

结合光遗传神经调控技术的仿生嗅觉传感系统的研究进展

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摘要 嗅觉系统是一个极其巧妙的化学感受器, 嗅觉感觉神经元对外界的气味刺激产生响应, 然后将此信号传递到嗅球进行信号的整合和处理, 最终传递到嗅皮层, 嗅皮层作为嗅觉感觉系统的最高级皮层识别出气味信息, 这种响应和传递过程具有高度的灵敏性、特异性和快速性. 光遗传学是近年来国际上一个新的发展方向, 通过光控制神经细胞可诱发细胞按照某一特定频率发放. 仿生嗅觉传感器是一种新型化学传感仿生技术, 它以嗅觉受体、细胞、组织等生物材料为敏感元件, 结合二级变换器实现对气味物质的特异性和高灵敏的检测, 从而达到类似生物体嗅觉的检测性能, 将其与光遗传技术结合, 可以更深入研究仿生嗅觉系统传递的机制. 本文概述了近年来国际上光遗传技术在嗅觉机制研究中的研究进展, 并结合光遗传在仿生嗅觉传感技术的研究和我们的研究工作, 展望了未来该领域可能的发展前景.

关键词 嗅觉, 光遗传, 仿生传感技术, 气味识别, 电子鼻

1 生物嗅觉系统气味识别机理

嗅觉是一种感觉. 脊椎动物的感觉系统相对比较复杂, 首先是外周神经元对周围环境中的刺激信号产生响应, 然后这些信号传递到感觉中枢进行更高层次的处理, 最后分辨出不同的感觉信息. 人的嗅觉系统可以分辨出上千种气味分子, 气味分子与鼻腔内嗅黏膜上的嗅觉感觉神经元(olfactory sensory neurons, OSNs)特异性结合, 嗅觉感觉神经元轴突将1000多种不同的气味分子的信息准确地传递到嗅球的1800个左右的嗅小球(glomeruli)中^[1], 嗅觉感觉神经元位于嗅觉上皮(olfactory epithelium, OE), 参与嗅小球在中枢神经系统最初的结构和功能的形成, 气味分子的信息通过嗅觉感觉神经元的轴突传递到嗅球

(olfactory bulb, OB)内, 嗅球是大脑中处理嗅觉信息的第一站, 在嗅觉信息的形成和传递过程中起着至关重要的作用. 每一类嗅觉感觉神经元表达某一种特定的嗅觉感觉受体(odorant receptors, ORs), 表达同种受体的嗅觉感觉神经元将轴突投射到嗅球特定的嗅小球结构中^[2,3]. 因此嗅觉感觉神经元在整个嗅觉系统中起着举足轻重的作用: 传递气味信息到神经系统和构成嗅球上嗅小球编码嗅觉信息分布图的起始端^[4].

嗅小球是嗅觉系统的重要组成部分, 是嗅觉感觉神经元和嗅球中僧帽细胞连接的中介, 它包含从嗅觉感觉神经元发出的1000多个神经轴突和来自至少20个僧帽细胞的树突, 参与着嗅觉信息的初级快速编码过程^[5,6]. 嗅小球在嗅球中同僧帽细胞和丛状细胞(mitral and tufted neurons, M/T cells)通过不同的角色

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Gao K Q, Zhuang L J, Qin Z, et al. Advances in research on combining the optogenetics with bionic olfaction sensor (in Chinese). Chin Sci Bull, 2017, 62: 3619–3630, doi: 10.1360/N972016-01453

共同对嗅觉信息进行加工处理,最后通过同一路径将整合后的嗅觉信息传递给嗅觉皮层 (olfactory cortex, OC)^[7,8](图1)

大脑皮层中主要有4个区域与嗅觉信息的传递和处理相关:梨状皮层(piriform cortex, PC)、杏仁核(amygdala)、嗅前核(anterior olfactory nucleus, AON)和内嗅皮层(entorhinal cortex, EC).从嗅球上的一个嗅小球中连接的僧帽细胞支配着梨状皮层比较大的区域(图2)^[5,9,11],相反地,梨状皮层神经元直接通过突触连接与大量的僧帽细胞的轴突相连接传递嗅觉信息并且反向支配着大量分散的嗅小球^[10].嗅觉神经系统,包括感觉神经元以及处理和传递嗅觉信息的中间各种神经元,共同作用着感知外界化学环境的变化并通过反馈来驱动着动物的行为.

2 光遗传技术在嗅觉系统研究中的进展

2.1 光遗传原理以及光遗传技术的运用

光遗传学(optogenetics)全称为光刺激基因工程(optical stimulation plus genetic engineering),它结合了现代遗传学、微生物学、神经科学、生物工程等多种技术,实现对特定细胞活动的定向调控.光遗传学首先由斯坦福大学 Karl Deisseroth教授2005年提出,在2010年分别获得 Golden Brain Award和*Nature Methods*年度奖项.光遗传学最关键和核心的部分就

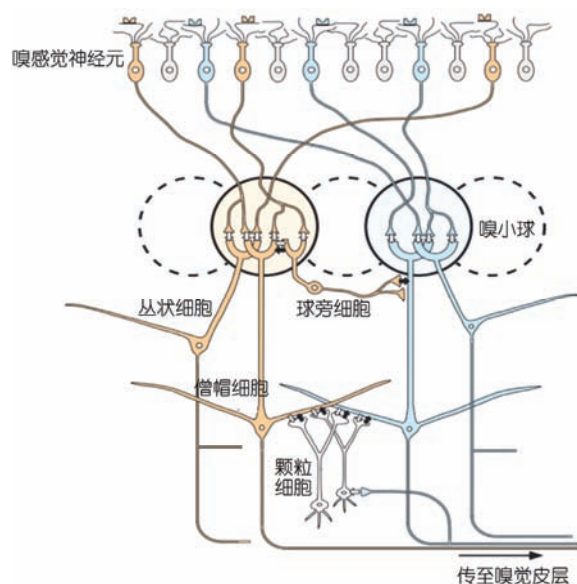


图1 (网络版彩色)用简单图解来概括脊椎动物嗅球上的嗅觉相关的主要的突触环路大致结构.两种不同的嗅小球分别代表两种不同类型的气味接收体,白色的箭头表示兴奋性递质,黑色箭头表示抑制性递质^[1]

