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Piezo2介导模拟直升机低频振动致腰痛大鼠的机械性痛觉超敏

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[摘要] **目的** 探究机械敏感离子通道Piezo2在模拟直升机低频振动诱导大鼠腰痛和机械性痛觉超敏中的作用和机制。**方法** 采用三维六自由度的低频振动(low-frequency vibration,LFV)诱导坐姿大鼠腰痛模型。将24只8周龄雄性SD大鼠用随机数字表法分为对照(Ctrl)组和低频振动(LFV)组。在多裂肌损伤评估基础上,von Frey实验检测疼痛敏感性,旷场实验评估自主活动和焦虑水平,ELISA、Western blot及免疫荧光检测神经生长因子(nerve growth factor,NGF)、TrkA及下游分子Piezo2的表达。原代培养SD大鼠背根神经节(dorsal root ganglia,DRG)神经元,经免疫荧光鉴定后设置Ctrl组、LFV组和LFV+D-G4(D-GsMTx4,Piezo2抑制剂)组,以Western blot检测Piezo2的表达,结合钙离子荧光探针检测细胞内Ca^{2+}水平。使用NGF(50 ng/mL)预处理神经元1 h,设置LFV组、LFV+NGF组和LFV+NGF+D-G4组,利用钙离子荧光探针观察胞内Ca^{2+}变化。**结果** LFV组大鼠多裂肌形态异常并伴有炎性细胞浸润,行为学结果显示,其缩足反射阈值降低($P<0.05$),旷场总活动时间、中心区域活动时间和中心区域路程减少($P<0.05$),总静止时间、周围区域静止时间以及周围区域路程增加($P<0.05$),DRG组织Piezo2、NGF和TrkA表达增加($P<0.05$);细胞实验显示,与Ctrl组相比,LFV组神经元Piezo2表达增加($P<0.05$),细胞内Ca^{2+}水平明显升高($P<0.05$)。与LFV组相比,LFV+NGF组Ca^{2+}水平升高($P<0.05$),使用D-G4后,NGF对Piezo2的敏化作用被逆转($P<0.05$)。**结论** 持续低频振动通过NGF-TrkA/Piezo2通路诱导大鼠腰痛和机械性痛觉超敏。

[关键词] 振动;腰痛;Piezo2;机械性痛觉超敏
[中图法分类号] R-332;R363.21;R441.1 [文献标志码] A

Piezo2 mediates mechanical allodynia in rats with low back pain induced by simulated helicopter low-frequency vibration

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[**Abstract**] **Objective** To explore the role and mechanism of mechanically sensitive ion channel Piezo2 in mechanical allodynia of rats with low back pain induced by simulating the low-frequency vibration of a helicopter. **Methods** Low-frequency vibration (LFV) model with 3-dimensional 6 degrees of freedom was used to induce low back pain in awake rats in a sitting position. Twenty-four male SD rats (8 weeks old) were randomly divided into control (Ctrl) group and LFV group. HE staining was used to evaluate the injury of the

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multifidus muscle. Von Frey test was carried out to detect pain sensitivity. Open field test was employed to assess the spontaneous activity and anxiety. ELISA, Western blotting and immunofluorescence staining were performed to detect the expression of NGF, TrkA and downstream molecule Piezo2. Dorsal root ganglia (DRG) neurons was isolated from SD rats and primarily cultured. After identified with immunofluorescence staining, the neurons were divided into the Ctrl group, the LFV group, and the LFV+ D-GsMTx4 (D-G4, an Piezo2 channel antagonist) group. Western blotting was used to detect the protein expression of Piezo2, and a calcium ion fluorescent probe was utilized to detect the intracellular Ca^{2+}. The DRG neurons were pretreated with 50 ng/mL NGF for 1 h. Calcium ion fluorescent probe was used to observe the changes in intracellular Ca^{2+} in the LFV group, the LFV+NGF group, and the LFV+NGF+D-G4 group. **Results** The rats of the LFV group showed abnormal morphology in multifidus muscles, accompanied with inflammatory cell infiltration, decreased paw withdrawal reflex threshold ($P<0.05$), and shortened total active time and active time in the centre, and decreased distance traveled in the centre ($P<0.05$), while prolonged total stationary time, stationary time in the periphery, and increased distance traveled in the periphery ($P<0.05$), and moreover, enhanced expression of Piezo2, NGF and TrkA in the DRG tissues ($P<0.05$). Cell experiments showed that compared with the Ctrl group, the expression of Piezo2 in the neurons was increased ($P<0.05$), and the intracellular Ca^{2+} level was significantly elevated in the LFV group ($P<0.05$). Compared with the LFV group, the Ca^{2+} level was higher in the LFV+NGF group ($P<0.05$), and the sensitization effect of NGF on Piezo2 was reversed after D-G4 treatment ($P<0.05$). **Conclusion** Sustained low-frequency vibration induces low back pain and mechanical allodynia in rats through the NGF-TrkA/Piezo2 pathway.

