Australian-German collaboration in translational neuroscience of stress

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für Psychiatrie

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ABOUT THE AUTHOR

Between January and April 2018, I spent 12 weeks as a “Wissenschaftliche Mitarbeiterin” (Researcher) at the Max Planck Institute of Psychiatry in Munich, Germany.

My interest in Germany is rooted in the German language. I learnt German at high school and formed long-lasting connections with the country and its people, first while visiting for three weeks in 2009, and then while staying as an exchange student for 3 months with Scholarships for Australian-German Student Exchange (SAGSE) in 2011. Since then, I have been back almost every winter, consuming more baked goodies than should be humanly allowed, and always returning weighing at least 8-10kg heavier.

I studied neuroscience and languages at the University of Melbourne, obtaining first-class honours in my Bachelor of Science (Honours) and Diploma in Languages. One semester of my undergraduate degree was completed at the University of Granada, in Spain. My Honours project investigated a candidate gene for fear memory in a mouse model of post-traumatic stress disorder, which developed my interest in stress neurobiology.

My fluency in German allowed me to forgo the language course and maximise the research component of my AGA Fellowship. This extra time also allowed me to travel to meet with several people working in the international science policy and research management sector – an area I wanted to learn more about. As a result of these meetings I found my next career position and currently work in developing international research partnerships between the University of Melbourne and various institutions, with a particular focus on those in (you guessed it!) Germany.

ABBREVIATIONS

ACTH Adrenocorticotropic hormone (a stress hormone)

AGA Australian German Association

cDNA Copy DNA

CpG Cytosine-phosphate-Guanine (a site where methylation can occur)

DEL Deletion

DEX Dexamethasone (a stress-inducing drug)

GAPDH Glyceraldehyde 3-phosphate dehydrogenase (a housekeeping gene)

GR Glucocorticoid Receptor

INDEL Insertion/Deletion region

INS Insertion

ISQ Important Science Question

LCL Lymphoblastoid Cell Lines

MPI Max Planck Institute

mRNA messenger RNA

PCR Polymerase Chain Reaction

qPCR quantitative Polymerase Chain Reaction

SNP Single Nucleotide Polymorphism

TBS Targeted Bisulphite Sequencing

VEH Vehicle (a control substance)

CONTENTS

RESEARCH INTRODUCTION

My research project proposal was pitched at advancing knowledge and Australian-German collaboration in mental health research by working in the Binder lab at the Max Planck Institute (MPI) of Psychiatry. I actually came across the Binder group during my reading in my Honours studies and working with my previous Australian laboratory. The Binder group are established experts in stress neuroscience and epigenetics; the former being an area I had already delved into, and the latter being a fascinating area I wanted to explore further.

Over my 12-week research stay at the MPI, I worked together with molecular biologists, clinicians and bioinformaticians to develop our understanding of complex psychiatric disorders, particularly looking at the mechanism of stress. I completed the research component of my Fellowship under the guidance of Dr Elisabeth Binder (not only my laboratory head, but also a Director of this MPI) in the Department of Translational Research.

Translational Psychiatry is a field at the intersection of research and clinical application. It aims to translate findings from the laboratory bench-side to the clinical bed-side. While most of my research took place at the lab bench, as part of my research stay I participated in a 3-day seminar run by the International Max Planck Research School and designed for researchers to observe the clinical aspects of psychiatric disorders.

Psychiatric disorders are among the most debilitating and stigmatized conditions, which could not have been made more blatantly apparent than in this seminar: together with their psychiatrists, patients from different psychiatric wards openly and honestly discussed and answered questions about the symptoms and experiences associated with their diagnoses. Among the patients who presented their stories were those suffering from schizophrenia, depression, mania, borderline personality disorder, substance abuse, and autism spectrum disorder. It was a very unique, humbling and eye-opening experience for me, because it made me realise how detached I had become from the bigger picture. Realising the scope of raw, difficult, and untreatable experiences was a brutal reminder of the purpose and necessity of mental health research. Witnessing the patients' recollections of experiences in dealing with their world and their disorder was a reminder that this research was contributing to something meaningful.

PROJECT BACKGROUND

"All mental processes are brain processes, and therefore all disorders of mental functioning are biological diseases"

-Psychiatrist and Nobel Prize laureate, Eric Kandel

Psychiatric disorders are caused by the brain. This does not mean that all biological changes physically occur in the brain; sometimes the brain sends out faulty signals which create changes in other parts of the body.

The hormonal stress response

Some of the signals sent by the brain are hormones generated in a brain region called the hypothalamus. Under stress, the hypothalamus releases a hormone (ACTH) which acts on the adrenal gland (located just above the kidneys). In response to ACTH, the adrenal gland then releases other hormones (glucocorticoids) that circulate in our bloodstream, some of them acting on blood cells, and some feeding back onto brain activity (Figure 1A).

