Chapter 6

Research in behavioural genetics involving animals

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Introduction

6.1 One method of investigating human behaviour is to examine similar traits in animals. Although they have obvious limitations, animal 'models' of human behaviour have frequently been an effective tool for scientists. Such models are useful in understanding disease, but can also be informative about the contribution of genetic factors to some normal behavioural traits. Many species are used in both genetic and psychological research into behaviour, including primates, mice, rats, birds, fish and fruit flies. The different traits being studied in these animals include: intelligence/learning,[1] novelty seeking,[2] anxiety,[3] impulsivity,[4] aggression,[5] hyperactivity,[6] addiction,[7] social interaction,[8] sexual orientation,[9] emotionality,[10] depression and neuroticism.[11]

6.2 This chapter sets out various types of model that researchers use and points to possible advantages and problems with the use of animal models that should be borne in mind when evaluating the results of such research. The chapter focuses predominantly on research involving mice, since much research in behavioural genetics uses mouse models of human behaviour. Although primates are much closer to humans in terms of their behaviour, there are various reasons why non-human primates have not been used as often to study the genetics of human behaviour. Research involving primates tends to pose

[1] Dobkin, C. *et al*. (1997). FMR1 knockout mouse has a distinctive strain-specific learning impairment. *Neurosci*. **100**, 423–9; Fisch, G. S., Hao, H. K., Bakker, C. & Oostra, B. A. (1999). Learning and memory in the FMR1 knockout mouse. *Am. J. Med. Genet*. **84**, 277–82; Tang, Y. *et al*. (1999). Genetic enhancement of learning and memory in mice. *Nature* **401**, 63–9.

[2] Tang, Y. *et al*. (1999). Genetic enhancement of learning and memory in mice. *Nature* **401**, 63–9; Dulawa, S. C., Grandy, D. K., Low, M. J., Paulus, M. P. & Geyer, M. A. (1999). Dopamine D4 receptor-knock-out mice exhibit reduced exploration of novel stimuli. *J. Neurosci*. **19**, 9550–6.

[3] König, M. *et al*. (1996). Pain responses, anxiety and aggression in mice deficient in pre-proenkephalin. *Nature* **383**, 535–8; Smith, G. W. *et al*. (1998). Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. *Neuron* **20**, 1093–102.

[4] Cardinal, R. N. *et al*. (2001). Impulsive choice induced in rats by lesions of the nucleus accumbens core. *Science* **292**, 2499–501.

[5] De Felipe, C. *et al*. (1998). Altered nociception, analgesia and aggression in mice lacking the receptor for substance P. *Nature* **392**, 394–7; DeVries, A. C., Young, W. S. III & Nelson, R. J. (1997). Reduced aggressive behaviour in mice with targeted disruption of the oxytocin gene. *J. Neuroendocrinol*. **9**, 363–8; Ledent, C. *et al*. (1997). Aggressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A2a receptor. *Nature* **388**, 674–8.

[6] Accili, D. *et al*. (1996). A targeted mutation of the D3 dopamine receptor gene is associated with hyperactivity in mice. *Proc. Natl. Acad. Sci. USA* **93**, 1945–9.

[7] Crabbe, J. C. (1996). Elevated alcohol consumption in null mutant mice lacking 5-HT1B serotonin receptors. *Nat. Genet*. **14**, 98–101; Ledent, C. *et al*. (1999). Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science* **283**, 401–4; Maldonado, R. *et al*. (1997). Absence of opiate rewarding effects in mice lacking dopamine D2 receptors. *Nature* **388**, 586–9; McBride, W.J. & Li, T.K. (1998). Animal models of alcoholism: neurobiology of high alcohol-drinking behaviour in rodents. *Crit. Rev. Neurobiol*. **12**, 339–69; Nestler, E. J. (2000). Genes and addiction. *Nat. Genet*. **26**, 277–81; Rocha, B.A. *et al*. (1998). Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. *Nature* **393**, 175–8.

[8] Ferguson, J. N. *et al*. (2000). Social amnesia in mice lacking the oxytocin gene. *Nat. Genet*. **25**, 284–8; Gendreau, P. L., Petitto, J. M., Petrova, A., Gariepy, J. & Lewis, M. H. (2000). D(3) and D(2) dopamine receptor agonists differentially modulate isolation-induced social-emotional reactivity in mice. *Behav. Brain Res*. **114**, 107–17; Lijam, N. *et al*. (1997). Social interaction and sensorimotor gating abnormalities in mice lacking Dvl1. *Cell* **90**, 895–905.

