

出國報告（出國類別：進修）

2022 美國於神經病變痛動物模式鞘內注射 反義寡核苷酸之效果

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派赴國家：美國

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摘要

慢性神經病變痛是一個複雜，使人衰弱的健康問題，它影響了全世界 6.9-10%的人口的生活品質。近年來，對於慢性神經病變痛的致病機轉多有探討研究。在慢性神經病變痛的背根神經節中，與疼痛相關的基因在轉錄與轉譯過程中調控失調是目前被認為是導致產生慢性神經病變痛的分子機轉。美國新澤西州立大學羅格斯新澤西醫學院麻醉部疼痛醫學中心研究部副主任陶元祥教授本身是研究慢性神經病變痛這方面的專家，藉著到陶教授實驗室擔任訪問學者，學習慢性神經病變動物模式，其致病分子機轉與相關分子生物實驗技術。

關鍵字

動物模式，慢性神經病變痛

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一、目的

學習多種神經性病變慢性疼痛動物模式，並研究之發展與維持的細胞分子機轉。

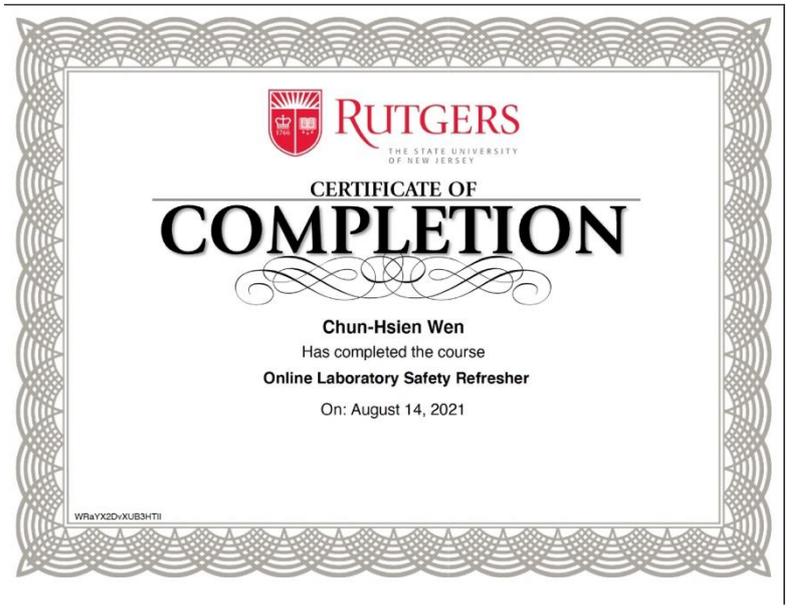
二、過程

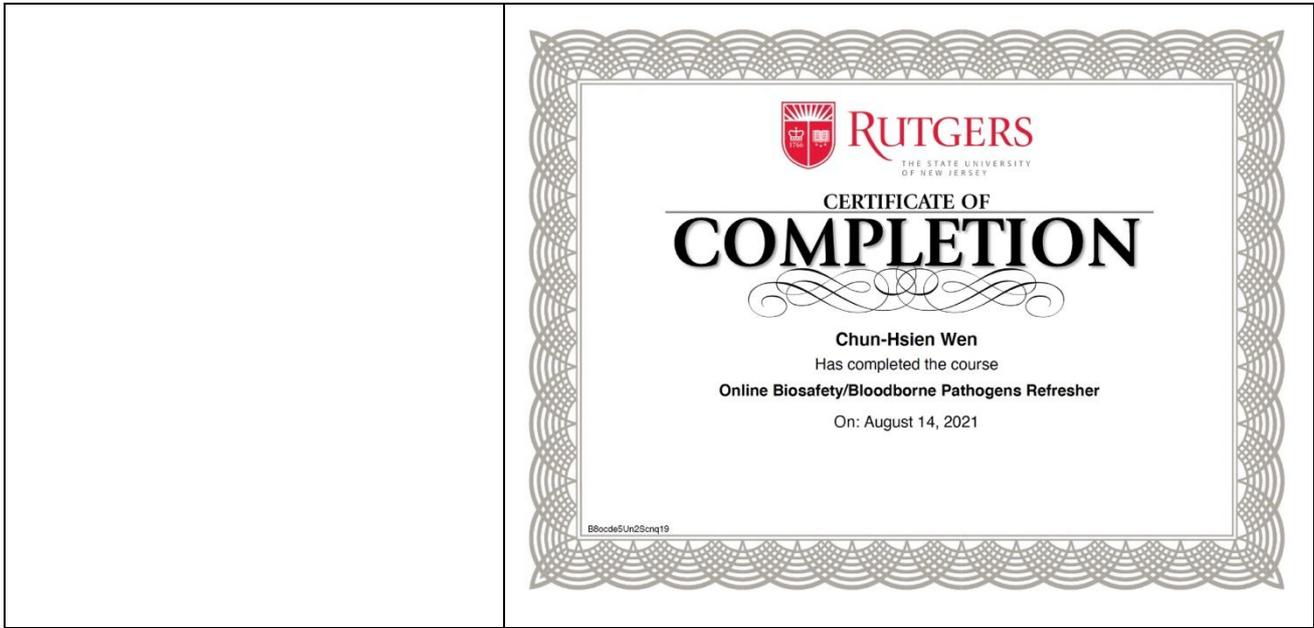
2021/08

初到實驗室，開始熟悉環境與實驗室作息。



開始進行動物實驗之前，須完成相關課程才能進行動物實驗。包括實驗室安全，生物安全等課程。





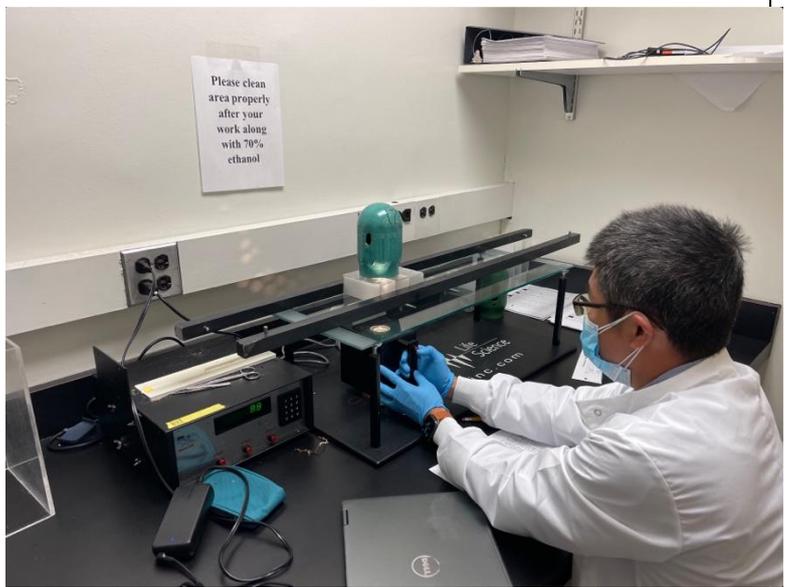
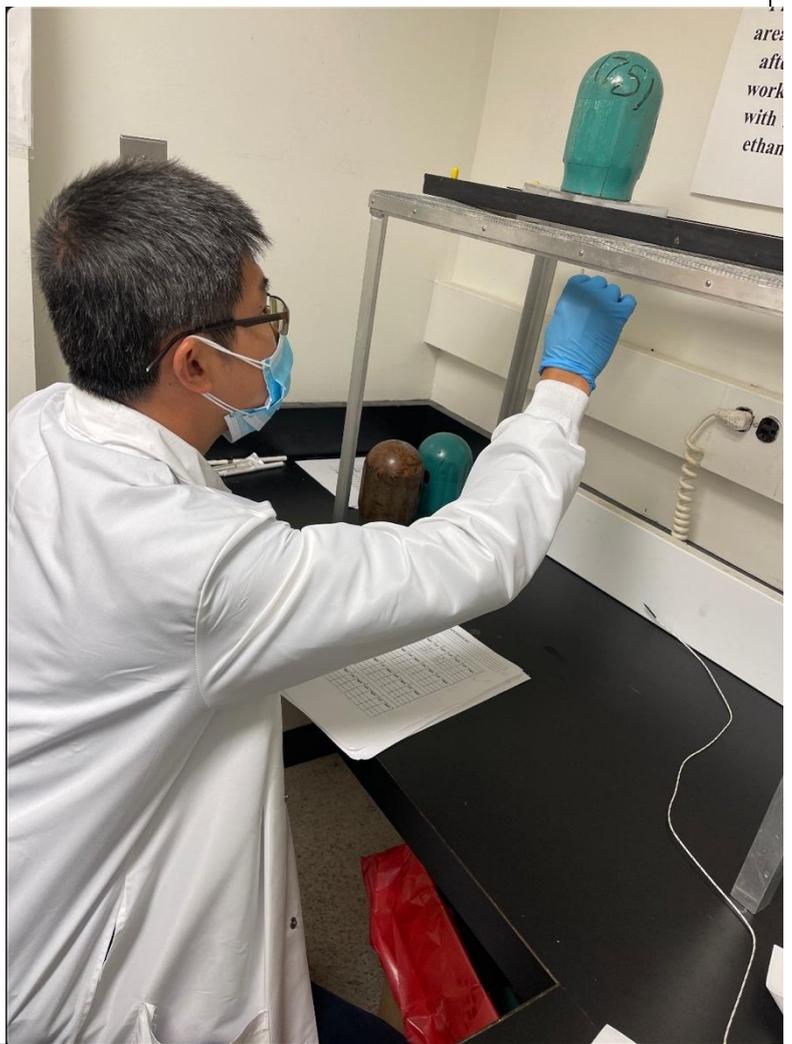
2021/09

