TE WHARE WĀNANGA O TE ŪPOKO O TE IKA A MĀUI
VICTORIA
UNIVERSITY OF WELLINGTON

NATURE, NURTURE, NEUROSCIENCE

A research profile of the

Behavioural Neurogenetics Group

School of Psychology, Victoria University of Wellington

November 2014

Introduction

Disorders of the central nervous system (CNS) are among the most prevalent disorders, especially in the developed world. A study performed in the European Union, several years ago, found that roughly 1 in 3 people will suffer from a CNS disorder in their life time (Andlin-Sobocki *et al.*, 2005). Moreover, the recent global burden of disease study identified CNS disorders as the most debilitation group of diseases, as measured in years lived with disability (see fig 1). This is due to the fact that many CNS disorders (such as autism, drug addiction, schizophrenia, depression etc.) start fairly early in life, and patients rarely fully recover (Vos *et al.*, 2012). As a result, brain diseases are the most costly diseases in the world. In fact a recent study showed that the total costs for brain disorders in Europe total almost 800 billion Euro, more than the costs of diabetes, pulmonary, cardiovascular and oncological disorders combined (Gustavsson *et al.*, 2011). These enormous costs, combined with the fact that for many disorders adequate treatment is still lacking show how imperative research into the neurobiology of CNS disorders is.

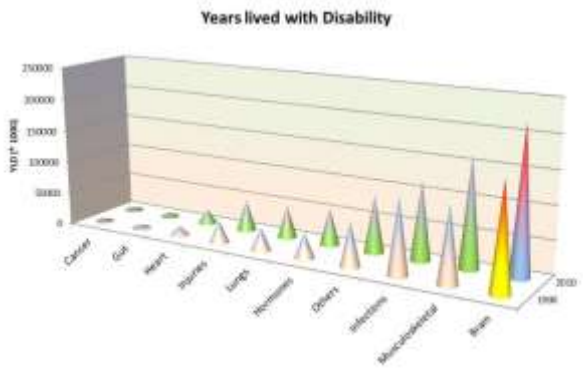

Fig 1: Years lived with disability for different disease categories (data from Vos et al, 2012)

This research is all the more important, as recent analyses have shown that the success rate of developing new drugs, especially in the field of CNS disorders, by the pharmaceutical industry is very low (Kola *et al.*, 2004) and in fact declining (Pammolli *et al.*, 2011), mainly because of lack of efficacy (Kola *et al.*, 2004; Arrowsmith *et al.*, 2013). Given that predictions about the (potential) effectiveness of novel drugs are made on the basis of animal models, this clearly indicates that the current generation of animal models lack translational value.

The problem with animal models

There are several important reasons why animal models for brain disorders, especially in the field of psychiatry have been notoriously poor predictors of therapeutic efficacy. Without wanting to be exhaustive, the main reasons are (Willner, 1984; Ellenbroek, 2003; Geyer *et al.*, 2003; Ellenbroek, 2010; Kaffman *et al.*, 2012):

- The aetiology and pathology of most psychiatric disorders is mostly unknown. As traditional animal models are based on mimicking either the pathology or (ideally) the aetiology, animal models for psychiatric disorders generally have to rely on (at best) presumptive aetiologies or pathologies.
- Many of the symptoms of psychiatric disorders can only be determined after an interview with the patients. Hence, symptoms such as hallucination, delusions, suicidal ideation or feeling of worthlessness cannot be assessed in animals.

