Εύρηκα

探討巨噬細胞對神經疼痛的影響

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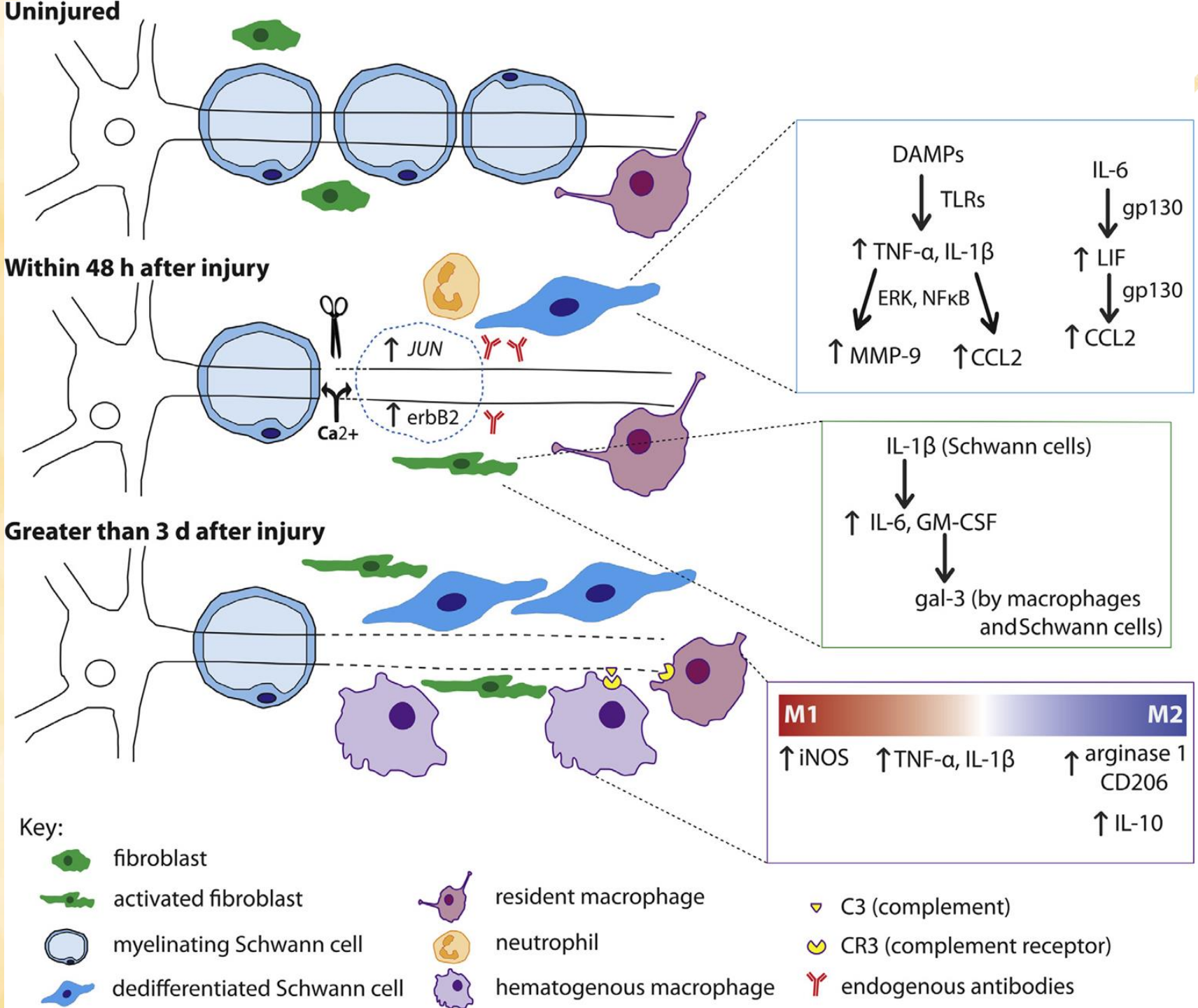
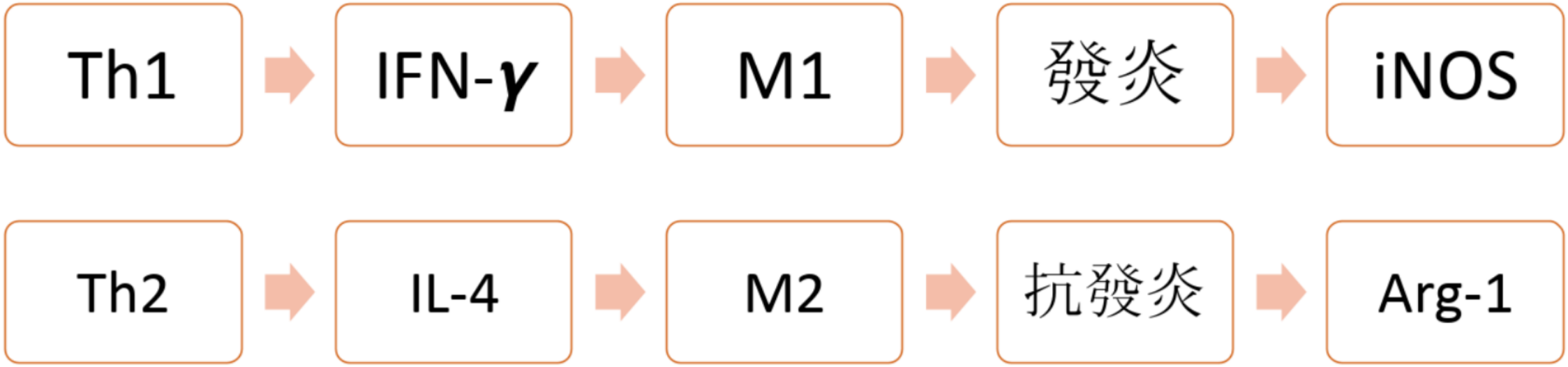
壹、 研究動機與背景

