30th International Biology Olympiad
SZEGED, HUNGARY

Practical Exam 4. 實作考試 4

Bioinformatics 生物資訊

Neurobiology 神經生物學

16^{th} July 2019

COUNTRY 國家
LANGUAGE 語言

Practical Exam 4. 實作考試 4

General instructions 一般說明

This exam consists of two subtasks:

- Subtask 1. Practical Task in Bioinformatics (50 points)
- Subtask 2. Examination of Neuronal Activity (50 points)

此考試包含兩個子實作：

- 子實作 1. 生物資訊學的實作任務（50 分）
- 子實作 2. 神經元活動試驗（50 分）

1. **Please remember to attach your barcode sticker to all pieces of paper on the answer sheet.**
2. Write your answers in the separate answer sheet provided. **Only answers given in the answer sheet will be considered**.
3. Ensure you received all necessary materials and equipment listed on the next page. If any items are missing indicate this by raising your red card within 10 minutes following the start of the exam.
4. During experiments ensure all materials and equipment is handled properly. Any spilled solutions or broken equipment will not be replaced.
5. Stop answering and put down your pencil immediately when the bell rings signalling the end of the exam.
6. No paper, materials or equipment should be taken out of the laboratory.

1. 請記得將你的條形碼貼紙貼在答案紙上的所有紙張上。
2. 在提供的單獨答案表中寫下你的答案。只在答案紙中寫出的答案會被計分。
3. 確保你已收到下一頁列出的所有必要材料和設備。如果有缺少任何項目，請在考試開始後 10 分鐘內舉起你的紅卡
4. 在實驗期間，確認你的所有材料和設備是正確的。任何翻倒溢出的溶液或破損的設備都不會被更換。
5. 當結束鈴聲響起，表示此考試結束，請立即放下鉛筆並停止作答。
6. 不得將紙張、材料或設備帶出實驗室。

IMPORTANT TECHNICAL NOTES ON CHEATING 關於作弊的重要技術說明

- **During the practice task, you will use the Geneious Prime and Neurophysiology (Neurofiziológia) software that are already running on your computer.**
- **Please do not use any other software, except for the Geneious and Neurophysiology software that you will use for the completion of the exam.**
- **Your computer records all of your activities, so if you open any other software program, or if you connect your computer to the internet, you will be excluded from this task and lose all points that you scored here.**

請注意以下說明，違者視為作弊

- 在回答本實作題時，你將使用已裝載於電腦上的神經生理學（Neurofiziológia）軟體。
- 除了使用於生物資訊學實作的 Geneious 軟體，**請不要使用任何其他軟體。**
- 你的電腦會記錄所有活動，因此，如果你打開任何其他軟體，或者試圖連上網路，本題將不予計分。

Materials and Equipment 材料和設備

- PC running Geneious Prime and Neurophysiology (Neurofiziológia)
- PC 運行 Geneious Prime 和神經生理學軟體（Neurofiziológia）

SUBTASK 1. PRACTICAL TASK IN BIOINFORMATICS 子任務 1. 生物資訊學中的實際的任務

Modern biological research in the 21st century is inconceivable without bioinformatic analyses. In this practical task, we present an example of how bioinformatic data are processed in order to investigate evolutionary issues of bacterial resistance and susceptibility to antibiotics.

如果沒有生物資訊學分析，21 世紀的現代生物學研究是無可想像的。在此實作中，我們提供了一個使用生物資訊學資料來研究細菌對於抗生素的抗藥性和易感性的演化議題的案例研究。

Please do not start using the Geneious Prime software until you have completely read and understood the instructions. Otherwise, you may cause changes to the data that cannot be restored. The lab assistant will not assist you in replacing lost or corrupted data, so you should proceed step by step only.

在未完全了解此說明前，請不要使用 Geneious Prime 軟體。否則你將造成資料數據之改變，以致無法回復。實驗助教將不會協助你回復已遺失或毀損的數據，所以你應慎重地依據指示，一步步進行分析。

IMPORTANT TECHNICAL NOTES ON CHEATING

- **During the practical, you will use the Geneious Prime software that is already running on your computer.**
- **Please do not use or even open any other software, except for this Geneious software and the Neurophysiology software that you will use for the other practical that is also already running.**
- **Your computer records all your activities, so if you open any other software, or if you connect your computer to the internet, you will be excluded from this task and lose any points that you scored here.**

重要的技術提醒，以防作弊!

- 在實作過程中，你會用到 Geneious Prime 軟體，其已安裝在你的電腦中。
- 請別使用或打開任何軟體，只能打開你會用到的 Geneious Prime 及神經生理學的軟體。你的電腦會記錄你所有的動作，如你打開任何其它的軟體，或是連結到網際網路，你在此部分的實作將會被排除，並失去此部分的分數。

Chloramphenicol acetyltransferase (CAT) is responsible for chloramphenicol resistance in Escherichia coli. The CAT enzyme is made up of 219 amino acids and encoded by a gene consisting of 660 nucleotides It is a well-known phenomenon that antibiotic resistance spreads rapidly across pathogens. This is why many scientific studies are looking at ways to prevent this. One approach is to explore whether the inverse of resistance, that is, recovered antibiotic susceptibility, can also be passed on.

氯黴素乙醯轉移酶 (CAT) 是造成大腸桿菌中氯黴素抗藥性的原因，CAT 酵素由 219 個氨基酸組成，並帶有 660 個核苷酸編碼組成的基因。眾所周知，抗生素抗藥性在病原體中快速傳遞，這是現今眾多科學研究試圖防止此種現象的主因，一種方法是嘗試是否可以進行逆向抗藥性反應，也就是回復抗生素的易感性。

In this task, you will be required to analyse the results of such a model experiment. An E. coli strain originally resistant to chloramphenicol was exposed to periodic heat stress, and strains were isolated after each stress plus inoculation cycle. Some of these strains remained resistant, while others have lost their defences against the antibiotic.

在此實作中，你將被要求分析此類模式實驗的結果。

最初對氯黴素具有抗藥性的大腸桿菌菌株進行週期性熱刺激處理，並且在每次週期性熱刺激處理後分離純化菌株。這些菌株中一部分仍然具有抗藥性，而其他菌株則喪失了對抗生素的防禦能力。

Freshly inoculated liquid cultures of 16 strains from the experiment were grown with chloramphenicol for 2 days. 10 mL of each culture was diluted by 100X. Then we determined the population density of each culture and measuring optical density at 600nm with a spectrophotometer. This value is directly proportional to the population density. Table 1 shows OD_{600} information of the 16 strains.

將來自此實驗的 16 菌株新鮮液體培養物與氯黴素共同培養 2 天。將每種培養物取 10mL 後稀釋 100 倍，然後使用分光光度計以 600nm 波長測量光密度方式測定每種培養物的族群密度。此數值與族群密度成正比。表 1 顯示 16 菌株的 OD600 資料。

1.	0.087	5.	0.915	9.	0.869	13.	0.784
2.	0.873	6.	0.852	10.	0.923	14.	0.858
3.	0.110	7.	0.077	11.	0.093	15.	0.102
4.	0.098	8.	0.859	12.	0.920	16.	0.931

When those E.coli strain were cultured in medium without Chloramphenicol, it is no difference of growth rate among each strain.

當這些大腸桿菌菌株在不含氯黴素的培養基中培養時，各菌株之間的生長速率無差異。

Q.4.1.1 Study Table 1 carefully and identify which strain(s) lost its/their resistance. Indicate your answer by putting an X in the appropriate box on the answer sheet.
依據表 1 資料研究並確定哪些菌株失去其抗藥性。在答題紙上的相應框中輸入”X”來代表你的答案。

In order to examine whether strains that became susceptible have evolved independently from each other or they have a genetic relationship, we will analyse the sequence of a segment of their CAT gene. Since CAT is an enzyme, it can be assumed that its loss of function can be primarily caused by mutations in the active site of CAT.