Figure 1 (Color online) Basic circuit diagram summarizing the synaptic organization of the mammalian MOB (main olfactory bulb). Two glomerular modules represent two different types of odorant receptors. Mitral cells and tufted cells are output neurons, and granule cells and periglomerular cells are local interneurons. Short white arrows denote excitatory synapses, and short black arrows denote inhibitory synapses^[1]

是“光感基因”,光感基因是一类存在于自然界绿色植物及光能微生物体内的遗传信息序列,它们能够

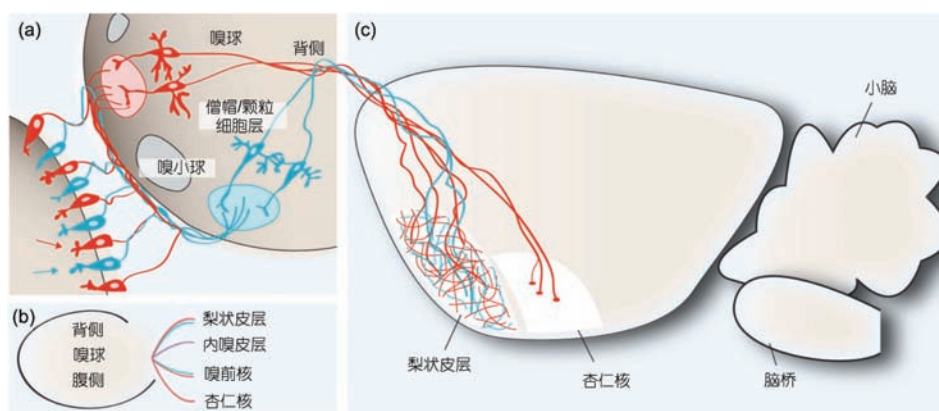


图2 (网络版彩色)嗅小球相关神经元将嗅觉信息投射到嗅觉皮层.(a) 在小鼠体内,嗅感觉神经元投射到特定的嗅小球中,嗅球上的僧帽细胞和丛状细胞又将嗅小球的相关信息投射到嗅觉皮层不同区域;(b) 嗅小球投射区域的起源范围,主要包括梨状皮层、内嗅皮层、嗅前核和杏仁核;(c) 嗅小球投射到梨状皮层和杏仁核的大体示意图

Figure 2 (Color online) Glomerular projections to the piriform cortex are not spatially restricted. (a) In mice, olfactory sensory neurons project to specific glomeruli innervated by mitral and tufted cells that project to different brain regions; (b) schematic showing the origin and targets of the glomerular projections including the piriform cortex, entorhinal cortex, anterior olfactory nucleus and amygdala; (c) projections to the amygdala are spatially restricted, while those to the piriform are distributed over the entire brain region

表达相应的光敏感离子通道蛋白, 这些蛋白通常附着于细胞膜表面, 能够将特定波长范围的光转化为细胞膜表面电位及突触电流, 从而引发细胞整体的电活动. 不同的光感基因所对应的可见光吸收频谱有较大的差异, 目前已被发现的典型的应用于神经科学领域的光感基因主要来源于低等藻类和简单微生物, 主要包括ChR2(channelrhodopsin-2), 来源于某种藻类, 是最先被应用于光遗传学神经调控的光感基因体系, ChR2在473 nm激光下被最大限度的激活, 可以将毫秒脉宽的光刺激转换为神经元的动作电位, 其最高发放频率可以达到30~50 Hz^[12]. 相对于ChR2来说, VChR1(volvox channelrhodopsin-1)有着更宽的光波吸收频谱, 使其最大程度激活的光波长在535 nm^[13]. 氯离子通道蛋白NpHR(natronomonas pharaonis halorhodopsin)来源于某种嗜盐古细菌, 其主要特点是能够实现神经元的抑制调控作用, 它对应的最大限度吸收光波则为580 nm附近波长的黄光, 在这种黄光下NpHR的活动将导致细胞膜的超极化, 从而外在表现为细胞整体电活动的抑制. 光遗传学技术的运用包括4个步骤: (1) 找到和课题最合适的光敏蛋白, 有的蛋白是具有天然的光敏性, 有的是需要经过化学修饰而具有光敏性; (2) 通过转染、转基因动物系的建立等方式将光敏蛋白的遗传信息传递给目标细胞; (3) 通过在时间和空间上控制激光的特定性, 实现对细胞活动的精确调控; (4) 读取研究结果的时候可采用电极通过检测细胞膜内外电压来分析变化值, 也可用荧光性生物传感器来检测不同细胞的读出值, 最后, 也可通过行为测试来评估光遗传技术细胞活动对整个动物的影响. 目前光遗传技术除了大量应用于神经调控机制方面的研究, 也在药物筛选^[14]、瘫痪治疗^[15]等领域应用并取得突破性进展.

2.2 光遗传应用于嗅觉机理的研究

嗅小球是整个嗅觉系统的最主要通道, 连接着嗅感觉神经元和大脑嗅觉皮层. 一个特定的嗅小球会接收到同种类型基因编码的嗅感觉受体传送的嗅觉信号^[2], 然而给予特定的气味刺激会引起大量的嗅小球发生响应^[16~20], 光遗传技术出现之前还没有技术能够有效地检测到单个嗅小球在气味感知过程中的作用, 为了检测单个嗅小球在气味感知过程中的作用, 美国霍华德·休斯医学研究所联合纽约大学医学中心以及西北大学神经生物研究所通过光遗传技

术对其进行了探索研究, 他们将光敏感蛋白ChR2表达在一种特定的嗅感觉神经元中, 并且利用其投射到单个特定嗅小球上内的轴突的集合, 发现单个嗅小球受到刺激也可以引起嗅觉感知. 另外, 不同的嗅小球在不同时段的刺激都可以被辨别区分, 并作出推测可以利用单个嗅小球响应的不同的波段, 振幅及其实时相应的编码信息来驱动小鼠行为学的相应变化^[21].

