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用于哺乳动物行为研究的多通道光遗传系统

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摘要 为了实现在活体小鼠脑中多部位的光纤记录和区域可选择的光刺激，开发了一套集成度高、参数可独立调控的多通道光遗传系统，以满足光遗传学领域中大规模神经动力学研究需求。在该系统中，设计了一款1转7扇出多模光纤束，将光纤束与扫描振镜相结合，并利用时分复用技术调制不同波长的光源，以实现高质量的多通道记录和通道可选择的光刺激。评估了多光纤通道的参数稳定性，在小鼠前额叶、杏仁核、腹侧被盖等脑区同步记录了4个通道的钙信号。通过振镜靶向特定的光纤通道，可对自由移动的小鼠进行光遗传干预。该系统可用于活体多脑区光遗传干预与记录，为神经环路研究和行为学实验提供了有力工具。

关键词 生物光学；光遗传学；光刺激；多通道；多模光纤

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1 引 言

光遗传学[1-5]将遗传技术与光学方法相结合，在细胞、组织、活体动物中实现对神经元的高速精准调控。近年来该技术被广泛应用于高分辨神经调控和多脑区同步实验，为病理机制探索和疾病治疗研究提供了支持[3,6-9]。光遗传学通常使用多模光纤（MMF）传输光[10-12]，但多模光纤尺寸较小，限制了神经元编解码的区域范围。而动物的某些行为是整个大脑神经元网络综合作用的结果，目前，可用于评估动物大规模神经动力学的技术仍然有限[13-15]。动物记忆、焦虑、睡眠等复杂行为是大脑不同区域的神经元在执行特定任务期间作出的协调反应，需要多根光纤对不同类型的神经元或不同脑区进行同步实验[16-18]。此外，多只动物的特定行为研究也需要进行多光纤通道同步测试，例如研究社交互动的神经相关性[19-21]。因此，多通道、多波长、多功能的光遗传系统将成为神经环路与复杂行为研究的有效工具，为光遗传学实验探究提供了更多的选择。

近年来，研究者对多通道光遗传技术愈发关注。Adelsberger等[22]开发了双通道记录系统，通过增加光学传感器的数量，同时记录了两个脑区的神经活动，并证明小鼠运动皮层的不同子区控制着特定的运动。Paukert等[23]使用电荷耦合器件（CCD）相机成功记录了多通道光纤束的钙信号，在小鼠的不同脑区均发现了钙离子的激活信息。Kim等[24]开发了投影式独立光纤光度法，它可以同时记录来自多个脑区的荧光信号，成功量化了社交行为期间各个脑区的实时活动联系。Guo等[19]设计了可长期植入的高密度光纤阵列，该阵列能够实现哺乳动物大脑中跨区域的光纤光度记录。多通道光遗传系统通常利用大面积照明同时点亮所有光纤通道，并结合多个光学传感器独立采集各通道信号，但大部分多通道光纤及连接件需要定制，这给各个光纤通道参数的独立调整、通道数目的增减、系统的集成和多通道光纤的推广应用带来不便，在一定程度上限制了多通道光遗传实验的自由度。

本文采用扫描振镜来快速切换和精准靶向特定的光纤通道，通过优化扫描方案，可实现光刺激过程中多通道光纤数目、次序、频率、占空比等参数的独立调整；选取MMF定制1转7扇出多模光纤束，使用一台科学级互补金属氧化物半导体（sCMOS）相机作为光学传感器，有力降低了系统的集成与推广难度。可通过振镜扫描灵活选择实验通道，各个光纤通道参数稳定，串扰极小。小鼠光遗传实验表明，该系统稳定、灵活且易于使用。

2 系统开发

2.1 光学系统设计

图1展示了基于振镜开发的多通道光遗传系统示意图，该系统的功率、频率、光纤通道数目及通道扫描次序均可依据需求灵活设定。以功率为200 mW

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的两台 405 nm、473 nm 半导体激光器作为记录光源，支持双晶体管逻辑（TTL）调制输出，此过程类似于通信中的时分复用。由消色差透镜组成的望远镜系统用于准直激光束，并将光束扩展到实验所需的尺寸。以功率为 200 mW 的 589 nm 半导体激光器作为刺激光源，通过调节振镜的偏置电压来改变光束偏转角，实现目标平面的光斑扫描。值得注意的是，入射光相对于透镜光轴的角度变化会引起光学畸变，可以通过共轭系统进行校正，该共轭系统由远心扫描透镜和管透镜组成。扫描镜与管透镜组成的系统可以产生对称的成像平面，且光斑尺寸失真最小，无需进行大量的后处理。结合具有无限远校正功能的物镜，可以将光束精准耦合到指定的光纤通道，实现光传输与记录。多通道光纤采用 1 转 7 扇出多模光纤束，刺激光被多模光纤传输到小鼠的不同脑区进行刺激。同时，反射荧光被相同的光纤收集，并且通过二向色镜将分离的荧光成像到 sCMOS 相机上。系统所有器件均集成在 900 mm×600 mm×300 mm 空间内。虽尺寸较大，但系统工作时，仅需连接光纤与小鼠，无需对系统主体进行移动。多通道光纤扇出端为 7 根独立通道[图 1(b)]，与小鼠多个脑区的植入光纤连接后，可进行多脑区实验，较长的光纤扇出端也支持与不同小鼠的脑区连接。系统振镜电压与光斑位置具有良好的线性关系，如图 1(c)所示，有利于光斑位置的精准调控，实现 1 转 7 扇出多模光纤束通道的准确选择。同时，输出光频率与程序设定值保持一致，在时序上可有效保证系统的准确率与灵活性[图 1(d)]。与传统的多通道系统相比，本系统可以通过灵活切换光源、优化振镜扫描方案来更改系统参数并增加功能的多样性，同时解决了传统多通道系统存在的光路结构体积庞大、成本高昂以及同步控制复杂等问题。