- The psychiatric disorders are, by their very nature, heterogeneous. A patient is generally diagnosed on the basis of a checklist of symptoms, and if the patients has a specific number of symptoms (say 5 out of a total of 9), he/she will receive a diagnosis. However, this implies that patients can end up with the same diagnosis, but very different symptoms (see fig 2A).
- Related to this, some disorders can have diametrically opposite symptoms. For instance, in the DSM-V classification of major depression, patients can have either significant weight loss **or gain**, and can suffer from either insomnia or **hypersomnia**. It seems obvious that a single patient is unlikely to suffer from both, and by analogy, an animal model cannot encompass both extremes at the same time either.
- Many psychiatric disorders share similar symptoms. For instance, anhedonia is a common symptom in depression, bipolar disorders and schizophrenia. Likewise, deficits in social communication can occur in schizophrenia, autism and ADHD. This means that we may patients may have different diagnoses but (at least in part) the same symptoms (see fig 2B).
- In relation to this, it is now well established that co-morbidity is very frequent among psychiatric diagnoses. For instance, many patients with depression also suffer from anxiety disorders, a significant proportion of patients with PTSD fulfil the criteria for substance abuse disorders, and a large number of patients with ADHD show autism-like features.

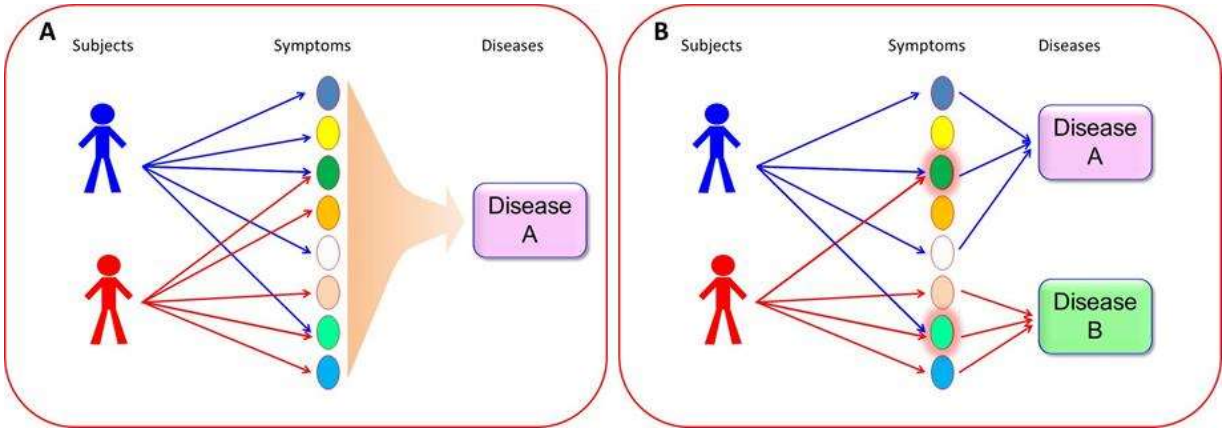

Fig 2: Some problems with psychiatric diagnoses impacting on animal modelling (see also text). A: Subjects can be diagnosed with the same disease, yet show (in part) different symptoms. B: Subjects can be diagnoses with different disorder, yet share (in part) the same symptomatology.

Overall, these reasons make it obvious that in order to improve the translational value of our animal models, we will need to abandon the concept of animal models for specific psychiatric disorders, and rather focus on the signs and symptoms, irrespective of the diagnostic categorization.

Our approach – General Rationale

In line with this last statement, within the Behavioural Neurogenetics Group (BNG), we aim to increase our understanding of the aetiology and neurobiology of symptoms and endophenotypes related to several psychiatric disorders. At present we strongly focus on cognition, drug self-administration, anxiety and social behaviour and communication. As the clinical literature more and more clearly shows that most of these features are strongly influence by both genetic and (early) environmental challenges, we try to mimic this in our models by using several specific genetic rat models alone, or in combining with early (often prenatal) manipulations. In the remainder of this profile, we will describe some of methods that we use in our research approach.

Our approach – Manipulations

As discussed above, our focus is on investigating how genetic and/or environmental challenges alter brain and behaviour in rats. We use several different experimental approaches within our group to induce these alterations. In addition to using drugs to change brain processes, we use both genetic and early environmental manipulations. The most important ones are described below.

