MEA

总结

在这份论文中，研究团队通过使用高密度电极阵列（MEA）与虚拟游戏世界进行交互，探索神经元集团的学习行为。以下是实验的主要步骤和方法：

1. **MEA 设置和准备**：
   - 使用MaxOne多电极阵列，包含26000个铂电极，分布在8平方毫米的区域上。
   - 电极阵列基于CMOS技术，能够从1024个通道进行记录，理论上可以从32个电极进行刺激，但实际上由于空间限制和控制需求，只能从8个独立控制的电极进行输入刺激。
   - 电极和玻璃载玻片表面涂有促进细胞粘附的物质，如聚乙烯亚胺（PEI）或聚D-赖氨酸。

2. **虚拟游戏环境的表示**：
   - 根据电极记录的神经元放电“尖峰”，每10毫秒更新一次虚拟游戏（如乒乓球游戏）中的状态。
   - 定义两个活动区域，这两个区域尖峰计数后，不同区域计数的不同，判断用来控制游戏中的“球拍”上下移动。
   - 游戏中的球在固定速度下移动，碰到边界和球拍会反弹。
   - 每次球拍未接住球时，游戏环境会利用 8个 输入电极，以提供不同类型的反馈（随机、静默或无反馈）。

3. **输入配置**：
   - 刺激通过8个预定义的感觉区域电极以特定频率和电压传递。
   - 刺激类型包括编码球的位置的“感觉刺激”和四种反馈协议（不可预测、可预测、静默、无反馈）。

   a. **感觉刺激**：
      - 使用75毫伏的电压刺激八个电极中的其中一个来编码球相对于球拍的位置。
      - 频率从4Hz（球在对面墙壁附近时）线性增加到40Hz（球接近球拍时）来组合编码球相对于球拍的位置。

   b. **反馈类型**：
      - **不可预测刺激**：当“未接住”球时，以150毫伏和5赫兹在随机时间和位置提供刺激。
      - **可预测刺激**：当“接住”球时，以75毫伏和100赫兹提供刺激，持续100毫秒。
      - **静默反馈**：在后续研究中，用无刺激替换不可预测刺激，不提供可预测刺激。
      - **无反馈**：在无反馈条件下，不基于任何结果或动作向培养物提供反馈，只提供标准的感觉刺激。

4. **输出配置**：
   - 在HD-MEA上配置了1024个电极，用于记录活动。
   - 活动记录分布在几个预定义的运动区域，这些区域的活动决定了虚拟环境中球拍的移动方向。
   - 系统中增加了“增益”功能，以校正不同区域的活动差异，确保即使在自发活动不平衡的情况下也能控制球拍的移动。

原文

MEA setup and preparation

MaxOne Multielectrode Arrays (MEA; Maxwell Biosystems, AG, Switzerland) were used for this research. The MaxOne is a high-resolution electrophysiology platform featuring 26,000 platinum electrodes arranged over an 8 mm2. The MaxOne system is based on complementary meta-oxide-semiconductor (CMOS) technology and allows recording from up to 1024 channels. Stimulation was theoretically possible up to 32 electrodes. In practice it was not possible to route 32 electrodes through independent stimulation units to facilitate independent electrode level control, especially if these electrodes were spatially proximate to each other. This meant that for the actual setup of input stimulation described below a subset would be limited by the desired spatial configuration – in this case to 8 individually controlled electrodes. MEAs and chambered glass slides are coated with either polyethyleneimine (PEI) in borate buffer for primary culture cells or Poly-D-Lysine for cells from an iPSC background before being coated with either 10 µg/ml mouse laminin or 10 µg/ml human 521 Laminin (Stemcell Technologies Australia, Melbourne, Australia) respectively to facilitate cell adhesion.

Representation of the gameplay environment

Spikes are themselves optionally recorded in binary files, and regardless of recording are counted over a period of 10 milliseconds (200 samples), at which point the game environment is given the number of spikes detected in each of the configured electrodes in predefined motor regions as described below. These spike counts are interpreted as motor activity depending on which motor region the spikes occurred in, thereby moving the ‘paddle’ up or down in the virtual space. At each of these 10ms intervals the pong game is also updated, with a ball moving around a play area at a fixed speed, ‘bouncing’ off the edges of the play area and off the paddle, until it hits the edge of the play area behind the ‘paddle’, which marks the end of one ‘rally’ of pong. At the end of the rally, the game environment will instead configure the stimulation sequencer to apply one of three types of feedback described below: random, silent or none. Under the standard stimulus condition, feedback is also provided when the ball contacts the paddle as described below. As described in detail below, during each rally the location of the ball relative to the paddle is encoded as stimulation to one of eight stimulation sites, which is tracked in an internal ‘stimulation sequencer’ module. The stimulation sequencer is updated 20,000 times a second, once every time a sample is received from the MEA, and once the previous lot of MEA commands should have finished, it constructs another sequence of MEA commands based on the place-code and rate-code information that it has been configured to transmit. The stimulations take the form of a short square bi-phasic pulse that is a positive voltage, then a negative voltage. A Digital to Analog Converter (or DAC) on the MEA will read and apply this pulse sequence to the given electrode. Figure S5C shows an image of the game visualiser, and a real-time interactive version is available Video S2 at https://spikestream.corticallabs.com/. There was also the option to record cells at ‘rest’ where a gameplay environment was initiated and activity was recorded to move the paddle, but no stimulation was delivered, with corresponding outcomes still being recorded. This acted as a baseline control to determine the gameplay characteristics of a culture based on spontaneous activity alone.

