



鸣禽模型在语言学习中承担独特的生物学角色*

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摘要 鸣禽鸣唱与人类语言相似, 是一种复杂的发声学习行为, 并受脑中一组相互联系的神经核团调控。该组核团与人类发声控制相关脑区具有一定程度的结构同源性, 并可能共享某些发声学习调控机制。因此, 鸣禽成为研究发声学习神经机理的重要模式动物, 不仅对鸟类语言学习, 也可揭示人类语言学习的神经过程和语言障碍的治疗提供重要参考借鉴。本文基于本课题组长期坚持的研究方向, 较系统地概述了国内外鸣禽鸣唱行为研究的历史、重要发现和进展, 及其为相关中枢神经系统疾病治疗带来的启示。

关键词 鸣禽, 鸣唱行为, 语言学习, 鸣唱控制系统, 语言障碍
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斑胸草雀 (*Taeniopygia guttata*), 成年雄鸟善鸣, 可用作开展发育生物学、神经生物学、语言机理、医学等领域研究的模型动物。

以模式动物进行科学研究是生物学的一个传统。从线虫到果蝇, 从小鼠到鸣禽, 模式动物功不可没。将斑胸草雀 (zebra finch) 等鸣禽用作模型动物, 得到全球同行的认可, 并在发育生物学、神经生物学、语言机理、医学等领域取得重要进展。

鸣禽是鸟类中数量最为庞大的一支, 属于雀形目, 体态小巧玲珑, 鸣唱婉转动听 (附件 Movie S1, 附件 Audio S1)。鸣禽的发声出自鸣管, 位于气管与支气管交界处, 由一组鸣肌环绕, 在脑神经支配下收缩引起气道内气体震荡产生优美的旋律 (附件 Movie S1)。更重要的是, 鸣禽被认为是自然界中除人类外具有最为复杂发声行为的动物。鸣禽鸣唱与人类语言相似, 是一种发声学习行为^[1-2]。人类和鸣禽都是在生命早期的关键阶段学习发声, 也都需通过听觉反馈来纠正并完善自身发声^[3]。在进化上, 鸣禽某些鸣唱控制核团与人类语言发声脑区共享类似的转录谱, 且具有和人类相似的发声调控神经通路, 包括对发声学习至关重要的皮层-纹状体-丘脑-皮层环路和前脑发声运动控制

区域投射至脑干发声运动神经元的运动通路^[4]。此外, 鸣禽鸣唱可以通过声学软件进行量化分析, 更加准确地评估行为与神经活动之间的关系。因此, 鸣禽为发声学习行为研究提供了理想的动物模型^[5-6], 并对理解人类语言学习机制具有重要意义^[7]。

1 国内外鸟鸣研究历史回顾

鸣禽鸣唱研究已有近百年历史, 但将鸣禽作为模式动物只有几十年。在国内, 东北师范大学蓝书成教授是中国鸟鸣生理学的开拓者和奠基人, 率先开展鸟类 (家禽) 及鸣禽发声行为机制研究并形成

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“具有特色和独创性”的比较生理学研究领域^[8]。1958年首次发现刺激鸟类中脑可以引起鸣叫反应及植物性反应^[9]。1962年在清醒状态下刺激鸟类中脑可以引起鸣叫反应及躯体运动反应^[10]。1980年后,左明雪及其团队、李东风及其团队、张信文课题组分别在东北师范大学、北京师范大学、华南师范大学、海南师范大学接续传承,继续开展鸣禽鸣唱行为机制的研究。2016年,孟玮和王松华博士在江西科技师范大学组成团队继续坚持鸟鸣研究,并尝试探索鸣禽模型对语言障碍治疗的意义。