完成動物實驗相關課程後，取得
進出動物房權限，可以自行取老
鼠做實驗。

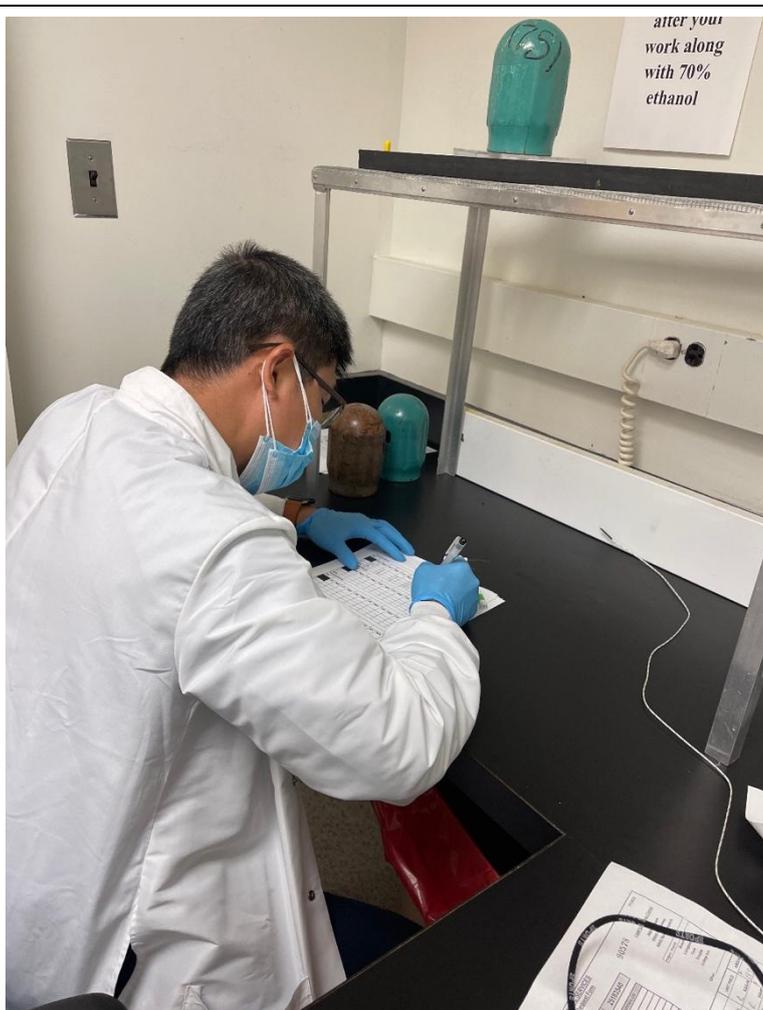




練習與熟悉測量動物疼痛行為，
包含觸痛覺與冷熱感覺。



將測量結果紀錄並與教授報告，
討論測量技巧需改進之處。





14930 Mechanical Test - Mice

ID		1	2	3	4	5	6	7	8	9	10	%
1	low	left	0	0	0	X	0	0	0	0	X	20
	right	0	0	0	0	0	0	0	0	X	0	10
X = Positive Response, O = Negative response												
high	left	0	0	0	0	X	X	X	X	0	X	40
	right	X	X	0	0	0	X	X	0	0	X	40

ID		1	2	3	4	5	6	7	8	9	10	%
2	low	left	0	0	0	0	0	X	0	0	0	10
	right	0	0	0	0	0	0	0	0	0	X	10
high	left	0	0	0	0	0	0	X	X	X	X	40
	right	0	0	0	0	0	0	X	X	0	X	40

ID		1	2	3	4	5	6	7	8	9	10	%
3	low	left	0	0	X	0	0	0	0	0	X	20
	right	0	X	0	0	0	0	0	0	0	0	10
high	left	0	0	0	0	0	0	X	X	X	X	40
	right	0	0	0	X	X	0	0	X	0	X	40

ID		1	2	3	4	5	6	7	8	9	10	%
4	low	left	0	0	0	0	0	X	X	0	0	20
	right	0	0	0	0	0	0	0	X	X	0	10
high	left	X	0	0	X	0	0	X	X	X	X	60
	right	0	X	0	0	X	0	X	X	X	X	60

ID		1	2	3	4	5	6	7	8	9	10	%
5	low	left	0	0	X	0	0	0	0	0	0	20
	right	0	0	0	0	X	0	0	0	0	0	10
high	left	X	0	X	X	0	X	X	0	0	0	60
	right	0	X	0	0	X	0	X	X	X	X	60

Room Temperature: F Date: 2018- Time: Investigator:

NO.	1		2		3		4		6023 1		2	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
1	12.44	10.3	7.30	8.13	13.39	7.49	5.18	13.03	4.86	6.40	6.06	4.68
2	6.35	7.35	13.34	12.34	5.47	10.46	10.77	16.6	13.37	3.85	11.43	5.30
3	12.34	11.16	17.37	8.38	12.02	8.22	11.27	8.84	10.37	8.03	9.42	16.67
4	6.47	9.89	11.22	6.36	2.72	15.27	12.70	3.88	14.57	10.99	4.85	15.11
5	9.12	7.98	9.76	7.33	8.71	10.88	9.29	15.28	3.55	8.66	3.80	5.66
Mean	8.33	8.69	11.16	8.67	8.24	10.06	10.47	11.07	9.35	7.94	6.75	9.99

Cold

Room Temperature: F Date: 2018- Time: Investigator:

