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变间隔的脉冲改变高频刺激对于脑神经元的作用*

胡汉汉　封洲燕**　王兆祥　郑吕漂　黄　璐
（浙江大学生物医学工程与仪器科学学院，生物医学工程教育部重点实验室，杭州 310027）

摘要　通常采用恒定电脉冲间隔的高频刺激（high-frequency stimulation，HFS），进行深部脑刺激治疗帕金森氏症等运动障碍疾病．为了开发适用于不同脑疾病治疗的新刺激模式，近年来脉冲间隔（inter-pulse-interval，IPI）变化的变频刺激模式受到关注．已有研究表明，即使具有相同的平均电脉冲频率，变频刺激与恒频刺激的治疗效果也不同．我们推测，变频刺激的短小IPI变化就足以改变HFS对于神经元的作用．为了验证此推测，本文在大鼠海马CA1区锥体神经元的输入轴突纤维上交替施加恒频刺激（100或133 Hz，即IPI = 10 ms或7.5 ms）和随机变频刺激（100~200 Hz，即IPI = 5~10 ms，平均频率为133 Hz），记录并分析刺激下游神经元群体的诱发电位，用于定量评价神经元对于恒频和变频刺激的响应．实验结果表明，持续的恒频刺激使得神经元的响应从最初的同步发放形成的群峰电位（population spike，PS）转变为非同步的动作电位发放（即单元锋电位）．但是，当刺激切换为变频模式时，却又可以诱发神经元群体同步产生动作电位，重新形成PS波．并且，变频刺激诱发的PS幅值和神经元发放的同步程度可达基线的单脉冲刺激诱发波的水平．但是，PS的发生率只有脉冲刺激频率的7%左右，表明在持续的变频刺激时，多个脉冲累积的作用才能诱发这种同步的神经元发放．而且PS的出现与前导IPI的长度之间存在一定关系．神经元的轴突和突触等结构对于高频刺激的非线性响应可能是变频刺激诱发同步活动的原因．这些结果表明，变频刺激序列中短小的间隔变化可以产生与恒定间隔不同的调控作用．本文的结果对于揭示脑刺激的作用机制，促进新型刺激模式的开发及其在不同类型脑疾病治疗中的应用具有重要意义．

关键词　高频刺激，脉冲间隔，随机频率，群峰电位，同步发放
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20世纪80年代法国Benabid教授等[1]通过脑内植入的电极施加高频电脉冲刺激，成功地控制了帕金森病患者的震颤．此后40多年里，这种深部脑刺激（deep brain stimulation，DBS）技术不断发展，在临床上已能够安全有效地治疗帕金森病、肌张力障碍和原发性震颤等运动障碍疾病[2]．而且，在治疗癫痫、抑郁症、强迫症等其他神经和精神疾病上，DBS也展现出良好的应用前景[3-4]．

DBS所产生的作用与许多因素相关，包括电刺激波形和频率等参数[5]．目前临床上采用的刺激波形是电荷平衡的双相窄脉冲，不同的刺激频率可以产生不同的作用效果．脉冲频率在90~185 Hz范围内的刺激可以抑制帕金森氏症的震颤[6-7]，130 Hz左右的脉冲刺激也可以控制癫痫发作[8-9]．临床上通常采用的脉冲频率均大于90 Hz，因此称为高频刺激（high-frequency stimulation， HFS）．

常规DBS采用的HFS脉冲频率恒定（即脉冲间隔恒定）．为了开发新的DBS模式，提高DBS疗效，近年来研究人员在动物实验、计算机仿真和临床试验中尝试了脉冲间隔（inter-pulse-interval，IPI）变化的变频刺激[10-12]．这些研究结果表明，即便平均频率相同，变频刺激也会产生与恒频刺激不同的效果[10, 13]．这些研究中所采用的刺激序列包含较长的IPI（如50 ms），因此有人认为这种长IPI可能削弱了HFS抑制神经元原有病理性活动的能力[10, 12]．但是，也有研究表明，即使恒频刺激

* 国家自然科学基金资助项目（30970753）．
** 通讯联系人．
Tel：13515711296，E-mail：fengzhouyan@139.com
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期间偶尔插入少量的延长IPI，也会产生不同于恒频刺激的作用[12, 14]．而且，即使延长的IPI只有一二十毫秒（15~25 ms），变频HFS的作用也不同于恒频刺激[12]．这些少而短的IPI间隔似乎难以允许神经元原有的病理性活动“复活”．因此我们推测，变频刺激不是削弱了对于神经元原有活动的抑制，而是可能诱发出另一种神经元活动．而且，这种活动的诱发并不一定需要IPI的绝对延长，而是只要存在IPI的相对变化即可．即使变频刺激所包含的IPI长度都不超过恒频刺激的IPI，短小的IPI变化也可以改变HFS对于神经元的作用．