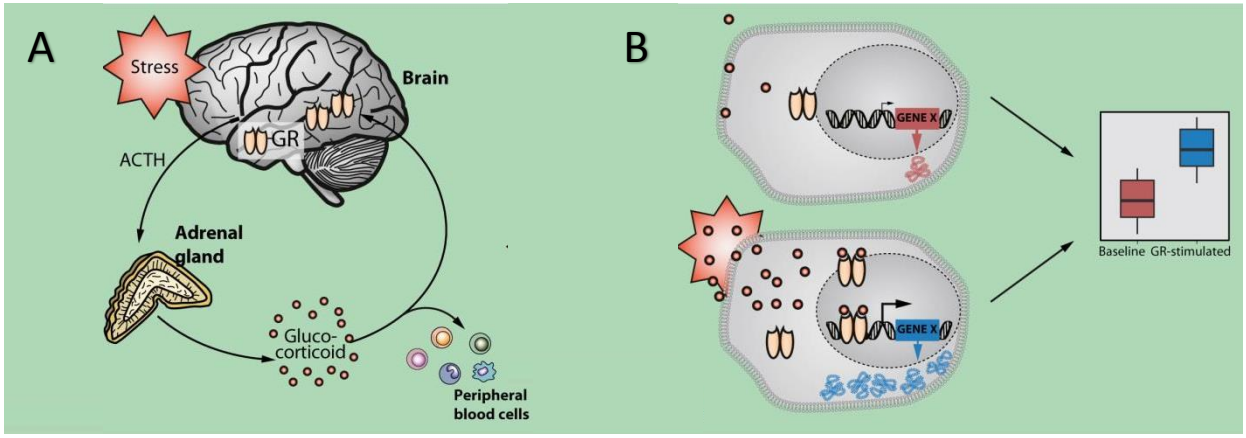


Figure 1: Hormonal stress response modified from Arloth *et al*., 2015 (Neuron).
ACTH: Adrenocorticotropic hormone, GR: Glucocorticoid receptor.

Glucocorticoids act by binding to their glucocorticoid receptor (GR). The same cell can respond differently when exposed to different levels of glucocorticoids (Figure 1B). Small levels of glucocorticoids might not activate the glucocorticoid receptor, resulting in low levels of GR-stimulated gene expression and subsequent protein production. Larger levels of glucocorticoids may induce a high GR-stimulated gene expression response and subsequent protein production. The amount of glucocorticoid is not influencing the DNA or genes themselves, but it is influencing how the DNA is read, i.e. to what extent genes are expressed and subsequent proteins are made.

How is this happening? This process is an example of epigenetic change, which I find easiest to explain with a recipe-book and paperclip analogy.

Recipe books and paperclips

A family recipe book, passed down from generation to generation, represents your genetic make-up- your DNA. This recipe book contains all the recipes to make you who you are.

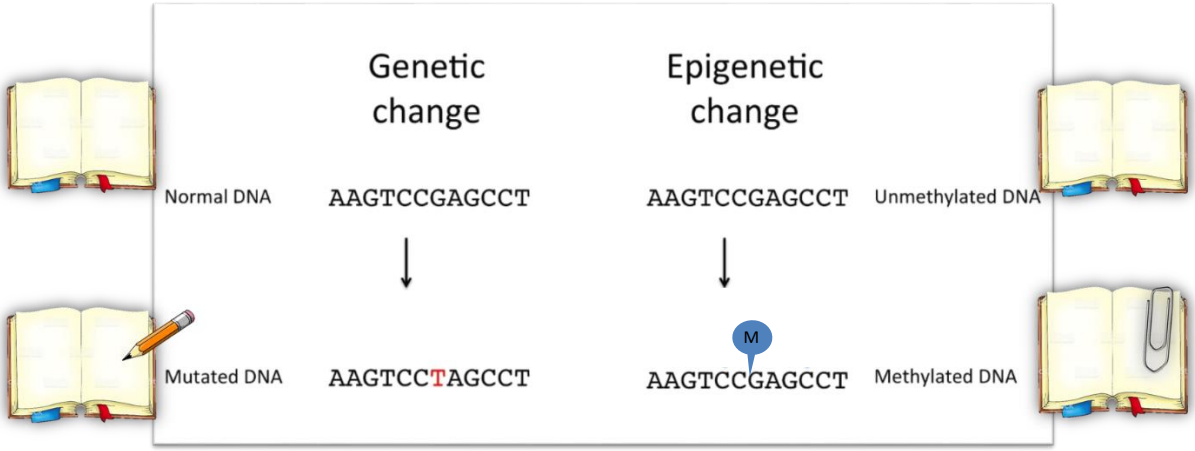
Within the recipe book, each individual recipe represents a gene. When a gene is read out, or a recipe is correctly followed, it makes a protein. These proteins are our building blocks. Proteins are involved in almost every bodily function including cell structure, communication between cells and protecting the body from foreign particles like bacteria and viruses. If a recipe is read out incorrectly, or it has a mistake, our proteins may not be able to function as they are supposed to, which can lead to different health problems.

Genetic changes are changes in our DNA sequence – in the recipe book analogy, like adding or removing ingredients. **Epigenetic changes** (literally "outside" "genetics") are changes in gene function (how our recipes are read) which occur outside of our genes (not changes to the recipe itself). Both genetic and epigenetic changes are heritable. But how?

One of the main epigenetic processes is methylation. A paperclip, put between several pages of the recipe book, holds that recipe closed. This paper clip represents methylation. Chemically speaking, methylation is simply adding a chemical compound called a methyl group to your DNA. Methylation isn't changing the recipe itself; it's just making the recipe unreadable.

Methylation can only occur at specific sites on a DNA molecule. Each strand of DNA is made up of a combination of four different types of nucleotides: A, T, C, and G. Methylation can only occur in places on the DNA where a C and G are next to each other on the DNA strand, called a CpG site.

When a CpG site in a gene is methylated, the gene becomes silenced and can no longer be read. This way, the protein that it was encoding can no longer be made by that cell. The process of removing a paperclip, called demethylation, can open up previously hidden recipes, so that they are now available for the cooks to use.



How is this all relevant to stress? Read on…

Brainless psychiatry

As the study of mental illness, psychiatric research often faces a limitation of not being able to look directly into sick people's brains to measure or evaluate brain function (it's expensive, invasive, and people generally need their brains to live). Dead people's brains, in addition to being hard to come by in a useable state for research, are confounded by any combination of the never-ending variables between their previous owners – genetics, environment, medications, diseases, the list goes on. So, with no human brains, how do we study psychiatry brainless?