1948年,英国剑桥大学Willams Thorpe率先开始了鸟鸣研究,发现苍头燕雀可以通过学习获得鸣唱行为,开创性运用声谱仪记录了鸣禽的发声行为。随后他的学生Peter Marler^[11]在美国加利福尼亚大学伯克利分校以白冠雀为研究对象,发现幼年关键学习期对鸣禽鸣唱学习的重要性。此后,Marler的学生们发表了一系列关于鸣禽鸣唱行为及机制研究的论文。其中,1969年麻省理工学院Masakazu Konish^[12]阐述了听觉反馈对鸣禽发声学习及产生的重要意义。1976年洛克菲勒大学Fernando Nottebohm^[13]探明了金丝雀鸣唱神经通路,包括发声运动通路和前端脑通路,这为后续鸣禽鸣唱神经生物学研究奠定了解剖学基础。同年,Nottebohm和加州大学洛杉矶分校Arthur P Arnold^[14]发现鸣禽鸣唱控制系统存在性别二态性,开启了探索性激素对鸣禽鸣唱行为调控作用的研究方向。1985年,Nottebohm实验室^[15-17]发现成年鸣禽高级发声中枢(high vocal center, HVC)存在新生神经元,并替换旧神经元加入已有的发声通路,这是首次在哺乳动物外发现成年新生神经元。1998年至今,洛克菲勒大学Erich D Jarvis^[18]深入研究了鸣禽发声学习的分子和遗传机制,参照人脑结构对鸣禽脑结构重新命名^[19],将鸟类纹状体与人类皮层相对应,进而探究人类复杂语言行为的进化历程^[5, 20]。2000年,加州大学旧金山分校Michael S Brainard和Allison Doupe^[21]发现鸣禽基底神经节X区调控鸣唱可塑性的机制,从此启动大量关于鸣禽基底神经节对发声行为调控的研究。同年,芝加哥大学Daniel Margoliash实验室^[22]首次发现睡眠在鸣禽鸣唱感觉运动学习过程中的重要作用,为支持睡眠参与学习记忆的假设提供了重要例证。2001年,Doupe实验室^[23]在与鸣唱学习相关前端脑通路中的巢型皮质前部巨细胞核外侧部(lateral portion of the magnocellular nucleus of the

anterior neostriatum, LMAN)发现长时程增强(long term potential, LTP)现象对鸣唱学习是必须的,这是首次证实鸣唱控制系统内存在长时程突触可塑性。2012年,杜克大学Richard Mooney实验室^[24]首次将光遗传技术应用在鸣禽脑操纵鸣唱行为,为鸣禽鸣唱行为研究带来了新的视角。2021年,Brainard与Todd F Roberts等^[25]合作,运用单细胞RNA测序技术检测鸣禽鸣唱运动通路的神经元特性,首次提供了鸣禽鸣唱核团神经元的RNA表达谱,并揭示鸣禽鸣唱通路和哺乳动物发声通路具有不同的发育起源但具有相似的转录神经元。

2 鸣禽模型在发声行为学与神经生物学跨学科研究中的应用

2.1 鸣曲可塑性机制

鸣禽为揭示动物行为和神经可塑性的关系提供了一个有趣的研究系统^[26]。在鸣禽鸣唱的习得过程中,鸣曲从高度变化的可塑期逐渐进入稳定的固化期。即使在成年鸣曲完全固化之后,鸣曲也具有一定的可塑性。近些年研究发现,鸣曲可塑性的产生机制主要受控于基底神经节及相关联区域。Woolley等^[27]首次发现,X区的苍白球神经元电活动编码鸣禽鸣唱类型。Mooney实验室^[28]利用光遗传技术研究发现,抑制X区棘神经元的活动可调控鸣禽鸣唱学习过程中的鸣曲可塑性。另外还发现,激活腹侧被盖区(ventral tegmental area, VTA)-X区通路足以引起幼鸟鸣唱学习^[29-30],其中VTA是神经递质多巴胺(dopamine, DA)的重要来源之一,提示VTA投射至X区的多巴胺能神经纤维在对鸣唱学习行为的调控中起重要作用。同时有研究表明,X区的下游核团LMAN电活动调控鸣曲音节基频的波动^[31]。此外,研究证实投射到X区的HVC神经元(HVC_X)活动对幼鸟鸣唱学习行为是必需的,但与成年期鸣曲可塑性无关^[32]。且近年有研究表明,鸣禽连接小脑和基底神经节的皮层下回路,参与了鸣唱学习过程^[33],提示小脑在调控鸣唱学习中的作用。

2.2 神经递质及性激素等生物活性物质对鸣禽鸣唱行为的调控

DA系统是脑内重要的神经递质系统之一,在神经调节中起关键作用,因此一直是国内外研究的热点。在哺乳动物中,DA参与运动、认知、动机、奖赏、成瘾、学习等活动,并与人类的帕金森病、精神分裂症和亨廷顿病等密切相关^[1, 34]。研

