PSYCHOLOGY MISCELLANY

No.85 - July 2016

Kevin Brewer

ISSN: 1754-2200

Orsett Psychological Services
PO Box 179
Grays
Essex
RM16 3EW

orsettpsychologicalservices@phonecoop.coop

This document is produced under two principles:

1. All work is sourced to the original authors. The images are all available in the public domain (most from http://commons.wikimedia.org/wiki/Main_Page). You are free to use this document, but, please, quote the source (Kevin Brewer 2016) and do not claim it as you own work.

2. Details of the author are included so that the level of expertise of the writer can be assessed. This compares to documents which are not named and it is not possible to tell if the writer has any knowledge about their subject.

Kevin Brewer BSocSc, MSc

An independent academic psychologist, based in England, who has written extensively on different areas of psychology with an emphasis on the critical stance towards traditional ideas.

A complete listing of his writings at http://psychologywritings.synthasite.com/ and http://kmbpsychology.jottit.com.

CONTENTS

1. THE MOUSE MODEL OF HUMAN DEPRESSION AND ANXIETY, ANIMAL EXPERIMENTS AND DEPRESSION GENERALLY

1.1. MOUSE MODEL

From evolutionary theory has come the belief that biology and behaviour in non-human animals can be studied to gain insight into that of humans. By today, we have the popularity of such studies as in the mouse model of human depression and anxiety.

The high cost of clinical trials for pharmaceutical companies has contributed to this popularity - "pharmaceutical companies and research funding agencies increasingly seek assurance that any specific biological target is relevant to the disease. So, there is a growing emphasis on first obtaining proof that a new chemical entity designed to alter the function of a specific target will do so in a predictable and safe manner" (Cryan and Holmes 2005 p776) [1].

Cryan and Holmes (2005) reported the existence of around eighty different transgenic lines of mice linked to anxiety-related and depression-related behaviour at that time. For example, mice with mutations in neurochemistry related to GABA (gamma-aminobutyric acid type A) have been used in the development of anti-anxiety drugs (anxiolytics) (Cryan and Holmes 2005).

How valid is the mouse model? Put another way, how applicable are the findings to humans?

One key challenge is how to model the symptoms of depression and anxiety in mice. Some symptoms are easier

[1] This fits with what has been called "neurochemical selves" (appendix 1A).

to model than others (table 1.1).

SYMPTOM	MOUSE BEHAVIOUR
Major depression:	
• Anhedonia (diminished interest in pleasure)	• Reduced response to gain positive reward
• Changes in appetite/weight	• Changes in weight after exposure to chronic stress
• Recurrent thoughts of death/suicide	• No equivalent
Anxiety disorders:	
• Phobia	• Avoidance of certain things associated with unpleasant consequences
• Re-experiencing of a traumatic event (PTSD)	• Freezing response to fear-conditioned cues
• Difficulty concentrating	• Sustained attention task difficulties
• Feelings of losing control (panic disorder)	• No equivalent

(After Cryan and Holmes 2005 tables 1 and 2)

Table 1.1 - Examples of symptoms of depression and anxiety and mouse equivalents.

Cryan and Holmes (2005) stated: "It goes without saying that mice are not simply miniature versions of human beings. We can never fully recapitulate human depression or anxiety in the mouse and, indeed, cannot truly know whether a mouse is depressed or feeling anxious. Given the considerable differences in brain anatomy between humans and mice, particularly the greatly elaborated human cerebral cortex and the resultant capacity for processing complex psychological concepts, certain aspects of disease symptomatology, such as low self-esteem, suicidal ideation or 'fear of going crazy', are impossible to model in mice" (p779) [2].

However, they go on to emphasise the common sub-cortical brain physiology, and that "there are many fundamental physiological and behavioural responses that have been evolutionarily conserved between species" (Cryan and Holmes 2005 p779) [3].

McKinney and Bunney (1969) proposed that a valid

[2] Generally the mouse model cannot capture the personal experience of depression including its cultural manifestations (appendix 1B).

[3] The central nervous system evolved from a common ancestor (appendix 1H).

animal model should be "'reasonably analogous' to the human disorder in its manifestations or symptomatology; causes a behavioural change that can be monitored objectively; produces behavioural changes that are reversed by the same treatment modalities that are effective in humans; and is reproducible between investigators" (Cryan and Holmes 2005 pp779-780).

In particular, these sort of criteria can be applied to stress, which is known to be a predisposing factor in major depression. Chronic stress is induced in mice in experiments with procedure like the forced swim test (FST), where an animal is placed in an inescapable cylinder filled with water. Initially, they try to escape and then become immobile. The amount of time before immobility is measured, and anti-depressants increase the length of escape-directed behaviours, for example (Cryan and Holmes 2005). But is the immobility the same as depression?

Likewise, the tail suspension test (TST) involves hanging the mouse by its tail, and immobility occurs after "futile struggling" (Cryan and Holmes 2005) [4].

Mild stress is induced by food deprivation or constant lighting, and mice exposed to these conditions in the long-term show a deterioration in coat condition through lack and grooming, and this "phenomenon has been considered analogous to the observation that depressed patients execute even the smallest tasks with great effort, often leading to poor personal hygiene" (Cryan and Holmes 2005 p781).

An alternative is "pleasure-seeking", which is the willingness to work for a reward, and a reduction in the motivation here can be compared to anhedonia (the loss of pleasure) in major depression.

In relation to modelling anxiety disorders, Cryan and Holmes (2005) noted that "the state of anxiety is a normal, adaptive response to danger, and as such is distinct from depression, which is by definition pathological. Working from the assumption that the same neurobiological systems mediate both normal and abnormal anxiety, many animal models of anxiety disorders have therefore tapped knowledge of the natural behavioural patterns of rats and mice to develop ethologically based behavioural tasks" (pp781-782). The most popular procedure is the exploratory approach-avoidance task. Mice tend to explore new environments (approach), but keep away from well-lit, open spaces (avoidance). So, for example, a box is divided into a dark and light part, and the willingness to investigate the lit area is taken as a

[4] Amphetamine withdrawal increases mouse immobility in the FST and the TST (Cryan and Holmes 2005).

measure of anxiety-like behaviour. Mice given the tranquilliser diazepam, for instance, are more willing to explore the aversive areas (Cryan and Holmes 2005).

But Cryan and Holmes (2005) warned that "because these tests are based on the interplay between approach and avoidance, they do not satisfactorily dissociate decreased anxiety-related avoidance from increased novelty-seeking or impulsivity- related approach behaviour, as both will manifest as increased time spent in the novel, aversive area. Such ambiguity could be confounding in cases where a manipulation has potential effects on both emotion and novelty-seeking.. Behavioural performance on exploration-based tests is also heavily reliant on intact sensory and motor function in the mouse" (p782).

The main alternative is the punished conflict test (eg: Vogels et al 1971). Water-deprived animals were provided with a drinking spout, but received a mild electric shock after a certain number of sips. Thus, there is a conflict between the motivation to drink and the fear of the punishment with "anxiety" animals drinking less. Tranquillisers encourage more drinking despite the punishment. This type of test has "generally fallen out of favour" (Cryan and Holmes 2005).

Anxiety disorders as a category covers a number of different conditions, including phobias, panic disorder, and post-traumatic stress disorder (PTSD), and modelling each of these is challenging.

Studies have tried to model the effects of experiences for newborns on their adult behaviour. Meaney (2001), for example, reared pups with poor maternal care (or none at all), and they developed behavioural abnormalities relevant to anxiety and depression as adults. While mice genetically engineered with serotonin deficient in early life showed similar behaviours, but not if the deficiency occurred in adulthood (Gross et al 2002).

Cryan and Holmes (2005) ended with a general word of warning about the mouse model and the genetics of anxiety and depression (appendix 1C):

> Not surprisingly, fear and anxiety-like behaviours in mice also seem to be polygenic and epistatic in nature. Therefore, studies in mutant mice are not going to discover the "gene for anxiety" or the 'gene for depression'. More realistically, mouse behavioural research can provide valid model systems to explore the role of a given gene in molecular pathways that influence depression- and anxiety-related behaviours. However, although mutant mice are often discussed as "genetic models", they are in actuality rarely used to model directly the role of a human candidate gene variant in depression or anxiety. Rather, genetically

> modified animals have largely been used as a means to study the consequences of altering a specific gene product much in the same way as a highly selective pharmacological agent would activate or antagonise the function of a protein such as a receptor (p787).