為了檢驗易感性的菌株是否為獨立演化或它們具有遺傳關係，我們將分析其 CAT 基因片段的序列。由於 CAT 是一種酵素，可以推測其功能的喪失主要是由 CAT 活性位點的突變引起的。

- Log in to your computer and click on the Geneious Prime icon in the bottom bar:

登錄到您的電腦然後按底欄中的 Geneious Prime 圖標：

- cDNA sequence segments of mRNAs from the CAT genes of the examined 16 strains have previously been uploaded.

  16 品系菌株的 CAT 基因之 mRNA 的 cDNA 序列區段已經預先上傳。

- You can display them on your screen by clicking on the ‘+’ sign next to the folder entitled ‘IBO2019’ in the left pane, which will show the folders of this subtask. Clicking on ‘Subtask1’ will display the sequences.

  你可以經由按左側長方格名為“IBO2019”的文件夾旁邊的“+”符號而在螢幕上顯示，該文件夾為此子實作的文件夾。按“Subtask1”將顯示序列。

- Your first task will be to compare the sequences.

  你的第一項實作是比較序列。

- By ticking the boxes to the left of the sequence names, select all 16 sequences, then click on ‘Multiple Align’ command in the ‘Align/Assemble’ drop-down menu. In the pop-up window, do not change any settings, and click on OK. The alignment of the sequences can take up to 1 minute, so be patient.

  勾選序列名稱左側的框，選擇所有 16 個序列，然後選取“排列/組裝”選項下拉選單中“多個排列”命令。在跳出視窗中，不要更改任何設定，然後按“OK”。序列的排列約需要 1 分鐘，請耐心等待。

- When the alignment process is completed, the progress bar will disappear and an additional line will appear under the original sequences. Double-click on this new line. You should see a lot of grey bars with some scattered tiny coloured spots on them.

  當排列過程完成後，進度顯示將消失，而在原始序列下將顯示額外一行。雙擊這一新行。你應該看到很多的上面具有一些分散小色斑點的灰色條紋。

- Zoom the sequence segments with the Ctrl + mouse scroll key combination. The letter identifying the bases will appear. Above the sequences, the consensus sequence is shown which appears to be the most common base sequence during the alignment of the examined instances.

  使用 Ctrl + 滑鼠滾動按鈕組合來縮放序列片段。將出現字母標示的鹼基。在序列上方，所顯示的共有序列為在序列排列比對後最共通的鹼基序列。

- Use the scroll button of the mouse to move left and right along the sequences.

  使用滑鼠的滾動按鈕沿序列向左和向右移動。

Answer the following questions in the answer sheet by writing your answer in the appropriate boxes.

在答案卷相對應的位置填寫你的答案來回答以下問題

Q.4.1.2.a Specify the alignment position in sequence segment 250-350 in which Strain1 has a base different from the consensus sequence. **Write this ordinal number in the corresponding box on the answer sheet.**
在序列區段 250-350 中，指出菌株 1 不同於共識序列的鹼基之位置。**請將此序號填入答案紙上對應的空格中。**

Q.4.1.2.b Specify the base in Strain1 at the position identified in Q4.1.2.a **by writing the one-letter code of the base in the appropriate box on the answer sheet.**
指出在 Q4.1.2.a 中菌株 1 的鹼基，將該鹼基的單一字母代碼**填入答案紙上對應的空格中。**

Q.4.1.2.c Specify the two alignment position(s) where the maximum number of strains showing a difference from the consensus sequence. **Write the ordinal numbers of these two positions in the corresponding boxes on the answer sheet.**
指定兩個校準位置，找出與共識序列有最大菌株數量的位置。**在答案紙上對應的空格中，分別寫出這兩個位置的順序號碼。**

Q.4.1.2.d Specify the three strains that are likely to carry a deletion mutation in the examined gene segment. **Write these ordinal numbers in the corresponding boxes on the answer sheet.**
在所檢視的基因區段中，指出此三個菌株中可能攜帶缺失突變的**順序號碼**。**請將此序號填入答案紙上對應的空格中。**

Q.4.1.2.e Specify the alignment positions of adjacent-double base changes, wherein both bases were replaced by their **complementary** ones. **Write the ordinal numbers of these two adjacent bases in the corresponding boxes on the answer sheet.**
相鄰雙鹼基改變的特定位置，此特定位置為兩個鹼基被其互補鹼基所取代。**在答案紙上的相對應方框中寫下這兩個相鄰鹼基的序列號碼。**

Q.4.1.2.f Specify the alignment positions where at least four of the strains specified in your response to question Q4.1.1 have the same changes? **Write each of these ordinal numbers in the corresponding boxes on the answer sheet.**
指定校准位置，其中對問題 Q4.1.1 的回答中指定的至少四個菌株具有相同的變化？**將每一個順序號碼填寫在答案紙上的相應方框中。**

Your answer to question Q4.1.2.f. is relevant because all the mutations in question can form the basis for the loss of resistance. Your next task will be to check which mutation causes amino acid substitution in the CAT enzyme.

你對問題 Q4.1.2.f 的回答為相關的，因為在問題中所有突變都可成為失去抗藥性的基礎。你的下一個實作是檢查哪種突變導致 CAT 酵素中的氨基酸取代。

- Close the window where you have worked before (NOT the GENEIOUS program, this window only) by clicking on the ‘X’ button in the upper right corner.
- 按右上角的“X”按鈕來關閉之前工作的視窗（不是 GENEIOUS 程序，僅此視窗）。
- Select the 16 DNA sequences by ticking the boxes to their left. Click on the ‘Sequence/Translate…’ command in the menu to see the amino acid sequences in the image that appears at the bottom or in a separate window. The consensus amino acid sequence is shown in the top line. Similarly to the DNA sequence, the image can be zoomed using the Ctrl+scroll key combination.
- 勾選左側的方框來選擇 16 條 DNA 序列。按選單中的“序列/轉譯...”命令，可在下方或是單獨視窗中顯示的氨基酸序列。共有氨基酸序列將顯示在上方行。與 DNA 序列類似，可以使用 Ctrl + 滑鼠滾動鍵組合來縮放圖像。
- Note that if you zoom into the image to a high magnification, one-letter amino acid codes will be replaced by three-letter codes. In spite of this, ALWAYS USE THE ONE-LETTER AMINO ACID CODES when providing your answers.
- 請注意，如果你將圖像放大到高倍率時，則單字母氨基酸代碼將被三字母胺基酸代碼替換。儘管如此，在填寫答案時，**需使用單字母氨基酸代碼。**
- Use the scroll button of the mouse without pressing Ctrl to move left and right along the sequences.
- 使用滑鼠滾動按鈕而不按 Ctrl 鍵可沿序列向左和向右移動。

Answer the following questions on the answer sheet.

在答案紙上回答以下問題。

Q.4.1.3.a

Q.4.1.3.b Let us suppose that you want to know which amino acid is encoded by the bases 121–123 of the mRNA from the encoding region of the peptide gene in question Q4.1.3.a. Specify the ordinal number of amino acids in the amino acid sequence of the peptide that need to be checked in order to answer the question. **Write the ordinal number in the corresponding box on the answer sheet.**

假設你想知道由問題 Q4.1.3.a 的胜肽基因的編碼區的 mRNA 的鹼基 121-123 編碼的氨基酸為何。在胜肽的氨基酸序列的指定的氨基酸的順序號碼需要檢查以回答問題。**在答案紙上的相應方框中填寫入順序號碼**

Based on your answers to questions Q4.1.3.a and Q4.1.3.b., check the amino acid positions corresponding to the mutations found in question Q4.1.2.f.