究表明, DA可通过调控鸣唱相关核团及听觉区域, 促进鸣禽幼年期鸣曲学习以及成年期鸣曲保持^[1]。近年发现, DA可通过作用于X区参与调控鸣禽幼年期鸣曲学习, 以及成年鸣禽的鸣唱维持和求偶性鸣唱行为^[30, 35-36]。对X区的上游核团HVC研究发现, 中脑投射至HVC的DA能神经纤维有助于教习曲的习得和正常鸣唱行为的形成^[37]。同时, DA还作用于听觉皮层调控鸣禽听觉区域可塑性^[38-39]。本课题组近年对鸣唱前运动核团弓状皮质核(robust nucleus of the arcopallium, RA)的研究发现, DA可经D1样DA受体(D1-like dopamine receptor, D1R)提高成年鸣禽RA投射神经元的兴奋性^[40], 同时DA通过突触前机制经D1R降低RA投射神经元接受的兴奋性突触传递^[41]。由于RA投射神经元活动变化决定了鸣曲特征的变化^[42], 基于该结果有理由推测, 生理条件下, DA可通过改变RA投射神经元兴奋性影响其活动, 进而调控鸣唱行为。

另一种儿茶酚胺类神经递质去甲肾上腺素(norepinephrine, NE)在调控鸣禽鸣唱习得的过程中也发挥重要作用。研究表明, 幼年期减少NE合成可抑制鸣禽鸣曲学习^[43], 其中可能的原因是NE通过调控听觉皮层, 提升幼鸟习得鸣曲与教习曲的相似性^[44]。在成年期, NE可增强复杂听觉刺激的信号检测^[45]和听觉前脑的编码功能^[46], 提升鸣曲稳定性。

鸣唱运动核团HVC和RA还接受中枢胆碱能系统的投射和调控^[47]。研究证实, 非选择性乙酰胆碱受体激动剂卡巴可(carbachol)渗透到HVC中, 可以提高鸣曲的音调、振幅、节奏和稳定性, 并增加HVC神经元的活动^[48]。本课题组近年研究了胆碱能信号对成年鸣禽RA投射神经元兴奋性和内在膜特性的影响。结果表明, 胆碱能信号可通过引起膜电位超极化, 增大后超极化电位和膜电导, 降低RA投射神经元兴奋性, 减少其活动。进一步研究证实, 胆碱能信号主要通过毒蕈碱型乙酰胆碱受体影响RA投射神经元的兴奋性和内在膜特性, 而尼古丁型乙酰胆碱受体不参与该作用^[49-50]。胆碱能信号调控鸣禽鸣唱行为的机制仍有待揭示。

除神经递质外, 鸣禽脑内的性激素在调节鸣唱稳定性方面发挥关键作用。其中雄激素已被证实可以通过调控细胞增殖^[51]、神经元电生理特性^[52]及突触传递^[53]来影响鸣唱控制核团, 进而导致鸣唱

行为改变^[54-55]。而鸣禽脑内的雌激素主要是雌二醇, 由脑内睾酮通过芳香化酶的作用转化而来。现有研究表明, 雌激素对鸣禽听觉编码^[56]、脑损伤修复^[57]、空间记忆^[58]及鸣唱行为等方面^[59]具有重要影响^[60]。

研究提示, 脑源性神经营养因子(brain-derived neurotrophic factor, BDNF)在鸣禽鸣唱运动通路HVC-RA对鸣唱行为的调控中起重要作用。Dittrich等^[61]研究发现, 短暂提升斑胸草雀雄性幼鸟HVC中BDNF表达, 可显著提高感觉运动期鸣曲与教习曲的匹配度, 促进鸣曲习得。而Kittelberger等^[62]研究发现, 在鸣曲结构已固化的成年雄性斑胸草雀RA内注入BDNF, 可使已高度稳定的鸣曲序列发生可逆性改变, 并伴随HVC-RA突触数量显著增加。近期, Miller等^[63]研究表明, 在成年雄性季节性繁殖鸣禽白冠雀RA附近注射BDNF, 可使RA神经元自发动作电位发放频率增加, 并提示BDNF该作用经HVC-RA跨突触起效。而最近本课题组在成年雄性斑胸草雀离体脑片上发现, BDNF可提升RA投射神经元的兴奋性, 与此同时, BDNF还可增强RA投射神经元接受的兴奋性突触传递(未发表数据)。