1.2. ANIMAL EXPERIMENTS TODAY

"The genomics era opened a door to understanding genetic changes in susceptibility to diseases, such as single nucleotide polymorphisms, gene copy number variations, and gene deletions and insertions... The subsequent explosion of related 'omics' approaches, including transcriptomics, metabolomics, and proteomics, have provided more details of how gene regulation and protein production are implicated in human disease mechanisms" (Langley et al 2015 pA268) [5]. The environment is also relevant to many human illnesses, as seen in epigenomics ("the study of changes in gene activity not attributable to DNA sequence alterations"; Langley et al 2015).

Langley et al (2015) argued that these developments in the 21st century offer an opportunity for a new paradigm for researching human diseases (and behaviour) that does not include animal experiments [6].

The idea of adverse outcome pathways (AOPs) (figure 1.1) describes "the sequence of changes between the molecular initiating event (eg: a chemical binds to a cell receptor) and adverse outcomes at the molecular, cellular, organ, organism, and population levels" (Langley et al 2015 pA268). This allows for the study of biological mechanisms in a larger framework rather than in terms of isolated factors (which encourage animal experiments). In other words, developing a big picture of human diseases (and behaviour).

Russell and Burch (1959) proposed the "3Rs" model as a way to perform experiments on animals in a more "humane" way. The "3Rs" are:

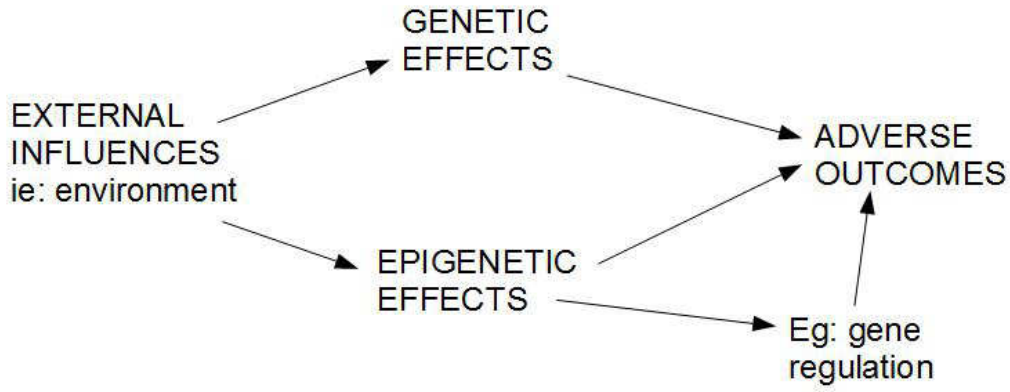
- Replacement - find an alternative method. Russell and Burch (1959) distinguished between complete Replacement and relative Replacement, where the use of animals is still necessary.

- Reduction - aiming to use the smallest number of animals if Replacement is not possible.

---

[5] The use of transgenic and "knock-out" mice has grown (appendix 1E), for instance as in example in appendix 1F. Transgenic animals have a new gene, either a human one or an extra copy of a gene, while knockout animals have a gene "affectively deleted" (Holmes and Crawley 2000).

[6] This is growing evidence that animal experiments are confounded by the laboratory conditions in which the animals are kept, which are usually ignored (appendix 1G).



(Based on Langley et al 2015 figure 1 pA269)

Figure 1.1 - Adverse outcome pathways.

- Refinement - refining experimental procedures to minimise, alleviate, or eliminate pain and suffering for the experimental animals (Venerosi and Vitale 2007).

Referring to the study of epilepsy, Jones et al (2016) stated: "There is no doubt that a best alternative to animal models would be to conduct basic functional and mechanistic studies in a completely homologous 'model' ie: living human brain tissue. This is becoming increasingly possible using tissue resected during neurosurgery for refractory epilepsy" (p222). The researchers then discussed the issues related to the use of human brain tissue. Though they were talking in relation to epilepsy, the points are relevant to any such study looking for alternatives to the use of animals.

Human brain slices ("in vitro" study) includes the study of cells and their response to electrical stimulation, and this requires tissue that is removed with minimal damage by the neurosurgeon, and that can be stored effectively. There are advancements all the time in these processes (Jones et al 2016).

The greatest advantage of "in vitro" study over "in vivo" (ie: studying the brain in a living individual) is the "number of techniques and manipulations that would not be possible in vivo" (Jones et al 2016 p224) - eg: recording high frequency network oscillations (HFOs) in cells, or localised electrical activity ("micro-seizures") (Jones et al 2016).

"A major drawback to the use of living human tissue obtained through brain resection is the limited and often sporadic availability of tissue" (Jones et al 2016).

Talking specifically about Alzheimer's disease (AD),

Pistollato et al (2015) pointed out that over 100 potential drugs that have advanced to human trials have failed since 1998. This is translational failure [7], where treatments tested and modelled on animals do not work with humans.

Pistollato et al (2015) argued that "animal models consistently fail to accurately recapitulate human AD causes, complex molecular and cellular dynamics, clinical manifestations, and drug responses. This is primarily due to anatomical, biochemical, and physiological as well as genetic and epigenetic interspecies differences between animals and humans" (p858). Thus, the authors proposed human-based methods with cellular and tissue models, medical technology, and epidemiological data. The latter, for example, includes lifestyle and environmental risk factors, like diet, smoking, socio-economic status, and air pollution. This is the "exposome" - "the totality of environmental exposures from gestation onwards" (Pistollato et al 2015).

While neuroimaging (or "in vivo" imaging) of live patients is developing in technology and expertise (eg: 3D reconstruction of neuronal networks in the brain - human connectomics).

Pistollato et al (2015) summed up: "combining data derived from a wide range of studies, also accounting for neuropsychological/ cognitivity tests, neuroimaging, the analysis of patient-derived CSF [cerebro-spinal fluid]- and plasma-related biomarkers, together with computational models and high-throughput readouts applied to patient-derived cell-based models to assess signalling pathways, posttranslational, translational, and transcriptional events, represent an invaluable and more reliable strategy to better understand AD pathology, predict long-term sequelae, and develop successful treatments" (p864).

1.3. APPENDIX 1A - NEUROCHEMICAL SELVES

Rose (2003) asked how we become "neurochemical selves" - ie: "the belief that variations in neurochemistry underlie variations in thought, mood and behaviour, and that these can be modulated with drugs" (p46). Key to the answer is the increasing dependence on commercially produced pharmaceuticals in what he called "psychopharmacological" societies. "In such societies, in many different contexts, in different ways, in relation to a variety of problems, by doctors, psychiatrists,

[7] Mice physiology, for example, is different to human (eg: mice heartbeat is ten times higher) (Bogeholz et al 2014). Venerosi and Vitale (2007) suggested that there were "some gaps in the face validity of the rodent behaviour that are difficult to fill up: for example, many behavioural tasks which involve visual and language competencies are not modelled in rodent models" (p10).

parents and by ourselves, human subjective capacities have come to be routinely re-shaped by psychiatric drugs" (Rose 2003 p46).

Taking the example of the UK, figures show the increase in the use and prescription of psychotropic drugs. For example, tranquilliser (minor and major) prescriptions increased four-fold between 1960 and 1980, and anti-depressants by 200% between 1980 and 2000. In the USA, prescription of SSRI [8] anti-depressants (eg: Prozac) rose over 1300% in the decade of the 1990s (Rose 2003).

A number of explanations have been proposed for the increase in prescriptions:

i) There is a real increase in mental disorders in recent years due to social and economic factors.

ii) Campaigns to increase awareness of mental disorders by pharmaceutical companies, professionals, and patient groups. This includes the "co-production of the disease, the diagnosis and the treatment" (Rose 2003). Pharmaceutical companies, directly and indirectly, emphasise the misery of undiagnosed conditions, particularly when a treatment is available, with the aim of shaping "fears and anxieties into a clinical form" (Rose 2003).

iii) A new way of conceptualising the self in the West, "involving the obligation of freedom: responsibility, choice and active self-fulfilment. The continual incitements to action, to choice, to self-realisation and self improvement act as a norm in relation to which individuals govern themselves and are governed by others, and against which differences are judged as pathologies" (Rose 2003 p54).