嗅觉系统编码气味信息是通过嗅觉相关神经元对气味产生的反应^[22,23], 在哺乳类动物体内, 每一种气味都能通过嗅感觉神经元引起一种极其精确而且具有特异性的反应. 同样地, 在神经系统中, 从外周系统的感觉到神经中枢的知觉过程, 不同的神经元都会有实时的响应模式, 并最终在嗅觉系统中呈现特定的嗅觉信息^[24,25], 然而还没有相关研究证明嗅觉系统可以有识别对特定气味感知的记忆. 为了证明这个猜想, 有学者^[26]利用光遗传技术进行验证, 并得到了初步的结论: 他们在转基因小鼠上利用光刺激作为一种固定的、实时可控的可充当气味刺激的输入信息, 通过小鼠行为变化来观察嗅感觉神经元对光刺激的反应(图3), 发现小鼠嗅觉系统可以识别10 ms内相应的光刺激变化, 这与整个嗅觉系统对特定气味刺激相应的时间^[27,28]相似, 利用电生理技术在清醒小鼠的嗅球内记录光敏感蛋白刺激引起的实时的电信号变化, 他们发现脊椎动物小鼠, 嗅觉系统可以很快地辨别和区分不同时间给予的相同刺激并及时形成感知.

光敏基因除了可以表达在嗅感觉神经元和其轴突延伸到达的嗅小球以外, 也可以表达在嗅觉皮层中, 利用其可以对嗅觉系统进行更深层次的研究. 嗅觉信息通过嗅小球传到僧帽细胞, 并通过外侧嗅束(lateral olfactory tract, LOT)最终传递到嗅觉皮层的锥体细胞中^[29], 皮层的锥体细胞又可以反向引起嗅球颗粒细胞的兴奋, 也就是说僧帽细胞在嗅觉皮层反馈回路传导过程中主要起兴奋抑制性颗粒细胞的作用. 曾有实验证明在嗅球及皮层切片上, 给予嗅觉皮层胞外刺激使嗅觉皮层产生兴奋性并且将其兴奋性通过递质传递到嗅球颗粒细胞层, 同时传递到僧帽细胞层, 通过树突连接起到兴奋抑制性通路的作用^[30,31](图4). Boyd等人^[32]将光敏感蛋白转染到小鼠嗅觉皮层锥体细胞中, 然后通过刺激嗅觉皮层来展示皮层到嗅球的反馈回路上不同种类嗅球神经元的

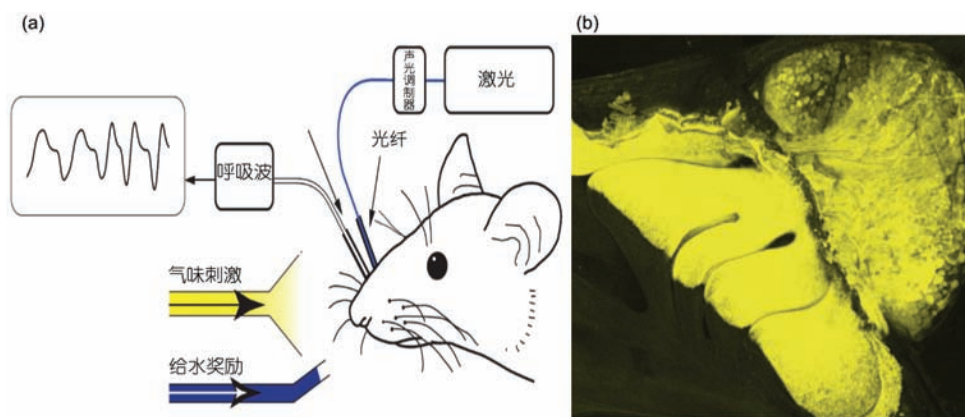


图3 (网络版彩色)利用光遗传技术实现对特定气味的感知和训练。(a) 实验基本原理图。小鼠鼻腔内植入一个光纤(optical fibre stub, OFS)来接收传递光刺激, 另外一个PC(pressure cannula)连接一个传感器来检测呼吸频率变化; (b) 携带荧光蛋白的转基因小鼠的嗅上皮和嗅球图片, 在OMP-ChR2 转基因小鼠, ChR2-YFP标记的嗅感觉神经元和它们伸到嗅小球内的轴突^[26]

Figure 3 (Color online) The detection and training of specific odor by optogenetics technology. (a) Schematic of experimental set-up. Mice were implanted with a nasal optical fibre stub to deliver light, gated by an acousto-optic modulator, a nasal pressure cannula coupled to a pressure sensor measures sniffing, inverted intranasal pressure signal is shown at top left. (b) Sagittal view of wholemount olfactory epithelium and bulb, in OMP-ChR2 mice, ChR2-YFP labels OSNs and their axons in the bulb^[26]

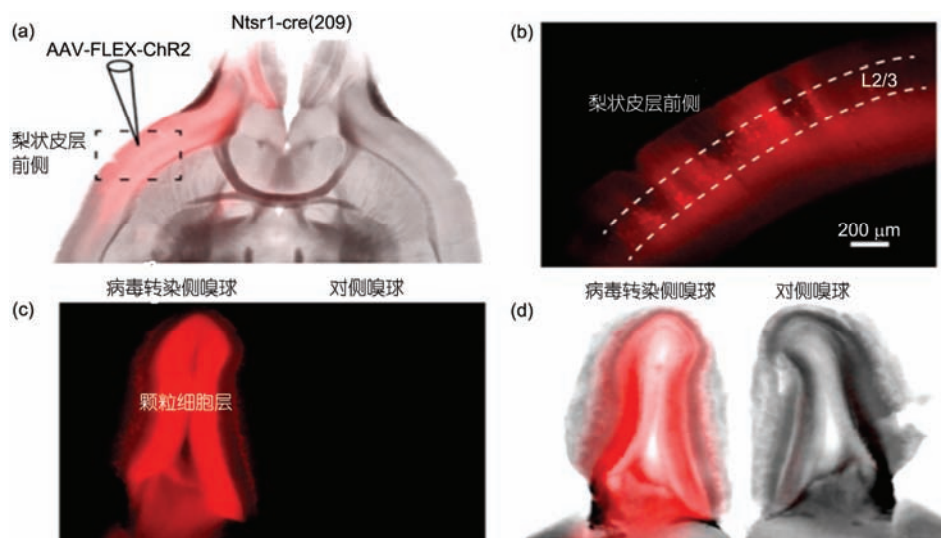


图4 (网络版彩色)在梨状皮层锥体细胞表达ChR2来研究嗅觉皮层到嗅球的回路。(a) 荧光覆盖区域是转基因小鼠Ntsr1-cre在梨状皮层前侧的ChR2-mCherry的表达, 梨状皮层前侧, 侧嗅束嗅球; (b) 为(a)图梨状皮层第2/3层锥体细胞ChR2-mCherry的表达区域; (c) ChR2-mCherry只表达在嗅球的单侧, 在同一只小鼠对侧不表达, 颗粒细胞的细胞层(granule cell layer, GCL); (d) 嗅球内ChR2-mCherry荧光以及明场成像图^[32]