Genetic models – The Serotonin transporter (SERT) knock-out rat

Several years ago, in collaboration with professor Edwin Cuppen from the Hubrecht Laboratory (Smits *et al.*, 2006), we were among the first in the world to develop several genetic rats models using the mutagen N-ethyl-N-nitrosourea (ENU). One of the point mutations induced with this technique was a nonsense mutation in the SERT, leading to a premature stopcodon. As a result the homozygous SERT knock-out (KO) rat completely lacks the SERT protein, while the heterozygous rats has approximately 50% of the wild type (WT) levels (Homberg *et al.*, 2007). Since the first identification, we have extensively characterised this animal and have found that the SERT KO rats show signs of depression and anxiety (Olivier *et al.*, 2008), and are hypersensitive to the rewarding properties of cocaine (Homberg *et al.*, 2008) and MDMA (Oakly *et al.*, 2014).

Genetic models – The dopamine D1 mutant rat

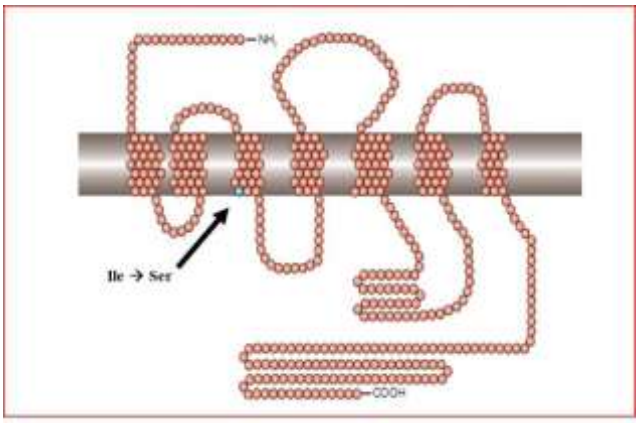

Fig 3: The point mutation in the D1 mutant rats.

In addition to the SERT KO rat, the ENU mutagenesis screen also led to a missense mutation in the dopamine D1 receptor. In contrast to a nonsense mutation (where a premature stopcodon is introduced) a missense mutation leads to a single amino acid change. In our example a isoleucine to serine mutation in the third transmembrane domain (see fig 3).

Although the functional consequences of this mutation have not been as extensively characterized as for the SERT KO, data so far show that rats homozygous for this mutation are insensitive to dopamine D1 agonists and antagonists. Moreover, a first analysis indicates that the D1 mutation may actually led to an internalization of the receptor.

Prenatal environmental manipulation – LPS

Within our lab, we use several different prenatal environmental manipulations. In order to mimic a bacterial infection, we use the compound LPS (Lipopolysaccharide). This compound is a constituent of the outer membrane of gram-negative bacteria, such as E. coli. As a result of this, when injected with LPS, rats (or humans) show a strong immune response. The advantage of LPS over a "true infection" is several fold. First of all, by using LPS, we prevent the risk of an infection spreading throughout the entire colony. Secondly, we have more control over the intensity of the immune response (by using different doses of LPS). Thirdly, we have more control over the timing of the immune response. This is very important, as results from our and other labs has shown that the functional consequences of this immune challenge crucially depends on the developmental stage. For instance, in figure 4 we see that a maternal immune response only affects prepulse inhibition (see below) in the offspring when LPS was given at gestational day (GD) 10 and 11, but not 15 and 16 or 18 and 19).

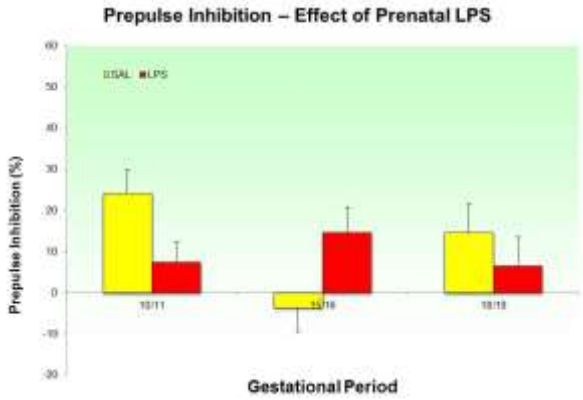

Fig 4: The effects of prenatal exposure to LPS on prepulse inhibition in adulthood.