[9] McGraw, K. J. & Hill, G. E. (1999). Induced homosexual behaviour in male house finches (Carpodacus maxicanus): the 'prisoner effect'. *Ethol. Ecol. Evol*. **11**, 197–201.

[10] Flint, J. *et al*. (1995). A simple genetic basis for a complex psychological trait in laboratory mice. *Science* **269**, 1432–5.

[11] Flint, J. *et al*. (1995). A simple genetic basis for a complex psychological trait in laboratory mice. *Science* **269**, 1432–5.

greater ethical problems than animal models using rodents.[12] Monkeys do not breed rapidly nor do they have large numbers of offspring. The time and costs involved in producing and rearing them would be prohibitive. Many strains of mice already exist that have been bred selectively to display particular traits. For these reasons, the mouse currently remains the most commonly used organism for studying the genetics of human behaviour.

6.3 Many genes involved in fundamental biological processes have been conserved as species have evolved. In other words, many genes are similar in different species. Due to the fact that many genes that play an important role in development and the proteins they produce tend to be very similar in mice and humans, considerable evidence has been gathered from research involving mice as a guide to the human case. However, when considering the findings of various studies, it is vital to remember that conservation of a gene across the two species does not necessarily mean that the gene itself, the timing of its expression in the organism or its function will be exactly equivalent in mice and humans.[13]

How are animal models created?

6.4 Before it became possible to manipulate specific genes in animals, researchers examined the effect on behaviour of preventing particular parts of an animal's brain from influencing its behaviour in the normal way. Today, changes in an animal's genetic make-up can be produced in several ways, either by selecting animals which show natural variation or by inducing variation through genetic manipulation.[14] The main methods are:

- variation induced in individual animals by surgery, conditioning, diet and so on, which is not due to the animals' genotype;
- selective breeding (i) of naturally-occurring traits, where animals are specifically chosen for mating based on an observed behavioural trait; and (ii) of animals exposed to pre- or post-natal rearing environments which are either enriched or impoverished;
- variation induced in specific genes by (i) the deletion or 'knocking out' of a gene; (ii) the under- or over-expression of a gene; (iii) transferring a gene to create a transgenic animal; (iv) exposure to radiation or drugs. Variation can be as subtle as changing just one base pair; this sometimes has effects as profound as those of knocking out the whole gene.

6.5 Selective breeding capitalises on the genetic variation that is present either in unmodified mice or in those which have been produced by cross-breeding two or more inbred strains. Measurements of behavioural traits may be used to divide groups of animals which do not have similar genotypes into sub-groups with, for example, high or low aggression, or high or low levels of exploratory activity. Selective breeding from animals with the most extreme manifestations of the trait in question is then undertaken for a number of generations.

[12] Some of these issues were discussed in *Animal to human transplants: the ethics of xenotransplantation*, Nuffield Council on Bioethics (1996). The issue of the ethics of research involving animals is outside the scope of this Report, but will be the subject of a future Report by the Council.

[13] Fougerousse, F. *et al*. (2000). Human–mouse differences in the embryonic expression patterns of development control genes and disease genes. *Hum. Mol. Genet*. **9**, 165–73.

[14] See for example Flint, J. (1996). Annotation: behavioural phenotypes: a window on the biology of behaviour. *J. Child Psychol. Psyc*. **37**, 355–67; Heintz, N. (2000). Analysis of mammalian central nervous system gene expression and function using bacterial artificial chromosome-mediated transgenesis. *Hum. Mol. Genet*. 9, 937–43; Hunter, A. J., Nolan, P. M. & Brown, S. D. M. (2000). Towards new models of disease and physiology in the neurosciences: the role of induced and naturally occurring mutations. *Hum. Mol. Genet*. **9**, 893–900; Kempermann, G., Georg Kuhn, H. & Gage, F. H. (1997). More hippocampal neurons in adult mice living in an enriched environment. *Nature* **386**, 493–5.

Often, by the end of a selection experiment, animals may have become inbred. Groups of animals that have similar genotypes but that differ in terms of a particular phenotype can also be created by inbreeding animals that have little genetic ancestry in common. These are powerful methods for identifying genetic differences between strains; however, the genetic diversity that is present is limited, compared to that which occurs naturally, as a result of using inbred mice.

