Brain asymmetry and long-term memory

Fruitflies that have structurally similar brain hemispheres forget within a matter of hours.

The asymmetrical positioning of neural structures on the left or right side of the brain in vertebrates[1,2] and in invertebrates[3,4] may be correlated with brain laterality, which is associated with cognitive skills[5]. But until now this has not been illustrated experimentally. Here we describe an asymmetrically positioned brain structure in the fruitfly *Drosophila* and find that the small proportion of wild-type flies that have symmetrical brains with two such structures lack a normal long-term memory, although their short-term memory is intact. Our results indicate that brain asymmetry may be required for generating or retrieving long-term memory.

Detailed inspection of the *Drosophila melanogaster* wild-type brain revealed an unknown structure present in the right hemisphere (asymmetrical body: 'AB' in Fig. 1). This asymmetrical body is round (diameter about 10 μm), expresses the neural protein fasciclin II (FasII)[6] and is localized near the fan-shaped body, which connects the left and right hemispheres[7].

We searched for natural exceptions to this asymmetry in the wild-type population and identified the FasII-expressing structure in both brain hemispheres in a small proportion of flies (7.6%; $n=2{,}550$) (Fig. 2a, b). These wild-type flies showed no other anatomical differences compared with flies whose brains were asymmetrical in this respect. In particular, the specific brain regions involved in long-term memory[8] appear to be normal in flies with symmetrical brains (see supplementary information). The distribution of symmetrical and asymmetrical brains is similar in the wild-type strains *Canton-Special* and *Berlin*, and in males and females.

We tested the memory of flies that had a symmetrical brain. Wild-type flies were trained to associate an odour with an electric shock in two experimental protocols: one in which a single training cycle was used to induce short-term memory[9], and another in which intensive conditioning, consisting of five individual training sessions interspersed with 15-min rest intervals, was used to induce protein-synthesis-dependent long-term memory[8,10].

Flies were tested after three hours for their short-term memory, and after four days for long-term memory. Flies that made correct choices during the test were processed separately for staining with an anti-FasII antibody from those that made incorrect choices. We then recalculated a memory-performance index post mortem for wild-type flies with asymmetrical and symmetrical brains.

Flies with symmetrical and asymmetrical brains performed comparably in the test after three hours (Fig. 2c), which indicates that brain asymmetry is not required to establish short-term memory. It also shows that flies with symmetrical brains are not defective in learning, or in odour or shock perception. However, four-day long-term memory was not evident in wild-type flies with a symmetrical brain (Fig. 2c).

Our findings indicate that structural asymmetry is important in the formation or retrieval of long-term memory in *Drosophila*. Further investigation should clarify the position of the asymmetrical body in the neural circuitry of the olfactory memory process.