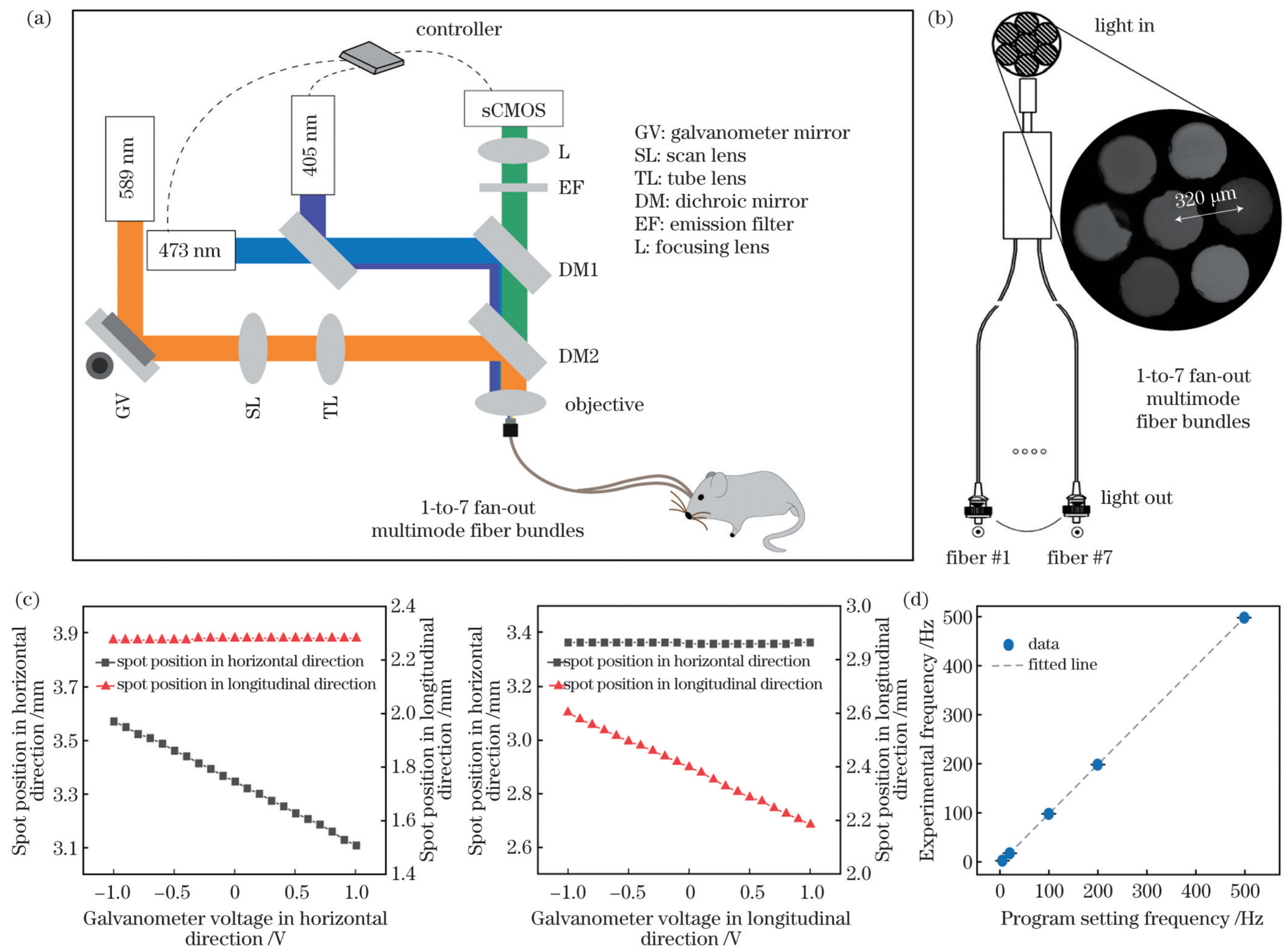

图 1　多通道光遗传系统示意图。(a)光学系统设计图；(b) 1 转 7 扇出多模光纤束及光纤入射端图像；(c)振镜电压对光斑位置的影响；(d)刺激光输出频率与程序设定频率的测试结果