Well, we *could* use animal brains (but, Maria, that sounds completely unethical, plus do animals even get psychiatric disorders??… Yes, dear reader, you raise valid points, and there will be more on that later). Another option to find out what is happening in the brain is by using human blood cells. Patient blood is relatively easy to obtain and can give a biological read-out. Not all molecules cross the barrier between the brain and the rest of the body (aptly named the blood-brain barrier), so the blood isn't really a reflection of what cocktail of proteins are circulating in the brain. Blood does, however, show a snapshot of a patient's genetic and epigenetic signatures and can be used to discover how stress affects these.

http://getdrawings.com/cell-cartoon-drawing

A superhero technique: immortal cells

Human blood contains different types of blood cells, and in my research I used lymphoblastoid cell lines, or LCLs: a type of white blood cell sourced from patients involved in the international 1000 Genomes Project, and transformed with magical superpowers (the Eppstein-Bar virus; yes the very same that causes glandular fever) which makes them replicate forever.

Essentially, LCLs are a never-ending supply of cells – each batch of replicating LCLs contains the same genetic and epigenetic signature as the very first white blood cells donated by the patient. The immortality of these cell lines allows researchers to undertake a range of experiments on cells with a controlled genetic and epigenetic background.

The mystery gene (mystified by complexities of intellectual property)

The focus of the Binder lab is a *mystery gene* (which for the purposes of presenting yet unpublished data in this report, I have left de-identified). A few things I can note about this mystery gene are:

- The mystery gene codes for a mystery protein which modulates the glucocorticoid receptor
- The mystery gene has different splicing variants. This means that the gene can be cut into different lengths (long and short) which affects the proteins it produces (more on that below)
- The mystery gene is not the same in all people: it differs in a "Single Nucleotide Polymorphism" (or SNP; meaning that one letter in the genetic sequence varies between individuals) **and** it also differs in an Insertion/Deletion region (or INDEL; meaning that a section of the genetic sequence is either present or missing)
- The mystery gene also contains various methylation sites in different regions. This means that the DNA could be epigenetically modified in multiple different areas.

Getting to the Important Science Questions

Over my time at the MPI I worked to answer the following Important Science Questions (ISQs):

ISQ 1. What kind of genetic information of the mystery gene can predispose risk to stress?

a. How do the short and long variants of the mystery gene influence the stress response?
b. How do different types of genetic information (SNP and/or INDEL) influence the stress response?

ISQ 2. In measuring epigenetic effects on stress responsiveness, does our methylation-measuring technique work?

LABORATORY METHODS

DNA carries our genetic information, but genes are being expressed at different levels in different situations at any given time. For example our gene expression is different when we're trying to escape a stressful situation compared to when we are relaxing on the beach to the sound of waves and looking up at stars. Because our genetic information in DNA is static, we need to look at something else to measure the levels of gene expression in response to stress. What could that be? DNA's closest friend: RNA.

RNA is made in a process called transcription in which a particular segment of our DNA (a transcription unit) is copied into RNA. If the transcription unit contains genes which encode protein, the transcription produces messenger RNA (mRNA) which serves to transmit genetic information from the nucleus, where the DNA is located, to the cell compartments where proteins are being made, through a process called translation. The different lengths of RNA splicing variants can affect the downstream variation of protein.

ISQ 1: Lymphoblastoid Cell Lines (LCLs) as a model system for genotype experiments

To find out how the short and long variants and SNP and INDEL genotypes of the mystery gene can predispose risk to stress, and answer Important Science Question 1 (ISQ 1), I conducted three sets of experiments using similar procedures (Figure 2).

To begin, I cultured LCLs (allowed them to replicate under laboratory conditions) with different genetic backgrounds, either varying in SNPs or INDELs until I had the same number of cells for each genotype. For each lot of cells, half were given a drug, dexamethasone (or DEX), and half were given a control substance, also termed "vehicle" (or VEH). DEX is a glucocorticoid receptor (GR) agonist, which means that it activates GRs, essentially acting as a pharmacological stressor to the cells.

To demonstrate how LCLs respond to stress over time, I compared responses of cells treated with DEX and with VEH, measured over different time-points. I separated these LCLs into groups, and allowed them to react with either DEX or VEH for different times. After "treating" LCLs with either the control (VEH) or the drug (DEX), they were ripped apart with Trizol and chloroform (science can be more ferocious than a crime show sometimes!) in the process of RNA extraction.

I then quantified mRNA to estimate the mystery gene's expression after stress. Although DNA is directly measurable, mRNA isn't. To measure the amount of mRNA I used an enzyme called "reverse transcriptase" (naturally occurring in retroviruses) to turn mRNA into copy DNA (cDNA). I then used a process called "quantitative Polymerase Chain Reaction" (qPCR) to detect fluorescence from sequence-specific probes that I attached to my cDNA, thereby telling me how much cDNA there is, thereby telling me how much mRNA there is, thereby telling me how much stress impacts our mystery gene expression!

My first two experiments were designed to assess the broad effects of either SNP or INDEL differences over time (Figure 2; Experiment 1 with SNP differences, Experiment 2 with INDEL differences). For each experiment I used 6 different cell lines. For the SNP experiment, these 6 LCLs were split between two possible SNP variants for the mystery gene region of interest (TT/CC) and for the INDEL experiments these were split between the two possible INDEL variants (INS/DEL). For both experiments, I waited 1, 2,

3, 4, 8, and 24 hours before extracting their RNA and measuring gene expression to assess how stress (via DEX) affected the LCLs' expression of our mystery gene over time.

For my third experiment (Figure 2; Experiment 3 with genotype combinations) I focussed on the 8-hour time-point to look at the differences between the mystery gene expression of SNP and INDEL genotypes in more detail. I used 13 different lines in all three possible genotype combinations: CC-INS, CC-DEL, and TT-INS. We had no LCLs with the TT-DEL genotype as this combination doesn't exist (most likely a lethal combination).