This can be linked to Rose's (2003) concept of "somatic individuality" - "the tendency to define key aspects of one's individuality in bodily terms, that is to say to think of oneself as 'embodied', and to understand that body in the language of contemporary biomedicine. To be a 'somatic' individual, in this sense, is to code one's hopes and fears in terms of this biomedical body, and to try to reform, cure or improve oneself by acting on that body [9]. At one end of the

[8] Selective serotonin reuptake inhibitor (SSRI).

[9] For example, individuals with depression can struggle with concentration and decision-making. Are these cognitive problems an offshoot of the mood disorder & so will clear up when the depression is treated or separate to the depressive condition? Whatever the answer, "interest in cognitive drugs for people with depression is building as more & more anti-depressants become available in cheap, generic forms and pharmaceutical companies seek to carve out niches for newer, more expensive offerings" (Ledford 2016 p17). Cognitive dysfunction as a treatable symptom of depression would appeal to them.

spectrum this involved reshaping the visible body, through diet, exercise, and tattooing. At the other end, it involves understanding troubles and desires in terms of the interior 'organic' functioning of the body, and seeking to reshape that - usually by pharmacological interventions. While discontents might previously have been mapped onto a psychological space - the space of neurosis, repression, psychological trauma - they are now mapped upon the body itself, or one particular organ of the body - the brain" (Rose 2003 p54).

These explanations can be seen as working together in many ways. Rose (2003) summed up:

> By the 1990s a fundamental shift had occurred in psychiatric thought and practice. No matter that there was little firm evidence to link variations in neurotransmitter functioning to symptoms of depression or any other mental disorder in the living brains of unmedicated patients - although many researchers are seeking such evidence and occasional papers announce that it has been found. And no matter that most of the new smart drugs are no more effective than their dirty predecessors for moderate or severe depression - they are favoured because they are claimed to be safer, and to have fewer 'unwanted effects'. A way of thinking has taken shape, and a growing proportion of psychiatrists find it difficult to think otherwise. In this way of thinking, all explanations of mental pathology must 'pass through' the brain and its neurochemistry - neurones, synapses, membranes, receptors, ion channels, neurotransmitters, enzymes, etc (p57).

This, in a nutshell, is how we become "neurochemical selves".

1.4. APPENDIX 1B - EXPERIENCE OF DEPRESSION

It is important to emphasise that "depression is not a singular, universal phenomenon, and that various forms of dysphoria are culturally conceptualised and experienced in diverse ways" (Olszewska 2015). Working with refugee Afghan poets in Iran, Olszewska (2015) was clear about the terminology: "When I refer to depression, melancholy, and dysphoria in this article, I refer not to a singular mental disorder with clear diagnostic criteria, but to a constellation of subjective experiences and cultural discourses describing negative affects, some of which are pathologised and others of which, on the contrary, are positively valorised in contemporary Iran" (p85).

The feelings of "depression" were grounded in the experience of being a refugee, including harassment and

racism, the "limbo" of their status, socio-economic conditions, and personal trauma. One "depressed" poet summed up the experience:

> I think that being a refugee [*mohājerat*] really has an effect. It's a feeling inside a person that he can't tell anyone about, and nobody can understand it. You live in this society, but you don't belong to this society. This feeling of not-belonging always exists in my mind, its pulse keeps beating. On the other hand, you don't remember anything from your own past. You say Afghanistan, but which Afghanistan? You have no background, you have nothing from your own country. Except war – and even that you hear on the news – war, conflict, and so on, and so on. And your childhood memories are marked by *mohājerat*, by labour, sometimes by insults, sometimes by restrictions – in particular by restrictions. All these things in a way lead one to take refuge in something, something is created inside you (Olszewska 2015 p90).

Olszewska (2015) described the functions of the poetry as individual catharsis, and as testimony. She summed up: "we neglect a profound dimension of human experience if we accept wholesale the 'negative model' that came to dominate in the twentieth century – that emotional suffering is a purposeless annoyance – at the expense of older religious and philosophical traditions that see it as a vehicle for spiritual growth and greater insight. Even for refugees living in a state of poverty and marginality, it can be the key to great imagination, self-improvement, solidarity, and creativity" (Olszewska 2015 pp100-101).

1.5. APPENDIX 1C - GENES AND DEPRESSION

Sullivan (2015) admitted that "of all complex human illnesses, major depressive disorder (MDD) has arguably proved the trickiest to understand". Part of the reason is that the same MDD symptoms may have a genetic basis for some individuals, but an environmental cause for others [10]. This is the heterogeneity of the disorder, and it is a challenge to those seeking the genetic basis.

A recent study (CONVERGE consortium 2015 [11]) decided to concentrate on the more-severe cases of MDD, as it was assumed that these were more likely to be genetic. This was manifest in the focus on female sufferers [12], who had

[10] "The current consensus is that depression may be a collection of partly distinct diseases, with overlapping causal pathways" (CONVERGE consortium 2015 p588).

[11] China, Oxford and Virginia Commonwealth University Experimental Research on Genetic Epidemiology (CONVERGE).

[12] 5303 cases in fifty-eight hospitals vs 5337 controls.

experienced two or more episodes that required hospitalisation [13] in China [14]. Sullivan (2015) questioned whether this sample was more heterogeneous than previous studies.

Comparison of the cases and controls led to two genetic regions being identified (close to SIRT1 [15] and LHPP genes on chromosome 10). Previous studies in Europe and the USA have not found these genetic regions associated with MDD (Sullivan 2015).

Concentrating of particular populations is one source of study of genetics. For example, the "Plain" communities in North America (eg: Amish, Mennonite) live in small, isolated groups separate from modern society. Individuals tend to marry within their community, which gives a high chance of genetic relatedness, a limited "gene pool", and high rates of certain genetic conditions. Many conditions are recessive which means that both parents must carry the gene for the offspring to show the disease (Strauss 2015) (figure 1.2).

For example, a mutation in a gene called KCNH7, which encodes proteins in the cell membrane related to the flow of potassium ions, has been linked to bipolar disease from work with Amish families (Strauss et al 2014).

PARENT A	PARENT B	OFFSPRING
RR	NN	All RN (carrier)
RN	NN	1/2 RN; 1/2 NN (normal)
RN	RN	1/4 RR (affected); RN; 1/4 NN
RN	RR	1/2 RR; 1/2 RN

R = recessive gene; N = normal gene

Figure 1.2 - Example of recessive gene and inheritance.

There is also growing interest in the source of such genes. Studies of the human (Homo sapiens or anatomically modern human; AMH) genome have found that hominins relatives are a small proportion (eg: 2-4% Neanderthal;

[13] Mean number of episodes was 5.6.

[14] MDD is recorded as lower in China than the USA, for example (3.6 vs 16.2% respectively) because of a reluctance to report the disorder, so hospitalised cases will be the most severe (CONVERGE consortium 2015).

[15] Links to this gene could implicate abnormalities in mitachondria (ie: energy-producing centres in cells) (CONVERGE consortium 2015).

Green et al 2010) [16], but the "sequences may have had an outsize effect on human biology" (Callaway 2015). For example, using anonymous genome data and medical records of 28 000 hospital patients in the USA [17], Simonti et al (2016) compared individuals with a particular Neanderthal gene version and those with a Homo sapiens version of the same gene. The former version was linked to a number of conditions like nicotine addiction and osteoporosis, and depression and obesity (Callaway 2015) [18].

Simonti et al (2016) stated: "Depression risk in modern human populations is influenced by sunlight exposure, which differs between high and low latitudes, and we found enrichment of circadian clock genes near the Neanderthal alleles that contribute most to this association... It is possible that some Neanderthal alleles provided a benefit in early AMH populations as they moved out of Africa, but have become detrimental in modern Western environments" (pp739; 741).