Figure 4 (Color online) (a) Conditional expression of ChR2 in piriform cortex pyramidal cells reveals cortical feedback projections to the olfactory bulb overlay of bright-field and fluorescence image of a horizontal section (300 μm) of forebrain from an Ntsr1-cre mouse showing ChR2-mCherry expression in olfactory cortex; (b) blow-up of region in (a) indicating expression of ChR2 in layer 2/3 pyramidal cells; (c) ChR2 is expressed in the ipsilateral, but not contralateral OB from the same mouse; (d) overlay of fluorescence and bright field images of the bulbs^[32]

兴奋性变化, 通过大量的实验他们发现刺激嗅皮层也能影响到气味刺激引起的僧帽细胞的自发放电, 从而发现这个反馈回路对僧帽细胞的调节在嗅觉信息的传递过程中起着重要的作用。

在嗅球中, 气味刺激分别在两个层面上引起具有气味特异性的时间模式上的相应活动, 他们分别

是嗅神经信息的输入(嗅小球的响应)^[33,34]和输出(僧帽细胞和丛状细胞的锋电位发放)^[33,35~37], 这个过程发生在200 ms左右的一个嗅觉周期之内, 在这几十个毫秒内就会有大量的嗅觉信息被提取出来^[38], 因此, 在理论上, 嗅觉信息在嗅球上的短暂响应被编码, 其特异性的功能也已经被确定, 如果这个暂时的

编码是有效的,那么接受者就会迅速接受并且破译^[26,39]。有研究表明,在嗅觉皮层上,典型的嗅觉皮层在整个嗅觉周期的发放率与传送大多数气味信息的锋电位发放并不是同时的^[40]。另外,早前有研究使用混合气体^[41,42],利用脑片上采用电生理记录^[43,44]以及通过在体检测嗅球谷氨酸释放技术^[45]都检测到了梨状皮层神经元(piriform cortex neurons, PCNs)是如何整合来自嗅球的不同种类的气味信息,这些研究都显示特异性嗅觉信息的输入会使梨状皮层产生超线性和亚线性的响应,然而来自嗅小球特异性嗅觉信息的传递是否重要还是未知,有学者^[46]就利用光遗传技术来验证这个假说(图5):来自嗅球的实时特异性的嗅觉信息传递到梨状皮层是可以产生不同的发放频率的,他们在转基因小鼠 Omp-ChR2 上用特定频率的光刺激嗅小球,并同时用电生理技术分别记录嗅球上僧帽/丛状细胞(M/Ts),前侧梨状皮层(anterior of the piriform cortex, APC)和后侧梨状皮层(posterior of the piriform cortex, PPC),他们发现梨状皮层神经元的频率发放相对于嗅球来说更加敏感,说明来自嗅小球内部神经元相关的锋电位发放在气味编码过程中起着重要的作用。

3 仿生嗅觉系统的研究进展

从古至今,人类利用自身和动物的嗅觉辨识各

种气味。随着社会文明的进步,人类对气体成分检测的准确度和灵敏度提出了更高的要求,天然的生物化学感受系统在很多领域都受到了不同程度的限制,不少科研工作者一直致力于研制更客观准确地检测仪器来替代生物的感觉。随着对嗅觉机理研究的不断深入以及生物仿生传感器研究的不断进步,人们以生物活性材料作为敏感元件,耦合各种二级换能器,分别从分子、细胞和细胞网络3个层面对仿生嗅觉传感器进行了系统深入的研究,研制出多种仿生嗅觉传感器(biomimetic olfactory-based biosensors)。仿生嗅觉传感器主要由两个部分构成,首先是作为敏感元件的生物活性组分,能与目标分析物发生相互作用以产生特异响应,可以是嗅觉受体蛋白(olfactory receptor proteins, ORPs)、嗅感觉神经元、嗅上皮等;另一部分是作为换能器的物理化学探测器,能把敏感元件产生的响应转换为更易于处理和分析的物理信号,比如电信号。最后仿生嗅觉传感器与信号处理以及相关的电子电路和仪器部分相结合构成一个完整的检测系统,使得检测结果能以更好的方式呈现出来,因此这些仿生嗅觉传感器获得了部分生物嗅觉系统具有的特点,比如灵敏度高、检测限低、选择性好等,具有很大的潜力,可用于开发检测气味的生物电子鼻(bioelectronic nose),广泛应用于生物医学、环境监测、药物开发、食品和水质控制等

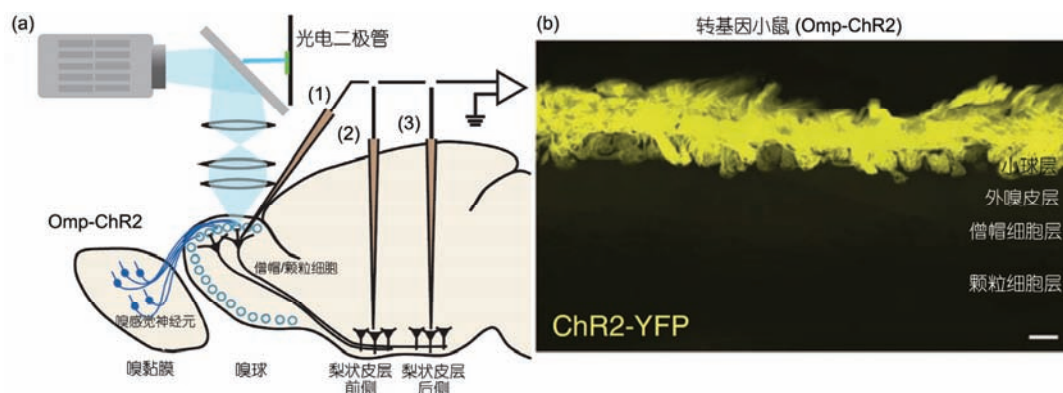


图5 (网络版彩色)通过单根光纤光刺激嗅神经的响应特征。(a) 实验设置。光聚集在嗅球表面,转基因小鼠(Omp-YFP)的嗅感觉神经元表达了ChR2光感蛋白,接受到光刺激会产生响应,然后分别在嗅球上僧帽/丛状细胞(M/T)。(b) 转基因小鼠(Omp-YFP)嗅球上表达了光感蛋白的嗅觉神经元的荧光成像,ChR2被标记荧光蛋白,全部位于嗅小球内,图片显示嗅小球层(glomerular layer, GL)、外嗅皮层(external plexiform layer, EPL)、僧帽细胞层(mitral cell layer, MCL)、颗粒细胞层(granule cell layer, GRL)的大体区域,参考标尺是100 μm ^[46]

