articles

Recent Developments in Science and Medicine

Marilyn Monk

Religious disbelief linked to analytical thinking

Psychologists Will Gervais and Ara Norenzayan (University of British Columbia, Vancouver, Canada) are interested in the cognitive processes that promote religious belief. They have demonstrated that people have a lower tendency to believe in the supernatural following experimental interventions that encourage analytical thinking. During the tests, volunteers were either engaged in a task that surreptitiously elicited analytical thinking, or were given a control task. They were then asked if they concurred with a series of statements about religion, such as "I believe in God".

The authors do not devalue religious belief nor do they argue that analytical thinking is the enemy of religious belief. These studies simply indicate that analytic processing is one factor that may promote some forms of religious disbelief. They also support the view that scientific training might reduce some forms of religiosity. Obviously, there are difficulties in subjecting religious belief to scientific scrutiny. Moreover, religiosity is difficult to define and it takes many forms. For some it is belief in a personal and interventionist God, in the devil and in angels (in a Judaeo-Christian context which is analysed in this study). Others acknowledge the teachings of Jesus, the power of prayer, the expression of gratitude in celebration, and believe in God as a higher power, maybe synonymous with the totality of existence. It is noteworthy that the beliefs of the great Christian philosophers survived their analytical appraisal.

Reference

Gervais WM and Norenzayan A (2012) Analytic thinking promotes religious disbelief. *Science* 336: 493–496.

Windows of learning in neurodevelopment

Neural circuits establishing our ability to interpret and interact with the world around us are set up during critical windows in time during early childhood (e.g., the ability to learn a language, to recognise faces, or to locate objects in space). In the early 1960's, Hubek and Wiesel (Harvard Medical School) demonstrated the neural basis of a critical period for the development of sight. If they covered one eye of a kitten during a critical period after birth then cells in the visual cortex in the brain that would normally respond to that covered eye were diverted to respond only to the open eye (a model for adult amblyopia). Covering the eye of an adult cat did not change anything.

Once a developmental window is shut it becomes difficult, if not impossible, to learn anything new in that particular realm. Takao Hensch and colleagues (Boston Children's Hospital, Massachusetts) hope that the molecular understanding of this crucial programming of the brain during these critical developmental windows may open the way to interventions in various neurological disorders that 'unlock' the brain and open it to new learning - for example, in adult amblyopia (in which information from one eye is not correctly processed by the brain) and possibly even in autism. In the cat model of amblyopia, a drug known to increase inhibitory interneuron activity in the critical period prevented the silencing of the neurons corresponding to the covered eye. Further work involving collaborators at the RIKEN Brain Science Institute, Japan (M Fagiolini) and College de France (A Prochiantz) identified drugs that enhance and inhibit the effects of interneurons, inhibitory neurotransmitters and regulatory proteins.

The critical periods are normally shut down to protect the newly optimized brain circuits from disruption by further input. However, the researchers found that a new critical period can be reset by transplanted interneurons. The interest now is to investigate drugs that re-open the critical period and a phase I clinical trial for treating amblyopia with a drug that increases the amount of acetylcholine in the brain has been approved. One may imagine the advent of treatments for severe brain injury, or complex disorders such as autism.

Reference

Beurdeley M, Spatazza J, Lee HH, Sugiyama S, Bernard C, Di Nardo AA, Hensch TK and Prochiantz A (2012). Otx2 binding to perineuronal nets persistently regulates plasticity in the mature visual cortex. *J Neurosci*. 4:9429-9437.

Species extinction in Brazilian Amazon – sad predictions for future

Current species extinction in the Brazilian Amazon due to deforestation is only a prelude of what is to come. Even though today just over half of Brazil's rainforest is under protection, the effects of habitat destruction take time to take their toll. Ewers and colleagues (Imperial College, London) derived mathematical calculations to correlate species extinction with rate and magnitude of habitat loss and thus were able to predict future extinctions. They found that local extinctions of forest-dependent vertebrate species in the Brazilian Amazon have so far been minimal (1 per cent of species by 2008). However, even with a scenario that the Brazilian government continue to apply some regulation of deforestation, 80 per cent of extinctions expected to be caused from past habitat loss are still to come - two mammals, five birds and one amphibian per 2,500 square kilometres. and a further 16 vertebrate species committed to extinction by 2050. Although the findings seem bleak, knowledge of where the highest extinction debt is expected provides an opportunity to target these particular areas and conserve the species that are still there.