为了验证此推测，本文利用大鼠在体实验比较了神经元群体对于恒频刺激（100或133 Hz，相应的IPI为10或7.5 ms）和随机变频刺激（100~200 Hz，IPI = 5~10 ms，平均频率为133 Hz）的响应．将刺激脉冲施加于大鼠海马CA1区的输入轴突纤维上，记录并分析刺激下游的神经元群体诱发电位，用于定量评价神经元对于变频和恒频刺激的响应．海马脑区致密且层次分明的结构有利于评价神经元群体的同步性活动．本文的研究结果对于揭示DBS的作用机制、开发新的DBS刺激模式用于治疗更多的神经系统疾病具有指导意义．

1　材料与方法

1.1　动物手术和电极植入

成年雄性Sprague-Dawley大鼠10只（体重（320 ± 44） g），购自浙江省医学科学院实验动物中心．腹腔注射乌拉坦（Urethane，1.25 g/kg）麻醉后，固定于大鼠脑立体定位仪上．电极植入手术和定位详见已有的报道[15]．简言之，开颅后，将一根16通道阵列记录电极（#Poly2，NeuroNexus Technologies Inc.，USA）植入至海马CA1区，再将一根同芯双极刺激电极（#CBCSG75，FHC Inc.，USA）插入到记录位点上游的CA1区传入轴突纤维Schaffer侧支上，施加的刺激顺向激活下游记录区域的神经元，最后根据单脉冲刺激诱发的电位波形，微调电极位置，使两根电极定位准确[16]．

1.2　刺激脉冲序列的产生与神经电信号的采集

高频刺激（HFS）的脉冲序列由自行设计的LabVIEW程序触发刺激器（Model 3800，A-M Systems Inc.，USA）产生．采用脉宽为0.1 ms、强度为0.3 mA的双相对称电流型脉冲．该强度的单脉冲刺激诱发的群峰电位幅值达到最大值的75%左右．脉冲频率分为两种：100/133 Hz的恒频和100~200 Hz范围内的变频．变频脉冲的间隔（IPI）随机且均匀地分布于5~10 ms范围内，其平均频率为133 Hz．每个刺激脉冲序列的时长共3 min，包括起始50 s恒频，紧跟10 s变频，此后再重复此60 s序列2次．

记录电极采集的细胞外神经电位首先用放大器（Model 3600，A-M Systems Inc.，USA）放大100倍，频带范围设为0.3~5 000 Hz，再用PowerLab数据采集系统（Model PL3516，AD Instruments Inc.，Australia）以20 kHz的速率采样并保存，用于离线分析．

1.3　群峰电位检测与分析

在16通道记录信号中，选取位于海马CA1区胞体层的一路信号．首先去除刺激伪迹[17]，然后用阈值法检测刺激期间诱发的群峰电位（population spike，PS），检测阈值设定为1.0 mV．再计算PS波的幅值、半高宽和面积等参数．PS波是许多神经元同时产生动作电位时叠加而成的电位，其幅值的大小与发放动作电位的神经元数量和发放的同步性都相关，其面积与神经元数量相关，半高宽则与同步性相关[18]．如果PS幅值和面积较大、半高宽较窄，则表明参与动作电位发放的神经元数量较多且同步性较高．

统计数据均以“均值 ± 标准差”的形式表示．采用配对t检验确定同次实验中变频与恒频刺激之间差异的显著性．“n”表示实验大鼠个数．

2　结　　果

2.1　随机变频的高频脉冲刺激诱发下游神经元群体产生同步发放

如图1a所示，在海马CA1区的输入纤维Schaffer侧支上施加电脉冲刺激时，刺激诱发的兴奋可以沿轴突传至下游锥体神经元的顶树突层，再经突触传递后激活神经元群体产生动作电位发放，形成群峰电位（PS）．由此，在100 Hz恒频HFS的起始时刻，首个刺激脉冲诱发了大幅值PS_1（~10 mV），潜伏期（即PS波谷与刺激脉冲之间的时间差）为（4.2 ± 0.98）ms（n = 7，见图1b左下）．但随后，PS波幅值减小，并在HFS持续（23.6 ± 5.63）s（n = 7）后消失，而非同步的神经元单元锋电位（unit spike）仍然持续．这种持续恒频刺激期间PS的消失，可能是由于HFS引起的轴突阻滞减弱了刺激的兴奋作用[19]．