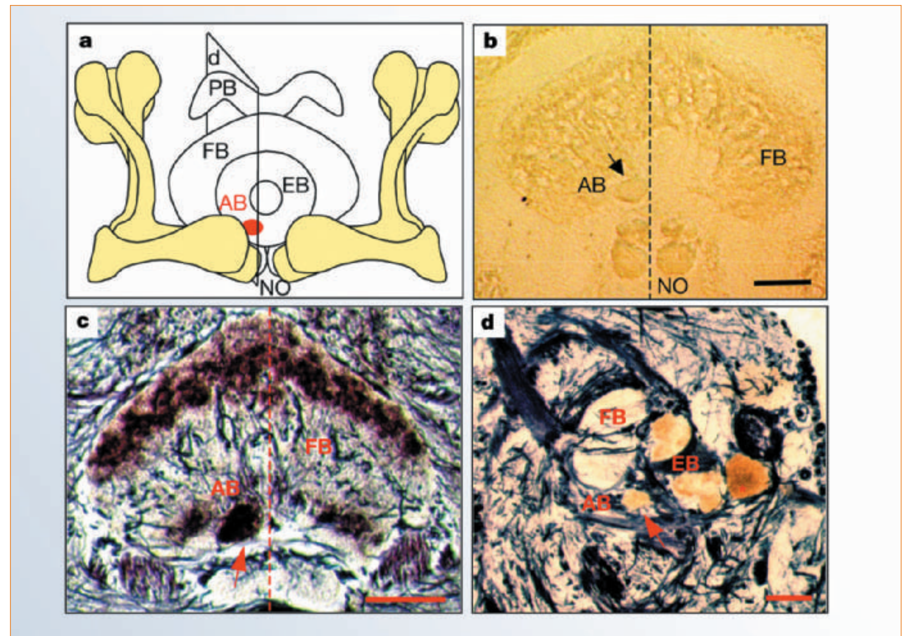

Figure 1 A structural asymmetry in the *Drosophila* brain. **a,** Central brain (dorsal, above; anterior, foreground); the sagittal-cut plane is shown ('d'). The mushroom-body lobes (yellow) and the ellipsoid body (EB), the fan-shaped body (FB), the noduli (NO) and the protocerebral bridge (PB) are shown; the asymmetrical body (AB) is in red. **b,** Frontal paraffin section, showing frontal structural brain asymmetry (arrow). **c,** Frontal section of a wild-type brain double-stained with anti-FasII antibody (brown) and Bodian staining (black and pale red). **d,** Sagittal section of wild-type brain stained as in **c**. The FasII antibody reveals the AB, the EB and the tip of the β-lobe of the mushroom body (red asterisk). Scale bar, 20 μm. Dashed lines in **b** and **c** indicate the midline.

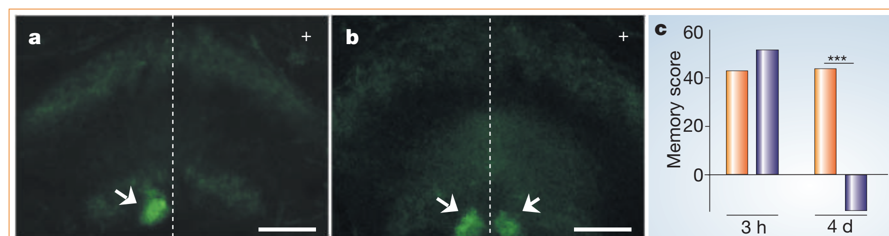

Figure 2 An asymmetrical brain is required to form or retrieve long-term memory in *Drosophila*. **a,** Single confocal section showing the asymmetrical body (arrow) in a wild-type brain, labelled by using anti-FasII antibody. **b,** In a few wild-type flies, the brain is symmetrical, presenting a double structure (arrows). Dashed lines in **a** and **b**, midline. Scale bar, 20 μm. **c,** Memory was measured three hours after a single conditioning cycle (3 hours, 1 cycle) and four days after five spaced cycles (4 days, 5 cycles). The 3-hour memory of flies with symmetrical brains (purple bars) was not significantly different from that of flies with an asymmetrical brain (orange bars; χ^2 test, $P>0.3$). However,long-term memory was significantly decreased in wild-type flies with a symmetrical brain in comparison with flies with an asymmetrical brain (χ^2 test, $P<10^{-7}$). For full methodological details, see supplementary information.

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Evolutionary genetics

CCR5 mutation and plague protection

A recent and prevalent mutation in the chemokine receptor CCR5 in humans of northern European ancestry has been proposed to provide protection against bubonic plague[1,2]. Here we infect both normal and CCR5-deficient mice with the bacterium *Yersinia pestis*, the cause of the plague epidemics that wiped out one-third of Europeans in the Middle Ages[3], and find no difference in either bacterial growth or survival time between the two groups. Unless the pathogenesis of *Yersinia* infection differs markedly between mice and humans, our results indicate that CCR5 deficiency in people is unlikely to protect against plague.

A 32-base-pair deletion in the coding region of *CCR5*, a mutation designated as *CCR5Δ32*, was first identified in individuals who had been exposed to HIV but who seemed to be resistant to infection[4]. This *CCR5Δ32* allele is mainly confined to caucasians[5,6], and its protective effect in homozygotes against transmission of the most common HIV-1 isolates arises because both CCR5 and CD4 are needed as co-receptors for entry of the virus into the cell.

Because the *CCR5Δ32* allele shows evidence of very strong selection, it has been suggested that it may protect against another disease associated with high mortality[1,2]. A candidate agent is *Yersinia pestis*, which emerged shortly after the estimated origin of the *CCR5Δ32* mutation (about 800 years ago), and killed some 25 million people in the Black Death plague of 1346–52.

If plague was responsible for driving this selection, the *CCR5Δ32* genotype should alter the host response to *Y. pestis* infection to improve the survival rate. But, because plague is no longer common among caucasians, the allele has not been tested for a protective effect. We therefore compared the susceptibility of two groups of mice, with and without CCR5 deficiency, to infection and death following challenge with *Yersinia*.

The homozygous *CCR5Δ32* genotype is associated with intracellular retention of a truncated CCR5 protein and ablation of the chemokine response to macrophage inflammatory protein-1β (ref. 6), whereas heterozygotes have a reduction in surface expression of CCR5 and slower progression from HIV infection to AIDS[5]. The lack of CCR5 at the cell surface has no obvious deleterious effect in humans. Mice with homozygous deletion of CCR5 have subtle immunological abnormalities, including in controlling infection by intracellular organisms such as *Listeria*, *Cryptococci* and *Leishmania*[7–9].