6.6 Mutations can be induced at random in the genome through irradiation using X-rays, or by the use of mutagenic chemicals. Several large-scale projects are under way, in which thousands of mice are exposed to mutagenic chemicals. Their offspring are then screened for a wide range of characteristics, including behavioural and neurobiological abnormalities. After finding such mutants, they can be bred to create a line of animals with the characteristics in question. Mutagenesis is of relevance when it produces animals that have abnormal phenotypes similar to complex traits of interest to the investigator (for example, increased anxiety). Once such a mutant line is established, further research may be able to identify the mutation that causes the trait. It is not yet clear how frequently complex traits can be mimicked by induced mutations, nor how useful such experiments will be in uncovering the genetic basis of the complex trait itself.

Box 6.1: The 'Doogie mice'

Here, we provide a brief description of a much-publicised research project investigated memory and learning in genetically modified mice.[*] The study was undertaken to investigate a potential treatment for the memory and learning problems of patients with Alzheimer's disease, but its extrapolation to the enhancement of normal variation in these traits, which are components of intelligence, was focused on both by scientists and the popular press. The genetically modified mice were nicknamed 'Doogie' after the intellectually precocious star of a popular TV programme, Doogie Howser MD.

The mice were genetically altered so that they over-expressed a gene that has an effect in the brain and is thought to be involved in learning. The modified mice were then compared to unmodified mice in various tasks. These tested the ability of the mice to recognise objects they had previously seen, to remember events that had caused an emotional response, to learn relationships between an electric shock and a particular outcome and to succeed in spatial learning. The genetically modified mice were normal in all respects, except for learning and memory. They showed normal growth, normal body weight and normal mating behaviour, but appeared to have enhanced learning capacity. When tested three to six months after birth, the genetically modified mice showed a greater tendency for exploratory behaviour, a stronger preference for novel situations and superior abilities to code and store information. The researchers concluded that over-expression of the gene resulted in a better long-term memory. However, it is very difficult to measure the precise effects of the over-expression of the gene in the brain. Moreover, the enhancement effects in one experiment lasted only three days, and in others for merely a few hours, so the claims should be treated with great caution.

Nevertheless, the scientific and popular press was rapid in hailing these results as pointing to the existence of a 'gene for learning', a 'gene for intelligence' or even simply 'the IQ gene'[†] that might subsequently be enhanced in humans. Even the original press release, issued by Princeton University, to which the researchers were affiliated, claimed that 'the finding also

shows that genetic improvement of intelligence and memory in mammals is now feasible, thus offering a striking example of how genetic technology may affect mankind and society in the next century'. This was based on the hypothesis that overexpression of the gene might help the brain to retain the extensive capacity for learning that young children possess naturally early on but gradually lose with age. This example attracted media coverage of increasing exaggeration throughout the world and points to the risks of generalising the tentative results of a relatively restricted experiment on mice to the human case.

More recent experiments on the same strain of genetically modified mice have suggested that there is an additional, unintended effect of this manipulation, namely an increased susceptibility to persistent pain. This illustrates that attempts at genetic enhancement may have unexpected side effects.[‡]

[*] Tang, Y. *et al*. (1999). Genetic enhancement of learning and memory in mice. *Nature* **401**, 63–9.

[†] See, for example, Lemonick, M. D. (1999). Smart Genes? *Time Magazine* 13 Sept.

[‡] Wei, F. *et al*. (2001). Genetic enhancement of inflammatory pain by forebrain NR2B overexpression. *Nat. Neurosci*. **4**, 164–9.

What are the benefits of using animals to study the genetics of human behaviour?

6.7 One of the obvious advantages of research involving mice is that the human and mouse genomes are very similar, so that many human genes have counterparts in mice. Mice breed very rapidly and plentifully, so programmes of breeding can be more easily implemented. Many organs in mice are also very similar to those in humans. Thus, discovering the parts of the body and brain in which a known or candidate gene is expressed (called expression analysis) can be done in the laboratory with a reasonable expectation that this will usually be similar in the human case. A particular advantage is that scientists can study gene expression throughout the development of the mouse before birth and in early postnatal life, which makes it possible to chart where and when genes are expressed. Some genes are expressed early in development and never again, whereas others are expressed later. The earlier in development that a gene is expressed, the greater the ability to understand its function. This may enable the planning of timely intervention during periods at which the brain is most receptive to alteration. New treatments can be tried out when the function of genes and their products are fully identified. For example, using mouse models in which genes related to the human hearing system have been knocked out, researchers may be able to establish the best period of time for inserting cochlear implants.

