Sex on the brain

Reports of morphological differences between the brains of humans with different sexual orientation or gender identity have furthered speculation that such behaviours may result from hormonal or genetic influences on the developing brain. However, the causal chain may be reversed; sexual behaviour in adulthood may have caused the morphological differences. I report how adult sexual experience alters the appearance of rat motor neurons as revealed by Nissl staining, the same technique used in post-mortem human studies.

Male Sprague-Dawley rats were castrated at 117–123 days of age and I implanted 5-mm-long testosterone-filled Silastic capsules subcutaneously, to produce a much lower than normal concentration of circulating androgens while sustaining continued male copulatory behaviour. One week later I gave each rat a cage-mate as an ovariectomized female that had been subcutaneously implanted with either a 5-mm capsule containing 10% oestradiol or an empty capsule.

Male rats provided with oestrogen-treated cage-mates began copulating shortly after the introduction of the receptive female and were therefore labelled 'copulators'. The males caged with untreated (and therefore unreceptive) females were never observed to copulate and are designated 'non-copulators'. Females were replaced from a reserve pool every 3 days (with confirmation that only the oestrogen-treated females were receptive) for 27 days.

I stained the spinal cords from the males with thionin to reveal motor neurons in the spinal nucleus of the bulbocavernosus (SNB). These motor neurons and their striated target muscles are active during male copulatory behaviour, and shrink after castration unless replacement testosterone is provided. Changes in the size of SNB somata and nuclei are accompanied by changes in the number and size of synaptic inputs to the motor neurons. An observer, blind to group membership, determined the number and cross-sectional area of SNB somata and nuclei.

As expected, SNB somata and nuclei were smaller than in gonadally intact males, and there was no difference between the groups in the number of SNB motor neurons. The number of neurons, which is reported in only a few human studies, appears stable. The ten copulator males had significantly smaller SNB somata (P < 0.04, two-tailed t-test) and nuclei (P < 0.02) than the nine non-copulators. The bulbocavernous muscles innervated by the SNB were also lighter in copulators (711±37 mg; mean±s.e.m.) than in non-copulators (686±50; P < 0.02). The animals received equivalent androgen exposure as shown by the lack of difference in either body mass (P > 0.20) or the mass of the highly androgen-sensitive seminal vesicles (168.5±12.9 mg, copulators; 177.8±11.9 mg, non-copulators).

Copulatory experience can therefore alter the size of neurons, as revealed by Nissl staining. Whether the sensory experience or motor activity of copulation induced these morphological changes, interpretations of correlations between human behaviour and neural morphology must acknowledge that the two are reciprocally related. It is possible that differences in sexual behaviour cause, rather than are caused by, differences in brain structure.

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Antibiotic resistance spread in food

Nutritive and therapeutic treatment of farm animals with antibiotics, amounting to half of the world's antibiotic output, has selected for resistant bacteria that may contaminate the food produced. Antibiotic-resistant enterococci and staphylococci from animals are found in food when they survive the production processes, as in raw cured sausages and raw milk cheeses. The broad host ranges of some plasmids and the action of transposons in many bacteria will allow antibiotic-resistance genes to be communicated by conjugation between different species and genera. A multi-antibiotic resistance plasmid from a lactococcus found in cheese provides a historical record of such events.

Lactococci function as starter cultures for lactic fermentation in cheese and have conjugative 'metabolic' plasmids. The streptomycin-, tetracycline- and chloramphenicol-resistant Lactococcus lactis strain K214 was isolated by us in 1993 from a raw milk soft cheese (2 × 10⁶ colony-forming units (c.f.u.) per g). A circular 29,871-base-pair plasmid (pK214) transferred all three resistances to Enterococcus faecalis strain JH2-2 by electroporation. We determined the complete nucleotide sequence of pK214 and identified homologous molecular structures in other organisms (Table 1).