Clinical studies have shown that a maternal infection increases the risk of offspring to develop, among others, schizophrenia (Brown *et al.*, 2010) and autism (Atladottir *et al.*, 2010), though it is as yet unknown whether this is related to a specific subset of symptoms. In this

respect it is interesting that in a study in which schizophrenic patients with and without exposure to maternal infection were compared, those with a history of maternal infection showed significantly more cognitive symptoms, especially in the executive functioning domain (Brown *et al.*, 2010).

Prenatal environmental manipulation – Valproate

In addition to maternal immune challenges, we often use the model of prenatal valproate administration. Epidemiological evidence shows that prenatal exposure to valproate (an antiepileptic drug) in humans enhances the risk of the offspring to develop autistic like features (Rasalam *et al.*, 2005; Bromley *et al.*, 2008), such as decreased sociability and anxiety. In rats, we find very similar changes in behaviour, when a single dose of valproate is administered at gestational day 12. As shown in figure 5, offspring of rats prenatally treated with 400 mg/kg valproate show an enhanced latency to start eating in a novel open field in the so-called novelty suppressed feeding test (an animal model for anxiety, see below).

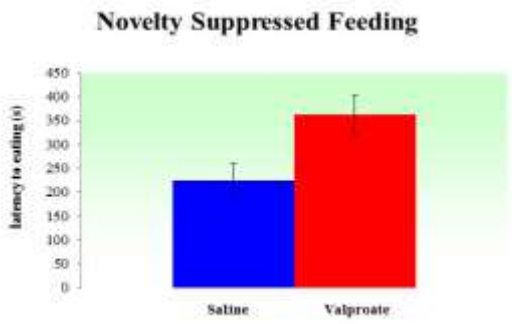

Fig 5: The effects of prenatal exposure to valproate on anxiety in adulthood.

Postnatal environmental manipulation – Maternal Deprivation

In addition to prenatal manipulations, we also use several different (early) postnatal manipulations, including short-term (15 min) repeated and single more prolonged (24 h) mother-pup separations. These latter separations are known as maternal deprivation and are known to strongly affect both the dopaminergic and the serotonergic system (Rots *et al.*, 1996; Ellenbroek *et al.*, 2003; Rentesi *et al.*, 2013).

Our approach – Measuring behaviour

In order to determine the functional consequences of our manipulations, we use a variety of different approaches, and we keep adding new analyses as well. This section is therefore far from complete but it does describe some often used approaches.

Measuring the rewarding properties of drugs of abuse

We use several different measures to assess the rewarding properties of drugs of abuse, including models for self-administration and conditioned place preference.

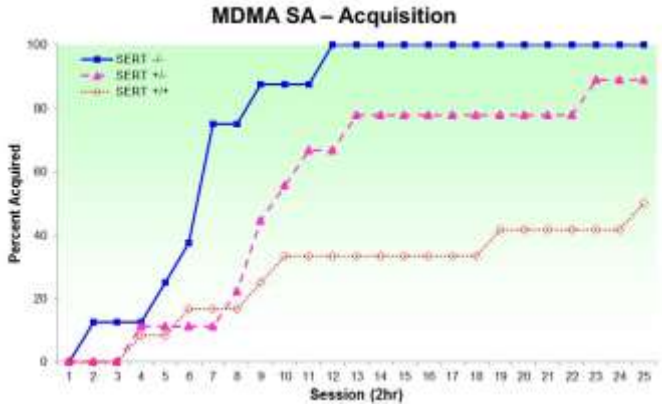

Fig 6: Homozygous (SERT-/-) and heterozygous (SERT+/-) KO rats are more sensitive to the rewarding properties of MDMA than WT (SERT+/+) rats.

