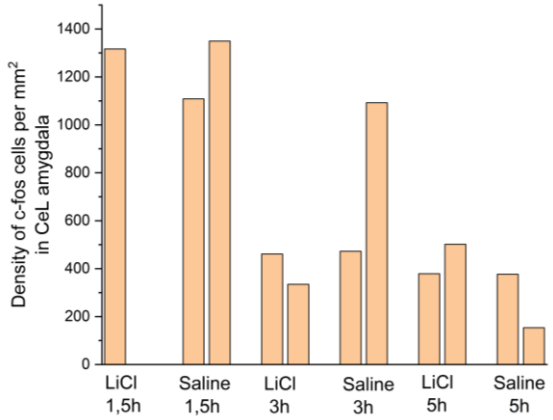
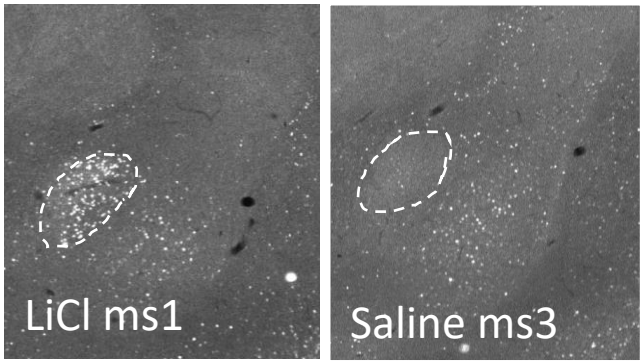
Final scientific report

The time period for the report includes from 02/12/2019 to 31/08/2020, as from 01/12/2018 to 01/12/2019 I was on maternity leave. In this 9-month-long period, we focused our research in designing the workflow for future experiments and adapting it to the current tools available. Due to the Covid19 pandemic, a significant amount of time has been spent in home office, during which no experimental work was done. In this period, I analysed the results of preliminary experiments that were done either prior to the maternity leave, or during the reported period.

First, as there is no previous data in the literature about the immediate early gene (IEG) expression by vPAG/DR VIP+ neurons, we made a preliminary experiment to try to find out if these neurons express c-fos or Ziff upon visceral malaise, and if they do, at what point after the intraperitoneal (i.p.) LiCl injection. We injected either saline or LiCl in groups of 2 wild type mice, and perfused either at 1.5h, 3h or 5h. We couldn't find c-fos or Ziff labelling in the area where vPAG/DR VIP+ neurons are located in any of the groups.

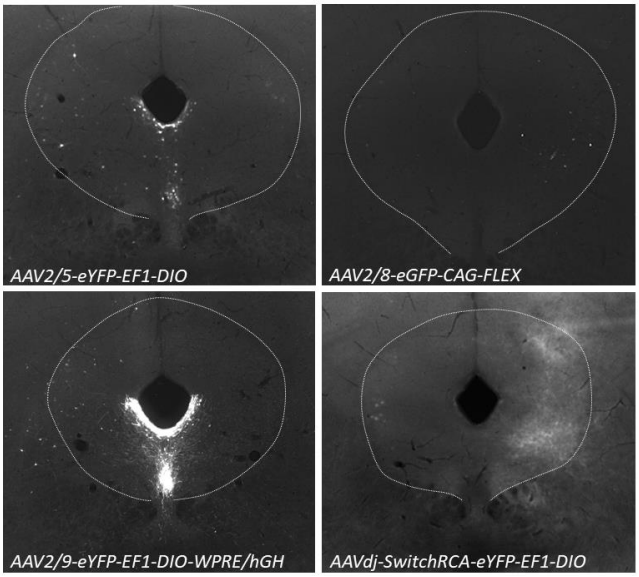
As it had been previously described an induction of c-fos expression after LiCl i.p. injection in the CeA, we also quantified the density of c-fos+ cells in this area, to verify that the visceral malaise model was working in our hands. In these conditions, we could replicate the induction of c-fos in the CeA, suggesting that the LiCl-induced malaise model is working in our hands.



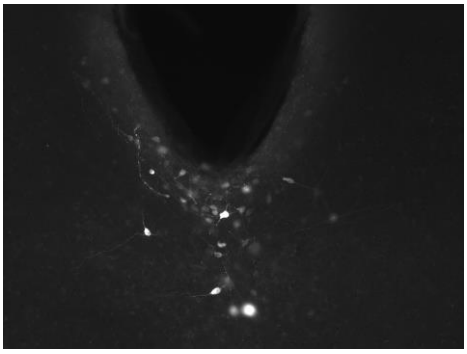


As we cannot discard that the activation of this neuronal population is independent of these IEGs (c-fos and Ziff), but we know that the LiCl-induced malaise model is working in our hands, this line of research will be continued in the future.

Another preliminary experiment with different behavioural paradigms was done previously in the laboratory with a cohort of animals using the DREADD approach. Briefly, we injected bilaterally in the vPAG/DRN region AAV2/8-hSyn-DIO-mCherry or AAV2/8-hSyn-DIO-hM4D(Gi)-mCherry. As the labelling of the VIP neurons was very scarce or inexistent using AAV8 serotype, even injecting 20x larger volumes and similar coordinates than previously, we established as a new goal to assess the viral serotypes that work best, to selectively manipulate the activity of VIP+ neurons in the vPAG/DRN. We found that AAV2/9 was the best serotype to label VIP+ neurons in the PAG/DR, followed by AAV5. AAV8 or AAVdj serotypes, however, could label very few, if any, VIP neurons.



Because the DREADD approach included viruses of the AAV2/8 serotype, we had to change our strategy and use a different virus. We tried a different approach, which consists in selectively expressing the tetanus toxin light chain (TeLC) in PAG/DR VIP+ neurons. TeLC cleaves vesicle-associated membrane protein synaptobrevin, thereby permanently inhibiting neurotransmitter release. We injected either an AAV1/2-Cre(on)-GFP or AAV1/2-Cre(on)-TeLC-GFP, kindly provided by Prof. Peer Wulff (Kiel University, Germany). With this virus, we could effectively target and label the VIP+ neurons in the PAG-DRN.



Therefore, future behavioural experiments will be performed using the TeLC expression approach. The project will not be interrupted but will be continued by Müller Kinga in the same laboratory.