Geneticist Sriram Sankararaman noted: "Exactly how these genes affected Neantherdals themselves is not always clear. This doesn't mean that Neanderthals were depressed..." (quoted in Gibbons 2016).

1.5.1. Inheriting Suicidal Tendencies

Reardon (2015) observed: "Suicide is a puzzle. Less than 10% of people with depression attempt suicide, and about 10% of those who kill themselves have never been diagnosed with any mental health conditions" (p19) [19].

However, the biological relatives of adopted children who kill themselves are more likely to commit suicide than the general population (Brent and Mann 2005). This suggests a genetic influence to suicide [20] [21]. It is unlikely that there is a gene or genes for suicide, so an inherited characteristic may be involved. One possibility relates to impulsivity and judgment. Hoehne et al (2015) found that close relatives of suicide completers were more impulsive in a gambling game than controls.

The heritability of cognitive deficits represent

---

[16] Neanderthals (Homo neanderthalensis) diverged from AMHs (Homo sapiens) between 750 000 and 500 000 years ago (Kuhlwilm et al 2016). This means that the two groups are classed as separate, but that did not stop mating (and gene flow).

[17] Electronic Medical Records and Genomics (eMERGE) Network in nine cities in the USA.

[18] Also found gene variants related to blood clots, skin lesions, and metabolising of carbohydrates in the gut, as well as other studies showing positive changes to the immune system (Gibbons 2016).

[19] Self poisoning is a major means of suicide (appendix 1D).

[20] The heritability of suicidal behaviour is estimated at 50% (Hoehne et al 2015).

[21] Niculescu et al (2015) identified six genes whose expression is different in individuals who kill themselves.

endophenotypes of suicidal acts - ie: a stepping stone between the observable characteristic and the underlying gene). Hoehne et al (2015) concentrated on deficits in cognitive inhibition and decision-making.

They recruited seventeen biological first-degree relatives of depressed suicide completers (with no history of suicide themselves), eighteen relatives of individuals with major depression but no history of suicide, and nineteen healthy controls with no family history of suicide or mental disorders.

All the participants performed a number of neuropsychological tests including the Stroop Colour Test (Stroop 1935) to measure cognitive inhibition, and the Iowa Gambling Task (IGT) (Bechara et al 1999) for decision-making. The Stroop Test involves naming the colour of the ink of words, but the interference condition uses names of colours (eg: the word "red" written in blue ink). The IGT is a computerised game where participants choose from different packs of cards in order to win points, but half the packs give small gains or losses (safe decision - net gain) and the other half large gains or losses (risky decision - net loss). The participants are not told these rules, but must learn them over a number of rounds.

The suicide relatives made significantly more errors on the Stroop Test (ie: saying word and not colour of ink), and showed less learning of the rules of the IGT leading to poorer decision-making (though this was subtle). The former provided evidence of sensitivity to interference, which Jollant et al (2011) saw as part of the cognitive vulnerability to suicidal acts.

1.6. APPENDIX 1D - SELF-POISONING AND ADOLESCENTS

Self-poisoning by adolescents is the most common cause of death for that age group, as well as a major form of deliberate self-harm (DSH) (Tyrrell et al 2016).

Studies tend to use admissions or visits to hospital emergency departments to calculate the incidence of such behaviour, but this misses cases that do not present at hospital. It is also difficult to determine how representative the cases are of the age group (Tyrrell et al 2016). An alternative source of data is official poisoning records (eg: collected by the National Poisons Information Centre in the UK), but they also have gaps. For example, in the UK, the general public cannot contact the National Poisons Information Centre, only clinicians, and "so will tend to include more severe cases or those from rarer substances" (Tyrrell et al 2016). It is also important to distinguish between intentional (eg: DSH) from unintentional (eg: alcohol-related) poisonings.

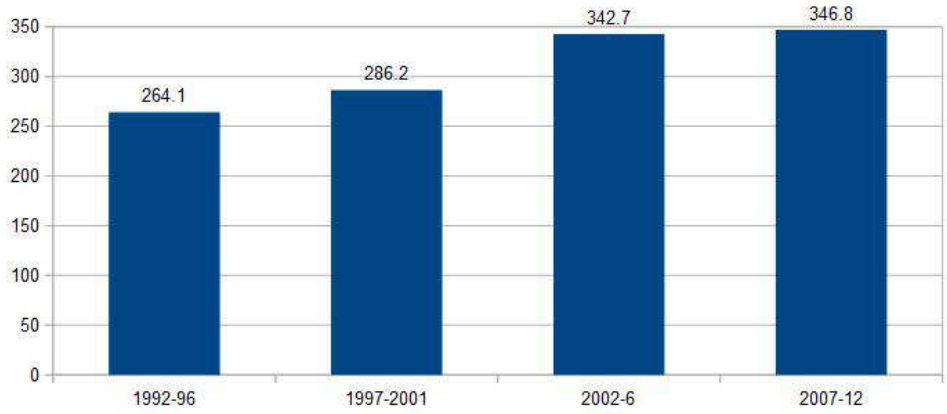
Bearing these limitations in mind, Tyrrell et al

(2016) used data from The Health Improvement Network (THIN) in the UK, which is a database from general practice patient records. This gave a cohort of over 1.3 million 10-17 year-olds for the study period 1992 to 2012.

Poisonings were categorised as intentional, unintentional, unknown intent, or alcohol-related based on details recorded by the general practitioner (GP). "Alcohol related poisonings... were categorised separately as classifying their intent as complex and, as such they are not accurately described by the other three intent categories" (Tyrrell et al 2016).

The incidence rate (IR) was calculated per 100 000 person-years (PY), which is "the total number of events divided by the total person time at risk". Overall, 15 647 individuals had at least one poisoning in the study period.

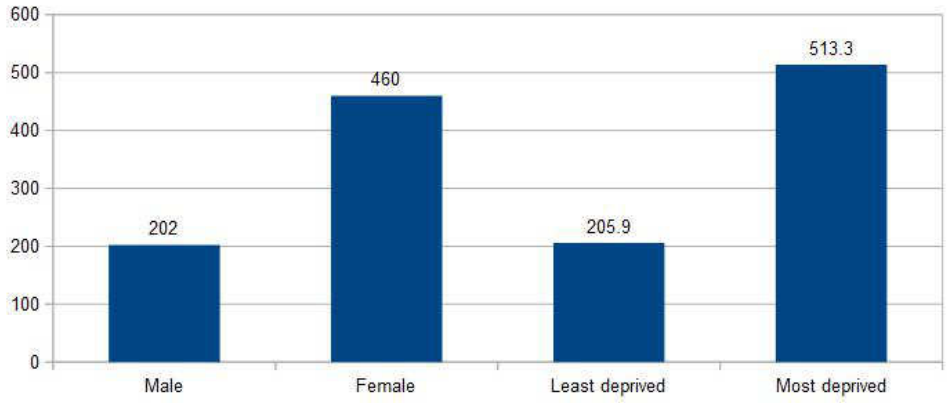
The study period was divided into four parts for time comparison, and there was a 27% increase from the beginning to the end (ie: IR of 264 in 1992-6 and 347 in 2007-12) (figure 1.3). Intentional and alcohol-related poisonings showed the largest increase.



(Data from Tyrrell et al 2016 table 1)

Figure 1.3 - Overall incidence rate (per 100 000 person years) for parts of study period.

The IR was over twice as high for females over males, and overall incidence increased with lower socio-economic status/socio-economic deprivation (figure 1.4). The largest increase in the study period was intentional poisonings by 16-17 year-old females and alcohol-related poisonings by 15-16 year-olds females, but unintentional poisonings for females 12-17 years old decreased. The rates for males were stable.



(Data from Tyrrell et al 2016 table 1)

Figure 1.4 - Overall incidence rate (per 100 000 person years) based on gender, and socio-economic deprivation.

Tyrrell et al (2016) asked whether their data "reflects real changes, increased health seeking behaviour or changes in GP coding practices or popular trends, such as clinicians perceiving intentional poisonings as more frequent and therefore recording events as such". They answered: "The simultaneous reduction in unintentional poisonings among females alongside the increase in intentional events suggests this may be a partial explanation. However, the increase in intentional poisonings was much larger than the reduction in unintentional poisonings, indicating that this is only a partial explanation" (Tyrrell et al 2016).

