**ISN International Career Development Grant (CDG) Report**

**(Deadline: two months after completion of the project)**

**Beneficiary:**

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| **Name** | Christina Perry |
| **Email address** | Christina.perry@mq.edu.au |
| **Institutional address:** | |
| * Department | School of Psychological Sciences |
| * Institution | Macquarie University |
| * City | Sydney |
| * Country | Australia |

**Project title:**

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| **Using animal models to understand motivated behaviour** |

**Project duration:**

|  |  |
| --- | --- |
| **From (DD/MM/YY)** | **To (DD/MM/YY)** |
| **01/01/21 31/12** | **31/12/23** |

**Report about the outcome of the grant and future perspectives**

(3 pages max.)

**(1) What were the major goals of the project?**

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| Aim 1 – to describe the connectome and neurochemical phenotype of RXFP3+ cells in the Zona Incerta (ZI).  Aim 2 – to describe the function of ZI RXFP3+ neurons during fear extinction and memory retrieval. |

**(2) What was accomplished under these scientific goals and objectives?**

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| Over 2021-2022 I worked with a Masters student on the major aims of this grant. At the beginning of 2021 I received an appointment at Macquarie University in Sydney, and relocated my lab from Melbourne (Florey Institute of Neuroscience and Mental Health) to Sydney. My student continued work on Aim 2 while completing his degree in Melbourne, and then applied successfully to the PhD program at Macquarie University, where he has since been working on the other aims of the grant. I was able to use the pilot data from this grant to secure a major competitive government grant to complete and extend the aims of this project. Current progress is outlined below:  **Aim 1a –** Characterising the neurochemical phenotype of RXFP3+ neurons in the ZI (and adjacent Lateral Hypothalamus - LH). This aim has been completed thanks to the ISN career development grant.  ***Outcomes***: The ZI is known to have heterogeneous neurochemistry. Therefore, our first aim was to identify whether RXFP3+ neurons (ZIRXFP3+) were preferentially co-expressed with other neurotransmitters or neuropeptides. We used fluorescent in-situ hybridisation (RNAScopeTM) to assess co-localisation of mRNA transcripts for RXFP3+ receptor (*Rxfp3*) together with *slc17a6*, *gad1, pvalb, sst,* and *th.* This allowed us to characterise ZIRXFP3+ as primarily excitatory or inhibitory, as well as whether the receptor was expressed on neurons that also expressed parvalbumin, somatostatin or dopamine. We have conducted these analyses in both the ZI and the LH. For brevity, I will summarise only the data obtained from the ZI, however we have a manuscript in preparation that will describe the findings from the study in detail.  *GAD1* and *slc17a6* The majority of ZIRXFP3+ were GABAergic, although most ZIGAD1+ neurons did not express *Rxfp3*. For *slc17a6* the opposite pattern was observed, whereby only a small proportion of ZIRXFP3+ were glutamatergic, but more than half of ZIslc17a6+  neurons also expressed *Rxfp3.* Furthermore, the rostral ZI contained a higher proportion of glutamatergic:GABAergic RXFP3+ neurons when compared with the intermediate and caudal zones.  *TH* and *Pvalb* Distribution of ZIRXFP3+ co-expressing *TH* (dopaminergic), or *Pvalb* (parvalbumin) transcripts showed clear topographical distribution (figure 1). Taking the entire ZI into account, ~14% (± 2%) of *Rxfp3*+ cells were *TH*+ (Figure 6A), however, spatial mapping revealed that most of these cells were in the A13, with *Rxfp3*+/*TH*+ cells sparsely scattered throughout the rest of the ZI. In the A13 area, ~77% (± 3%) of *Rxfp3*+ cells were *TH*+, and ~48% (± 4%) of identified *TH*+ A13 cells also expressed *Rxfp3* mRNA (Figure 6C). This indicates that a large subset of TH+ cells in the A13 express RXFP3, but RXFP3 is also expressed in other populations within the A13 area.  **Figure 1: Co-localisation of *Rxfp3* with *TH* and *Pvalb***  Overall, ~37% (± 3%) of ZI *Rxfp3+* cells co-expressed *Pvalb*, with spatial mapping revealing that Rxfp3+/Pvalb+ cells predominantly populated the ZIv, though were ubiquitously present throughout the entire ZI. The proportion of *Rxfp3*+ cells expressing *Pvalb* increased in a rostrocaudal gradient. Most strikingly, the average proportion of Rxfp3+ cells expressing Pvalb in the caudal ZId/ZIv and ZIc was ~76% (± 3%). However, the average proportion of Pvalb+ cells expressing Rxfp3+ in the caudal ZId/ZIv and ZIc was between 5 and 40%.  *SST* ZIRXFP3+ co-expressing *SST* transcript (Figure 2) likewise showed a distinct topography, that was opposite in trend to that observed with *Pvalb.* Overall, ~35% (± 2%) of Rxfp3+ cells were SST+, and ~29% (± 1%) of SST+ cells were Rxfp3+, however the proportion of Rxfp3+ cells expressing SST in the ZIr was significantly greater than all other sectors. Furthermore, spatial mapping revealed that a distinct dense Rxfp3+/SST+ cluster was apparent in the caudal A13.  **Aim 1b –** although not yet completed, I have been able to make a significant start on this the aim. With funding described below, I hope to be able to complete this aim.  ***Outomes:*** Initial tracing experiments had quite large injections sites, which covered a large proportion of the ZI, as well as parts of the LH. We found that LH/ZI RXFP3+ (LH/ZIRXFP3) cells projected strongly to fear learning, stress, and arousal centres, notably, the periaqueductal gray, lateral habenula, and nucleus reuniens. These cells did not express hypocretin/orexin or melanin-concentrating hormone but displayed putative efferent connectivity with LH hypocretin/orexin+ neurons and dopaminergic A13 cells.  We have now optimised our surgery techniques so as to be able to make very small, localised injections of tracer in discrete subregions identified in Aim 1a. This will allow us to identify the topology connectivity of ZIRXFP3+ neurons. Together with the data from Aim 1a, this will help us to begin to understand how specific groups of neurons within the ZI might activate different downstream pathways, potentially leading to different behavioural responses.  **Figure 2: Co-localisation of *Rxfp3* with *SST.***  **Aim 2 –** to describe the function of Zona Incerta RXFP3+ neurons during fear extinction and memory retrieval. We have completed the initial pilot experiment. Following Pavlovian fear conditioning, chemogenetically activating LH/ZIRXFP3 cells reduced fear expression (freezing) overall, but this was not dependent on context. Activating these cells also induced jumping behaviour and increased locomotor activity. Therefore, the decreased freezing is more likely to reflect enhanced arousal rather than reduced fear. Indeed, stimulating these cells increased the activation of several motor, stress, and arousal regions, as measured by Fos expression. These results suggest that activating LH/ZIRXFP3 cells generates brain-wide activation patterns that augment behavioural arousal.  We have now optimised a behavioural protocol (auditory loom), that allows us to measure innate defensive responses to perceived threat from predation under different levels of arousal. We are conducting a study that will allow us to first understand whether ZIRXFP3+ neurons are differentially activated under these different levels of arousal, and then whether manipulating them changes these responses. Together with findings from learned fear experiments we hope that this information will help build an understanding of how ZIRXFP3+ neurons mediate appropriate responding to learned and innately threatening stimuli under different conditions. |