叉头框P(fork head box P transcription factor, FoxP)基因是与人类语言及言语障碍相关的基因, 亦称语言基因。在鸣禽脑中, FoxP蛋白大量表达。研究证实, FoxP蛋白对幼年鸣禽神经环路发育和鸣唱学习, 以及成年鸣禽的鸣曲稳定都有重要作用^[64]。幼年鸣禽X区缺失FoxP1、FoxP2或FoxP4基因, 将导致鸣唱学习能力受损, 最终鸣曲不能发育完善^[65-66]。最新研究发现, 若敲除HVC的FoxP1基因, 可阻止幼鸟鸣曲的习得^[64], 提示FoxP蛋白在鸣唱学习过程中起关键作用。

2.3 听觉反馈与鸣禽鸣唱行为

鸣禽需要通过听觉反馈来学习和维持鸣唱^[67]。鸣禽脑中的听觉系统主要包括与哺乳动物听皮质同源的L区(field L complex)、巢状皮质尾内侧区(caudomedial nidopallium, NCM)和旧皮质尾部(caudal mesopallium, CM)等结构^[67]。鸣唱系统和听觉系统之间的联系相对有限, 大多数听觉核团投射至HVC周围的HVC壳(HVC shelf)和RA周围的杯区(RA cup), 只有界面核(nucleus interfascialis nidopallii, Nif)能把来自丘脑葡萄形核(uvaeformis, Uva)的听觉和感觉信息直接上

传至HVC^[68]。在鸣禽听觉系统中研究比较多的是NCM区域, 其与雄性鸣唱学习有关^[69]。研究提示, 早期的听觉经验可形成NCM神经元回路, 通过招募 γ -氨基丁酸抑制回路来形成对教习曲的记忆^[70]。而在雌鸟中, NCM主要作用是对雄鸟鸣曲的识别^[71], 短暂致NCM失活可导致雌鸟对雄鸟鸣曲偏好的特异性降低^[71]。

致聋可破坏听觉反馈, 导致鸣曲快速退化。研究表明, 致聋后HVC_x神经元树突棘体积下降^[67, 72], 并导致HVC_x神经元的兴奋性和抑制性突触传递下降^[72-73]。孙颖郁等^[6, 74]近年研究发现, 致聋可导致斑胸草雀鸣唱控制核团X区和RA内树突棘数目增多, 提示X区和RA的神经可塑性与致聋引起鸣唱行为的改变相关。

2.4 光遗传技术和基因组学在鸣禽鸣唱行为研究中的应用

Mooney实验室^[24]首次将光遗传技术应用在鸣禽鸣唱行为研究中, 发现通过光遗传抑制幼年期HVC的神经活动会导致幼鸟鸣唱学习能力下降。更有趣的是, Roberts团队^[75]利用光遗传技术通过控制斑胸草雀与听觉经验学习有关的两个区域, 成功对其大脑植入了一段记忆编码, 让受试斑胸草雀产生了一段之前没有学习过的鸣曲。另外, Ausra等^[76]最近开发了一种无线无电池的轻携光遗传平台, 可以实现记录自由活动状态下的鸣禽鸣唱行为, 为鸣禽鸣唱行为学研究提供了更有利的工具。

近几十年来, 基因组学取得了长足的发展, 广泛应用于生命科学各个领域。近年, Mello团队^[77]应用微阵列及原位杂交技术对斑胸草雀鸣唱控制系统中的380个基因进行了测序, 为解析鸣唱控制通路功能特性的可能分子决定因素提供了新的见解。同时进一步利用比较基因组学技术检测了鸣唱控制系统内钠、钾、钙和氯离子通道及其调节亚基, 发现这些基因家族在鸟类和哺乳动物谱系之间高度保守, 为深入探索离子通道如何促进鸣唱控制系统的神经元兴奋性等特性奠定了基础^[78-79]。近年发展起来的单细胞基因组学是理解神经元多样性的最有力工具之一。Colquitt等^[25]利用单细胞RNA测序技术首次发现, 鸣禽鸣唱前运动核团HVC和RA与功能上具有相似性的哺乳动物新皮层进化起源并非一致。而Ivana等^[80]首次利用鸣禽原始生殖细胞, 通过慢病毒载体转导成功获得转基因斑胸草雀。这些研究进展将为后续深入研究鸣禽的功能基因组学奠定了坚实的基础。

