

Research Topic	Virus-based gene transfer technology	Graduate School of Engineering
Host University	Western University / London, Ontario / Canada	Applied Chemistry, Environmental and Biomedical Engineering
Duration	From October 1 to December 28, 2022	NAKATA Ayato

Summary of the Research Activities

I studied virus-based gene transfer technology at Western University, where Cre-loxp and FLP-FRT systems and virus-based gene transfer can be used to express specific genes in specific cells. Cre-loxp and FLP-FRT systems are site-directed recombination technologies. The mice used in the study were CRH-iPs. The mice used are a cross between CRH-ires-CRE and Slc32a1-2A-FlpO-D knock-in. CRH-ires-CRE is a mouse in which CRE is inserted downstream of the CRH promoter, while Slc32a1-2A-FlpO-D knock-in is a mouse in which FLP is inserted into VGAT. Mice in which FLP was inserted into VGAT. The AAVs used were Virus(Cre):AAV2/2-EF1a-DIO-hChR2(H134R)-eYFP and Virus(flp):pAAV-hSyn-fDIO-hM4D(Gi)-mCherry-WPREpA(AAV Retrograde)CNO Receptor. Virus(Cre): AAV2/2-EF1a-DIO-hChR2(H134R)-eYFP is a virus that can introduce hChR2, and Virus(flp):pAAV-hSyn-fDIO-hM4D(Gi)-mCherry-WPREpA(AAV Retrograde)CNO Receptor is a virus that introduces hM4D.

In this experiment, mice carrying the above two genes were injected with 240 nL of a mixture of the two viruses into the PVN of the brain. The purple area is PVN (Figure 1).

Figure 2 shows the results after administration. Expression of ChR2 (green), hm4D (red), and cfos (purple) was observed in the vicinity of PVN. This indicates that the viral gene transfer was successful. The presence of red at the end of green suggested a relationship between the CRH neurons exiting the PVN and the surrounding GABAergic neurons (Figure 3).

Mouse, Adult, 3D Coronal

Acronym: PVH
Name: Paraventricular hypothalamic nucleus

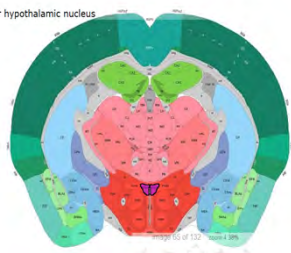


Figure 1, PVN location (Quoted from mice brain atlas)

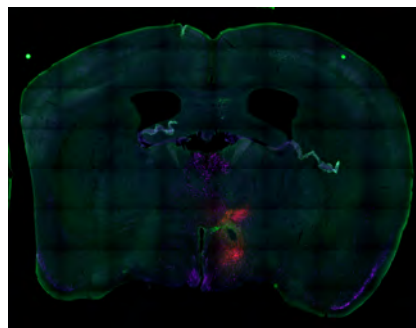


Figure 2, image of transgene

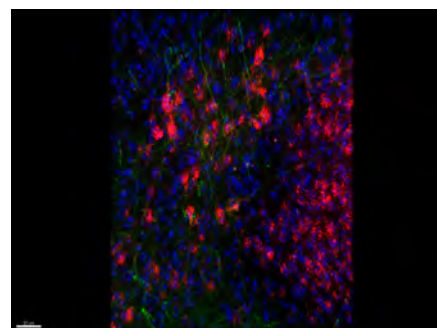


Figure 3, Enlarged image

College Life, Friends and Others

Western University is a Canadian state university with 11 faculties and 400 different specializations. People from different countries learned in the university, and therefore I was able to experience the culture of many countries. We had a home party for Thanksgiving and a Secret Santa for Christmas.



指導教員講評

無害なウイルスを利用して、特定の神経細胞に遺伝子導入をする技術を習得した。この手法は今後の脳研究に重要なものである。留学先では充実した時間をすごしたことがうかがえる。この留学経験を今後に活かしてほしい。

指導教員氏名: 松村潔