Figure 5 (Color online) Characterization of responses to single spot optogenetic activation of olfactory nerve input. (a) Experimental setup. Light was focused on the surface of the olfactory bulb, mice expressing ChR2 in ORNs (Omp-ChR2) were used. Spiking activity was recorded extracellularly from mitral and tufted (M/T) cells in the olfactory bulb (1) and neurons in the APC (2) and PPC (3). (b) Fluorescence image of the olfactory bulb in an Omp-ChR2 mouse. It indicates the location of ChR2 tagged with a yellow fluorescent protein (ChR2-YFP). ChR2 was located exclusively in the glomerular layer. Scale bar represents 100 μm . Sections from $n>20$ mice were examined^[46]

诸多领域具有广阔的应用前景和商业开发潜力。

3.1 基于体外嗅觉受体分子及细胞传感器的研究

基于光伏效应的光寻址电位传感器(light-addressable potentiometric sensor, LAPS)在生物传感技术上最早的应用是检测细胞的代谢率,细胞在代谢过程中会产生酸性物质,使得外环境酸化, LAPS可以检测细胞外微环境的pH变化,定量分析质子排出速率从而分析出细胞的代谢率.本实验室(图6)在LAPS的基础上,以嗅觉感觉神经元作为敏感元件构建了一种用于检测化学分子的仿生嗅觉传感器,将嗅觉感觉神经元、僧帽细胞和丛状细胞等有效地培养在半导体芯片上,使用LAPS作为敏感芯片检测神经元的细胞外电位,记录神经元在气味分子或者神经递质刺激下的响应.与此同时,以微电极阵列(microelectrode array, MEA)芯片为检测平台,研究细胞在芯片上可以特异性固定以及细胞网络的可控性的生长模式,MEA传感器是指在玻璃或硅基底上,用微电子加工技术将Au, Ir或Pt等金属沉积在上面形成电极和引线,采用

钝化层保护引线,在电极上暴露与细胞接触的区域,传输并记录细胞外动作电位频率、幅度、波形以及细胞网络间信号传播速度等参数的细胞传感器,通过对MEA芯片的电极位点进行特殊的化学表面处理,能够特异性地将细胞捕获在电极表面,通过细胞培养,神经细胞长出轴突和树突连接后形成细胞网络,在加工MEA芯片时可以通过设计特定的电极布局,从而实现控制细胞网络的生长模式.

本实验室在前期的仿生传感器研究中引入嗅觉受体神经元,天然或异源表达的嗅觉受体蛋白等材料作为敏感元件,制作出LAPS单细胞传感器^[47,48]和36通道MEAs(36-channel microelectrode arrays)^[49],并且在此基础上制作出64通道MEAs(64-channel microelectrode arrays)^[50],检测出了海马神经元对从0.1 $\mu\text{mol/L}$ 到100 $\mu\text{mol/L}$ 的多浓度的5-HT的电位变化,以确定传感器的响应特征,作为神经递质,5-HT能通过激活五羟色胺能受体来影响神经元电生理活动,而海马神经元中内源性表达了丰富的五羟色胺能受体,且海马区具有神经电兴奋能力,因此通过构建海马神经元网络的

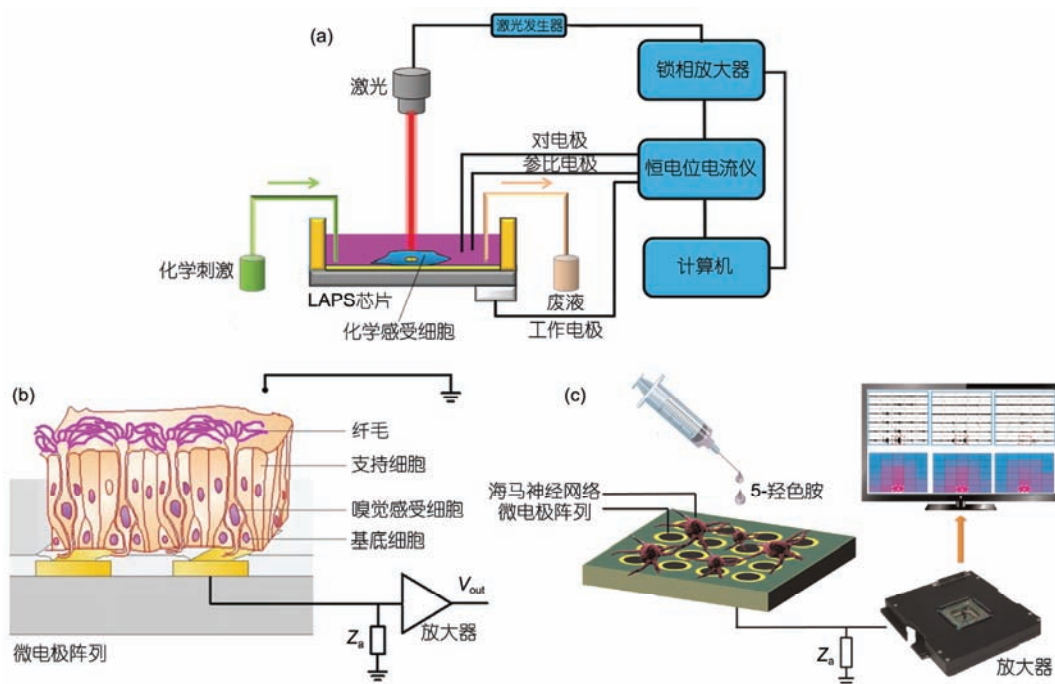


图6 (网络版彩色)基于嗅觉受体的分子及细胞传感器基本装置示意图。(a) 基于LAPS的仿生嗅觉传感器的原理和系统示意图;(b) MEA记录完整嗅黏膜上的嗅觉感觉神经元的胞外电位示意图;(c) 基于海马神经网络的64通道MEA传感器系统^[48-50]

Figure 6 (Color online) (a) Schematic diagram of a bioengineered cell-based sensor for label-free functional assays of chemical receptors based on localized extracellular acidification measurement; (b) recording extracellular potentials of olfactory receptor neurons in intact epithelium by microelectrodes; (c) schematic of multi-site. Recordings of hippocampal neuron networks with MEAs. Hippocampal neuron networks (HNNs) cultured on MEAs surface. Signals detected by microelectrodes were amplified by the amplifier and then displayed on computer screen^[48-50]

传感器,可以有效方便地观测5-HT对神经网络活动的抑制效应,并且增加了该传感器的特异性.