但是，如图1b所示，当50 s的100 Hz恒频刺

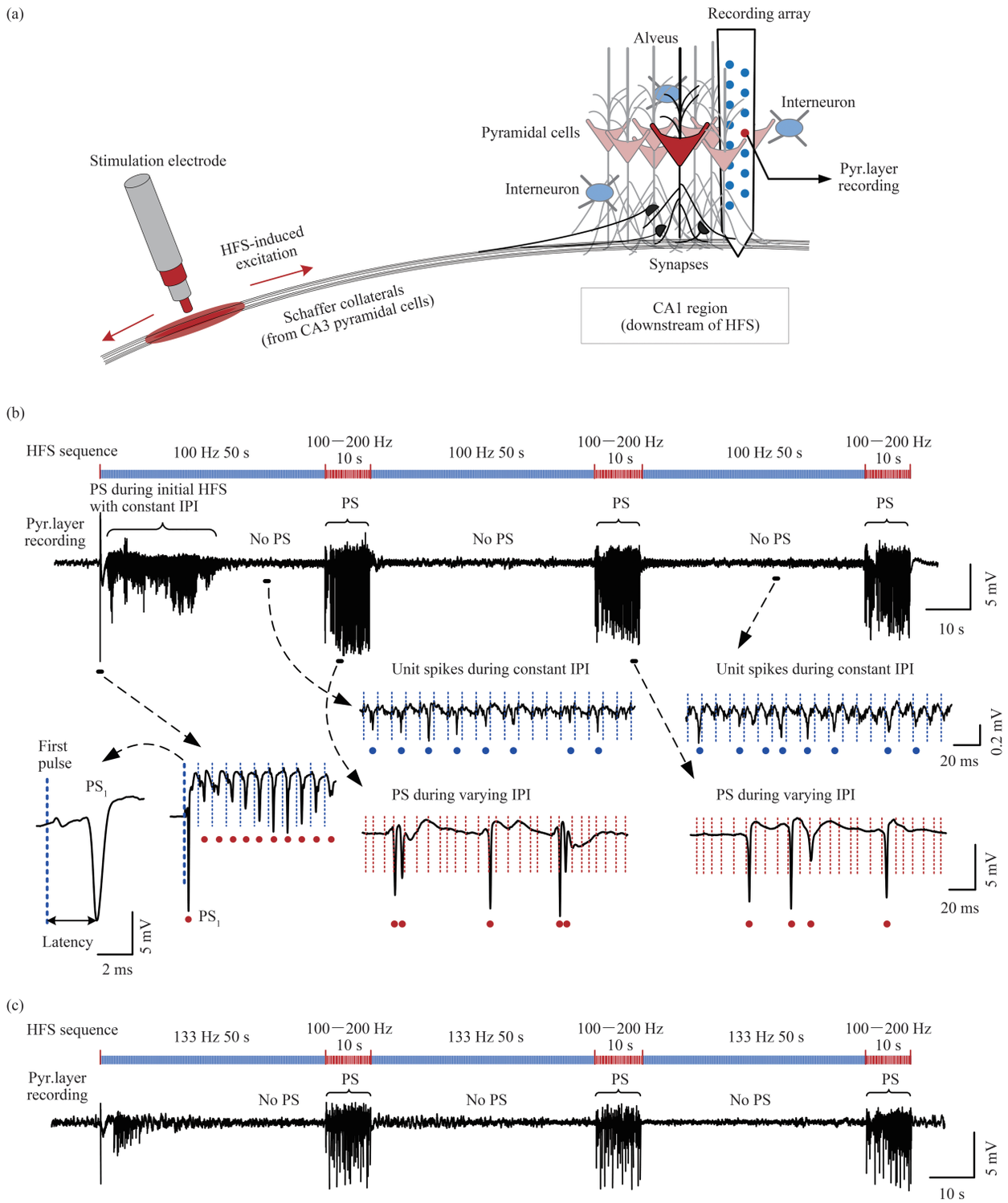

Fig. 1　Different responses of neuronal populations to orthodromic stimulation with constant and varying inter-pulse-intervals

（a）Schematic diagram of the locations of stimulation electrode in the Schaffer collaterals and recording electrode array in the CA1 region. （b）Example recording of neuronal responses to a high-frequency stimulation（HFS）sequence switched between constant IPI（10 ms，100 Hz for 50 s） and varying IPI （5-10 ms，100-200 Hz for 10 s）. Typical signals during the different periods of stimulation are given in the expanded insets. Blue and red dashed lines denote the removed stimulation artifacts with constant IPI and varying IPI，respectively. Blue and red dots denote unit spikes and population spikes （PS），respectively.（c）Example of HFS similar to （b）except the constant IPI decreased from 10 to 7.5 ms，i.e.，100 to 133 Hz.

