

國家科學及技術委員會專題研究計畫申請書

申請條碼：112WIC5010167

一、基本資料：



計畫類別(單選)	一般研究計畫			
研究型別	整合型			
計畫歸屬	生科處			
申請機構/系所(單位)	財團法人國家衛生研究院生技與藥物研究所			
本計畫主持人姓名	葉修華	職稱	副研究員	身分證號碼
本計畫名稱	中文	利用高效率頭戴式顯微鏡平台及人工智慧演算法加速開發組織蛋白酶S抑制劑治療巴金森氏症引發的運動功能障礙		
	英文	Utilizing a high-efficiency head-mounted miniscope platform and artificial intelligence algorithms to accelerate the development of cathepsin S inhibitors for treating Parkinson's disease-induced motor dysfunction		
整合型總計畫名稱	利用高效率頭戴式顯微鏡平台及人工智慧演算法加速開發組織蛋白酶S抑制劑治療巴金森氏症引發的運動功能障礙			
整合型總計畫主持人	葉修華		身分證號碼	S12225****
全程執行期限	自民國 112 年 05 月 01 日起至民國 116 年 04 月 30 日			
研究學門	學門代碼	學門名稱		
	B90A002	腦科學專案研究計畫		
【請考量己身負荷，申請適量計畫】				
本年度申請主持本會各類研究計畫(含預核案)共 <u>1</u> 件。(共同主持之計畫不計入)				
本計畫是否同時有其他單位提供補助項目： <input checked="" type="checkbox"/> 否； <input type="checkbox"/> 是，請務必填寫表CM05*。				
近三年內是否有執行非國科會補助之其他(含國內外、大陸地區及港澳)計畫： <input checked="" type="checkbox"/> 否； <input type="checkbox"/> 是，請務必填寫表CM14-1。				
本計畫是否為國際合作研究： <input checked="" type="checkbox"/> 否； <input type="checkbox"/> 是，請加填表IM01~IM03				
本計畫是否申請海洋研究船： <input checked="" type="checkbox"/> 否； <input type="checkbox"/> 是，請務必填寫表CM15。				
本計畫是否申請高效能計算資源： <input checked="" type="checkbox"/> 否； <input type="checkbox"/> 是，請另於國網中心網站進行申請 (https://rac.nchc.org.tw)。				
1. 本計畫是否有進行下列實驗/研究：(勾選下列任一項，須附相關實驗/研究同意文件)				
<input type="checkbox"/> 人體試驗/人體檢體 <input type="checkbox"/> 人類胚胎/人類胚胎幹細胞 <input checked="" type="checkbox"/> 基因重組實驗 <input type="checkbox"/> 基因轉殖田間試驗 <input type="checkbox"/> 第二級以上感染性生物材料 <input checked="" type="checkbox"/> 動物實驗(須同時加附動物實驗倫理3R說明)				
2. 本計畫是否為人文處行為科學研究計畫： <input type="checkbox"/> 是(請檢附已送研究倫理審查之證明文件)； <input checked="" type="checkbox"/> 否				
3. 本計畫是否為臨床試驗研究計畫： <input type="checkbox"/> 是(請增填性別分析檢核表CM16)； <input checked="" type="checkbox"/> 否				
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計畫主持人簽章：_____

日期：_____

二、研究計畫中英文摘要：請就本計畫要點作一概述，並依本計畫性質自訂關鍵詞。

計畫中文關鍵詞	利用高效率頭戴式顯微鏡平台及人工智慧演算法加速開發組織蛋白酶S抑制劑治療巴金森氏症引發的運動功能障礙
計畫英文關鍵詞	Utilizing a high-efficiency head-mounted miniscope platform and artificial intelligence algorithms to accelerate the development of cathepsin S inhibitors for treating Parkinson's disease-induced motor dysfunction
計畫中文摘要	<p>理由：巴金森氏症是由於腦中多巴胺神經元死亡所造成的疾病，目前的治療方針以增加腦中多巴胺濃度為主。由於造成神經元死亡的機轉尚不明瞭，目前並無有效防止多巴胺神經元死亡的治療方針。另外，以傳統動物行為實驗進行巴金森氏症治療方針的概念驗證工作不僅費時耗工，且難以量化複雜行為參數反應療效指標。所以，發展有效反應行為表現的方法是加速開發有效治療方針的關鍵策略。最近研究指出分析及量化頭戴式顯微鏡平台收集的腦細胞鈣離子訊號似乎是一個有效的解決方案。</p> <p>假設：根據初步結果，我們推論若能開發具活體活性的高選擇性組織蛋白酶S抑制劑，應能保護多巴胺神經元免於死亡，防止或治療巴金森氏症。我們計畫開發一個高效率頭戴式顯微鏡平台及針對鈣離子影像開發人工智能演算法，協助加速該藥物研發計畫。</p> <p>初步結果：我們已建立頭戴式顯微鏡平台，成功紀錄清醒小鼠腦細胞鈣離子影像。另外，已在小鼠模型證實組織蛋白酶S隨病程增加，在離體及活體模型中證實組織蛋白酶S抑制劑能有效抑制多巴胺神經元死亡並減緩運動功能失調。</p> <p>具體目標：本計畫擬基於建立一套高效率頭戴式腦細胞鈣離子影像平台，進一步以此平台數據建立人工智能演算法，加速開發高選擇性組織蛋白酶S抑制劑用以治療巴金森氏症。分工如下：一)：與電子工程專家及設備商合作，製造高精度自動水平校正立體定位儀，快速建立頭戴式顯微鏡平台及增加成功率。二)：與電腦模擬學家，神經藥理學家合作開發基於腦神經鈣離子活性與運動功能的人工智能演算法，能協助設計藥物及快速分析藥物療效。三)：與藥物化學家合作，開發具備高效價的組織蛋白酶S抑制劑，並對該系列化合物進行動物藥效測試。</p> <p>新穎性及應用：此計畫預計開發的高精度自動水平校正立體定位儀為市場首見。此產品問世預計造福全球行為神經科學家，並使得頭戴式顯微鏡平台技術普及化。成功開發針對腦神經鈣離子活性與運動功能的人工智能演算法亦有助於縮短相關藥物的臨床前研發時程及更精準預測藥效。開發該演算法的經驗亦有助於開發其他神經疾病演算法如失智症，慢性疼痛等。最後，有別於傳統療法，開發組織蛋白酶S抑制劑可能經由保護多巴胺神經，提供巴金森氏症的另一個治療方針。</p>
計畫英文摘要	<p>Rationale: Parkinson's disease is a condition caused by the death of dopamine neurons in the brain. Currently, the main treatment strategy is to increase the dopamine concentration in the brain. The underlying mechanism of dopamine neuronal death is still unclear, and there is no effective treatment strategy to prevent it. Furthermore, the concept validation work for the treatment strategy of Parkinson's disease using traditional animal behavior experiments is not only time-consuming and labor-intensive, but also difficult to quantify the response index of complex behavioral parameters. Therefore, the development of effective methods for measuring behavioral performance is a key strategy for accelerating the development of effective treatment strategies. Recent research indicates that analyzing and quantifying the calcium signals collected by the head-mounted miniscope platform seems to be an effective solution.</p> <p>Hypothesis: If a highly selective inhibitor of Cathepsin S (CTSS) with in vivo activity can be created, it could protect dopamine neurons from death and prevent or treat Parkinson's disease. Our plan is to create a high-efficiency head-mounted miniscope platform and develop artificial intelligence algorithms for</p>

	<p>calcium imaging to speed up the drug development process.</p> <p>Preliminary results: We have developed a head-mounted miniscope platform and successfully captured calcium activity images of brain cells in awake mice. Additionally, it has been confirmed in mouse models that the level of CTSS increases as the disease progresses. Through both in vitro and in vivo studies, we have confirmed that inhibitors of CTSS effectively prevent the death of dopamine neurons and slow down motor dysfunction.</p> <p>Specific goals: Our plan is to establish a high-efficient head-mounted miniscope platform for recording calcium images from awake brain, and to develop artificial intelligence algorithms based on the platform data to accelerate the development of a highly selective Cathepsin S inhibitor for the treatment of Parkinson's disease. The plan is divided into three parts: 1) Collaborate with electronic engineering experts and equipment providers to manufacture a high-precision automatic leveling stereotaxic system and establish a high-efficient head-mounted microscope platform to increase the success rate. 2) Collaborate with computer scientists and neuropharmacologists to develop artificial intelligence algorithms based on brain neural calcium activity and motor function, which can assist in designing drugs and rapidly analyzing drug efficacy. 3) Collaborate with medicinal chemists to produce a highly efficient Cathepsin S inhibitor and conduct relative animal studies of Parkinson's disease.</p> <p>Novelty and application: The high-precision automatic leveling stereotaxic system is expected to be the first of its kind in the market. This product is expected to benefit global behavioral neuroscientists and popularize the technology of head-mounted miniscope platform. The successful development of artificial intelligence algorithms based on brain neural calcium activity and motor function will also help shorten the preclinical research and development time of relevant drugs and predict drug efficacy more accurately. The experience of developing this algorithm will also be helpful in developing algorithms for other neurological diseases such as Alzheimer's and chronic pain. Finally, unlike traditional therapies, developing Cathepsin S inhibitors may provide another therapeutic approach for Parkinson's disease by protecting dopamine neurons.</p>
	<p>請概述執行本計畫之目的及可能產生對人文、社會、經濟、學術發展等面向的預期影響性(三百字以內)。</p> <p>※此部分內容於獲核定補助後將逕予公開</p>
計畫概述	<p>巴金森氏症是老年人常見的疾病，耗費巨大的社會資源。經由實行這個計畫，我們將開發一個全自動水平立體定位系統，以惠及全球使用立體定位儀器的神經科學家，特別使得頭戴式顯微鏡平台技術得以普及化。我們還將開發兩種人工智能演算法，AutoPD和AutoMolGen，以協助藥物開發和預測帕金森病藥物的動物療效。開發演算法期間所得到的經驗也能夠應用在其他神經退行性疾病的藥物開發策略。我們還將宣布一種治療帕金森病的CTSS抑制候選藥物，經由保護神經來達到治療目的。預期將彌補目前市售藥效果上的不足之處。</p>

三、研究計畫內容：

計畫性質表

重點主題	<p>■開發臨床可驗證之產品(例如新穎生物標記及其平台技術、數位技術、醫療影像等)，應用於神經系統疾病相關預防、診斷、預後、藥物評估或行為與活動介入之有效性等。</p> <p>□開發侵入式或非侵入式技術應用於治療腦與神經疾病(例如失智症、帕金森氏症、癲癇、中風、腦瘤等)、精神疾病 (憂鬱症、自閉症、藥酒癮、睡眠障礙等)、疼痛(例如偏頭痛、慢性疼痛、神經性疼痛等)之產品。</p> <p>□結合神經科學及行為決策模式，開發及推廣 (例如教育、學習及認知決策)之應用產品。</p>
所跨領域*(必填)	動物藥理，分子生物，化學，電腦科學，機械及自動控制
產業鏈結	合作產業/廠商：南州精密工業有限公司

*所跨領域自行填寫，例如生醫、工程、數理、資訊、認知、心理、統計、人文社會、影像分析、模擬計算與通訊科技產業等。

Specific Aims

Parkinson's disease is a neurodegenerative disease caused by the death of dopamine neurons in the brain. The market size of drugs for the treatment of Parkinson's disease is showing a growing trend year by year. Most treatment approaches are based on enhancing the function of the dopamine system in the brain or increasing the concentration of dopamine in the brain. **However, in the terminal stages of the disease, dopamine neurons have already died off in large numbers, and the effectiveness of this therapy will be very limited.** Currently, there are very few methods to safeguard dopamine neurons from dying. Our team found that the (Cathepsins S) CTSS inhibitor **CT001** can protect dopamine neurons from death and improve motor dysfunction in cellular models and animal models of Parkinson's disease. However, Parkinson's disease-related animal experiments often need to be carried out for more than half a year, which consumes a lot of time, manpower and material resources. Moreover, current evaluation strategies for animal behavior are difficult to establish a simple and intuitive detection method, which leads to the inability to accelerate drug development. Because motor dysfunction only occurs when the dopamine system has degenerated to a certain extent (60-80%). **If a method could be developed to detect changes in neural system activity, this could lead to the development of an early diagnostic indicator, which would benefit to the development of related drugs.** This research project will jointly develop a high-efficient head-mounted miniscope platform with electronic engineers and industry equipment manufacturers, provide a large amount of *in vivo* neural activity data and corresponding animal behavior data to computer scientists, and create artificial intelligence algorithms to identify motor impairments associated with Parkinson's disease and aid in drug development. Then with the assistance of neuropharmacologists and medicinal chemists, use animal models and drugs to verify and adjust these algorithms, which is expected to help accelerate the development of CTSS inhibitors or related drugs to treat Parkinson's disease, shorten the drug R&D timeline and cost of development (Figure 1).

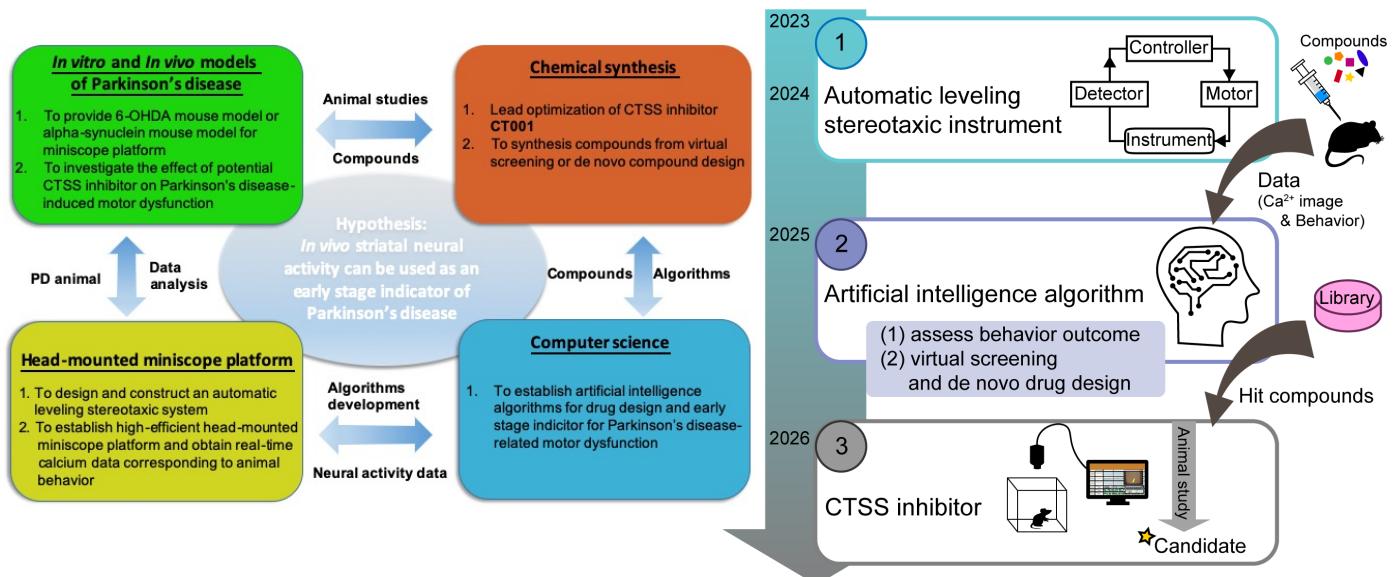


Figure 1. Plan and expected output of research team

The overall goal of the team is to develop a rapid and effective diagnostic method to accelerate Parkinson's disease drug development in collaboration with industry, computer scientists, neuropharmacologists, and medicinal chemists.

- 1) Develop a high-efficient head-mounted miniscope platform to record behavioral changes of living mice and synchronized brain neural activity images.

- 2) Based on a large amount of brain neural activity data, we plan to develop artificial intelligence algorithms to automatically detect motor dysfunction related to Parkinson's disease and de novo drug design.
- 3) Test the accuracy of the algorithm using animal models of Parkinson's disease and a series of CTSS inhibitors, and make necessary adjustments.
- 4) Using algorithms and a high-efficient head-mounted miniscope platform to assist in drug design and prediction of drug efficacy.
- 5) The expected output of this project is a high-precision automatic leveling stereotaxic system, artificial intelligence algorithms for detecting motor dysfunction and de novo drug design, and a CTSS inhibitors patent for technique transfer.

Sub-project 1. To establish high-efficient head-mounted miniscope platform

- Aim 1: To design the highly precision stereotaxic system
- Aim 2: To design the closed-loop control system for automatic leveling
- Aim 3: To construct the prototype of an automatic leveling stereotaxic system
- Aim 4: To establish high-efficient head-mounted miniscope platform by using automatic leveling stereotaxic system

Sub-project 2. To develop artificial intelligence algorithms for automatic detection of motor dysfunction related to Parkinson's disease and drug design

- Aim 1: Development of an automatic pipeline to extract and preprocess neural activity from image data
- Aim 2: Development of machine learning algorithms for detecting motor dysfunction related to Parkinson's disease
- Aim 3: Development of prediction models for therapeutic effects of molecules for Parkinson's disease
- Aim 4: Development of methods for virtual screening and de novo drug design

Sub-project 3. Using artificial intelligence algorithms to assist in drug design and develop an efficient go/no-go strategy of CTSS inhibitor development for treating Parkinson's disease

- Aim 1: Lead optimization of CTSS inhibitor **CT001**, molecular data bank, de novo
- Aim 2: To assess the effectiveness of CTSS inhibitors in Parkinson's disease animal experiments beforehand using a high-efficient head-mounted miniscope platform and artificial intelligence algorithm
- Aim 3: Gram scale synthesis of potent CTSS inhibitors
- Aim 4: To perform the Parkinson's disease animal experiments of CTSS inhibitors selected from artificial intelligence algorithm

Background

Parkinson's disease

Parkinson's disease is a progressive nervous system disorder that affects movement, caused by the degeneration of dopamine-producing neurons in the brain, leading to motor skill symptoms including bradykinesia, tremors, rigidity, vocal symptoms, rigidity and postural instability, difficulty with balance and coordination, and other nonmotor skill symptoms including mental issues, sense of smell problem, sweating, gastrointestinal issues, and pain. Most people diagnosed with Parkinson's disease are over the age of 65¹. However, about 10-15% of patient onset with the disease before the age of 40². The Parkinson's disease drug market is a rapidly growing market, driven by the increasing prevalence of Parkinson's disease worldwide and the growing demand for effective treatments. In 2020, the global Parkinson's disease therapeutics market generated revenues over \$5 billion. The expected annual growth rate of 6.5% from 2021 to 2027. In fact, the global therapeutics market for Parkinson's disease is expected to reach sales of \$ 8 billion by 2027.