In each of these experiments, the VEH/DEX stimulation and qPCR quantification steps were repeated up to 3 times to account for technical variation and ensure outcomes were consistent.

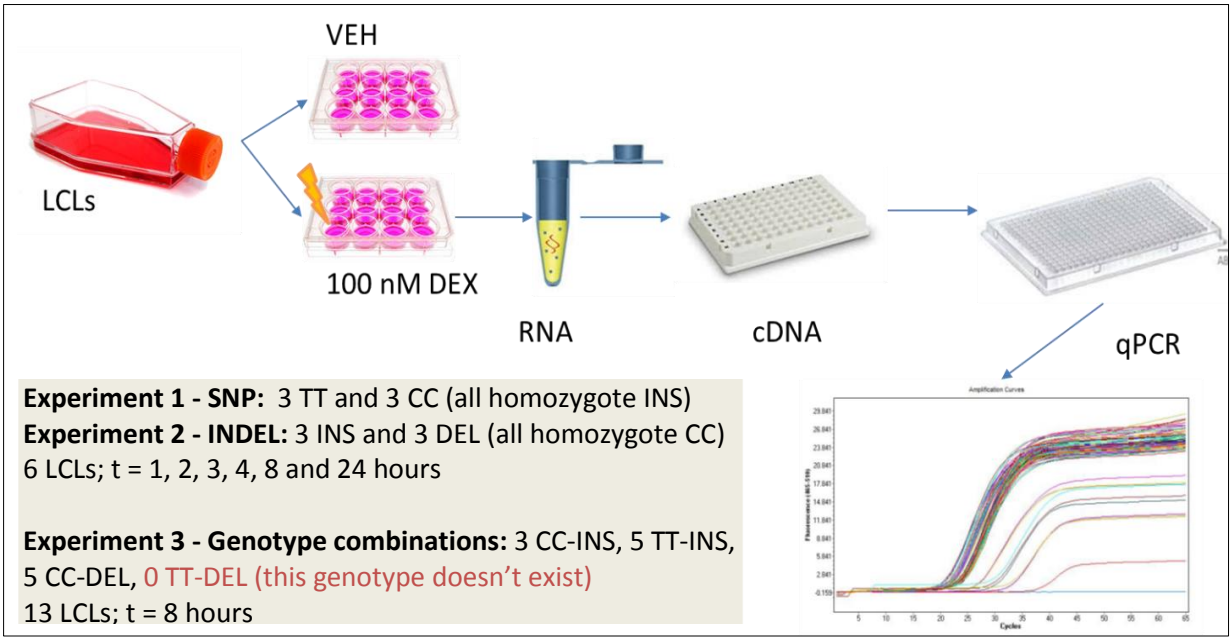


Figure 2: Experimental method schematic for LCL SNP and LCL INDEL experiments over time, and LCL genotype combinations experiment at t=8 hours.
For the SNP experiment, 3 TT and 3 CC LCLs were used (total 6 LCLs), with the same INDEL background (INS). For the INDEL experiment, 3 INS and 3 DEL LCLs were used (total 6 LCLs), with the same SNP background (CC). For both experiments, LCLs were stimulated for 1, 2, 3, 4, 8 or 24 hours with VEH or DEX. For the genotype combinations experiment, 3 CC-INS, 5 TT-INS, and 5 CC-DEL LCLs were used (total 13 LCLs). LCLs were stimulated for 8 hours with VEH or DEX. The concentration of DEX in all LCL experiments was 100 nanomolar (nM).

ISQ 2: Human and mouse DNA methylation

The first part of my experiments (in answering Important Science Question 1) were focussed on genetic risk to stress. The second part of my experiments (in answering ISQ 2) formed the beginning of epigenetic tests.

Before measuring methylation changes in response to stress, it was important determine whether our methylation technique was working. To do so, I measured methylation at known levels and established a

control methylation curve (comparing my actual measurements with the expected levels) using mouse and human DNA.

Why mouse and human? Well, an important aspect of translational research is the ability to use a number of models and techniques, including those relying on a mouse model. Studies involving mouse models let scientists dig deeper than they could with humans because of ethical issues, as well as short generation times, which let scientists test for transgenerational effects. Knowing how well both species respond to our methylation technique is an important step in inferring data from mouse models and designing experiments appropriately.

Mouse DNA was already extracted from tails pooled together across a number of mice by another lab member to increase the amount of DNA for the experiment. I extracted human DNA from my LCLs, pooled from my previous experiments (in ISQ 1). The same technique was used for both species. I used a kit called Repli-G to de-methylate (remove all methyl groups in) the DNA. Then another bio tool-kit was used on half of that de-methylated DNA for in-vitro methylation, which added methyl groups on all possible CpG sites. I then had a pool of non-methylated DNA (0% methylation) and a pool of completely methylated DNA (100% methylation), which was then combined in the correct ratios to generate 25%-, 50%- and 75% - methylated DNA in a graduated "standard curve" of methylation.

For the purposes of ISQ 2, I was only interested in specific regions of DNA within my mystery gene which could be influenced by stress. To identify these regions, I used Targeted Bisulphite Sequencing (TBS), a technique developed at the Max Planck Institute, which uses an open-source website (the UCSC genome browser) to identify methylation sites with predicted glucocorticoid receptor interactions. These sites can be targeted by designing appropriate primers used in a process called "bisulphite conversion", which results in methylated and non-methylated DNA having different sequences. These sequence differences are maintained through the next steps of the experiment- involving another polymerase chain reaction (PCR) which prepares the samples for methylation detection.

