Magnetic minerals

Keystone-like crystals in cells of hornet combs

The hexagonal brood-rearing cells inside the nest combs of the hornet *Vespa orientalis* are uniform in both their architecture and orientation. We have discovered that each cell contains a minute crystal that projects down from the centre of its domed roof and has a composition typical of the magnetic mineral ilmenite[1,2]. These tiny crystals form a network that may act like a surveyor's spirit-level, helping the hornets to assess the symmetry and balance of the cells and the direction of gravity while they are building the comb.

The keystone-like crystal we describe here is glued with hornet saliva to the centre of the roof on the inside of each cell (Fig. 1); it is positioned away from the egg, which is fastened onto a side wall[3,4], the site at which the hatching larva also develops. The crystal is opaque, round, about 100 μm in diameter, and consists of polydomains. Diffraction analysis of the element composition (data not shown) reveals that these crystals contain large quantities of titanium, iron and oxygen, with some carbon and trace amounts of manganese (58 out of 60 comb cells we inspected contained a crystal with the following composition in terms of number of atoms: 25–35% Ti, 15–20% Fe, 34–38% O, 12–18% C). This composition is similar to that of ilmenite crystals ($FeTiO_3$). The carbon component may originate from the hornet's saliva.

Comb building in social wasps occurs in stages. First, the hornet (whether a queen in the spring or workers thereafter) builds three to five concentric bands, which ultimately form the roof of the comb cell. Once the roof is completed, the hornet polishes it from the inside and deposits another construction layer of small particles or organic fibres. At the centre of the dome, the builder leaves a hollow where it attaches the crystal and which it fills with saliva that rapidly hardens into a polymer.

As the roof of the cell moves, the hanging crystals move in response to gravity. The downward-sloping cell walls that separate each domed roof from its six neighbours prevent the crystal from being shaken loose by workers or larvae communicating with other members of the colony, either acoustically or by tapping.

We presume that hornets collect these crystals from the local environment, but it is possible that they secrete them themselves, as titanium and iron are present in the hornet body[5].

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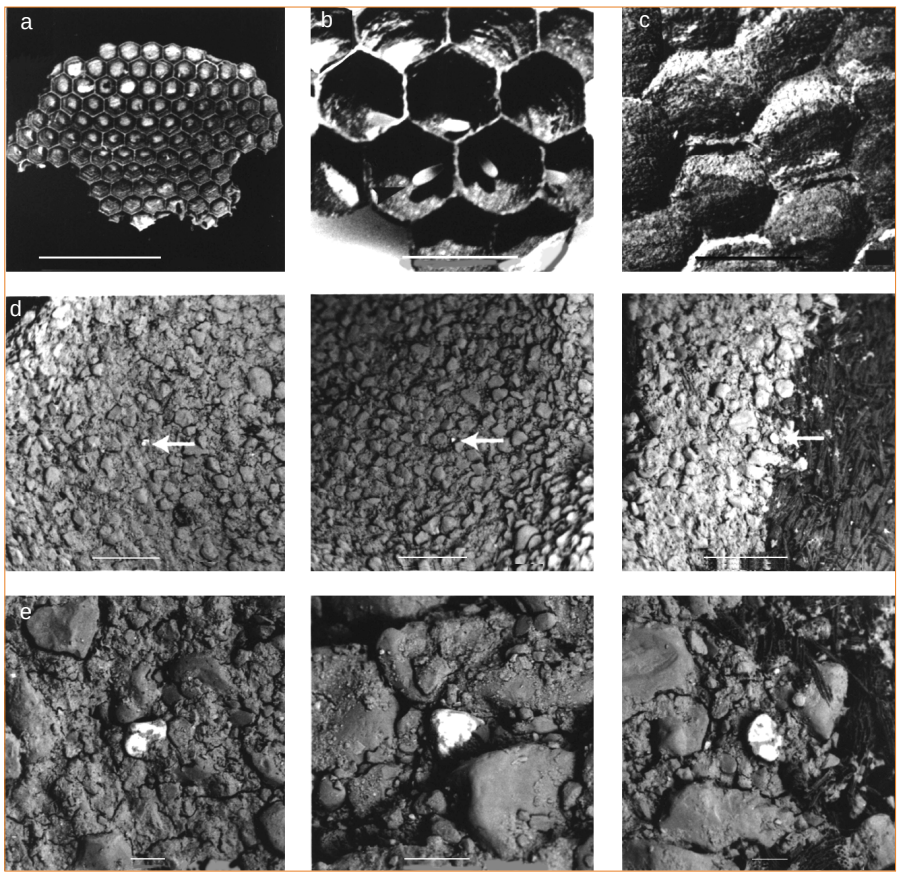

