STUDYING GENETIC ORIGINS OF BEHAVIOUR: FRUIT FLIES, KNOCKOUT MICE, HUMANS AND SLEEP

The twenty-first century seems to be the age of studying the genetic origins of behaviour. The ever-increasing knowledge about genes fuels more research into genetic origins.

Generally genetic origins of behaviour can be studied in three ways:

i) Manipulation of genes in fruit flies;
ii) Use of "knockout mice";
iii) Genetic history and human case studies.

Each method has advantages and disadvantages, or they can be combined to gain a more detailed picture of behaviour, in this case, sleep.

Generally animal models for human behaviour are used in two ways (Maxson 2003):

a) To identify and map genes with effects on human behaviour: eg alcoholism and mice;

b) To develop hypotheses about the biological causes of human behaviour: eg memory in aplysia (mollusc), fruit flies, and mice.

FRUIT FLIES

The fruit fly (Drosophila) has become the stalwart of genetic research in all areas [1], mainly because they have only four pairs of chromosomes (Taylor 2007) (table 1).

Fruit flies produce a large amount of saliva, and this contains the chromosomes which are one thousand times larger than normal (Brookes 2001).

Fruit fly studies have been most helpful in understanding the genes involved in circadian rhythms over the last thirty years [2]. These genes have names like "period", "timeless", "Clock", and "doubletime" (Wager-Smith and Kay 2000). Findings are then moved to mice, though the circadian process is genetically slightly different in mice to flies.

[1] Database of research at http://flybase.bio.indiana.edu.
[2] First gene ("period") identified by Konopka and Benzer 1971).

Recently, Ganguly-Fitzgerald et al (2006) found differences in length of sleep depending upon social activity during waking. Those flies with an enriched social environment slept longer, particularly in the day, than those with an impoverished one. The length of sleep was linked to pathways in the brain related to learning and memory, and to seventeen different genes (of forty-three tested).

Socially enriched environments contained thirty flies, while socially impoverished flies were kept alone. The effect of interaction on sleep did not occur when groups of vision, olfactory, or hearing impaired flies were used.

Yuan et al (2006) investigated the role of the neurotransmitter, serotonin, in the sleep. Fruit flies with three genetically altered expressions of serotonin receptors were used, and one of these mutations (d5-HT1A) had shorter and fragmented sleep compared to others. The significant differences ($p<0.01$) were:

- Less than 600 minutes per 24 hours of total sleep versus approximately 800 minutes in controls;
- Average length of sleep bouts less than 20 minutes versus nearly 30 minutes in controls;
- Equal amounts of daytime sleep, but less at night;
- Over twenty bouts of sleep per night versus fifteen in controls;
- In situations of constant darkness, 30% reduction in sleep amount.

In a slightly different type of study, using wild-type strains, Cirelli et al (2005) screened 9000 different lines of flies to find one that slept much less than the average (called "minisleep") (eg 4-5 hours per day versus 9-15 hours). The researchers isolated the cause to a recessive [3] gene mutation on the X chromosome through selective breeding over five generations.

Greenspan et al (2001) are optimistic about the use of fruit flies to study sleep: "Once again flies are proving that they are more like us than one might think" (p145).

[3] Recessive genes require both copies before manifesting the effect, while dominant genes only need one copy from either the biological mother or father.

ADVANTAGES

1. Similarity between fruit-fly and mammalian sleep.
Fruit flies show following criteria used to define mammalian sleep: reduced responsiveness to external stimuli, circadian rhythms of sleep and waking patterns, "rebound" after sleep deprivation (ie increased sleep to compensate for loss), age differences (eg young sleep longer, old have fragmented and less sleep), and caffeine increases waking (Greenspan et al 2001).

2. Possible to manipulate genes in a way not possible with humans.

3. Short life allows observation across whole lifespan and many generations.
Males become sexually mature 12 hours after birth and females three days with lifespan of approximately 15 days (five as adults) (Taylor 2007).

4. Limited number of genes to study, and large chromosomes in saliva.

5. Generates findings that can be tested and applied to humans, and opens the way to studying difficult questions.
"Genetics are the shock-troops of biology" (Edgar and Epstein 1965).