**(3) Career Advancement of the beneficiary**

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| **1) Award of a major competitive Australian government grant to beneficiary.**  The data collected thanks to ISN award put me in a position where I could apply successfully for one of the major Australian grant rounds (the Australian Research Council, ARC) as lead investigator. This is the first time I have led a significant project, and represents an important milestone in my career development. With this additional funding I was able to recruit a PhD and a masters student, as well as a full-time research assistant and a postdoc. I now have a small but established team of researchers working with me, and my recognition of my lab is growing.  **2) Career development at Macquarie University.**  Since award of this grant I relocated from Melbourne to Sydney to take up a fixed contract at Macquarie University. Thanks to the ISN award and flow on ARC grant, I was in a position to negotiate better terms and a longer contract. I was also able to apply successfully for a large internal infrastructure grant with enabled me to purchase equipment to set up my own behavioural lab at Macquarie University. I am in the processes of developing an application for a major mid-career fellowship award, and will shortly be applying for promotion to associate professor. |

**(4) How and when was audience informed on the ISN and on the benefits of an ISN membership?**

I work closely with a number of researchers in the Macquarie Medical School and Macquarie School of Psychological Sciences, and have informed many graduate students working in the field of neuroscience and neurochemistry regarding benefits of attending ISN meeting. Through this at least three graduates students have applied successfully for ISN travel awards to attend the meetings in 2022 and 2023.