根據你對問題 Q4.1.3.a 和 Q4.1.3.b 的回答，檢查對應於問題 Q4.1.2.f 中所發現的突變之氨基酸位置。

Q.4.1.3.c Specify the position of the amino acid(s) that changed due to mutations detected in question Q4.1.2.f. **Write this(these) position number(s) in the corresponding boxes on the answer sheet.**

指定由問題 Q4.1.2.f 中檢測到因突變而改變的氨基酸的位置。**將這些位置寫在答案紙上的相應方框中。**

Q.4.1.3.d Specify the position of the amino acid(s) in which the mutation(s) detected in question Q4.1.2.f. remain(s) silent. **Write this(these) position number(s) in the corresponding boxes on the answer sheet.**

指定在問題 Q4.1.2.f 中檢測到的靜默突變的氨基酸的位置。**將位置寫在答案紙上的相應方框中。**

Q.4.1.3.e Which is the amino acid substitution that appears in all of the chloramphenicol sensitive strains detected in question Q4.1.1.? Specify the position of that amino acid. **Write the position number of this amino acid in the corresponding box on the answer sheet.**

在問題 Q4.1.1 中檢測到的所有氯黴素易感性菌株中出現的氨基酸取代是什麼？指定該氨基酸的位置。**將位置寫在答案紙上的相應框中。**

We have identified which amino acid substitutions are likely to cause chloramphenicol sensitivity. Since the examined DNA segment has changed several times and in several loci during the microevolution of the strains, it is also possible to build a DNA-based phylogenetic tree of the 16 strains. You have to do this with the help of Geneious Prime, as specified below.

我們已經發現了上述導致易感性的原因，即某種氨基酸取代可能引起易感性。由於檢測的 DNA 片段在菌株的微演化過程中已經多次改變並且在幾個基因座中發生，因此可以建構基於 DNA 資料的 16 個菌株之親緣關係樹。你需要在 Geneious Prime 的協助下完成這項工作，如下所述。

- If you performed the previous examinations in a new window, close this window (NOT the entire Geneious Prime program!) or if the amino acid sequence appeared in the split bottom pane of the screen, untick the box next to ‘Translation of 16 sequences’ in the top pane.
- 如果你在新視窗中完成了之前的檢測，關閉此視窗 (不是整個 Geneious Prime 程序！) 或者如果氨基酸序列出現在螢幕的分割底部視窗格，請在頂部視窗中取消勾選“16 個序列的轉譯”旁邊的框。
- If you can only see the DNA segments again, tick/select the tested 16 DNA sequences, then click on the’ Tree’ icon in the top menu bar. In the pop-up window, accept the default settings by clicking on’ OK’. It may take a few seconds for the software to generate the phylogenetic tree. If it appears below only, click the upper right corner of the area displaying the phylogenetic tree to enlarge the image so that you can see the window in full screen.
- 如果你只能再次看到 DNA 片段，勾選/選擇測試的 16 個 DNA 序列，然後按頂部選單欄中的“Tree”圖標。在彈出視窗中，按“OK”接受默認設置。軟體可能需要數鐘才能生成親緣關係樹。如果它僅顯示在下方，請按顯示親緣關係樹的區域之右上角以放大圖像，以便可以全螢幕觀看視窗。
- The obtained tree is unstructured (unrooted), so you need to transform it so that it can serve as a basis for conclusions. The first step is to select the oldest (most divergent) strain, which will be the root, also known as the outgroup, of the structured tree.
- 你所獲得的樹形圖是非結構化的 (無根的) ，所以你需要對它進行轉換才能作為結論的基礎。第一步是選擇最老的 (最分歧的) 品系，它將是此結構樹的根，亦稱為外群。
- Since the original phylogenetic tree does not provide accurate information about the oldest strain, you will have to find it in a different way.
- 由於原本的系統發育樹無法提供有關最老菌株的準確信息，因此你必須以不同的方式找到它。
- Click on the’ Distances’ tab in the second textual menu bar in the upper left. Thus, a distance matrix is obtained that shows the degree of divergence between each pair of DNA sequences. .In the upper middle menu, change’ Styles’ to’ Heatmap’. The darkness of rectangles corresponds to the extent of divergence.
- 單擊左上角第二個文本菜單欄中的“距離”選項卡。因此，獲得距離矩陣，其顯示每對 DNA 序列之間的發散程度。在中上方選單中，更改“Styles”為“Heatmap and Numbers”。矩形的暗度對應於分歧的程度。

Q.4.1.4.a Specify the ordinal number of the strain that probably has the oldest sequence among the 16 strains. **Write this ordinal number in the corresponding box on the answer sheet.** (For example, if your answer is Strain-23, write '23' on answer sheet.)
在 16 個菌株中可能具有最古老序列的菌株的順序號碼為何。將此順序號碼寫在答題紙上的相應方框中。(例如，如果你的答案是 Strain-23，請在答題紙上寫上“23”。)

Based on the above data, you can already start structuring the phylogenetic tree.

基於上述的資料，你已經可以開始構建親緣關係樹。

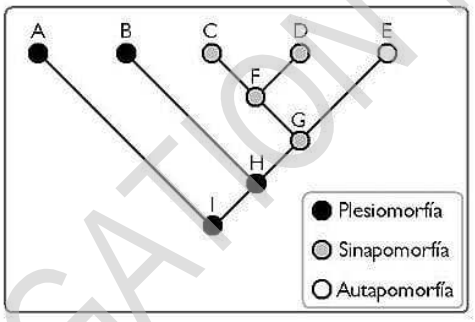
- To do this, switch back to the ' Tree view' view by clicking on the appropriate tab in the second menu bar from the top.
- 執行此操作，請經由按頂端第二個選單欄中的相應選項切換回“Tree view”視窗。
- Subsequently, click on the endpoint nearest to the name of the strain identified in question Q4.1.4.a., and then click on the ' Root' button in the third menu bar that is activated at this time. Thus, an already structured tree is obtained where the common ancestor is the strain identified in question Q4.1.4.a.
- 隨後，按最接近 Q4.1.4.a 中確定菌株名稱的終點，然後按第三個選單中的“Root”按鈕。因此，可獲得一已經結構化的親緣關係樹，其中共同的祖先是 Q4.1.4.a 中確定的菌株。
- For a simpler view, tick the ' Transform branches' box in the right menu bar (Formatting submenu)
- 要獲得更簡單的視圖，勾選右側選單中“Transform branches”框（格式化子選單）
- Change the scaling from “Equal” to “Cladogram” using the scroll down menu just below.
- 使用下面的向下滾動選單將縮放從“Equal”更改為“Cladogram”。

Q.4.1.4.b Find the branch containing the sensitive mutants and specify the strain-number of the resistant strain(s) that is most closely related to these strains. **Write these numbers in the corresponding boxes on the answer sheet. (For example, if your answer is Strain-23, write '23' on the answer sheet.)**
找到具有易感性突變株的分支，並指定與易感性菌株最相近的抗藥性菌株的號碼。**將這些號碼寫在答案紙上的相對應方框中。**（例如，如果你的答案是 Strain-23，請在答題紙上寫上“23”。)

Q.4.1.4.c Specify the strain-number of the two sensitive strains that may have evolved most recently. **Write these numbers in the corresponding boxes on the answer sheet.** (For example, if your answer is Strain-23, write '23' on the answer sheet .)
指定可能是近期演化來的兩種易感性菌株的號碼。**將這些號碼寫在答案紙上的相對應方框中。**（例如，如果你的答案是 Strain-23，請在答題紙上寫上“23”。)