[**Key words**] vibration; low back pain; Piezo2; mechanical allodynia

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腰痛(low back pain,LBP)严重影响人类健康和生活质量。研究表明2050年将有8亿多人患有腰痛[1],部分腰痛患者因出现机械性痛觉超敏而伴有抑郁、焦虑等不良情绪[2]。军事人员中,直升机飞行员群体的腰痛患病率为48.1%[3],研究表明低频振动的职业暴露环境是飞行员患病的重要原因之一[4]。因此,亟需构建良好模拟飞行员作业特征的动物模型以探究振动所致腰痛的发病机制。BAIG等[5]首创振动相关啮齿类动物模型,发现俯卧位水平振动麻醉大鼠可致其前后爪痛觉敏感性升高。ZEEMAN等[6]利用加速度计和视频成像系统,证实大鼠脊柱在8~10 Hz共振频率下DRG与脊髓的疼痛相关神经免疫反应显著增强。本研究采用三维六自由度的低频振动模型[7],成功诱发清醒的坐姿大鼠腰部多裂肌炎症和后爪机械性痛觉超敏。

机械性痛觉超敏又称为机械性异常性疼痛,是由轻触觉等无痛机械刺激诱发的痛觉反应[8]。近来发现机械敏感的阳离子通道蛋白Piezo2是介导机械性痛觉超敏的关键分子。Piezo2主要表达于外周初级感觉中枢的DRG神经元,早期研究表明,Piezo2介导触觉、本体感觉以及振动感知,在机械转导中发挥重要作用[9-11]。现有研究发现在病理情况下,Piezo2参与伤害性的机械痛觉感受,介导机械刺激后异常性疼痛[12]。MURTHY等[13]利用神经损伤和炎症模型,发现Piezo2条件性敲除小鼠对机械刺激的敏感性显著降低,野生型小鼠出现痛觉超敏反应。LIU等[14]利用膀胱炎模型,表明敲低Piezo2能够显著抑制机械刺激诱发的大鼠膀胱机械痛觉超敏和膀胱过度活动。然而,Piezo2介导机械性痛觉超敏的分子机制亟待深入阐明。

既往研究证实,NGF通过TrkA高亲和力受体调控外周神经元敏化从而介导痛阈改变[15]。啮齿类动物腰背肌群或关节腔注射NGF可诱发持续性痛觉敏化[16-17]。特别是新近研究揭示Piezo2是NGF敏化外周神经元并导致机械性异常性疼痛的下游靶点之一[18]。为此,本研究推测低频振动致腰痛大鼠的机械性痛觉超敏可能通过Piezo2依赖性途径实现。故基于模拟直升机低频振动的腰痛大鼠模型,聚焦NGF-TrkA/Piezo2调控轴,探究振动模型下腰痛相关机械性痛觉超敏的分子机制,以期为直升机飞行员职业性腰痛的治疗提供新的靶点。

1 材料与方法

1.1 实验动物及分组

8周龄雄性SD大鼠24只,体质量200~250 g,由陆

军军医大学实验动物中心提供[动物生产许可证:SCXK(京)2024-0003;动物使用许可证号:SYXK(渝)2022-0003]。实验动物依据国际实验动物护理评估和认证协会的建议标准进行饲养管理,12 h/12 h昼夜交替循环,温度环境适宜,自由获取食物和水。采用随机数字表法将24只大鼠分成Ctrl组和LFV组,每组各12只。本研究经实验动物福利伦理委员会批准(AWUMEC20237402)。

1.2 主要试剂

NGF检测ELISA试剂盒(中国晶美生物,JM-01594R1);Piezo2兔多克隆抗体(Novus Biologicals,NBP1-78624);TrkA山羊多克隆抗体(R&D Systems,AF1056);NGF兔多克隆抗体(武汉菲恩,FNab10116);Piezo2通道非特异性抑制剂D-GsMTx4(MCE,HY-P1410B);钙离子探针Fluo-4AM(MCE,HY-P1410B);NGF(Sigma,N0513)。

1.3 实验方法

1.3.1 模型构建 根据前期方法制备动物模型[7],即将乙醚麻醉的大鼠置于振动台上的坐姿固定器中,待大鼠清醒后开机造模。振动仪(深圳洪钐仪器科技有限公司)参数设置为:振动频率:9.90~10.10 Hz;振动强度:20%;重力加速度:0.27×g;振幅:1.5~1.7 mm。LFV组大鼠将进行每天9 h,连续14 d的振动造模。Ctrl组不做振动处理,其余与LFV组处理方法一致,正常饲养。

利用原代DRG神经元制备细胞振动模型,将LFV组置于无菌环境中的振动仪,Ctrl组置于同一空间非振动处,参数设置同上。为避免原代神经元长时间处于非适宜生长环境,参照文献时间点行振动造模[19],造模0.5 h后进行Ca^{2+}检测。

1.3.2 大鼠DRG神经元的培养及鉴定 参照文献方法[20-21],取新生1 d的SD大鼠8只,全身经75%乙醇消毒后,断头处死,剪开背部皮肤,游离出脊柱,小心修剪脊柱周围的肌肉和组织。沿着棘突侧剪开脊柱,去除脊柱内的脊髓以及被膜,于椎间孔内找到DRG并用眼科镊取出,剪去两端神经丝并去除被膜,以预冷D-Hanks溶液洗涤2~3次,置于0.25% EDTA胰酶溶液,37 ℃下消化30 min。终止消化后,用1 mL移液枪轻柔吹打10~15次,使用70 μm滤网过滤,收集细胞悬液1 000 r/min离心5 min,弃上清,加入复温的Neurobasal培养基轻柔吹打,单细胞重悬后计数,接种于预先用多聚赖氨酸包被的孔板中,轻柔摇晃至细胞均匀分布,然后放入5% CO_2、37 ℃孵箱中培养,4 h后换液,24 h后加入阿糖胞苷抑制非神经元细胞增殖,每2天半量换液。第4天以神经元标志性蛋白β3-tubulin鉴定。