Fig. 1　Schematics of multi-channel optogenetic system. (a) Design drawing of optical system; (b) 1-to-7 fan-out multimode fiber bundles and fiber incident end image; (c) influence of galvanometer voltage on spot position; (d) test results of stimulation light output frequency and program setting frequency

2.2 自动程序开发

图 1(b)展示了系统拍摄的光纤端面图像，基于 LabVIEW 的图像识别算法可在 1 s 内导出各个光纤通道的中心位置。结合图 1(c)中光斑位置与电压的测试结果，便可得到每个通道对应的振镜电压信号，振镜可精准靶向到特定的单根光纤通道。数据采集卡将标定电压信号加载到振镜上并设置扫描时序，系统可以根据设定的参数自动运行。数据采集卡能够同时控制 3 台不同波长的激光器和 1 台 sCMOS 相机，既支持多通道信号记录，也支持光遗传干预，其刺激通道可任意选择，如图 2(a)、(b)所示。光电探测器检测系统的输出参数，振镜电压根据输出参数进行反馈，通过微调可获得更加准确的输出结果。将物镜与光纤之间的距离调整为物镜的工作距离，选择具有更长工作距离的物镜以减少不同入射角对不同光纤通道耦合效率的影响。基于 C 语言的控制程序[图 2(c)]将方案设定、参数监测和信号分析集于一体，降低了系统的操作复杂度。系统控制界面可实时显示光纤端面荧光强度[图 2(c)上]和感兴趣区域(ROI)信号记录的结果[图 2(c)下]。系统双色荧光记录结果显示在图 2(d)中，通过信号分析与处理，便可得到目标通道对应脑区的 $\Delta F/F$ 曲线，其中 ΔF 表示感兴趣区域基线校正后的荧光强度，F 表示刺激前荧光强度的平均值。开发了针对各个光电子元件的控制与数据采集的集成化软件程序，系统工作时利用计算机控制全部硬件仪器并进行信号分析，仅需要连接多通道光纤与动物脑区，动物实验操作空间大。