Q.4.1.4.d For each of the following groups of strains, specify whether it is monophyletic (A), or NON-monophyletic (B). **Indicate your answer by putting an X in the appropriate box on the answer sheet.**
關於下列每群的菌株，指定它是單系群（A）、或非單系群（B）。**在答案紙上的相應方框中輸入”X” 來代表你的答案。**
Groups:
I. 8; 12
II. 5; 6; 16
III. 3; 7; 10
Options:
A –monophyletic
A –單系群
B –NON-monophyletic
B –非單系群

Q.4.1.4.e Based on the phylogenetic tree obtained above, you can find autapomorphic, synapomorphic, and plesiomorphic mutations in certain nucleotide positions in the sequences examined in the first part.
根據上述親緣關係樹，你可在第一部分序列檢測中某些核苷酸位置發現” 獨有衍徵的” 、” 祖徵的” 和” 共衍徵的” 突變。



Return to the 16-line DNA sequence alignment obtained in the first part by double-clicking on the ‘Nucleotide alignment’ line and specify the types of mutations in the following positions from the perspective of the phylogenetic tree. **Indicate your answer by putting an X in the appropriate box on the answer sheet.**
經由雙擊“Nucleotide alignment”返回第一部分中獲得的 16 條 DNA 序列比對，並從親緣關係樹的角度來指定下列位置的突變類型。**在答案紙上的相應方框中輸入”X” 來代表你的答案。**
Positions:
位置：
51
144
312
447
531
Options:
A –autapomorphic
A –獨有衍徵的
B –plesiomorphic
B –祖徵的
C –synapomorphic
C –共衍徵的

SUBTASK 2. EXAMINATION OF NEURONAL ACTIVITY PATTERN ON MOVING ANIMALS

IMPORTANT TECHNICAL NOTES ON CHEATING

- **During the practice task, you will use the Neurophysiology (Neurofiziológia) software that is already running on your computer.**
- **Please do not use any other software, except for the Geneious software that you will use for the other bioinformatics task.**
- **Your computer records all of your activities, so if you open any other software program, or if you connect your computer to the internet, you will be excluded from this task and lose any points that you scored here.**

請注意以下說明，違者視為作弊

- 在回答本實作題時，你將使用已裝載於電腦上的神經生理學（Neurofiziológia）軟體。
- 除了使用於生物資訊學實作的 Geneious 軟體，請不要使用任何其他軟體。
- 你的電腦會記錄所有活動，因此，如果你打開任何其他軟體，或者試圖連上網路，本題將不予計分。

Please, do not start the software yet. Read the detailed descriptions on the next pages first.
不要啟動軟體，先閱讀下一頁的詳細說明。

Your task is to clarify the role of neurons involved in navigation. Navigating space is a complex problem involving many neurons. The 2014 Nobel prize was awarded to O' Keefe, and the 2011 Brain prize was awarded to the Hungarian Gyorgy Buzsaki, for developing techniques to study this problem. We can observe the individual activity of hundreds of neurons. Multiple electrodes are inserted into rodent brains. After the surgery, the animal recovers and adapts to the device on its head. Next, the animal is placed into experimental situations, and measurements are performed (Figure 1).

本題將闡明神經元在導航過程中所扮演的角色。空間導航是涉及許多神經元的複雜問題，2014 年諾貝爾獎頒給了 O' Keefe，而 2011 年 Brain 獎頒給了匈牙利 Gyorgy Buzsaki，以表彰其開發研究該問題的技術。利用在嚙齒類動物腦中植入多個電極，我們可以觀察並記錄數百個神經元的個體活動。植入電極手術後，休息幾天以讓動物恢復並適應頭部的裝置，接下來進行相關實驗並量測（圖 1）。

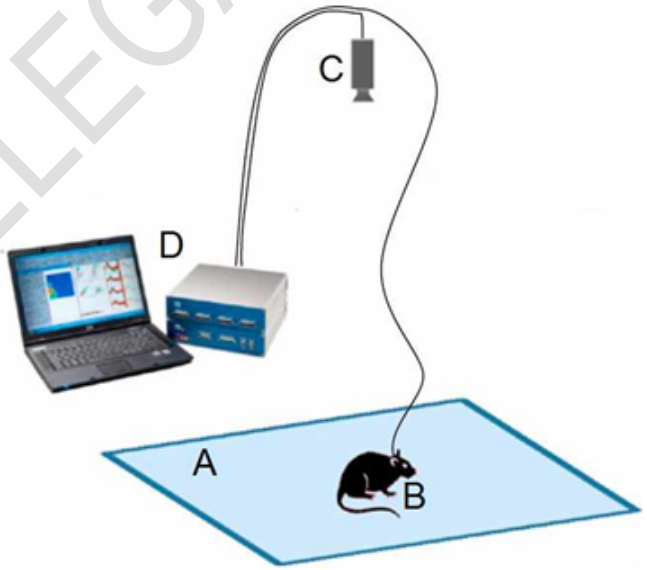


Figure 1. Experimental set-up. A: Exploration field. **B:** Animal with electrodes after surgery. **C:** Camera recording the path of the animal. **D:** Recording unit (records potential changes of brain regions and animal movements simultaneously)

圖 1. 實驗裝置。A：勘探領域。 **B：**手術後植入電極的動物。**C：**記錄動物活動軌跡路徑的錄影機。 **D：**記錄單元（可同時記錄大腦各區域電位變化和動物的移動）

Using similar techniques to you, O'Keefe investigated the hippocampus and found that many of its neurons fire when the animal is standing on or passing through a given point of its exploration field. These cells were named place cells. The results of such an experiment are shown on Figure 2.

O'Keefe 使用類似的技術研究海馬迴處神經元的變化，並發現當動物站立或穿過其探索區域的特定點時，有許多神經元會活化，這些細胞被命名為地區細胞 (place cell) 。這種實驗的結果如圖 2 所示。

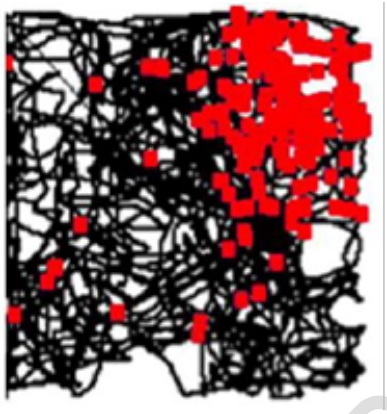


Figure 2. Place cell behaviour. Black line: The path a rodent took as it explored a square field. **Red dots:** places where a certain neurone fired.