NO.	1		2		3		4		6023 1		2	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
1	7.20	17.26	19.01	3.87	16.83	12.12						
2	5.18	10.46	16.40	3.27	8.15	11.68						
3	7.18	11.22	10.40	13.37	14.25	13.34						
4	11.22	6.67	8.83	11.97	14.57	11.37						
5	8.24	7.33	10.00	8.38	7.42	12.88						
Mean	8.44	10.92	11.85	9.18	12.23	12.08						

Cold

Room Temperature: F Date: 2018- Time: Investigator:

NO.	1		2		3		4		6023 1		2	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
1	9.10	13.85	14.92	6.72	9.18	11.59	10.69	12.88	6.00	13.27		
2	6.33	7.88	10.46	9.07	7.19	5.39	12.10	9.99	6.75	16.45		
3	6.22	5.76	14.73	9.16	5.03	3.38	12.99	11.83	15.91	4.89		
4	15.22	16.40	5.88	10.70	18.77	6.83	11.40	13.82	17.66	16.91		
5	12.87	7.16	10.86	7.87	11.82	1.76	4.43	14.03	12.84	9.38		
Mean	10.07	10.1	11.9	8.7	9.79	9.19	12.32	12.48	11.68	13.1		

Cold

Room Temperature: F Date: 2018- Time: Investigator:

NO.	1		2		3		4		6023 1		2	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
1	15.22	9.3	5.47	12.2	8.86	9.11	10.57	12.51				
2	13.25	9.19	16.64	7.85	7.25	6.91	16.44	12.4				
3	7.22	9.42	5.76	7.26	8.68	9.32	7.51	6.43				
4	12.96	15.28	10.22	11.6	5.84	14.53	8.37	15.71				
5	5.32	14.70	11.20	17.80	10.39	7.24	9.42	8.68				
Mean	9.38	11.88	9.86	12.3	8.10	11.17	10.47	12.3				

Cold

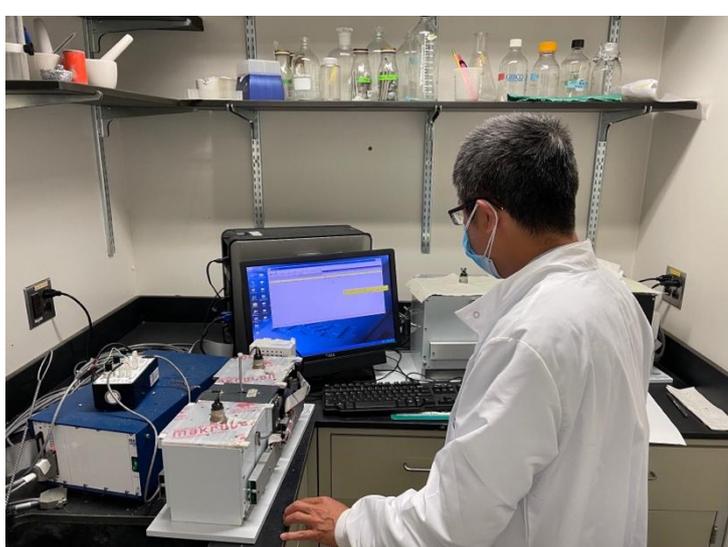
Room Temperature: F Date: 2018- Time: Investigator:

NO.	1		2		3		4		6023 1		2	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
1	6.84	5.62	11.29	15.68	6.17	17.16	13.9	12.99				
2	16.85	13.02	4.5	6.0	5.71	11.6	5.01	8.02				
3	13.25	5.21	8.07	4.11	15.08	14.3	6.28	8.2				
4	8.23	8.03	6.35	8.38	7.42	7.35	7.85	14.35				
5	17.62	10.32	5.15	5.73	5.79	6.93	16.43	8.34				
Mean	12.17	8.46	7.11	8.58	9.23	11.84	11.83	11.34				

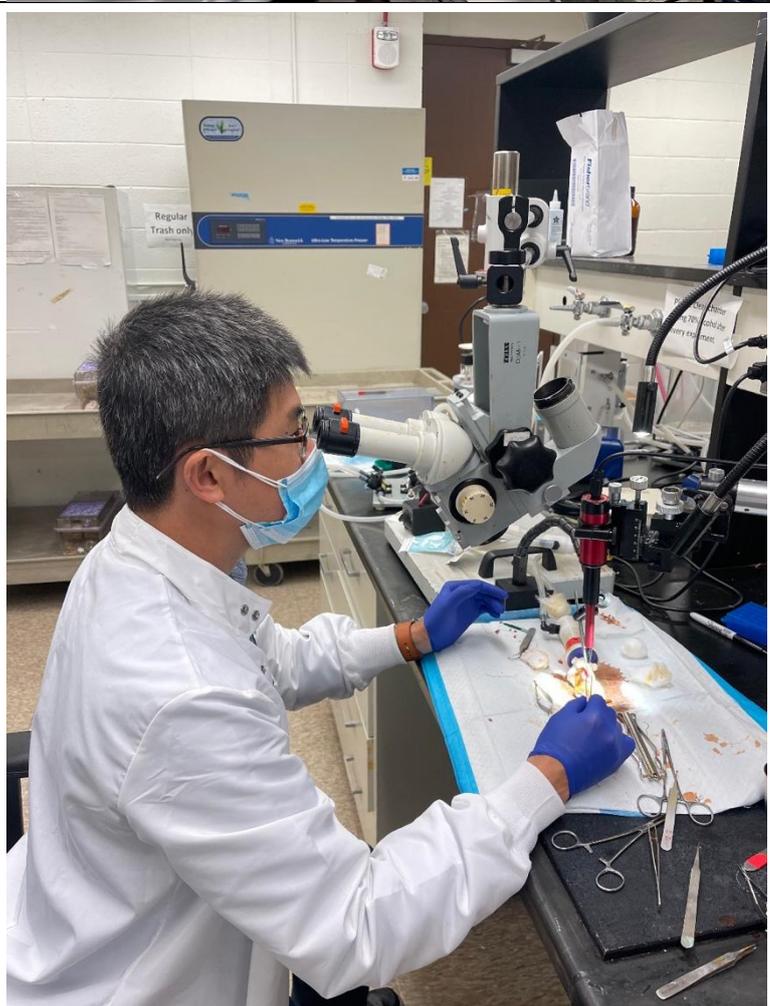
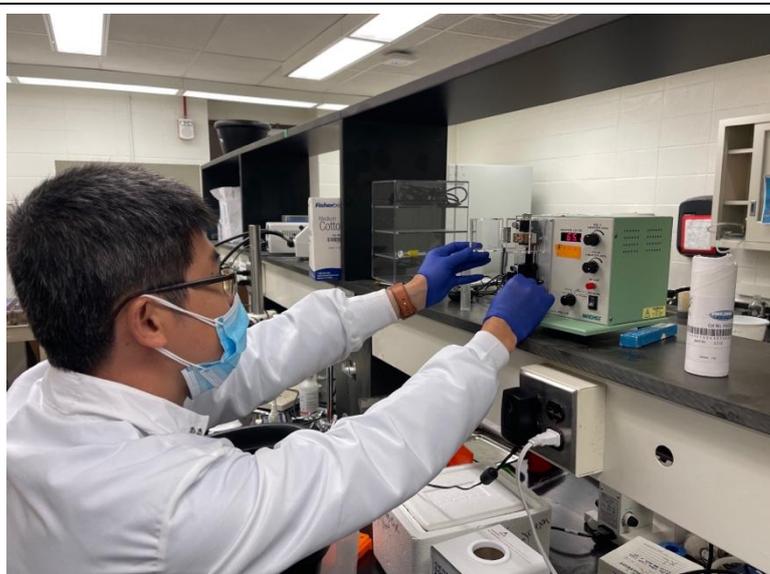
Cold