In the *self-administration paradigms*, rats are usually implanted with a jugular vein catheter and after a recovery period are placed in a two lever operant chamber. Pressing one lever leads to the administration of a specific amount of a drug directly in the jugular vein, while pressing the other lever has no consequences. Using this approach we can investigate how rapidly rats learn to self-administer specific drugs of abuse (Oakly *et al.*, 2014). As an example, we recently found that SERT KO rats are much more sensitive to the rewarding properties of MDMA (3,4-Methylenedioxy-methamphetamine, "ecstasy").

In addition to looking at the acquisition of self-administration, this paradigm also allows us to directly assess the magnitude of the rewarding properties by using a paradigm known as Progressive Ratio (PR). In this paradigm, the amount of effort the animal needs to exert becomes progressive larger after each reward obtained (Richardson *et al.*, 1996). A typical schedule would be 2 lever presses for the first injection, 4 for the second, followed by 9, 12, 15, 20, 25 etc.

Conditioned place preference (CPP) is another paradigm to assess the rewarding properties of drugs (Tzschentke, 2007). In this paradigm rats are given a free choice between two compartments that differ in floor texture, and wall patterns (and sometime smells). After having established the innate preference for each rat, animals then go through a series of conditioning trials (once daily typically for 6 to 8 days). On the odd days they receive the drug of choice and are placed in the least preferred compartment. On the even days they receive vehicle (placebo) and are placed in the most preferred compartment. On the final test they, the animals are again allowed to explore both compartment and an increase in time spent in the least preferred compartment is indicative of the rewarding properties of the drug.

Measuring social behaviour and communication

Many psychiatric disorders are associated with changes in social behaviour and communication, most notably schizophrenia and autism. As rats are very sociable animals, they are ideally suited for measuring changes in social behaviour. Within our group, we have developed a number of different paradigms to assess social behaviour and social communication.

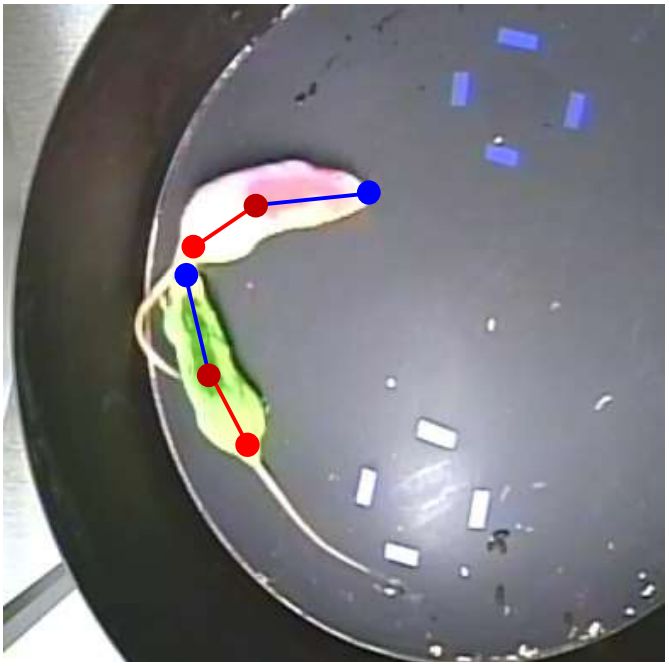

Fig 7: Using specialized software and paints to dye the rats, we can automatically obtain detailed information about the social interactions between rats.

The most direct way of measuring *social interaction* is to place two rats together in an open field. In addition to recording and offline observational analysis (using a pre-defined ethogram, a list of possible behaviours), we use sophisticated video-tracking analysis. The program we use (Ethovision®) allows us to detect three points on a rat (tip of the nose, middle of the body and base of the tail, see fig 7) and by using a special dye to distinguish two rats, we can even assess these points independently for each rat. This has the great advantage of measuring social interaction more objectively than normal visual analysis and might allow for more detailed analyses to uncover normally hidden aspects of social behaviour.