So far, there is no cure for Parkinson's disease, but medications and other therapies can help manage symptoms including tremors, rigidity, slow movement, and difficulty with balance and coordination. Currently, there are several drugs aim to increase dopamine concentration in brain to treat Parkinson's disease, including levodopa, dopamine receptor agonists, monoamine oxidase B (MAO-B) inhibitors, catechol-O-methyltransferase (COMT) inhibitors, and anticholinergics³. There is also a growing interest in developing new strategies to treat Parkinson's disease, including those aimed at slowing or preventing the progression of the disease, and therapies that address the underlying causes of the disease.

Cathepsin

Cathepsin belong to lysosomal proteases that are found in many different types of cells and tissues and play important roles in a variety of physiological processes. There are three types of cathepsins, including serine proteases (cathepsins A and G), aspartic proteases (cathepsins D and E), and cysteine proteases (cathepsins B, C, F, H, K, L, O, S, V, X and W)⁴. In the nervous system, cathepsin have been implicated in several neurological diseases, including Alzheimer's disease, Parkinson's disease, multiple sclerosis, and other age-related inflammatory processes⁵.

In Parkinson's disease, alterations in calcium homeostasis have been implicated in the degeneration of dopamine-producing neurons in the brain, which is the hallmark of the disease. Studies have shown that increased intracellular calcium levels can lead to oxidative stress and neurotoxicity, which can contribute to the degeneration of dopaminergic neurons⁶. Additionally, alterations in calcium signaling have been shown to affect the activity of cathepsins, lead to changes in neuronal function, and contribute to the development or progression of Parkinson's disease. Furthermore, the C-terminal truncated α -synuclein, enriched and aggregated in Lewy bodies, is associated with the altered expression of cathepsins^{7, 8}.

Inhibitors targeting CTSS are frequently utilized in cancer treatment, but have never been used in the field of neurodegeneration such us Parkinson's disease. In our preliminary results, CTSS was upregulated in substantia nigra after 6-hydroxydopamine (6-OHDA) bundle lesioning. Selective CTSS inhibitor **CT001** was able to inhibit dopaminergic neuron lose both in cellular and animal models of Parkinson's disease. **CT001** also able to improve locomotor activities and sensory functions in animal models of Parkinson's disease.

However, animal experiments need to be performed for 6 to 12 months to complete. Such long-term requirement for animal study and evaluation of drug efficacy will seriously affect the progress of preclinical drug development. Furthermore, the choice of behavioral experiment used to evaluate the effectiveness of a drug can also lead to bias or incorrect conclusions.

Rationale

In Parkinson's disease, dopaminergic neuronal death can occur before the onset of behavioral symptoms. These symptoms can emerge when approximately 60-80% of dopamine-producing neurons in the substantia nigra have already been lost. **The miniscope platform has the capability to monitor alterations in the activity of living neural cells^{9,10}, implying that this research approach has the potential to identify degenerative changes in neurons at an early stage. This strategy is also suitable for the disease that lack of precise measurement methods.** A previous study successful create an artificial intelligence algorithm for chronic ongoing pain using *in vivo* calcium activity and deep learning. **Such observations indicate that, an early stage indicator can also be generated for Parkinson's disease-induced motor dysfunction based on the *in vivo* neural activity. This indicator can predict the efficacy of Parkinson's disease drugs in a timely manner, avoid resource waste, and accelerate the drug discovery program of Parkinson's disease.** In the present preliminary study, we established a miniscope platform and collected synchronized animal behavior and neural activity images. In order to efficient collect enough neural activity data for developing artificial intelligence algorithms. We plan to design and construct an essential surgery instrument for miniscope platform. The created artificial intelligence algorithms will then be field test for animal models of Parkinson's disease and CTSS inhibitors. This method should speed up the drug discovery program and help medicinal chemists to optimize CTSS inhibitors for further preclinical studies.

Preliminary results

Neural activity images in awake mice

We have established a head-mounted miniscope technology. This technology will play a crucial role in this project, assisting in the development of artificial intelligence algorithms and drugs. The standard procedure for this experiment was to perform stereotaxic surgery, injecting a virus carrying the GCaMP6 gene into a specific brain area (such as the hippocampus or striatum) so that the cells in that area expressed this protein. This protein significantly enhanced fluorescence intensity when bound to calcium, making it an indicator of cell activity. One month later, another stereotaxic surgery was performed, where a head-mounted fluorescent miniscope weighing approximately 2 grams was implanted into the same location as the previous virus injection (Figure 2A). If the location is accurate, after fixing the miniscope, **it was able to record the activity changes of over 500 neurons and corresponding mouse behavior data for at least three months while the mouse was awake**. Further observation showed a significant correlation between specific neural activity and specific mouse behavior (Figure 2B). However, one of the keys to the success of this experiment was very accurate stereotaxic surgery. When leveling the mouse skull, the deviation of each reference point must be less than 50 micrometers. Therefore, to significantly increase the success rate of the head-mounted miniscope technology to meet the needs of this project, it is necessary to redesign and manufacture an easy-to-operate stereotaxic system that meets the accuracy requirements of this technology.

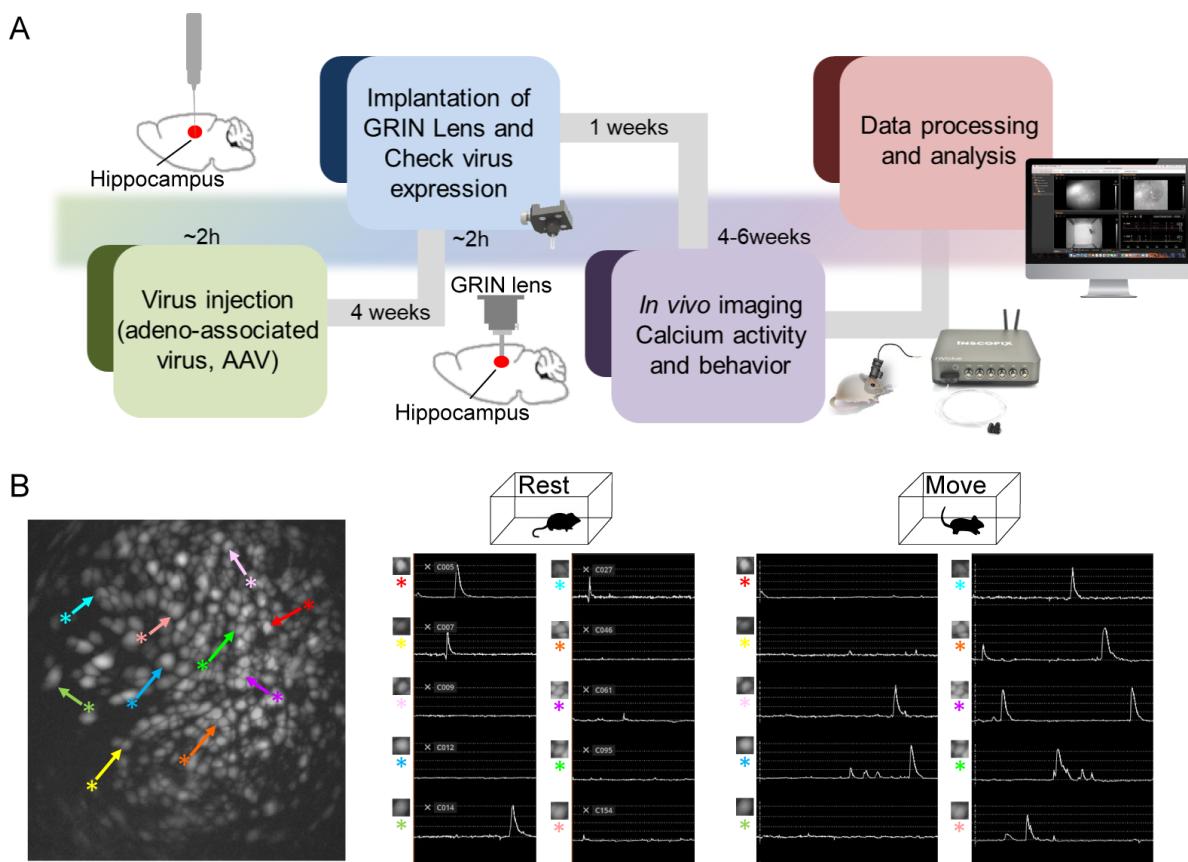
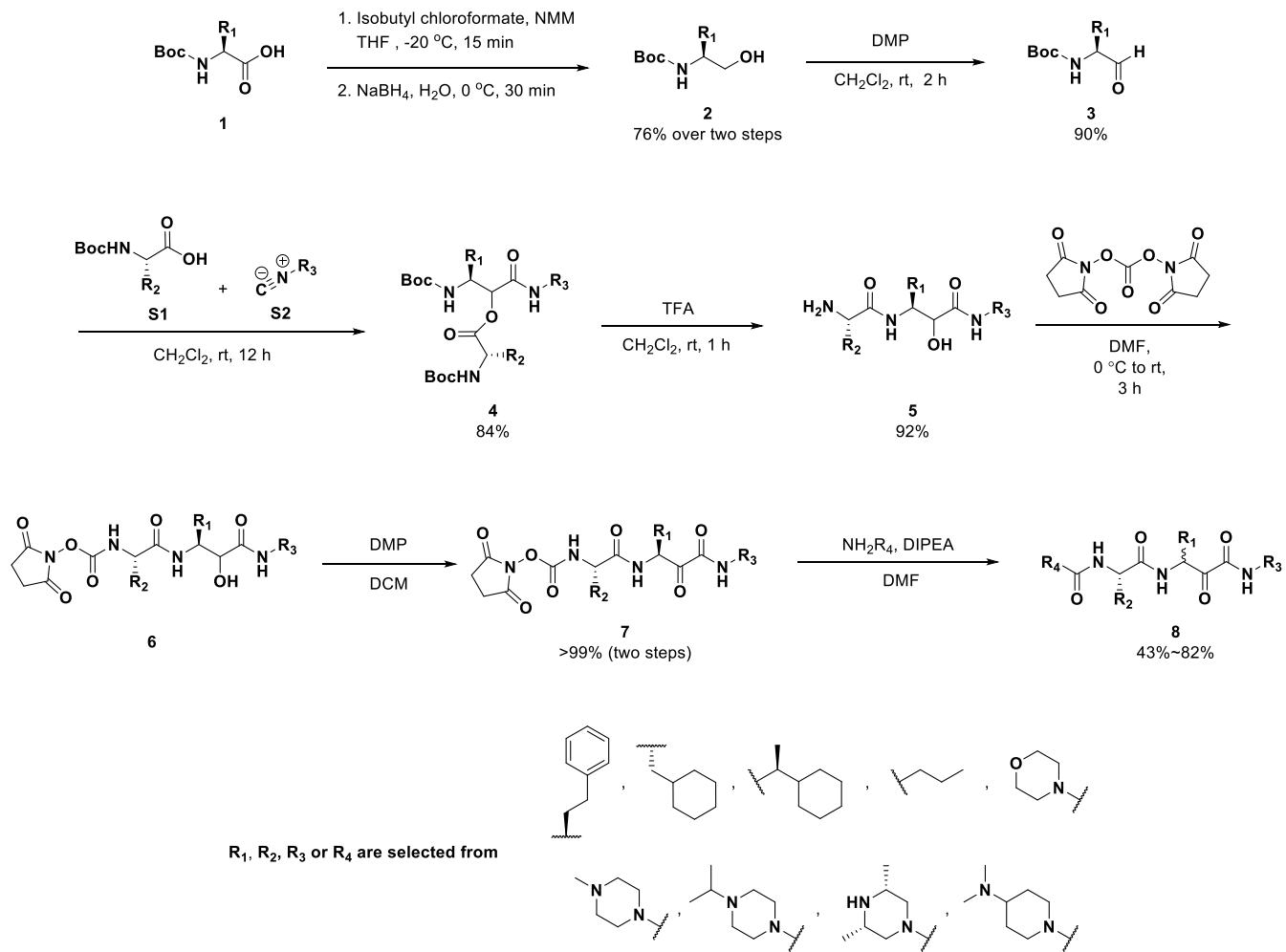


Figure 2. Head-mounted miniscope experiment A. Flow chart of the experiment. B. All neural activity images recorded by the miniscope within 1 hour. Highly correlation observed between individual neural activity and specific behavior.

Discovery of selective CTSS inhibitors

Dr. Chun-Cheng Lin and Dr. Hsing-Pang Hsieh's laboratory jointly developed a highly specific CTSS inhibitor **CT001** which has demonstrated nanomolar inhibitory efficacy against CTSS both *in vitro* and *in vivo*, and against pancreatic cancer cell line SUIT-2. Dr. Lin's graduate student's thesis has revealed the chemical structure of **CT001**. The general synthesis process of **CT001** and its analogues was described in Scheme 1, which involved seven steps and took 23-43% total yield. The synthesis started from different uncommon amino acid, which was activated by isobutyl chloroformate, and followed by reduction with NaBH₄ to give alcohol **2**. Oxidation of **2** with Dess-Martin periodinane (DMP) provided aldehyde **3**. Key Passerini reaction was utilized in which aldehyde **3**, carboxylic acid **S1** and isocyanide **S2** were combined to afford the intermediate **4**. The deprotection of Boc group using TFA led to an intramolecular rearrangement and give dipeptide **5**. The terminal NH₂ of dipeptide **5** was capped with succinimidyl carbonate, followed by the oxidation of DMP to give **7**. By reacting with various amines, the succinimidyl carbonate of **7** was transformed to yield the target tripeptide **8**. Using this in-house established method, we have already synthesized five analogues of **CT001** with different amino acids and amines.



Scheme 1. Synthetic process of CT001 and its analogues

Furthermore, the preliminary *in vitro* results of **CT001** and other five analogues (**CT002~CT006**) showed dose-dependent inhibition in the CTSS enzyme assay (Figure 3). All compounds have an IC₅₀ less than 100 nM. Among them, **CT003** may have a stronger potency and efficacy than **CT001**.

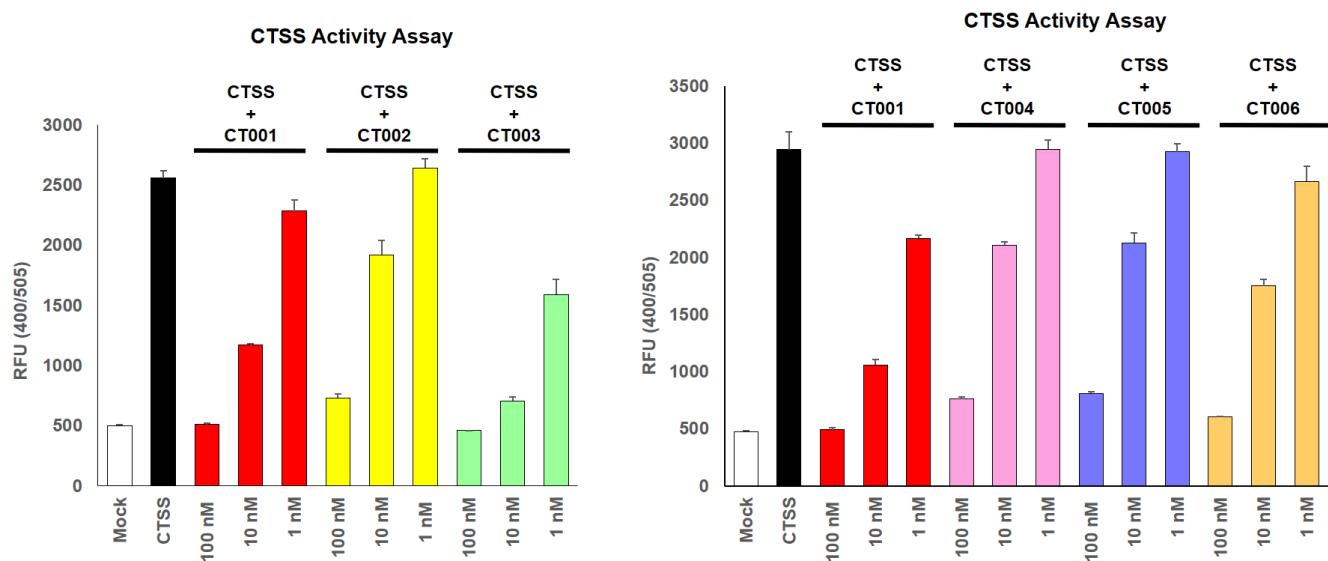


Figure 3. CTSS enzyme assay of CT001~CT006

Specific CTSS inhibitor CT001 against 6-OHDA-mediated dopaminergic neuronal loss in primary ventromesencephalic neuronal culture.

The protective effects of **CT001** were examined in rat primary ventromesencephalic neuronal culture (Figure 4A). Treatment with 6-OHDA (100 μ M) significantly reduced tyrosine hydroxylase immunostaining (TH-ir) (Figure 4B, p<0.001). **CT001** (10 nM, 100 nM, or 1000 nM; Figure 4C-4E) significantly antagonized 6-OHDA-mediated changes in TH -ir (p<0.001, one-Way ANOVA+NK test; Figure 4F)). Furthermore, **CT002** and **CT003** showed slightly higher potency compared to **CT001** in the cell assay (Figure 5)

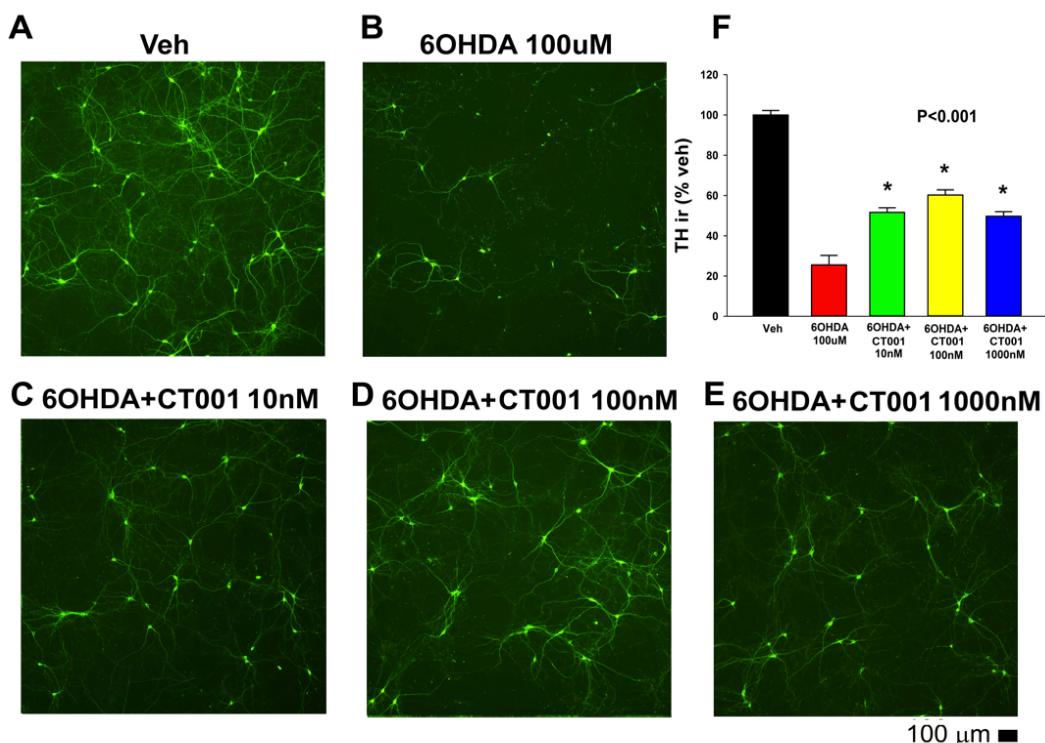


Figure 4. Effects of specific CTSS inhibitor CT001 on 6-OHDA-mediated dopaminergic neuronal loss in primary ventromesencephalic neuronal culture A. primary ventromesencephalic neuron. B. 6-OHDA induced cell loss. C-E. CT001 (100nM) showed the best protective effect. F. Statistical graph.