激结束并转换为10 s变频刺激（100~200 Hz）时，PS波立即重新出现，并且在整个10 s的变频期间持续存在．直至刺激重新切换回恒频后，PS波随即消失，仍然仅存在单元锋电位的发放．在随后2次重复的恒频与变频切换期间，神经元的响应重复同样的变化．这表明，虽然变频期的IPI（5~10 ms）均未超过恒频期的IPI（10 ms），但是变频刺激能够诱发下游神经元的同步发放，与持续的恒频刺激不同．

那么，变频刺激是否由于其平均频率（133 Hz）较高而引起神经元的同步发放呢？在3只大鼠实验中将恒频刺激的频率提高至133 Hz（图1c），即，整个3 min刺激期间的平均频率保持不变．结果与上述100 Hz恒频时相似，当恒频刺激无法诱发同步发放时，3次变频刺激期间均能诱发大幅值的同步PS波．已有报道也表明[20-21]，在100~200 Hz刺激频率范围内，恒频刺激的频率较高时，反而不利于诱发神经元的同步活动．因此，变频期诱发的同步活动并不是由于刺激频率提高引起的．

2.2　变频刺激诱发的PS波特性

HFS序列的首个刺激脉冲诱发的PS波（简称PS_1）具有较大的幅值和较窄的半高宽，相当于神经元群体对于单个测试脉冲的同步响应（图1b左下），本文下面将其作为对照来研究变频刺激诱发的神经元同步活动．与PS_1相比，变频刺激期间诱发的PS波平均幅值显著减小，平均半高宽显著增加，但平均面积与PS_1无明显差异（图2）．这表明，在平均水平上，变频刺激期间PS波所包含的发放动作电位的神经元数量与单脉冲刺激诱发的相似，但动作电位的同步性降低．不过，变频期间PS的最大幅值、最小半高宽以及最大面积均与PS_1无显著差别，这表明变频刺激期间神经元群体发放的同步性也能够达到单脉冲诱发的水平．

此外，变频刺激期间PS的平均发放率为（9.9 ± 3.2）counts/s（n = 7），仅为刺激脉冲的平均频率133 Hz的7.4%左右，这表明，只有少数刺激脉冲之后跟随了PS波．为了研究变频期间这些PS波出现的规律，下面分析各PS的出现与前导刺激脉冲之间的关系．

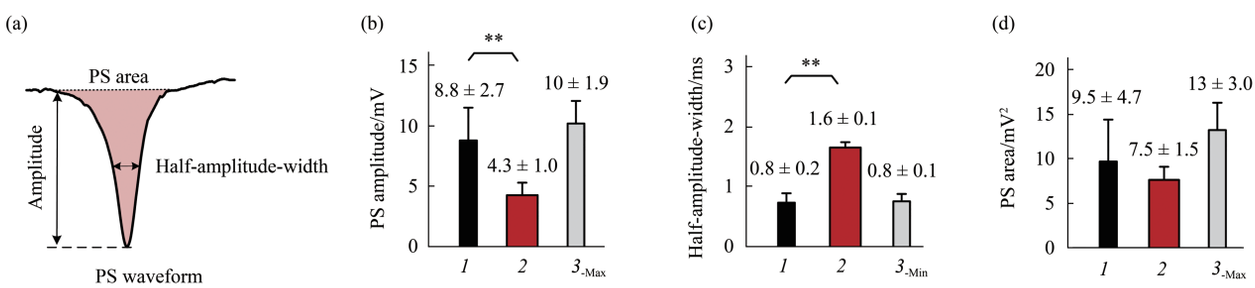

Fig. 2　Comparison of population spikes induced by the first stimulation pulse and the pulses with varying IPI

（a）Typical PS waveform with the definitions of three parameters：amplitude，half-amplitude-width and area.（b−d）Comparisons of the three PS parameters among PS_1 evoked by the first pulse，mean value and maximum/minimum value of PS evoked during stimulations with varying IPI. $^{**}P$<0.01，paired t-test，n=7 rats. *1*：PS_1；*2*：Mean during varying IPI；$3_{\text{-Max}}$：Maximum during varying IPI；$3_{\text{-Min}}$：Minimum during varying IPI.

2.3　变频脉冲的间隔长度与PS诱发之间的关系

为了研究变频期间PS诱发的规律，将3段共30 s变频刺激的神经元响应合并在一起分析．图3a为一个示例，设每个PS波之前的4个前导刺激脉冲依次为Sti_1、Sti_2、Sti_3和Sti_4，并将相应的4个前导脉冲间隔（IPI）设为IPI_1、IPI_2、IPI_3和IPI_4．变频刺激的IPI在5~10 ms范围内服从均匀的随机分布（图3b）．散点图和概率分布图都显示PS在IPI_1和IPI_4的长度上分布较为均匀（图3c左列和右列），这表明PS的出现及其幅值大小与IPI_1和IPI_4之间不存在明显的关系．但是，IPI_2较大、IPI_3较小时，PS出现的概率较大（图3c中间2列），表明PS的出现与IPI_2和IPI_3的长度之间存在一定关系．PS幅值、IPI_2和IPI_3组成的三维图也显示了这种关系（图3d）．

将IPI对半分成5~7.5 ms和7.5~10 ms一小一大两个长度分区．统计结果表明，在这两个分区上，PS在IPI_2的大分区中的分布显著多于小分区（约多1倍），而PS在IPI_3的小分区中的分布显著多于大分区（图3e）．此结果表明，PS的诱发与IPI_2和

IPI_3的长度变化存在相关性，即PS波由脉冲Sti_2或Sti_3诱发的可能较大，而不是由紧邻的前导脉冲Sti_1诱发的（与前导IPI_1无关）.