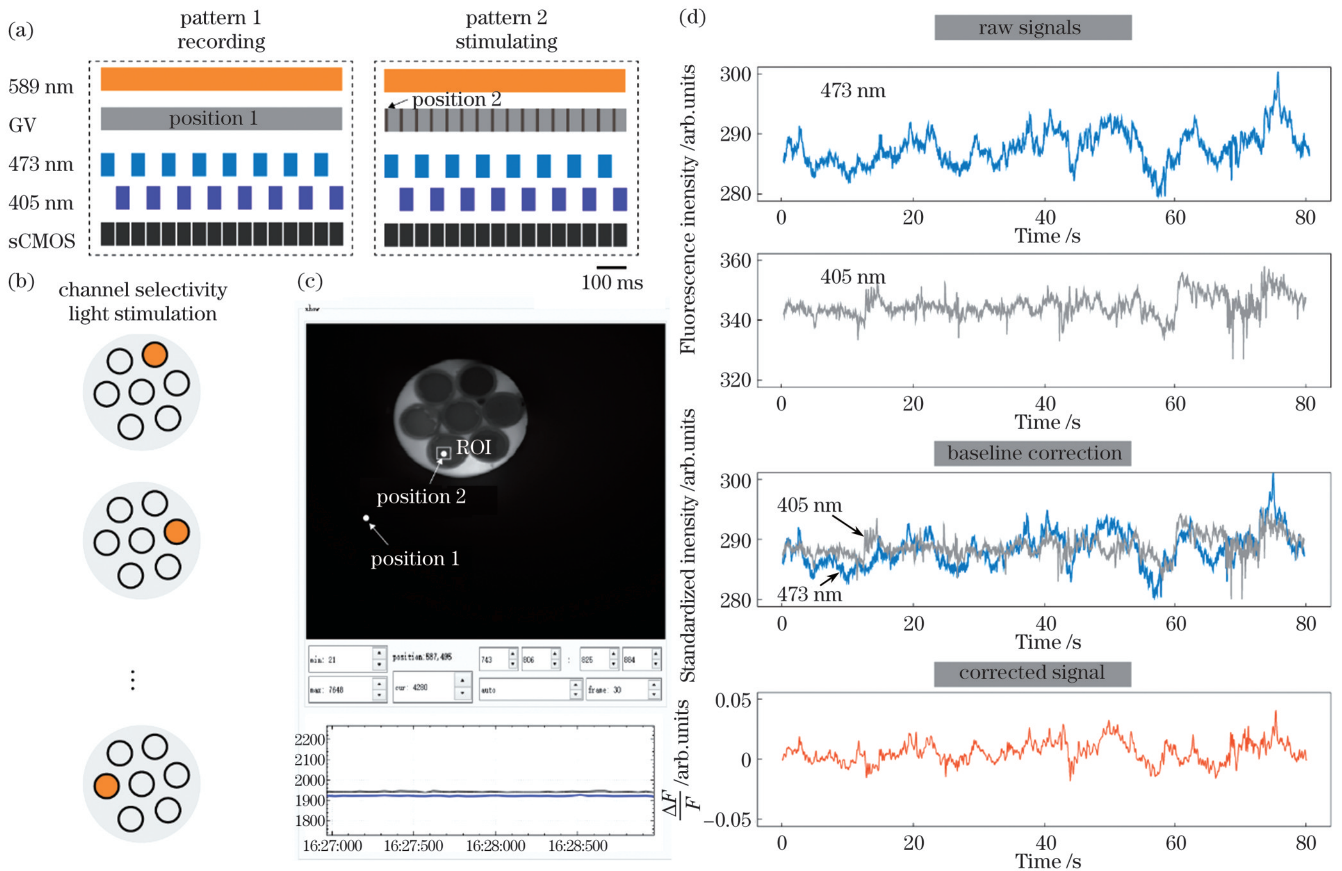

图 2 多通道光遗传系统控制方案。(a)系统的多样性实验方案；(b)灵活的光纤通道选择与次序设定；(c)软件界面；(d)双色荧光记录结果

Fig. 2 Control scheme of multi-channel optogenetic system. (a) Diversity experiment scheme of system; (b) flexible fiber channel selection and sequence setting; (c) software interface; (d) results recorded double-color fluorescence

3 结果与讨论

3.1 系统稳定性

在多通道实验探究中，保持各个通道功率的一致性至关重要，因此我们对每个通道的功率值进行了可靠性研究，以验证系统的稳定性与准确性。1 转 7 扇出多模光纤束能传导刺激光和记录光，也能收集生物荧光信号。我们分别测试了光遗传刺激和信号记录时 7 个光纤通道的功率稳定性(标准差与均值的比值)。光刺激通常需要 mW 级别较高的功率[25]，0～90 mW 激光功率梯度下各个光纤通道的输出功率测试结果如图 3(b)所示。当 589 nm 刺激光耦合到多通道光纤中时，各个光纤通道的输出功率与激光器输出功率呈正相关，7 通道的功率波动为 6.02%。同时，

μW 级别的光功率通常能够满足信号记录需求[12]，473 nm 记录光的测试结果表明，在小功率下系统仍能维持较高的稳定性，7 通道的功率波动为 5.04%。图 3(d)测量了 473 nm 记录光在 100～1000 μm 厚度小鼠脑组织离体切片中的透过率，当脑组织厚度为 100 μm 时，透射光功率约为入射光功率的 1/2，当脑组织厚度增加到 1 mm 时，透射光功率仅为入射功率的 10%，这与之前的研究结果[25]基本一致。光功率的测试结果证明了系统的稳定性，多通道间的功率差异在较小的范围内。在选择任意通道进行实验时各个通道输出功率均能维持一致，进而达到生物对照实验要求。