圖 2. 地區細胞之行為。黑線: 嚙齒動物在探索正方形區域時所採取的路徑。紅點：特定神經元活化的位置。

The place cells described by O'Keefe are not measured in your data!
They are not a cell-type you should look for!
你的數據並未測量 O'Keefe 描述的地區細胞！
它們不是你應該尋找的細胞類型！

Summary of Tasks 實作摘要

Your task is to analyse the function of neurons in the entorhinal cortex. The task will consist of two parts:

本題是分析內嗅皮質中神經元的功能，包括兩部分：

- **Part 1.** You will have to analyse recordings of 30 cells. You will identify which neurons are responsible for perceiving the same aspects of movement and/or position in space. At first, you do not have to state which aspects of movement or position each neuron responds to, you only have to identify groups of cells performing similar functions.
- 第 1 部分。請分析 30 個神經元的數據。請指出哪些神經元負責感知相同的移動及 (或) 空間位置。首先，你不必說明每個相對應的運動或位置的哪個方面，你只需要指出執行類似功能的細胞群。
- **You may only begin the second part after completing the first part.**
- 完成第一部分後才可開始第二部分。
- **Part 2.** When beginning the second part, the lab assistant will provide you additional sheets with a short description of the exact function of the cells in part 1. After studying these descriptions, you will have to state the precise aspects of movement/space which six neurons respond to.
- 第 2 部分。在開始第二部分時，實驗室助教將提供您提供額外說明，其上有第一部分細胞實際功能的簡單描述。仔細閱讀後，你必須清楚說明對應動物移動或空間認知的六個相對應神經元的表現。

The background of your experiment:

實驗所需知識背景：

1. A rat implanted with electrodes was allowed to explore a 2m x 2m square field. 大鼠植入電極後置於 2m×2m 的正方形區域。
2. We recorded the animal’s movement and the activity of different neurons. 我們記錄了動物的運動和不同神經元的活性。
3. Results are displayed in the Neurophysiology software. 結果顯示在神經生理學軟體中。
4. You can switch between the NEUROPHYSIOLOGY and the Geneious Prime softwares by clicking on the system tray or using the Alt+Tab key combination. 你可以直接點擊系統鍵 (system tray) 或同時按下 Alt + Tab 組合鍵以切換 NEUROPHYSIOLOGY 和 Geneious Prime 軟體。
5. **Do not use the software before reading the tasks. 沒閱讀題目及說明之前，請勿使用軟體。**

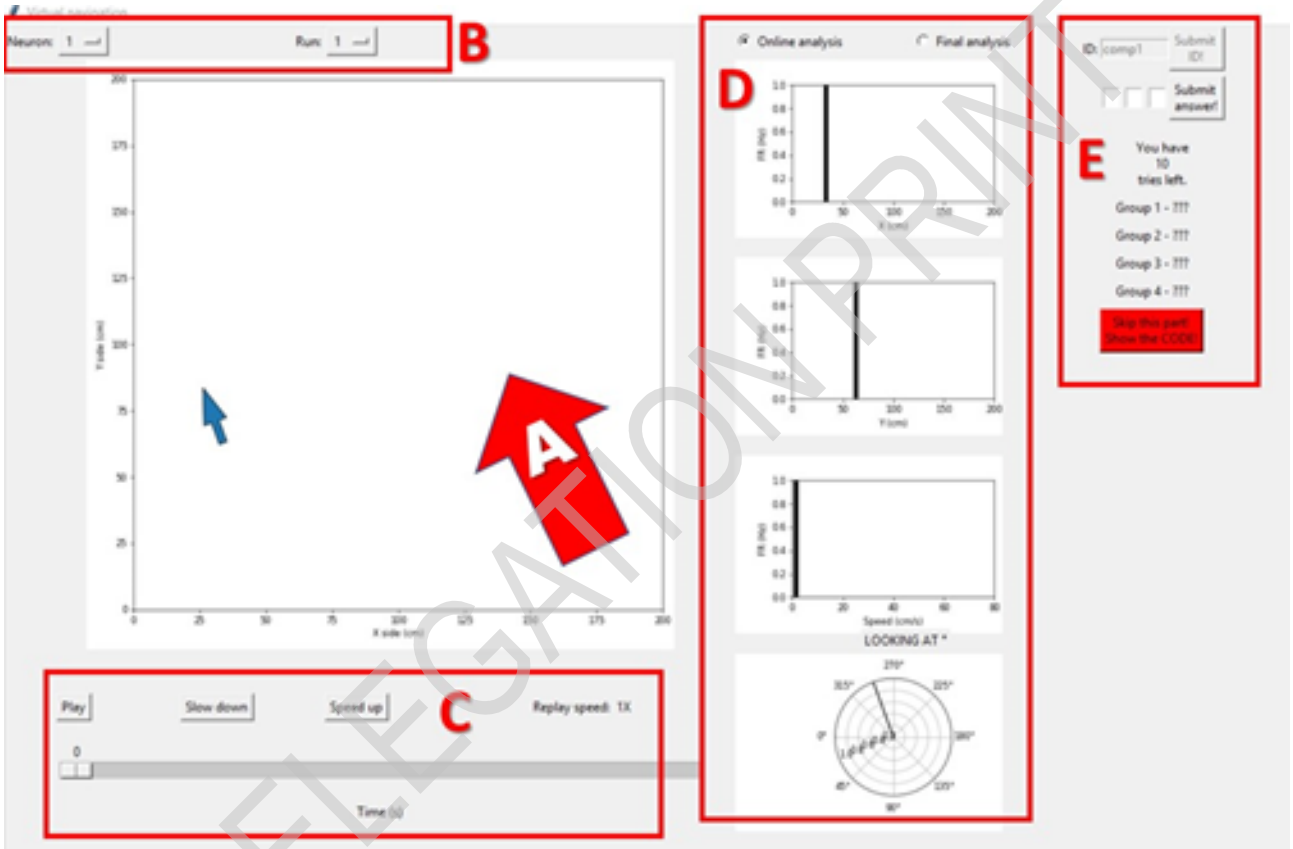


Figure 3. Software overview. A: Exploration field. B: Selection of neuron and run. C: Playback control. D: Statistical information on the actual run. E: Answer block

圖 3. 軟體使用說明。A：探索場域。B：選擇神經元並執行。C：回放一次 (playback)。D：實際執行的統計數據。E：答案區

Part 1 第 1 部分

Your software displays the route of the experimental animal continuously in time. You are able to select individual neurons, and red dots appear on the animal’s route when the firing of this neuron changes significantly. Each animal was recorded doing three separate runs, so three data sets are available for each neuron. (Figure 4-6).

你的軟體會持續並即時顯示實驗動物的運動路線。您可以選擇單個神經元，當該神經元的活性發生顯著變化時，動物的路徑上會出現紅點。每隻動物將進行三次實驗並記錄之，因此每個神經元可獲得三個數據組。(圖 4-6)。

In Part 1, the animal’s route will fade with time, so you can only observe a portion of the rat’s movements.

在第 1 部分中，動物的路徑會隨著時間而消退，因此你只能觀察到大鼠的部分移動。

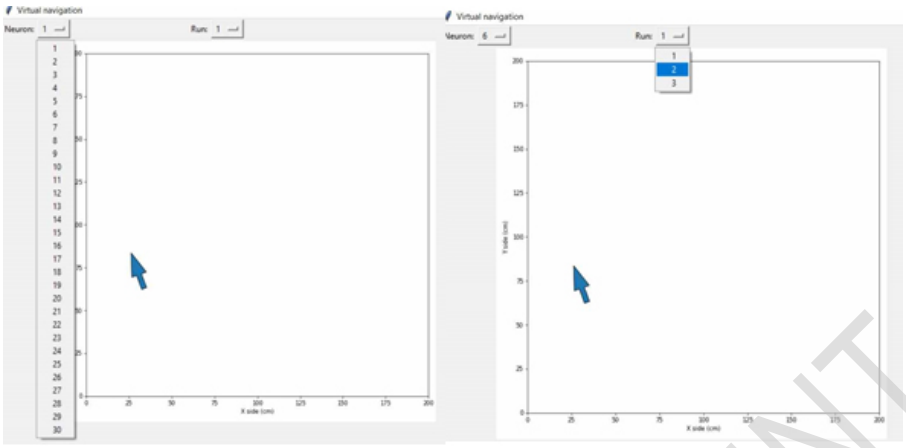


Figure 4. Selecting neurones and runs. In this menu, you can switch between individual neurons (“neurons” 1-30). Click on “run” and choose 1-3 from the drop-down menu.