此外，变频期间也会出现数个PS连续发放的情况，且PS幅值逐渐减小（图3a右下），它们是部分小幅值PS的来源．连续的PS发放可能与锥体神经元具有的爆发式（burst）发放特性有关[22-23].

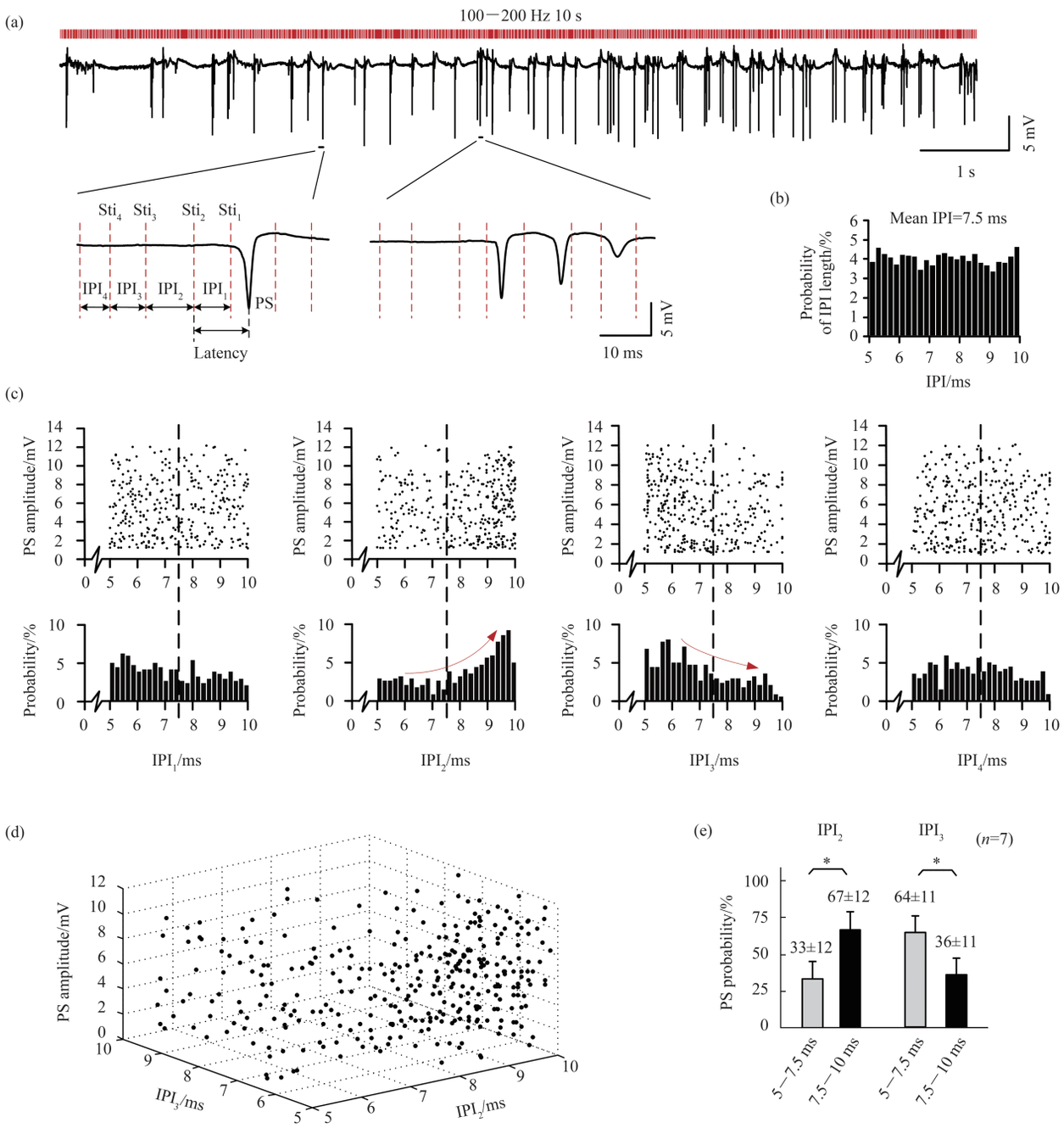

Fig. 3　Relationship between the appearance of population spikes and the lengths of inter−pulse−intervals during stimulation with varying IPI

（a）Example of 10 s recording during stimulation with varying IPI （100−200 Hz），together with expanded insets illustrating the definitions of preceding IPIs and stimuli. Red dashed lines denote the removed stimulation artifacts.（b）Probability distribution of IPI lengths during stimulation with varying IPI.（c）Scatter diagrams illustrating the relationship between the PS amplitudes and the lengths of four preceding IPIs （upper row），and the probability distribution of PS against one of the four preceding IPIs （lower row）. The dashed line divides the IPI （5−10 ms）into two interval ranges，equally.（d）Three-dimensional scatter diagram of PS amplitudes，the lengths of IPI_2 and IPI_3.（e）Comparisons of PS appearance probability in the two interval ranges of varying IPI：5−7.5 ms and 7.5−10 ms. $^*P < 0.05$，paired t-test，$n = 7$ rats.