Next I measured the methylation in my DNA regions of interest using a very dramatic-looking (and somewhat stressful) technique called Pyrosequencing (literally "fire sequencing" because it uses the firefly luciferase enzyme). This is a method of synthetising DNA molecules from a template (of the methylated sequence), identifying the actual sequence of nucleotides, and whether nucleotides are methylated, by detecting light emitted by a molecule, "pyrophosphate" released during synthesis. A "matched" nucleotide to the methylated template sequence emits light and the Pyrosequencer measures its intensity. A non-matched nucleotide (and a negative control, such as water), does not emit any light. Comparing light peaks between the DNA of interest and fully methylated DNA shows how much methylation is present in each region.

EXPERIMENTAL RESULTS

LCL experiments

Along measuring the responses to stress of our short and long mystery gene variants, I also measured the response of a house-keeping gene, GAPDH. The house-keeping gene does none of the cleaning, but all of the standardising – as a gene which does not generally vary with stress, it is used to "normalise" our mystery gene results.

Long variant is ~12 times more abundant than the short variant

Coming out of the qPCR, the raw data is measured in Cp values which correspond to the number of cycles that the cDNA had gone through (doubling with each cycle) before achieving a statistically significant increase in fluorescence. Cp values are inversely proportional to abundancy. Simply put, a lower Cp value by 1 mean there is double of that variant being expressed.

Looking purely at the Cp values of our mystery gene variants and GAPDH (Figure 3), we can see that GAPDH is most abundant and has very little variation between VEH, DEX or any of the time points (exactly what we would expect, because we use it as the standard to compare our other expression data to). The long variant of our mystery gene is next abundant (with VEH Cp values averaging ~24), and roughly 12x more abundant than the shorter variant (with VEH Cp values averaging ~30).

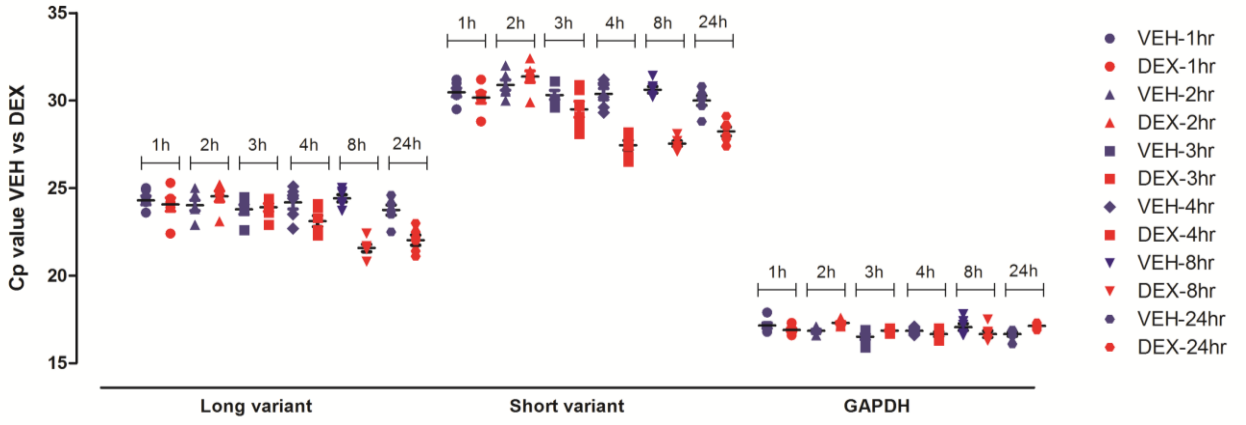


Figure 3: Mystery gene and GAPDH Cp values across time, where t= 1, 2, 3, 4, 8 and 24 hours after treatment (pooled genotypes)
Cp values are inverse to abundancy, doubling for each value. The long variant is ~12 times more abundant than the short variant.

After comparing raw Cp values, I calculated the difference in response between our DEX- and VEH-treated cells to determine the effect of drug-induced stress on each mystery gene genotype at each time-point. I then normalised this difference to the DEX-VEH difference of our GAPDH Cp values for each time-point. This gave me an indication of DEX effect on the different variants of our mystery gene expression (induction) as a ratio compared to GAPDH.

Short variant has an earlier DEX-induction point

Looking firstly at the overall variant effect of DEX treatment, I pooled data from our 6 SNP-differentiated LCLs together (Figure 4A) and our 6 INDEL-differentiated LCLs together (Figure 4B). I showed, in the SNP and INDEL data, that the long and short variants have a different time-course of DEX-induction. More specifically, I found that the short variant responds more quickly to DEX-induced stress than the long variant of our mystery gene.

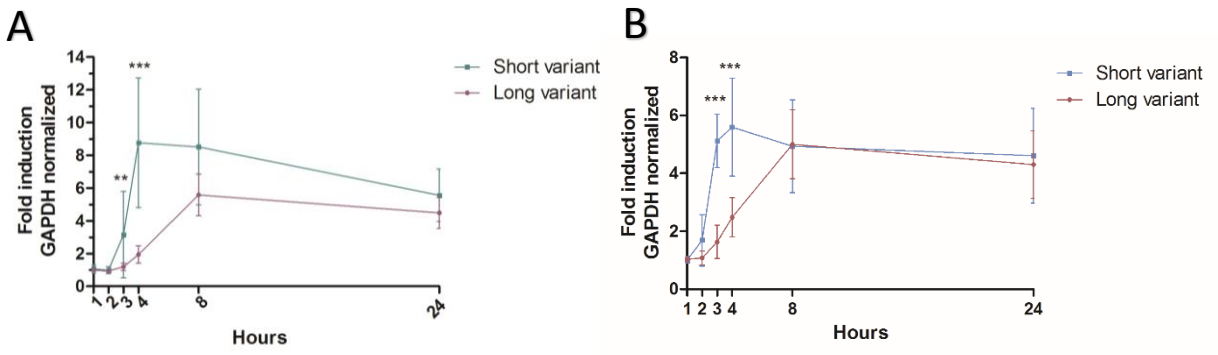


Figure 4: Broad variant effect on DEX treatment over time, where t= 1, 2, 3, 4, 8 and 24 hours after treatment

A) pooled SNP genotypes and B) pooled INDEL genotypes. The short variant has an earlier DEX-induction point than the long variant in SNP and INDEL genotypes.

