浓度多西环素长期暴露能提高斑马鱼认知灵活性

环境浓度多西环素长期暴露提高斑马鱼认知灵活性。Y迷宫是一种重要的检测动物认知记忆能力的方法。Ahmad 等[28]将抗生素环丙沙星以饮用水的形式进入小鼠体内, 经Y迷宫方法检测后, 环丙沙星的暴露降低了小鼠的认知记忆能力。同样的, Y迷宫也可以评估斑马鱼的学习记忆和认知灵活

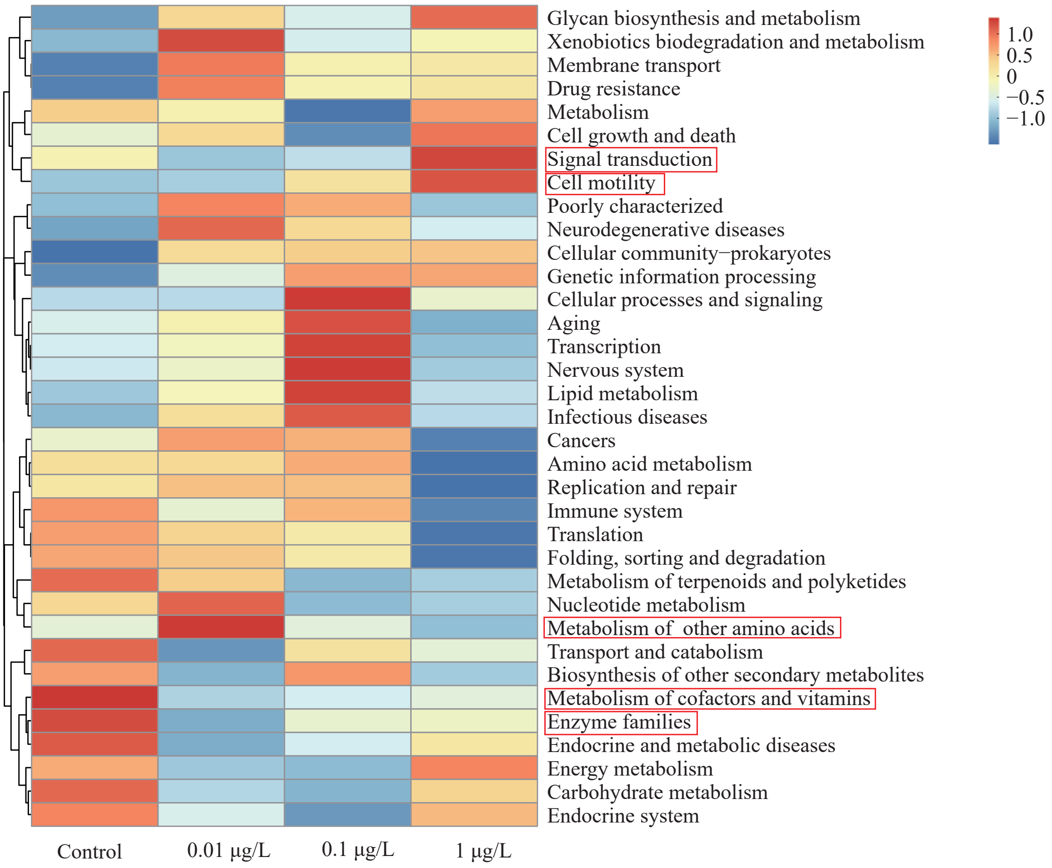

图 10　基因数据库变化显著通路

Fig. 10　Significant in change pathwaygene database

性, 斑马鱼在探索臂停留时间越长、进入探索臂的次数越多, 表明其认知灵活性和记忆能力的增加[18]。在本实验中, 0.01 μg/L浓度组斑马鱼在探索臂停留时间特别显著, 进入探索臂次数比对照组相比升高。这说明较低浓度0.01 μg/L浓度组多西环素长期暴露能够提高斑马鱼的短期学习记忆能力, 但是随着多西环素浓度的升高, 学习记忆能力降低。研究表明, 可进入中枢的药物分子量普遍小于450 Da[29], 而多西环素标准品的分子量为444.435 Da, 这意味着多西环素具备通过血脑屏障的能力。在本实验中, 多西环素在同一体积中, 浓度越小, 其能够通过血脑屏障进而影响大脑的可能性增加。同时, 低浓度组在提高了斑马鱼学习和认知记忆能力的同时, 也产生了焦虑行为, 具体机制有待进一步研究。