1.3.3 von Frey检测 参照前期方法[7],将大鼠置于铁丝网上,利用不同直径和硬度的von Frey纤维丝检测大鼠后爪的异常性疼痛和疼痛敏感性变化,从4.0 g纤维丝开始检测,垂直向大鼠后爪足底的中心皮肤刺激,按照up-down method原则继续完成后续测试。用Up-Down Reader软件处理数据[22],取左右后肢平均数并剔除无效数据后分析。检测时间为造模前1 d和造模后1、4、7、14 d。

1.3.4 旷场实验 大鼠置于正方形的实验区域并进行5 min无干预行为观察。采用红外追踪系统和摄像头记录大鼠的轨迹和行为。统计指标包括总活动时间、总静止时间、中心区域路程、周围区域路程、中心区域活动时间和周围区域静止时间。每只大鼠测试完后用酒精溶液擦拭,以减少不同大鼠气味对嗅觉的干扰。

1.3.5 多裂肌组织HE染色 取大鼠L4-5节段多裂肌置于4%的多聚甲醛中固定,行石蜡包埋和切片。将切片依次完成脱蜡、染色、脱水、透明以及封片等步骤,光学镜下观察多裂肌病理变化。

1.3.6 细胞膜红色荧光探针(1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine, DiI)逆行示踪对大鼠的L4-5多裂肌注射红色染料DiI进行逆行示踪,标记支配多裂肌的DRG神经元,DiI染料稀释参照文献[14],大鼠以2.5%的异氟烷吸入麻醉,在其背部切小口,将DiI微量注入L4-5脊柱两侧的多裂肌中,每侧多裂肌注射上下两个不同部位(每个部位2 μL),缝合背部皮肤,待麻醉大鼠清醒后回笼饲养。

1.3.7 免疫荧光染色 取L3-4节段的DRG组织置于4%的多聚甲醛中固定2 d,经蔗糖梯度脱水后进行包埋和冰冻切片。进行荧光染色时,将切片于室内复温30 min,PBS洗涤8 min×3次,然后用0.3%的triton X-100通透30 min,PBS洗涤8 min×3次,使用免疫组化笔在组织周围画圈,加入免疫染色封闭液孵育60 min,吸去液体,加入一抗(Anti-Piezo2,1∶200),4 ℃过夜孵育。次日,切片室内复温30 min,吸去一抗,PBS洗涤8 min×3次,加入相应的荧光二抗,室温孵育1 h,PBS洗涤8 min×3次,加入DAPI孵育3 min,PBS洗涤8 min×3次,待组织吹干后滴加抗淬灭封片剂,封片后置于共聚焦显微镜观察和拍摄。

1.3.8 蛋白质印迹实验法(western blot)检测蛋白表达 提取DRG蛋白后,BCA测定蛋白浓度,电泳并将蛋白电转至PVDF膜上。5%的脱脂奶粉封闭1.5 h,置于Piezo2兔抗(1∶1 000)中,4 ℃过夜孵育。次日,使用TBST洗涤条带8 min×3次,置于二抗中孵育1 h,TBST洗涤8 min×3次,化学显影后,用Image J软件行灰度值分析。

1.3.9　神经元胞内钙离子浓度检测　使用Fluo-4 AM检测DRG神经元内的钙离子浓度[23]。提前用DMSO配制成1 mmol/L储备液,-80 ℃避光保存。细胞造模后,吸除培养基,加入100 μL终浓度为1 μmol/L工作液,孵育10 min后用培养基洗涤5 min×3次,用荧光显微镜观察和拍摄。

1.4　统计学方法

GraphPad Prism 9.5软件对实验数据统计分析,计量数据以$\bar{x}\pm s$表示,两组间比较采用t检验,多组比较行单因素方差分析,P<0.05时差异有统计学意义。