2021/10

除了誘發疼痛行為以外，也會測試動物持續性疼痛行為反應。會使用『制約場域偏好反應 conditioned place preference (CPP) 』設備加以測量老鼠是否具有持續性神經疼痛。完整測試需 5-6 天的時間。



學習神經痛動物模式，包括：『慢性壓迫脊神經傷害 chronic constriction injury, CCI』與『脊神經結紮 tight ligation of spinal nerves, SNL』模式。學習使用顯微鏡與微注射 (micro injection)設備進行背根神經節(dorsal root ganalion, DRG)內注射與脊髓鞘內注射化學 (intrathecal injection, IT)。



2021/11

依照實驗室排程，與教授，同事進行線上會議報告，包含：實驗結果進度報告與期刊報告。

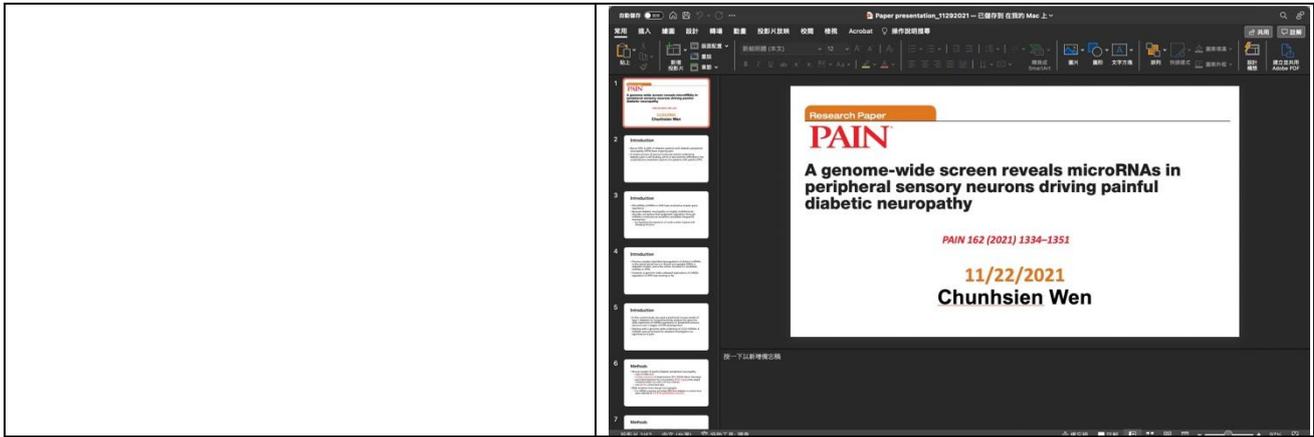
Dr. Tao's Lab Meeting Schedule

Date	Data Presentation	Paper Presentation
Sep 7	Gokula/Tolga	Bing Wang
Sep 13	Shibin/Sharma	Li Zhang
Sep 20	Bing Wang	Sharma
Sep 27	Xiaozhou/Li Zhang	Shibin
Oct 4	Gokula/Tolga	Ma
Oct 11	Shibin/Sharma	Xiaozhou
Oct 18	Bing Wang/Ma	Gokula
Oct 25	Xiaozhou/Li Zhang	Tolga
Nov 1	Gokula/Tolga	Bing Wang
Nov 8	Shibin/Sharma/Wen	Li Zhang
Nov 22	Bing Wang/Ma	Gokula
Nov 29	Xiaozhou/Li Zhang	Wen

Virtual Lab Meeting starts at 8:30 am on Monday
Paper should be emailed to each member 3-4 days before presentation.

The screenshot shows a Zoom meeting interface. The main window displays a slide titled "Pre-microinjection of AAV-Inc-eRNA rescued the ISL1 knockdown-induced neuropathic pain." The slide contains four line graphs (a, b, c, d) showing PWF (%) over time for different groups: AAV-NC, AAV-Inc-eRNA, and AAV-Inc-eRNA + ISL1. The graphs show that AAV-Inc-eRNA treatment significantly reduces PWF compared to AAV-NC, and this reduction is partially rescued by the addition of ISL1. The bottom right of the screenshot shows a list of participants in the meeting, including Chun-hsien Wen, Bing Wang, ruijing ma, Dilip Sharma, Hui xing Wang, Tolga Berkman, Wanhong Zuo, Xiaozhou Feng, Yingping Liang, and Yuanxiang Tao.