随着仿生嗅觉传感器的不断发展,纳米囊泡的概念出现在生物电子鼻的研究中,纳米囊泡可以传递一些信号,类似细胞的功能一样,通过和基底连接将信号传递到不同种类的二级换能器.二级换能器通常有场效应晶体管(field-effect transistor, FET)、石英晶体微天平(quartz-crystal microbalance, QCM)、表面等离子共振敏感元件(surface plasmon resonance sensors, SPRs)、电化学阻抗传感元件(electrochemical impedance spectroscopy, EIS)、碳纳米管(carbon nanotubes)以及石墨烯(graphene)等等很多种类,纳米囊泡的组合比较简单,主要是嗅觉受体的多肽链在去离子液体里通过自组装被固定在单壁式碳纳米管上,气味分子结合到特定的嗅觉受体蛋白上产生化学反应信号经过纳米囊泡传递到二级换能器,同时,纳米囊泡展现出很多蛋白质复合材质的优点,可以大批量生产以及易于储藏^[51,52].随着生物电子鼻的灵敏性和特异性逐渐提高,可以被应用于很多的领域^[53]:某些疾病的早期诊断;通过发酵检测、细菌水平、主成分以及添加剂成分分析和气味释放进行食物的质量安全监控;酒、咖啡、香料以及化妆品的鉴定;环境的分析与监测;爆炸品和毒品的探测以及气味的成分分析等(图7^[54]).

3.2 基于在体生物仿生电子鼻的研究

在神经检测技术日新月异的今天,电生理方法

在嗅觉的产生机制、传导通路、编码解码等问题的研究上发挥了重要的作用,因此,本实验室近期在前期研究的基础上建立了研究嗅觉神经电生理的实验平台,将多通道微丝电极阵列植入麻醉大鼠嗅球及皮层区域,分别检测记录麻醉以及清醒状态下用不同气味在不同浓度刺激下的神经元活动,总结分析嗅觉神经元对化学刺激的响应规律^[55-57].最重要的是,我们提出了基于植入式电极的在体的生物电子鼻系统的概念,利用大鼠灵敏的嗅觉作为气味传感单元,对其嗅球神经信号进行解码,初步实现气味分类的识别.与此同时,在前期研究的基础之上,我们还新研制出基于Wi-Fi技术的动物嗅觉机器人,包括一种穿戴式的8通道无线神经信号记录系统,利用Wi-Fi技术传输大鼠的嗅觉神经信号,成功用于复杂环境中气味检测和识别的神经记录系统有着非常大的应用价值^[58],相信在未来的缉毒、搜爆、反恐等社会防范方面能够发挥重要的作用(图8).

离子分子细胞传感器将生物嗅觉材料替代传统的物理传感器,使得气味检测系统在特异性、灵敏度和响应时间上有所突破,但这种传感器面临的最大挑战是其较短的使用寿命.因此,如何利用生物嗅觉系统检测气体,并提高使用寿命?随着在体神经信号记录技术的发展,长时的在体记录成为可能. Strauch等人^[59]利用在体钙成像技术,记录果蝇触角嗅觉神经元对癌细胞和非癌细胞产生的挥发性物质的响应,通过多元分析方法,显示多个神经元阵列对正常细胞和不同乳腺癌细胞的响应不同.这项结果

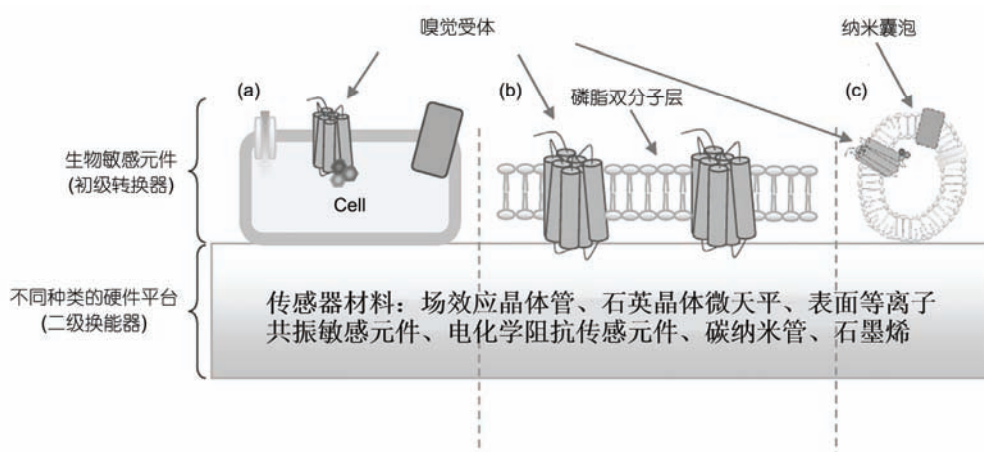


图7 生物电子鼻传感器设计示意图. 3种不同种类的与基底结合的生物敏感材料. (a) 细胞; (b) 多肽; (c) 纳米囊泡^[54]

Figure 7 Schematic presentation of sensors design of bioelectronic nose. Three basic types of bio-noses differ in the elements, which are deposited on suitable substrate. (a) Cells; (b) peptides; (c) nanovesicles^[54]

证实了利用生物敏感材料, 结合人工嗅觉和生物嗅觉技术在临床诊断的可行性. 类似地, Nowotny 等人^[60]利用玻璃电极在体记录果蝇嗅觉神经元, 结合标准线性支持向量机分类算法, 实现气味响应分类, 结果显示这种方法可以很好区分36种不同的酒类气体以及35种工业气体. 将气味受体应用于人工嗅觉系统, 结合长时在体技术, 实现气味检测, 相比于离子嗅觉传感器具有更强的可行性.