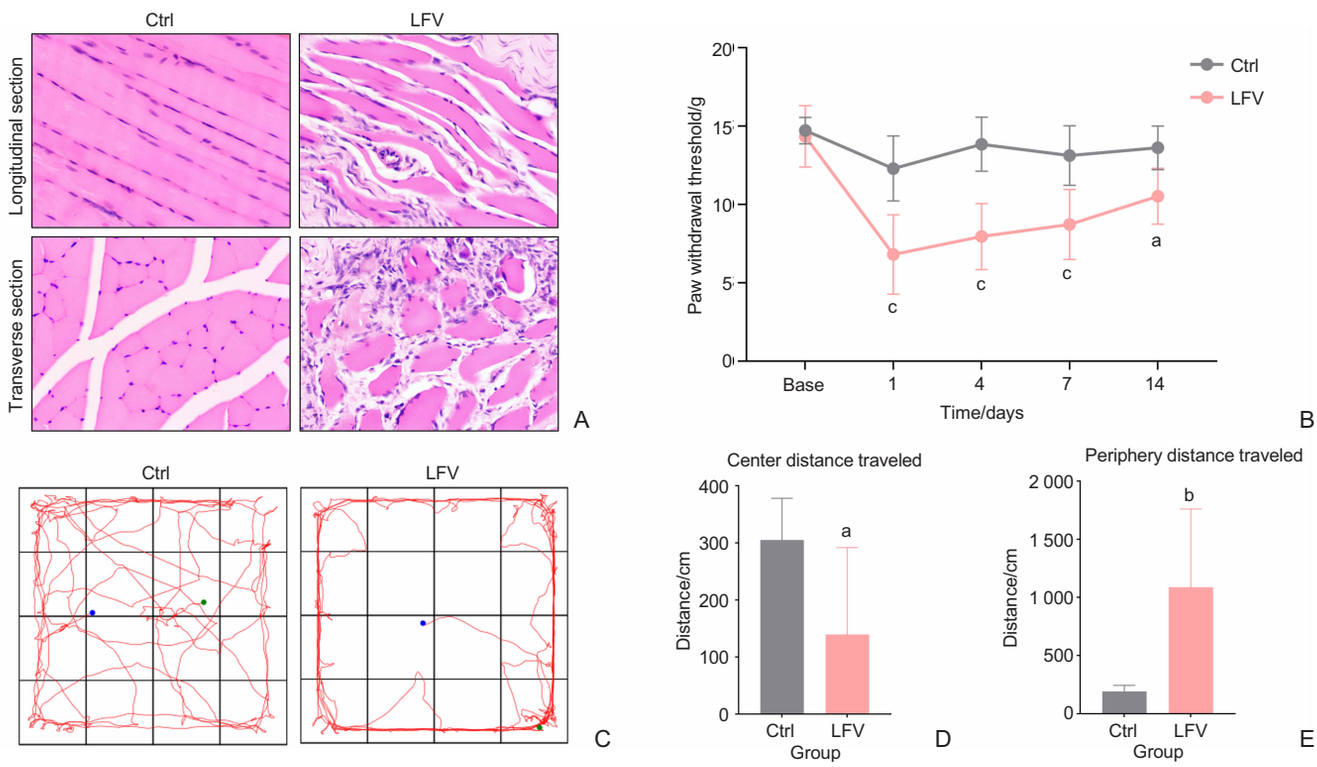
2　结果

2.1　低频振动导致大鼠L4-5多裂肌损伤和诱发大鼠机械性痛觉超敏

多裂肌HE染色结果显示,与Ctrl组相比,LFV组多裂肌纵切面肌纤维排列紊乱、分裂、大小不一,肌横纹消失且肌间隙增大;横切面可见炎细胞浸润,肌萎缩且形态异常,肌纤维粗细不等以及出现环形空泡(图1A)。为观察大鼠的疼痛行为改变,利用von Frey纤维丝检测大鼠后爪的疼痛阈值变化,结果显示造模后大鼠出现机械性痛觉超敏,表现为疼痛敏感性增加,痛阈降低并可持续至造模后两周(P<0.05,图1B)。旷场实验显示,与Ctrl组相比,LFV组大鼠总活动时间、中心区域活动时间和中心区域路程减少,而总静止时间、周围区域静止时间以及周围区域路程增加,提示LFV组大鼠因为疼痛而表现出自主活动水平下降,焦虑水平升高(P<0.05,图1C~I)。

2.2　Piezo2通道表达于支配L4-5多裂肌的DRG神经元

为确认L4-5多裂肌的感觉神经支配,用DiI逆行示踪剂标记支配L4-5多裂肌的DRG神经元,结果发现L4-5多裂肌主要受L3-4 DRG神经元的支配,并表明Piezo2在L1-6 DRG神经元中均大量表达(图2)。

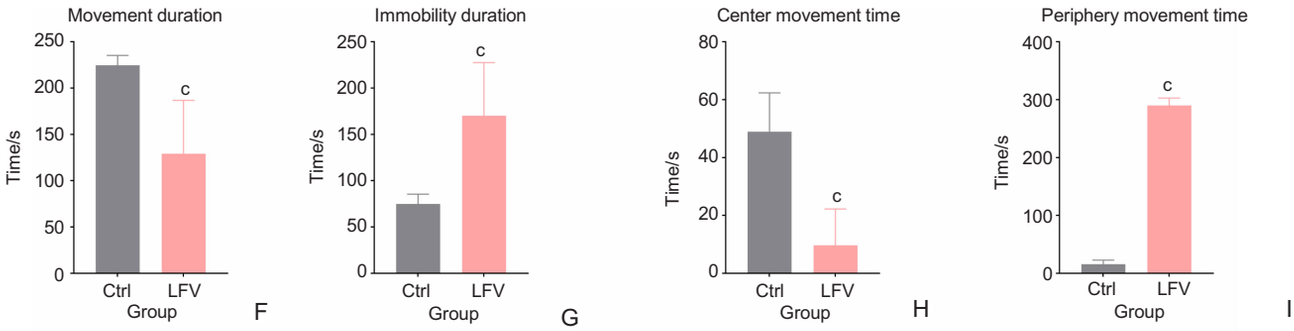


A: HE staining of the L4-5 multifidus muscle; B: assessment of mechanical paw withdrawal threshold in rats using von Frey filaments　a: P<0.01, b: P<0.001, c: P<0.000 1 *vs* Ctrl group. C: trajectory map in the open field test, blue dot: starting point; green dot: endpoint; D: statistical analysis of open field test parameters (center distance traveled); E: statistical analysis of open field test parameters (periphery distance traveled); a: P<0.01, b: P<0.001 *vs* Ctrl group

图1　低频振动后大鼠多裂肌损伤及疼痛相关行为学变化(n=9)

Figure1　Low-frequency vibration-induced multifidus muscle injury and pain-related behavioral changes in rats (n=9)