Data presentation
11/08/2021
Chunhsien Wen

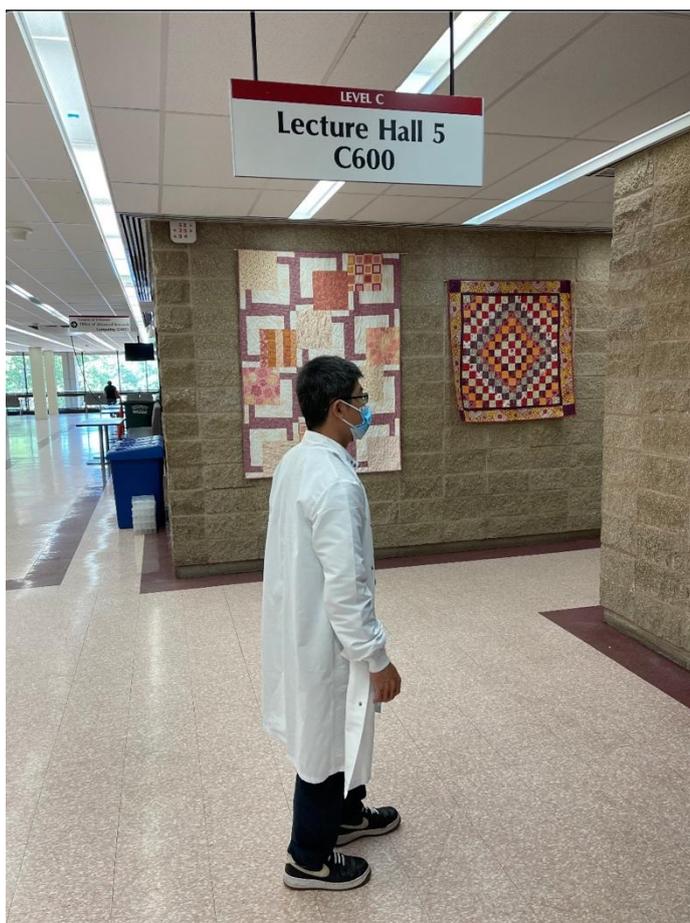


2021/12

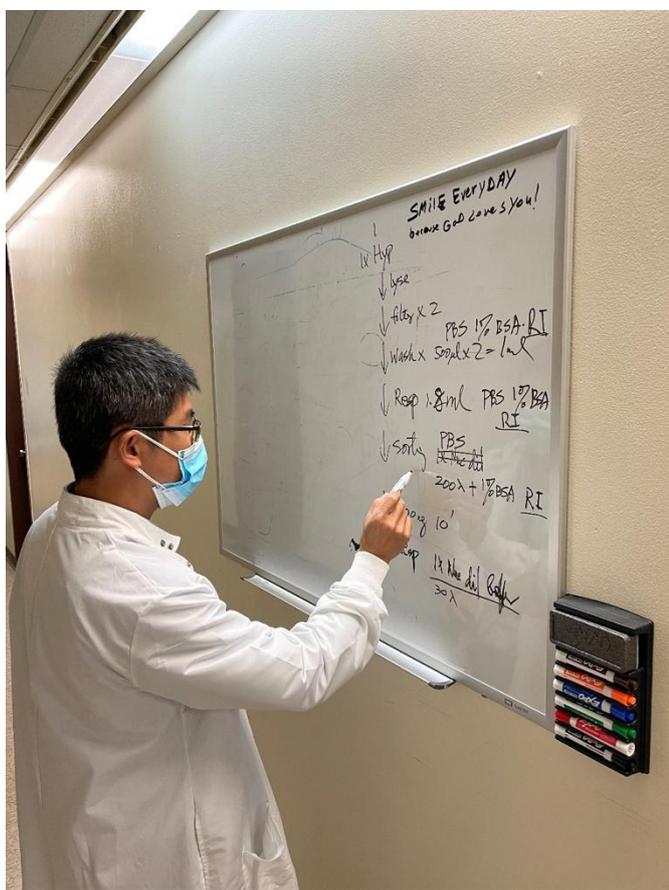
與教授就實驗結果做電話討論



參與 Rutgers medical school 羅格斯醫學院舉辦之演講



動物行為測試即將告一段落，與教授討論後續實驗進度安排



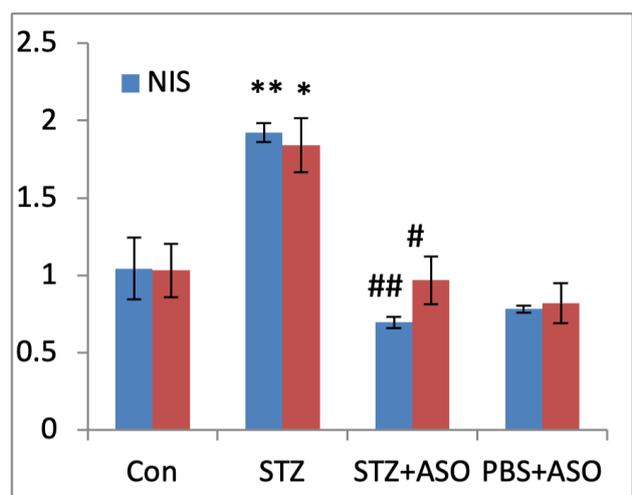
2022/01

開始分批取動物檢體（脊髓與背根神經節 spinal cords and dorsal root ganglions），取下之檢體冰至-80 C 冰箱做後續分析。



老鼠背根神經節做檢體分析（定量即時聚合酶連鎖反應 qPCR）

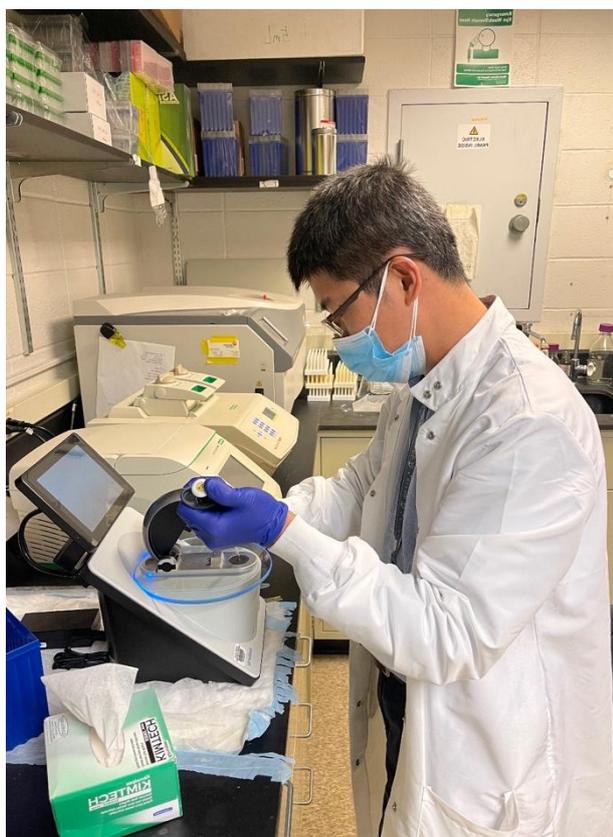
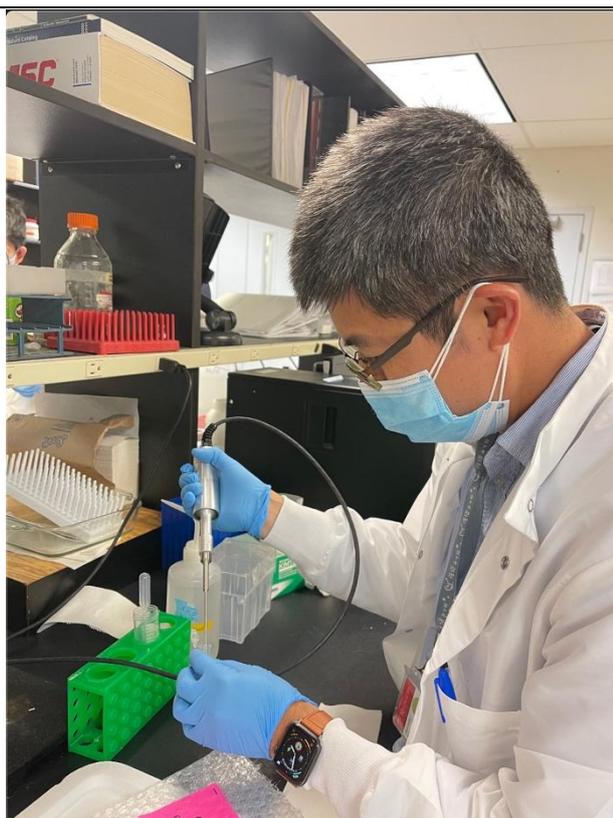




2022/02

老鼠檢體分析，脊髓(spinal cords)

做西方墨點法(western blot)

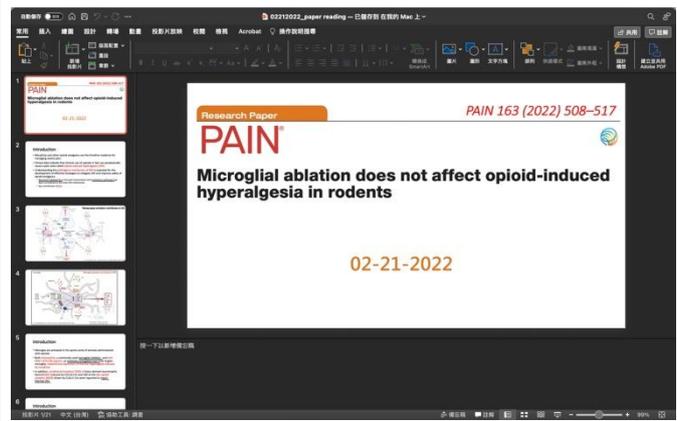


於研究室會議上期刊報告

Dr. Tao's Lab Meeting Schedule

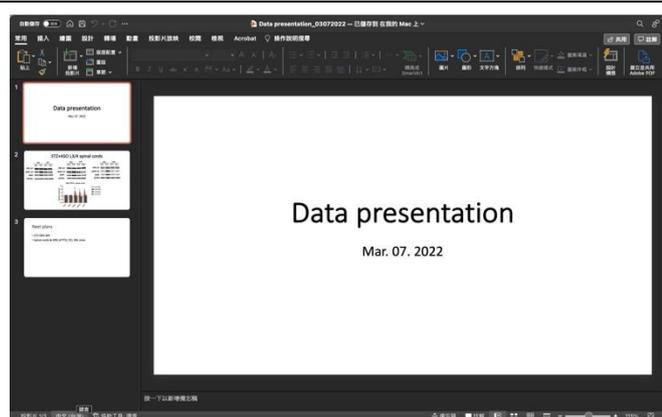
Date	Data Presentation	Paper Presentation
Feb 14	Bing Wang/Ma	Li Zhang/Sharma
Feb 21	Xiaozhou/Li	Wen
Feb 28	Gokula/Tolga	Ma
Mar 7	Sharma/Wen	Xiaozhou
Mar 14	Bing Wang/Ma	Tolga
Mar 21	Xiaozhou/Li	Gokula
Mar 28	Gokula/Tolga	Wenhong
Apr 4	Sharma/Wen/Liang	Bing Wang
Apr 11	Bing Wang/Ma	Huixin
Apr 18	Xiaozhou/Li/Huixin	Liang
Apr 25	Gokula/Tolga	Sharma
May 1	Sharma/Wen/Liang	Li