In addition to social interaction, we often use the *social approach/avoidance* paradigm. In this paradigm a rat is placed in an open field three times in brief succession for a short period of time (typically 10 minutes). In the first period, the open field is empty and the rats get a chance to habituate to the novel environment. In the second exposure, the open field contains two cylinders, under cylinder 1 a young rat is placed, while cylinder 2 remains empty. In the third phase, the original young rat remains under cylinder 1, while a novel young rat is placed under cylinder 2. Using this paradigm we can assess whether rats show a preference for a social compared to a non-social stimulus (in phase 2) and whether rats

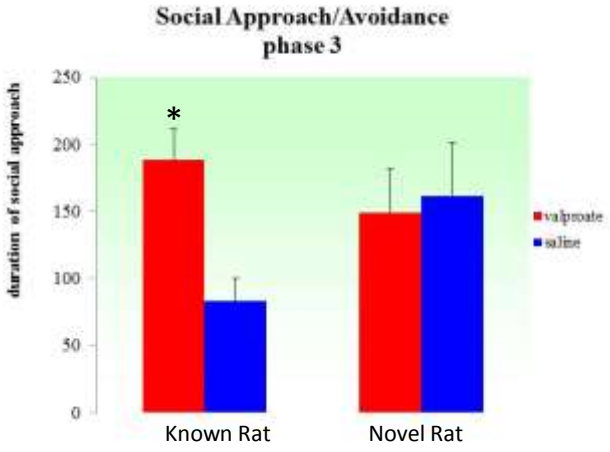

Fig 8: Offspring of mothers prenatally treated with valproate prefer a known to a novel rat in the social approach/avoidance paradigm

prefer a novel vs. a known social stimulus (in phase 3). Figure 8 shows an example of an experiment where we found that offspring of mothers treated prenatally with valproate (400 mg/kg) showed less interest in a novel social stimulus in phase 3.

Recently, we have also stated to investigate different aspects of *social communication*. Rats communicate with each other predominantly using auditory and olfactory signals. Within the auditory domains, most of the important communication takes places in the ultrasonic range (i.e. above 20 kHz) and is therefore inaudible for humans. However, using sophisticated hard- and software, it has been shown that rats distinguish communicate in broadly three different frequency ranges (Wohr *et al.*, 2013):

- 20 – 25 kHz: these calls are especially related to fear, anxiety and stress;
- 30 – 50 kHz: these calls are predominantly made by juvenile pups upon separation from their mothers
- 50 – 60 kHz: these calls are especially related to positive emotional states (social grooming, play behaviour etc.).

Within the olfactory domain, rodents communicate effectively through scent marking. Although this has been reported especially in mice (Arakawa *et al.*, 2008; Wohr *et al.*, 2011), we have recently successfully adapted the paradigm to rats. In this experiment, rats are placed in an open field for a period of 20 minutes of habituation. During this period two round pieces of filter-paper (32 cm in diameter) are placed on opposite sides of the open field. In phase 2, the two pieces of filter paper are replaced with two new one. However, on one of these a small amount of a non-social odour (almond of lemon scent) is placed in the centre, while on the other one a small amount of a social odour (female urine) is placed. The rats are now allowed to freely move around the open field again. After the experiment, the pieces are filter paper are sprayed with ninhydrin spray, which selectively dyes amino acids, to visualize the urine marks the rats have placed on the two pieces of paper. A selective decrease in the number (and surface) of scent marks on the filter paper with the social stimulus can be interpreted as a reduction in social communication/social interest (see fig 9).