Reference

Wearn OR, Reuman DC and Ewers RM. (2012). Extinction debt and windows of conservation opportunity in the Brazilian Amazon. *Science* 337, 228–232.

Selfish or altruistic? – games people play

Does selfishness pay? Scientists, Press and Dyson (University of Texas, Austin, and Princeton, New Jersey, USA) use game theory (a branch of mathematics that examines competitive decision making) to question the accepted view of the evolution of altruism. In the game, Iterated Prisoner's Dilemma, a player may be tempted to freeload – to take the spoils while allowing the other(s) to do the work and take the risks. But if the game is played often enough, the selfish player learns that it is best in the long run to behave altruistically. It has been generally assumed that, in an evolutionary sense, co-operation emerges with time. However, Press and Dyson now claim to show, unexpectedly, that strategies do exist for one player to enforce a claim to an unfair share of rewards. The multiple ways in which the game can be played allows for a number of outcomes depending on different strategies. If both players are playing Tit for Tat – each co-operating when the other co-operates but defecting where the other defects - then in the long run it becomes clear to the players that co-operation is best. The new

finding claims to demonstrate that the game can be played so as to be successfully selfish – instead of Tit for Tat the selfish player can calculate his move employing a probabilistic strategy according to the last player's decision to co-operate or defect. More recently, scientists Adami and Hintze claim that the Press and Dyson selfish strategy is still evolutionarily unstable and will eventually be outcompeted by more generous players. The argument depends on what sort of game your opponent is playing and certainly individual people will vary in their approach. Perhaps it is not an either/or answer. Either altruistic or selfish strategies could be operating at different times, influenced by the different players, and according to different circumstances.

Reference

Press WH and Dyson FJ (2012). Iterated Prisoner's Dilemma contains strategies that dominate any evolutionary opponent. *Proc Natl Acad Sci U S A.* 109:10409-10413.

Increase in mutation in sperm with increasing age of father.

Mutations accumulate in cellular DNA during rounds of DNA replication at cell division. Therefore, since sperm are derived from continuous rounds of cell division (mitosis) in the testis, mutations accumulate in sperm DNA with increasing age of the father. This is not the case with the eggs from the mother because the entire egg population is already formed in the female foetus during development in the womb. Dr Kari Stefansson, and colleagues (deCODE Genetics, Reykjavik, Iceland) investigate mutation rate with age in 78 Icelandic parents and their offspring. The team searched for mutations in the child that were not present in either parent and that must therefore have arisen spontaneously in the egg, sperm or embryo. Fathers pass on nearly four times as many new mutations as mothers. Moreover, the number of mutations per year increase as the fathers age - a 36-year-old man will pass on twice as many mutations to his child as a man of 20, and a 70-year-old eight times as many Most mutations identified are effectively neutral so the effect is not enormous. Also, mutations provide sequence diversity and provide a substrate for selection and very occasionally this might happen to advantage. However, it is significant that some of the mutations identified are in genes that have been indicated in diseases like autism and schizophrenia. The father's age does need to be taken into account with the recent increase in these diseases that are thought to result from mutations in several genes. For this, and other, reasons men and women should not delay parenthood.

Reference

Kong A, Frigge ML, Masson G, Besenbacher S, Sulem P, Magnusson G, Gudjonsson SA, Sigurdsson A, Jonasdottir A, Jonasdottir A, Wong WS, Sigurdsson G, Walters GB, Steinberg S, Helgason H, Thorleifsson G, Gudbjartsson DF, Helgason A, Magnusson OT, Thorsteinsdottir U and Stefansson K. (2012). Rate of de novo mutations and the importance of father's age to disease risk. *Nature* 488:471-475.