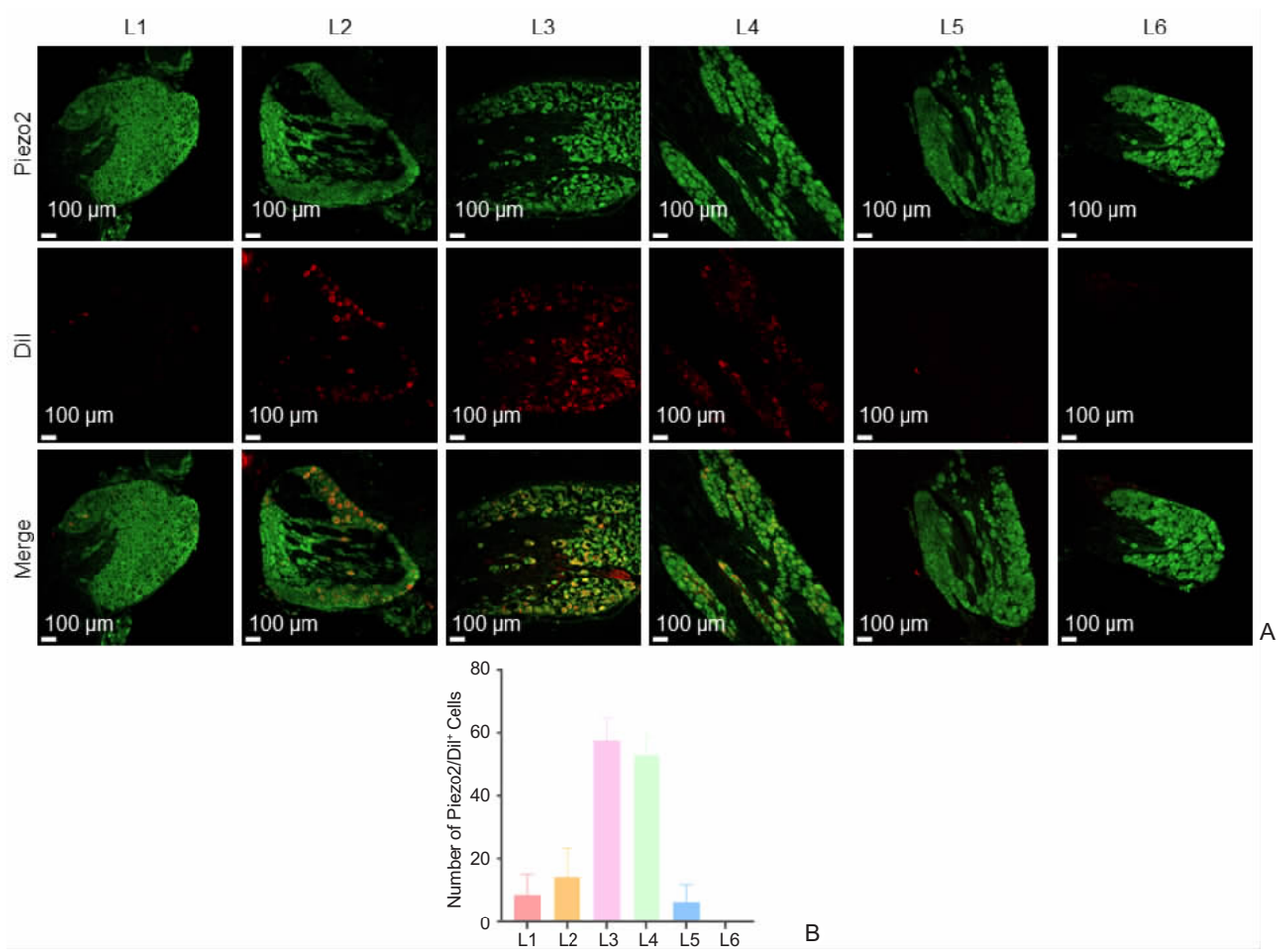
图1F~I续下页



F: statistical analysis of open field test parameters (total movement duration); G: statistical analysis of open field test parameters (total immobility duration); H: statistical analysis of open field test parameters (center movement time); I: statistical analysis of open field test parameters (periphery movement time); c: $P<0.000\ 1$ *vs* Ctrl group

续图1 低频振动后大鼠多裂肌损伤及疼痛相关行为学变化(n=9)

Figure1 Low-frequency vibration-induced multifidus muscle injury and pain-related behavioral changes in rats (n=9)



A: Representative image showing Piezo2 immunostaining in DiI-retrogradely labeled DRG neurons innervating the L4-5 multifidus muscle; B: quantitative analysis of Piezo2/DiI co-localization in L1-L6 DRG neurons

图2 Piezo2表达于支配L4–5多裂肌的DRG神经元(n=3)

Figure 2 Piezo2 expression in DRG neurons innervating the L4-5 multifidus muscle (n=3)