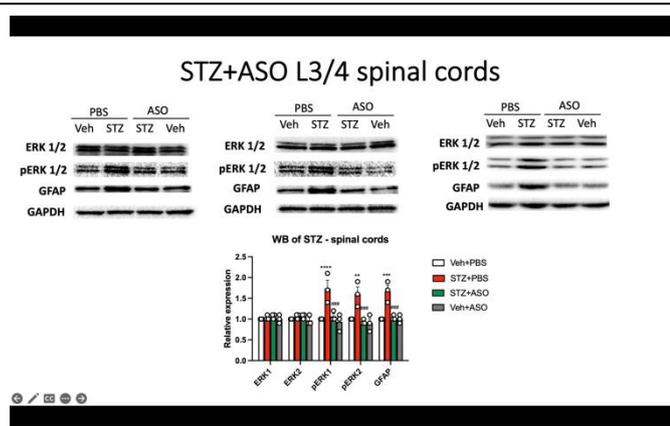
Virtual Lab Meeting starts at 8:30 am on Monday
Paper should be emailed to each member 3-4 days before presentation.



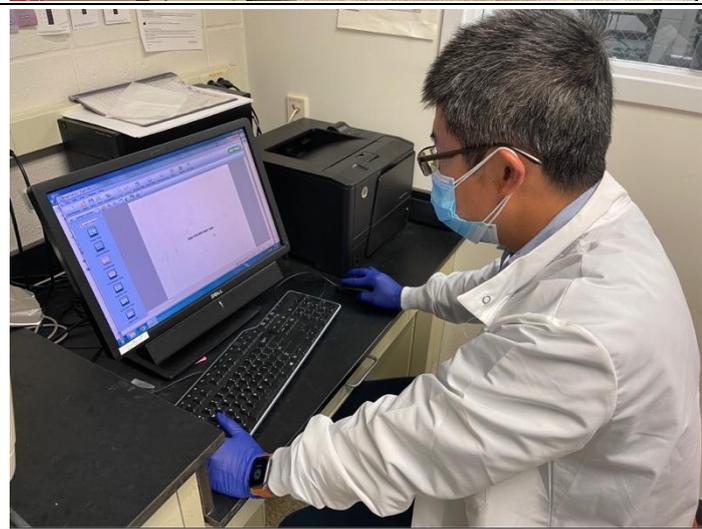
2022/03

於研究室會議上做進度報告，報告第一批老鼠 (STZ group) 檢體脊髓(spinal cords)做西方墨點法 (western blot)之初步成果



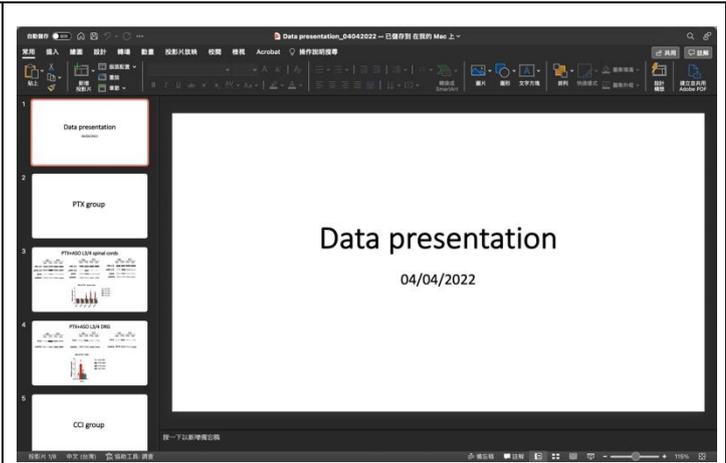


繼續做其他批老鼠 (STZ group)
 檢體脊髓(spinal cords)與背根神經節(dorsal root ganglion)西方墨點法分析

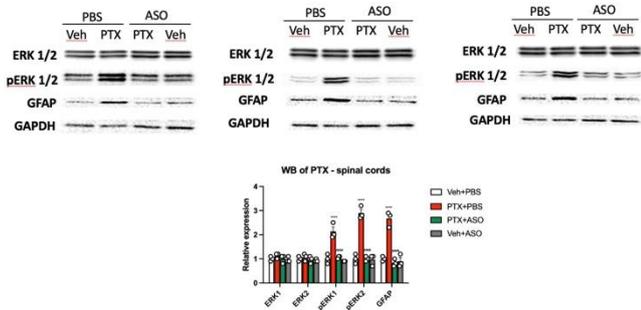


2022/04

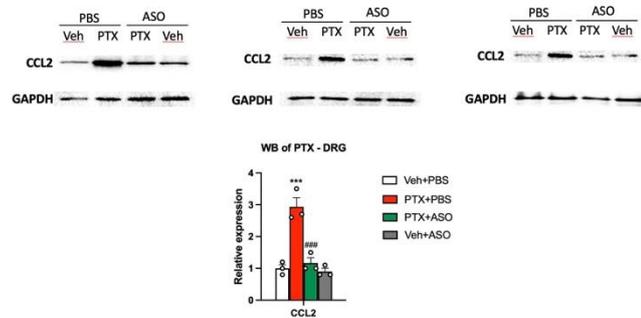
於研究室會議上做進度報告，報告第二批老鼠 (PTX group) 與第三批老 (CCI group) 檢體脊髓 (spinal cords) 與背根神經節 (dorsal root ganglion) 西方墨點法 (western blot) 之結果