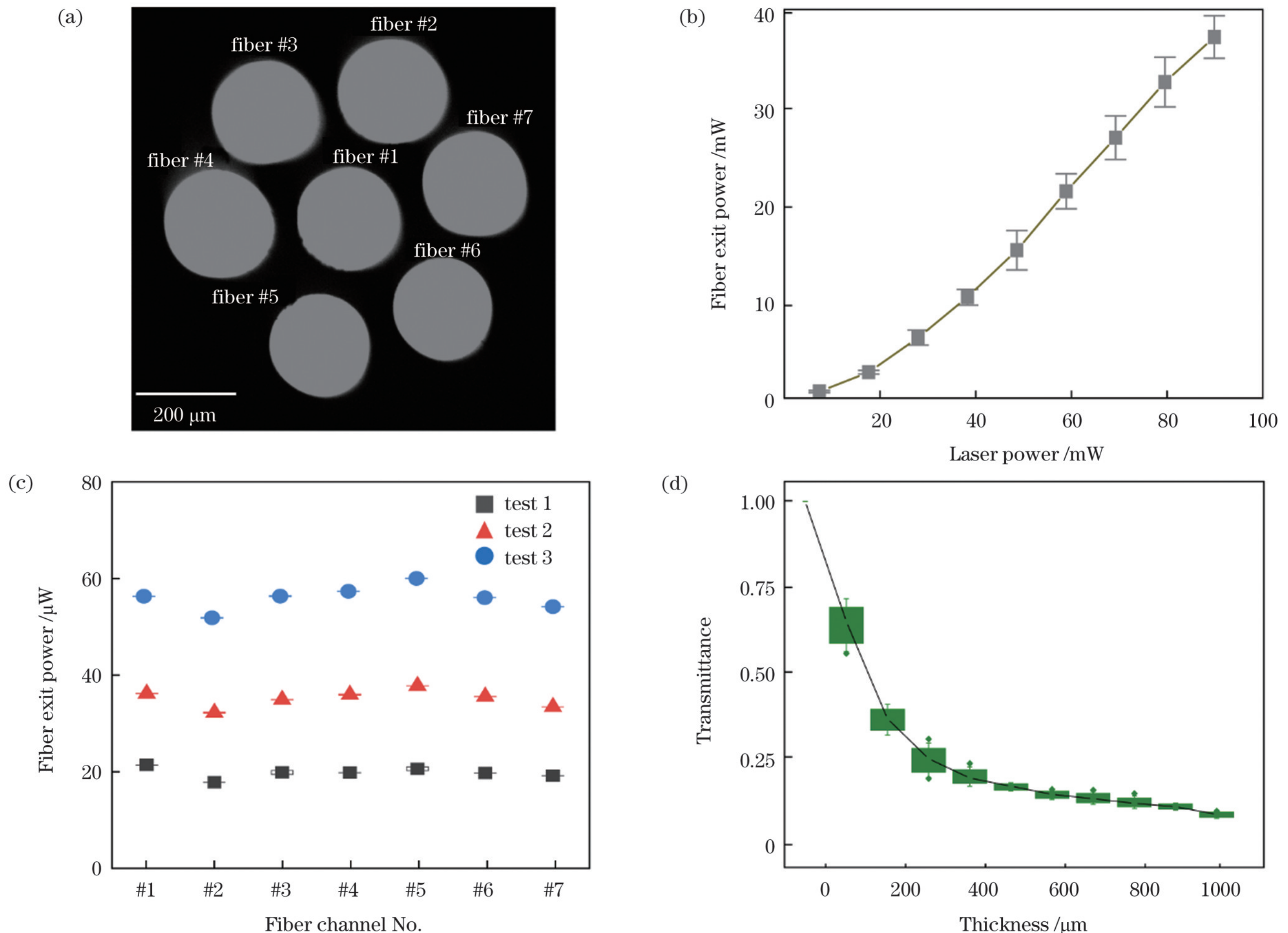

图 3 多通道光纤功率稳定性及透过率。(a)多通道光纤端面图像及通道编号；(b)不同功率梯度下 589 nm 刺激光 7 通道功率稳定性；(c)不同功率梯度下 473 nm 记录光 7 通道功率稳定性；(d)不同厚度小鼠离体脑片的透过率

Fig. 3 Multi-channel fiber power stability and transmittance. (a) Multi-channel fiber end face image and channel No.; (b) 7-channel power stability at 589 nm stimulation light under different power gradients; (c) 7-channel power stability at 473 nm recording light under different power gradients; (d) transmittance of mouse *in vitro* brain slices with different thicknesses

除了光学参数的一致性外，光纤通道之间没有串扰也是关键[13,24]。串扰是由较强刺激光的反射和光纤通道间的荧光扩散造成的。选择 1、2、3、4 号光纤[图 4(b)]分别连接表达光敏蛋白 ChrimsonR 和钙指示剂 GCamP6s 的内侧前额叶皮层前缘区(PL)、脊柱背侧区(Str)、基底外侧杏仁核(BLA)和中脑腹侧被盖区(VTA)。首先单独进行系统刺激模式下的抗串扰能力测试，在未打开 473 nm 记录光的状态下，无生物荧光信号。1 号光纤通道在 5、30、50 s 处的刺激光会引起该通道强度值的小幅提升，但强度值提升是规律的，方便降噪处理，且此时刺激光对相邻的 2 号光纤通道没有影响，如图 4(c)、(d)所示。之后打开记录光，进行不同通道的单通道刺激实验，同时采集另外 3 个通道的荧光信号，在长达 30 s 的采集过程中其他 3 个通道不同的钙反应显示了信号基本不受刺激光通道较强光的影响，如图 4(e)、(f)所示，其中刺激光波长为 589 nm，功率为 3 mW，频率为 20 Hz，光刺激持续 2 s。因此，该系统较强的刺激光不会干扰信号记录，多通道信号记录时通道串扰可忽略不计，系统能够进行多通道信号记录，具有良好的区域特异性与信噪比。使用多通道光遗传系统可以记录同一只小鼠的多个脑区，且通道间无明显串扰，能对标多台单通道信号记录系统的协同使用效果。