In terms of real change, Hawton et al (2003) found an increase in DSH (including poisonings) among 12-18 year-old females in Oxford between 1990 and 2000. The increase in alcohol-related poisonings among females fits with other studies showing increased binge drinking among female adolescents in the 21st century (eg: Healey et al 2014). Tyrrell et al (2016) stated: "One potential explanation for the increase in alcohol poisonings over time is increased availability, with the relative affordability of alcohol in the UK increasing steadily between 1980 and 2012, licensing hours having increased since 2003 and numbers of outlets increasing alongside alcohol harm".

Though THIN database is representative of the UK demographically and includes a complete picture of an individual's medical history, there is concern that DSH is under-recorded (eg: by up to 30% compared to hospital admissions data; Thomas et al 2013). The accuracy of GP reporting can also be a limitation (Tyrrell et al 2016).

1.7. APPENDIX 1E - TRANSGENIC ANIMALS

The genes of animals have been altered in the past by selective breeding. This approach is limited to individuals of the same species, it takes time, and "fate has a major role in determining the results obtained" (Branchi and Alleva 2007). Transgenic techniques are quick, and allow the focus on specific genes.

Specifically, the transgenic mouse allows (Branchi and Alleva 2007):

i) Targeting of the gene of interest which limits the role of fate.

ii) A specified combination of genes in one generation (approximately two months) [22].

iii) Flexibility, in the sense of transferring genetic material between species.

iv) The whole process for low cost.

On the other side, the main problems are ethical and practical. The latter includes transgenic mice escaping the experimental facilities and mating in the wild. The ethical issues include pain to the animal [23] and unexpected side-effects. The "alterations induced by the genetic manipulation may lead also to less subtle and less evident effects such as a modification of the response to stress or of the pain threshold. For instance, iper- or ipo-algesia (respectively increased or reduced sensitivity to pain), motor or sensory weakness, low social interaction capacities, etc., can make the animal especially susceptible to stressful conditions" (Branchi and Alleva 2007 p3).

Branchi and Alleva (2007) also raise concerns about

---

[22] "In general, regional development of the rodent brain proceeds on a timeline of days versus weeks to months in humans, although gross development of the brains of rodents and human is similar" (Venerosi and Vitale 2007 p9).

[23] Establishing that animals experience pain is not without controversy. For example, Elwood and Adams (2015) felt that shore crabs experience pain because of their reactions to electric shocks. Stevens et al (2016) challenged this conclusion, and argued that the electrical shocks caused physiological stress which is not pain. They said that pain is a conscious experience and it could be over-interpreted in species like such crabs.

Elwood (2016) responded that the original research did not conclude that crabs experienced pain as "absolute proof of pain is not possible in any animal" (Elwood and Adams 2015). But criteria have been suggested "that should be fulfilled before the possibility of pain in an animal taxon is entertained" (Elwood 2016), and the crabs fulfilled "the criteria expected of pain" (Elwood and Adams 2015). "Physiological stress has been put forward as one of many criteria of pain. It is not the same as pain..." (Elwood 2016). Furthermore, Elwood (2016) said: "I cannot agree that we should reject pain as a possibility until conscious processing is shown. To do so would be an invitation to withdraw protection of animals in a wide variety of situations and that would be highly retrogressive" (p1).

the interpretations of findings from studies with genetically modified animals:

- "Special attention should be paid to accurately control the environment in which the modified gene is expressed in order to control for possible pleiotropic effects: same gene modifications produces markedly different results when expressed in different mouse strains, and thus in different genetic backgrounds" (Branchi and Alleva 2007 p4).

- "The time of expression of the gene of interest should be monitored to avoid misinterpretations. For instance, changes rising as a consequence of the absence of the gene of interest at early ontogenic phase may heavily interfere with the normal developmental program, leading to physiological and/or behavioural modification that can be erroneously interpreted as consequences of the lack of the gene at adulthood..." (Branchi and Alleva 2007 p4).

- ..."the insertion of genes adjacent to the one of interest, the so-called 'flanking' genes, which can introduce unexpected variability hard to control" (Branchi and Alleva 2007 p4).

- The mouse train used - eg: the C57B6 strain has congenital deafness, and the FVB mouse retinal degeneration.

1.7.1. Gene Editing

Transgenic mice are part of the many changes in the work with genomes (like gene editing as well as in humans).

The possibility of genome editing was increased by Jinek et al's (2012) introduction of CRISPR-Cas9 [24], which made DNA editing easier [25]. Previously, techniques based on zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) were being used (Corbyn 2015).

All three techniques are based on enzymes called programmable nucleases that can be made to cleave to any nucleotide sequence, and this causes the cell to repair the break in the DNA with two possible options - non-homologous end joining or homologous recombination. The

[24] Clustered regularly interspaced short palindromic repeat - CRISPR associated (CRISPR-Cas).

[25] Gewin (2015) referred to "the CRISPR craze" "now in full swing, and the platform's ability to treat a range of diseases - from severe combined immunodeficiency (SCID) to muscular dystrophy - is being put to the test" (pS10).

former is used for gene "knock-out", and the gene can no longer be translated. Homology-directed repair or homologous recombination allows gene "knock-in", and an altered or inserted gene is the upshot (Corbyn 2015).

ZFNs and TALENs use a DNA-cutting enzyme called "Fokl" (from the bacterium Flavobacterium okeanokoites), while CRISPR-Cas9 uses the enzyme Cas9 (part of the adaptive immune system of bacteria). Other CRISPR enzymes have subsequently been detailed (Corbyn 2015).

Other than the different strengths and weaknesses of the three techniques for gene editing, there are wider issues to consider, including:

i) The ownership of intellectual property (Corbyn 2015).

ii) The ethics of human germline editing - Doudna (2015) summed up: "When genomic changes are made in fully developed non-reproductive cells, they affect only the treated organism or person and do not become heritable. But if genomic changes are made to germ cells such as those that develop into eggs or sperm, or to developing embryos, the changes are incorporated into the cells of the organism that grow from them - including its own germ cells. Hence the changes can be passed on to future generations" (pS6).

On the other hand, Church (2015) wanted to emphasise the benefits rather than the risks - "germline editing is unlikely to cause dangerous, unforeseen mutations. In the best case scenario so far, CRISPR-Cas9 seems capable of less than 1 error per 300 trillion base pairs..." (pS7).

The benefits can be seen in the example of HIV/AIDS. Certain individuals have been found with a mutation in their cell surface protein which means the HIV cannot attach to the cell to enter it. The upshot is that these individuals are "essentially resistant to most commonly transmitted strain of HIV" (Eisenstein 2015). Gene editing of this cell surface protein mutation could be used with HIV-positive individuals. Research is ongoing (Eisenstein 2015).

iii) The "delivery hurdle" - "One strategy is to extract cells, edit their genomes, check that there are no unintended genetic changes, known as 'off-target effects', and then reintroduce them to the body so that they can operate as healthy cells" (Gewin 2015 pS10).

iv) Monogenic diseases (ie: linked to a single gene) are the "low-hanging fruit", but these are still a challenge, let alone diseases with genetic complexity (Gewin 2015).

v) The challenge of epigenetics - "Where the genetic

code offers simplicity and stability, with its four bases of DNA, passed down stably from one generation to the next, the epigenetic code is gnarly and dynamic. Dozens of different chemical modifications decorate both the DNA and the histones - proteins that package the DNA into chromosomes. All of these marks can vary from cell to cell, influenced by age, developmental stage and the environment" (Ledford 2015 ppS12-S13).

Tauxe (2015) summarised the "known unknowns" [26] of gene editing as:

- The off-target effects
- The diseases appropriate for gene editing
- The phenotypic effects (ie: the physical effects of the DNA edit)
- Editing the germline to prevent heritable diseases [27].