Growing an eye (and other parts of the brain) in a dish

One of the most amazing and elegant developments in the field of coaxing stem cells into different tissues in a culture dish, is the growth of an optic cup (back of the eye in a developing embryo). The optic cup structure forms in culture by self-organisation of human embryo stem cells (ESC) into the convex multilayered neural retina containing both rods and cones. The accumulation of photoreceptors in human ESC culture could be the prelude to a treatment or cure for eye disease such as retinitis pigmentosa or macular degeneration. The delicate and thoughtful approach of Takano and Sasai (RIKEN Center for Developmental Biology, Kobe, Japan) is noteworthy. Instead of over-supplementing the culture media with cocktails of growth factors, blood serum and supporting tissues, they relied on the stem cells themselves knowing what to do with only a little help from physical and chemical cues. The stem cells form the structure of their own accord. Sasai discovered that what is important is the reduction of confusing outside signals rather than throwing in more of them. Embryonic stem cells tend to differentiate towards neural tissue as a first option by default and very little else is needed to encourage this. In addition, the scientists grew the human embryonic stem cells in suspension in matrigel instead of restricting their potential due to their tendency to attach to the surface of the dish. Sasai and co-workers are also growing different parts of the brain in culture. They have succeeded in growing a cerebral cortex, the beginnings of a pituitary gland (making hormones), and the beginnings of a cerebellum. In the case of the eye, although the stem cells can develop into a convincing looking optic cups in the culture dish, the research is a long way off the stage of transplanting an eye in a patient. It is difficult to imagine how the structure could make proper nerve connections to reach and wire the visual cortex at the back of the brain. At this stage, the scientists are busy working towards implanting lab-grown retinas into mice, monkeys and humans.

Reference

Nakano T, Ando S, Takata N, Kawada M, Muguruma K, Sekiguchi K, Saito K, Yonemura S, Eiraku M and Sasai Y (2012). Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell.*10:771-785.

Mutation in a single gene allows the pacing gait in horses

Horses exhibit three main forms of movement – walk, trot and gallop. In all these gaits hindleg and foreleg on opposite sides move forward together. Also, in humans, as one leg moves forward, the arm on the opposite side swings forward. Horses however, may also exhibit the unusual gait of pacing, an advantage in harness racing. The pacing horse moves the front and back leg on the same side in unison rather than the usual diagonal movements. Andersson and colleagues (Department of Animal Breeding. Uppsala. Sweden) have studied the genomes of pacing horses to show that this unusual gait is due, surprisingly, to a mutation in a single gene (called DMRT3). Mice made deficient for this gene were also able to move their front and back legs on the same side in unison. It is surprising that such complex co-ordination of movement is controlled by a single gene. Co-ordination of limb movement in mammals is regulated by the arrangement of spatial interneurons in the spinal cord during development. The left-right alternation of limbs is controlled by the co-ordinated activation of flexor and extensor muscles. The mutation in DMRT3 is essentially permissive for alternative gaits. This work may lead to a greater understanding of movement disorders in humans.

Reference

Andersson LS, Larhammar M, Memic F, Wootz H, Schwochow D, Rubin CJ, Patra K, Arnason T, Wellbring L, Hjälm G, Imsland F, Petersen JL, McCue ME, Mickelson JR, Cothran G, Ahituv N, Roepstorff L, Mikko S, Vallstedt A, Lindgren G, Andersson L and Kullander K (2012). Mutations in DMRT3 affect locomotion in horses and spinal circuit function in mice. *Nature* 488: 642–646.