6.8 Fairly complex behaviours in mammals can be dependent on the presence and functioning of the chemicals produced by specific genes. For example, studies in prairie voles have suggested that two chemicals in the brain, the neuropeptides oxytocin and vasopressin, 'play important roles in behaviours associated with monogamy, including affiliation, paternal care and pair bonding'.[15] Some prairie vole species are monogamous. They have been shown to have a higher density of oxytocin receptors in specific parts of the brain than do closely related non-monogamous species. The specific behaviours related to the

[15] Young, L. J., Lim, M. M., Gingrich, B. & Insel, T. R. (2001). Cellular mechanisms of social attachment. *Horm. Behav*. **40**, 133–8.

development of social bonds, but not other general behaviours, appear to be altered when animals are treated by injection of substances that block the binding of these neuropeptides to the relevant cells in the brain.[16] What is much less clear, currently, is the extent to which similar mechanisms may or may not exert some influence on human behaviour, even with regard to disease, let alone within the normal range. It is important to emphasise that the absence of current evidence of this nature is not evidence that such effects could not exist. On the contrary, if animal evidence suggests a neurobiological mechanism underlying certain complex behaviours, it is entirely plausible that at least some residue of these mechanisms will eventually also be discovered in human beings. For example, the sleep disorder narcolepsy has been found to have the same chemical basis in mice, dogs and humans.[17]

What are the problems with using animals to study the genetics of human behaviour?

6.9 While human genes have many homologues in mice, their patterns of expression are often dissimilar both spatially and temporally.[18] Indeed, time-dependent processes differ significantly between the two species.[19] Although specific genes may be similar, interactions between genes, as well as with the internal and external environments, may be different. Identical genes may have different functions within the development of the brain in different species and may be expressed at varying times and at different developmental stages. It may be that haploinsufficiency (having one instead of the normal two copies of a gene) in mice turns out to be less detrimental than in humans. Such potential differences must always be taken into account.[20] Generalisations from mouse to human can sometimes, therefore, be premature and need to be examined with caution.

6.10 In scientific experiments, caution should always be exercised in interpreting the results. Unless replicated, reported findings cannot be taken at face value, because sometimes outcomes differ even in ostensibly identical conditions. For example, one group of researchers set up a comparison of results from three different laboratories that studied genetically modified mice in which the gene involved in regulating a chemical in the brain (a neurotransmitter called serotonin), had been knocked out.[21] Each of the three laboratories had identical strains of mice and conducted the experiments starting at exactly the same time, on the same day and under the same laboratory conditions, using the same mouse feed and the same behavioural tests. The results were, in some respects, surprising. For example, in a simple test of anxiety (a maze in which animals could either stay 'safe', relatively hidden in areas with high walls, or else venture out into 'more dangerous' open areas), the differences between genetically modified mice and the controls, varied as a

[16] Insel, T. R. (1997). A neurobiological basis of social attachment. *Am. J. Psychiat*. **154**, 726–35.

[17] Overeem, S., Mignot, E., Gert van Dijk, J. & Lammers, G. J. (2001). Narcolepsy: clinical features, new pathophysiologic insights, and future perspectives. *J. Clin. Neurophysiol*. **18**, 78–105.

[18] Fougerousse, F. *et al*. (2000). Human–mouse differences in the embryonic expression patterns of development control genes and disease genes. *Hum. Mol. Genet*. **9**, 165–73.

[19] Doyle, J. L., DeSilva, U., Miller, W. & Green, E. D. (2000). Divergent human and mouse orthologs of a novel gene (WBSCR15/Wbscr15) reside within the genomic interval commonly deleted in Williams syndrome. *Cytogenet. Cell Genet*. **90**, 285–90.

[20] Keverne, E. B. (1997). An evaluation of what the mouse knockout experiments are telling us about mammalian behaviour. *BioEssays* **19**, 1091–8.