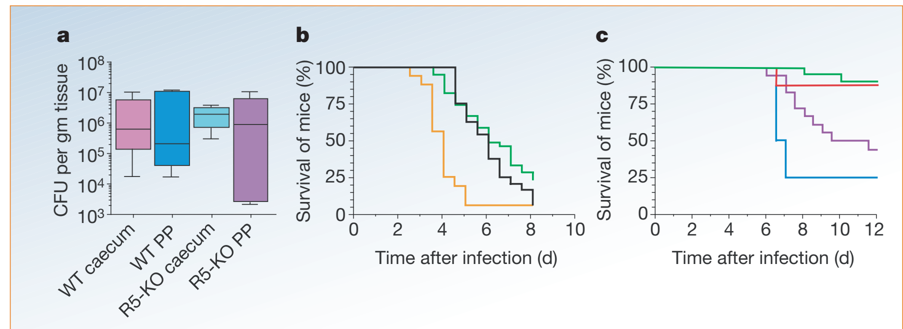

Figure 1 Impact of CCR5 deletion on the growth of *Yersinia pseudotuberculosis* and survival of mice after infection with *Y. pestis*. **a,** Bacterial growth in the caecum or in Peyer's patches (PP) of mice infected by orogastric lavage (D. Monack) with 2×10^9 *Y. pseudotuberculosis* YPIIIYV. Bacterial colony-forming units (CFU) are per g tissue at 4 days after infection. Plot shows the median, the range from 25th to 75th percentile (boxed) and the data range. WT, normal wild-type C57BL/6 mice; R5-KO, CCR5-deficient C57BL/6 mice. **b,** Comparison of survival of BALB/c mice with and without CCR5 ($n=40$ per group), over 10 days following intravenous challenge with 10^2 *Y. pestis* KIM, substrain D27 (Pgm−, LcrV+). Green, CCR5 WT females; orange and black, CCR5-deficient males and females, respectively. **c,** Survival of mice after intravenous challenge with about ten *Y. pestis* organisms, which is close to the LD_{50} dose in this strain. Green, CCR5 WT BALB/c females; purple, CCR5 WT BALB/c males ($n=20$ per group); red, CCR5 WT C.B-17 SCID females ($n=8$); blue, CCR5 WT C.B-17 SCID males ($n=4$).

To test whether CCR5 deficiency protects mice against *Yersinia* infection, we challenged them with lethal inocula of wild-type *Y. pseudotuberculosis* YPIIIpYV (C57BL/6 CCR5-deficient mice) or Pgm− *Y. pestis* KIM substrain D27 (BALB/c CCR5-deficient mice). There was no significant difference in the bacterial load in the caecum or Peyer's patches at two or four days post-infection between C57BL/6 CCR5-deficient and CCR5-expressing mice following oral infection (Fig. 1a). Macrophages from CCR5-deficient animals showed little to no difference in bacterial growth of *Y. pseudotuberculosis* or *Y. pestis* compared with those from CCR5-expressing mice.

These results argue against CCR5 being essential for infection by *Y. pestis* or *Y. pseudotuberculosis*. However, they do not eliminate the possibility that a protective effect caused by CCR5 deletion may reduce mortality without changing bacterial spread. We therefore evaluated the effect of CCR5 deficiency on survival after *Y. pestis* infection in the more susceptible BALB/c mouse strain, but found no significant difference in survival between CCR5-deficient BALB/c female mice and BALB/c females with normal CCR5 expression (Fig. 1b).

Male CCR5-deficient mice survived for a shorter time (Fig. 1b; $P<0.0001$). We therefore challenged male and female BALB/c mice with a lower dose of *Y. pestis* and again found that the males showed significantly reduced survival (Fig. 1c; $P=0.0006$). As increased susceptibility was also seen in limited numbers of male SCID mice (Fig. 1c), this gender-related defect could be affected by innate immunity. The poorer survival of CCR5-deficient male mice (Fig. 1b) is therefore likely to be related to their gender and not to CCR5 deficiency. Gender may also be a factor in *Yersinia* infection in humans, because males seem to be more susceptible to bubonic plague than females[10].

Our results show that CCR5 deficiency in mice does not protect against infection or death caused by experimental *Yersinia* infection, making it unlikely that the *CCR5Δ32* allele protects against plague. A modelling study[11] reaches a similar conclusion, with smallpox instead of plague being proposed as the disease that selected for the *CCR5Δ32* allele.

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