**(5) Have the results been disseminated to communities of interest? Future plan to present the findings at a scientific meeting (oral/poster mode) or publish in scientific journals (specify the tentative title)**

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| Manuscript under review at Neuropsychopharmacology:  Richards BK, Ch’ng SS, Simon AB, Kim JH, Lawrence AJ, **Perry CJ.** *Relaxin family peptide receptor 3 (RXFP3) expressing cells in the zona incerta/lateral hypothalamus augment behavioural arousal*  Manuscript in preparation:  Richards BK, Kim JH, Lawrence AJ, **Perry CJ.** *A comprehensive analysis of neurochemical phenotype and connectivity of relaxin family peptide receptor 3 (RXPF3) expressing cells in the zona incerta and lateral hypothalamus*  Oral presentations:   * **Perry CJ** (2021) The Zona Incerta regulates encoding of conditioned fear extinction. UNSW Behavioural Neuroscience Seminar Series (Sydney, Australia). * Richards BK, Ch’ng SS, Simon AB, Kim JH, Cornish JL, Lawrence AJ, **Perry CJ** (2022). Investigating the properties and behavioural roles of RXFP3+ zona incerta/lateral hypothalamus neurons. Sydney Postgraduate Psychology Conference (The University of New South Wales, Sydney, Australia)   Poster Presentations:   * Richards BK, Ch’ng SS, Simon AB, Kim JH, Cornish JL, Lawrence AJ, **Perry CJ** (2023). *Investigating the properties and behavioural roles of RXFP3+ zona incerta/lateral hypothalamus neurons*. International Society of Neurochemistry- European Society of Neurochemistry (ISN-ESN) Meeting 2023 (Porto, Portugal) * Richards BK, Ch’ng SS, Simon AB, Kim JH, Cornish JL, Lawrence AJ, **Perry CJ** (2022). *Investigating the properties and behavioural roles of RXFP3+ zona incerta/lateral hypothalamus neurons.* Sydney Sensory Neuroscience Symposium (Western Sydney University, Sydney, Australia) * Richards BK, Ch’ng SS, Simon AB, Kim JH, Cornish JL, Lawrence AJ, **Perry CJ** (2022). *Investigating the properties and behavioural roles of RXFP3+ zona incerta/lateral hypothalamus neurons.* Biological Psychiatry Australia (BPA) 12th Annual Scientific Meeting (The University of Newcastle, Newcastle, Australia) |

(Please note that in any publications resulting from the grant ISN funding shall be acknowledged.)

**Financial report**

Please provide a brief overview how funds have been used.

**Total costs** (in USD)**:**

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| --- | --- | --- |
|  | **Costs** | **Total** (USD) |
| **1** | Small instrumentation |  |
|  |  |  |
| **2** | Animal costs | 3500 |
|  |  |  |
| **3** | Measuring costs\* | 600 |
|  |  |  |
| **4** | Consumables | 5900 |
|  |  |  |
| **5** | Other project costs |  |
|  |  |  |
| **6** | ISN conference participation# |  |
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| **Total costs of the project:** | | **10,000** |
| **Total CDG application sum:** | | **10,000** |

\* for equipment use, e.g. microscopy MRI, spectroscopy facilities, and others.

# max. 20% of the total grant, only for the applicant.

**Other external and local funds used for the completion of the project**

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| **Source** | **Amount** (USD) | **Used for** |
| **Home institution** | **70,000**  **25,000pa (ongoing)**  **20,000** | **Purchase of fear conditioning chambers**  **Stipend for PhD student**  **Stipend for Masters student** |
| **Host lab** |  |  |
| **Other sources** | **500,000** | **ARC grant** |

**Photograph showing the beneficiary and his laboratory** (please indicate copyright)

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I agree that portions of this report, and a copy of the supplied photograph, may be published on the ISN homepage to inform ISN members of this ISN-supported CDC activity.

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| **Signature of beneficiary:** |  |
|  |  |
| **Place, Date:** | **Sydney, Australia 20/10/2023** |