圖 4. 選定神經元並執行。在選單中，你可以在單一神經元之間切換 (“神經元” 1-30)。按下“執行鍵 (run)” 後從下拉式選單中選擇 1-3。

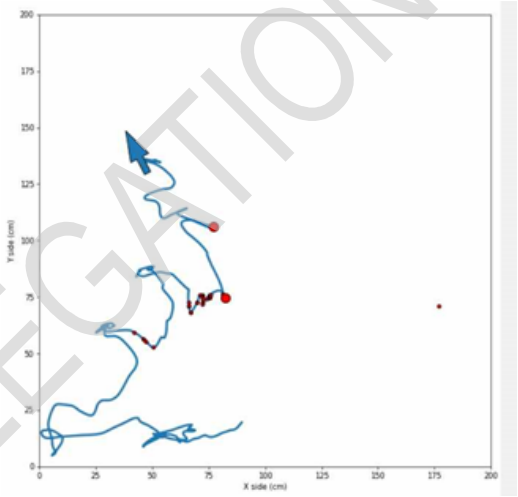


Figure 5. Exploration field. Blue shows route of animal, red dots show neurone activity and the arrow shows the direction of the head.

圖 5. 探索場域。藍色顯示動物的路線，紅色點顯示神經元的活性，而箭頭顯示頭部的方向

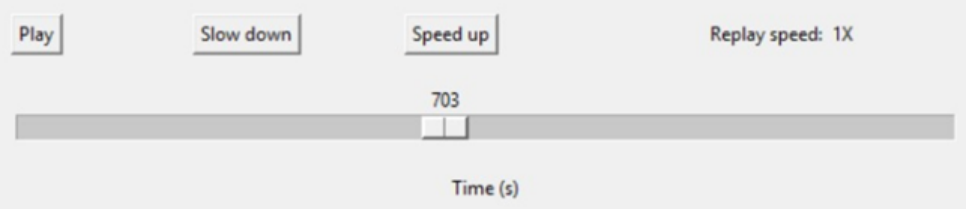


Figure 6. Playback control. You can start (‘Play’) or pause (‘Pause’) the run, or you can speed up (‘Speed Up’) or slow down (‘Slow Down’) the playback. Using the slider, you can jump forward or backward in a run. **Please be patient when using the slider, the software may take a few seconds to load.**

圖 6. 回播控制。你可以開始（‘Play’）或暫停（‘Pause’）程式執行，或者您可以加速（‘speed up”）或減緩（‘slow down’）執行。利用游標，您可以在向前或向後移動。**使用游標時請耐心等待，軟體可能需要幾秒鐘才能下載與執行。**

You will see aggregated data of the activity of the neuron (y-axis) plotted against aspects of its movement (Figure 7).

圖 7 為神經元活動的整合數據（y 軸）與動物移動的關係圖（圖 7）。

‘**Online Analysis**’: the graphs show aggregated data collected until the current time point of the current playback. The rat’s current position is represented by black lines on the graphs.

“即時 (on line) 分析”：圖表顯示當次實驗目前所收集的所有數據。圖表上的黑線表示大鼠的目前位置。

‘**Final Analysis**’: the graphs show aggregated values of the entire current run. The black line returns to the 0 positions.

“最終分析”：圖表顯示整個程式執行的匯總值，此時黑線返回位置 0 處。

FR(Hz) vs. X (cm): the firing frequency of the neuron is plotted against the animal’s horizontal distance measured from the lower left corner of the exploration field.

神經活性 (FR:Hz) 與水平移動距離 (X: cm) 的關係: 神經元的活化頻率與動物水平移動距離的關係圖，水平移動距離可由實驗區左下方測得。

FR(Hz) vs. Y (cm): the firing frequency of the neuron is plotted against the animal’s vertical distance measured from the lower left corner of the exploration field.

神經活性 (FR:Hz) 與垂直移動距離 (Y: cm) 的關係: 神經元的活化頻率與動物垂直移動距離的關係圖，垂直移動距離可由實驗區左下方測得。

FR (Hz) vs. Speed (cm/s): the firing frequency of the neuron is plotted against the animal’s linear speed.

神經活性 (FR:Hz) 與移動速度 (S: cm/s) 的關係：神經元的活化頻率與動物線性移動速度的關係圖。

LOOKING AT: the firing frequency of the neuron mapped against the angle between the animal’s direction of gaze and the straight line connecting the centre of the exploration field with its lower left corner.

關注重點：” 神經元活化頻率” 對” 動物注視方向及其連接探索場域左下角之中心線所成角度” 所製作的圖。

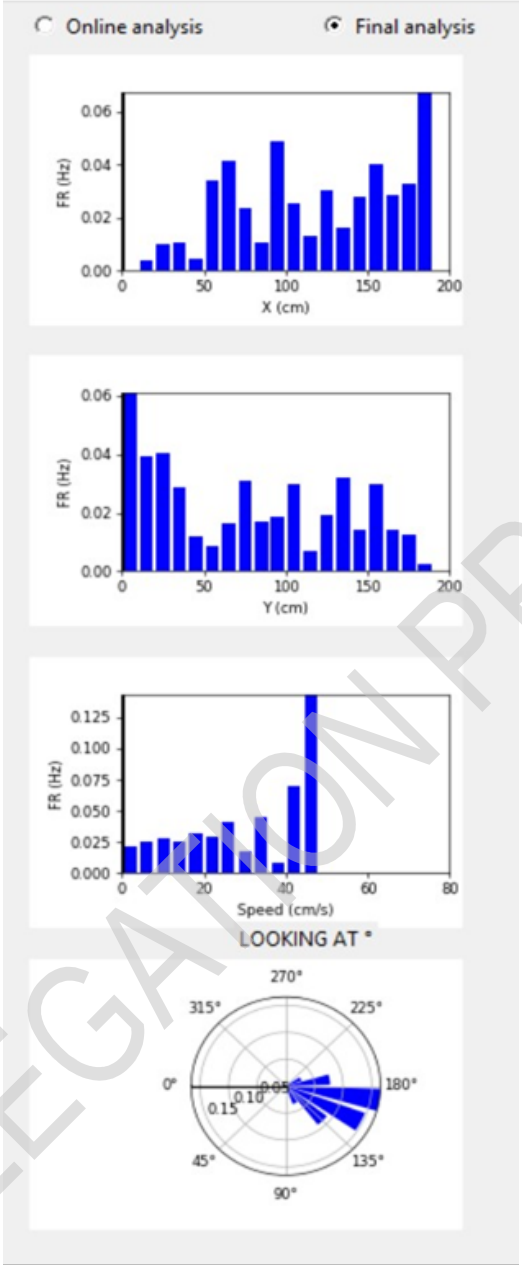


Figure 7. Statistical information on the run

本次測試的統計數據

4.2.1.Q You will have to use the exploration field and statistical information to determine whether each neuron has a function in the perception of movement and position characteristics. You will then have to find cells with similar function.
你必須使用探索場域實驗及統計數據，以釐清每一個神經元在運動及位置認知過程中所扮演之角色，接下來找出具類似功能的細胞。

- When you have an idea what the function of a certain neuron is, use the software to look for cells with similar function.
- 當你了解某個神經元的所具有的功能時，繼續使用該軟體來尋找具有類似功能的細胞。
- **The cells measured in your data are divided into FOUR groups based on the different charac-**

teristics of movement and position they respond to.