Time-course of DEX-induced SNP LCLs identifies CC as a protective genotype

Looking further into the genotype split (Figure 5), I found that a TT SNP (dotted line) is more highly expressed than a CC genotype in both variants. This means that DEX-induced stress has a higher effect on a TT genotype, suggesting that a CC genotype is more protective against reacting to stress.

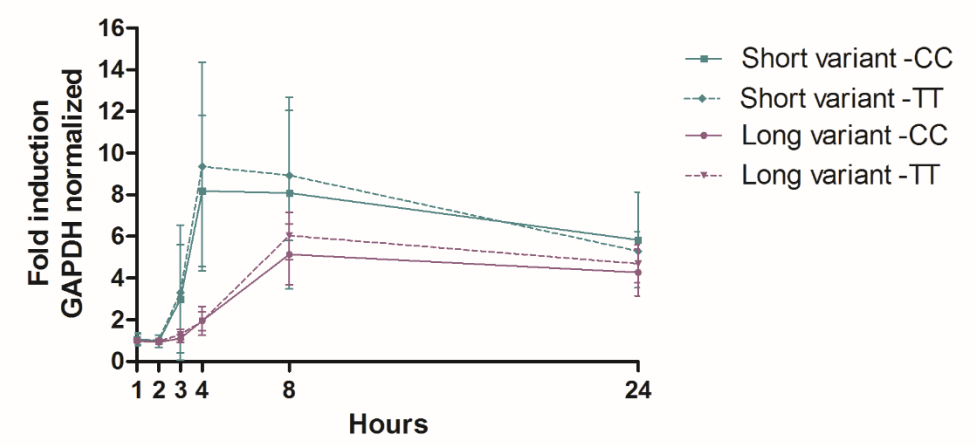


Figure 5: SNP effect on DEX treatment over time, where t= 1, 2, 3, 4, 8 and 24 hours after treatment

3 LCLs/SNP, all with an INS background. The TT genotype is more highly expressed than CC in the short and long variants.

Time-course of DEX-induced INDEL LCLs suggests INS is a protective genotype in the later time-points

Looking at the INDEL data (Figure 6), I found differing patterns in DEX-induction of the two variants by genotype. In the time-curves of the short variant, the deletion (DEL) was mostly induced higher than the insertion (INS) genotype. In the longer variant, there appears to be a similar levels of induction with INS being more highly induced prior to 8 hours, with a swap at the 8-hour time-point between the INS and DEL. Overall, there was a greater difference in DEX-induction of INS or DEL genotype in the shorter variant than the longer variant. Between 8 and 24 hours, the DEL genotype was more highly induced, suggesting it is less protective against reacting to stress.

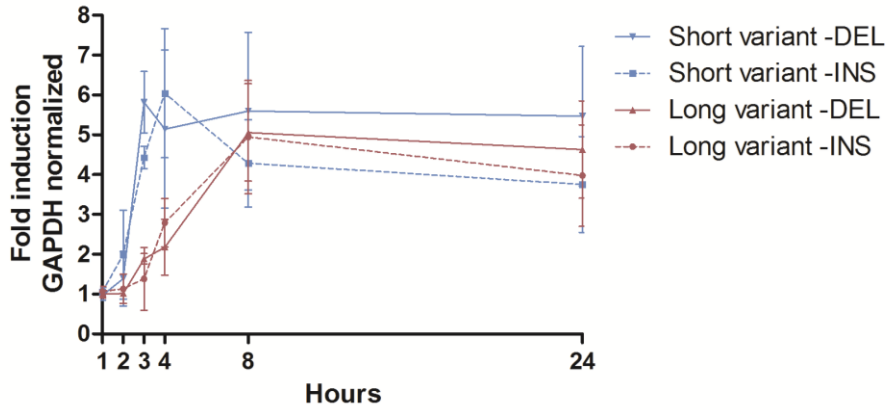


Figure 6: INDEL effect on DEX treatment over time, where t= 1, 2, 3, 4, 8 and 24 hours after treatment
3 LCLs/INDEL, all with a CC background. Between 8 and 24 hours, DEL genotype was more highly induced in both variants.

Combination of SNP and INDEL of the mystery gene is most important in determining genetic risk/predisposal to stress

My third LCL experiment was designed to elucidate the difference and interaction between SNP and INDEL effects on DEX-induction of the two mystery gene variants, at the 8 hour time-point (with the largest differences in the SNP and INDEL experiments); Figure 7. Comparing the DEX-induced values of CC-INS (blue) and TT-INS (green) shows the effect of SNP. Comparing the DEX-induced values of CC-INS (blue) and CC-DEL (red) shows the effect of INDEL. Comparing the DEX-induced values of TT-INS (green) and CC-DEL (red) shows the effect of both.

In both long and short variants, the greatest difference was observed when both SNP and INDEL were varied. This difference was significant in the short variant (*$p<0.05$). These results suggest that the most "protective" genotype against stress, the one that is least-induced by DEX, is a CC DEL genotype.

Although the TT genotype being more highly induced by stress than CC is consistent with previous results (Figure 5), the observation of the INS genotype being more highly induced than DEL is not (Figure 6). The small sample size and large spread of data points for the CC INS genotype, suggest that this experiment should be repeated with a larger group of genotypes for a more conclusive result.