2.3 低频振动通过NGF-TrkA/Piezo2诱导腰痛大鼠机械性痛觉超敏

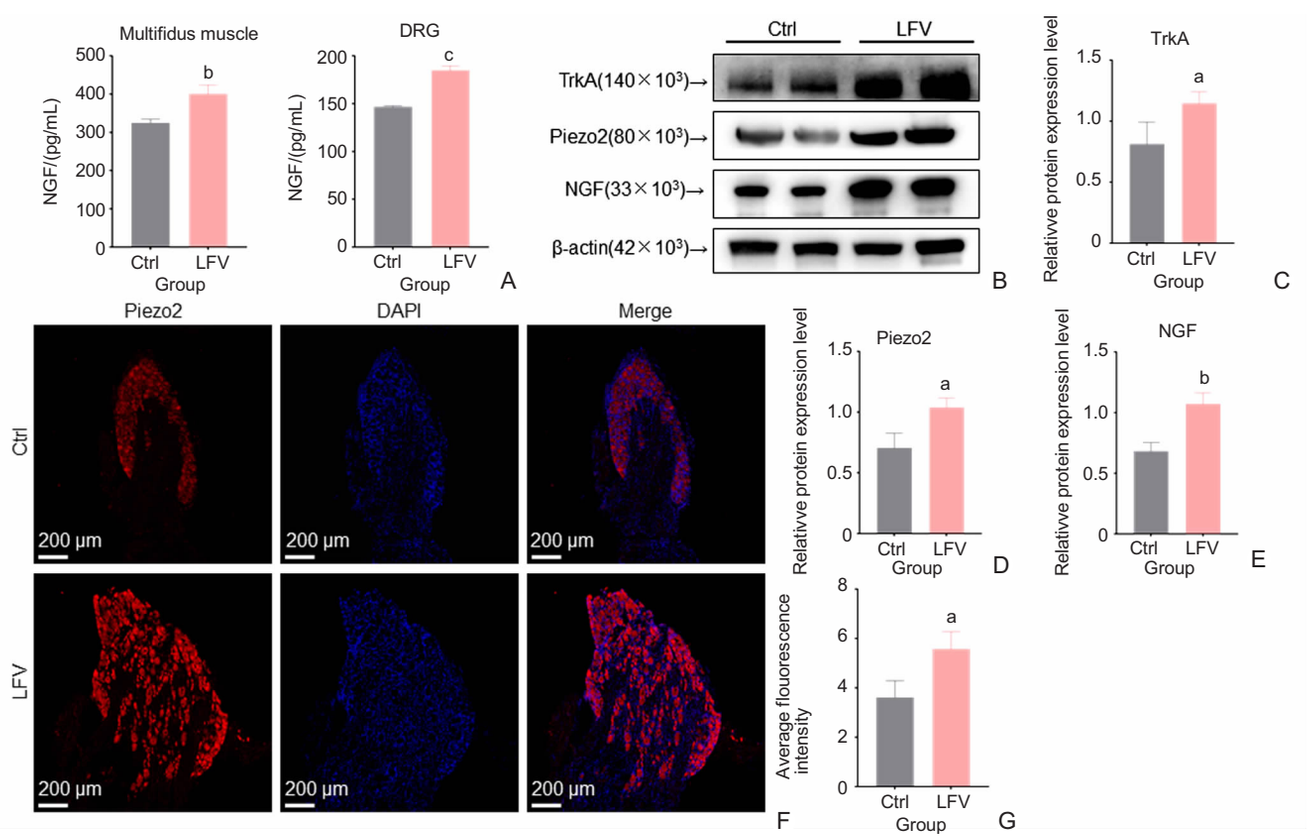
为了验证NGF是否通过上调Piezo2参与腰痛大鼠机械性痛觉超敏,首先采用Elisa实验检测多裂肌和DRG组织NGF水平,结果发现与Ctrl组相比,LFV组NGF水平明显升高(P<0.05,图3A)。Western blot结果显示,造模后大鼠DRG组织中NGF、TrkA和Piezo2的蛋白表达水平上调(P<0.05,图3B~E)。免疫荧光结果显示,LFV组中Piezo2的平均荧光强度高于Ctrl组(P<0.05,图3F、G)。这些结果初步提示低频振动可能通过NGF-TrkA/Piezo2通路诱导大鼠腰痛和机械性痛觉超敏。

2.4 低频振动促进体外DRG神经元Piezo2蛋白的激活和表达

为了在细胞水平进一步验证以上结果,本研究提取大鼠DRG神经元进行体外培养(图4A),利用免疫荧光染色对DRG神经元进行鉴定,结果显示神经元标志性蛋白β3-tubulin呈阳性表达(图4B)。采用多重免疫荧光染色技术进一步观察Piezo2在神经元中的分布(图4C)。然后通过Western blot技术,观察各组细胞中Piezo2的表达变化,结果显示,与Ctrl组相比,LFV组细胞Piezo2表达上调(P<0.05,图4D、E),提示低频振动促进Piezo2的表达。采用Fluo-4 AM检测各组细胞内Ca^{2+}水平,结果显示LFV组的Ca^{2+}水平高于Ctrl组(P<0.05,图4F、G);使用10 μmol/L的D-GsMTx4阻断Piezo2后,发现与LFV组相比,LFV+DG-4组Ca^{2+}水平明显降低(P<0.05,图4F、G),提示Piezo2的激活参与低频振动诱发的胞内Ca^{2+}水平升高。

2.5 NGF增强Piezo2介导的低频振动诱发的胞内Ca^{2+}水平升高

为了探讨Piezo2在机械性痛觉超敏中的机制,在细胞层面上验证经NGF孵育后神经元中Piezo2介导



A: NGF level in multifidus muscle and DRG detected by ELISA; B: expression levels of TrkA, Piezo2, and NGF in DRG detected by Western blotting; C~E: statistical analysis of relative protein expression of TrkA, Piezo2, and NGF; F: immunofluorescence staining of Piezo2 in DRG; G: quantitative analysis of mean fluorescence intensity of Piezo2. a: P<0.05, b: P<0.01, c: P<0.001 *vs* Ctrl group