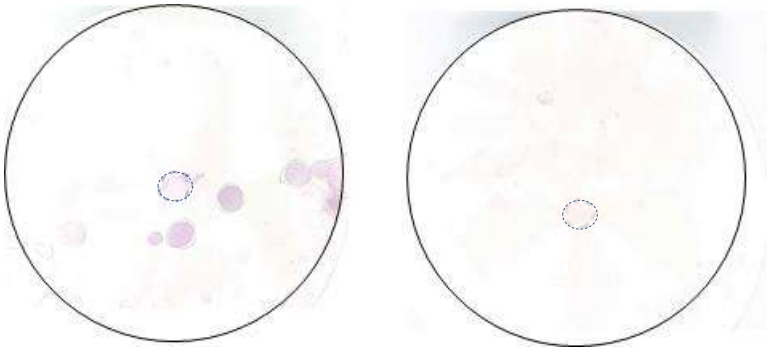

Fig 9: Scent markings (in purple) around a social (left) and a non-social (right) stimulus. The original stimuli are encircled.

Measuring anxiety

Anxiety is a core symptom of a variety of disorders, including post-traumatic stress disorder, schizophrenia, depression and autism. There are a large number of different paradigms in rats

to measure anxiety, most of which are based on a conflict. For instance in the elevated plus maze and home cage emergence test, the rats have a conflict between wanting to explore a novel environment and remaining in a safe place. In addition to these tests, we also use the novelty suppressed feeding paradigm (Olivier *et al.*, 2008). In this paradigm rats are made hungry by taking away their normal food for a few hours. They are then placed along the wall, in a round open field while in the middle a small pellet of food is placed. Again, the rat has a conflict. On the one hand it would prefer to remain close to the wall, as this is the safest place. On the other hand, it is hungry and wants to eat. The latency to start eating is an indication of this conflict. Assuming our manipulation does not affect the level of hunger in the animals, it is directly related to the level of anxiety. As an example, in fig 8, we see that rats prenatally treated with valproate have an increased latency to start eating, indicating that they are more anxious.

Measuring other behaviours

We have given an overview of a number of different paradigms that we routinely use within our group. However, there are many more tasks that we use, and we constantly try to develop novel paradigms based on the literature if we think they will be valuable assets to our repertoire. Students often participate in the development of such paradigms.

Further information and contact details

If you are interested in our research and want to participate, feel free to contact us. You can find more information on our website:

https://behaviouralneurogenetics.com/

In addition, please feel free to email me or give me a call for more information:

Professor Bart Ellenbroek
Behavioural Neurogenetics Group
School of Psychology
Victoria University of Wellington
PO Box 600
Wellington
New Zealand
Tel: +64 – 4 – 463 6159
Fax: +64 – 4 – 463 5402
Email: bart.ellenbroek@vuw.ac.nz

References

Andlin-Sobocki P, Jonsson B, Wittchen HU, *et al.* (2005). Cost of disorders of the brain in Europe. *European Journal of Neurology* 12**:** 1-27.
Arakawa H, Blanchard DC, Arakawa K, *et al.* (2008). Scent marking behavior as an odorant communication in mice. *Neurosci Biobehav Rev* 32**:** 1236-1248.