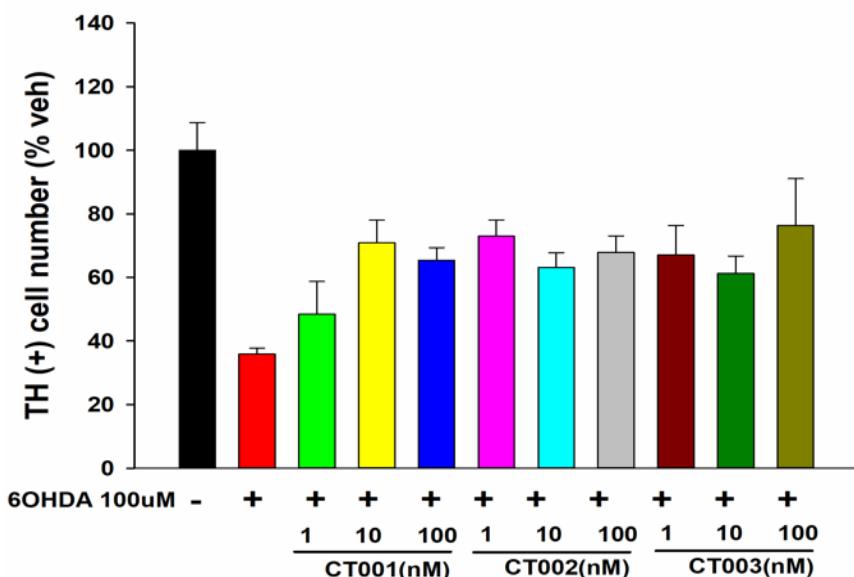


Figure 5. Protection effect of CT001~CT003 on 6-OHDA-mediated dopaminergic neuronal loss

The expression of CTSS or CTSD in 6-OHDA rat model of Parkinson's disease

Increasing evidences support that Cathepsins are closely involved in neurodegeneration, however, the role of CTSS in dopaminergic neurodegeneration has not been reported. Cathepsins D (CTSD) has been proposed to be the major lysosomal protease involved in α -synuclein degradation, its deficiency has been linked to the presence of insoluble α -synuclein conformers in the brain of mice and humans as well as to the transcellular transmission of α -synuclein aggregates¹¹. In our data, we have examined chronic upregulation of CTSS and transient upregulation of CTSD in substantia nigra after 6-OHDA lesioning at medial forebrain bundle (Figure 6). Similarly, CTSS and CTSD was significantly upregulated in the substantia nigra after 6-OHDA lesioning at striatum (Figure 7). Taken together, we have confirmed that 6-OHDA caused upregulation of CTSS and CTSD in the substantia nigra.

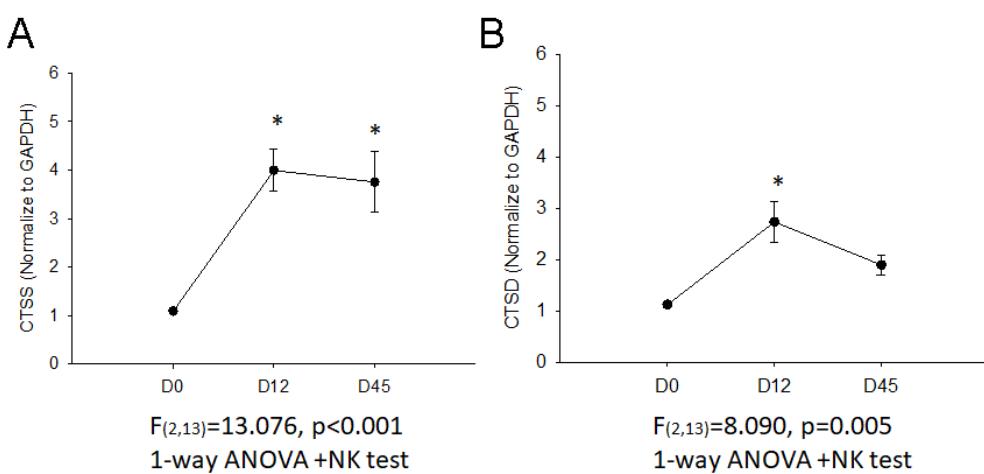


Figure 6. Differential upregulation of CTSS and CTSD in substantia nigra after 6-OHDA bundle lesioning A and B. expression of CTSS mRNA (A) and CTSD mRNA (B).

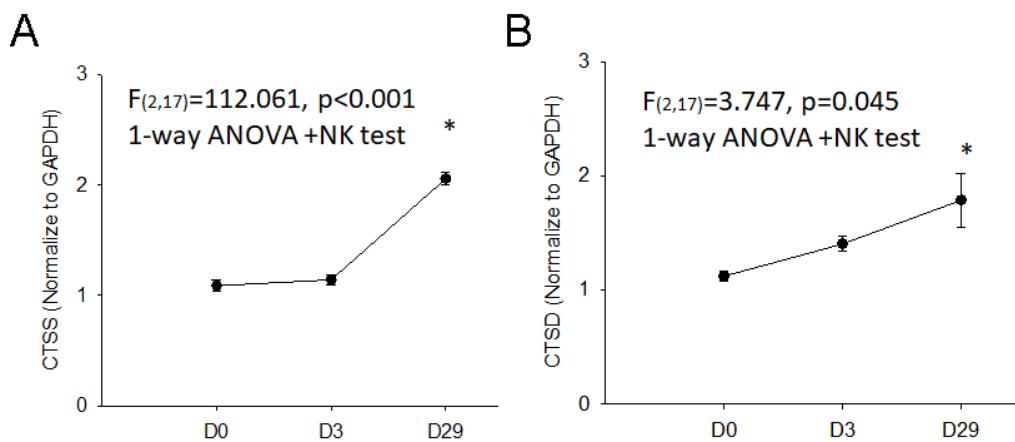


Figure 7. Lesioning at striatum induced significant CTSS and CTSD mRNA upregulation in substantia nigra A and B. expression of CTSS mRNA (A) and CTSD mRNA (B).

CT001 treatment improved locomotor activities and grip strength in 6-OHDA rat model of Parkinson's disease.

CT001 has been administered (15mg/kg/d, i.p.) after 6-OHDA lesioning for 4 weeks. Animals were received behavioral test from 6 weeks after 6-OHDA lesioning (naïve n=4; 6-OHDA n=4, 6-OHDA + **CT001** n=6). Our data demonstrated that **CT001** treatment significantly improved locomotor activity such as horizontal activity, total distance traveled, and movement time compared to 6-OHDA treated animals (Figure 8). In addition, it has been known that increasing severity of Parkinson's disease was associated with weaker grip. We measured the grip strength on 12 weeks after 6-OHDA lesioning. We found that **CT001** treatment significantly increased the strength (Figure 8).

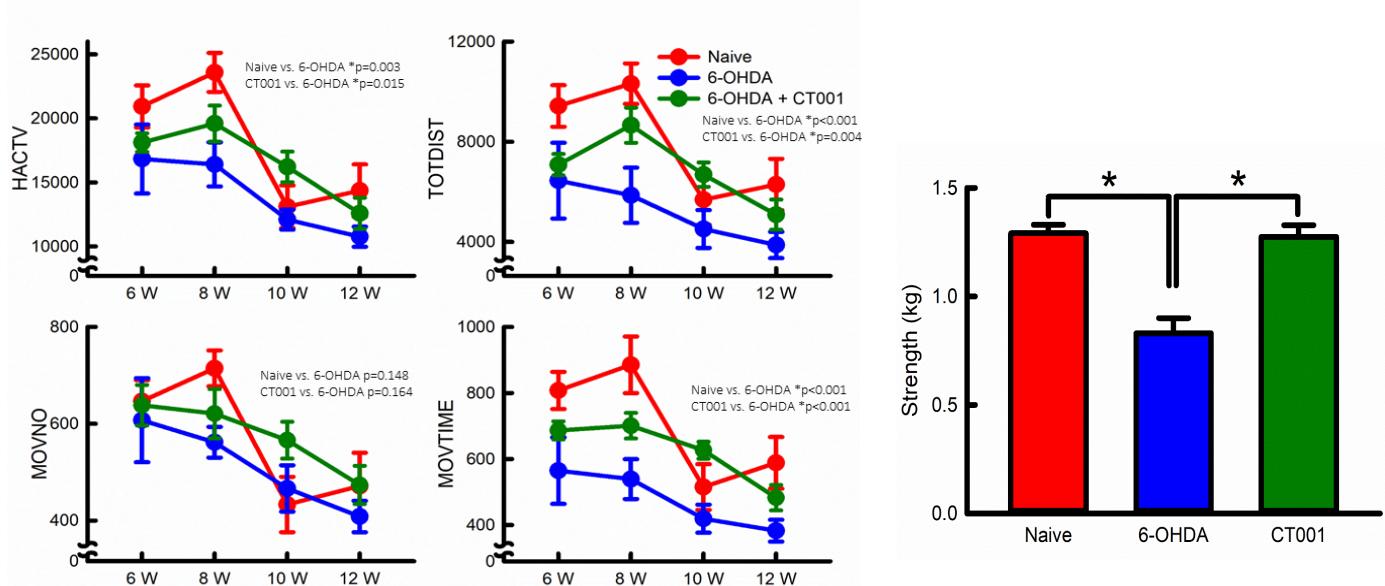
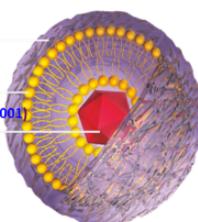


Figure 8. CT001 treatment improved locomotor activities and grip strength in 6-OHDA rat model of Parkinson's disease.

Preformulation studies of CT001

Despite the positive results from the preliminary *in vitro* and *in vivo* studies, **CT001** still has poor pharmacokinetic properties, which results in the issue of a high dosing frequency (daily dosing is required). Therefore, we also developed a formulation of **CT001** combined with liposome to improve its pharmacokinetics. Preliminary data showed that this combination has the potential to significantly improve the pharmacokinetic of **CT001** in mice, which is **a positive outcome with the potential for a formulation patent.**

Although the use of liposome formulation has enhanced the pharmacokinetics of **CT001** in mice, there remain obstacles that need to be overcome. The poor hydrophilicity of **CT001** currently limits the amount of **CT001** that can be encapsulated to only 4% (Table 1). Although the encapsulation efficiency is 53.5%, the overall amount of **CT001** encapsulated is still relatively low. Therefore, there is still scope for improvement in terms of ensuring the stability and managing the release of the liposomal form of the drug.



Samples	Z-average (nm)	PDI ^a	Zeta potential (mV)	EE% ^b	Liposomal CT001 (mg/mL)	Mean Drug-to-lipid (mole %)
1 2%, 0.9% ^c , S	94.39	0.112	-	-	-	-
2 2%, 0.9%, F	92.47	0.102	- 4.38	38.7	0.14	0.8
3 2%, 5% ^d , S	145.6	0.208	-	-	-	-
4 2%, 5%, F	142.8	0.117	- 10.5	43.2	0.16	0.9
5 3%, 5% ^e , S	122.9	0.127	-	-	-	-
6 3%, 5%, F	122.0	0.126	- 11.2	44.6	0.25	1.3
7 4%, 5% ^f , S	126.2	0.110	-	-	-	-
✓ 8 4%, 5%, F ^g	124.6	0.098	- 11.9	53.5	0.40	2.1

^a Polydispersity index
^b Encapsulation efficiency
^c 2% CT001, 0.9% MPEG2000-DSPE
^d 2% CT001, 5% MPEG2000-DSPE
^e 3% CT001, 5% MPEG2000-DSPE
^f 4% CT001, 5% MPEG2000-DSPE
^g Liposome sample for pharmacokinetic study

S: Sonicated liposome sample
F: Sonicated liposome sample was filtered through a 0.45 µm membrane filter

Table 1. Physical properties of CT001 liposomes

Experimental Design

The preliminary results suggest that inhibitors of CTSS could potentially be used to treat Parkinson's disease. However, due to the long duration and resource-intensive nature of animal experiments for Parkinson's disease, we plan to establish an efficient head-mounted microscope platform, accompanied by corresponding artificial intelligence algorithms, to evaluate the efficacy of the inhibitors in early stage, avoiding waste of resources.

Sub-project 1. To establish high-efficient head-mounted miniscope platform

Sub-project 2. To develop artificial intelligence algorithms for automatic detection of motor dysfunction related to Parkinson's disease and drug design

Sub-project 3. Using artificial intelligence algorithms to assist in drug design and develop an efficient go/no-go strategy of CTSS inhibitor development for treating Parkinson's disease

Sub-project 1

To establish high-efficient head-mounted miniscope platform

The main purpose of this sub-project is to pioneer a high-precision automatic leveling stereotaxic instrument. This instrument will greatly increase the success rate of head-mounted miniscope experiments, making it a high-efficient platform that can generate a large amount data of neural activity and corresponding behavioral data for Dr. Chun-Wei Tung to establish artificial intelligence algorithms (sub-project 2), and then make accurate predictions of treatment strategies for animal models of Parkinson's disease.

Stereotaxic instruments are critical for performing any brain surgery of animal. For miniscope experiment, adeno-associated virus (AAV) carrying a neuron-specific promotor driving GCaMP6 must be injected at the same location one month prior to the implantation of the head-mounted microscope, so that the calcium signal of neuron can be detected by miniscope. According to our experience, the deviation of stereotaxic coordinates between the two operations must be less than 50 microns. Since the focusing depth of field of the lens is only 200 microns, the inaccurate surgical positioning will directly lead to the failure of the experiment, and *in vivo* calcium signal of neuron cannot be observed.

As mentioned above, two precise stereotaxic operations must be performed consecutively in the head-mounted miniscope platform experiment to place the miniature microscope accurately on the nerve cells expressing GCaMP6. Looking at the current top stereotaxic manufacturers, the precision of their micro-manipulator arms is very high (to one micron). Some models, such as 71000 Automated Stereotaxic Instrument (RWD) or Robot Stereotaxic instrument (Neurostar), can also operate fully automatically. However, a skull rotation device that aligns the skull in the 71000 Automated Stereotaxic Instrument (RWD), and the 69100 The Rotational Digital Stereotaxic Frame is manual, and there is no precision gear to fine-tune the skull position to satisfy the minimal requirement of horizontal in miniscope experiment (bregma and lambda; reference points 2 mm left and right relative to bregma; Z-axis deviation < 50 microns). In addition, the mechanical alignment indicator lack of precision and sensitivity, and the contact point must be judged by the naked eye, which will greatly increase the deviation. The Robot Stereotaxic instrument (Neurostar) does not have a skull rotation device, instead of software to compensate and correct the stereotaxic coordinates according to the tilt of the skull. This strategy is indeed feasible under the premise of a single operation,

however, if more than two operations are performed, software compensation and correction errors may easily cause positioning inaccuracy. Furthermore, the micro-manipulator arm may reach the target area at different angles, which will further increase the area of tissue damage.

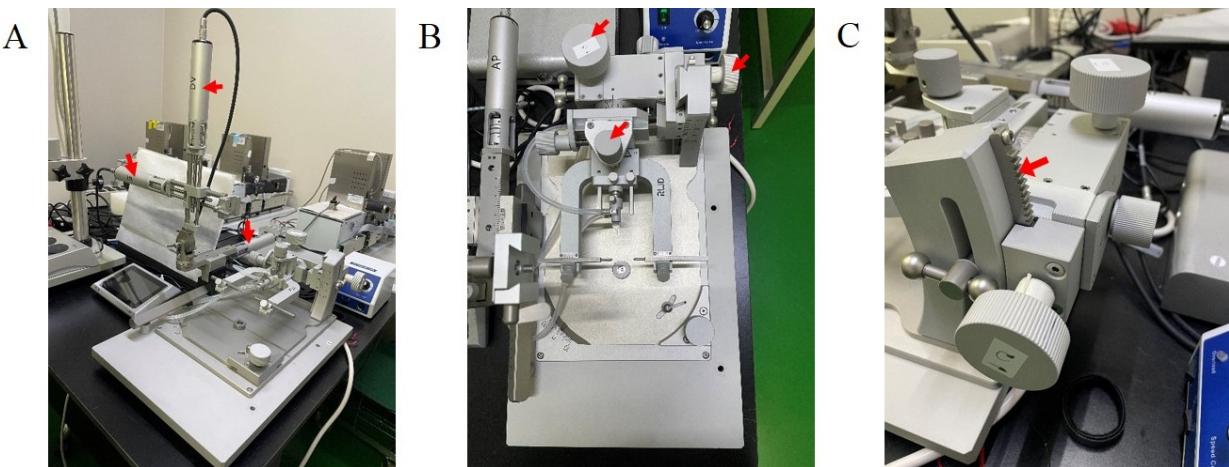


Figure 9. High-end stereotaxic instrument A. Comes with a 3-axis precision electric arm. B. Cranial adjustment device is still manually operated. C. The precision of the adjusting gear is insufficient.

Hence, we plan to develop a high-precision skull displacement and rotation device (Aim 1), closed-loop controlled by using non-contact contour sensor, logic program controller and stepping motor (Aim 2). After designing, a prototype machine will be manufactured for testing, and necessary modifications are made (Aim 3). Finally, this prototype machine will be field tested for miniscope experiment (Aim 4). This automatic leveling stereotaxic system should quickly correct the mouse skull level (less than 5 minutes), and avoid the common downsides of the current commercially available models. The accuracy rate (Z-axis deviation less than 10 microns) will eventually greatly increase the success rate of the experiment.