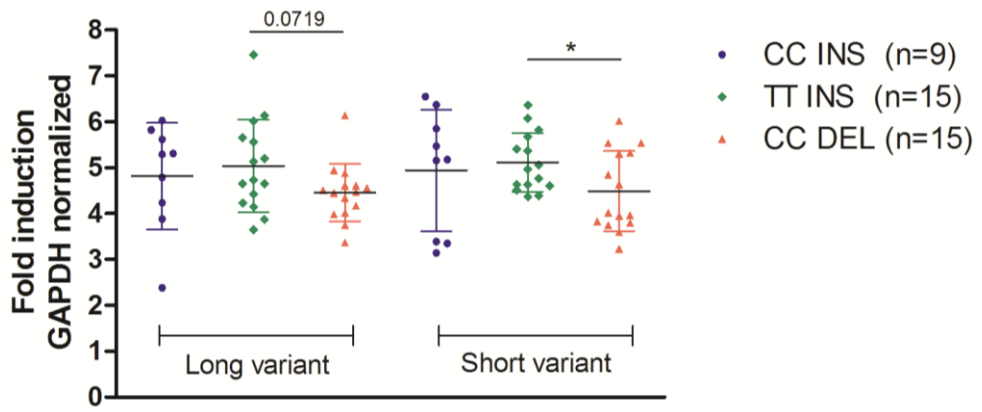


Figure 7: Genotype combination (SNP x INDEL) effect on DEX treatment
Significant differences were observed in the short variant, between the TT-INS and CC-DEL genotypes.

Mystery gene methylation

The methylation protocol was designed for human DNA. I therefore measured human DNA methylation (Figure 8) at one singular CpG site which was, as expected, well-aligned with the perfect methylation curve. Because we did not know whether the same protocol would efficiently apply to mouse DNA, I measured mouse DNA methylation at three CpG sites in triplicates, and took the average methylation percentages to plot the curve. Mouse control DNA methylation levels were slightly lower than the perfect methylation curve (Figure 8). Nevertheless, these data show that this process of methylation assessment works almost equally well across humans and mice, a useful demonstration for further translational studies.

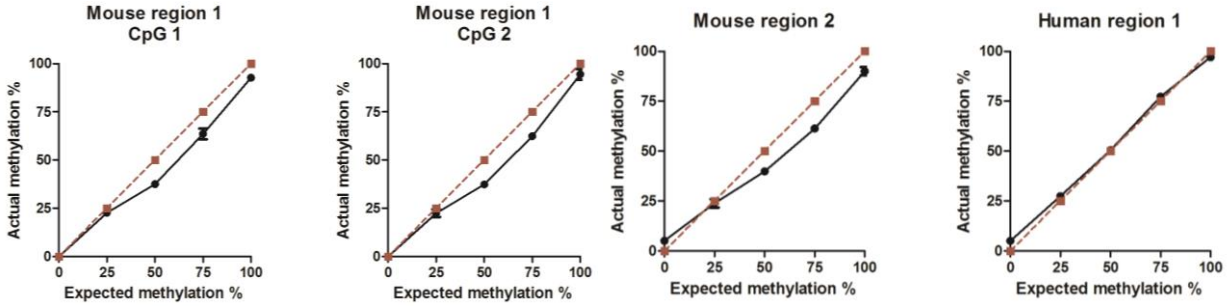


Figure 8: Methylation control curves for mystery gene regions in mouse and human DNA
The red dotted line represents the hypothetical "perfect" curve where expected and observed values align exactly. The black dotted line represents the observed methylation values.

EXPERIMENTAL MEANING AND RELEVANCE

One of the biggest challenges in psychiatry is to correctly diagnose the patient. Many patients spend years being treated for one disorder before being re-diagnosed with another. Diagnosis affects the treatment and as a result, many patients don't receive the appropriate treatment.

Identifying a person's genetic risk or predisposition to developing stress can help scientists tailor the treatment to them appropriately, something termed "personalised medicine". Answering Important Science Questions aids in developing the greater scientific body of knowledge which will lead to personalising treatment for mental health disorders in the future.

The results of my LCL experiments are a promising start for understanding genetic predispositions to stress. Identifying the abundance of the different gene variants, time-course of induction, and protective genotypes for stress risk are all be important aspects to consider for therapeutic development. These studies will need to be expanded to generate more substantive and conclusive results.

Validating animals as a model for stress-related research helps scientists overcome the limitation of patients for basic research. Because human and mouse genomes are similar, many genes (including the mystery gene) are found in both species. Despite this similarity, there are some differences in DNA which may impact on how well a technique, developed for one species, is translated across to another. The significance of my methylation study was demonstrating that the methylation assay technique designed for humans can be used for analysing mouse DNA – a promising finding that will contribute to further mouse methylation experiments in the future.

These data contribute to the growing body of knowledge which will help to demystify the questions about genetic predispositions to stress-related disorders and the epigenetic effects of stress, to develop personalised medicine for more effective patient treatment. This understanding will be especially useful in designing better pharmaco- and psycho- therapies for people with psychiatric disorders.

BILATERAL (AUSTRALIAN-GERMAN) RESEARCH COLLABORATION

The work undertaken through this Fellowship combined my previous experience and expertise by contrasting experimental approaches of forward and reverse genetics. Forward genetics is a "phenotype to genotype" approach which formed the basis of my Australian stress research (Figure 9): first identifying a behavioural difference (in my instance, in mice), then, through cross-breeding narrowing this behaviour down to a subset of genes that regulates this behaviour, and then further identifying a candidate-gene.