1.8. APPENDIX 1F - MEMORY LOSS AND MARIJUANA

The major psychoactive ingredient in marijuana is delta-9-tetrahydrocannabinol (THC), and it produces memory impairment (Robinson 2015). Vinals et al (2015) showed that the impairment in memory could be reduced by blocking a certain pathway in the brain, without the loss of the beneficial effect of pain relief (analgesia) of THC [28]. This was done with "knockout" (or transgenic) mice [29], who were bred with particular genes "turned off". It seems that activation of a particular serotonin receptor in the brain produces the memory problems with THC, and mice with alterations to these receptors have no memory problems after THC (Robinson 2015).

1.9. APPENDIX 1G - LABORATORY ENVIRONMENT CONFOUNDERS

Experimental studies with mice, for example, traditionally ignore environmental factors like their food, bedding, or lighting, unless it is part of the research focus. Such differences in environmental

---

[26] These are aspects of the future that are conceived as possible to the observer now, but it is not known how or whether they will manifest, as opposed to "unknown unknowns" (aspects of the future not conceived by the observer at this point in time).

[27] Liu et al (2016) reported a study with transgenic macaque monkeys with a particular human gene, who showed autism-like behaviours, and this was found in germline transmission (ie: in offspring).

[28] The analgesic effect was measured with the tail-immersion test. The mouse's tail in placed in water heated to 50 SYMBOL 176 \f "Times New Roman" \s 10C, and the speed of removal is measured (up to ten seconds to avoid tissue damage).

[29] Mice are most commonly used because their reproductive cycle is short "promising a higher rate of success when performing the transgenic operation and a higher number of animals to be used for experimentation" (Bogeholz et al 2014 p1332).

conditions may explain why findings cannot be replicated between laboratories in some cases (Reardon 2016). For example, diet influences gut bacteria [30], and this has been linked to differences in anxiety levels (Reardon 2016) [31].

There are emerging wider issues when comparing like with like [32], including microchimerism. This is the presence of cells in an individual from another person - mostly commonly the child in the mother or vice versa. This is because cells move from one bloodstream to the other during pregnancy (Ridgway 2016).

Studies of women who died during pregnancy or giving birth have found cells from their child in different organs of the body, while microchimeric cells have been found to survive many years (Ridgway 2016).

In a study with mice, foetal cells helped in repairing the pregnant mother's damaged heart tissue after an induced heart attack (Ridgway 2016).

One evolutionary-based explanation is that the child's cells remain in the mother to aid her survival after birth, and thus to be alive to care for the newborn. Another possibility relates to parent-child conflict. The individual child wants all the maternal care and has no interest in future siblings, whereas the mother will care for the current offspring to a point but wants future offspring. Microchimeric cells have been found in the mother's breasts, possibly encouraging lactation and thus reducing fertility (Ridgway 2016).

1.10. APPENDIX 1H - EVOLUTION OF CENTRAL NERVOUS SYSTEM

Explaining the evolution of the central nervous system (CNS) in different species is one of a number of conundrums, argued Strausfeld and Hirth (2016). They noted that one possibility is the evolution of neurons twice independently (eg: Moroz and Kohn 2016; table 1.2) [33] [34] [35]. There is "an intriguing landscape of questions and

---

[30] Richard Jefferson used the term "hologenome" to cover how an individual had evolved in symbiosis with microbes in their gut (Ridgway 2016).

[31] Interestingly, "the high-fat, high-sugar food used in obesity studies goes rancid quickly; when it does, mice may stop eating and lose weight without researchers realising why" (Reardon 2016 p264).

[32] Significant differences have been found between inbred strains of laboratory mice in mating behaviour, and exploratory behaviour (Barnard 1983).

[33] For example, differently between vertebrates and invertebrates.

[34] The number of neurons in an organism can range from hundreds in nematodes to hundreds of billions in humans (Moroz and Kohn 2016).

[35] The common ancestor of all animals is an organism called Urmetazoa "without defined neurons and synapses" (Moroz and Kohn 2016). The Initial Darwinian Ancestor (IDA) is the first material on Earth to become living, and this led to the Last Universal Common Ancestor (LUCA) ("a molecule that stored information as genetic code, and gave rise to all life on Earth") (Brahic 2016).

hypotheses punctuated by enormous gaps, in which our knowledge is still profoundly deficient", they stated.

- There is a debate as to whether neurons had a single origin or multiple origins. With the latter, neurons evolved in ctenophores (eg: comb jellies) separately to other animals. One difference is in the evolution of neurotransmitters in cniderian (eg: corals, sea anemones) and bilaterian [36] lineages as compared to secretory peptides in ctenophores. Specifically, Moroz and Kohn (2016) argued that "both electrical and chemical synapses evolved more than once".

Table 1.2 - Moroz and Kohn (2016).

Historically, the idea of a single evolution of the CNS has dominated in the time since Darwin's work [37]. For example, Guiseppe Bellonci in the late nineteenth century described physiological similarities in the olfactory area of the brain of a crustacean, an eel, and a cricket (Strausfeld and Hirth 2016).

This idea is challenged today by recent discoveries like the unique organisation of the octopus CNS (Albertin et al 2015), which "would seem to define the CNS of cephalopod molluscs as wholly distinct from all other taxa" (Strausfeld and Hirth 2016).

Fossil evidence is key in searching for the origins of the CNS. For example, organisms from the late Ediacaran period (around 550 million years ago [38]) appear to have extremely simple avoidance-like behaviours, while it seems to be more developed by the Cambrian period [39] (Strausfeld and Hirth 2016) [40].

Movement by the earliest animals would require sensory-motor organisation. The simple action potentials of unicellular eukaryotes [41] could have become mechano-receptive cells in multi-cellular organisms, "from which evolved true neurons and muscles" (Strausfeld and Hirth 2016).

Strausfeld and Hirth (2016) summed up the debate about the evolution of the CNS thus: "Homology and convergence are two conceptual frameworks for discussing

---

[36] Covers most animals, that have bilateral symmetry in the body shape.

[37] Known as homology - " a correspondence of parts due to common ancestry" (Strausfeld and Hirth 2016) - as opposed to convergence.

[38] Ediacaran period - 635 to 540 million years ago - dominated by cyanobacteria on shallow sea floors (Fox 2016)

[39] The Cambrian period - 540 to 485 million years ago - saw the arrival of anthropods with legs and compound eyes, and predators (known as "Cambrian explosion") (Fox 2016).

[40] Life emerged three billion years ago, but dominated by single-celled organisms for a large part of the time (Fox 2016).

[41] Organisms with, usually, a single cell within a membrane.

correspondences and to identify genealogical order amongst the many different types of nervous systems that characterise extant species across large phylogenetic distances. Both the concepts of homology and convergence can be united for a common aim: that of identifying the 'geometry of life' [Conway Morris 2015] whose algorithms, if uncovered, would enable an explanation for the many similarities observed, for example, between the brains of arthropods [42] and chordates [43] and the stunning differences exemplified by the brains of cephalopods [eg: squid]" (p4).

The nature and extent of the evolution of the CNS can come up against the view that humans are unique, for instance. Rene Descartes in the seventeenth century held the view that humans were distinct from other animals, that "people were creatures of reason, linked to the mind of God, while animals were merely machines made of flesh - living robots" (Staff 2015 p65). Charles Darwin (1872) offered a contrary view in "The Expression of Emotions in Man and Animals", though Behaviourists throughout the twentieth century were not interested in the mind as it could not be studied like other behaviour. Progress in studying animals means that "most scientists now feel they can say with confidence that some animals process information and express emotions in ways that are accompanied by conscious mental experience" (Staff 2015 p66). For example, "mirror neurons", which are involved in imitation of another's action in humans, in other mammal brains.