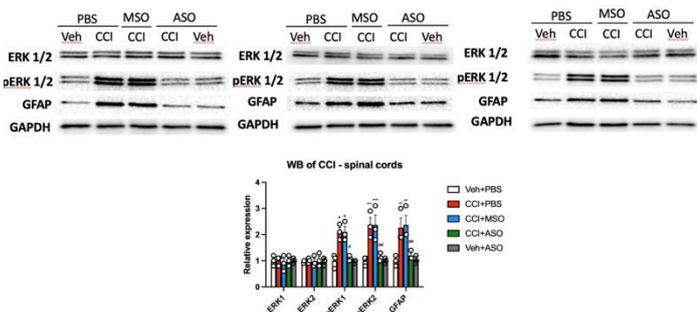
PTX+ASO L3/4 spinal cords



PTX+ASO L3/4 DRG



CCI+ASO L3/4 spinal cords



2022/05

於研究室會議上做進度報告，與期刊報告。

檢體分析資料（包含定量即時聚合酶連鎖反應 qPCR 與西方墨點法

western blot）搜集分析告一段

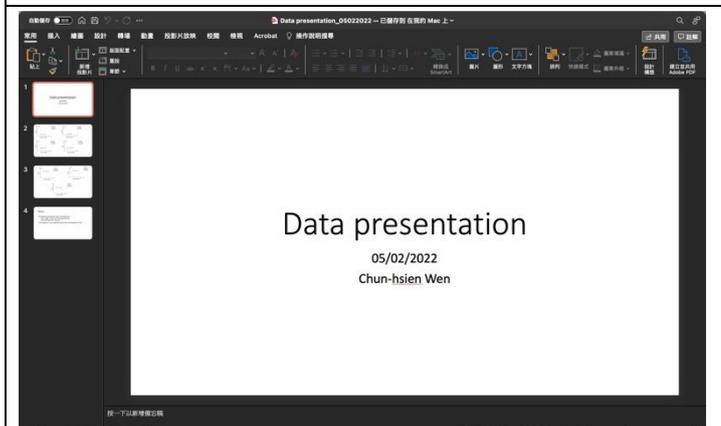
落，接續開始預備做切片尼氏染色

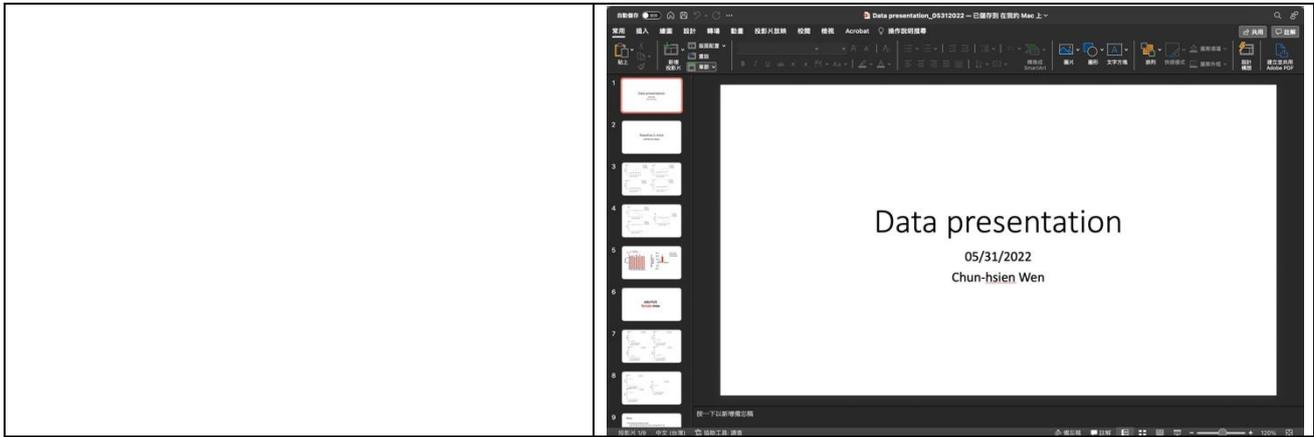
法(Nissl Staining)與撰寫論文。

Dr. Tao's Lab Meeting Schedule

Date	Data Presentation	Paper Presentation
May 9	Bing Wang/Ma	Li Zhang/Sharma
May 16	Xiaozhou/Li	Wen
May 23	Gokula/Tolga/Jerry	Ma
May 30	Sharma/Wen/Liang	Xiaozhou
Jun 6	Bing Wang/Ma	Tolga
Jun 13	Xiaozhou/Li	Gokula
Jun 20	Gokula/Tolga/Jerry	Wenhong
Jun 27	Sharma/Wen/Liang	Jerry
July 5	Bing Wang/Ma	Huixin
July 11	Xiaozhou/Li/Huixin	Liang
July 18	Gokula/Tolga/Jerry	Bing Wang
July 25	Sharma/Wen/Liang	Li

Virtual Lab Meeting starts at 8:30 am on Monday
Paper should be emailed to each member 3-4 days before presentation.

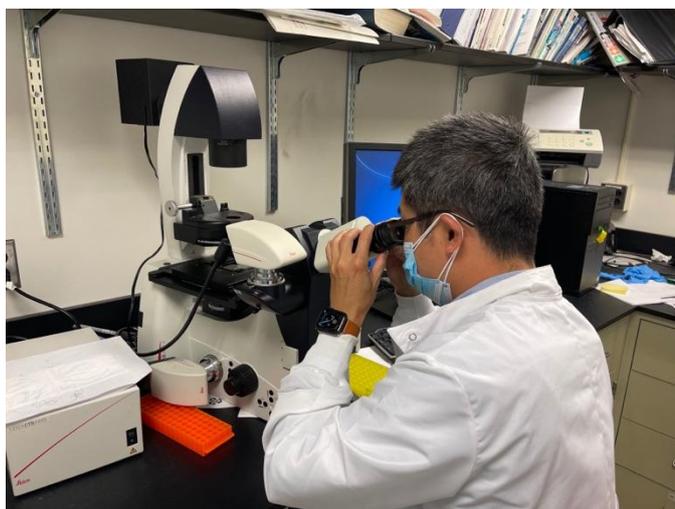




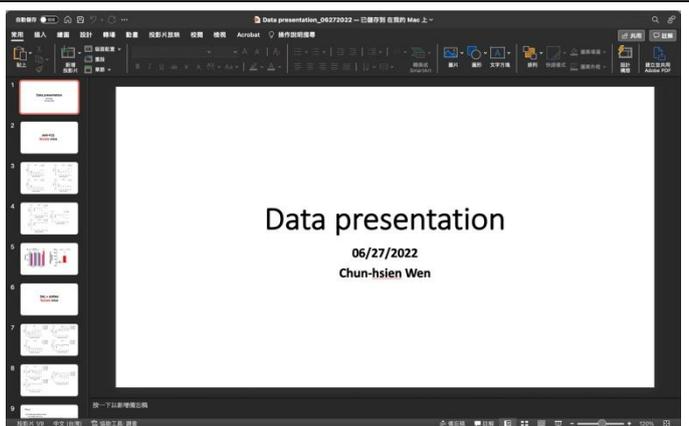
2022/06

做檢體脊髓(spinal cords)與背根神經節(dorsal root ganglion)切片尼氏染色法(Nissl Staining)。藉此觀察神經元內的細胞結構，來了解神經元的損傷情況。