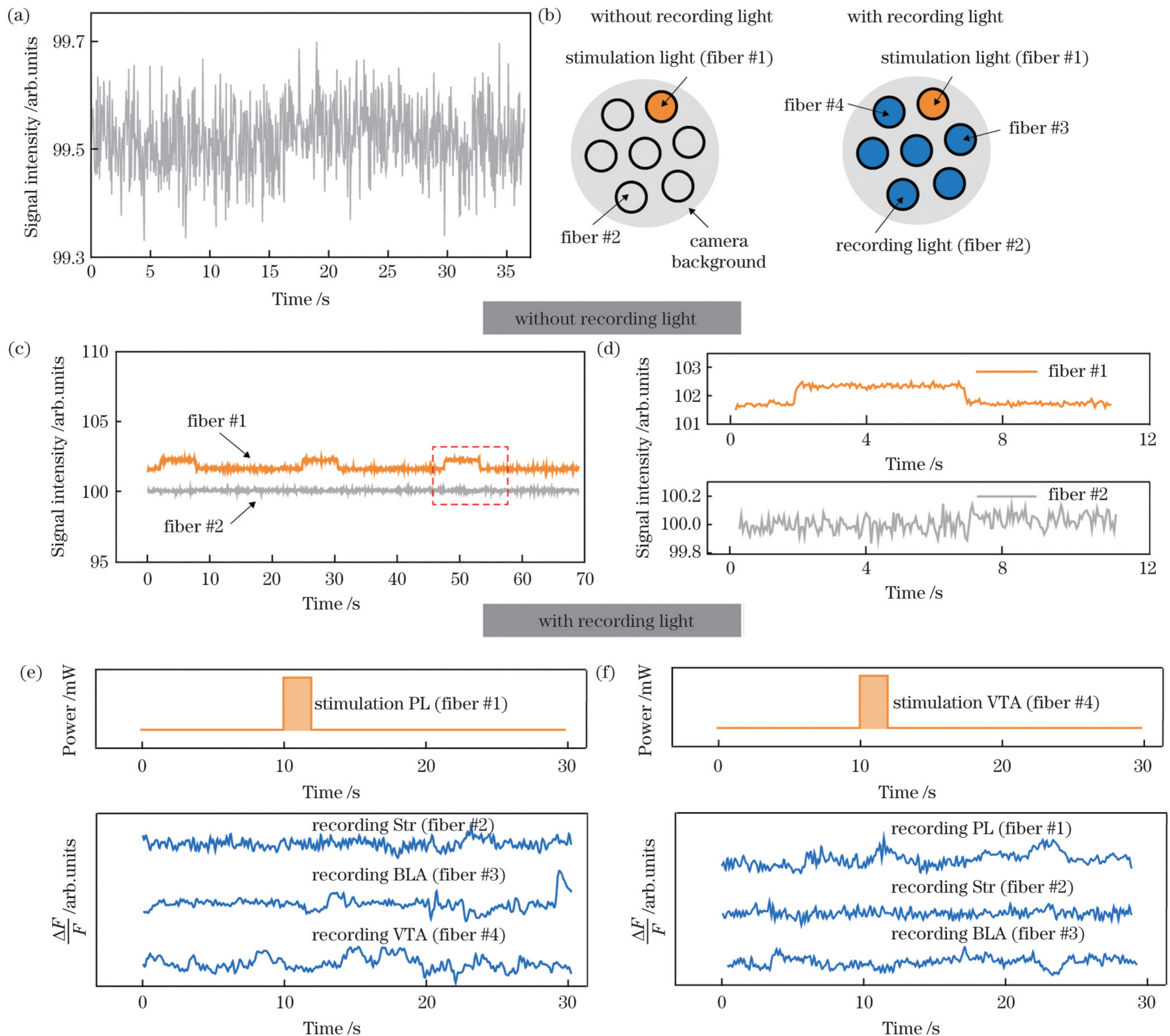

图 4　不同光纤通道的串扰测试。(a)相机基底噪声；(b)不同模式下多通道串扰测试示意图；(c)单通道刺激时的双通道串扰测试结果；(d)图 4(c)局部放大；(e)PL 脑区单通道刺激时周围通道的串扰评估结果；(f)VTA 脑区单通道刺激时周围通道的串扰评估结果

Fig. 4　Crosstalk test of different fiber channels. (a) Camera base noise; (b) schematics of multi-channel crosstalk test under different modes; (c) test result of two-channel crosstalk during single-channel stimulation; (d) local magnification of Fig. 4(c); (e) crosstalk evaluation results of peripheral channels during single-channel stimulation in PL brain region; (f) crosstalk evaluation results of peripheral channels during single-channel stimulation in VTA brain region

3.2　行为学实验

我们利用多通道光遗传系统对 Thy1-Cre 转基因小鼠进行了光遗传干预。小鼠 VTA 脑区注射荧光蛋白 ChrimsonR 和钙指示剂 GCaMP6s，注射 3 周后对小鼠进行单根光纤埋置，待小鼠术后恢复 2 周进行行为学实验，同时采用植入光纤但未表达光敏蛋白的小鼠作为对照。用功率为 5 mW、频率为 40 Hz 的 589 nm 脉冲光（占空比为 1∶9，持续时间为 10 s）刺激 VTA 脑区，可以诱使小鼠发生单侧偏转运动。多次实验（N 为重复实验次数）显示：小鼠单侧偏转运动均在脉冲光停止时结束，之后进行无规律运动[图 5(a)、(b)]。从量化统计结果图 5(c)～(f)可以看到：光刺激时小鼠的运动距离、速度、角度和头部偏转角度等参数均明显大于无光刺激时，运动距离和速度几乎是无光刺激时的 2 倍，对照组小鼠并未对光刺激表现出强烈可靠的反应（模型可靠性统计检验值 $P>0.05$）。通过免疫组化的方式进一步验证神经元激活的准确性，目标脑区 VTA 表达光敏蛋白的神经元 c-fos 表达更多，即系统选择性激活了目标脑区兴奋性神经元。利用多通道光遗传系统可以进行生物活体行为学研究，其 1 转 7 扇出多模光纤束的 7 个扇出端具有高的功率稳定性和通