- 瓦勒氏退化(Wallerian degeneration)
- 神經痛
- 巨噬細胞
- clodronate liposome


Uninjured
Within 48 h after injury
↑ JUN
↑ erbB2
Ca2+
Greater than 3 d after injury
DAMPs
TLRs
↑ TNF-α, IL-1β
ERK, NFκB
↑ MMP-9
↑ CCL2
IL-6
gp130
↑ LIF
gp130
↑ CCL2
IL-1β (Schwann cells)
↑ IL-6, GM-CSF
gal-3 (by macrophages and Schwann cells)
M1
M2
↑ iNOS
↑ TNF-α, IL-1β
↑ arginase 1
CD206
↑ IL-10
Key:
fibroblast
activated fibroblast
myelinating Schwann cell
dedifferentiated Schwann cell
resident macrophage
neutrophil
hematogenous macrophage
C3 (complement)
CR3 (complement receptor)
endogenous antibodies

極化作用 (polarization)

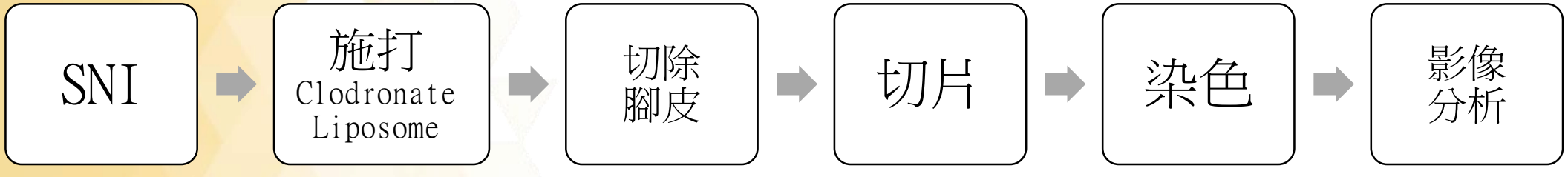
- M1 發炎、吞噬、攻擊相關
- M2 抑制免疫、纖維化、組織修復



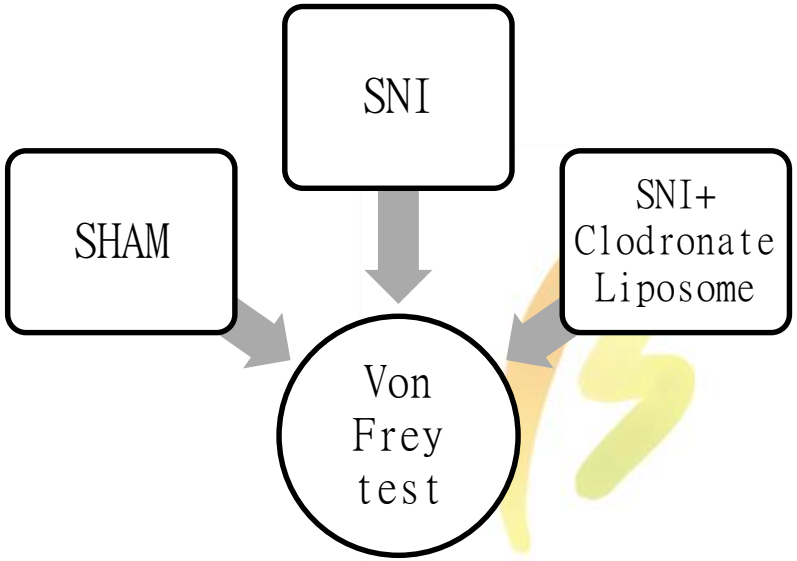
貳、 研究目的

- 利用 SNI 技術做出坐骨神經受傷的老鼠
- 取老鼠腳皮組織切片觀察觀察巨噬細胞數量變化
- 試驗 clodronate liposome 對免疫細胞的抑制作用