实际上, 本实验室已早于上述研究组发表在体生物电子鼻相关工作. 2012年, 提出在体植入大鼠嗅球的气味传感器用于识别和确定肺癌气体标志的方法, 利用哺乳动物嗅觉系统搭建了用于气味检测的生物电子鼻平台, 采用多通道植入式电极记录了嗅球神经元电生理信号并进行分析, 结果显示大鼠嗅球神经元对不同气味刺激的电生理响应信号具有一

定的敏感性和特异性, 并初步实现了气味识别. 之后, 希望通过清醒大鼠嗅球的气味响应记录, 并选用肺癌患者呼出气体作为刺激气味, 分析大鼠在肺癌呼出气体刺激前后的嗅球神经元响应信号建立有效的肺癌标志物挥发性有机化合物 VOCs(volatile organic compounds)检测模型: 当大鼠检测呼出气体时, 根据VOCs检测模型来判断被测者呼出气体中是否含有肺癌标志性VOCs, 从而实现临床肺癌检测目的. 相比于用训练的动物嗅觉进行癌症检测的方法, 节省了训练动物需要的高成本, 并且VOCs检测模型适用于同种动物的不同个体, 为此大大节约了时间成本, 尤其是对于不易训练的动物. 然而建立VOCs检测模型需要大量的数据和有效的建模算法, 要真正实现基于哺乳动物嗅觉的肺癌呼吸检测生物电子鼻仍面临很大挑战.

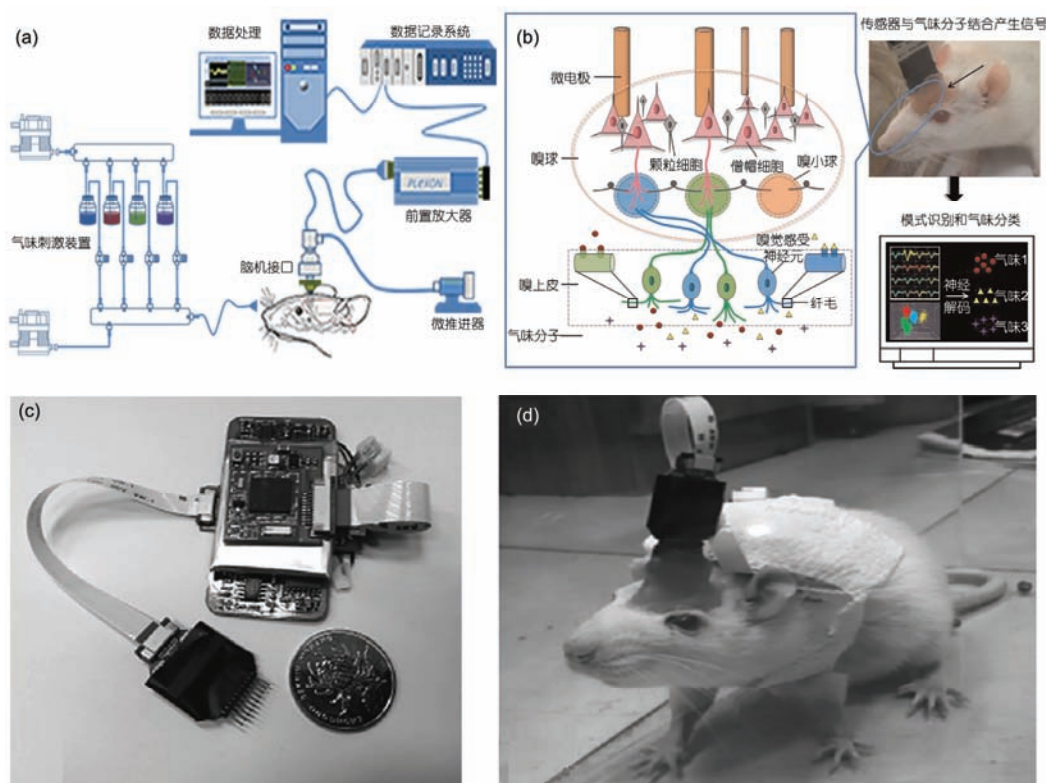


图 8 (网络版彩色)基于植入电极生物电子鼻结构示意图. (a) 由多通道嗅觉计、SD大鼠、微电极以及信号采集系统构成, 微电极阵列直接采集大鼠嗅球输出的整合后的信号, 及僧帽细胞的胞外动作电位. (b) 微电极记录装置及信号采集示意图. 通过大鼠脑图谱精确计算僧帽细胞层具体位置并利用定位仪进行电极植入. (c) 基于Wi-Fi技术的系统硬件实物图. (d) 嗅觉神经记录过程中的大鼠^[55,56,58]

Figure 8 (Color online) Schematic of the novel bioelectronic nose. (a) In rats' olfactory system, odors are presented to a distributed array of semiselective receptors in the cilia of olfactory sensory neurons in olfactory epithelium. The mitral/tufted cells in OB receive synaptic input from OSNs and generate odor-specific action potential patterns; (b) the neural activity is recorded by microelectrode and then fed into a computer-based pattern recognition algorithm for identification of the input odors; (c) schematic of hardware which based on the technical of Wi-Fi; (d) the rats in the process of olfactory nerve recording^[55,56,58]

4 光基因技术在嗅觉仿生研究中的应用

仿生嗅觉传感器和植入式电极在体仿生电子鼻系统都具有高灵敏性和特异性的特点。目前嗅觉仿生研究进展迅速,在各方面的应用也都取得了飞速的进展,但是,也出现了一定的问题,光遗传技术的出现在一定程度上可以更有利于嗅觉仿生的发展。前期研制的36通道MEAs可以有效地利用嗅感觉神经元实现对气体的检测和分类,64通道MEAs通过检测神经元电位变化来推测神经细胞内5-HT的浓度变化,为神经退行性疾病的研究提供了先进的技术手段。如果结合光基因技术(图9(a)),用光刺激代替气味刺激

结合我们的MEAs,构建体外病理模型并且进行体外光敏感蛋白的病毒转染,在神经退行性疾病尤其是在阿兹海默症(alzheimer disease, AD)的探究方面会更深一个层次。另外,在前面提到光基因小鼠证明嗅觉系统可以对特定气味感知形成特定的记忆,就可以利用特定频率刺激产生的记忆来训练小鼠,并且利用一些行为学奖励方式进行多次训练,然后利用植入式电极去实现更高灵敏度和特异性的气体检测(图9(b))。同时由于转基因小鼠都携带不同的荧光蛋白,有利于植入式电极的准确定位,同时气味的检测的特异性也会有所提高。