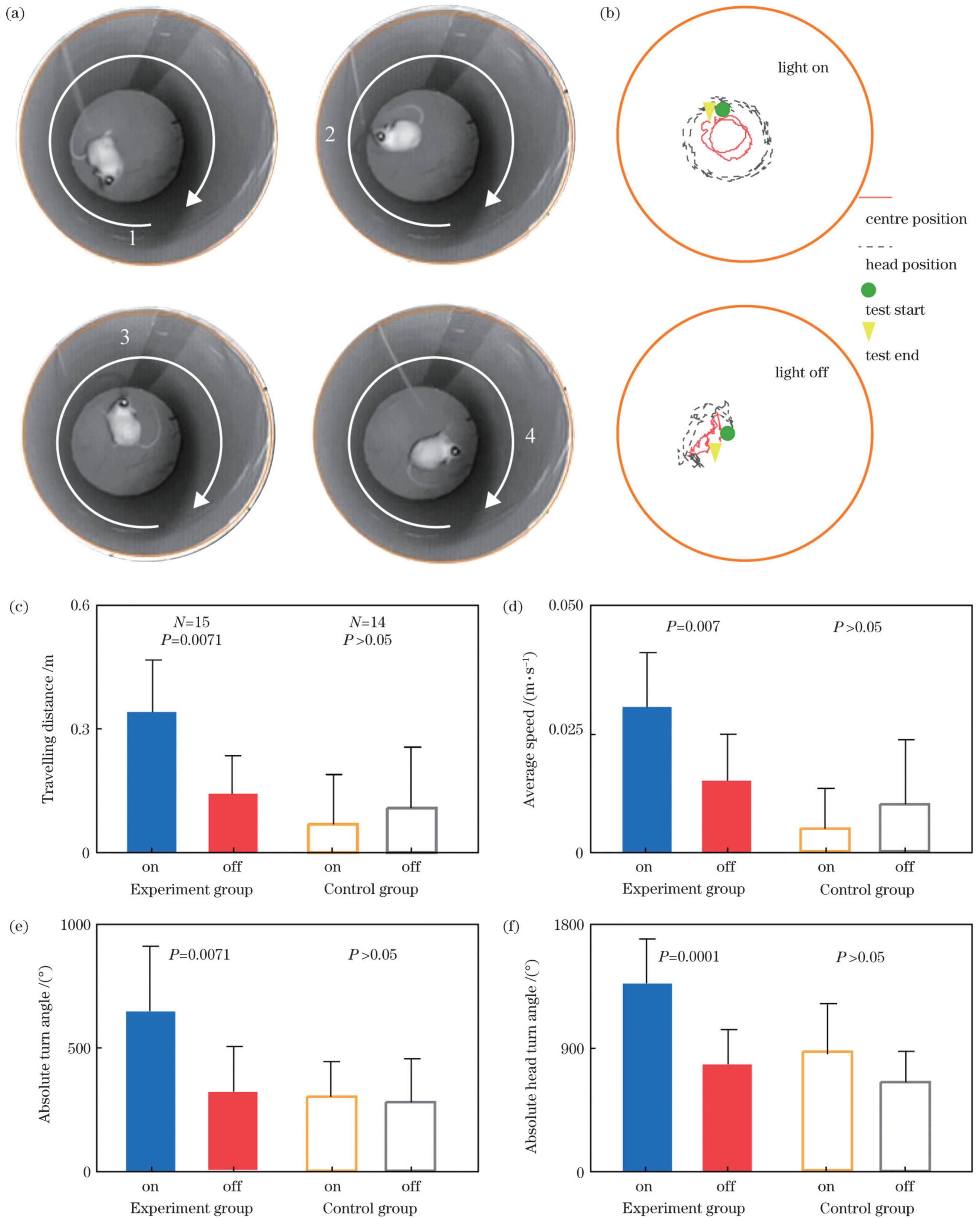

图 5　小鼠行为学实验图像。(a)小鼠 VTA 脑区的行为学实验；(b)有无光刺激下小鼠行为学实验中的运动轨迹；有无光刺激下小鼠(c)运动距离、(d)速度、(e)角度、(f)头部角度的统计结果

Fig. 5　Mice behavior experiment images. (a) Behavior experiment of mice VTA brain region; (b) movement tracks in mice behavior experiment with or without stimulation light; statistical results of (c) travelling distance, (d) speed, (e) angle, and (f) head angle of mice with or without stimulation light