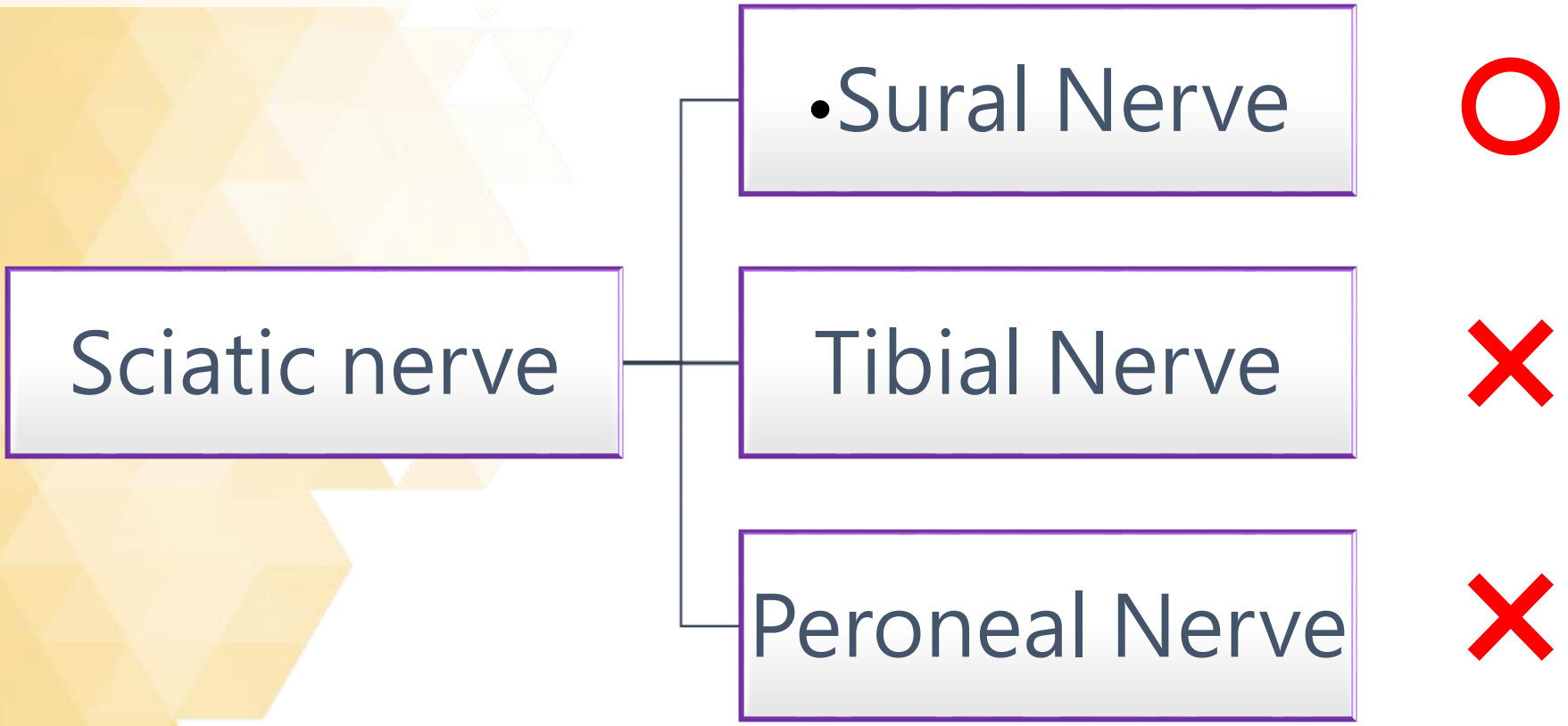
參、 研究流程


SNI
施打
Clodronate
Liposome
切除
腳皮
切片
染色
影像
分析


SNI/
SHAM
切除
腳皮
切片
染色
影像
分析


SNI
SHAM
SNI+
Clodronate
Liposome
Von
Frey
test

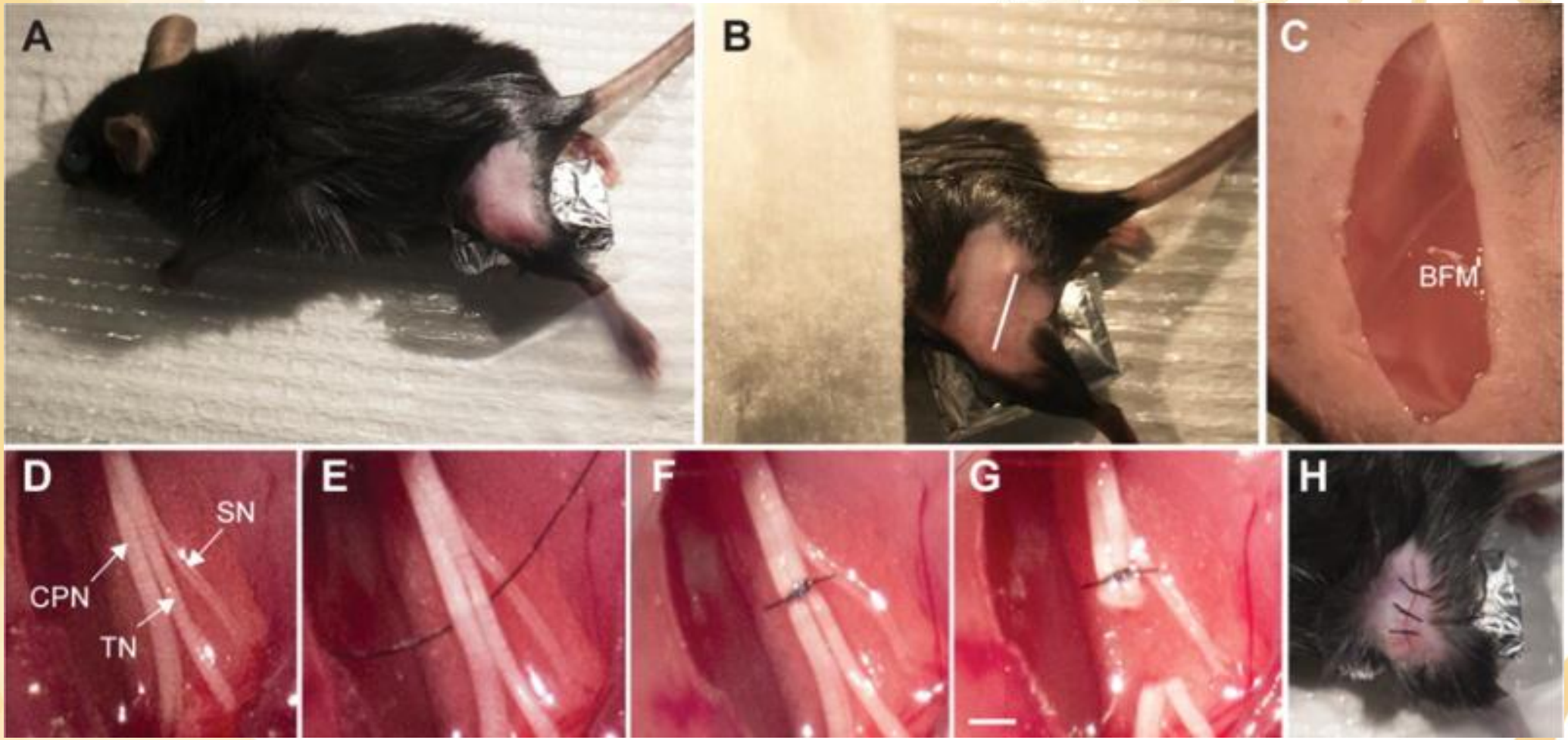
什麼是SNI手術呢?



○

×

×



(Joseph Cichon et al., 2018)