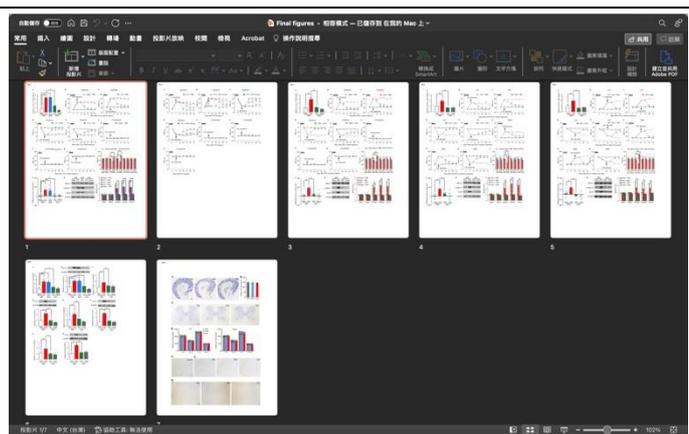


於研究室會議上做進度報告



2022/07

經過幾次修改後，論文圖表製作完成



<p>論文撰寫完成，與教授討論修改後，預備投稿</p>	<p>Effect of intrathecal <i>NIS-IncRNA</i> antisense oligonucleotides on neuropathic pain caused by nerve trauma, chemotherapy, or diabetics ^{1,2,3,4}</p> <p>Chun-Hsien Wen¹, Tolga Berkman¹, Xiang Li¹, Shibin Du¹, Gokulapriya Govindarajulu,¹ Haijun Zhang¹, Alex Bekker¹, Steve Davidson², Yuan-Xiang Tao^{1,3,4}</p> <p>¹Department of Anesthesiology, New Jersey Medical School, Rutgers, The State University of New Jersey, Newark, NJ07103, USA ¹</p> <p>²Department of Anesthesiology, Pain Research Center, and Neuroscience Graduate Program, University of Cincinnati College of Medicine, Cincinnati, OH45267, USA ²</p> <p>³Department of Physiology, Pharmacology & Neuroscience, New Jersey Medical School, Rutgers, The State University of New Jersey, Newark, NJ07103, USA ³</p> <p>⁴Department of Cell Biology & Molecular Medicine, New Jersey Medical School, Rutgers, The State University of New Jersey, Newark, NJ07103, USA ⁴</p>
<p>通訊作者（實驗室教授）投稿至英國麻醉學雜誌 British Journal of Anaesthesia（影響指數 IF=9.166）</p>	<p>Journal: British Journal of Anaesthesia Title: Effect of intrathecal NIS-IncRNA antisense oligonucleotides on neuropathic pain caused by nerve trauma, chemotherapy, or diabetes Corresponding Author: Prof. Yuanxiang Tao Co-Authors: Chun-Hsien Wen¹ MD, Tolga Berkman, Bachelor; Xiang Li, MD; Shibin Du, MD; Gokulapriya Govindarajulu, PhD; Haijun Zhang, PhD, MD; Alex Bekker, PhD, MD; Steve Davidson, PhD Manuscript Number: BJA-D-22-01003</p> <p>Dear Wen, Prof. Yuanxiang Tao submitted this manuscript via Elsevier's online submission system, Editorial Manager, and you have been listed as a Co-Author of this submission. Elsevier asks Co-Authors to confirm their consent to be listed as Co-Author and track the papers status. In order to confirm your connection to this submission, please click here to confirm your co-authorship: https://www.editorialmanager.com/bja1.asp?i=5621681=1862CPM1 If you have not yet registered for the journal on Editorial Manager, you will need to create an account to complete this confirmation. Once your account is set up and you have confirmed your status as Co-Author of the submission, you will be able to view and track the status of the submission as it goes through the editorial process by logging in at https://www.editorialmanager.com/bja1 If you did not co-author this submission, please contact the Corresponding Author directly at y1211@nims.rutgers.edu</p> <p>Thank you, British Journal of Anaesthesia</p>

三、心得及建議

本人此次出國進修針對當初設定的出國目標『學習慢性疼痛動物模式及其分子機轉，盼望歸國後能複製並獨立操作所學得之動物模式。』有所學習與收穫。在這一年中，在實驗室陶元祥教授的督導之下，研究形成與維持慢性疼痛的分子與細胞機轉，學習設計與執行實驗，蒐集與分析資料，準備與撰寫文章以及報告實驗結果。實驗室團隊彼此分工，互相幫忙，實在是值得學習效法。本院教研部也積極安排多樣提升研究能力教育訓練課程，課程範圍多領域，相信對有意參與研究

的同人幫助甚大。為課程時間大多在上班時間，建議是否也同步提供線上課程，方便課後學習。

附錄

陶教授實驗室會議排程表

Dr. Tao's Lab Meeting Schedule

Date	Data Presentation	Paper Presentation
Sep 7	Gokula/Tolga	Bing Wang
Sep 13	Shibin/Sharma	Li Zhang
Sep 20	Bing Wang	Sharma
Sep 27	Xiaozhou/Li Zhang	Shibin
Oct 4	Gokula/Tolga	Ma
Oct 11	Shibin/Sharma	Xiaozhou
Oct 18	Bing Wang/Ma	Gokula
Oct 25	Xiaozhou/Li Zhang	Tolga
Nov 1	Gokula/Tolga	Bing Wang
Nov 8	Shibin/Sharma/Wen	Li Zhang
Nov 22	Bing Wang/Ma	Gokula
Nov 29	Xiaozhou/Li Zhang	Wen

Virtual Lab Meeting starts at 8:30 am on Monday
Paper should be emailed to each member 3-4 days before presentation.

Dr. Tao's Lab Meeting Schedule

Date	Data Presentation	Paper Presentation
Nov 15	Bing Wang/Ma	Gokula
Nov 22	Xiaozhou/Li Zhang	Wen
Nov 29	Gokula/Tolga	Bing Wang
Dec 6	Shibin/Sharma	Li Zhang
Dec 13	Bing Wang	Sharma
Dec 20	Xiaozhou/Li Zhang	Shibin
Jan 3	Gokula/Tolga	Ma
Jan 10	Shibin/Sharma	Xiaozhou
Jan 18	Bing Wang/Ma	Gokula
Jan 24	Xiaozhou/Li Zhang	Tolga
Jan 31	Gokula/Tolga	Bing Wang
Feb 7	Shibin/Sharma/Wen	Li Zhang

Virtual Lab Meeting starts at 8:30 am on Monday
Paper should be emailed to each member 3-4 days before presentation.

Dr. Tao's Lab Meeting Schedule

Date	Data Presentation	Paper Presentation
Feb 14	Bing Wang/Ma	Li Zhang/Sharma
Feb 21	Xiaozhou/Li	Wen
Feb 28	Gokula/Tolga	Ma
Mar 7	Sharma/Wen	Xiaozhou
Mar 14	Bing Wang/Ma	Tolga
Mar 21	Xiaozhou/Li	Gokula
Mar 28	Gokula/Tolga	Wenhong
Apr 4	Sharma/Wen/Liang	Bing Wang
Apr 11	Bing Wang/Ma	Huixin
Apr 18	Xiaozhou/Li/Huixin	Liang
Apr 25	Gokula/Tolga	Sharma
May 1	Sharma/Wen/Liang	Li

Virtual Lab Meeting starts at 8:30 am on Monday
Paper should be emailed to each member 3-4 days before presentation.

Dr. Tao's Lab Meeting Schedule

Date	Data Presentation	Paper Presentation
May 9	Bing Wang/Ma	Li Zhang/Sharma
May 16	Xiaozhou/Li	Wen
May 23	Gokula/Tolga/Jerry	Ma
May 30	Sharma/Wen/Liang	Xiaozhou
Jun 6	Bing Wang/Ma	Tolga
Jun 13	Xiaozhou/Li	Gokula
Jun 20	Gokula/Tolga/Jerry	Wenhong
Jun 27	Sharma/Wen/Liang	Jerry
July 5	Bing Wang/Ma	Huixin
July 11	Xiaozhou/Li/Huixin	Liang
July 18	Gokula/Tolga/Jerry	Bing Wang
July 25	Sharma/Wen/Liang	Li

Virtual Lab Meeting starts at 8:30 am on Monday
Paper should be emailed to each member 3-4 days before presentation.

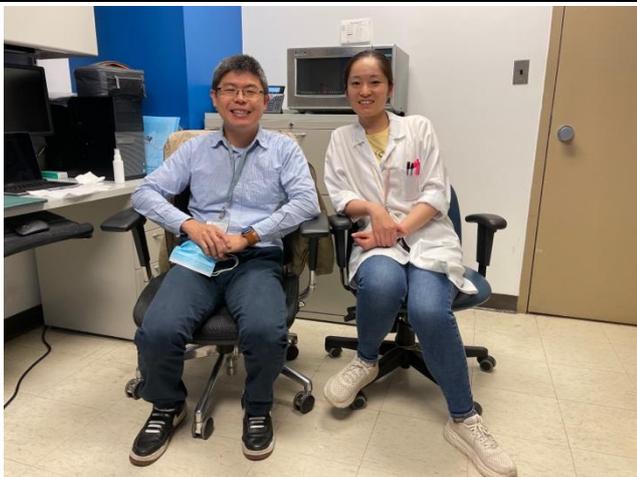
與陶元祥教授合影



與實驗室同仁合影_1



與實驗室同仁合影_2



與實驗室同仁合影_3



與實驗室同仁合影_4



與實驗室同仁合影_5