1.11. REFERENCES

Albertin, C.B et al (2015) The octopus genome and the evolution of cephalopod neural and morphological novelties Nature 524, 7564, 220-224

Barnard, C.J (1983) Animal Behaviour: Ecology and Evolution London: Croom Helm

Bechara, A et al (1999) Different contributions of the human amygdala and ventromedial prefrontal cortex to decision-making Journal of Neuroscience 19, 5473-5481

Bogeholz, N et al (2014) Advantages and limitations of transgenic mice: The role of the NA+/CA2+ exchanger in cardiac electrophysiology and arrhythmia Current Medical Chemistry 21, 11, 1330-1335

Brahic, C (2016) Desperately seeking: Initial Darwinian Ancestor New Scientist 19/3, p32

Branchi, I & Alleva, E (2007) Bioethical considerations on the use of genetically modified animals in biomedical research. In Vitale, A et al (eds) Human and Non-Human Animals Interaction: Contextual, Normative and Applicative Aspects Rome: Istituto Superiore di Sanita

[42] Invertebrates with external skeleton (eg: scorpion).
[43] Vertebrates.

Brent, D.A & Mann, J.J (2005) Family genetic studies, suicide and suicidal behaviour American Journal of Medical Genetics Part C: Seminars in Medical Genetics 133C, 1, 13-24

Callaway, E (2015) Neanderthals had outsize effect on human biology Nature 523, 7562, 512-513

Church, G (2015) Encourage the innovators Nature 528, 7581, S7

CONVERGE consortium (2015) Sparse whole-genome sequencing identifies two loci for major depressive disorder Nature 523, 7582, 588-591

Conway Morris, S (2015) The Runes of Evolution West Conshohocken, PA: Templeton Press

Corbyn, Z (2015) Biology's big hit Nature 528, 7581, S4-S5

Cryan, J.F & Holmes, A (2005) The ascent of mouse: Advances in modelling human depression and anxiety Nature Reviews: Drug Discovery 4, 9, 775-790

Darwin, C (1872) The Expression of Emotions in Man and Animals London: Murray

Doudna, J (2015) Embryo editing needs scrutiny Nature 528, 7581, S6

Eisenstein, M (2015) Closing the door on HIV Nature 528, 7581, S8-S9

Elwood, R.W (2016) Stress was never said to be pain: Response to Stevens et al (2016) Biology Letters 12, 20160126

Elwood, R.W & Adams, L (2015) Electric shock causes physiological stress responses in shore crabs, consistent with prediction of pain Biology Letters 11, 20150800

Fox, D (2016) What sparked the Cambrian explosion? Nature 530, 7590, 268-270

Gewin, V (2015) Expanding possibilities Nature 528, 7581, S10-S11

Gibbons, A (2016) Neanderthal genes linked to modern diseases Science 351, 648-649

Green, R.E et al (2010) A draft sequence of the Neanderthal genome Science 328, 710-722

Gross, C et al (2002) Serotonin 1A receptor acts during development to establish normal anxiety-like behaviour in the adult Nature 416, 396-400

Hawton, K et al (2003) Deliberate self-harm in adolescents: A study of characteristics and trends in Oxford, 1990-2000 Journal of Child Psychology and Psychiatry 44, 1191-1198

Healey, C et al (2014) Under-age drinking in the UK: Changing trends, impact and interventions. A rapid end synthesis International Journal of Drug Policy 25, 124-132

Hoehne, A et al (2015) First-degree relatives of suicide completers may have impaired decision-making but functional cognitive control Journal of Psychiatric Research 68, 192-197

Holmes, A & Crawley, J.N (2000) Promises and limitations of transgenic and knockout mice in modelling psychiatric symptoms. In Myslobodsky, M.S & Weiner, I (eds) Contemporary Issues in Modelling Psychopathology New York: Springer

Jinek, M et al (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity Science 337, 816-821

Jollant, F et al (2011) The suicidal mind and brain: A review of neuropsychological and neuroimaging studies World Journal of Biological Psychiatry 12, 319-339

Jones, R.S.G et al (2016) Human brain slices for epilepsy research: Pitfalls, solutions and future challenges Journal of Neuroscience Methods 260, 221-232

Kuhlwilm, M et al (2016) Ancient gene flow from early modern humans into Eastern Neanderthals Nature 530, 7591, 429-433

Langley, G et al (2015) Lessons from toxicology: Developing a 21st century paradigm for medical research Environmental Health Perspectives 123, 11, A268-A272

Ledford, H (2015) The genome unwrapped Nature 528, 7581, S12-S13

Ledford, H (2016) Drugmakers target fog of depression Nature 530, 7588, p17

Liu, Z et al (2016) Autism-like behaviours and germline transmission in transgenic monkeys overexpressing MeCP2 Nature 530, 7588, 98-102

Meaney, M.J (2001) Maternal care, gene expression, and the transmission of individual differences in stress reacting across generations Annual Review of Neuroscience 24, 1161-1192

McKinney, W.T.J & Bunney, W.E.J (1969) Animal model of depression. I. Review of evidence: Implications for research Archives of General Psychiatry 21, 240-248

Moroz, L.L & Kohn, A.B (2016) Independent origins of neurons and synapses: Insights from ctenophores Philosophical Transactions of the Royal Society B 371, 20150041

Niculescu, A.B et al (2015) Understanding and predicting suicidality using a combined genomic and clinical risk assessment approach Molecular Psychiatry 20, 11, 1266-1285

Olszewska, Z (2015) The poet's melancholy Medicine Anthropology Theory 2, 3, 83-104

Pistollato, F et al (2015) A human-based integrated framework for Alzheimer's disease Journal of Alzheimer's Disease 47, 857-868

Reardon, S (2015) Brain study seeks roots of suicide Nature 528, 7580, p19

Reardon, S (2016) A mouse's house may ruin studies Nature 530, 7590, p264

Ridgway, A (2016) The others inside you New Scientist 9/1, 27-29

Robinson, R (2015) Selective reduction of THC's unwanted effects through serotonin receptor inhibition PLoS Biology 13, 7, e1002193 (Freely available at http://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1002193)

Rose, N (2003) Neurochemical selves Society November/December, 46-59

Russell, W.M.S & Burch, R.L (1959) The Principles of Humane Experimental Technique Wheathampstead: Universities Federation for Animal Welfare

Simonti, C.N et al (2016) The phenotypic legacy of admixture between modern humans and Neanderthals Science 351, 737-741

Staff (2015) Animals think, therefore... Economist 19/12, 65-70

Stevens, E.D et al (2016) Stress is not pain. Comment on Elwood and Adams (2015) "Electric shock causes physiological stress responses in shore crabs, consistent with prediction of pain" Biology Letters 12, 20151006

Strausfeld, N.J & Hirth, F (2016) Introduction to homology and convergence in nervous system evolution Philosophical Transactions of the

Royal Society B 371, 20150034

Strauss, K.A (2015) Genomics for the people Scientific American December, 59-65

Strauss, K.A et al (2014) A population-based study of KCNH7 p.Arg395His and bipolar spectrum disorder Human Molecular Genetics 23, 23, 6395-6406

Stroop, J.R (1935) Studies of interference in serial verbal reactions Journal of Experimental Psychology 18, 643

Sullivan, P.F (2015) Associations with depression Nature 523, 7582, 539-540

Tauxe, W (2015) Genome editing: Four big questions Nature 528, 7581, S17

Thomas, K.H et al (2013) Validation of suicide and self-harm records in the General Practitioner Research Datalink British Journal of Clinical Pharmacology 76, 145-157

Tyrrell, E.G et al (2016) Changes in poisonings among adolescents in the UK between 1992 and 2012: A population based cohort study Injury Prevention (http://injuryprevention.bmj.com/content/early/2016/04/01/injuryprev-2015-041901.abstract)

Venerosi, A & Vitale, A (2007) Application of the "3Rs" model in biomedical experimentation: The case of the study of the neurobehavioural toxicity effects after developmental exposure to anti-retroviral agents. In Vitale, A et al (eds) Human and Non-Human Animals Interaction: Contextual, Normative and Applicative Aspects Rome: Istituto Superiore di Sanita

Vinals, X et al (2015) Cognitive impairment induced by delta-9-tetrahydrocannabinol occurs through heteromers between cannabinoid CB, and serotonin 5-HT2A receptors PLoS Biology 13, 7, e1002194 (Freely available at http://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1002194)

Vogel, J.R et al (1971) A simple and reliable conflict procedure for testing anti-anxiety agents Psychopharmacologia 21, 1-7

2. ANIMALS DETECTING HUMAN DISEASE

2.1. DOGS

The dog's sense of smell is reported as over 100 000 times more sensitive than humans (Gordon et al 2008) [44].