Input configuration

Stimulation is delivered at a given Hz and voltage as appropriate for the required input type across 8 predefined electrodes in a sensory area, as shown in Figure 4B. A total of 5 types of input were able to be delivered. This consisted of either “Sensory Stimulus” that encoded ‘ball’ position, or one of four feedback protocols, either Unpredictable, Predictable, Silent, or No-feedback.

Sensory stimulus

Given that cells appeared robust to voltage stimulation, the decision was made to base voltage levels on existing evidence of neurological function. Therefore, to prevent forcing hyperpolarised cells from firing, 75 mV was chosen as the sensory stimulation voltage that would relate to where the ball was relative to the paddle as described in the main text to key electrodes. For the main study, place coding was combined with a rate coding that delivered stimuli at 4 Hz when the ball was closest to the opposing wall and increased in a linear fashion to a max of 40 Hz as the ball reached the paddle wall.

Unpredictable stimulus

For the standard stimulus feedback condition unpredictable stimulation was delivered to the cultures when a ‘miss’ occurred – i.e., when the culture failed to line the ‘paddle’ up to connect with the ‘ball’. In order to add unpredictable external stimulus into the system, this feedback stimulus was set at 150 mV voltage and 5 Hz. This stimulation occurred at random sites at a random timescale over the 8 predefined input electrodes, for a period of four seconds, followed by a configurable rest period of four seconds where stimulation is paused, followed then by the next rally. Theoretically the higher voltage than that used for the Sensory Stimulus would be sufficient to force action potentials in cells subjected to the stimulation regardless of the state the cell was in, thereby being even more disruptive to the culture.

Predictable stimulus

For the standard stimulus feedback condition a predictable stimulation was delivered to cultures when a ‘hit’ occurred – i.e., when the cultures successfully lined up the ‘paddle’ to connect with the ‘ball. This was delivered at 75mV at 100Hz over 100ms. This occurred at the instant of when the simulated ball impacted the paddle and replaced other sensory information for the 100ms period. Predictable stimulation occurred at this frequency and period across all 8 stimulation electrodes simultaneously.

Silent feedback

Silent feedback only occurred for follow up studies in the Silent condition. This feedback replaced the Unpredictable Stimulus described above with no stimulation for the same length of time. Predictable Stimulus feedback was also removed during Silent Feedback sessions. This feedback is still distinct from No-Feedback as described below as it is a change in the culture environment that is tied to culture activity in a closed-loop manner and therefore a form of feedback.

No feedback

This condition only occurred for follow up studies in the No-feedback condition. This condition was designed to assess whether sensory stimulation was sufficient to drive learning in cultures and was an open-loop condition. This means that no feedback of any kind was delivered to the cultures based on any outcome or action. Standard Sensory Stimulus as described above was delivered to the cultures and the outcome was measured on the same metric, however when a ‘miss’ would normally occur, instead the ball continued the same trajectory bouncing off the wall behind the paddle – still recorded as a ‘miss’ – that would otherwise result in the end of a rally. When the ‘ball’ connected with the simulated paddle a ‘hit’ would be recorded. As such, under No-Feedback the entire gameplay session is essentially a single rally with the final position of the simulated ball being predictable from the initial vector, but with the scoring occurring as normal otherwise.

Output configuration

A total of 1024 electrodes were routed on the HD-MEA to record activity in a pattern as shown in Figure 4B. The 'Sensory' area, where stimulation electrodes were embedded as described above consisted of 626 electrodes. The remaining output electrodes were divided into predefined motor regions on the MEA, consisting of four regions that were defined either as motor region 1 or motor region 2 as shown in Figure 4B. As described above, this configuration was selected as it offered the possibility for biologically relevant features and minimized the chance of apparently successful performance through bias alone—as it precludes a direct relationship between input stimulation and output activity recording. Only activity in motor regions contributed towards paddle movement. Activity in motor region 1 moved the paddle 'up' and activity in motor region 2 moved the paddle 'down'. Activity was measured over these two regions, where the region with higher activity would move the paddle in a corresponding direction. This was found to be extremely sensitive to culture characteristics, where asymmetrical spontaneous spiking activity in cultures would cause the paddle to move swiftly in only one direction. However, due to the technical difficulty of culturing neurons with precisely balanced activity in both these regions it was found to be necessary to add 'gain' into the system. This gain function measured activity in both regions and added a multiplier to a target of 20 Hz. Activity >20 Hz was weighted by a correction factor >1, while activity <20 Hz was weighted by a correction factor <1. This would allow changes in activity in each given region to influence the paddle position, even if they displayed different latent spontaneous activity. No other filtering or machine learning style weights were applied to decode motor region activity, meaning there was no need for regularization or risk of over fitting as all learning was required to occur within the biological neural cultures.