3 讨　论

本文的同一刺激序列内恒频与变频反复切换的实验结果清楚地表明，在恒频刺激无法诱发下游神经元群体同步发放形成PS波时，包含微小脉冲间隔变化的100~200 Hz变频刺激却能够诱发PS波．其中的可能机制分析如下．

刺激脉冲在轴突束上诱发的兴奋需要经过轴突的传导和突触的传递之后才能激活下游投射区的神经元．刺激同步激活的神经元数量足够多时，就可以形成PS波．但是，持续的恒频HFS会导致轴突产生间歇性阻滞[19-20, 24]，或者导致突触神经递质耗竭等[25-26]，从而减弱电刺激对于下游神经元的兴奋作用，使得脉冲刺激仅能诱发单元锋电位，而非PS波（图1b）．而且，恒频HFS的脉冲频率越高，刺激诱发的轴突阻滞或神经递质耗竭等的程度就越深[14, 25, 27]．然而，本文的研究却表明，即使变频刺激的平均脉冲频率高于恒频刺激，其中微小的脉冲间隔的变化（≤5 ms）就可以诱发大幅值PS波．不过，PS的发生率仅占脉冲频率的7%左右，表明多个脉冲刺激累积的作用才能诱发这种同步的神经元发放，神经元响应的非线性特性可能是这种同步活动产生的原因．

根据变频刺激期间PS的出现与前导脉冲IPI之间的关系（图3），Sti_2和Sti_3诱发PS的可能性较大，因为轴突上的脉冲刺激诱发下游神经元产生动作电位时存在潜伏期[18]．正常情况下，单脉冲刺激诱发的PS潜伏期大于4 ms，而持续HFS的作用会延长刺激诱发PS的潜伏期[14, 20]．在变频HFS期间，PS的潜伏期会超过5~10 ms的IPI．因此，PS由脉冲Sti_1诱发的概率很小，可能由Sti_2或Sti_3诱发．如果由Sti_3诱发PS，那么潜伏期大于IPI_2与IPI_1之和，即大于10 ms．之前的研究表明，持续的轴突HFS期间下游神经元发放的潜伏期超过10 ms的较少[28]．因此，由Sti_3诱发PS的概率也较小，只有Sti_2诱发PS的可能性最大（见图3a左下）．

IPI_2较长且IPI_3较短时易于诱发PS的数据（图3c~e）支持上述推测．其中的机制分析如下．如果是Sti_2诱发PS，那么其前导IPI_2越长越有利于HFS诱导的轴突阻滞等状态的恢复，因此，IPI_2较长时易于诱发PS．此外，在刺激脉冲诱发的兴奋传导过程中，轴突细胞膜、突触传递和突触后电位的整合等过程随时间的变化都存在非线性的特性．因此轴突阻滞等状态的间歇性恢复也具有非线性的阈值效应[24]．Sti_3的前导间隔IPI_3较短时，神经元的恢复达不到响应阈值，从而将神经元的发放推迟到Sti_2之后，可以累积形成多神经元同步的动作电位发放；因此，IPI_3较短时有利于Sti_2诱发PS．

总之，恒频HFS可以诱发下游神经元的非同步的动作电位发放[28-29]，而本文的研究发现，仅含微小IPI随机变化的变频HFS却可以导致下游神经元的同步发放．这种与恒频刺激截然不同的变频刺激的作用为开发DBS的新刺激模式提供了重要线索．同步的神经元发放对于投射区具有更加强有力的作用．已有的大鼠实验表明，服从泊松分布的变频刺激只需要使用比恒频刺激小的刺激强度，就可以获得与恒频刺激相同甚至更好地减少慢性癫痫发作次数的作用[30]，从而可以降低DBS的电能消耗、延长电池的使用寿命．而本文的变频刺激研究结果为揭示其中的机制提供了新思路．变频刺激诱发的同步活动可能通过“以毒攻毒”的方式控制癫痫活动[31]．此外，变频刺激的同步作用还可能有利于意识障碍、抑郁症、记忆衰退等神经退行性疾病的治疗[32-33]．本文的结果对于揭示深部脑刺激的作用机制、促进其在临床中的推广应用具有重要意义．

参　考　文　献

[1] Benabid A L, Pollak P, Louveau A, *et al*. Combined (thalamotomy and stimulation) stereotactic surgery of the VIM thalamic nucleus for bilateral Parkinson disease. Applied Neurophysiology, 1987, **50**(1-6): 344-346