Fruit, vegetables, exercise and abstinence from alcohol and cigarettes combine to triple chances of healthy old age

Severine Sabia and coworkers (University College London) are interested in behaviours leading to healthy ageing. They investigated the health of 5,100 individuals who were previously classified as free from cancer, heart disease and stroke in the period 1991-1994 when they were aged 42-63 years. Those that were still alive (549 had died) were re-assessed in the period 2007-2009 and their current health correlated with their lifestyles during the interval period (average 16.3 years). Those that never smoked, drank only moderate amounts of alcohol,

exercised regularly, and ate a daily diet including fresh fruit and vegetables, were shown to have a three times greater expectancy of healthy ageing. Healthy ageing (953 individuals) is defined as good cognitive, physical, respiratory and cardiovascular functioning, in addition to the absence of disability, mental health problems and chronic disease (coronary artery disease, stroke, cancer and diabetes). Others engaging in only one or two of the healthy behaviours during the interval since first assessment aged normally. The important message is that the combined impact of these four healthy behaviours is clearly substantial - the greater the number of healthy behaviours, the greater the benefit. Since we live longer today it is important that we are as healthy as possible for as long as possible.

Reference

Sabia S, Singh-Manoux A, Hagger-Johnson G, Cambois E, Brunner EJ and Kivimaki M (2012). Influence of individual and combined healthy behaviours on successful aging.
Canadian Medical Association Journal.Oct 22.

Honeybees programmed reversibly for different roles

All bee cells have the same complement of genes. Different caste phenotypes (e.g., forager bees, warrior bees, worker bees, nurse bees) are created from the same genotype by epigenetic programming, i.e., modification of gene DNA to programme which genes are active. Differential DNA methylation regulates different bee behaviours. The origin of different behaviours, whether the larva will grow into a queen bee or a worker bee, is associated with care of the larva and how much royal jelly it is fed by the worker bees. Scientists Herb, Feinberg and colleagues (Johns Hopkins University School of Medicine, Baltimore, USA) have shown that worker bees exhibit different roles at different ages, i.e., the programming of behaviour in bees changes with developmental age and may be reversed and changed to another behavioural type. They found differences in methylation of bee DNA between nurses and forager subcastes. Nurse bees can switch to foraging later in life and this was shown to be associated with changes in methylation of 150 regions of the DNA. Thus, in bees, reversible epigenetic changes are associated with changes in behaviour.

Reference

Herb BR, Wolschin F, Hansen KD, Aryee MJ, Langmead B, Irizarry R, Amdam GV and Feinberg AP (2012). Reversible switching between epigenetic states in honeybee behavioral subcastes. Nat Neurosci. 15:1371-1373.

Increasing number of journal retractions are implicated with fraud

Since 1975 there has been a tenfold increase in the number of life science journal retractions associated with fraud. A recent survey by Fang, Steen and Casadevall (University of Washington, Seattle, USA) of over 2,000 papers retracted and removed from academic record, found that only 21.3 per cent of retractions were attributable to error, whereas 67.4 per cent were due to scientific misconduct (43.4 per cent fraud or suspected fraud, and a further 24 per cent due to duplicate publication or plagiarism). Moreover the distribution of fraud cases is uneven with respect to Journal and country of origin. Journals with higher impact factors are more susceptible and the USA and Germany are the major culprits. Recurring offenders were also identified in the study. It is not known whether the increase in fraud is due to greater vigilance by the higher-ranking journals or improved methods of detection. The increased insecurity of careers in science with the reduced availability of grant money and the decrease in tenured appointments may also be playing a part together with the increase of media hype. In the 'olden days' the idea of scientific fraud was regarded with considerable horror. It is hoped that the journals will be more open and clear about the criteria underlying their retraction notices. It will be interesting to see such studies carried out to discover the frequency of fraud in other fields of research. A retractions database based on studies such as the Fang et al study must be established so that scientists will avoid wasting time trying to replicate retracted work.