6. Can be controlled and kept in lab conditions.

7. Similarity of genes to humans: eg 74% of human disease-causing genes (Reiter 2003).

8. Similarity of biological pathways in invertebrates and vertebrates.

9. Prolific and easy to breed, particularly as genetically identical.

10. Processes found in flies and in humans suggest an evolutionary basis.

11. Allows testing of sleep mechanisms independently of circadian rhythms: ie mutants mean that surgery to parts of the brain is not needed as in mammals.

12. Ability to isolate genes in way not possible with knockout mice (Greenspan et al 2001).

DISADVANTAGES

1. Limited applicability of results to humans, particularly as only four pairs of chromosomes compared to 23 pairs in humans.

2. Ethics of using animals in such ways. However, this is less of an issue because few people feel as strongly against the use of fruit flies as with mammals.
Fruit flies with different genetic variations can be ordered from suppliers' catalogues by stock number and name: eg Yuan et al (2006) used catalogue number/name: e01363/5HT2RB among others.

3. There are genetic differences: eg 1 X sex chromosome produces a male and XX produces a female compared to XX (female) and XY (male) in humans.

4. Only small number of genes will make a difference if genes work by their interactions rather than individually.

5. Genetic manipulation can produce unexpected results, particularly if genes have more than one function or role.

6. Ignores the ability of humans to learn and adapt.

7. Problems in observing sleep in small insects. It requires using visual, infrared, and ultrasound equipment (Greenspan et al 2001).

8. Genes varying in animals may not vary in humans or vice versa: ie same genes but different roles in animals and humans (eg "dunce" gene involved in memory formation in flies, but in mood in humans; Davis 2005).

9. If sleep is a whole brain process, then isolating individual genes is of limited use.

Table 1 - Advantages and disadvantages of using fruit flies to study genetics of sleep in humans.

KNOCKOUT MICE

"Knockout mice" are those animals with specific genes "turned off" in order to see the effect. The gene has been inactivated by replacing it with an artificial piece of DNA. The observed effect is then used to understand the normal role of the gene (table 2).

Breeding programmes then produce more animals with that gene turned off. Newman (2007) noted that the International Mouse Knockout Consortium's desire to have 20 000 different knockout mice (each with one gene turned off) will need a breeding programme of seven million animals to maintain it.

Knockout mice are made by taking embryonic stem cells from a four day old embryo. An artificial piece of DNA is inserted into the cells in a process called gene targeting or homologous recombination, and then the cells are injected back into the embryo (National Human Genome Research Institute 2007). An alternative known as gene trapping places random DNA rather than non-active pieces into the cells.

In terms of studying sleep disorders, Chemelli et al (1999) turned off a gene related to orexin production and produced behaviour in the mice similar to narcolepsy (eg sudden onset of sleep; unusual EEG patterns).

HUMAN CASE STUDIES

Work with humans involves studying specific individuals or groups and then constructing a genetic history to isolate specific genes from blood samples (table 3).

ADVANTAGES

1. Humans and mice share many genes.

2. Saves time for researchers and allows them to focus on the particular gene of interest.

3. Involves studying live animals.

4. Can be controlled and bred relatively easily in labs.

5. Exact details of the effects of the genes can be ascertained from observation from life and from post-mortem studies of the brain.

6. Allows elaboration on observation from studying humans.

7. Can lead to discovery of causes of behaviour (which can then be tested on humans).

8.. Whole lifespan and subsequent generations can be studied.

9. Good way to test hypotheses when it is not known which gene involved in behaviour.

10. Processes found in mice and in humans suggest an evolutionary basis.

11. Human sleep disorders, like advanced sleep phase syndrome, show similarities to lab mice with mutations to a particular gene ("dbt"; "doubletime") controlling circadian rhythms (Wager-Smith and Kay 2000).

DISADVANTAGES

1. Turning off a gene and seeing the effect is not necessarily the same as understanding the gene turned on. Assumptions have to be made about what is happening (ie deducing from the phenotype [4]). Other genes may compensate for the loss in some way.