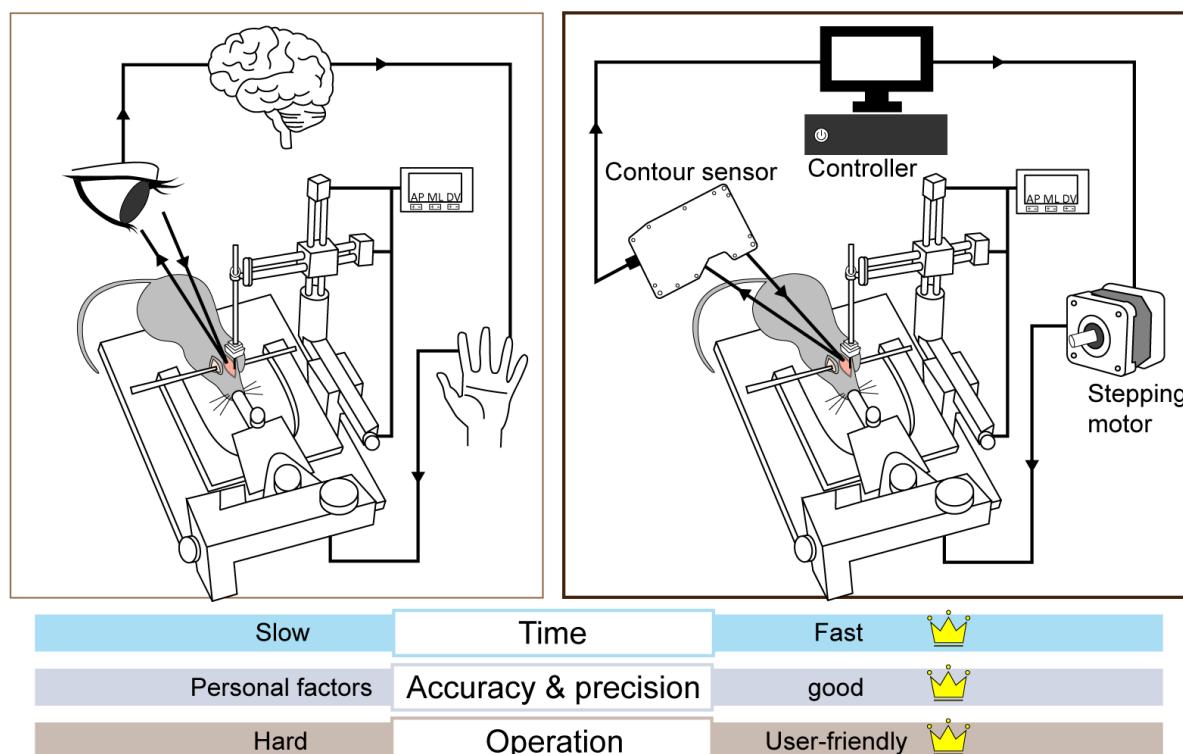


Figure 10. The automatic leveling stereotaxic system The latest automatic leveling stereotaxic system has the ability to automatically detect any inclination in the skull and make precise adjustments, leading to a noticeable improvement in the accuracy of locating the target.

Timetable

Year & Month Work Item	Year 2023		Year 2024		Year 2025		Year 2026	
Aim 1: To design the highly precision stereotaxic system	<input type="checkbox"/>							
Aim 2: To design the closed-loop control system for automatic leveling	<input type="checkbox"/>							
Aim 3: To construct the prototype of an automatic leveling stereotaxic system			<input type="checkbox"/>					
Aim 4: To establish high-efficient head-mounted miniscope platform by using automatic leveling stereotaxic system			<input type="checkbox"/>					

Aim 1: To design the highly precision stereotaxic system (Shiu-Hwa Yeh, NHRI/ Lun-De Liao, NHRI/ Nanzhou Precision Industry Co., Ltd.)

We will discuss with the equipment manufacturer, redesign and manufacture the stereotaxic instrument. The goal is to make the skull rotation device meet the precision requirements and integrate various automatic control components, including non-contact 2D or 3D contour sensor, and stepping motors. The hardware components will be manufactured by Nanzhou Precision Industry Co., Ltd. with a computer-controlled precision lathe. The company has many years of experience in precision component manufacturing and has completed many commission projects from Academia Sinica and National Synchrotron Radiation Research Center.

Aim 2: To design the closed-loop control system for automatic leveling (Shiu-Hwa Yeh, NHRI/ Lun-De Liao, NHRI/ Nanzhou Precision Industry Co., Ltd.)

The hardware part of the closed-loop automatic control unit will consist of a 2D or 3D contour sensor, a programmable logic controller, and stepping motors. The contour sensor can real-time record the position displacement changes of two reference points synchronously. The logic controller will receive the signal from 表 CM03

contour sensor and output the corresponding signal to the stepping motors for compensation. Electronic engineers (Dr. Lun-De Liao) in this team and equipment manufacturers will use programming support software to integrate PROTOCOL STUDIO, KV REPLAY VIEWER, KV MOTOPN+ and other application programs to meet customer needs, such as feedback of specific position displacement signals, horizontal precision adjustment and setting, etc.. The automatic control system will be handed over to Nanzhou Precision Industry Co., Ltd. to be responsible for the construction.

Aim 3: To construct the prototype of an automatic leveling stereotaxic system (Shiu-Hwa Yeh, NHRI/ Lun-De Liao, NHRI/ Nanzhou Precision Industry Co., Ltd.)

The electrical engineers of our team will hold close discussions with Nanzhou Precision Machinery Co., Ltd. (equipment) in order to optimize the configuration of the prototype based on the following conditions: including mechanical accuracy (including repeatability, resolution, and precision), information about the machine's control system (type of control algorithm used, input/output interfaces, and control software), Drive System (type of motor used, transmission mechanism, and maximum motion speed), environmental conditions (operating temperature range, humidity range, etc.), power requirements, physical dimensions, and safety features (emergency stop buttons, interlocks, safety sensors, etc.). The produced prototype will be evaluated on durability, accuracy, repeatability, and user-friendliness after actual application, and necessary modifications will be made.

Aim 4: To establish high-efficient head-mounted miniscope platform by using automatic leveling stereotaxic system (Shiu-Hwa Yeh, NHRI)

We will field test the prototype of automatic leveling stereotaxic system to conduct head-mounted miniscope experiments, and evaluate whether this prototype can significantly shorten the overall experimental time, reduce the difficulty of the experiment, and improve the success rate of the experiment. The successful development of this prototype machine is not only the key to generate enough *in vivo* neural activity data with miniscope instrument for sub-project 2 to build artificial intelligence algorithms, but also can benefit behavioral neuroscientists around the world, especially the potential of using head-mounted miniscopes laboratory. According to Martin Verhoef, chief commercial officer at Inscopix Biotech, the company has sold about 1,500 miniature microscopes to more than 650 laboratories since 2011¹². The advent of the automatic leveling stereotaxic system is expected to popularize the technology and attract more neuroscientists to this field. Furthermore, the high-efficient head-mounted miniscope platform is also critical for sub-project 3 because each test drug must be transformed into a neural activity data through this platform before being evaluated by the artificial intelligence algorithm.

Overall, we are developing a new stereotaxic system that is user-friendly and capable of achieving micrometer level calibration in minutes. With the existing miniscope instrument, we will have enough capability to support the work of sub-project 2 and sub-project 3 (Figure 11).

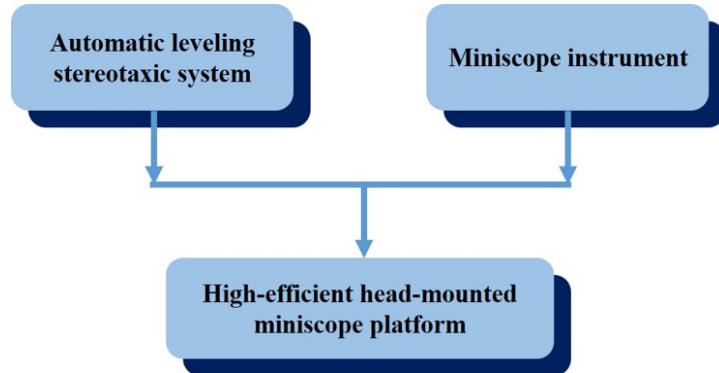


Figure 11. Summary of sub-project 1

Sub-project 2

To develop artificial intelligence algorithms for automatic detection of motor dysfunction related to Parkinson's disease and de novo drug design

The aim of sub-project 2 is to develop algorithms for identifying indicators for motor dysfunction related to Parkinson's disease and facilitating drug design. The identification of indicators for motor dysfunction related to Parkinson's disease includes two steps. The first step is to collect and process brain neural activity data of striatum from image recorded based on the system developed in sub-project 1 (Aim 1). The second step is to analyze the *in vivo* neural activity data by using machine learning algorithms and applying feature selection algorithms to identify indicators of motor deficit related to Parkinson's disease. The indicator identified in this step will be utilized to develop a prediction model for drug development (Aim 2).

For de novo drug design, we will first develop a prediction model for therapeutic effects of molecules for Parkinson's disease based on in-house and publicly available experimental data. The structure and activity data generated from sub-project 3 will be continually collected and utilized to retrain the model to improve the performance of the model that will be utilized to evaluate chemical structures before synthesis and measurement of bioactivity (Aim 3). Subsequently, de novo design algorithms will be developed based on reinforcement learning and evolutionary algorithms. The proposed algorithm will generate potential chemical structures for Parkinson's disease giving the highest score on the model developed in Aim 3 (Aim 4).

Timetable

Year & Month Work Item	Year 2023		Year 2024		Year 2025		Year 2026	
Aim 1: Development of an automatic pipeline to extract and preprocess neural activity from image data	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Aim 2: Development of machine learning algorithms for detecting motor dysfunction related to Parkinson's disease			<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Aim 3: Development of prediction models for therapeutic effects of molecules for Parkinson's disease	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Aim 4: Development of methods for virtual screening and de novo drug design				<input checked="" type="checkbox"/>				

Aim 1: Development of an automatic pipeline to extract and process neural activity from image data (Shiu-Hwa Yeh, NHRI/ Chun-Wei Tung, NHRI/Seong-Jin Yu, NHRI)

1-1 Implementation of a pipeline for extracting neural activity from striatum and detection of spike events as baseline features

First, Parkinson's disease mouse models such as 6-OHDA injection, alpha-synuclein overexpression, and their corresponding control groups will use the high-efficient head-mounted miniscope platform (sub-project 1) to record neural activity imaging sets of striatal neurons and corresponding behavioral data. Recording will be done for half an hour per week for a total of three months. To avoid the impact of individual differences, each group is expected to use data from 50 mice. The two Parkinson's disease animal models are time-consuming. As an alternative, we will also consider using drug-induced Parkinson's disease models such as dopamine D1 receptor antagonism or dopamine D2 receptor antagonism animal models to generate sufficient calcium imaging and behavioral data for algorithm development.

The images data of each time point will then be manually checked before the extraction of calcium activity. The data will be motion corrected if necessary. Regions of interest (ROIs) will be firstly identified by using the CNMF-E algorithm¹³ and followed by manual review with assistance from sub-project 1. The average fluorescence in each ROI and amplitude and frequency of spike events will be calculated by using ImageJ 表 CM03

(<https://imagej.nih.gov/ij/>) and MLSpike¹⁴ that have been proved to be effective for calcium activity calculation and event detection, respectively¹⁰. To reduce the noise of the extracted calcium traces, a Gaussian window method will be utilized. The de-noise activity value will be normalized by dividing the activity value (dF) by the mean (F0) of the activity values below the 70th percentile in each ROI. The above mentioned pipeline will be implemented for the extraction of calcium activity as baseline. The system flow of signal processing is shown in Figure 12.

1-2 Pipeline optimization for automatic calcium activity extraction

Recently, there are several new methods that have been proposed for motion correction, de-noising, source localization, source extraction and activity estimation. As there is a trade-off between efficiency/interpretability and accuracy, and the performance of methods is dataset dependent¹⁵, it is worthy to examine the performance on real cases and to optimize each component in the pipeline to develop an automatic pipeline for calcium activity calculation. For example, a recent deep learning model¹⁶, trained on extensive 2-photon calcium image data, is shown to provide a high correlation (0.8) to ground truth for spike estimation. The adaptation of the newly developed model could benefit the analysis of calcium activity from two-photon calcium images.

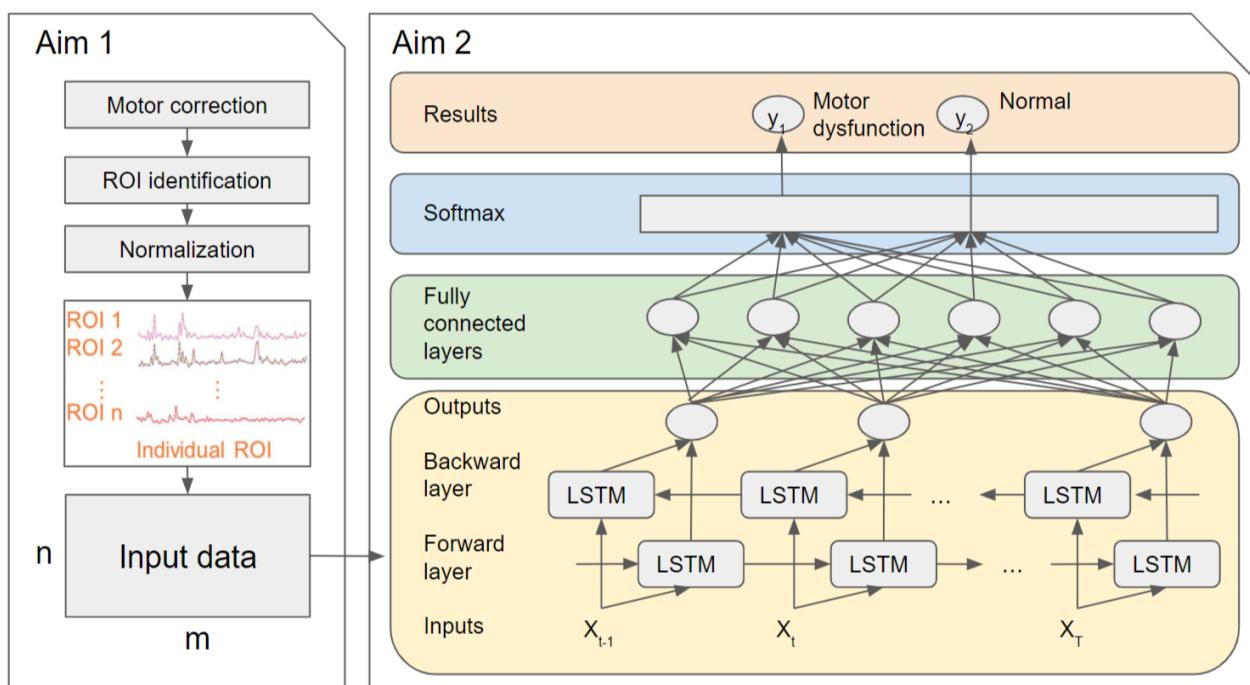


Figure 12. The system flow of signal processing and machine learning algorithms for detecting motor dysfunction related to Parkinson's disease

Aim 2: Development of machine learning algorithms for detecting motor dysfunction related to Parkinson's disease (Shiu-Hwa Yeh, NHRI/ Chun-Wei Tung, NHRI)

2-1 Development of a baseline model for detecting motor dysfunction related to Parkinson's disease

This project will develop a baseline model according to the successful machine learning application to the detection of spontaneous pain¹⁰. The baseline model will be developed by using a recurrent neural network (RNN)-based algorithm whose input is a two-dimensional matrix of $n \times m$, where n is the number of ROIs and 表 CM03

m is the number of frames. The models will be trained for optimizing the binary classification of motor dysfunction related to Parkinson's disease. The input layer will be fed to bidirectional long short-term memory RNN with a hyperbolic tangent activation function, and then connected to two dense layers with a rectified linear unit (ReLU) activation function and a sigmoid activation function, respectively. Finally, a dense layer with a softmax function will be utilized to calculate the final prediction. The system flow of machine learning algorithms is shown in Figure 12.

2-2 Development of machine learning algorithms optimized for detecting motor dysfunction related to Parkinson's disease

While the reference method provides good performance on detecting spontaneous pain¹⁰, its dataset is small ($n < 30$) and the performance measurements include no independent test dataset due to data scarcity that might overestimate the performance. One of the aims of this project is to collect a relatively large dataset with at least 150 samples from sub-project 1 and split the data into a training and an independent test dataset. Models will be trained and validated on the training dataset and tested on the independent test dataset to give a reliable estimation of prediction performance. Furthermore, feature selection techniques will be applied to identify the informative ROIs for detecting motor dysfunction. A sequential feature selection algorithm will be applied to iteratively include or exclude features for identifying the feature set giving the highest performance. As there are also several other methods for signal processing. Promising methods such as convolutional neural networks (CNN)¹⁷ will be implemented to compare their performance. The final outcome of this item is to develop a machine learning algorithm (AutoPD) optimized for detecting motor dysfunction related to Parkinson's disease.

Before proceeding to formally assist in evaluating the efficacy of potential drugs for Parkinson's disease in sub-project 3, the AutoPD algorithm will first be verified for accuracy using neural imaging in animal models of Parkinson's disease. We will evaluate which of the animal models, such as 6-OHDA injection, alpha-synuclein overexpression, dopamine D1 receptor antagonism, or dopamine D2 receptor antagonism, have better discrimination compared to the control group, and at which time points in the animal model have the best discrimination. This information will help establish the methodology for drug efficacy assessment in sub-project 3.

Aim 3: Development of prediction models for therapeutic effects of molecules for Parkinson's disease (Shiu-Hwa Yeh, NHRI/ Chun-Wei Tung, NHRI/ Hsing-Pang Hsieh, NHRI)

3-1 Development of prediction model from in-house and publicly available chemical structures with known experimental outcome

This item will develop a prediction model based on previously generated in-house chemical from sub-project 3 and neural activity data from sub-project 1 and publicly available data to guide early drug development for Parkinson's disease. A chemical structure will be encoded as a feature vector consisting of physicochemical properties and fingerprints. Alternatively, modern chemical encoding such as Mol2Vec¹⁸, Chemformer and an improved version of Chemformer called MegaMolBart (<https://catalog.ngc.nvidia.com/orgs/nvidia/teams/clara/models/megamolbart>) will be tested for their predictivity. The prediction target will be the binary therapeutic effect. Algorithms including LightGBM, CatBoost, XGBoost, random forest, extra trees, k-nearest neighbors and neural networks will be utilized to

develop individual models. Subsequently, feature selection and stacking algorithms will be utilized to identify the most relevant features and the optimal combination of machine learning classifiers for maximizing prediction accuracy. The resulted prediction model can facilitate the drug design of sub-project 3 by selecting the most potential chemical structures to synthesize and test.

3-2 Improvement of prediction model by integrating new chemical structures with experimental outcome

While the model developed in 3-1 can be useful for drug design, the dataset may not be easily extended and validated. To improve the drug design process, the model for detecting motor dysfunction related to Parkinson's disease developed in Aim 2 will be applied to calculate the motor dysfunction score as labels for training a new prediction model. In this way, machine learning algorithms will learn to predict motor dysfunction scores based on the physicochemical properties, fingerprints and other features. The data of chemical structure and corresponding motor dysfunction score will be obtained from the collaboration of all three projects. The developed model (AutoMolGen) is expected to provide a more specific indicator for guiding drug design.