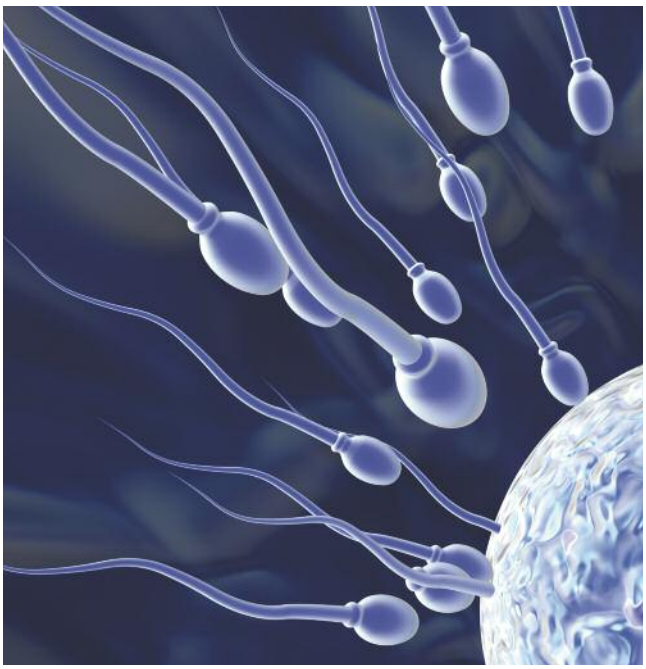
Reference

Fang FC, Steen RG and Casadevall A (2012).
Misconduct accounts for the majority of retracted scientific publications.
Proc Natl Acad Sci U S A. 109:17028-17033,

Making sperm and eggs in the lab

Embryonic stem cells can be isolated from an early embryo (embryonic stem cells, ESC) or created in the lab by the introduction into a somatic (body) cell of four genes that are known to be specifically expressed in early embryos (induced pluripotent stem cells, iPSC). ESC and iPSC can be coaxed to develop into different specific cell types in culture since they are pluripotent (potential to develop into all cell types of an individual).

Recently, Hayashio and Saitou and colleagues (Department of Anatomy and Cell Biology, Kyoto University, Japan) have coaxed ESC and iPSC to develop into sperm or eggs by appropriate stepwise manipulation of the culture conditions. The cells were



first encouraged to develop into implantation stage embryonic stem cells, which then become capable of development into primordial germ cells (PGCs). The culture procedures were carefully monitored to reflect the changes that occur in a normal developing embryo *in vivo*.

The male PGCs (XY) created in the lab could be injected directly into the testes of infertile male mice to mature into sperm. In the case of female cells, the cultured cells taken stepwise to the primordial germ cell stage, were incubated in a dish with fragments of mouse ovary devoid of their own germ cells. Given this environment the cultured female (XX) stem cells colonised the ovary and made eggs. The colonised ovary was transplanted into the ovary of a female mouse where the cultured cells underwent final stages of differentiation into mature eggs. Following fertilisation of these eggs, created from somatic cells, they gave rise to viable offspring which themselves were fully fertile.

Saitou's group is now trying to make PGCs from human somatic cells.

Reference

Hayashi K, Ohta H, Kurimoto K, Aramaki S. and Saitou M (2011)). Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells. *Cell* 146: 519–532.

Hayashi K, Ogushi S, Kurimoto K, Shimamoto S, Ohta H and Saitou M. (2012). Offspring from oocytes derived from in vitro primordial germ cell-like cells in mice. *Science*.338:971-975.

Nobel Prize to Gurdon and Yamanaka for the origin of cloning and creation of embryonic stem cells in the lab

Cloning is the creation of a copy of an existing life form. It is carried out by taking a somatic cell (say a skin cell) from an individual and using that cell to make a new embryo and thence a new individual - a copy of the original life form. Cloning arose from wanting to know the difference between a somatic cell and an embryo or germ cell. Somatic cells of the body are mainly differentiated cells committed to performing a particular function. There are nerve cells, muscle cells, skin cells – there are about 100 different cell types performing particular tasks to serve the greater whole - you. Tissue-specific stem cells residing in our bodies constantly replenish different organs and tissues - for example, haemopoetic stem cells continually give rise to all the cells of the blood, neural stem cells give rise to the differentiated cells of the nervous system, and so on. These tissue specific stem cells, with limited potential, are called pluripotent. In contrast, the fertilised egg and early embryonic cells at the very beginning of development of the new individual are totipotent - they have the potential to make all the cells of the body. It has been clear that the totipotent, pluripotent, and differentiated cells with different developmental potential are differently programmed in some way. The differential programming superimposed upon the genes was given the name epigenetics by Waddington in the 50's but the mechanisms of epigenetic programming remained unknown for a further 20 to 30 years and are still being uncovered today.