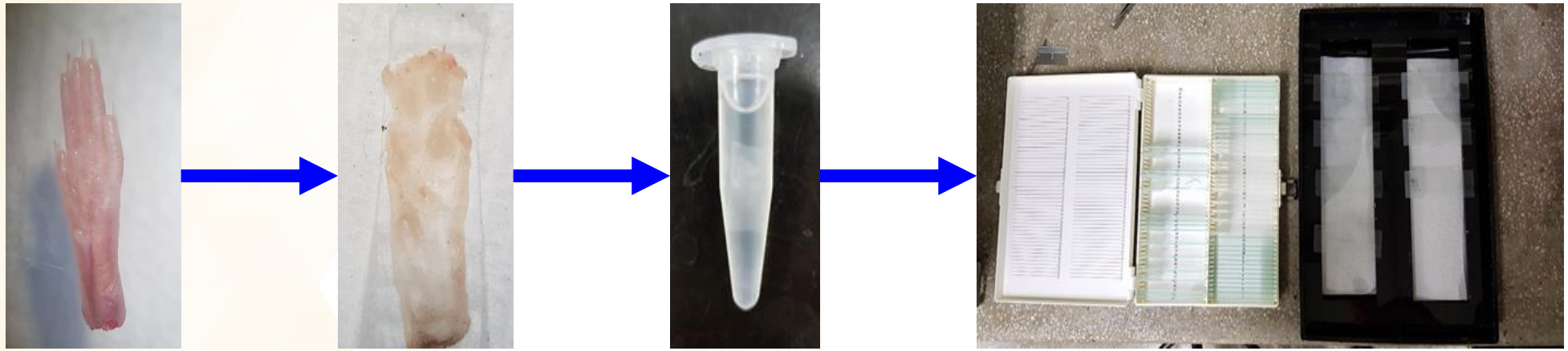
控制組

SHAM手術

- 保留所有神經一刀不剪，縫合皮膚

肆、 研究過程或方法

- 二、測試方法
- (一)切片染色



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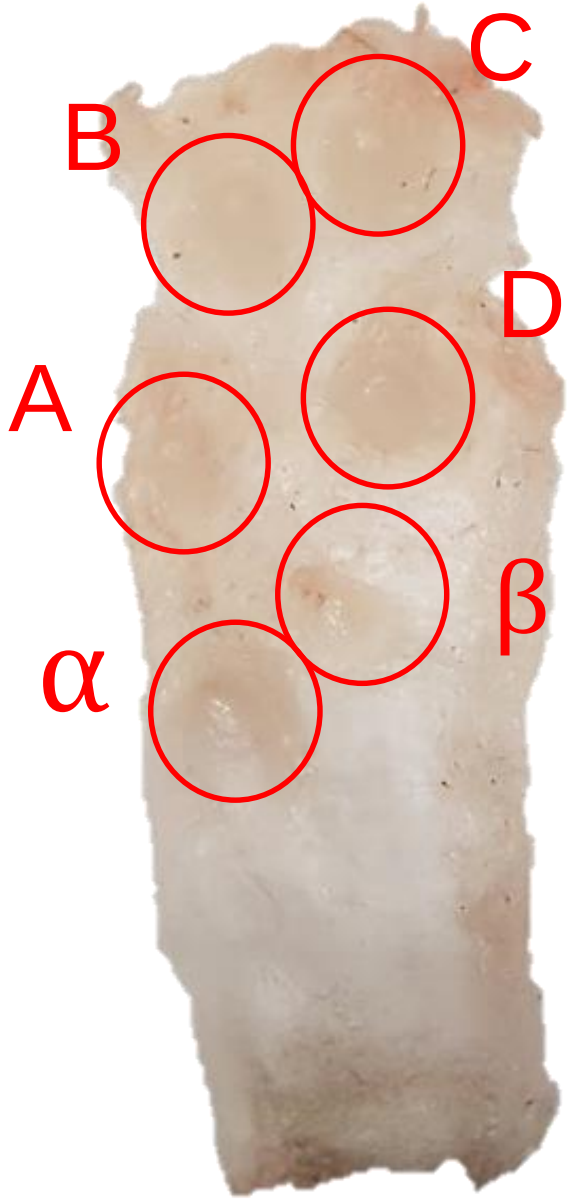

β

肆、 研究過程或方法

- 染色

Day 1		
1.fixation	ice-cold acetone	5min, on ice
2.washing	0.3%PBST	10min*3, RT
3.blocking	2%BSA in PBST	30min, RT
4.1ºAb	anti-mouse F4/80 1:500 in 1%BSA	o/n, 4ºC

Day 2		
5.washing	0.3%PBST	10min*3, RT
6.2ºAb	Gt*Rat A594 1:500,DAPI 1:100 in PBST	2hr, RT
7.washing	0.3%PBST	10min*3, RT

痛覺怎麼量化?

- 老鼠行為測試
- 1.對老鼠做進行 von frey 測試，從0.16g開始，之後依序為0.4g、0.6g、1.0g、1.4g，若老鼠可承受之，因而沒有做出立即反應，便增加力量，並紀錄圈號。反之，則紀錄叉號並減輕重量。測量左右兩腿，只要測到一次轉折 (即圈號或叉號不再連續)，後再測量四次即可停止，一隻腳一天測量三組數據。

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A

Starting from 0.6g, remain in the first row when testing mice until a different response occurs.

Then, test one filament strength in each row until either all five rows are filled out, or there is no further filament strength to test.

0.04	0.07	0.16	0.4	0.6	1	1.4	2	4
		O	X	X				
			X					
		O						
			X					
		O						

B

Beginning with the mid-range filament, record in the first row until a different response occurs.

End test when the animal fails to respond to the strongest filament (or if the weakest elicits a response).

0.04	0.07	0.16	0.4	0.6	1	1.4	2	4
				O	O	O	O	X
							O	
								O

C

If an error is made, strike through at least twelve boxes and proceed to a new table.

UDReader will process the mistake and display N/A in the readout for this error.