2. Turning off a gene can produce a different effect in mice than in humans, no effect in mice but in humans, or vice versa.

3. Effects observed may be due to the interaction of genes not just the one gene being turned off.

4. Genetic manipulation can produce unexpected results including pain and distress to the animal.

5. Ethics of using animals in this way, particularly as they are sold as products by bio-engineering companies.
For example, Tafti and Franken (2006) claimed the "creation" of four new strains of transgenic mouse to study sleep.

6. Genetically manipulated animals are not the same as "normal" ones.

7. Human sleep disorders may be genetically complex and not amenable to the single gene approach, and sleep may be a whole brain process.

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[4] Phenotype is the actual behaviour manifest by the gene.

8. Ignores the flexibility of humans to learn.

9. Some knockouts are lethal (eg 15%; National Human Genome Research Institute 2007), and the mouse does not live to adulthood. Some genes may serve different functions in adulthood than in embryos or infants, and this cannot be established.

10. Gene trapping is a random process and there is no guarantee that anything will happen.

11. Different to flies in some biological processes (eg circadian rhythms), so there must be differences to humans.

12. Same gene but different roles in animals and humans, and genes may have more than one role.

Table 2 - Advantages and disadvantages of using knockout mice to study genetics of sleep in humans.

In the case of the sleep disorder, narcolepsy, for example, first degree relatives (eg mother, father, siblings) of patients with narcolepsy have up to forty times greater risk of developing the condition (Taheri and Mignot 2002). A number of genetic factors have been studied in such families.

Twin studies, both monozygotic (MZ; identical) and dizygotic (DZ; non-identical), can be used. If one twin has a particular behaviour or disorder, how often the other twin has the same thing is calculated as the concordance rate. With MZ twins, who have the same genes, a concordance rate of 100% (1.0) would mean every time one twin has the behaviour or disorder so does the other twin. Thus it would be entirely inherited. A concordance rate of 0% would be the complete opposite. Furthermore, the concordance rate for MZ twins should be higher than of DZ twins if the behaviour or disorder is inherited.

In reality, the concordance rates vary in MZ twins for different aspects of sleep: eg 80% (0.80) for awake-resting EEG patterns, 50% (0.50) for sleepwalking and night terrors (vs 10-15% for DZ twins) (Taheri and Mignon 2002).

Xu et al (2005) constructed a family tree for advanced sleep phase syndrome to show the genetic basis (PER 2 gene). The sufferers fell asleep early in the evening (eg 7-8pm) and woke early the next morning (eg 4-5am). Onset of the condition occurred between early childhood and the mid-teens. In the family, the grandmother was a sufferer, and so were three of her four daughters, and one granddaughter.

Restless legs syndrome (RLS) (or Ekbom's syndrome; Ekbom 1960) is the involuntary movement of the legs

during non-rapid eye movement (NREM) sleep which disrupts the sleep.

In family studies, working with a sufferer (proband), the researchers try to discover how many first degree relatives also have the same condition. A few of the studies use control groups. The frequency of RLS in families of sufferers varies between 40-90% depending on the study (Winkelmann and Ferini-Strambi 2006).

Linkage studies [5] initially suggested genes on chromosome 12 (known as RLS1) in a South Tyrol family and an Icelandic sample, and then chromosome 14 (RLS2) (an Italian family), and 9 (RLS3) (two US families) (Winkelmann 2006; Winkelmann and Ferini-Strambi 2006).

ADVANTAGES
1. Studying humans sleeping is the best way to study human sleep behaviour.
2. Individuals with sleep disorders are better to study than "manufactured" animal versions.
3. Study specialist populations/families where sleep disorders more common.
4. Patient can talk about the experience of sleep or the disorder.
5. Can study patients with EEG or neuroimaging.
6. Can study sleep as a whole brain process.
DISADVANTAGES
1. Not possible to genetically manipulate.
2. Dependent on cases available, particularly for rare sleep disorders.
3. Limited knowledge of cases before came to notice of medical staff or came to the study.
4. Limitations on how they can be studied (eg length of sleep deprivation) compared to animals.
5. Cannot really know what is happening at the microscopic level in the brain.
6. Limitations of EEG and neuroimaging.