[2] Cury R G, Fraix V, Castrioto A, *et al*. Thalamic deep brain stimulation for tremor in Parkinson disease, essential tremor, and dystonia. Neurology, 2017, **89**(13): 1416-1423

[3] Fitzgerald P B, Segrave R A. Deep brain stimulation in mental health: Review of evidence for clinical efficacy. Australian and New Zealand Journal of Psychiatry, 2015, **49**(11): 979-993

[4] Li M C H, Cook M J. Deep brain stimulation for drug-resistant epilepsy. Epilepsia, 2018, **59**(2): 273-290

[5] Udupa K, Chen R. The mechanisms of action of deep brain stimulation and ideas for the future development. Progress in Neurobiology, 2015, **133**: 27-49

[6] Rizzone M, Lanotte M, Bergamasco B, *et al*. Deep brain stimulation of the subthalamic nucleus in Parkinson's disease: effects of variation in stimulation parameters. Journal of Neurology, Neurosurgery, and Psychiatry, 2001, **71**(2): 215-219

[7] Dayal V, Limousin P, Foltynie T. Subthalamic nucleus deep brain stimulation in Parkinson's disease: The effect of varying stimulation parameters. Journal of Parkinson's Disease, 2017,

7(2): 235-245

[8] Cukiert A, Cukiert C M, Argentoni-Baldochi M, *et al*. Intraoperative neurophysiological responses in epileptic patients submitted to hippocampal and thalamic deep brain stimulation. Seizure, 2011, **20**(10): 748-753

[9] Laxpati N G, Kasoff W S, Gross R E. Deep brain stimulation for the treatment of epilepsy: circuits, targets, and trials. Neurotherapeutics, 2014, **11**(3): 508-526

[10] McConnell G C, So R Q, Grill W M. Failure to suppress low-frequency neuronal oscillatory activity underlies the reduced effectiveness of random patterns of deep brain stimulation. Journal of Neurophysiology, 2016, **115**(6): 2791-2802

[11] Cassar I R, Titus N D, Grill W M. An improved genetic algorithm for designing optimal temporal patterns of neural stimulation. Journal of Neural Engineering, 2017, **14**(6): 066013

[12] Swan B D, Brocker D T, Hilliard J D, *et al*. Short pauses in thalamic deep brain stimulation promote tremor and neuronal bursting. Clinical Neurophysiology, 2016, **127**(2): 1551-1559

[13] Birdno M J, Cooper S E, Rezai A R, *et al*. Pulse-to-pulse changes in the frequency of deep brain stimulation affect tremor and modeled neuronal activity. Journal of Neurophysiology, 2007, **98**(3): 1675-1684

[14] Feng Z, Yu Y, Guo Z, *et al*. High frequency stimulation extends the refractory period and generates axonal block in the rat hippocampus. Brain Stimulation, 2014, **7**(5): 680-689

[15] 朱玉芳, 封洲燕, 王兆祥, 等. 高频电刺激改变神经元锋电位的波形. 生物化学与生物物理进展, 2016, **43**(8): 787-795
Zhu Y F, Fend Z Y, Wang Z X, *et al*. Progress in Biochemistry and Biophysics, 2016, **43**(8): 787-795

[16] 封洲燕, 光 磊, 郑晓静, 等. 应用线性硅电极阵列检测海马场电位和单细胞动作电位. 生物化学与生物物理进展, 2007, **34** (4): 401-407
Feng Z Y, Guang L, Zheng X J, *et al*. Progress in Biochemistry and Biophysics, 2007, **34**(4): 401-407

[17] Yu Y, Feng Z, Cao J, *et al*. Modulation of local field potentials by high-frequency stimulation of afferent axons in the hippocampal CA1 region. Journal of Integrative Neuroscience, 2016, **15**(1): 1-17

[18] Theoret Y, Brown A, Fleming S P, *et al*. Hippocampal field potential: a microcomputer aided comparison of amplitude and integral. Brain Research Bulletin, 1984, **12**(5): 589-595

[19] Jensen A L, Durand D M. High frequency stimulation can block axonal conduction. Experimental Neurology, 2009, **220**(1): 57-70

[20] Feng Z, Zheng X, Yu Y, *et al*. Functional disconnection of axonal fibers generated by high frequency stimulation in the hippocampal CA1 region *in vivo*. Brain Research, 2013, **1509**: 32-42

[21] Feng Z, Wang Z, Guo Z, *et al*. High frequency stimulation of afferent fibers generates asynchronous firing in the downstream neurons in hippocampus through partial block of axonal conduction. Brain Research, 2017, **1661**: 67-78

[22] Kamondi A, Acsády L, Buzsáki G. Dendritic spikes are enhanced by cooperative network activity in the intact hippocampus. Journal of Neuroscience, 1998, **18**(10): 3919-3928

[23] Andersen P, Morris R, Amaral D, *et al*. The Hippocampus Book. Oxford: Oxford University Press, 2007