0.04	0.07	0.16	0.4	0.6	1	1.4	2	4
				O	O	X		
					X			
			O	O				

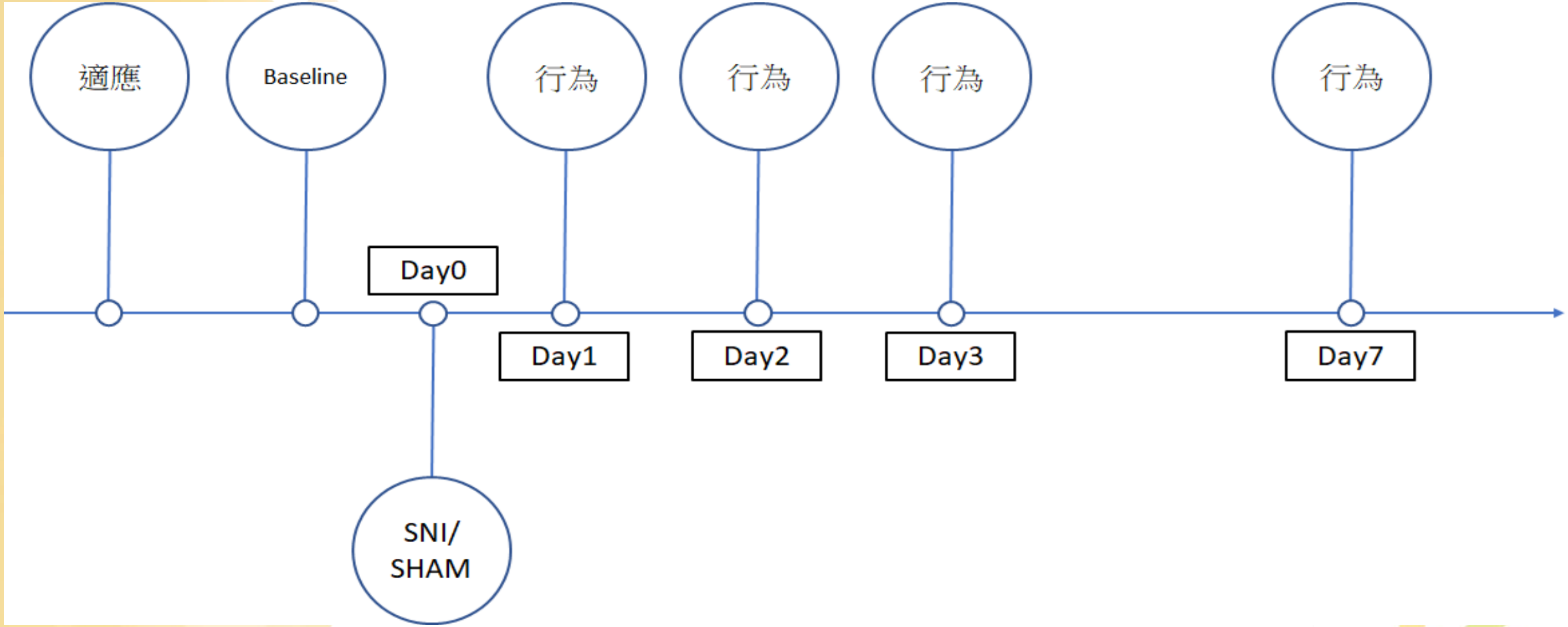
2.Von Frey 換算數據方法

50% g threshold(50%機率抬腳時的使用的Von Frey克數) = 10^(x+ka)/10,000，x為final克數對應的size，k為觀察數據對應的數值(例如OOOXOXO對應0.741)，a為所有Size間的interval之平均值
0.315(S.R.Chaplan et al.,2018)

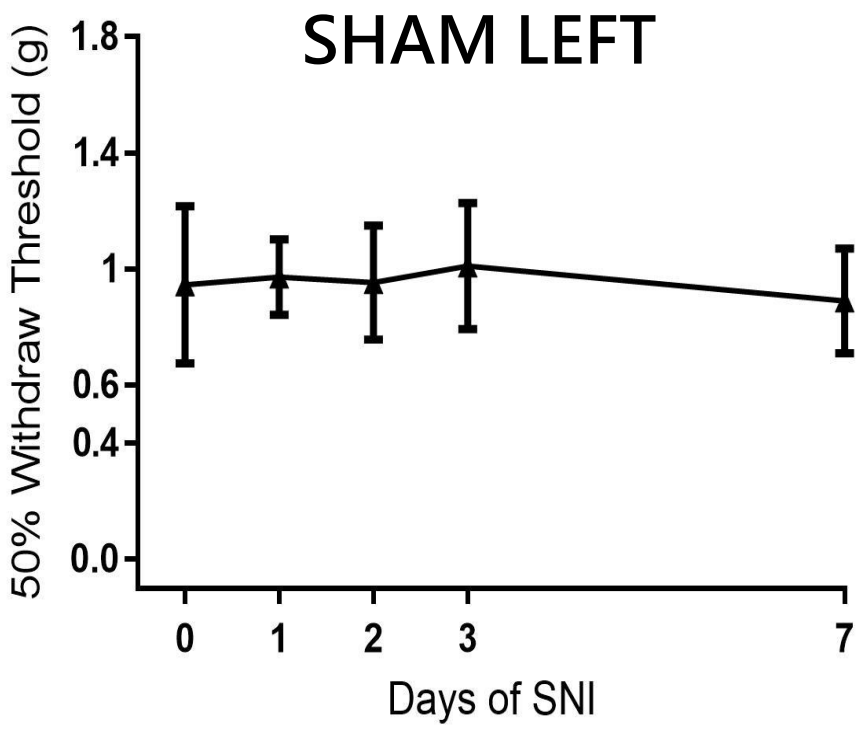
Force(g)	Size	interval
0.008	1.65	
0.02	2.36	0.71
0.04	2.44	0.08
0.07	2.83	0.39
0.16	3.22	0.39
0.4	3.61	0.39
0.6	3.84	0.23
1	4.08	0.24
1.4	4.17	0.09