Gurdon's brilliant contribution to the field in 1962 was to show that a differentiated cell nucleus could be turned into a totipotent nucleus. He transplanted a nucleus from a cell from a tadpole into a frog egg whose own nucleus had been removed. That egg then developed into another tadpole. This meant that the nucleus in the original tadpole's cell had not forgotten how to begin again and make a whole new frog, i.e., the programming in a somatic cell of a tadpole could be reversed to the totipotent state in the cytoplasm of the frog egg.

My own work was very much influenced by Gurdon's discovery. As a molecular biologist I wanted to know the molecular mechanism of differential programming of the nuclear DNA in the differentiated cell and how could it be erased to form an embryonic stem cell. In my earlier field of work in microbial genetics, it was known that bacteria modify their DNA by the addition of methyl groups to the DNA bases. In this way bacteria could identify their own DNA from foreign DNA such as bacterial viruses – a primitive immune system. In the early 80's we were able to show that DNA methylation was also involved in gene silencing in mammals (Lindsay et al, 1984). Following this we showed that erasure of this methylation (removal of methyl groups from the DNA) occurred in early embryonic development and that this was the mechanism of deprogramming (return to *tabula rasa*) to form the totipotent embryonic stem cell (Monk et al, 1987). Moreover, we showed that it was possible that some gene programming could escape erasure and thus influence expression of a gene in the next generation (providing a molecular basis for Lamarckian inheritance; Zuccotti and Monk, *Nature* 1995). Now epigenetic programming is being rediscovered everywhere – it is an exciting time in the understanding of differential gene function regulating different cell function and behaviour.

Following Gurdon's major discovery, cloning became rather quiet until the appearance of Dolly the sheep in 1996. This feat was essentially the same experiment that Gurdon achieved in the frog, i.e., the transplant of a nucleus from a skin cell of a sheep by Wilmut and colleagues into a sheep egg whose own nucleus had been removed and transfer of that egg into a surrogate mother to give rise to a cloned sheep. Cloning in this way is now reproducibly carried out in many animals (though banned in humans).

The important contribution of Yamanaka and colleagues in 2006 was to 'manufacture' mouse embryonic stem cells in the laboratory. Yamanaka introduced four genes known to be normally specifically expressed in the totipotent embryo stem cells into a differentiated mouse skin cell from an adult. This directly converted the skin cell into an embryonic stem cell (without the need for an egg). These cells are called induced pluripotent stem cells – or iPSC. He also showed that the induced pluripotent stem cells could give rise to new mice which are fertile and themselves can give rise to healthy offspring.

The importance of this work lies in the emergence of the field of regenerative medicine. Adult skin cells may be deprogrammed into the pluripotent state and reprogrammed into a specific tissue type for the treatment of disease or injury. This advance removes the necessity to repress the patient's own immune system since the transplanted cells are the patients' own cells.

Reference

Gurdon JB. (1962). The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *J Embryol exp Morphol* 10:622-640.

Takahashi K and Yamanaka S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126:663-676.

Monk M, Boubelik M and Lehnert, S. (1987). Temporal and regional changes in DNA methylation in the embryonic, extraembryonic and germ cell lineages during mouse embryo development. *Development* 99:371-382.

Marilyn Monk *is UCL Emeritus Professor of Molecular Embryology at the Institute of Child Health, and Honorary Professor at Melbourne and Monash Universities, researching gene expression and its regulation in development and cancer. She is also an Alexander Technique Teacher and Psychosynthesis Counsellor.*