[24] Guo Z, Feng Z, Wang Y, *et al*. Simulation study of intermittent axonal block and desynchronization effect induced by high-frequency stimulation of electrical pulses. Frontiers in Neuroscience, 2018, **12**: 858

[25] Xia R, Berger F, Piallat B, *et al*. Alteration of hormone and neurotransmitter production in cultured cells by high and low frequency electrical stimulation. Acta Neurochirurgica, 2007, **149**(1): 67-73

[26] Iremonger K J, Anderson T R, Hu B, *et al*. Cellular mechanisms preventing sustained activation of cortex during subcortical high-frequency stimulation. Journal of Neurophysiology, 2006, **96**(2): 613-621

[27] Anderson T R, Hu B, Iremonger K, *et al*. Selective attenuation of afferent synaptic transmission as a mechanism of thalamic deep brain stimulation-induced tremor arrest. Journal of Neuroscience, 2006, **26**(3): 841-850

[28] Wang Z, Feng Z, Wei X. Axonal stimulations with a higher frequency generate more randomness in neuronal firing rather than increase firing rates in rat hippocampus. Frontiers in Neuroscience, 2018, **12**: 783

[29] Medeiros D C, Moraes M F. Focus on desynchronization rather than excitability: a new strategy for intraencephalic electrical stimulation. Epilepsy & Behavior, 2014, **38**: 32-36

[30] Wyckhuys T, Boon P, Raedt R, *et al*. Suppression of hippocampal epileptic seizures in the kainate rat by Poisson distributed stimulation. Epilepsia, 2010, **51**(11): 2297-2304

[31] Gwinn R P, Spencer D D. Fighting fire with fire: brain stimulation for the treatment of epilepsy. Clinical Neuroscience Research, 2004, **4**(1-2): 95-105

[32] Kennedy S H, Giacobbe P, Rizvi S J, *et al*. Deep brain stimulation for treatment-resistant depression: follow-up after 3 to 6 years. American Journal of Psychiatry, 2011, **168**(5): 502-510

[33] Laxton A W, Tang-Wai D F, McAndrews M P, *et al*. A phase I trial of deep brain stimulation of memory circuits in Alzheimer's disease. Annals of Neurology, 2010, **68**(4): 521-534

The Altering Effect of High Frequency Stimulation on Brain Neurons With Small Changes in The Lengths of Inter−Pulse−Intervals*

HU Han-Han, FENG Zhou-Yan**, WANG Zhao-Xiang, ZHENG Lü-Piao, HUANG Lu

(*Key Laboratory of Biomedical Engineering of Education Ministry, College of Biomedical Engineering and Instrumentation Science, Zhejiang University, Hangzhou* 310027, China)

Abstract　Deep brain stimulation has been used to treat movement disorders such as Parkinson diseases by utilizing high-frequency stimulations (HFS) of electrical pulses with constant inter-pulse-intervals (IPI). To develop new stimulation paradigms for treating more brain diseases, HFS with varying IPI (i. e., varying-frequency) has been investigated. Previous studies have shown that the efficacy obtained by varying-frequency is different from that obtained by constant-frequency even with a same mean frequency. We hypothesized that small changes in IPI during HFS could substantially change the effect of HFS on neurons. To test this hypothesis, HFS sequences with constant IPI (IPI = 10 or 7.5 ms for a frequency of 100 or 133 Hz) and varying IPI (IPI = 5−10 ms with a mean frequency of 133 Hz) were alternately applied at afferent axon fibers of pyramidal cells in rat hippocampal CA1 region. The evoked potentials of downstream neurons were recorded and analyzed to quantitatively evaluate the neuronal responses to stimulations with constant IPI and varying IPI. The results showed that during persistent stimulation with constant IPI, the responses of downstream neurons changed from initial synchronized firing of population spikes (PS) into non-synchronized firing (i. e., unit spikes). However, once the stimulation switched to the sequence with varying IPI, synchronized firing reappeared with large PS events. Additionally, the amplitude of PS and the synchronization degree of firing induced by varying IPI were similar to those induced by single pulses at baseline. However, the incidence of PS was only ~7% of the pulse frequency, indicating a cumulative action of multiple pulses for generating such synchronized firing of neurons by stimulations with varying IPI. In addition, the appearance of PS was related to the length of proceeding IPI. Presumably, nonlinear responses of neuronal axons and synapses to high-frequency stimulation might cause the synchronized activity induced by varying-frequency. These results indicate that tiny differences in intervals of varying-frequency stimulation may generate a modulation effect on neurons very different from that of constant-frequency stimulation. The present study shows important results for revealing the mechanisms of brain stimulation and for advancing the development of new stimulation paradigms to treat various brain diseases.

Key words　high-frequency stimulation, inter-pulse-interval, random frequency, population spike, synchronized firing

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** Corresponding author.

Tel：13515711296，E-mail：fengzhouyan@139.com

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