3 鸣禽模型对中枢神经系统疾病及人类语言障碍治疗的启示

成年鸣禽鸣唱控制核团HVC和X区中加入的新生神经元可与原有鸣唱控制通路整合并行使功能, 促进新的鸣曲记忆形成^[81-82]。研究表明, 鸣唱控制核团损伤导致鸣曲受损后, HVC新生神经元可加速鸣曲恢复^[83]。且新生神经元可以重建成年鸣禽端脑退化后的功能性神经回路, 并显示非损伤诱导的退化和神经回路的重建对产生鸣唱习得行为至关重要^[84]。这些重要发现预示着成年神经发生对中枢神经系统损伤及神经退行性疾病的潜在治疗前景。

可导致语言障碍的疾病主要有自闭症、亨廷顿病、中风失语症、运动性失语症、感觉性失语症等。由于研究人脑的局限性及医学伦理的限制, 无法深入探索各类语言障碍的发生机制, 难以对症治疗。研究表明, 鸣禽高级发声中枢HVC与人类运动性语言中枢布洛卡区(Broca's area)同源, 鸣唱前运动核团RA与人类运动皮层第五层同源, 基底神经节X区与人类纹状体同源, 听觉系统中的NCM区则与人类听觉皮层同源^[85]。同时鉴于鸣禽鸣唱行为与人类语言功能的高度相似性, 鸣禽可为探索人类语言障碍的发生机制及治疗手段提供有价值的研究模型。以自闭症为例, 其主要表现为社会交往障碍、语言交流障碍、重复刻板的行为等。有研究显示, 鸣禽鸣唱控制系统高度表达与自闭症相关的接触蛋白相关蛋白2(contactin associated protein like protein 2, Cntnap2)^[86]、哺乳动物雷帕霉素靶蛋白(mechanistic target of rapamycin, mTOR)^[87]、活动依赖神经保护蛋白(activity-dependent neuroprotective protein, ADNP)^[88-89]及FoxP蛋白^[64]等。这些蛋白质失活将导致鸣禽幼年期鸣曲学习障碍及成年期发声障碍, 与人类自闭症语言障碍高度相似。除自闭症外, Liu等^[90-91]开创性利用转基因技术将亨廷顿病的致病基因转入斑胸草雀胚胎中, 孵化后可产生严重的鸣曲学习及发声障碍, 包括发声模仿不良、口吃、渐进的鸣曲句法和音节退化^[91]。而Mooney团队^[92]直接将亨廷顿病的致病基因转入斑胸草雀X区, 可使其鸣唱音节序列不稳定。上述鸣唱行为变化与人类亨廷顿病语言障碍具有一定程度的相似性。这些有益的探索将为揭示人类语言障碍的发生机制并找到对症的治疗手段带来借鉴。

4 展 望

作为与人类语言功能相似的发声学习研究理想动物模型，鸣禽鸣唱行为学从提出到与神经生物学跨学科深入研究已有数十年。现在对鸣禽鸣唱控制系统的解剖结构及其对鸣唱行为的调控作用有了较为全面的了解^[93]（图1）。光遗传学、单细胞测序、

CRISPR/Cas9 基因编辑^[94]等前沿技术已将鸣禽鸣唱行为研究领域推向更深层次水平。另外，神经递质等对鸣禽鸣唱控制核团神经网络调控及对鸣唱行为的影响仍有待深入研究。同时新型荧光探针的运用将有助于进一步揭示鸣禽鸣唱过程中神经递质的调控作用。鸣禽鸣唱行为的神经生物学研究定将为人类语言障碍相关疾病发生机制和治疗手段的探索带来重要启示和理论支持。