A putative Lactococcus region of the plasmid contains genes for a resolvase, a replication-associated protein, a DNA nickase, insertion sequence IS904 and a new insertion-sequence element. A new membrane-spanning efflux protein confers increased macrolide resistance (erythromycin and others) on Escherichia coli, but not on strain K214 itself. A repB gene and tandem repeats (inters) identify pK214 as a theta-type replicating plasmid.

A mainly Staphylococcus-derived segment contains the information for the streptomycin-inactivating adenylyase, the chloramphenicol-inactivating acetylase and replication protein RepD, known from staphylococcal plasmids. A mobilization gene is homologous to a gene from a Lactobacillus plantarum plasmid. The region is flanked on both sides by insertion-sequence elements from Enterococcus faecium.

The tetracycline-resistance gene (providing ribosome protection) is 99.8% homologous to tet(S) from Listeria monocytogenes. It is joined by sequences similar to a region of transposon Tn916 from E. faecalis which is involved in tet(M) expression. Another E. faecium insertion-sequence element complements the assembly.

Fifteen open reading frames (ORFs) including six insertion-sequence elements and DNA rearrangement, regulation, mobi-
zation and replication. Seven ORFs have to do with antibiotic resistance and seven are unknown, ill-defined or truncated. No known plasmid-transfer genes are present. Whether conjugative mobilization of plasmid pK214 can be triggered by the other three plasmids of strain K214 (sizes roughly 10, 20 and 50 kilobase pairs) remains to be established.

In plasmid pK214, Lactococcus K214 has, with the help of insertion-sequence elements, collected genetic information from four other species to construct an antibiotic survival kit that also works in E. faecalis. pK214 is a live record of previous genetic exchange between pathogenic and non-pathogenic bacteria in food-associated environments. It is further demonstration of the presence of transmissible antibiotic-resistance genes in the human food chain. The resistant bacteria probably originated from antibiotic treatment of the cows. As lactococci may be found together with enterococci and staphylococci as part of the cows microflora, resistance transfer and pick-up may have occurred in the animals or during cheesemaking, where enterococci, listeria and staphylococci survive and eventually multiply.

When analysed in 1991 and 1995, this cheese brand had also contained different enterococci resistant to tetracycline, chloramphenicol, gentamicin, penicillin, erythromycin, lincomycin and vancomycin (mechanism unknown but neither vanA nor vanB) at 10⁻⁸–10⁻⁹ c.f.u. g⁻¹ (ref. 1). This cheese was the source of a Listeria monocytogenes epidemic in 20 patients in 1995⁵. To preserve the life-saving potential of antibiotics, the spread of resistance genes at all levels must be stopped⁶. Distribution routes like those between animals, food and consumers have to be interrupted. In this example, it could be achieved by using pasteurized milk for cheese making. The situation might also be helped by stopping the inappropriate use of the selective antibiotics in animal husbandry. 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Targeted disruption in Arabidopsis

Homologous recombination has been used for two decades to target insertions into cloned genes in bacteria and yeast, and more recently has become a routine method of gene inactivation in mammals. Arabidopsis is one of several multicellular model organisms (along with Drosophila, Caenorhabditis and zebrafish) in which mechanisms controlling development have been studied. Previously, traditional genetic methods have been used, as targeted disruption by homologous recombination has not been successful in any of these organisms. We have now successfully disrupted the AGL5 MAD-box gene in Arabidopsis by homologous recombination, providing a useful tool for future analyses.

Experiments in cultured Arabidopsis cells using a root transformation procedure have indicated that targeted disruption of cloned genes should be possible⁷. But regeneration was not possible from this cell line, prohibiting the isolation of homozygous lines. Seven ORFs have to do with antibiotic resistance and seven are unknown, ill-defined or truncated. No known plasmid-transfer genes are present. Whether conjugative mobilization of plasmid pK214 can be triggered by the other three plasmids of strain K214 (sizes roughly 10, 20 and 50 kilobase pairs) remains to be established.

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