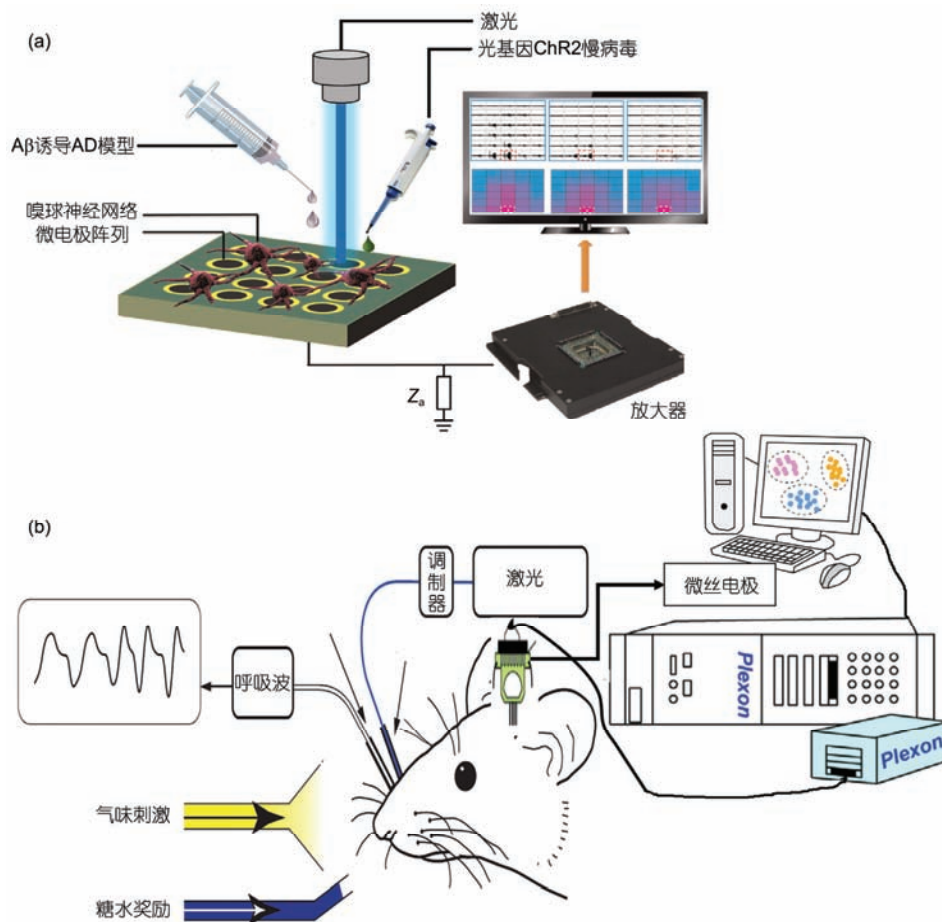


图9 (网络版彩色)光基因技术结合仿生嗅觉传感系统的体外(a)以及在体(b)的研究展望示意图

Figure 9 (Color online) Schematic of the prospect of research on optogenetics technology combined with the bionic olfactory sensor system *in vitro* (a) and *in vivo* (b)

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Summary for “结合光遗传神经调控技术的仿生嗅觉传感系统的研究进展”

Advances in research on combining the optogenetics with bionic olfaction sensor

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There are plenty of amazing wonders existing in nature, and the crisis-crossing connections between them are performing their best duties. For example, biological olfactory system is an ingenious biosensor, since the olfactory sensory neurons in peripheral nervous respond to the external stimuli, then the signals are transmitted to the olfactory bulb to be integrated and processed, the axonal connection is precisely organized signals from about 1000 different types of odorant receptors, then sorted out in 1800 glomeruli in the mouse olfactory bulb, after delivered to the most advanced central nervous system, the information can be distinguished eventually. Cortical regions underlying vision, audition, and somatosensation receive sensory information from the thalamus and also make corticothalamic feedback projections that influence thalamic sensory processing. This process is responding with particularly high sensitivity, specificity and rapidity. The ability to detect odorants is crucial for survival as it informs an organism with its environment: food, approaching predators, mates nearby, etc. During recently year, researchers have employed optogenetics technology to explore deeper in the mechanism of olfactory system. Microbial proteins that can be rapidly activated by light have been adapted for research in neuroscience, including ChR2 and NpHR, which permit millisecond-precision optical control of genetically defined cell types in intact neural tissue. This technology allows the use of light to alter neural processing at the level of single spikes and synaptic events, yielding a widely applicable tool for neuroscientists and biomedical engineers. An important question in the study of the olfactory system is the role of spatial factors in olfactory processing, but traditional methods have been relatively unsuccessful in elucidating their theories, odor stimulation evokes complex spatiotemporal activity in the olfactory bulb, suggesting that both the identity of activated neurons and the timing of their activity convey information about odors, the emergency of optogenetics technology can solve this problem efficiently. Biological olfactory systems can recognize and discriminate thousands of distinct odors with very high sensitivity and specificity, with the progress of research on olfactory mechanisms and the advancements of olfaction, many types of biomimetic olfactory-based biosensors have been developed by the combination of olfactory functional components with various secondary sensors. The bioinspired olfaction sensor is an innovative chemical sensing bionic system with far-reaching significance, it contains various biological components as the sensitive elements including olfactory receptors, olfactory cells and olfactory tissues, which has great applicable prospects in many fields for detecting specific odorants with high sensitivity, also by chronically coupling multiple microelectrodes to olfactory bulb of behaving rats, we extract an array of mitral/tufted cells which could generate odor-specific temporal patterns of neural discharge. They have great potential commercial prospects and promising applications in such fields as biomedicine, environmental monitoring, pharmaceutical screening and the quality control of food and water. This review interpreted the transferring mechanism of biological olfactory system and the applications of optogenetics in researches concerning the fundamental research of olfactory system. In addition, a combination of the work of our laboratory with other domestic and international researchers were presented here to predict the future scenario and the promising applications in disease diagnosis, environmental monitoring, military and food quality control.

olfaction, optogenetics, bionic olfaction sensor, odor detection, bioelectronic nose

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