[21] Crabbe, J. C., Wahlsten, D. & Dudek, B. C. (1999). Genetics of mouse behaviour. *Science* **284**, 1670–2.

function of the laboratory. One laboratory's modified mice showed more activity in the open areas of the maze, the second laboratory's modified mice showed less, and in the third laboratory there was no difference in activity between the modified mice and the controls. These results presumably result from uncontrolled differences, for example in handling, in the odour of the handlers or in the composition of the water supplied to the mice. Some of these kinds of differences, particularly handling, might well be expected to affect the emotional responses of the mice and hence change their behaviour in this simple anxiety test. Results that have been replicated under different conditions or using several different ways of assessing anxiety are therefore likely to be more reliable than those resulting from a single measurement.[22] Of course this caveat does not just apply to genetic studies. The results obtained are always likely to depend in part on the environment in which the test is conducted. Note also that knocking out a gene that is expressed in the brain, for example, might have no consequences of behaviour in mice, while damage to the equivalent genes might by contrast critically affect behaviour in the human case. This is because the effects of the expression of the same gene across two species may differ.

6.11 Genetic effects are usually very dependent on context (both in terms of other genes and of environmental factors), such that even after breeding for a specific behavioural change, it may be significantly altered by subsequent experience. In sum, environmental factors clearly interact with an animal's genotype to produce the final phenotype. Furthermore, genetic effects can be beneficial in one environment, but damaging in another. For example, in the fruit fly, a number of sites on particular chromosomes (QTLs) have been identified that contribute to variations in lifespan. However, research has revealed that the effects of these QTLs vary as a function of sex and of larval environment. Some even have antagonistic effects on life span in the different sexes and across different environments.[23]

6.12 Another factor relevant to the problems of generalising from mouse to human is the fact that the mouse repertoire of behaviour measurable in the laboratory is comparatively limited. Often the effects of genetically modifying an animal are only studied with respect to a single hypothesis about the function of the gene in question, despite the fact that the gene may be pleiotropic (that is, have more than one effect) and be expressed in several parts of the body and brain.

6.13 When investigating intelligence in mice, most researchers focus on spatial memory in a task called the water maze, in which the mice have to remember where a submerged platform is located in a tank of water. How comparable is the enhancement of the mouse's learning capacity and thus performance in the water maze task (which is by no means a natural environment for mice) to improvements in, say, human memory in all its multiple forms? Other measurements less often used, but perhaps more analogous to human behaviour, might be speed of processing or the time one takes to react to a novel stimulus.[24] Researchers are now tending to use a wider range of tests in comparing mice to humans.

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[22] For example, Turri, M. G., Henderson, N. D., DeFries, J. C. & Flint, J. (2001). Quantitative trait locus mapping in laboratory mice derived from a replicated selection experiment for open-field activity. *Genetics* **158**, 1217–61.

[23] Leips, J. & Mackay, T. F. C. (2000). Quantitative trait loci for life span in *Drosophila melanogaster:* interactions with genetic background and larval density. *Genetics* **155**, 1773–88.

[24] It is important to note that although mouse models have been used to make claims about enhanced performance, faster is not always better in the human case. One might, for instance, be able to speed up a mouse's search for hidden objects by altering one of its genes. Should it therefore be concluded that such enhancement would be beneficial in the human case? Not necessarily. People with autism, for instance, are significantly faster than matched controls on a visual search task (O'Riordan, M. A., Plaisted, K. C. & Baron-Cohen, S. (2001). Superior visual search in autism. *J. Exp. Psychol. Hum. Percept. Perform*. **27**, 719–30), but this enhanced speed is actually detrimental to cognition and contributes to the tendency of people with autism to focus on specific features at the expense of context and overall configuration.

Indeed, it is becoming increasingly clear that good genetic research requires one to define and measure the traits under study effectively.

Conclusion

6.14 In conclusion, we have seen that research involving animals is one method of investigating influences on behaviour. While there are many similarities, in terms of genetics, between some animals and humans, the results of studies involving animals cannot be taken to apply in a straightforward way to human behaviour. Box 6.2 summarises the central themes that have emerged in this chapter.

Box 6.2: Central points about animal models of human behaviour

- Animal models have greatly advanced our understanding of how genes have an effect in the organism and of how the brain develops.
- Animal models can be created by various techniques including selective breeding and the direct manipulation of specific genes.
- Although there are many similarities with regard to genetics between human and non-human animals, there are also considerable differences in the expression of their genes both within the organism and over time.
- It is difficult to equate directly the richness of complex human traits such as intelligence, personality and sexual orientation with the behaviour of animals. This may limit the potential value of the research.
- For these reasons, caution should be exerted when hypothesising that genes studied in research involving animals will have the same effect in humans.