Williams and Pembroke (1989) reported the first case in a medical journal of a pet dog who sniffed at the owner's mole on the leg, which turned out to be a malignant melanoma. Metabolic changes produced by cancer lead to "volatile organic chemicals" that the dog detected (Young 2016).

Church and Williams (2001) detailed a similar case. A sixty-six year-old man, whose pet labrador sniffed excessively at an apparent patch of eczema on the left thigh, actually had a basal cell carcinoma.

In terms of rigorous studies, Willis et al (2004) used blind testing of urine samples from patients with bladder cancer or controls (in a ratio of 1 of 6) with six dogs. The mean success rate was 41% which was significantly greater than chance (which was approximately 16%). While McCulloch et al (2006) (appendix 2A) found 90% and above success with five dogs detecting lung or breast cancer from breath samples.

Not all studies have been so successful. For example, Gordon et al (2008) investigated whether dogs could be trained to detect breast or prostate cancer from urine samples. Ten dogs were taught by their owners, using operant conditioning, to distinguish the urine for one of the cancers only. The test scenario involved nine samples for breast cancer and 54 controls, and eleven patients with prostate cancer and sixty-six controls.

In the former case, only two of six dogs were better than chance for specificity (accuracy on cases - ie: true positive vs false positive; table 2.1), but none of them were significant for sensitivity (accuracy for non-cases - ie: true negative vs false negative). For the prostate cancer samples, two of four dogs were significantly

[44] Comparing dogs to analytical instruments for detection of explosives by odour chemicals, Furton and Myers (2001) stated: "Overall, detector dogs still represent the fastest, most versatile, reliable real-time explosive detection device available. Instrumental methods, while they continue to improve, generally suffer from a lack of efficient sampling systems, selectivity problems in the presence of interfering odour chemicals and limited mobility/tracking ability" (p487).

better than chance for specificity, and none of them again for sensitivity. The overall success rate was 20% (which was not statistically significant compared to chance).

		TRUE SITUATION Cancer	Not cancer
DOG'S DECISION	Cancer	True positive	False positive
	Not cancer	False negative	True negative

Table 2.1 - Assessment of accuracy.

The difference between the three studies above may be due to methodological differences as shown in table 2.2.

Aspects of study	Willis et al (2004)	McCulloch et al (2006)	Gordon et al (2008)
Type of cancer	Bladder	Lung & breast	Prostate & breast
Source	Urine	Breath	Urine
Storage time of samples (up to months)	5	2	5
Dog training	Professionals	Professionals	Owners
Duration of training (months)	7	Less than 1	12-14
Testing ratio (cancer and controls)	1 of 6	1 of 5	1 of 6

(Based on Gordon et al 2008 table 2 p66)

Table 2.2 - Key methodological differences between three studies.

Maurer et al (2016) found success for dogs detecting bacteriuria and urinary tract infections. Dogs, who had been trained for eight weeks, were presented with five samples of urine, of which one was diseased (with E.coli bacteria). Of the samples, 231 were from individuals with the infections and 687 not in Hawaii. The five dogs had an overall accuracy above 90%, even when the positive samples were diluted to 1% or 0.1%.

The researchers noted two limitations to the first published study on canine scent detection of bacteriuria:

i) A limited number of confounding factors were measured (eg: age and gender), but not co-morbidity of illnesses, for instance.

ii) "Case samples were used only once in the testing phase, whereas control samples were used throughout each day of testing. It is possible that dogs may learn to identify that which is new in a line-up. If this occurred, the first runs of each day (with all new samples) would have lower accuracy rates than the subsequent runs. However, statistical testing confirmed the accuracy rates for the first runs of each day were identical to overall accuracy rates. In future work, we plan to use new control samples for each run" (Maurer et al 2016).

Gould et al's (2015) literature review (table 2.3) concluded: "Whilst canine detection may be unsuitable for clinical implementation they can, at least, provide inspiration for more traditional laboratory investigations" [45].

Cancer	Source of detection	Sensitivity	Specificity
Bladder	Urine	63-73	64-92
Prostate	Urine	91-99	91-97
Breast	Breath	86	98
Lung	Breath	56-99	30-99
Colorectal	Stool	91-97	97-99

(Source: Gould et al 2015)

Table 2.3 - Range of percentage of accuracy of dogs in detecting different cancers in different studies.

2.2. OTHER ANIMALS

In Tanzania the African giant pouched rat (Cricetomys gambianus) has been trained to detect tuberculosis (TB). In the breath and lung tissue there are compounds of the bacteria involved and the animal sniffs this. Between 2007 and 2015 rats screened over 300 000 lung tissue samples and identified over 9000 patients told by clinics than TB-free before (ie: increased screening by 40%) (Young 2016).

The rats are trained via operant conditioning, where a clicker is used to signal a food reward to reinforce

[45] Gould et al (2015) noted great variability between individual dogs used in the studies.

desirable behaviour. In this case, keeping their noses in a "sniffing hole" for five seconds when detecting TB in sputum samples. Over seven days, Weetjens et al (2009) tested eighteen of these rats on 819 samples, of which sixty-seven were known to be TB-positive in sets of ten at a time. The average accuracy (true positive) was around 90% with about 5% for false positives.

Cockroaches have been trained to detect TNT from buried land mines (Young 2016).

2.3. APPENDIX 2A - McCULLOCH ET AL (2006)

McCulloch et al (2006) recruited fifty-five patients with lung cancer and thirty-one with breast cancer along with 83 controls in California. Breath samples of these individuals were presented to five dogs who had been trained to identify cancer samples. Testing involved five samples to sniff, of which one was from a cancer patient.

A single-blinded experiment was performed where the dog handler did not know which sample was which, but the experimenter did, while in the double-blinded experiment, neither of these individuals knew. In the former, the experimenter told the handler if the dog was correct in their choice and a reward was given, but in the latter design the dog was rewarded at the end of the trial. The overall accuracy compared to biopsy-confirmed diagnosis was over 90% for the dogs (table 2.4).

	Lung cancer	Breast cancer
Sensitivity (true positives)	99	88
Specificity (true negatives)	99	98

Table 2.4 - Breakdown of accuracy (%) in McCulloch et al (2006).

McCulloch et al (2006) argued that the use of dogs to detect lung and breast cancers has great potential compared to traditional diagnostic methods:

- Chest x-rays and sputum analysis - high false-negative rate.
- Computerised tomography (CT) scans - false-positive risk as unable to detect very small lesions.

"Canine scent detection may possibly help overcome some of these drawbacks due to the extraordinary scenting ability of the dog's nose, which has detection thresholds

as low as parts per trillion" (McCulloch et al 2006 p31).

2.4. REFERENCES

Church, J & Williams, H (2001) Another sniffer dog for the clinic? Lancet 358, p930

Furton, K.G & Myers, L.J (2001) The scientific foundation and efficacy of the use of canines as chemical detectors for explosives Talanta 54, 487-500

Gordon, R.T et al (2008) The use of canines in the detection of human cancers Journal of Alternative and Complementary Medicine 14, 1, 61-67

Gould, O et al (2015) Canine olfactory differentiation of cancer - a review of the literature (http://arxiv.org/abs/1510.05495)

Maurer, M et al (2016) Detection of bacteriuria by canine olfaction Open Forum Infectious Diseases 3, 2, doi: 10.1093/ofid/ofw051

McCulloch, M et al (2006) Diagnostic accuracy of canine scent detection in early- and late-stage lung cancer and breast cancers Integrated Cancer Therapies 5, 1, 30-39

Weetjens, B.J et al (2009) African pouched rats for the detection of pulmonary tuberculosis in sputum samples International Journal of Tuberculosis and Lung Disease 13, 6, 737-743

Williams, H & Pembroke, A (1989) Sniffer dogs in the melanoma clinic? Lancet 333, p734

Willis, C.M et al (2004) Olfactory detection of human bladder cancer by dogs: Proof of principle study BMJ 329, 712-714

Young, E (2016) The rat detectives Guardian 23rd February, 25-27