- **根據細胞對運動和位置的不同特徵，將實驗所測得細胞分為四組。**
- **Each cell only responds to one of these four aspects of movement and position.**
- 每個細胞僅回應運動和位置四大特徵之一種特徵。
- **Approximately half of the 30 cells measured in your data do not respond to movement or position. These are NOT cells which you are interested in.**
- **在資料中測量的 30 個細胞中, 大約一半對個體的移動或位置沒有回應。這些細胞不必理會。**
- Your data includes at least FOUR neurons from each group.
- 你的數據每組至少包含四個神經元。
- **You must find THREE neurons from each group**
- **你必須從每組中找到三個神經元。**
  - I.e. you must identify four groups of three neurons
  - 即你必須識別四組，每組三個神經元。
  - All the neurons in each group you enter should have the same function
  - 你所輸入的每個組中的所有神經元應具有相同的功能 (同組神經元具相同功能)
  - All the groups you enter should have different functions
  - 你所輸入的所有組別彼此應具有不同的功能

You have a maximum of TEN ATTEMPTS at entering groups of three neurons to find all four categories
請注意，你最多只有十次機會 可以輸入三個神經元去找出所有的四個類別

- **You will receive fewer marks for each attempt**
- **測試次數越多，所得分數越少**
  - I.e. You should aim to find all four groups in your first four attempts
  - 即你應該試圖在前四次測試中找到所有四組
  - You can use table 1 to keep track of the attempts you make. It will NOT be marked.
  - 你可以使用表 1 來追蹤你的測試，注意，它不會被計分。

T1			
T2			
T3			
T4			
T5			
T6			
T7			
T8			
T9			
T10			

Table 1

When you identify three neurons from the same group, enter them into the answer block (Figure 8). Repeat for each group.

從同一組中識別出三個神經元時，將它們輸入到答案區中（圖 8），並重複此步驟於每組。

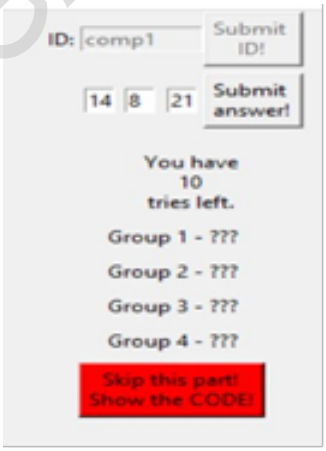


Figure 8. Answer block

圖八，答案區

Write the number of three neurons into the rectangles, then click “Submit answer”. The system counts down your attempts from 10 (“You have 10 tries left”)

將三個神經元的數目寫入矩形, 然後按一下” 答案送出: submit answer”。此時系統將顯示你僅剩之作答次數(從 10 遞減)(例如”你還有 10 次作答機會)。

If you answer is correct you will see this box (Figure 9). Click “OK” and continue to work.

如果你的答案是正確的，你就會看到這個畫面 (圖 9)，按一下” 確定” 並繼續答題。

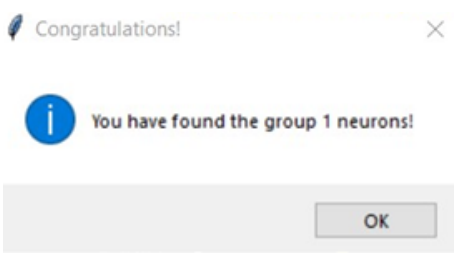


Figure 9. Correct answer

圖九，正確答案

If your answer is correct, but the neurons are in a group that you have already correctly submitted, then you will see this box (Figure 10). This will count as a false attempt and the number of remaining attempts will decrease. Click “OK” and continue to work.

如果你的答案是正確的，但是這些神經元在一個你已經送出正確答案的組別中，那麼你會看到這個框 (圖 10)。這將列為錯誤測試，並降低你剩下的測試次數。按一下” OK” 並繼續答題。

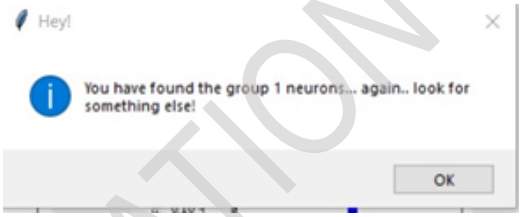


Figure 10. Re-discovery of a group

圖 10，再找找吧 重新尋找組別

If you submit three neurons which are not in the same group, you will see not see a pop-up box, but the number of remaining attempts will decrease, and this will count as a false attempt. Continue to work.

如果送出三個不在同一組中的神經元，你將不會看到快顯視窗，但剩餘測試次數將減少，此將視為錯誤嘗試。繼續作答。

When you correctly identify three neurons in each of the four groups, part 1 will end. Or, when you run-out of attempts, part 1 will end. You will see this box (Figure 11).

當你已正確地識別出四組，每組各三個神經元時，或你已用完十次測試機會時，即結束第 1 部分，你將看到此圖 (圖 11)。

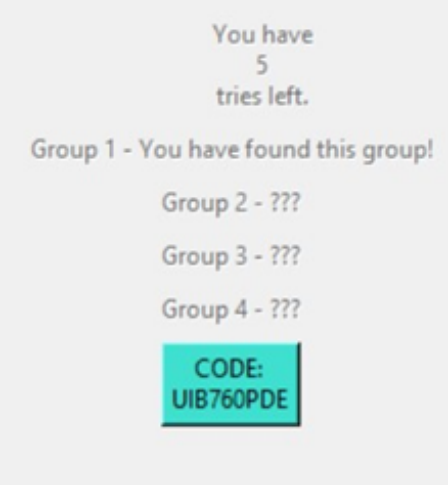


Figure 11. Completion of Part 1.

圖十一，第一部分結束

You MUST write the “CODE: #######” on your ANSWER SHEET.
你必須在” 答案表” 上寫上” 代碼: : #######”

Alternatively, you **may choose to end Part 1** before you run out of attempts to save time. Click “Skip this part! Show the CODE!” This box will appear (Figure 12). Click “YES” to end the task and reveal your code. This is irreversible, you will receive marks for the correct groups you have discovered but it will count as using all ten attempts. You must write your code on your answer sheet. Click “NO” to return to part 1 and continue working.

此外，你也可以選擇測試完十次機會前即**結束第 1 部分**以節省時間。點擊” 跳過此部分! 顯示代碼!”，螢幕即會顯示此圖 (圖 12)。按下” 是” 即結束第一部分之作答並顯示代碼。請注意，一旦按下，即結束第一部分作答且不可回復。你將得到你用了十次機會所發現正確組別之計分，您必須在答卷上寫上代碼。若按” 否” 則返回第 1 部分並繼續作答。

Figure 12. Giving-up

圖 12. 放棄作答!

After completing part 1 you MUST hold up your YELLOW CARD.
完成第 1 部分後，你必須立刻舉起黃卡。
An assistant will check that you have correctly written the code.
助教將檢查你是否正確寫出代碼。
An assistant will give you INFORMATION necessary for PART 2.
助教將為你提供第 2 部分作答所需的資訊。

Part 2

Read the information given to you by the assistant describing five different groups of neurons (A, B, C, D & E). Your software will now display the whole route of the animal without fading (Figure 13).

閱讀助教所提供描述五組不同的神經元 (A、B、C、D & E) 的資訊,。你的軟體目前將顯示動物的整個移動且未消退之路徑 (圖 13)

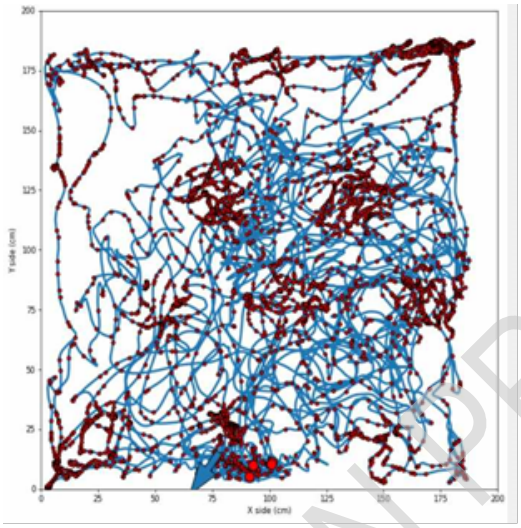


Figure 13. Exploration field for Part 2.