Table 3 - Advantages and disadvantages of human case studies to study genetics of sleep in humans.

[5] Linkage studies segregates the family members with or without the condition. It is possible to focus upon a particular loci (position on the chromosome), and to see which copies (allele) exists there for ill family members as opposed to healthy ones.

Humans can also be studied by looking at post-mortem brains (table 4), particularly for those with sleep disorders: eg less orexin neurons in narcoleptics (Thannickel et al 2000).

ADVANTAGES

1. Detailed look inside the brain.

2. Study parts of brain in microscopic detail.

3. Studying humans not animals.

DISADVANTAGES

1. Death may cause change to brain.

2. Confounding variables, like drug addiction, may distort findings.

3. Have to wait for patient to die.

Table 4 - Advantages and disadvantages of post-mortem human case studies to study genetics of sleep in humans.

CONCLUSIONS

Taheri and Mignon (2002) saw the benefits of animal models to study the genetics of sleep, but as complementary to large scale human studies.

Animal models are most effective for understanding disorders. For example, work on the genes that control circadian rhythms in both flies and mice have helped in the understanding of human sleep-wake disorders, like delayed sleep phase syndrome (Wager-Smith and Kay 2000).

REFERENCES

Brookes, M (2001) Fruit fly genetics Guardian 29/3 (Accessed at www.guardian.co.uk on 19/10/07)

Chemelli, R.M et al (1999) Narcolepsy in orexin knockout mice: Molecular genetics of sleep regulation Cell 98, 437-451

Cirelli, C et al (2005) Reduced sleep in Drosophila Shaker mutants Nature 28/4, 1087-1092

Davis, R.L (2005) Olfactory memory function in Drosophila: From molecular to systems neuroscience Annual Review of Neuroscience 28, 275-302

Edgar, R.S & Epstein, R.H (1965) The genetics of a bacterial virus Scientific American 212, 70-76

Ekbom, K.A (1960) Restless legs syndrome Neurology 10, 868-873

Ganguly-Fitzgerald, I et al (2006) Waking experience affects sleep need in drosophila Science 313, 1775-1781

Greenspan, R.J et al (2001) Sleep and the fruit fly Trends in Neuroscience March, 142-145

Konopka, R.J & Benzer, S (1971) Clock mutants of Drosphila melanogaster Proceedings of the National Academy of Sciences, USA 68, 2112-2116

Maxson, S.C (2003) Animal models of human behaviour. In Cooper, D.N (ed) Encyclopaedia of the Human Genome vol 1 London: Nature

National Human Genome Research Institute (2007) Knockout Mice (Accessed at www.genome.gov on 19/10/07)

Newman, C (2007) Top research grants for 2007 Alternative News Autumn, 4-6

Reiter, L.T (2003) Drosophila as a model for human diseases. In Cooper, D.N (ed) Encyclopaedia of the Human Genome vol 2 London: Nature

Tafti, M & Franken, P (2006) Using mice to elucidate genes controlling sleep and wakefulness Journal of Sleep Research 15, supplement 1, s28

Taylor, M (2007) Animal cameo Feedback September, p5

Taheri, S & Mignet, E (2002) The genetics of sleep disorders Lancet Neurology 1, 242-250

Thannickal, T.C et al (2000) Reduced number of hypocretin neurons in human narcolepsy Neuron 27, 469-474

Wager-Smith, K & Kay, S.A (2000) Circadian rhythm genetics: From flies to mice to humans Nature Genetics September, 23-27

Winkelmann, J (2006) Genetics of restless legs syndrome Journal of Sleep Research 15, supplement 1, s28

Winkelmann, J & Ferini-Strambi, L (2006) Genetics of restless legs syndrome Sleep Medicine Reviews 10, 179-183

Xu, Y et al (2005) Functional consequences of a CKI mutation causing familial advanced sleep phase syndrome Nature 31/3, 640-644

Yuan, Q et al (2006) A sleep-promoting role for Drosophila serotonin receptor 1A Current Biology 16, 1051-1062