Aim 4: Development of methods for virtual screening and de novo drug design (Shiu-Hwa Yeh, NHRI/ Chun-Wei Tung, NHRI/ Hsing-Pang Hsieh, NHRI)

4-1 Virtual screening for drug discovery

The proposed Aim 3 will develop prediction models for guiding drug discovery and design. Several chemical libraries including an in-house chemical library and the Maybridge library (Thermo Scientific, Thermo Fisher Scientific Inc.). will be screened for identifying the potential hits for Parkinson's disease. The in-house chemical library of IBPR NHRI consists of more than 200,000 chemicals. A virtual screening method will be applied to identify potential chemical structures for experimental validation.

4-2 Development of methods for de novo drug design

The virtual screening method may be limited by the diversity and size of the compound libraries. Considering the enormous chemical space, traditional virtual screening methods may explore only a small part of the chemical space. In this item, several strategies will be utilized for de novo drug design, including reinforcement learning, evolutionary algorithm and generative learning. The reinforcement learning techniques enable virtual modification of functional groups and atoms given the current best solution for achieving better scores obtained from the interacting environment. In this study, the models developed in the Aim 3 will be adopted as the environment for scoring the generated molecules. The Molecule Deep Q-Networks (MolDQN)¹⁹ will be developed for reinforcement learning-based de novo drug design. Alternatively, evolutionary algorithm-based de novo drug design²⁰ also showed advantage for reasoning the optimal structures for specific scoring functions. More recently, the emerging generative learning algorithm may also be useful for de novo drug design. For example, MegaMolBART utilized natural language processing techniques to learn latent representation and generate potential structures based on the latent features. The workflow of reinforcement learning and generative learning were illustrated in Figure 13A and Figure 13B, respectively. The present study will develop and implement the three strategies to explore the potential

chemical space for Parkinson's disease. The generated molecules will be synthesized and tested in sub-project 3 and sub-project 1.

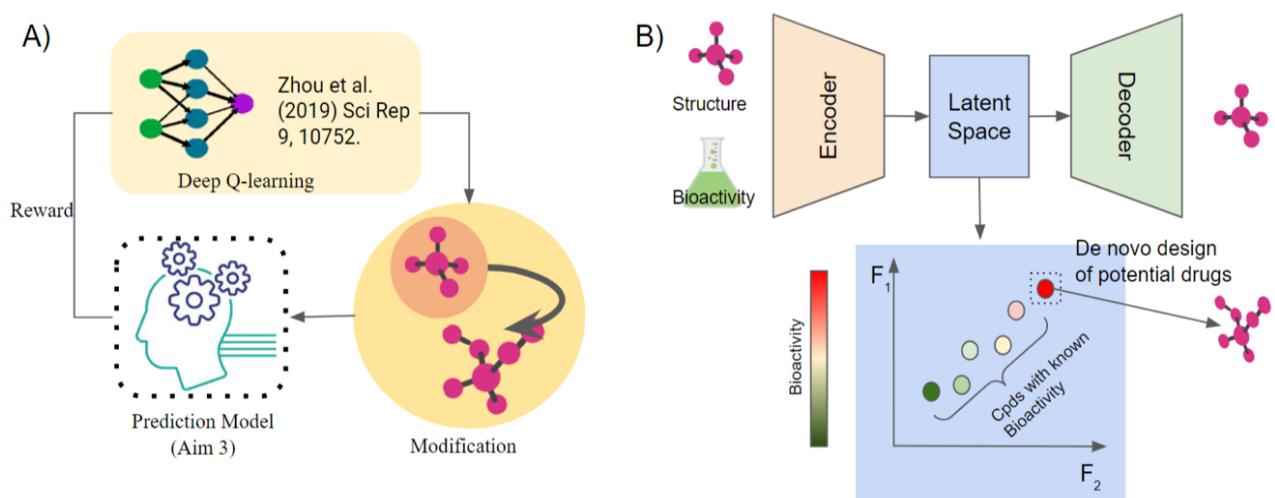


Figure 13. Illustration of methods for de novo drug design (A) reinforcement learning. (B) generative learning.

Overall, we plan to develop artificial intelligence algorithms for automatic detection of motor dysfunction related to Parkinson's disease (AutoPD) and de novo drug design (AutoMolGen), by using *in vivo* neural activity data generated by head-mounted miniscope platform (sub-project 1) (Figure 14).

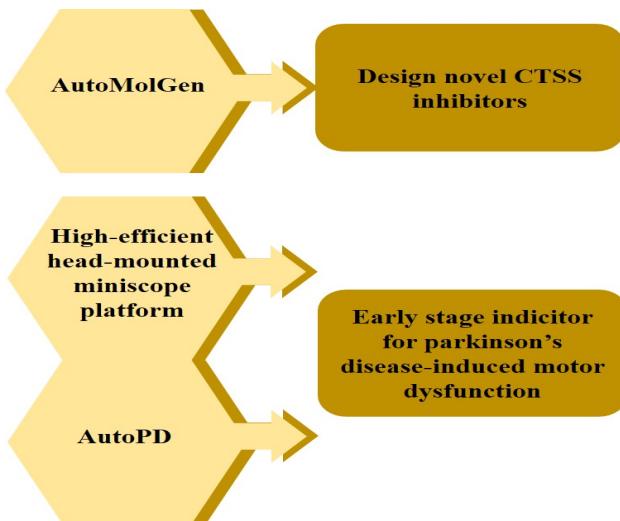


Fig 14. Summary of sub-project 2

Sub-project 3

Using artificial intelligence algorithms to assist in drug design and develop an efficient go/no-go strategy of CTSS inhibitor development for treating Parkinson's disease

The goal of sub-project 3 is to design highly selective CTSS inhibitors through continuous modification of the CT001 chemical structure, virtual screening, or with the help of the algorithm AutoMolGen (Aim 1). These compounds will then be evaluated for their *in vivo* activity using the head-mounted miniscope platform in 表 CM03

conjunction with the artificial intelligence algorithm AutoPD (Aim 2). Finally, the selected compounds will proceed gram scale synthesis (Aim 3) and undergo a series of cellular and animal experiments to thoroughly assess their efficacy (Aim 4).

Timetable

Year & Month Work Item	Year 2023			Year 2024			Year 2025			Year 2026		
Aim 1: Lead optimization of CTSS inhibitor CT001 , molecular data bank, de novo	<input type="checkbox"/>	<input checked="" type="checkbox"/>										
Aim 2: To assess the effectiveness of CTSS inhibitors in Parkinson's disease animal experiments beforehand using a high-efficient head-mounted miniscope platform and artificial intelligence algorithm				<input checked="" type="checkbox"/>								
Aim 3: Gram scale synthesis of potent CTSS inhibitors				<input checked="" type="checkbox"/>								
Aim 4: To perform the Parkinson's disease animal experiments of CTSS inhibitors selected from artificial intelligence algorithm							<input checked="" type="checkbox"/>					

Aim 1: Lead optimization of CTSS inhibitor CT001, molecular data bank, de novo (Hsing-Pang Hsieh, NHRI/ Chun-Wei Tung, NHRI)

1-1 Lead optimization of CTSS inhibitor CT001

Given that **CT001** has poor hydrophilicity resulting in low liposomal encapsulation efficiency, we aim to introduce ionizable functional groups to enhance the overall water solubility of the molecule while retaining the inhibition effect on CTSS. Based on preliminary bioactivity data, we infer that terminal R₄ groups in **8** (Scheme 1) should not significantly affect the inhibition effect on CTSS, hence we can start the structure modification from this point. Initially, tertiary amines were introduced to prepare salt-form structures in an attempt to increase the water solubility of the molecule, but the introduction of tertiary amine resulted in epimerization at the R₁ group. To overcome this, the oxygen atom in the morpholine group of **CT001** was planned to push out to form a hydroxyl group (**CT007**, Figure 15), which may provide a chance to introduce phosphate ester or acid-containing ester bonds as a prodrug²¹ (Figure 15).

Using this strategy may introduce other ionizable functional groups than tertiary amine, improve the overall water solubility of the molecule, and enhance the encapsulation efficiency in liposomes.

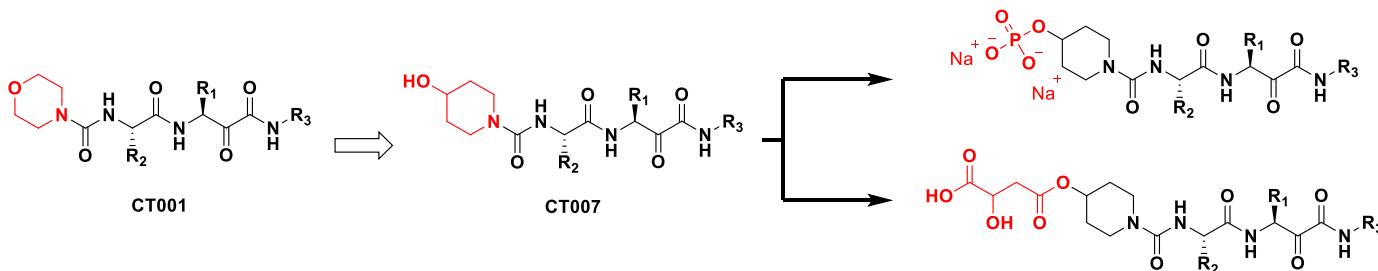


Figure 15. Prodrug scheme of CT001

1-2 Virtual screening or artificial intelligence algorithm for developing new compound structures

In addition to optimizing **CT001** and its chemical structure, we will also use the virtual drug screening method to screen our in-house compound library (approximately 200,000 compounds), or de novo design new compound structures using artificial intelligence algorithm AutoMolGen developed in sub-project 2 (Aim 4). The selected or designed compounds will be synthesized again and undergo initial CTSS enzyme tests to confirm their effectiveness ($IC_{50} < 10 \mu M$) before proceeding with further structural modifications.

Aim 2: To assess the effectiveness of CTSS inhibitors in Parkinson's disease animal experiments beforehand using a high-efficient head-mounted miniscope platform and artificial intelligence algorithm (Shiu-Hwa Yeh, NHRI/ Chun-Wei Tung, NHRI/ Seong-Jin Yu, NHRI/ Hong Chuang, NHRI/ Teng-Kang Yeh, NHRI)

2-1 In vitro activity testing of CTSS inhibitors

All newly synthesized compounds will first be observed for their ability to inhibit enzyme activity using the CTSS enzyme assay. Then, the ability of the compounds to protect dopamine neurons will be observed in primary ventromesencephalic neuronal culture. The enzyme assay and cell experiments will use **CT001** (10 nM or 100 nM) as a reference. All compounds that are equally or even better than **CT001** will be selected for further preliminary pharmacokinetic and solubility experiments, using **CT001** as the reference indicator.

2-2 Using artificial intelligence algorithm to pre-evaluate animal drug efficacy

We will use the methodology established in sub-project 2 (Aim 1&2) and a high-efficiency head-mounted miniscope platform to conduct short-term animal experiments with the test drugs. We will collect neural activity data from the striatum after drug treatment and use artificial intelligence algorithm AutoPD to evaluate them. In this step, **CT001** will also be used as a reference. Only CTSS inhibitors that pass the evaluation will move on to the next step, where the effect of the drugs on improving animal motor function, balance, and somatosensory will be evaluated in actual animal experiments (Aim 4).

Aim 3: Gram scale synthesis of potent CTSS inhibitors (Hsing-Pang Hsieh, NHRI)

Dr. Hsing-Pang Hsieh will implement pilot-scale synthesis of potent CTSS inhibitors which are selected from artificial intelligence algorithm (Aim 2) for further pharmacokinetic and animal studies.

Aim 4: To perform the Parkinson's disease animal experiments of CTSS inhibitors selected from artificial intelligence algorithm (Seong-Jin Yu, NHRI)

4-1 The neuroprotective effect of CTSS inhibitors will be examined in primary ventromesencephalic neuronal culture against 6-OHDA induced dopaminergic neuronal loss

To do that, primary cultures will be prepared from embryonic (E15) ventral mesencephalon (VM) tissues obtained from fetuses of timed-pregnant Sprague-Dawley rats. The whole-brain will be removed aseptically, and a small piece of tissue comprising the VM will be dissected. After removing the blood vessels and meninges, pooled VM tissues will be trypsinized (0.25%; Invitrogen, Carlsbad, CA) with gentle mixing for 15 min at 37°C. After rinsing off trypsin with pre-warmed DMEM/F-12 (Invitrogen), cells will be dissociated by trituration, counted, and plated into 96-well (6.0×10^4 /well) cell culture plates pre-coated with poly-D-lysine (Sigma-Aldrich). The culture plating medium consisted of Dulbecco's modified Eagle medium/F12 supplemented with 10% heat-inactivated fetal bovine serum, 1mM L-glutamine, and 2% B27 (Invitrogen). Cultures will be maintained at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. The cultures will be fed by exchanging 50% of media with feed media (Neurobasal medium, Invitrogen) with 0.5 mM l-glutamate and 2% B27 with antioxidants supplement on DIV (days in vitro) 3 and 5.

4-2 To investigate if the administration of CTSS inhibitors could alter Parkinson's-related behavioral deficits

To do that, adult rats will be used for this study. 6-OHDA will be stereotactically administered to the left medial forebrain bundle of rats to induce unilateral lesion of nigrostriatal pathway. Animals will develop rotational behavior after challenging with apomorphine or amphetamine. A prominent loss of TH immunoreactivity and DA will be found in the lesioned striatum. We will examine if test compounds normalize behavior at one month after 6-OHDA lesioning. Three neurological behavior will be examined:

- (a) Rotational behavior will be evaluated using an 8-channel rotometer system (RotoMax, AccuScan Instruments, Inc). Methamphetamine (2.5 mg/kg)-induced rotation will be evaluated.
- (b) Locomotor activity. Open field locomotor activity will be measured using an automated Accuscan activity monitor (Columbus, OH) after surgery. Each animal will be placed in a 42X42X31 cm 4 plexiglass open box for 1 day. Water bottles and food pellets will be provided in the chambers. Motor activity will be calculated using the number of infra-red beams broken by the animals.
- (c) Cylinder test. Unilateral injection of 6-OHDA can cause limb impairment. The cylinder test will be performed to investigate spontaneous forelimb lateralization, taking advantage of the natural exploratory instinct of rodents to a new environment. The test will be performed as follows: Rats were placed individually inside a glass cylinder (diameter, 22 cm; height, 26 cm). The video will be recorded for 5 min after rats first touched the walls of the cylinder with the impaired or unimpaired forelimb or both simultaneously. Each individual wall touch numbers will be counted by a blinded researcher.

4-3 The expression of multiple genes will be examined by Western blot, qRT-PCR or immunohistochemistry to find relevant mechanisms

All animals will be sacrificed after behavior test for molecular or histological examination (i.e., dopamine transporter or DAT, tyrosine hydroxylase or TH staining) and biochemical analysis (DAT, TH Western

analysis). In case of qRT-PCR, Total RNAs will be isolated from cells or brain tissues using TRIZOL Reagent (Life Technologies, #15596-026) and cDNAs will be synthesized from 2.5ug total RNA using SuperScript Vilo™ cDNA Synthesis Kit (Life Technologies, #11754 -050). The TaqMan® Gene Expression Assays (primer and probe set) for specifically detecting BDNF, GDNF, or others will be purchased from Life Technologies. Quantitative Real-Time PCR (qRT -PCR) will be carried out using TaqMan Fast Advanced Master Mix (Life Technologies, #4444557) and Applied Biosystems 7500 Fast Real-Time PCR System following the reaction condition recommended in the manufacturer's protocol. Expression and normalization of the target genes will be calculated relative to the endogenous reference gene (Beta-actin) by the use of qBase v 1.3.5, software for automated analysis of qRT-PCR data, with a modified delta-delta-Ct algorithm that takes specific gene-specific amplification efficiency into account for accurate calculation.

Overall, we will continuously modify the compound structure (SAR studies), design compounds with algorithm AutoMolGen, and perform virtual screening to obtain potent CTSS inhibitors. Then, by using the head-mounted miniscope systems with algorithm AutoPD, we will early assess the efficacy of these compounds in animal models (Figure 16). Our aim is to reduce resource waste and accelerate the development of Parkinson's disease drugs by using this strategy.

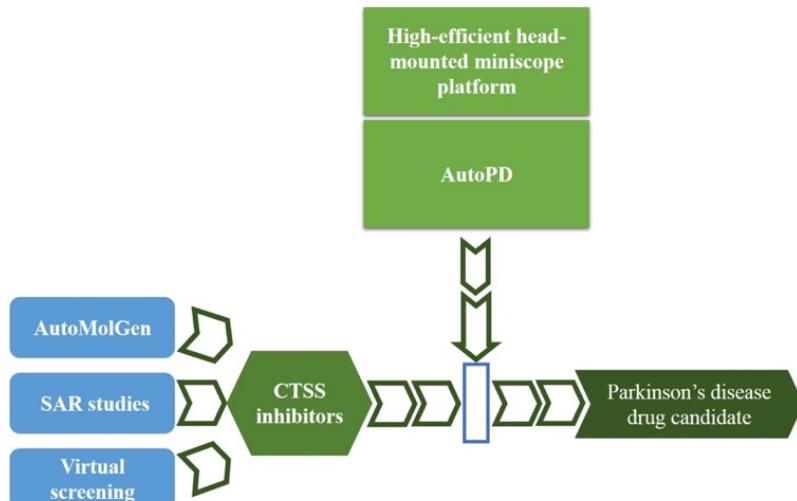


Figure 16. Summary of sub-project 3

Result Expectation

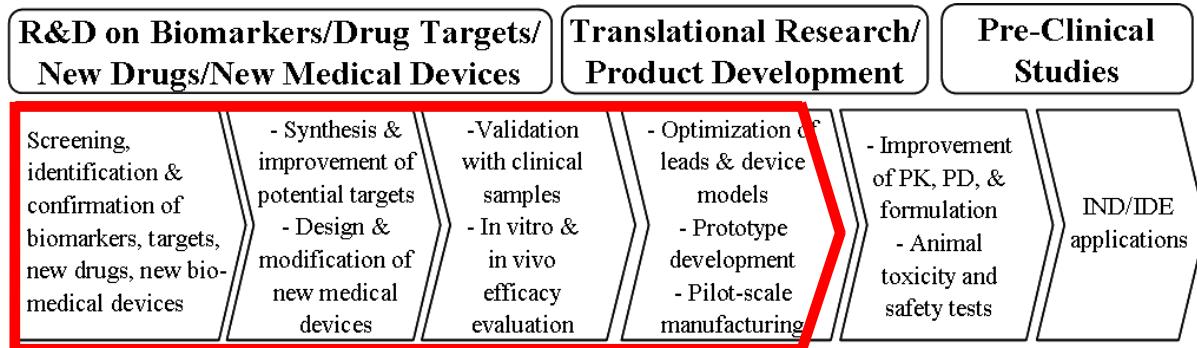
1. **The goal of Sub-project 1 is to produce a market-first automatic leveling stereotaxic system that caters to the large user base of global researchers utilizing head-mounted miniscope systems for neuroscience studies.** As previously mentioned, the success of such experiments depends on highly trained researchers who perform precise surgeries with an accuracy of less than 50 micrometers, twice every two weeks to a month. However, current high-end products such as the 71000 Automated Stereotaxic Instrument (RWD) or the Robot Stereotaxic Instrument (Neurostar) fail to meet this requirement. The automatic leveling stereotaxic system developed through this project will allow a researcher with moderate training to perform skull leveling correction within minutes with an accuracy of less than 10 micrometers. Currently, head-mounted microscopy technology is applied in fields such as neural circuit analysis,

optogenetics, behavioral neuroscience, neurodegenerative diseases, drug discovery, brain development, sleep research, and pain research. The well-known head-mounted microscopy company, Inscopix Biotech, has sold its products to over 650 laboratories worldwide since 2011. The industry of head-mounted miniscope is growing. The widespread adoption of automatic leveling stereotaxic system is predicted to increase the popularity of this technology and draw more neuroscientists to the field. We will collaborate with Dr. Lun-De Liao (NHRI), and Nanzhou Precision Industries Co., Ltd. to achieve this goal. It is expected to take two years to complete the design, manufacturing, and testing of the prototype machine. In the meantime, the product will be used to continuously drive the project in the third and fourth year, including artificial intelligence algorithms (sub-project 2) and accelerated evaluation of CTSS inhibitors in Parkinson's disease animal models (sub-project 3).