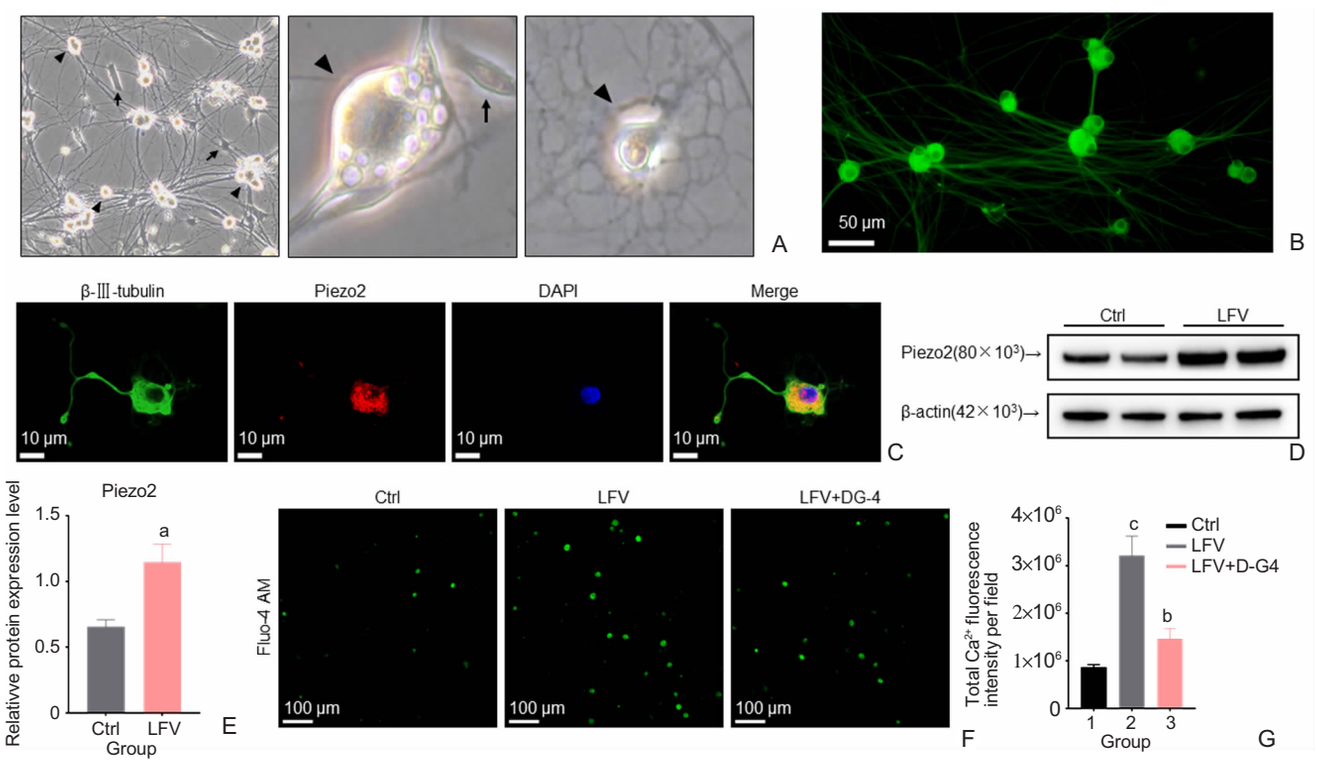
图3 低频振动后多裂肌及DRG组织相关分子表达变化

Figure 3 Low-frequency vibration-induced expression changes in related molecules in multifidus muscle and DRG

的机械刺激反应增强。钙离子荧光探针结果显示,与Ctrl组相比,LFV+NGF组Ca^{2+}水平显著升高,而LFV+NGF+D-G4组比LFV+NGF组Ca^{2+}水平降低(P<0.05,图5),结果提示NGF增强Piezo2介导的低频振动诱发的胞内Ca^{2+}水平升高。

3 讨论

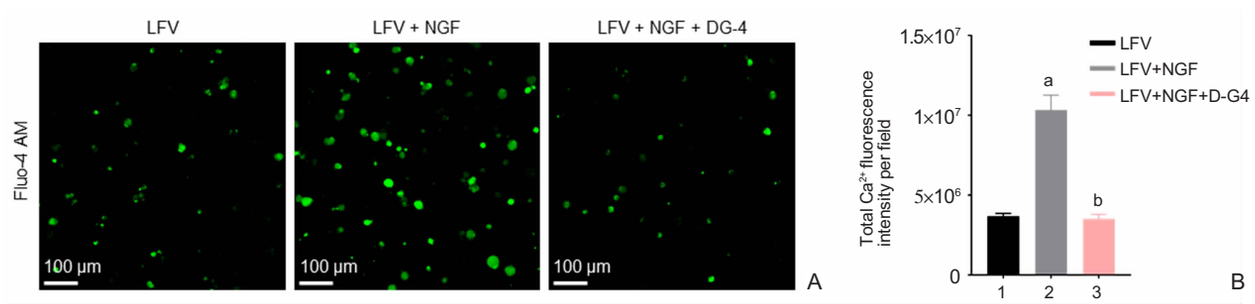
腰痛等脊柱相关疾病已成为我国现役直升机飞行员停飞的主要原因之一[24],其主要危险因素涉及长期低频振动暴露的职业环境[25]。然而,低频振动导致



A: morphology of living DRG neurons, arrow heads indicating neuron and arrows indicating glia; B: identification of DRG neurons by β3-Tubulin immunostaining; C: Piezo2 localization to neurons validated by immunofluorescence staining *in vitro*; D: Piezo2 protein expression in DRG neurons detected by Western blotting; E: relative protein expression level of Piezo2; F: intracellular Ca^{2+} level assessed using Fluo-4 AM; G: quantification of total fluorescence intensity; a: P<0.01, c: P<0.000 1 *vs* Ctrl group; b: P<0.001 *vs* LFV group

图4 振动刺激后体外培养DRG神经元中Piezo2的表达与功能变化

Figure 4 Vibration-induced alterations in Piezo2 expression level and functional properties of *in vitro* cultured DRG neurons



A: intracellular Ca^{2+} level monitored by Fluo-4 AM; B: quantification of total fluorescence intensity; a: P<0.000 1 *vs* LFV group; b: P<0.000 1 *vs* LFV+NGF group.