圖 13，第二部分之探索場域

Six specific neurons are listed on your answer sheet (Number 23, 17, 9, 25, 3 & 11). View the Exploration fields of runs 1-3 for each of these neurons. Identify which of the five different groups each neuron belongs to.

答案表上列出了六個特定的神經元 (編號 23、17、9、25、3 & 11)。查看每個神經元的前 3 次 (runs 1-3) 的探索場域圖。確定每個神經元屬於的五個不同組中的哪一組。

Q.4.2.2 Put an X on your ANSWER SHEET identifying which GROUP EACH NEURON belongs to.
在答案表上畫 X，以識別每個神經元隸屬於哪個組別。

Extra information on neuron types 有關神經元類型的額外資訊

A) Speed cells 速度細胞

Speed cells' firing rate is proportional to the linear speed of the animal

速度細胞的神經活性與動物的線性速度成比例關係

Researchers recorded neuronal activity whilst controlling rats' running speed. Rats were put in a bottomless frame which was moved at pre-set speeds, compelling them to run (Fig. 1a). Rats either ran with gradually increasing speed (Figure 1b left, middle), or with a sharp transition in the middle (Figure 1b right).

研究人員記錄到可控制大鼠運動速度的神經活性。老鼠被置於一個無底網架上以預設的速度移動，以強迫大鼠運動 (圖 1a)。大鼠除可逐漸加速運動外 (圖 1b 左, 中) ，亦可在中間快速轉換 (圖 1b 右)。

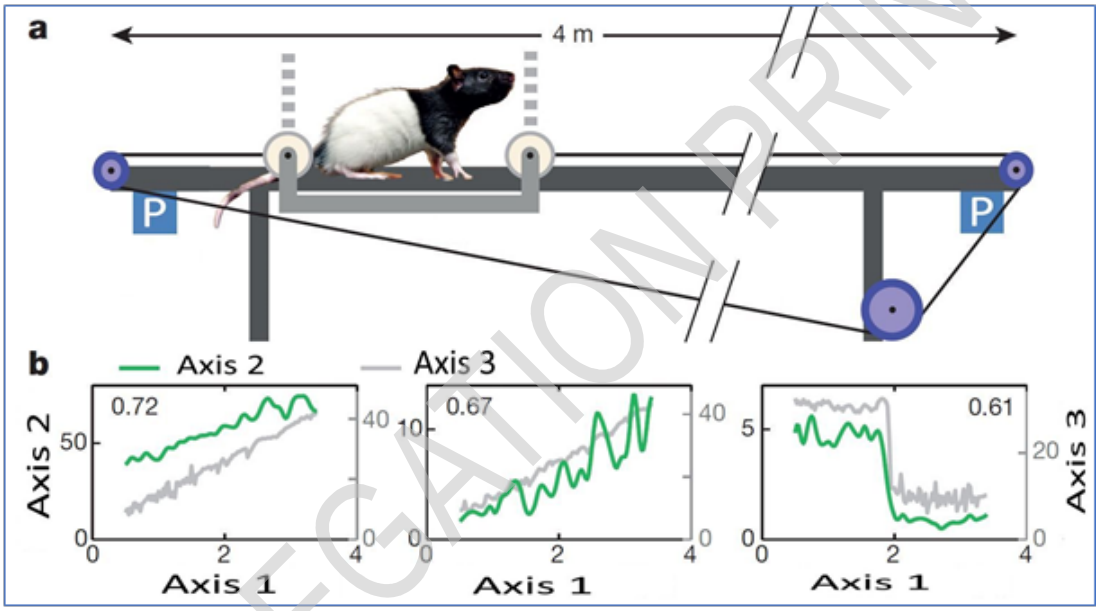


Figure 1. Speed set-up. Axis 1: Position in track (m). Axis 2: Firing Rate (Hz). Axis 3: Speed (cm/s)

圖 1. 速度測試裝置。軸 1: 在軌道 (m) 中的位置。軸 2: 神經元活化速率 (Hz)。軸 3: 速度 (cm/s)

The other cell-types were discovered with the experimental design shown in Part 1.
第 1 部分實驗所得其他細胞種類。
Results shown in Figure 2.
結果如圖 2 所示。

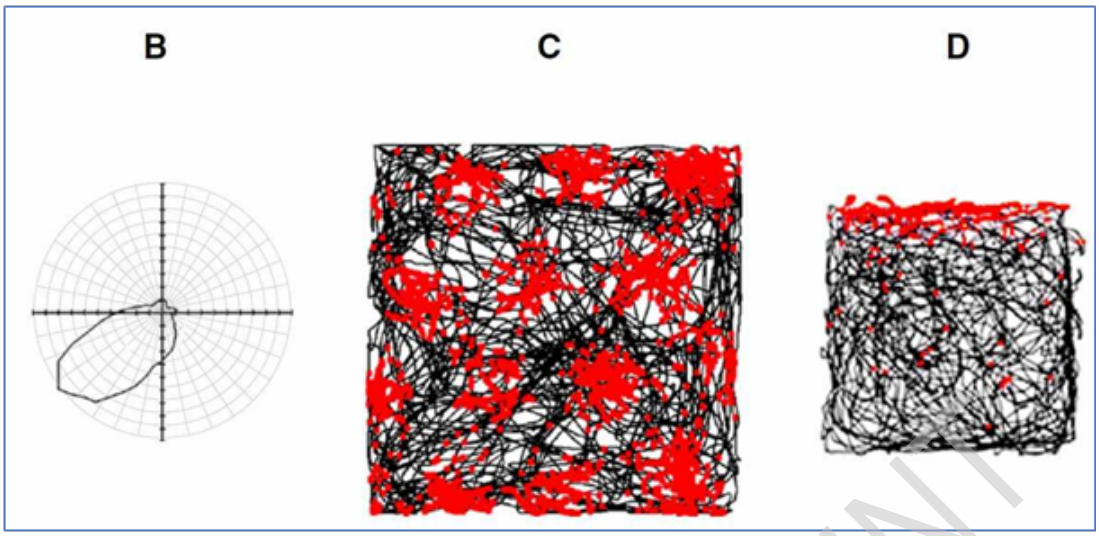


Figure 2

圖二

B) Head direction cells 頭部導向細胞

The diagram depicts the firing rate (distance from origin) as a function of the animal’s head direction. Head direction cells fire anywhere in an environment when the animal is looking in a certain direction.

本圖顯示了動物頭部的方向與神經活化速率 (距離從原點) 之關係圖。當動物向某個方向看時，會活化所有方向之頭部導向細胞。

C) Grid cells 網格細胞

Grid cells have firing areas evenly spaced in a close-packed hexagonal array across the surface of the field.

網格細胞的活化區域彼此間有規則的間距，並且在空間表面排成緊密堆積的六邊形陣列。

D) Border cells 邊界細胞

Border cells only fire when the rat is in close proximity to the wall of the field.

邊界細胞只有在大鼠靠近測試場域的四面牆壁時才會活化。

E) Bulk cells 其餘大部分細胞

These cells do not show a clear firing pattern when the animal is moving even though they are often firing during locomotion. This means we do not understand their function yet.

雖然在移動時，這些細胞通常會活化，但並無明確的神經活化模式。這意味著我們還不知道它們的確實功能。