2. **The goal of sub-project 2 is to develop artificial intelligence algorithms that can accurately detect movement impairments caused by Parkinson's disease and assist in drug design.** Traditional method in the development of drugs for neurodegenerative diseases, like Alzheimer's or Parkinson's disease, requires the use of multiple animal models to evaluate complex behavioral changes, which is time-consuming and can lead to misjudgments due to differences in behaviors between species. Neural activity data collected by head-mounted microscopy platforms are integrated information and relatively easy to read, with low species differences, making them ideal materials for creating relevant artificial intelligence algorithms. Dr. Seong-Jin Yu will provide Parkinson's mouse models and we will use them on the head-mounted miniscope platform to simultaneously record mouse behavior changes and corresponding neural calcium activity. Dr. Chun-Wei Tung will use this data to build the artificial intelligence algorithms. We expect to spend for at least two years to collect data, build and adjust the artificial intelligence algorithms, and then use the algorithm to evaluate the efficacy of the CTSS inhibitors in sub-project 3 in the third year. Additionally, this algorithm is expected to be valuable for Parkinson's drug development projects globally with commercial value. We will contact companies such as In-Vivo AI, PreClinical AI, BenevolentAI, NumeRhythm, and Recursion Pharmaceuticals that are skilled in conducting clinical trials using artificial intelligence algorithms to evaluate the possibility of technology transfer in the fourth year.
3. **The goal of sub-project 3 is to file a CTSS inhibitor patent for technique transfer and select a drug candidate for preclinical study.** The current treatment strategy for Parkinson's disease mostly through enhancement the function of dopamine system in the brain or supplements dopamine to increase its concentration. These drugs are very effective in the early stages of the disease, but their efficacy decreases significantly as the disease progresses, because of large numbers of dopaminergic neuron degeneration. This project adopts a different approach by inhibiting the activity of CTSS to protect dopamine neurons from death. This approach has been verified in cellular and animal models of Parkinson's disease. The discovery of drug candidates with novel chemical structures for the treatment of Parkinson's disease is patentable. The development of CTSS inhibitors will benefit the clinical treatment modalities of Parkinson's disease. Our research team has extensive experience in the development of drugs for neurological diseases and has been involved in the execution of National Research Program for Biopharmaceuticals (NRPB) and several NSC grants to develop novel painkillers with minimal adverse

effects. With the assistance of high-efficiency head-mounted miniscope platform and artificial intelligence algorithms, our team is confident in completing lead optimization, performing numerous animal studies, and developing drug candidates for preclinical studies within the next four years. The criteria of the drug candidate are: PSA = 2-5, cLogP < 85 Å², molecular weight < 400, cardiotoxicity > 100 mg/kg, water solubility > 200 µg/ml, and administration routes: oral or injection. Our developed CTSS inhibitors will be different from current drugs, and will prevent neuronal cell death in Parkinson's disease models, achieving the goal of treatment rather than just relieving the symptoms of the disease.

Milestone:



4. The development of drugs for neurodegenerative diseases such as Alzheimer's and Parkinson's disease are often hindered by long animal model execution time and complex evaluation methods. This project proposes an innovative approach by integrating live neural activity to establish artificial intelligence algorithms for rapid assessment of drug efficacy in animals. This project has a high potential impact as there are currently no competitors, and successful identification of a novel CTSS inhibitor for treating Parkinson's disease would serve as proof of concept. The results of this research have the potential to greatly impact fields such as neuropharmacology, medicinal chemistry, computer science, electronic engineering, and precision industry. The findings will be published in high-impact journals and will provide valuable information for future drug discovery efforts for neurodegenerative diseases like Parkinson's disease.

Aims	sub-project 1	sub-project 2	sub-project 3	Total
Paper	2	2	2	6
Conference Paper	2	2	2	6
Patent	2			2

Staff Training

The research staff involved in this project will be trained in the fields including neuropharmacology, medicinal chemistry, computer science, electronic engineering, precision industry, pharmacokinetics, formulation development, animal studies, and in the most important part: the logical thinking of drug discovery.

Relevance with other sub-projects

In this project, the manufacturing of an automatic leveling stereotaxic system is essential for establishing efficient head-mounted miniscope platform, which will be performed in sub-project 1 (Shiu-Hwa Yeh/Lun-De Liao/Nanzhou Precision Industry Co., Ltd.). This platform will be used to provide a large amount of live neural activity data and corresponding animal behavior data to establish artificial intelligence algorithms, which will be performed in sub-project 2 (Shiu-Hwa Yeh/Chun-Wei Tung/Seong-Jin Yu). Finally, with the assistance of the head-mounted miniscope platform and artificial intelligence algorithms, our team will be able to quickly identify and select potential CTSS inhibitors for critical animal experiments, which will be performed in sub-project 3 (Hsing-Pang Hsieh/Chun-Wei Tung/Shiu-Hwa Yeh/Seong-Jin Yu/Jang-Yang Chang). Lun-De Liao is an electronic engineer who will assist the team in cooperating with equipment vendors to complete the equipment for sub-project 1. Shiu-Hwa Yeh is a neuropharmacologist who will help establish a high-efficient head-mounted miniscope system and assist with all related work for sub-project 1, sub-project 2, and sub-project 3. Chun-Wei Tung is a computer scientist who will help establish the artificial intelligence algorithms for sub-project 2, and sub-project 3. Seong-Jin Yu is an animal behaviorist specializing in cellular and animal models related to Parkinson's disease, and will assist in animal experiments for sub-project 2 and sub-project 3. Hsing-Pang Hsieh is a medicinal chemist who has successfully completed many drug development projects and will assist with drug design and synthesis for sub-project 3. Jang-Yang Chang is a cancer biologist focused on CTSS inhibitor drug development and will perform CTSS enzyme activity assays for all CTSS inhibitors in sub-project 3. Teng-Kang Yeh is an expert in DMPK studies. They will evaluate the developability and preclinical formulation of CTSS inhibitors in sub-project 3. In order to ensure smooth and efficient progress of the projects, joint meetings will be held once every three weeks to exchange updated information and discuss follow-up strategies.

Business cooperation

This project will collaborate with Nanzhou Precision Industries Co., Ltd for 4 years to develop an automatic leveling stereotaxic system. (<https://www.findcompany.com.tw/%E5%8D%97%E5%B7%9E%E7%B2%BE%E5%AF%86%E5%B7%A5%E6%A5%AD%E6%9C%89%E9%99%90%E5%85%AC%E5%8F%B8>). The company has successfully completed several commission projects from Academia Sinica and National Synchrotron Radiation Research Center. We plan to complete the design and testing, and manufacture a prototype within two years. The prototype will then be put into use as a key system for a high-efficient head-mounted miniscope platform. Throughout the project period, user experience will be continuously fed back to the equipment manufacturer, and mass production will take place in the fourth year. The product will be released to the market in the fourth year, and the marketing and promotion work will be carried out by BIOCHIEFDOM INTERNATIONAL Co., Ltd (<http://www.bio-chief.com.tw/?%E9%A6%96%E9%A0%81,10>).

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四、整合型研究計畫項目及重點說明：

(一) 整合型研究計畫項目：

主持人及共同主持人	姓名	服務機構/系所	職稱	計畫中負責之任務及執行之項目	經費分配金額或原則
主持人	葉修華	國家衛生研究院/ 生技與藥物研究所	副研究員	Miniscope platform establishment and data analysis, develop automatic leveling stereotaxic system	40%
共同主持人 1	謝興邦	國家衛生研究院/ 生技與藥物研究所	研究員	Design and synthesis patentable Cathepsin S inhibitors	30%
共同主持人 2	童俊維	國家衛生研究院/ 生技與藥物研究所	研究員	Develop artificial intelligence algorithms for PD indicator and drug design based on brain neural activity data obtained from miniscope platform	30%

(二) 整合型研究計畫重點說明：

1. 整合之必要性：

1-1. 總體目標

The overall goal of this project is to develop a novel approach that combines a high-efficient head-mounted miniscope platform and corresponding artificial intelligence algorithms to assist with drug design and rapidly evaluate the potential efficacy of Parkinson's disease drugs in animal models, accelerate the validation process while avoiding resource waste. This unique approach will also be beneficial for other neurodegenerative diseases such as Alzheimer's disease. To achieve this goal, there is a need to integrate expertise from fields including medicinal chemistry, computer science, electronics engineering, mechanical engineering, cellular biology, animal pharmacology, and formulation development.

1-2. 整體分工合作架構及各子計畫間之相關性與整合程度:

The proposed study comprises three projects that will be conducted by a multi-disciplinary team consisting of researchers with complementary expertise. These researchers are associated with the Institute of Biotechnology and Pharmaceutical Research (IBPR), Center for Neuropsychiatric Research (CNR), and Institute of Biomedical Engineering and Nanomedicine (IBEN) of National Health Research Institutes (NHRI), Taipei Cancer Center (TCC) of Taipei Medical University (TMU), and Nanzhou Precision Industry Co., Ltd. Figure 1 indicates the organization of this program. The brief summary of which is given below:

Sub-project 1. To establish high-efficient head-mounted miniscope platform

The goal of this project is to develop a high-efficiency head-mounted miniscope platform. The head-mounted miniscope experiment requires excellent stereotaxic surgical skills and very precise leveling and positioning work. Currently, the stereotaxic instruments on the market cannot fully meet these requirements, often leading to time wasting or even experiment failure. The team, in collaboration with Dr. Lun-De Liao and Nanzhou Precision Industry Co., Ltd, will develop a high-precision automatic leveling stereotaxic system to make head-mounted miniscope experiments easier to perform and significantly reduce time waste and increase success rate. The establishment of a high-efficiency head-mounted microscope platform will fully meet the needs of establishing artificial intelligence algorithms (sub-project 2) and assist in the development of a CTSS inhibitor for the treatment of Parkinson's disease (sub-project 3).

Sub-project 2. To develop artificial intelligence algorithms for automatic detection of motor dysfunction related to Parkinson's disease and drug design

The goal of this project is to develop artificial intelligence algorithms for fast evaluation of the efficacy of Parkinson's disease drugs in animal studies and drug design. Establishment of these algorithms requires a large amount of *in vivo* striatal neural activity data ($n > 50/\text{group}$) and corresponding animal behavior data from sub-project 1. The established artificial intelligence algorithms will be used in sub-project 3 for rapid evaluation of drug efficacy in animal studies and de novo drug design.

Sub-project 3. Using artificial intelligence algorithms to assist in drug design and develop an efficient go/no-go strategy of CTSS inhibitor development for treating Parkinson's disease

The goal of the project is to design and select a CTSS inhibitor with therapeutic potential for Parkinson's disease. New compounds will first undergo CTSS enzyme assays and cell experiments to assess their efficacy as enzyme inhibitors and neuroprotective agents. The neural activity data of the selected compounds will be collected using the high-efficiency head-mounted miniscope platform from sub-project 1, and then be evaluated using the artificial intelligence algorithm established in sub-project 2. The results of these experiments will be used to select the most promising compounds for further animal testing. The results of the animal experiments will be fed back into sub-project 2 to optimize the algorithm.

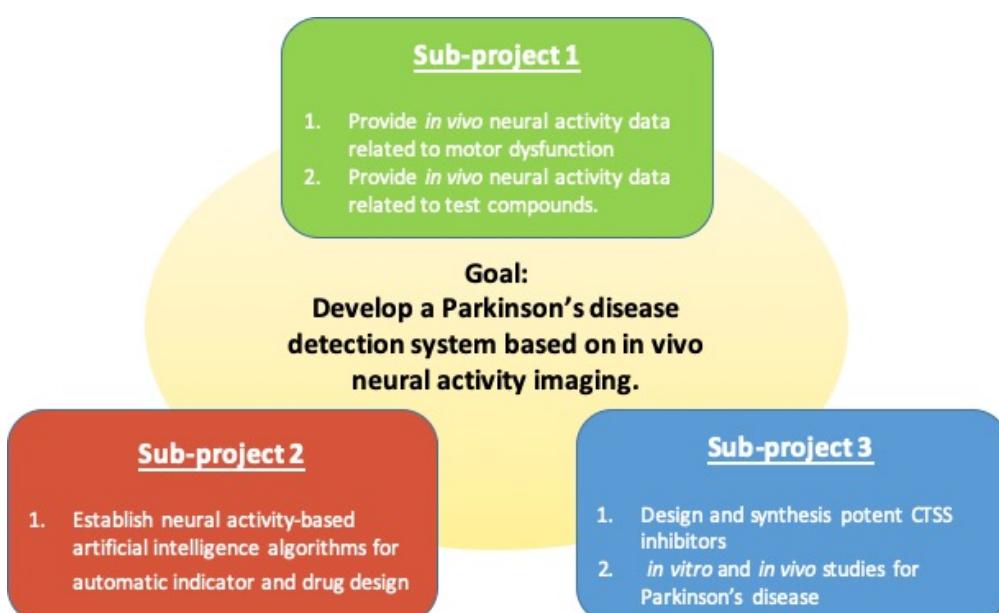


Figure 1. Aim and organization of this program

2. 人力配合度：

Shiu-Hwa Yeh (<https://ibpr.nhri.org.tw/zhtw/index.php/shiu-hwa-yeh/>) specializes in neuropharmacology and head-mounted miniscope experiments. Dr. Yeh has been focused on opioid-related painkiller research and the study of related neural pharmacodynamics for the past 12 years. His achievements have been published in several top journals, have received patents in multiple countries, and have been recognized with the 13th National Innovation Award (國家新創獎) (2016) and the 2020 Ministry of Science and Technology Future Tech Awards (未來科技獎) (2020). Lun-De Liao (<http://iben.nhri.org.tw/staffs-profile.php?staff=Lun-De+Liao>) specializes in biomedical electronic engineering, Dr. Liao specializing in medical electronics, neural imaging, multi-modal optical neuroimaging systems, small animal disease models, artificial intelligence in medical engineering applications, and integrating software and hardware design. Chun-Wei Tung (<https://ibpr.nhri.org.tw/zhtw/index.php/chun-wei-tung/>) is a computer scientist. Dr. Tung's lab focuses on the development of artificial intelligence and database techniques with applications to the prediction of function and toxicity of biological and chemical molecules. Several publicly available web servers have been developed including ChemDIS, SkinSensDB and SkinSensPred. He published more than 50 scientific papers and served as editorial board members for six journals including Scientific Reports and Current Computer-Aided Drug Design. Seong-Jin Yu (<https://np.nhri.org.tw/full-time-investigator/seong-jin-yu/>) is an animal pharmacologist specializing in Parkinson's disease animal pharmacology. Based on his research focus, Dr. Yu has published 59 scientific journals. Recently Dr. Yu used various methods for treating Parkinson's disease to define its detailed mechanisms, for example, Dr. Yu demonstrated the beneficial effect of human iPSC, antibody therapy using AAV-virus, glucagon-like peptide 1 agonist, Mu opioid receptors, 9-cis retinoic acid in cellular and animal models of Parkinson's disease. Dr. Yu has been studying brain diseases with animals for more than 20 years, thus, his experience and knowledge will be helpful to study neurodegenerative-related physiopathology in the brain. Hsing-Pang Hsieh (<https://ibpr.nhri.org.tw/zhtw/index.php/hsing-pang-hsieh/>) is a medicinal chemist. Dr. Hsing-Pang Hsieh is currently Distinguished Investigator/Director in Institute of Biotechnology and Pharmaceutical Research, NHRI. His lab specializes in drug discovery and developing on novel drugs "From Bench to Bedside". He has published 142 scientific journals including 30 papers published in J. Med. Chem., top-one journal in the field of medicinal chemistry, and obtained 63 composition patents granted worldwide. More importantly, I invented three clinical candidates (DBPR104, DBPR112 and DBPR114) in different clinical stages and also completed three technology transfer. In addition, Dr. Hsieh has been recognized and awarded by two-time MOST Outstanding Research Awards (科技部傑出獎) (2010 and 2017), Wang Ming-Ning Award (王民寧獎, 2016), TECO Award (東元獎, 2013), TienTe Lee Outstanding Award (永信李天德卓越醫藥科技獎, 2008). Jang-Yang Chang (<https://ibpr.nhri.org.tw/zhtw/index.php/jang-yang-chang-2/>) is a cancer biologist focused on CTSS inhibitor drug development. Dr. Chang is now Jointly Appointed Investigator of our institute and Dean of Research Center of Cancer Translation Medicine of Taipei Medical University (TMU). Teng-Kang Yeh's (<https://ibpr.nhri.org.tw/zhtw/index.php/teng-kuang-yeh/>) expertise is in pharmacokinetics. Shiu-Hwa Yeh and Lun-De Liao will work together with the equipment supplier Nanzhou Precision Industry Co., Ltd. to develop the high-precision automatic leveling stereotaxic system required for sub-project 1. They will discuss the hardware and software requirements such as system precision, sensitivity, durability, and software functionality with the developer. The developer will design and manufacture a prototype machine based on the requirements. After further optimization, the prototype will be used in sub-project 2 for developing artificial intelligence algorithms and in sub-project 3 for accelerating drug development.