道可选择特性，具备实现多部位同步刺激的能力，弥补了单通道系统通道不足的缺陷。

4 结 论

提出了一种多通道光遗传系统，能够支持 7 通道的光遗传研究，可灵活调整功率、频率、通道数目、通道次序和刺激模式，各个通道具有良好的稳定性。集控制、算法、分析于一体的软件系统也极大降低了系统的操作难度，为哺乳动物大脑中复杂环路的功能组织模式和行为相关动力学提供了有效的研究工具。未来可通过加入校正功能来实现物理参数的实时检测和闭环自动实验，结合轻量化技术减少多通道光纤重量对小鼠头部造成的负担，为多通道光遗传领域提供更加小型化、智能化的解决方案。

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Multi-Channel Optogenetic System for Mammalian Behavior Research

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Abstract

Objective Optogenetics combines genetic technology with optical methods to achieve high-speed and accurate regulation of neurons in living animals, cells, and tissues. Fiber photometry is a sensitive and simple method to stimulate and record neuronal activity in the

deep brains of animals. Multimode fibers can transmit stimulus light to neurons and collect their fluorescence. However, some animal behaviors result from the comprehensive action of the entire brain neural network, and the small size of the multimode fiber end face limits large-scale neuroscience research. Currently, techniques that can be used to evaluate large-scale animal neurodynamics are still limited. The previously proposed multi-channel optogenetic systems face three possible problems: a low degree of freedom of parameter adjustment, the need for independent customization of multi-channel fibers, and system integration problems caused by multi-sensors. In this paper, we report a multi-channel optogenetic system. The number, order, frequency, duty cycle, and other parameters of the multi-channel fibers can be independently adjusted by targeting specific fiber channels with a galvanometer. The system includes 1-to-7 fan-out multimode fiber bundles and only a scientific complementary metal-oxide-semiconductor (sCMOS) camera as the optical sensor, which effectively reduces the difficulty of the system integration. It provides a multi-channel, independent, flexible, and highly integrated solution for multi-channel optogenetic experiments.

Methods In this study, 1-to-7 fan-out multimode fiber bundles are used. First, a scanning galvanometer is used to target a selected fiber channel, and the time-division multiplexing technology is used to modulate lasers with different wavelengths. Subsequently, a beam with a selected wavelength is coupled to a selected fiber channel for the experiments. The fluorescence signal collected by the fiber channel is then imaged by the sCMOS camera to achieve multi-channel fluorescence recording. System debugging, instrument control, and data processing are then integrated into an automatic software system to simplify the operation process of the system and meet the requirements of the multi-channel, multiwavelength, and multifunctional optogenetics experiments. In the next step, stability and crosstalk experiments are carried out on the multi-channel optogenetic system to evaluate the uniformity and independence of the multi-channel fibers. Additionally, typical biological experiments are performed to prove the feasibility of the system in optogenetic experiments.

Results and Discussions The multi-channel optogenetic system has excellent parameter accuracy. The scanning galvanometer can accurately target the selected beam to a selected 200-μm diameter fiber channel. The test results of the fiber output frequency show that the average error of the stimulation frequency is 0.1% within the range of 5–500 Hz. Affected by the scanning angle of the galvanometer and the uniformity of illumination, the output powers of 7 channels are slightly different, but it still has excellent power stability, and the fluctuation does not exceed 6.02% (Fig. 3). The multi-channel crosstalk experiment results show that the strong stimulation light does not interfere with the fluorescence signals collected by other channels, and the fluorescence crosstalk between channels can be ignored (Fig. 4). Therefore, each channel of the multi-channel optogenetic system has excellent anti-interference capability, which helps control noise and ensure the accuracy of the experimental results. Finally, compared to the control mice, the photostimulation of the target region can induce unilateral rotation in mice, and the statistical results show more than twice the difference (Fig. 5). The experimental results show that the power value of every channel of the system is the same. Considering the microsecond response time of the scanning galvanometer, this system has the potential in special application scenarios such as multi-channel alternate outputs and ultrafast channel scanning.

Conclusions In this study, a multi-channel optogenetic system is designed, which includes a laser time-division multiplexing module, galvanometer scanning module, and 1-to-7 fan-out multimode fiber bundles. The system solves problems in previous multi-channel systems: inflexible parameter adjustment, difficult customization of multi-channel fibers, and difficult system integration caused by a large number of instruments. The system can be used in the dynamics at multiple sites across the mammalian brain. The galvanometer scans different fiber channels at a frequency of 1 kHz. The output power of a single fiber can be flexibly adjusted within the range of 0–40 mW. Additionally, this system can flexibly adjust the wavelength, frequency, duty cycle, channel number, and channel sequence. Every channel has high frequency accuracy and power stability. The software system that integrates the control, algorithm, and analysis also significantly reduces the difficulty of system operation, which provides a convenient tool for research on the functional organization and behavior-related dynamics of mesoscale circuits in the brain. In the future, a correction function can be added to realize the real-time detection of physical parameters and closed loop-automatic experiments during operation, and the weight burden of the multi-channel fiber on the head of mice can be reduced by combining lightweight technology, providing a more miniaturized and intelligent solution for the multi-channel optogenetics field.

Key words bio-optics; optogenetics; light stimulation; multi-channel; multimode fiber