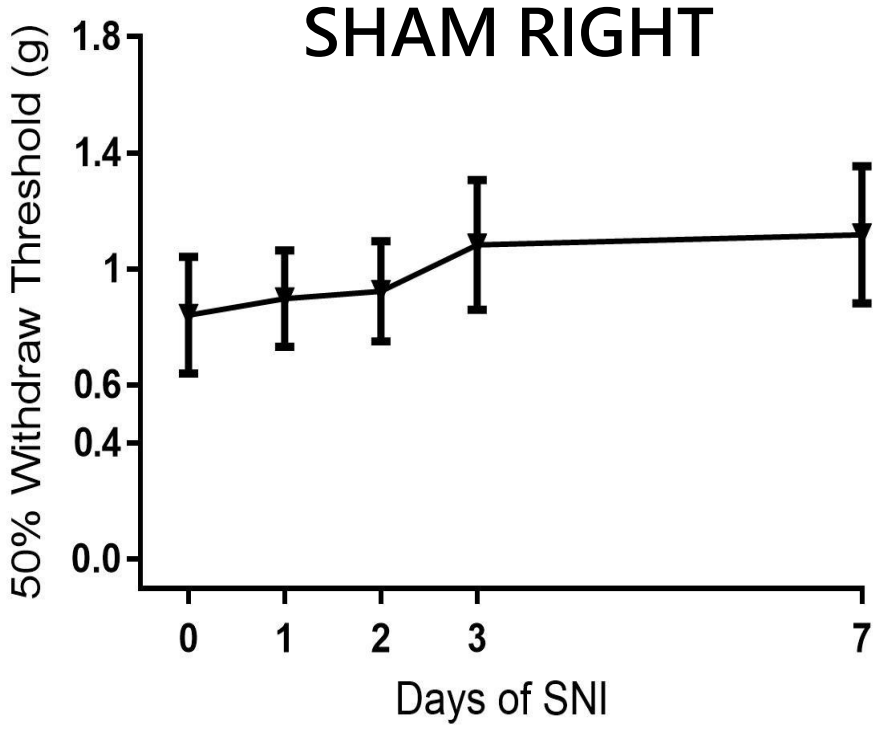
Pattern	Value for k	Pattern	Value for k	Pattern	Value for k	Pattern	Value for
OX	−0.5	OOOXOOOO	−0.547	XO	0.5	XXXOXXXX	0.547
OOX	−0.388	OOOOXOOOO	−0.547	XXO	0.388	XXXXOXXXX	0.547
OOOX	−0.378	OXOOOX	−1.25	XXXO	0.378	XOXXXO	1.25
OOOOX	−0.377	OOXOOOX	−1.247	XXXXO	0.377	XXOXXXO	1.247
OXO	0.842	OOOXOOOX	−1.246	XOX	−0.842	XXXOXXXO	1.246
OOXO	0.89	OOOOXOOOX	−1.246	XXOX	−0.89	XXXXOXXXO	1.246
OOOXO	0.894	OXOOXO	0.372	XXXOX	−0.894	XOXXOX	−0.372
OOOOXO	0.894	OOXOOXO	0.38	XXXXOX	−0.894	XXOXXOX	−0.38
OXX	−0.178	OOOXOOXO	0.381	XOO	0.178	XXXOXXOX	−0.381
OOXX	0	OOOOXOOXO	0.381	XXOO	0	XXXXOXXOX	−0.381
OOOXX	0.026	OXOOXX	−0.169	XXXOO	−0.026	XOXXOO	0.169
OOOOXX	0.028	OOXOOXX	−0.144	XXXXOO	−0.028	XXOXXOO	0.144
OXOO	0.299	OOOXOOXX	−0.142	XOXX	−0.299	XXXOXXOO	0.142
OOXOO	0.314	OOOOXOOXX	−0.142	XXOXX	−0.314	XXXXOXXOO	0.142
OOOXOO	0.315	OXOXOO	0.022	XXXOXX	−0.315	XOXOXX	−0.022
OOOOXOO	0.315	OOXOXOO	0.039	XXXXOXX	−0.315	XXOXOXX	−0.039
OXOX	−0.5	OOOXOXOO	0.04	XOXO	0.5	XXXOXOXX	−0.04
OOXOX	−0.439	OOOOXOXOO	0.04	XXOXO	0.439	XXXXOXOXX	−0.04
OOOXOX	−0.432	OXOXOX	−0.5	XXXOXO	0.432	XOXOXO	0.5
OOOOXOX	−0.432	OOXOXOX	−0.458	XXXXOXO	0.432	XXOXOXO	0.458
OXXO	1	OOOXOXOX	−0.453	XOOX	−1	XXXOXOXO	0.453
OOXXO	1.122	OOOOXOXOX	−0.453	XXOOX	−1.122	XXXXOXOXO	0.453
OOOXXO	1.139	OXOXXO	1.169	XXXOOX	−1.139	XOXOOX	−1.169
OOOOXXO	1.14	OOXOXXO	1.237	XXXXOOX	−1.14	XXOXOOX	−1.237
OXXX	0.194	OOOXOXXO	1.247	XXOOO	−0.194	XXXOXOOX	−1.247
OOXXX	0.449	OOOOXOXXO	1.248	XXOOO	−0.449	XXXXOXOOX	−1.248
OOOXXX	0.5	OXOXXX	0.611	XXXOOO	−0.5	XOXOOO	−0.611
OOOOXXX	0.506	OOXOXXX	0.732	XXXXOOO	−0.506	XXOXOOO	−0.732
OXOOO	−0.157	OOOXOXXX	0.756	XOXXX	0.157	XXXOXOOO	−0.756
OOXOOO	−0.154	OOOOXOXXX	0.758	XXOXXX	0.154	XXXXOXOOO	−0.758
OOOXOOO	−0.154	OXXOOO	−0.296	XXXOXXX	0.154	XOOXXX	0.296
OOOOXOOO	−0.154	OOXXOOO	−0.266	XXXXOXXX	0.154	XXOOXXX	0.266
OXOOX	−0.878	OOOXXOOO	−0.263	XOXXO	0.878	XXXOOXXX	0.263
OOXOOX	−0.861	OOOOXXOOO	−0.263	XXOXXO	0.861	XXXXOOXXX	0.263
OOOXOOX	−0.86	OXXOOX	−0.831	XXXOXXO	0.86	XOOXXO	0.831
OOOOXOOX	−0.86	OOXXOOX	−0.763	XXXXOXXO	0.86	XXOOXXO	0.763
OXOXO	0.701	OOOXXOOX	−0.753	XOXOX	−0.701	XXXOOXXO	0.753
OOXOXO	0.737	OOOOXXOOX	−0.752	XXOXOX	−0.737	XXXXOOXXO	0.752
OOOXOXO	0.741	OXXOXO	0.831	XXXOXOX	−0.741	XOOXOX	−0.831
OOOOXOXO	0.741	OOXXOXO	0.935	XXXXOXOX	−0.741	XXOOXOX	−0.935
OXOXX	0.084	OOOXXOXO	0.952	XOXOO	−0.084	XXXOOXOX	−0.952
OOXOXX	0.169	OOOOXXOXO	0.954	XXOXOO	−0.169	XXXXOOXOX	−0.954
OOOXOXX	0.181	OXXOXX	0.296	XXXOXOO	−0.181	XOOXOO	−0.296
OOOOXOXX	0.182	OOXXOXX	0.463	XXXXOXOO	−0.182	XXOOXOO	−0.463
OXXOO	0.305	OOOXXOXX	0.5	XOOXX	−0.305	XXXOOXOO	−0.5
OOXXOO	0.372	OOOOXXOXX	0.504	XXOOXX	−0.372	XXXXOOXOO	−0.504
OOOXXOO	0.38	OXXXOO	0.5	XXXOOXX	−0.38	XOOOXX	−0.5
OOOOXXOO	0.381	OOXXXOO	0.648	XXXXOOXX	−0.381	XXOOOXX	−0.648
OXXOX	−0.305	OOOXXXOO	0.678	XOOXO	0.305	XXXOOOXX	−0.678
OOXXOX	−0.169	OOOOXXXOO	0.681	XXOOXO	0.169	XXXXOOOXX	−0.681
OOOXXOX	−0.144	OXXXOX	−0.043	XXXOOXO	0.144	XOOOXO	0.043
OOOOXXOX	−0.142	OOXXXOX	0.187	XXXXOOXO	0.142	XXOOOXO	−0.187
OXXXO	1.288	OOOXXXOX	0.244	XOOOX	−1.288	XXXOOOXO	−0.244
OOXXXO	1.5	OOOOXXXOX	0.252	XXOOOX	−1.5	XXXXOOOXO	−0.252
OOOXXXO	1.544	OXXXXO	1.603	XXXOOOX	−1.544	XOOOOX	−1.603
OOOOXXXO	1.549	OOXXXXO	1.917	XXXXOOOX	−1.549	XXOOOOX	−1.917
OXXXX	0.555	OOOXXXXO	2	XOOOO	−0.555	XXXOOOOX	−2
OOXXXX	0.897	OOOOXXXXO	2.014	XXOOOO	−0.897	XXXXOOOOX	−2.014
OOOXXXX	0.985	OXXXXX	0.893	XXXOOOO	−0.985	XOOOOO	−0.983
OOOOXXXX	1	OOXXXXX	1.329	XXXXOOOO	−1	XXOOOOO	−1.329
OXOOOO	−0.547	OOOXXXXX	1.465	XOXXXX	0.547	XXXOOOOO	−1.465
OOXOOOO	−0.547	OOOOXXXXX	1.496	XXOXXXX	0.547	XXXXOOOOO	−1.496