Next, Shiu-Hwa Yeh will work with Seong-Jin Yu using a high-efficient head-mounted miniscope platform and Parkinson's disease animal models to provide large amounts of striatal neural calcium activity data and corresponding animal behavior data to Chun-Wei Tung. This data will be used to establish an algorithm to predict the therapeutic effect of Parkinson's disease in animals and another algorithm to assist in de novo drug design in sub-project 2. Hsing-Pang Hsieh will be responsible for all drug synthesis work in sub-project 3. The high-efficient head-mounted miniscope platform will be used to generate striatal neuronal activity data for the

artificial intelligence algorithm to predict the outcome of animal studies. The selected compounds will then be tested in multiple animal experiments by Seong-Jin Yu.

This method can quickly and effectively evaluate the efficacy of Parkinson's disease drugs, while avoiding wasting time and resources. The team is confident that they will develop a high-precision automatic leveling stereotaxic system, two commercially valuable artificial intelligence algorithms, and a CTSS inhibitor patent for commercial technology transfer within the next four years.

3. 資源之整合：

Lun-De Liao's laboratory will used to test the prototype of an automatic leveling stereotaxic system. Animal Center in NHRI and Shiu-Hwa Yeh's and Seong-Jin Yu's laboratories will be used to perform miniscope platforms and Parkinson's disease animal studies. Artificial intelligence algorithms will be established by Chun-Wei Tung's laboratory and the National Center for High-Performance Computing, Taiwan. All compounds will be synthesized in Hsing-Pang Hsieh's laboratory. All CTSS enzyme assays will be performed in Jang-Yang Chang's laboratory. All compounds pharmacokinetics will be performed in Teng-Kang Yeh's laboratory. Preformulation development of compounds will be performed in Hong Chuang's laboratory. Our team has conducted research together for years. Hence, we anticipate a good collaborative relationship.

4. 申請機構或其他單位之配合度。

All the institutions involved in this study, including the NHRI, TMU, and equipment supplier Nanzhou Precision Industry Co., Ltd, are willing to support this project. We have already signed an MOU (Memorandum of Understanding) with the equipment supplier.

5. 預期綜合效益。

- 1. Design and manufacture of a high-precision automatic leveling stereotaxic system will be transferred to industry. The anticipated advent of this system is expected to popularize miniscope technology and more neuroscientists will use this technology to explore brain functions.**
- 2. The algorithms developed in this project can assist in drug design and rapidly evaluate the effectiveness of animal experiments, with commercialization potential.**
- 3. Patents of CTSS inhibitors will be filed to protect intellectual property rights. CTSS inhibitors may be the next generation of drugs for treating Parkinson's disease by protecting dopamine cells.**
- 4. Upon completion of this study, the results will be published in high-impact journals.**
- 5. The novel methodology developed in this project can be applied to other neurodegenerative diseases such as Alzheimer's disease.**

(二) 年度 Milestone 及 End-Point 及預期達成目標

年度	年度 Milestone (查核點)	年度 End-Point (階段性目標)
112	<ol style="list-style-type: none"> 1. Design the structure of an automatic leveling stereotaxic system. 2. Design the automatic control hardware for the automatic leveling stereotaxic system. 3. Complete the automation process for handling neural activity images. 4. Start building a predictive model for the effect of CTSS inhibitors. 5. Modify the chemical structure of CT001. 6. Conduct solubility testing and pre-formulation studies for CT001 analogs 	<ol style="list-style-type: none"> 1. Complete the design drawings for the automatic leveling stereotaxic system. 2. Complete the prerequisite work for establishing an artificial intelligence algorithm. 3. Obtain at least one compound with equally <i>in vitro</i> potency with better solubility than CT001
113	<ol style="list-style-type: none"> 1. Design the automatic control software for the automatic leveling stereotaxic system. 2. Create a prototype machine and conduct tests. 3. Complete the pre-processing workflow for neural activity images. 4. Generate the first generation of AutoPD and perform validation. 5. Select at least two potent CTSS inhibitors to conduct pharmacokinetic studies. 6. Select at least two potent CTSS inhibitors to conduct pre-formulation. 	<ol style="list-style-type: none"> 1. Complete a prototype of the automatic leveling stereotaxic system. 2. Complete the artificial intelligence algorithm (AutoPD). 3. Develop at least one CTSS inhibitor with <i>in vivo</i> activity.

年度	年度 Milestone (查核點)	年度 End-Point (階段性目標)
114	<ol style="list-style-type: none"> 1. Complete the document writing of the patent application for the automatic leveling stereotaxic system. 2. File a patent for the automatic leveling stereotaxic system and commence technology transfer. 3. Generate the first generation of AutoMolGen and perform validation. 4. Conduct virtual screening using AutoMolGen. 5. Select at least one CTSS inhibitor to conduct gram scale synthesis. 6. Select at least one CTSS inhibitor to conduct animal experiments. 	<ol style="list-style-type: none"> 1. Complete the patent application for the automatic leveling stereotaxic system. 2. Complete the artificial intelligence algorithm (AutoMolGen). 3. Select at least one CTSS inhibitor to conduct a comprehensive animal study.
115	<ol style="list-style-type: none"> 1. Complete the technology transfer for the automatic leveling stereotaxic system. 2. Complete the application for marketing approval for the automatic leveling stereotaxic system. 3. Conduct de novo drug design using AutoMolGen. 4. Validate the efficacy of drugs designed by AutoMolGen. 5. Complete an animal experiment with a CTSS inhibitor and conduct analysis. 6. Conduct a preliminary toxicology study with a CTSS inhibitor. 	<ol style="list-style-type: none"> 1. Complete the technology transfer of the automatic leveling stereotaxic system and submit the application documents. 2. Using AutoMolGen to develop at least one compound with <i>in vitro</i> activity. 3. Announce a drug candidate for CTSS inhibition. 4. Complete the patent application for the CTSS inhibitors.
預期達成目標 (最終成果/效益)	<p>Through this project, we will develop an automatic leveling stereotaxic system to benefit neuroscientists worldwide who use stereotaxic instruments. Additionally, we will develop two artificial intelligence algorithms, AutoPD and AutoMolGen, to assist in drug development and predict the animal efficacy of Parkinson's disease drugs. We will also announce a candidate drug for CTSS inhibition to treat Parkinson's disease.</p>	

(三) 年度績效指標

112 年度：

編號	績效指標項目	預定目標值	質化說明
1	可商品技術	件數	
2	專利	1. 申請 件數 (國內 件；國外 件) 2. 獲證 件數 (國內 件；國外 件)	
3	原型品製作	件數	
4	技術移轉	1. 技轉 件數 2. 金額 千元	
5	促進投資	1. 促進投資 件數 2. 金額 千元	
6	產品上市許可申請	1. 申請 件數 2. 獲證 件數	
7	臨床研究案	1. 國內 件數 2. 國外 件數	
8	臨床應用案	件數	
9	研究論文	1 篇	Results of miniscope platform, artificial intelligence algorithms, and mechanisms of CTSS inhibitor on Parkinson's disease will be published in high-impact journals

編號	績效指標項目	預定目標值	質化說明
1	可商品技術	1 件數	Artificial intelligence algorithm (AutoPD)
2	專利	1. 申請 件數 (國內 件；國外 件) 2. 獲證 件數 (國內 件；國外 件)	
3	原型品製作	1 件數	Prototype of the automatic leveling stereotaxic system
4	技術移轉	1. 技轉 件數 2. 金額 千元	
5	促進投資	1. 促進投資 件數 2. 金額 千元	
6	產品上市許可申請	1. 申請 件數 2. 獲證 件數	
7	臨床研究案	1. 國內 件數 2. 國外 件數	
8	臨床應用案	件數	
9	研究論文	1 篇	Results of miniscope platform, artificial intelligence algorithms, and mechanisms of CTSS inhibitor on Parkinson's disease will be published in high-impact journals

編號	績效指標項目	預定目標值	質化說明
1	可商品技術	1 件數	Artificial intelligence algorithm (AutoMolGen)
2	專利	1. 申請 1 件數 (國內 1 件；國外 件) 2. 獲證 件數 (國內 件；國外 件)	patent application for the automatic leveling stereotaxic system
3	原型品製作	件數	
4	技術移轉	1. 技轉 件數 2. 金額 千元	
5	促進投資	1. 促進投資 件數 2. 金額 千元	
6	產品上市許可申請	1. 申請 件數 2. 獲證 件數	
7	臨床研究案	1. 國內 件數 2. 國外 件數	
8	臨床應用案	件數	
9	研究論文	2 篇	Results of miniscope platform, artificial intelligence algorithms, and mechanisms of CTSS inhibitor on Parkinson's disease will be published in high-impact journals

編號	績效指標項目	預定目標值	質化說明
1	可商品技術	件數	
2	專利	1. 申請 1 件數 (國內 件；國外 1 件) 2. 獲證 件數 (國內 件；國外 件)	patent application for the CTSS inhibitors
3	原型品製作	1 件數	
4	技術移轉	1. 技轉 1 件數 2. 金額 千元	technology transfer of the automatic leveling stereotaxic system
5	促進投資	1. 促進投資 件數 2. 金額 千元	
6	產品上市許可申請	1. 申請 1 件數 2. 獲證 件數	technology transfer of the automatic leveling stereotaxic system
7	臨床研究案	1. 國內 件數 2. 國外 件數	
8	臨床應用案	件數	
9	研究論文	2 篇	Results of miniscope platform, artificial intelligence algorithms, and mechanisms of CTSS inhibitor on Parkinson's disease will be published in high-impact journals

五、申請補助經費：

- (一) 請將本計畫申請書之第七項(表CM07)、第八項(表CM08)、第九項(表CM09)、第十項(表CM10)、第十一項(表CM11)、第十二項(表CM12\CM12-1)所列費用個別加總後，分別填入「研究人力費」、「耗材、物品、圖書及雜項費用」、「國外學者來臺費用」、「研究設備費」、「國外差旅費-執行國際合作與移地研究」及「國外差旅費-出席國際學術會議」等欄內。
- (二) 管理費為申請機構配合執行本計畫所需之費用，其計算方式係依本會規定核給補助管理費之項目費用總和及各申請機構管理費補助比例計算後直接產生，計畫主持人不須填寫「管理費」欄。
- (三) 依據本會「補助延攬客座科技人才作業要點」規定提出博士級研究人員申請，請依各年度申請之名額填入下表，如於申請時一併提出「補助延攬博士級研究人員員額/人才進用申請書」(表CIF2101、CIF2102)，若計畫核定僅核定名額者應於提出合適人選後，另向本會提出進用申請，經審查通過後，始得進用該名博士級研究人員。
- (四) 申請機構或其他單位(含國內外、大陸地區及港澳)補助項目，請檢附相關證明文件。

金額單位：新臺幣元

執行年次 補助項目	第一年 (112年5月 ~113年4月)	第二年 (113年5月 ~114年4月)	第三年 (114年5月 ~115年4月)	第四年 (115年5月 ~116年4月)	第五年
業務費	5,527,340	5,613,316	5,355,340	5,858,500	
研究人力費	2,421,840	2,479,676	2,553,440	2,611,220	
耗材、物品、圖書及雜項費用	3,105,500	3,133,640	2,801,900	3,247,280	
國外學者來臺費用	0	0	0	0	
研究設備費	0	0	0	0	
國外差旅費	148,000	148,000	148,000	148,000	
執行國際合作與移地研究	0	0	0	0	
出席國際學術會議	148,000	148,000	148,000	148,000	
管理費	829,101	841,997	803,301	878,775	
合計	6,504,441	6,603,313	6,306,641	6,885,275	
博士級研究人	國內、外區 共 0 名	共 0 名	共 0 名	共 0 名	共 _____ 名
	大陸地區 共 0 名	共 0 名	共 0 名	共 0 名	共 _____ 名

申請機構或其他單位(含國內外、大陸地區及港澳)補助項目(無配合補助項目者免填)

配合單位名稱	配合補助項目	配合補助金額	配合年次	證明文件

六、主要研究人力：

(一) 請依照「主持人」、「共同主持人」、「協同研究人員」及「博士級研究人員」等類別之順序分別填寫。

類別	姓名	服務機構/系所	職稱	在本研究計畫內擔任之具體工作性質、項目及範圍	*每週平均投入工作時數比率(%)
主持人	葉修華	財團法人國家衛生研究院生技與藥物研究所	副研究員	Miniscope platform establishment and data analysis, develop automatic stereotaxic alignment system	50%
共同主持人	童俊維	財團法人國家衛生研究院生技與藥物研究所	研究員	建立人工智慧演算法，資料處理工作	30%
共同主持人	謝興邦	財團法人國家衛生研究院生技與藥物研究所	研究員且兼任所長	小分子化合物設計及合成工作，預配方分析	40%
協同研究人員	廖倫德	財團法人國家衛生研究院生醫工程與奈米醫學研究所	副研究員且兼任國衛院光學生物核心實驗室指導PI	協助設計自動水平校正立體定位儀	30%
協同研究人員	劉誠珍	財團法人國家衛生研究院神經及精神醫學研究中心	副研究員	執行巴金森氏症離體及活體實驗	40%
協同研究人員	張俊彥	臺北醫學大學癌症轉譯研究中心	教授且兼任臺北癌症中心院長及癌症轉譯研究中心主任	建立CTSS 酵素分析平台及執行所有小分子的酵素分析實驗	25%
協同研究人員	葉燈光	財團法人國家衛生研究院生技與藥物研究所	副研究員	藥物動力學實驗	20%

※ 註：每週平均投入工作時數比率係填寫每人每週平均投入本計畫工作時數佔其每週全部工作時間之比率，以百分比表示（例如：50%即表示該研究人員每週投入本計畫研究工作之時數佔其每週全部工時之百分之五十）。

(二) 如依據本會「補助延攬客座科技人才作業要點」規定申請博士級研究人員，請另填表CIF2101及CIF2102(若已有人選者，請務必填註人選姓名，並將其個人資料表(表C301～表C303)併同本計畫書送本會)。

七、研究人力費：

(一) 凡執行計畫所需研究人力費用，均得依本會「補助專題研究計畫研究人力約用注意事項」規定，按所屬機構自訂敘薪標準及職銜，就預估專任、兼任人員或臨時工需求填寫，並請述明該研究人力在本計畫內擔任之具體內容、性質、項目及範圍，以利審查。專任人員不限學歷，包含博士級人員。

(二) 約用專任人員，請依其於專題研究計畫負責之工作內容，所應具備之專業技能、獨立作業能力、預期績效表現及相關學經歷年資等條件，綜合考量敘薪，並檢附各機構自訂之薪資支給依據，以為本會核定聘用助理經費之參考。

(三) 請分年列述。

第 1 年

金額單位：新臺幣元

類別	金額	請敘明在本計畫內擔任之具體內容、性質、項目及範圍 (如約用專任人員，請簡述其於計畫內所應具備之專業技能、獨立作業能力、預期績效表現及相關學經歷年資等條件)
專任人員	605,460	miniscope 研發 605,460元(含月支費用、年終獎金、勞健保費雇主負擔部分、勞工退休金雇主負擔部分) x 1名
專任人員	605,460	進行動物、細胞實驗 605,460元(含月支費用、年終獎金、勞健保費雇主負擔部分、勞工退休金雇主負擔部分) x 1名
專任人員	605,460	化學合成 605,460元(含月支費用、年終獎金、勞健保費雇主負擔部分、勞工退休金雇主負擔部分) x 1名
專任人員	605,460	建立人工演算法 605,460元(含月支費用、年終獎金、勞健保費雇主負擔部分、勞工退休金雇主負擔部分) x 1名
合計	2,421,840	

第 2 年

金額單位：新臺幣元

類別	金額	請敘明在本計畫內擔任之具體內容、性質、項目及範圍 (如約用專任人員，請簡述其於計畫內所應具備之專業技能、獨立作業能力、預期績效表現及相關學經歷年資等條件)
專任人員	619,919	miniscope 研發 619,919元(含月支費用、年終獎金、勞健保費雇主負擔部分、勞工退休金雇主負擔部分) x 1名
專任人員	619,919	進行動物、細胞實驗 619,919元(含月支費用、年終獎金、勞健保費雇主負擔部分、勞工退休金雇主負擔部分) x 1名
專任人員	619,919	化學合成 619,919元(含月支費用、年終獎金、勞健保費雇主負擔部分、勞工退休金雇主負擔部分) x 1名
專任人員	619,919	建立人工演算法 619,919元(含月支費用、年終獎金、勞健保費雇主負擔部分、勞工退休金雇主負擔部分) x 1名

合計	2,479,676	
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第 3 年

金額單位：新臺幣元

類別	金額	請敘明在本計畫內擔任之具體內容、性質、項目及範圍 (如約用專任人員，請簡述其於計畫內所應具備之專業技能、獨立作業能力、預期績效表現及相關學經歷年資等條件)
專任人員	638,360	miniscope 研發 638,360元(含月支費用、年終獎金、勞健保費雇主負擔部分、勞工退休金雇主負擔部分) x 1名
專任人員	638,360	進行動物、細胞實驗 638,360元(含月支費用、年終獎金、勞健保費雇主負擔部分、勞工退休金雇主負擔部分) x 1名
專任人員	638,360	化學合成 638,360元(含月支費用、年終獎金、勞健保費雇主負擔部分、勞工退休金雇主負擔部分) x 1名
專任人員	638,360	建立人工演算法 638,360元(含月支費用、年終獎金、勞健保費雇主負擔部分、勞工退休金雇主負擔部分) x 1名
合計	2,553,440	

第 4 年

金額單位：新臺幣元

類別	金額	請敘明在本計畫內擔任之具體內容、性質、項目及範圍 (如約用專任人員，請簡述其於計畫內所應具備之專業技能、獨立作業能力、預期績效表現及相關學經歷年資等條件)
專任人員	652,805	miniscope 研發 652,805元(含月支費用、年終獎金、勞健保費雇主負擔部分、勞工退休金雇主負擔部分) x 1名
專任人員	652,805	進行動物、細胞實驗 652,805元(含月支費用、年終獎金、勞健保費雇主負擔部分、勞工退休金雇主負擔部分) x 1名
專任人員	652,805	化學合成 652,805元(含月支費用、年終獎金、勞健保費雇主負擔部分、勞工退休金雇主負擔部分) x 1名
專任人員	652,805	建立人工演算法 652,805元(含月支費用、年終獎金、勞健保費雇主負擔部分、勞工退休金雇主負擔部分) x 1名
合計	2,611,220	

八、耗材、物品、圖書及雜項費用：

- (一) 凡執行研究計畫所需之耗材、物品(非屬研究設備者)、圖書及雜項費用，均可填入本表內。
 (二) 說明欄請就該項目之規格、用途等相關資料詳細填寫，以利審查。
 (三) 若申請單位有配合款，請於備註欄註明。
 (四) 請分年列述。