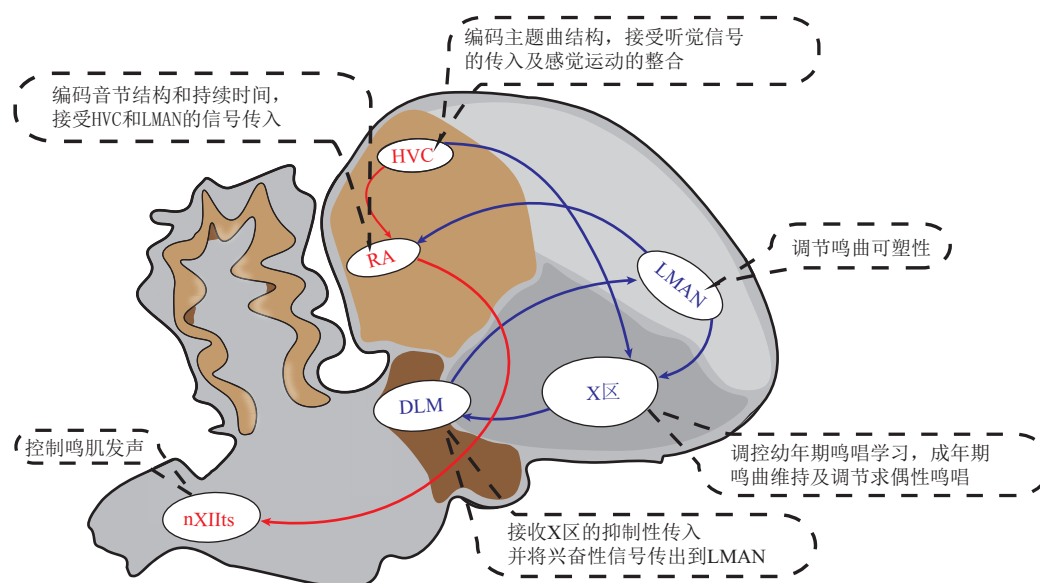


Fig. 1 The song control system of songbirds

图1 鸣禽鸣唱控制系统

红色代表控制鸣唱产生的发声运动通路（vocal motor pathway, VMP）：HVC→RA→nXIIIts；蓝色代表主导鸣唱学习和维持过程的前端脑通路（anterior forebrain pathway, AFP）：HVC→X区→DLM→LMAN→RA。虚线框表示目前对鸣唱控制系统内各核团功能的认识。HVC：高级发声中枢（high vocal center）；RA：弓状皮质核（robust nucleus of the arcopallium）；X区：Area X，鸟类基底神经节的一部分；DLM：丘脑背外侧核内侧部（medial portion of the dorsolateral nucleus of the anterior thalamus）；LMAN：巢型皮质前部巨细胞核外侧部（lateral magnocellular nucleus of anterior nidopallium）；nXIIIts：舌下神经气管鸣管亚核（tracheosyringeal part of hypoglossal nucleus）。

附件 PIBB_20220135_Movie_S1. mp4 和 PIBB_20220135_Audio_S1. wav 见本文网络版 (<http://www.pibb.ac.cn>或<http://www.cnki.net>)。

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Songbird Model Plays a Unique Biological Role in Language Learning*

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Abstract Songbirds' singing is similar to human language, which is a complex vocal learning behavior and is regulated by a group of interconnected nerve nuclei in the brain. These song control nuclei have a certain degree of structural homology with the brain regions related to human vocal control, and may share some basic regulation mechanisms of vocal learning. Therefore, songbirds have become an important model animal to study the neural mechanism of vocal learning, which can not only shed light on avian language learning, but also provide an important reference for understanding the neural process of human language learning and the treatment of language disorders. Willams Thorpe from University of Cambridge took the lead in bird singing research in 1948. Subsequently, many researchers in the United States used a variety of experimental techniques to carry out the study of birdsong neurobiology. In the 1960s, Chinese scholar Professor LAN Shu-Cheng first carried out the research on bird singing neurobiology in the Northeast Normal University, and then the researchers in Beijing Normal University, South China Normal University, Hainan Normal University and Jiangxi Science and Technology Normal University carried out the researches on the neural mechanism of bird singing using molecular biology and electrophysiological technology. In recent years, the application of songbird model in interdisciplinary research of vocal behavior and neurobiology mainly focuses on the mechanism of vocal plasticity; the neurotransmitters, sex hormones and other bioactive substances regulated the song behavior; the effect of auditory feedback on song behavior and application of photogenetic technology and genomics in this field. In the future, frontier technologies such as single cell sequencing and CRISPR/Cas9 gene editing have pushed these research fields to a deeper level. In addition, the effects of neurotransmitters on the neural network regulation of songbirds' song control nuclei and song behavior need to be further studied. At the same time, the application of some new fluorescent probes will help to further reveal the regulatory role of neurotransmitters in the singing process of songbirds. The neurobiological study of songbirds' song behavior will bring important enlightenment and theoretical support to the exploration of the pathogenesis and treatment of human language disorder related diseases. In conclusion, this paper systematically summarizes the research history, important findings and research progress of songbird song behavior, as well as the important enlightenment for the treatment of related central nervous system diseases.

Key words songbird, song behavior, language learning, song control system, language disorder

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