3.3 环境浓度多西环素长期暴露影响斑马鱼的肠道菌群丰度和多样性

抗生素的长期暴露, 影响斑马鱼肠道菌群的平衡。ALMEIDA等[21]将斑马鱼成鱼暴露于0、0.1、10、10000 mg/L的土霉素中2个月后, 肠道种群的多样性和丰度下降。Tian等[30]将恩诺沙星对斑马鱼的长期暴露28d后, 导致肠道菌群明显失调。本实验也得到了类似结果, 多西环素暴露影响了斑马鱼肠道种群的多样性和丰度。在本实验中, 不动杆菌属降低, 研究表明, 不动杆菌属能够抵抗肠应激[31], 肠应激同焦虑存在一定关联[32]。多西环素暴露组的香农指数、辛普森指数和Chao1指数同对照组相比整体上均有所下降, 香农指数同生物多样性呈正相关, 辛普森指数和Chao1指数同物种丰富度联系密切, 说明抗生素暴露组肠道菌群的丰富度和多样性下降。此外, 在多西环素暴露后, 随着暴露浓度的增加, 斑马鱼肠道菌群的丰度和数量逐渐减少, 斑马鱼的位置偏好发生改变, 与斑马鱼焦虑行为的参数变化趋势一致, 研究指出, 这种行为参数和位置偏好的变化能够视为一种斑马鱼的焦虑行为[22—25]。

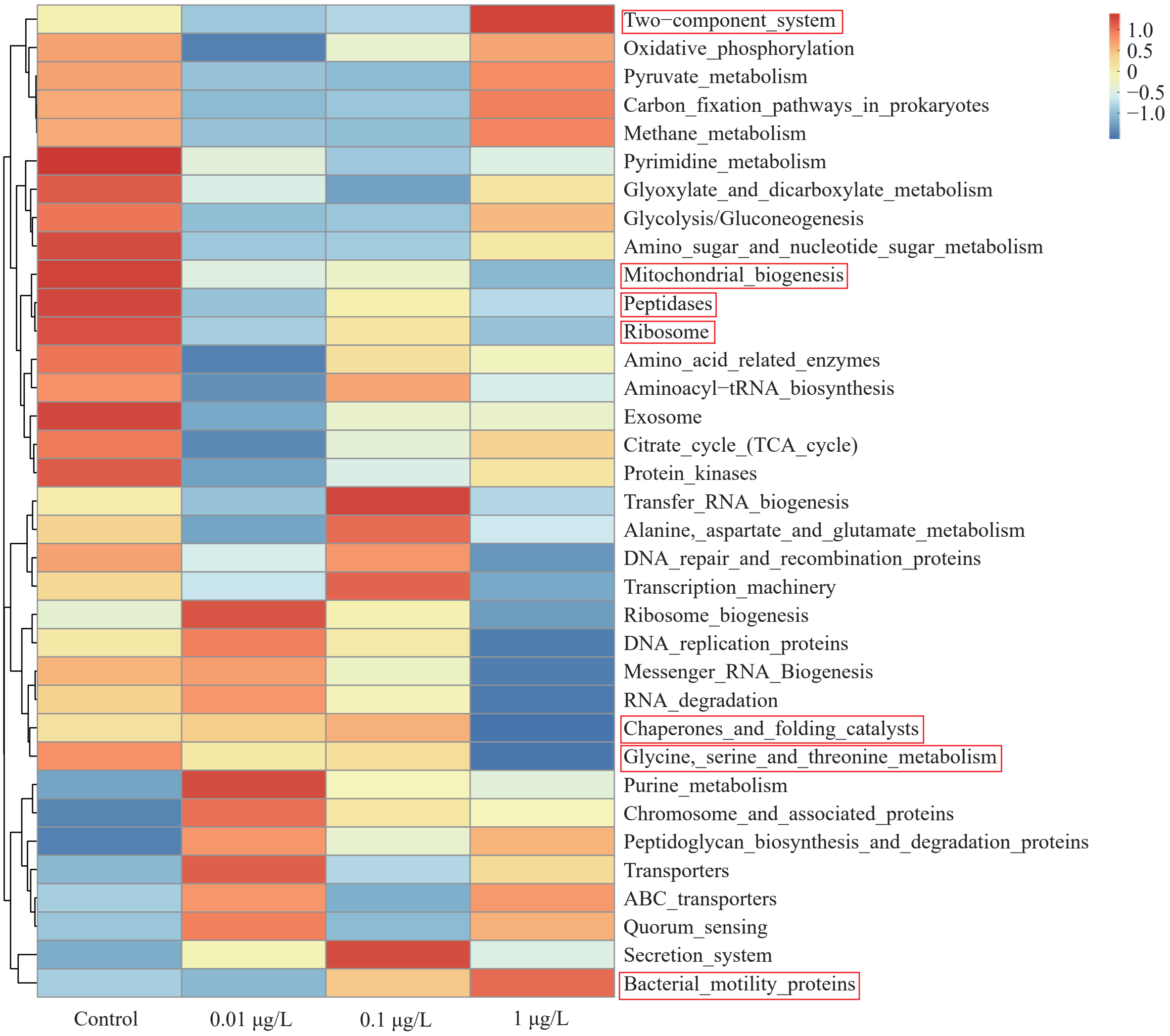

图 11 配体数据库中变化显著通路

Fig. 11 Significant pathways in the ligand database

在0.01 μg/L浓度组, 肠道菌群数量总体上符合随着多西环素浓度上升而下降的趋势, 但肠道菌群在种水平的数量的上升, 并且整体上在Y迷宫探索臂停留时间和Y迷宫探索臂转角度数该组别参数特别显著, KEGG通路中信号转导通路的水平增加或许与其学习记忆能力的增加有所关联。