第 1 年

金額單位：新臺幣元

項目名稱	說明	單位	數量	單價	金額	備註
消耗性器材	ProView™ Integrated Lens (5入/box)	box	25	38,400	960,000	
消耗性器材	200萬USB低照度攝像頭	set	5	18,000	90,000	
消耗性器材	Baseplate Cover	set	4	17,500	70,000	
消耗性器材	25G, 27G螺式針頭	box	20	5,000	100,000	
消耗性器材	調速刻磨機		1	7,500	7,500	
消耗性器材	化學合成-有機溶劑:丙酮、乙酸乙酯、正己烷、二氯甲烷、氘代溶劑等	batch	5	10,000	50,000	
消耗性器材	化學合成-有機與無機藥品	batch	2	150,000	300,000	
消耗性器材	化學合成-分析純化用品:管柱用矽膠、TLC 片(管柱色層分析片)	batch	5	100,000	500,000	
消耗性器材	化學合成-實驗用氣體:高純度氮氣、氬氣、氫氣等	batch	2	20,000	40,000	
消耗性器材	化學合成-實驗用消耗性用品:手套、活性碳口罩、防毒面具之活性碳罐、秤量紙等	batch	5	15,000	75,000	
消耗性器材	Human Cathepsin S ELISA Kit (CTSS)	kit	4	40,000	160,000	
消耗性器材	Cell culture consumables: FBS, DMEM, F12, glutamax, G418, Penicillin-Streptomycin, Trypsin-EDTA (0.05%), poly-llysine, etc.	bottle	4	15,000	60,000	
消耗性器材	96-Well Plates and Tips for CTSS enzyme assay	box	4	35,000	140,000	
消耗性器材	儀器維護與儀器耗材	time	1	25,000	25,000	

消耗性器材	細胞培養氣體：CO2	bottle	4	2,000	8,000	
消耗性器材	3D contour sensor		2	90,000	180,000	
消耗性器材	stepping motors		4	85,000	340,000	
合 計				3,105,500		

第 2 年

金額單位：新臺幣元

項目名稱	說明	單位	數量	單價	金額	備註
消耗性器材	ProView™ Integrated Lens (5入/box)	box	30	38,400	1,152,000	
消耗性器材	MOTICAM S1 影像擷取器 含 Motic Images Plus 3.0軟體		1	16,000	16,000	
消耗性器材	Automated Stereotaxic manipulator for Mouse		2	90,000	180,000	
消耗性器材	化學合成-有機溶劑:丙酮、乙酸乙酯、正己烷、二氯甲烷、氘代溶劑等	batch	1	20,000	20,000	
消耗性器材	化學合成-有機與無機藥品	batch	2	80,000	160,000	
消耗性器材	化學合成-分析純化用品 :管柱用矽膠、TLC 片 (管柱色層分 析片)	batch	3	100,000	300,000	
消耗性器材	化學合成-實驗用氣體:高純度氮氣、氬氣、氦氣等	batch	3	35,000	105,000	
消耗性器材	化學合成-實驗用消耗性用品:手套、活性碳口罩、防毒面具之活性碳罐、秤量紙、防護眼鏡、玻璃器皿等	batch	1	150,000	150,000	
消耗性器材	Human Cathepsin S ELISA Kit (CTSS)	kit	3	40,000	120,000	
消耗性器材	Cell culture consumables: FBS, DMEM, F12, glutamax, G418, Penicillin-Streptomycin, Trypsin-EDTA (0.05%), poly-llysine, etc.	bottle	3	15,000	45,000	
消耗性器材	C57BL/6 mice, animal shipping and feeding 動物購買 : 206*20*12=49,440 動		6	85,440	512,640	手術後動物需一隻一籠單獨飼養

	物飼養 ：150*30*12=36,000					
消耗性器材	96-Well Plates and Tips for CTSS enzyme assay	box	4	35,000	140,000	
消耗性器材	儀器維護與儀器耗材	time	1	25,000	25,000	
消耗性器材	細胞培養氣體：CO ₂	bottle	4	2,000	8,000	
消耗性器材	The automatic control system software		1	150,000	150,000	
消耗性器材	syringe with needle (27G, 26G, 22G) Insulin Syringe with Needle, 1.7 mL, 2.0 mL, 5.0 mL and 15 mL eppendorf	box	5	10,000	50,000	
合 計					3,133,640	

第 3 年

金額單位：新臺幣元

項目名稱	說明	單位	數量	單價	金額	備註
消耗性器材	ProView™ Integrated Lens (5入/box)	box	30	38,400	1,152,000	
雜支	stationer, postage, computer consumables, shipping fee, printing fee, rent for software, experimental equipments or databank	time	15	1,500	22,500	
消耗性器材	Rotational Stereotaxic for Mouse		2	75,000	150,000	
實驗動物	C57BL/6 mice, animal shipping and feeding 動物購買 ：206*20*12=49,440 動物飼養 ：150*30*12=36,000		10	85,440	854,400	手術後動物需一隻一籠單獨飼養
消耗性器材	Human Cathepsin S ELISA Kit (CTSS)	kit	5	40,000	200,000	
消耗性器材	Cell culture consumables: FBS, DMEM, F12, glutamax, G418, Penicillin-Streptomycin, Trypsin-EDTA (0.05%), poly-llysine, etc.	bottle	3	15,000	45,000	

消耗性器材	96-Well Plates and Tips for CTSS enzyme assay	box	2	35,000	70,000	
消耗性器材	儀器維護與儀器耗材	time	1	25,000	25,000	
消耗性器材	細胞培養氣體：CO2	bottle	4	2,000	8,000	
消耗性器材	Mouse Neocortex virus		5	15,000	75,000	
消耗性器材	Mouse Dorsal Striatum virus		5	15,000	75,000	
消耗性器材	Mouse Dorsal CA1 Hippocampus virus		5	15,000	75,000	
消耗性器材	syringe with needle (27G, 26G, 22G) Insulin Syringe with Needle, 1.7 mL, 2.0 mL, 5.0 mL and 15 mL eppendorf	box	5	10,000	50,000	
合 計					2,801,900	

第 4 年

金額單位：新臺幣元

項目名稱	說明	單位	數量	單價	金額	備註
實驗動物	C57BL/6 mice, animal shipping and feeding 動物購買 : 206*20*12=49,440 動物飼養 : 150*30*12=36,000	batch	12	85,440	1,025,280	手術後動物需一隻一籠單獨飼養
消耗性器材	Mouse Neocortex virus	kit	5	15,000	75,000	
消耗性器材	Mouse Dorsal Striatum virus	kit	5	15,000	75,000	
消耗性器材	Mouse Dorsal CA1 Hippocampus virus	kit	5	15,000	75,000	
消耗性器材	Nanoliter Injection Pump		1	95,000	95,000	
消耗性器材	syringe with needle (27G, 26G, 22G) Insulin Syringe with Needle, 1.7 mL, 2.0 mL, 5.0 mL and 15 mL eppendorf	box	10	10,000	100,000	
消耗性器材	ProView™ Integrated Lens (5入/box)	box	40	38,400	1,536,000	

消耗性器材	儀器維護與儀器耗材	time	1	25,000	25,000	
消耗性器材	Human Cathepsin S ELISA Kit (CTSS)	kit	3	40,000	120,000	
消耗性器材	Cell culture consumables: FBS, DMEM, F12, glutamax, G418, Penicillin-Streptomycin, Trypsin-EDTA (0.05%), poly-llysine, etc.	bottle	3	15,000	45,000	
消耗性器材	細胞培養氣體：CO2	bottle	3	2,000	6,000	
消耗性器材	96-Well Plates and Tips for CTSS enzyme assay	box	2	35,000	70,000	
合 計				3,247,280		

十二、國外差旅費-出席國際學術會議：

- (一) 計畫主持人及參與研究計畫之相關人員參加國際學術會議得申請本項經費。
- (二) 請詳述預定參加國際學術會議之性質、預估經費、天數及地點。
- (三) 機票費、生活費及其他費用之標準，請依照行政院頒布之「國外出差旅費報支要點」規定填列
(網址<https://law.dgbas.gov.tw/LawContent.aspx?id=FL017584>)。
- (四) 請詳述計畫主持人近三年參加國外舉辦之國際學術會議論文之發表情形。（包括會議名稱、時間、地點、發表之論文題目、補助機構，及後續收錄於期刊或專書之名稱、卷號、頁數、出版日期）
- (五) 請分年列述。

第 1 年

金額單位：新臺幣元

出席國際學術會議			
博士生人數	共 0 名	金額	148,000
費用說明	<p>Conferences title: The Society for Neuroscience annual meeting 2023 The Society for Neuroscience annual meeting is the premier venue for neuroscientists from around the world to debut cutting-edge research. Since 1971, the meeting has offered attendees the opportunity to learn about the latest breakthroughs and network with colleagues at top destinations throughout North America. Venue: Washington, Dist of Col, United States of America. ; Dates_ Nov 11-15, 2023 ; Budget_ airline ticket: 70,000, maintenance: USD300*7 (days)=63,000 NT ; registration fee: USD465=NT15,000 , total=148,000</p>		
近三年論文發表情形	<p>1. Title: BPR1M97, a dual mu opioid receptor/nociceptin-orphanin FQ peptide receptor agonist, produces potent antinociceptive effects with safer properties than morphine. Date: 2020 Apr Journal: Neuropharmacology. 166:107678.</p> <p>2. Title: Morphine produces potent antinociception, sedation, and hypothermia in humanized mice expressing human mu opioid receptor splice variants. Date: 2020 Jun Journal: Pain. 161: 1177-1190.</p>		

第 2 年

金額單位：新臺幣元

出席國際學術會議			
博士生人數	共 0 名	金額	148,000
費用說明	<p>Conferences title: The Society for Neuroscience annual meeting 2024 The Society for Neuroscience annual meeting is the premier venue for neuroscientists from around the world to debut cutting-edge research. Since 1971, the meeting has offered attendees the opportunity to learn about the latest breakthroughs and network with colleagues at top destinations throughout North America. Venue: Chicago, IL, United States of America. ; Dates_ Oct 05-09, 2024 ; Budget_ airline ticket: 70,000, maintenance: USD300*7 (days)=63,000 NT ; registration fee: USD465=NT15,000 , total=148,000</p>		

近三年論文發表情形	1. Title: BPR1M97, a dual mu opioid receptor/nociceptin-orphanin FQ peptide receptor agonist, produces potent antinociceptive effects with safer properties than morphine. Date: 2020 Apr Journal: Neuropharmacology. 166:107678.
	2. Title: Morphine produces potent antinociception, sedation, and hypothermia in humanized mice expressing human mu opioid receptor splice variants. Date: 2020 Jun Journal: Pain. 161: 1177-1190.

第 3 年

金額單位：新臺幣元

出席國際學術會議

博士生人數	共 0 名	金額	148,000
費用說明	Conferences title: The Society for Neuroscience annual meeting 2025 The Society for Neuroscience annual meeting is the premier venue for neuroscientists from around the world to debut cuttingedge research. Since 1971, the meeting has offered attendees the opportunity to learn about the latest breakthroughs and network with colleagues at top destinations throughout North America. Venue: San Diego, CA, United States of America. ; Dates_ Nov 15-19, 2025 ; Budget_ airline ticket: 70,000, maintenance: USD300*7 (days)=63,000 NT ; registration fee: USD465=NT15,000 , total=148,000		
近三年論文發表情形	1. Title: BPR1M97, a dual mu opioid receptor/nociceptin-orphanin FQ peptide receptor agonist, produces potent antinociceptive effects with safer properties than morphine. Date: 2020 Apr Journal: Neuropharmacology. 166:107678. 2. Title: Morphine produces potent antinociception, sedation, and hypothermia in humanized mice expressing human mu opioid receptor splice variants. Date: 2020 Jun Journal: Pain. 161: 1177-1190.		

第 4 年

金額單位：新臺幣元

出席國際學術會議

博士生人數	共 0 名	金額	148,000
費用說明	Conferences title: The Society for Neuroscience annual meeting 2026 The Society for Neuroscience annual meeting is the premier venue for neuroscientists from around the world to debut cuttingedge research. Since 1971, the meeting has offered attendees the opportunity to learn about the latest breakthroughs and network with colleagues at top destinations throughout North America. Venue: Washington, Dist of Col, United States of America. ; Dates_ Nov 14-18, 2026 ; Budget_ airline ticket: 70,000, maintenance: USD300*7 (days)=63,000 NT ; registration fee: USD465=NT15,000 , total=148,000		

近三年論文發表情形	<p>1. Title: BPR1M97, a dual mu opioid receptor/nociceptin-orphanin FQ peptide receptor agonist, produces potent antinociceptive effects with safer properties than morphine. Date: 2020 Apr Journal: Neuropharmacology. 166:107678.</p> <p>2. Title: Morphine produces potent antinociception, sedation, and hypothermia in humanized mice expressing human mu opioid receptor splice variants. Date: 2020 Jun Journal: Pain. 161: 1177–1190.</p>
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十四、近三年內執行本會之所有計畫

計畫名稱 (本會補助者請註明編號)	計畫內擔任之工作	起迄年月	補助或委託機構	執行情形	經費總額
利用基於結構的藥物設計策略開發一種獨特的mu-鴉片受體結構特異性促效劑(111-2320-B-400-008-MY3)	主持人	2022/08/01～2025/07/31	國家科學及技術委員會	執行中	4,800,000
開發新穎 mu-鴉片/痛敏肽受體雙效促效劑之安全止痛藥(110-2320-B-400-004-MY2)	共同主持人	2021/08/01～2023/07/31	國家科學及技術委員會	執行中	3,080,000
藉由人工智慧平台協助低副作用新穎mu-/痛敏肽鴉片受體雙效促效劑之小分子止痛藥物開發(109-2320-B-400-015-)	共同主持人	2020/08/01～2021/07/31	國家科學及技術委員會	已結案	1,300,000
探討嗎啡在人源性MOR受體基因轉殖小鼠的藥理作用(108-2320-B-400-014-MY3)	主持人	2019/08/01～2022/07/31	國家科學及技術委員會	已結案	4,920,000
發展創新的阿茲海默症早期診斷與治療—發展創新的阿茲海默症早期診斷與治療(108-2321-B-400-016-MY2)	共同主持人	2019/06/01～2021/12/31	國家科學及技術委員會	已結案	15,000,000
合 計					29,100,000

基因重組實驗申請同意書 收件證明

收件編號：IBC112022

收件日期：2023-02-10

計畫名稱：利用高效率頭戴式顯微鏡平台及人工智慧演算法加速開

發組織蛋白酶 S 抑制劑治療巴金森氏症引發的運動功能障礙

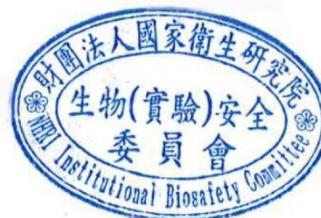
計畫主持人：葉修華

計畫執行期限：2023-05-01 ~ 2026-04-30

茲證明上列計畫業經本院生物安全會收件,刻正辦理審查中。

財團法人國家衛生研究院

生物安全會



中華民國

2023 年 02 月 10 日

動物實驗倫理 3R 說明：

本研究計畫所涉及動物實驗之相關內容，已充分考量「替代 (Replace)」、「減量 (Reduce)」及「精緻化 (Refine)」之 3R 精神，盡可能將實驗設計最佳化，說明如下：

(一)、3R 原則：

本實驗計畫所使用之實驗動物已經本人及機構內「實驗動物照護及使用委員會」詳實審查，無法以其他替代方案取代。業已預估使用最少數量之實驗動物，實驗也已做到精緻化設計，並要求動物福利最佳化，除了考慮並要求執行動物疼痛評估，並注重要求執行適當減輕動物痛苦之麻醉方式、適時投與止痛藥物、並設定人道安樂死時機。

(二)、人員之教育訓練：

為促進 3R 精神之落實，本研究實際負責進行動物實驗之相關人員均經歷實驗動物人道管理講座(如：動物福利、3R 原則)，並須接受實驗專業技術訓練，以減少實驗動物壓力或耗損。

(三)、使用動物之來源：

為確保本研究計畫實驗品質，本實驗之動物來源為 AAALAC 認證之繁殖機構「財團法人國家實驗研究院國家實驗動物中心」。

(四)、使用動物之監督機制：

為確保實驗品質與效益，本研究計畫相關動物實驗之監督機制堪稱周全，監督權責機構為國衛院設有之「實驗動物照護及使用委員會」，業已設置專責專職獸醫師，並參與計畫審查及動物照護與管理，計畫審查包括外部委員。

(五)、行政院農業委員會實地查核本機構「動物科學應用」之評比紀錄：

所屬實驗動物中心最近一次接受行政院農業委員會實驗動物利用機構監督查核，查核年度為107 年度，評比結果為優。

Receipt of the Application of Animal Use Protocol

「動物實驗計畫書」收件證明

Protocol Title :

English : Utilizing a high-efficiency head-mounted miniscope platform and artificial intelligence algorithms to accelerate the development of cathepsin S inhibitors for treating Parkinson's disease-induced motor dysfunction

中文 : 利用高效率頭戴式顯微鏡平台及人工智慧演算法加速開發組織蛋白酶S抑制劑治療巴金森氏症引發的運動功能障礙

Period of Validity Proposed :

From : 2023/05/01 To: 2026/04/30 (yyyy/mm/dd)

Principal Investigator (PI) : 劉誠珍 Seongjin Yu

Co-PI :

國家衛生研究院



收件日期 2023/02/14

備註：國家衛生研究院於111年度動物科學應用機構實地查核評比結果為「良」。

合作意向書

立合作意向書人：

財團法人國家衛生研究院（以下簡稱甲方）

南州精密工業有限公司（以下簡稱乙方）

緣甲乙雙方為合作「利用高效率頭戴式顯微鏡平台及人工智慧演算法加速開發組織蛋白酶 S 抑制劑治療巴金森氏症引發的運動功能障礙」，特簽訂本合作意向書，以做為雙方進一步合作之基礎。

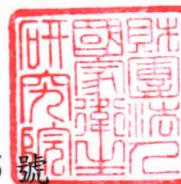
- 一、本合作意向書為表達雙方共同合作之意願，未得對方同意，雙方均不得公開宣稱雙方之合作夥伴關係相關合作細節。
- 二、雙方同意在平等互惠及誠信原則下，詳細及具體之合作條件於共同協商下，另以合約訂定之。其合約應明確規範合作計畫雙方之權利、責任及義務分工，並以不違反國家「科學技術基本法」及甲方相關法令規章為原則。
- 三、本意向書旨在明文敘述甲乙雙方合作之意願與共識，各條款不具任何正式合約之約束力。
- 四、本合作意向書自完成簽訂之日起生效至 116 年 4 月 30 日失其效力。
- 五、本意向書一式貳份，甲乙雙方各執乙份為憑。

立合作意向書人

甲 方：財團法人國家衛生研究院

代表人：司徒惠康 院長

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乙 方：南州精密工業有限公司

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