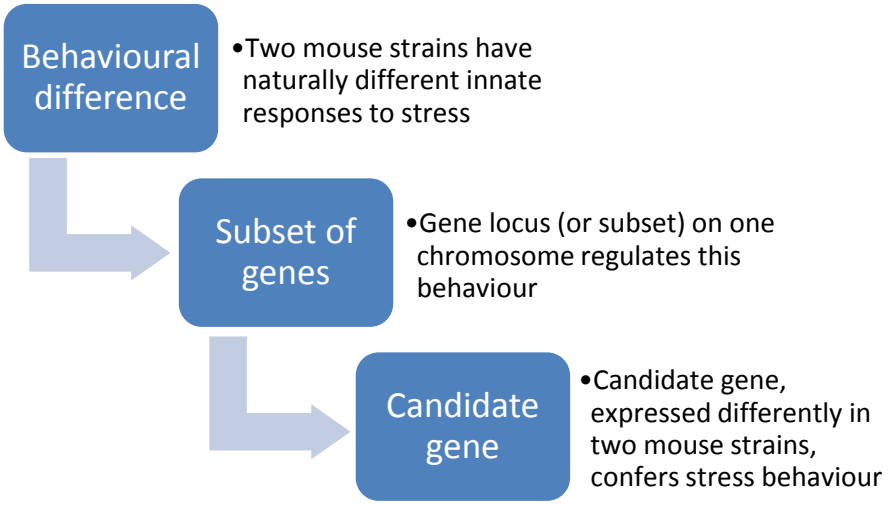


Figure 9: The "forward genetics" approach used in my previous Australian laboratory

At the Max Planck Institute (MPI), I used the reverse genetics, or "genotype to phenotype", approach (Figure 10). Using human cell lines, I studied the mystery (candidate) gene implicated in stress responsiveness through previous bodies of scientific literature. I then modelled the stress phenotype by inducing chemical stress to our model system (LCLs).

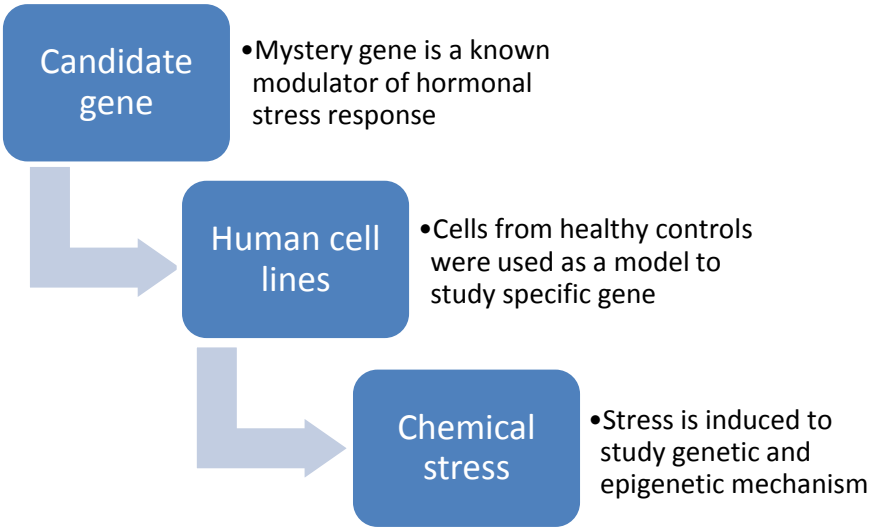


Figure 10: The "reverse genetics" approach used it my Max Planck laboratory

Incorporating these complementary techniques and overlapping subject matter, has identified a potential new direction of research. It would build on my biological understanding of epigenetic experimental design from the MPI and apply it to the candidate gene I studied in my previous Australian laboratory.

My work at the MPI generated data which will contribute to a peer-reviewed scientific paper about the mystery gene. The paper will add to the greater body of scientific knowledge about the mechanisms of stress. I am confident that, should I choose to continue my work in research, the people I met at the MPI would be able to help me in pursuing the new research direction and guide me further in their area of expertise.

In addition to identifying scientific complementarity between my host Australian and German laboratories with the help of this Fellowship, I was afforded the opportunity to contrast the Australian and German research systems more broadly. For example, PhD candidates at MPI in Germany were given significantly more freedom to design their experiments, while in Australia PhD supervisors appear to be more invested in the guidance of their students to align with pre-determined projects. This could be partially due to greater resources at MPI, enabling basic research of a more exploratory nature. On the other hand, this could be a result of a cultural expectation in Germany for graduate researchers (who are generally older than Australian PhD candidates, and treated as employees, rather than students) to be mature and independent from the project onset.

Despite the subtle differences in research systems, this Fellowship also highlighted the similarities of, and ease of conducting, research across borders. The knowledge and skills I had developed in Australia were relevant and easily transferrable to the lab in Germany. Language-wise, the MPI attracts many talented international scientists, and so the day-to-day conversations in the hallway were often held in English: speaking German was a bonus, but not a necessity.

The AGA-Goethe Fellowship is an excellent example of fostering people-to-people links between Australia and Germany. Cultivating a network of passionate alumni is an invaluable tool for soft diplomacy. This Fellowship, awarded to young professionals, inherently promotes the development of every recipient's future careers and interests, with a positive experience, network of contacts, and good-will for the bilateral relationship between Australia and Germany.

PROFESSIONAL BENEFITS

One of my professional goals for this fellowship was to gain clarity around the career direction I wanted to proceed in. My stay at the MPI afforded me an opportunity to network with people with different career backgrounds, including PhD students, post-docs, senior academics, clinicians, science policy-makers and international research management experts.

I have come to the conclusion that a PhD would improve my job prospects in Europe even outside of academia. It is something I am considering undertaking, with a project which would link an Australian stress-neurobiology laboratory with my new contacts at the Max Planck Institute.

My meeting with Australia's Science Counsellor to the EU, Dr Alex Cooke, also benefitted me professionally beyond what I had hoped for. He referred me to a colleague in Berlin who has since become my workmate in a tight-knit team developing international research partnerships with an immediate focus on Germany.

I would again like to thank the people at AGA, Goethe-Institut Australien, Max Planck Institute of Psychiatry, and the Binder lab, for making my research stay possible.