3.4　斑马鱼肠道菌群的变化同行为参数的联系

肠道菌群能够调控肠神经系统和中枢神经系统[33], 对于维持机体稳态起到重要作用。另外, 大脑和肠道微生物群之间的双向调节可表明肠道微生物群与中枢神经系统疾病之间存在联系[16], 而且肠道菌群的变化能够通过迷走神经联系大脑传递有关信息[34], 因此, 抗生素引起肠道菌群的变化, 能够诱发斑马鱼产生行为变化。

信号转导通路功能的增强有利于斑马鱼认知记忆能力的提升, 但同时也有研究在小鼠中表明, 焦虑期间, 也会引起信号转导水平的升高[35], 这与我们结果低浓度组斑马鱼产生的焦虑行为且认知记忆能力的提高类似。焦虑被认为是对慢性压力的不适应, 免疫系统通路和疾病内分泌代谢通路功能下降可能同焦虑关系密切, 免疫和内分泌系统在焦虑中起着主要作用[36]。此外, 线粒体是能量代谢、氧化应激和焦虑之间的重要纽带[37], 维生素D的缺乏则与患焦虑症的风险增加有关[38], 本实验中线粒体生物合成和维生素的代谢通路水平的下降提高了斑马鱼产生焦虑行为的可能性。环境浓度多西环素长期暴露能够影响斑马鱼的肠道菌群丰度和多样性, 并且影响多个通路的水平变化, 进而引起斑马鱼焦虑有关行为变化和提升认知记忆能力。

4　结论

环境浓度多西环素长期暴露能够引起斑马鱼行为改变, 产生焦虑样行为, 并引起肠道菌群丰富度和多样性的改变。0.01 μg/L多西环素能够提高斑马鱼短期学习记忆能力和认知灵活性。斑马鱼肠道菌群可能通过信号转导、线粒体生物合成和维生素的代谢通路水平影响斑马鱼行为。关于多西环素对斑马鱼神经行为作用机制可从菌群移植和代谢组方向进一步深入研究。

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ENVIRONMENTAL DOXYCYCLINE ON ANXIOUS BEHAVIOR, COGNITIVE MEMORY ABILITY AND INTESTINAL MICROFLORA OF ZEBRAFISH

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Abstract: The use of antibiotic can influence gut microbiota and impair memory function either positively or negatively. In order to investigate the effects of long-term exposure to doxycycline at environmental concentration on anxiety behavior, learning and memory, cognitive flexibility and intestinal flora, we took zebrafish as the animal model, and used doxycycline at different environmental concentrations (0, 0.01, 0.1 and 1 μg/L) to expose adult zebrafish to water for 21 days. Behavioral responses and intestinal flora alterations were assessed using the new tank test, Y-maze, light and dark test and zebrafish behavior high-throughput monitoring system. The results showed as follows: in the new tank experiment, the average swimming speed of zebrafish in 0.01, 0.1 and 1 μg/L concentration groups decreased significantly compared with the control group. Additionally, higher doxycycline concentrations correlated with reduced maximum swimming speed and total swimming distance, with a pronounced difference observed in the 1 μg/L group. Maze experiments indicated a significant increase in the residence time of zebrafish in the exploration arm at the 0.01 μg/L concentration, contrasting with decreased times in the 0.1 and 1 μg/L groups. The turning angle exhibited a similar pattern, increasing in the 0.01 μg/L group but decreasing in the higher concentration groups. In the light and dark test, zebrafish demonstrated an increased residence time in the light zone with rising doxycycline concentration. Zebrafish behavioral high-throughput monitoring system experiments further confirmed a significant decrease in total swimming distance with increasing doxycycline concentration, particularly notable in the 1 μg/L group. In terms of intestinal flora analysis, based on 16S rRNA sequencing technology, 1454814 original valid sequences were obtained from 12 intestinal samples, and 1314404 high-quality sequences were obtained after screening for subsequent analysis. At the same time, DADA2 method was used to reduce noise and cluster with 100% similarity. It was found that the abundance of intestinal flora of zebrafish in each concentration group decreased, and the abundance of bacteria genera such as Bosteria, Paracococcus and Acinetobacter decreased, while genera like Corynebacterium and Romatococcus increased. The Shannon index, Simpson index and Chao1 index were significantly lower in the exposed group, indicating decreased diversity. In conclusion, long-term exposure to doxycycline in environmental concentration induced behavioral changes in zebrafish, resulting in anxiety-like behavior. The 0.01 μg/L concentration improved short-term learning and memory ability and cognitive flexibility. Furthermore, doxycycline exposure led to changes in the abundance and diversity of zebrafish intestinal flora.

Key words: Environmental concentration; Doxycycline; Anxious behavior; Cognitive memory; Intestinal flora; Zebrafish