图5 NGF预处理后Piezo2通道的活性变化

Figure 5 Changes in Piezo2 channel activity after NGF pretreatment

腰痛的机制尚不清楚,早期研究表明低频振动可以导致大鼠出现机械性痛觉超敏和腰椎炎症反应[6],但鲜有分子机制研究。本研究通过体内外双向验证策略,初步探讨了机械敏感阳离子通道蛋白Piezo2在低频振动腰痛模型中的作用,结果显示,持续振动暴露通过激活NGF-TrkA/Piezo2信号轴诱导大鼠腰痛和机械痛觉超敏,为阐明直升机飞行员职业性腰痛的发病机制提供了新的视角,开发相关药物局部阻断Piezo2可能有助于该类患者的临床治疗。

3.1 多裂肌是直升机飞行员腰痛的关键肌肉

多裂肌位于脊柱肌群的最深层,对称分布于脊柱两侧,在腰部最发达,腰椎多裂肌是维持腰椎节段稳定的关键肌肉[26]。既往研究表明,腰痛患者的椎旁肌肉出现形态和结构上的变化,以腰椎多裂肌萎缩和脂肪浸润为突出症状,而竖脊肌和腰大肌等其它肌肉未发现明显差异[27-28]。值得注意的是,针对飞行员群体的MRI影像学研究证实,该类腰痛患者普遍存在多裂肌横截面积缩小以及脂肪浸润率升高的改变[29]。进一步研究显示,直升机飞行员进行躯干肌肉锻炼后,观察到多裂肌耐力和收缩力提高,患者的运动功能得到明显改善[30]。因此,本研究首先选择多裂肌进行形态学观察。HE染色显示振动造模后大鼠的腰椎多裂肌出现明显病变,包括炎性细胞增多、肌间隙增大和肌纤维排列紊乱等,表明持续性低频振动对腰椎多裂肌确能造成损伤。

3.2 Piezo2介导LFV诱导的腰痛大鼠机械性痛觉超敏

机械性痛觉超敏是神经病理性疼痛的突出症状,轻柔的触碰也会引起患者的疼痛反应,严重影响患者的心理情绪和日常生活[31]。然而,治疗痛觉超敏的策略进展缓慢,阻断与其直接相关的分子感受器可能是一个有效的潜在方向。Piezo2作为力敏感通道,在不同的疼痛模型中已被证明介导机械性痛觉超敏。例如,研究发现Piezo2通道上调参与了环磷酰胺诱导的膀胱炎大鼠的机械性痛觉超敏[14]。本研究发现,大鼠在接受连续14 d的低频振动暴露后,其后爪疼痛阈值降低并可持续到造模后两周,表明大鼠出现明显的机械性痛觉超敏症状。在旷场实验中发现,造模后的大鼠总活动时间、中心区域活动时间和中心区域路程减少,而总静止时间、周围区域静止时间和周围区域路程增多,提示大鼠因为疼痛导致焦虑水平升高。为探究目的节段多裂肌与DRG的支配关系,本研究使用DiI逆行示踪发现L4-5多裂肌主要受L3-4 DRG神经元的支配,并在解剖结构上验证了其支配的DRG神经元几乎都表达Piezo2。进一步研究发现,低频振动诱导的腰痛大鼠机械性痛觉超敏与多裂肌感觉传入中Piezo2的过度激活和表达上调有关。

3.3 NGF-TrkA/Piezo2通路可能是振动性腰痛机械性痛觉超敏的潜在机制

在炎症、损伤和某些疾病时,炎性细胞会释放与TrkA结合的NGF,NGF-TrkA复合体从外周神经末梢逆行运输至DRG胞体,激活胞内信号级联反应,导致膜表面的一些受体和离子通道敏化或表达增加[32]。近期研究发现,NGF可以上调Piezo2通道的表达,敏化感觉神经元,协同参与机械性痛觉超敏[17,33]。本研究结果发现,振动造模后大鼠多裂肌和DRG中NGF表达均增加,同时伴随DRG中TrkA与Piezo2的表达上调。Piezo2是非选择性的Ca^{2+}通透性阳离子通道[9],体外实验发现,通过钙离子探针检测到使用NGF预处理的神经元受振动刺激诱导的胞内Ca^{2+}水平显著升高,而在使用了Piezo2的阻断剂后,胞内Ca^{2+}水平降低,提示Piezo2是NGF促进胞内Ca^{2+}升高的关键通道。这些结果证实NGF可以过度激活Piezo2通道并促进其蛋白表达进而敏化感觉神经元。但NGF-TrkA信号轴调控Piezo2通道的胞内信号级联反应机制尚不明确,需进一步深入研究。此外,本研究存在一定的局限性:利用新生鼠制备的原代细胞模型难以完全模拟成年大鼠体内的复杂微环境,且细胞建模时长无法与动物模型保持一致。这主要源于两方面原因:①原代神经元本身缺乏增殖能力,但长期培养难以抑制胶质细胞的过度生长,进而影响实验结果;②细胞振动仪无法提供适宜的细胞生长环境,实验中细胞长时间暴露于室温条件会损害其活性;后续研究可从改进仪器设备入手,并在此基础上增加建模时间的探索,以完善体外细胞振动模型的构建与应用。

综上所述,本研究发现持续低频振动可能是通过NGF-TrkA通路过度激活Piezo2通道,从而诱导大鼠腰痛和机械性痛觉超敏,提示靶向Piezo2可能是治疗直升机飞行员职业性腰痛的有效方法。下一步研究中,亟待在动物水平Piezo2特异性敲低及探索NGF-TrkA/Piezo2信号轴的分子调控网络,以明确低频振动诱导的腰痛大鼠机械性痛觉超敏的潜在机制。

利益冲突 本文所有作者声明不存在利益冲突

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