Arrowsmith J, Miller P (2013). Trial watch: phase II and phase III attrition rates 2011-2012. *Nat Rev Drug Discov* 12**:** 569.
Atladottir HO, Thorsen P, Ostergaard L, *et al.* (2010). Maternal Infection Requiring Hospitalization During Pregnancy and Autism Spectrum Disorders. *Journal Of Autism And Developmental Disorders* 40**:** 1423-1430.
Bromley RL, Mawer G, Clayton-Smith J, *et al.* (2008). Autism spectrum disorders following in utero exposure to antiepileptic drugs. *Neurology* 71**:** 1923-1924.
Brown AS, Derkits EJ (2010). Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. *Am J Psychiatry* 167**:** 261-280.
Ellenbroek B (2010). Schizophrenia: Animal Models. In. *Encyclopedia of Psychopharmacology*, edn. Heidelberg: Springer. p^pp 1181-1186.
Ellenbroek BA (2003). Animal models in the genomic era: possibilities and limitations with special emphasis on schizophrenia. *Behavioural Pharmacology* 14**:** 409-417.
Ellenbroek BA, Riva MA (2003). Early maternal deprivation as an animal model for schizophrenia. *Clinical Neuroscience Research* 3**:** 297-302.
Geyer MA, Ellenbroek B (2003). Animal behavior models of the mechanisms underlying antipsychotic atypicality. *Progress In Neuro Psychopharmacology And Biological Psychiatry* 27**:** 1071-1079.
Gustavsson A, Svensson M, Jacobi F, *et al.* (2011). Cost of disorders of the brain in Europe 2010. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology* 21**:** 718-779.
Homberg JR, De Boer SF, Raaso¸ HS, *et al.* (2008). Adaptations in pre- and postsynaptic 5-HT1A receptor function and cocaine supersensitivity in serotonin transporter knockout rats. *Psychopharmacology* 200**:** 367-380.
Homberg JR, Olivier JDA, Smits BMG, *et al.* (2007). Characterization of the serotonin transporter knockout rat: A selective change in the functioning of the serotonergic system. *Neuroscience* 146**:** 1662-1676.
Kaffman A, Krystal JH (2012). New frontiers in animal research of psychiatric illness. *Methods Mol Biol* 829**:** 3-30.
Kola I, Landis J (2004). Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov* 3**:** 711-715.
Oakly AC, Brox BW, Schenk S, *et al.* (2014). A genetic deletion of the serotonin transporter greatly enhances the reinforcing properties of MDMA in rats. *Molecular Psychiatry* 19**:** 534-535.
Olivier JDA, Van Der Hart MGC, Van Swelm RPL, *et al.* (2008). A study in male and female 5-HT transporter knockout rats: An animal model for anxiety and depression disorders. *Neuroscience* 152**:** 573-584.
Pammolli F, Magazzini L, Riccaboni M (2011). The productivity crisis in pharmaceutical R&D. *Nat Rev Drug Discov* 10**:** 428-438.
Rasalam AD, Hailey H, Williams JH, *et al.* (2005). Characteristics of fetal anticonvulsant syndrome associated autistic disorder. *Dev Med Child Neurol* 47**:** 551-555.
Rentesi G, Antoniou K, Marselos M, *et al.* (2013). Early maternal deprivation-induced modifications in the neurobiological, neurochemical and behavioral profile of adult rats. *Behav Brain Res* 244**:** 29-37.
Richardson NR, Roberts DCS (1996). Progressive ratio schedules in drug self-administration studies in rats: A method to evaluate reinforcing efficacy. *Journal of Neuroscience Methods* 66**:** 1-11.
Rots NY, de JJ, Workel JO, *et al.* (1996). Neonatal maternally deprived rats have as adults elevated basal pituitary-adrenal activity and enhanced susceptibility to apomorphine. *J.Neuroendocrinol.* 8**:** 501-506.

Smits BMG, Mudde JB, van de Belt J, *et al.* (2006). Generation of gene knockouts and mutant models in the laboratory rat by ENU-driven target selected mutagenesis. *Pharmacogenetics and Genomics* 16**:** 159-169.
Tzschentke TM (2007). Measuring reward with the conditioned place preference (CPP) paradigm: Update of the last decade. *Addiction Biology* 12**:** 227-462.
Vos T, Flaxman AD, Naghavi M, *et al.* (2012). Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 380**:** 2163-2196.
Willner P (1984). The validity of animals models of depression. *Psychopharmacology* 83**:** 1-16.
Wohr M, Schwarting RKW (2013). Affective communication in rodents: ultrasonic vocalizations as a tool for research on emotion and motivation. *Cell and Tissue Research* 354**:** 81-97.
Wohr M, Roullet FI, Crawley JN (2011). Reduced scent marking and ultrasonic vocalizations in the BTBR T+tf/J mouse model of autism. *Genes, brain, and behavior* 10**:** 35-43.