Εύρηκα

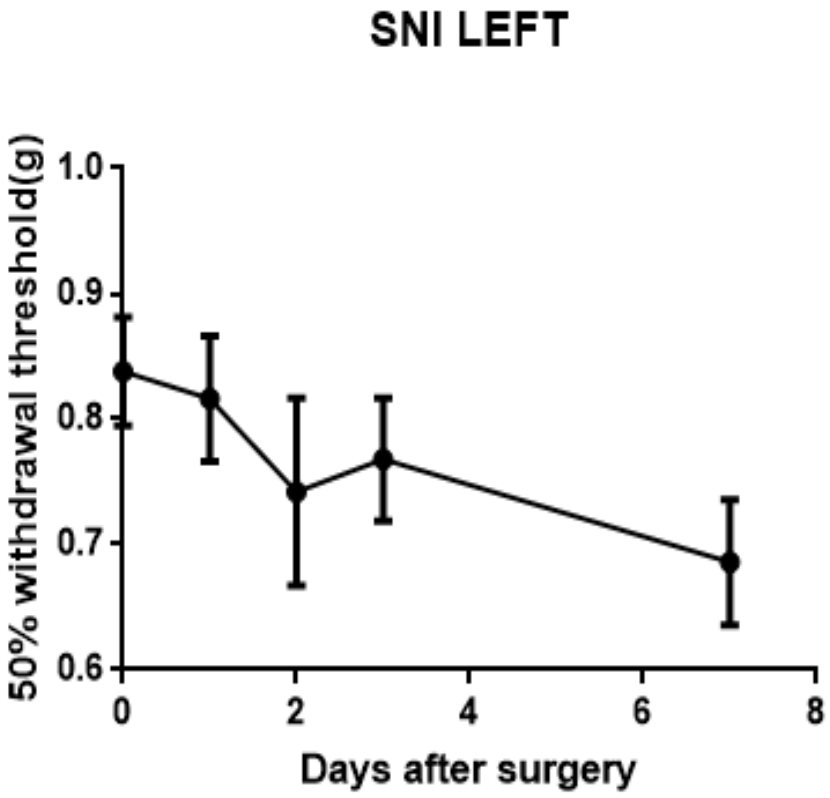


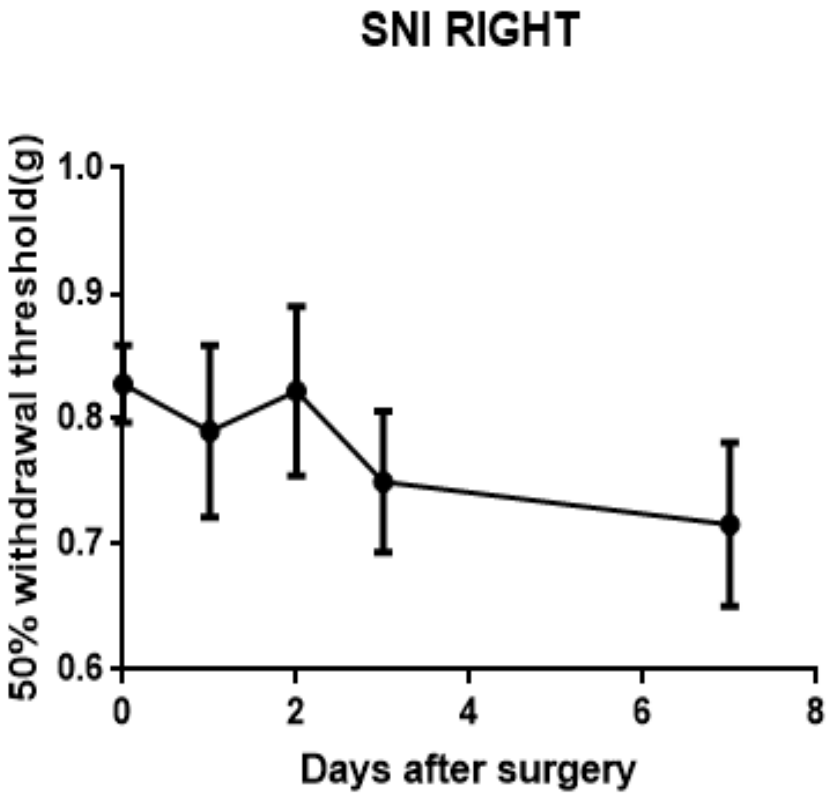
伍、實驗結果


SHAM LEFT
50% Withdraw Threshold (g)
1.8
1.4
1
0.6
0.4
0.0
0
1
2
3
7
Days of SNI

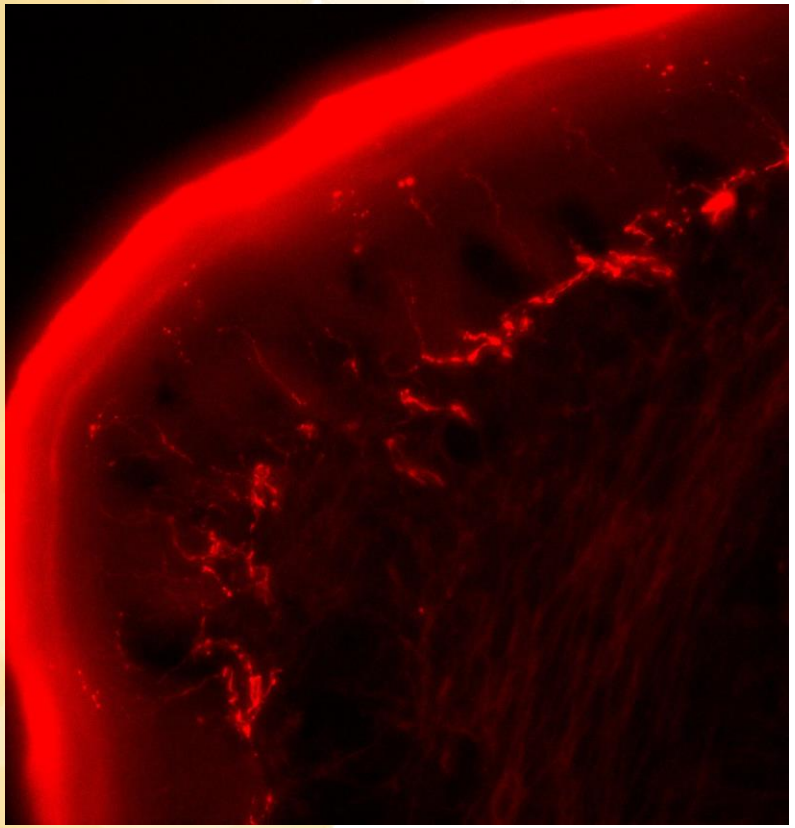

SHAM RIGHT
50% Withdraw Threshold (g)
1.8
1.4
1
0.6
0.4
0.0
0
1
2
3
7
Days of SNI

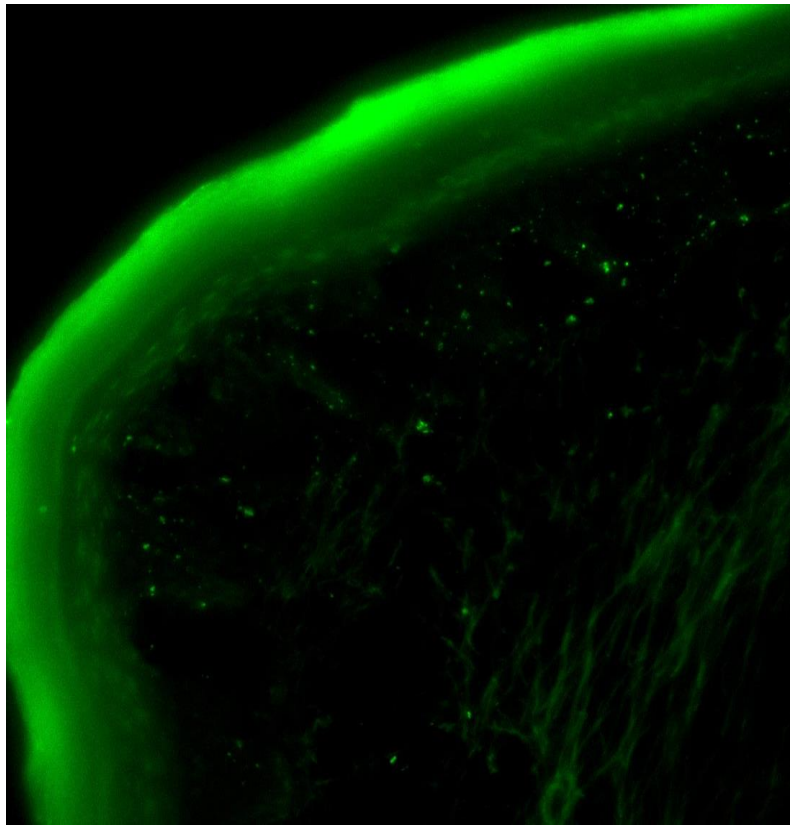
伍、實驗結果


SNI LEFT
50% withdrawal threshold(g)
1.0
0.9
0.8
0.7
0.6
0
2
4
6
8
Days after surgery


SNI RIGHT
50% withdrawal threshold(g)
1.0
0.9
0.8
0.7
0.6
0
2
4
6
8
Days after surgery

伍、實驗結果





六、結論

- 一、目前進度
- 　　已有五隻經過 SNI 手術處理的老鼠及五隻經過 SHAM 手術處理的老鼠進行行為測試。
- 二、正在進行
- 　　(一) 施打 clodronate liposmome的SNI老鼠五隻的行為數據。
- 　　(二) 收集共軛焦顯微鏡拍攝之影像，來觀察巨噬細胞在受傷神經纖維周圍的聚集與附著情形。
- 三、目前結論
- 　　根據行為數據，我們比較之後發現經過SNI手術處理的老鼠比經過SHAM手術處理的老鼠對外力的刺激更敏感。

柒、參考資料及其他

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  - 最棒的魏宏仁老師
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  - 支持我們的家長
  - 台下所有的聽眾