Figure 1 Macroscopic images and electron micrographs showing the locations of ilmenite crystals in cells of the comb of the oriental hornet. **a,** Inverted comb (with respect to the normal gravity-orientated position), showing the interior of comb cells and the inner side of the domed roof in each cell. **b,** Inverted hexagonal cells, some of which contain eggs. **c,** Small section of the comb at higher magnification, showing hexagonal roofs from the outside. **d, e,** At higher magnifications, an ilmenite crystal is seen (arrows in **d**) in the centre of the roof of different cells. For electron microscopy, the tops of cell walls were removed so that the roof could be seen; samples were prepared as described[6]. Scale bars: **a,** 5 cm; **b, c,** 1 cm; **d,** 1 mm; **e,** 100 μm.

Alzheimer's disease

Molecular consequences of presenilin-1 mutation

Alzheimer's disease is characterized by accumulation in the brain of a family of insoluble amyloid peptides (Aβ peptides)[1], which are produced as a result of the normal processing of β-amyloid precursor protein (β-APP). Russo *et al.*[2] claim that a truncated Aβ peptide that lacks the first ten amino acids accumulates in the brains of patients carrying a mutant form of presenilin 1 (PS1), a protein that is involved in cleavage of β-APP. However, we have found that this same species is also overrepresented in Alzheimer's patients with mutations in β-APP itself[3]. Our findings do not support the conclusion of Russo *et al.* that pathogenic PS1 mutations may control cleavage of β-APP by β-secretase[4,5].

Harmful mutations can occur at residue 717 in β-APP as well as in PS1, favouring generation of the Aβ species $Aβ_{1–42}$ (refs 6, 7). Tandem missense mutations at residues 670 and 671 of β-APP promote cleavage at the Aβ amino terminus[8], the rate-limiting step in producing Aβ.

Russo *et al.*[2] characterized Aβ species in the brains of patients suffering from sporadic or familial Alzheimer's disease due to mutations in PS1 or β-APP. Because N-terminally truncated Aβ species (particularly those starting with glutamate at residue 11) were overrepresented in PS1-mutant brains of familial Alzheimer's sufferers, they inferred that pathogenic PS1 mutations affect β- as well as γ-secretase reactions.

We used size-exclusion chromatography and electrospray ionization mass spectrometry to study extractable amyloid in brains from elderly, non-demented individuals and from cases of either sporadic or familial Alzheimer's disease resulting from K670N/M671L or V717I mutations[3] (where K, M and V represent the amino acid at that position in normal β-APP, and N, L and I the respective substituent in the mutant). We investigated their total Aβ content, whereas Russo *et al.*[2] looked primarily at soluble Aβ species.

Of the Aβ species we found, $Aβ_{1–42}$ was the most abundant in four of five non-demented elderly brains, and $Aβ_{1–40}$ was the most abundant in eight of ten brains with sporadic Alzheimer's. N-terminally truncated Aβ species were frequently identified in all patients: $Aβ_{11–42}$ was the most abundant in one of ten sporadic patients and in the single β-APP(V717I) familial case, and the second most abundant in two of five non-demented individuals.

However, $Aβ_{11–42}$ was more abundant than $Aβ_{1–42}$ in four of ten sporadic patients and in those with V717I and K670N/M671L β-APP mutations. The K670N/M671L mutations at the Aβ amino terminus help β-secretase to cleave β-APP, yielding Aβ with aspartate as its first residue[8], but in post-mortem tissue from a patient bearing these mutations, $Aβ_{11–42}$ was the predominant species recovered from the deposited amyloid plaque, which is inconsistent with the conclusions of Russo *et al.*[2].

The Glu-11 Aβ species is the main peptide generated from endogenous β-APP in rodent neurons[9] and in PS1-deficient mouse neurons[10]. The peptide bond at $Aβ_{10–11}$ is also the preferred alternative cleavage site[11] for BACE, the protease that is responsible for most β-secretase reactions. These results argue against the model of Russo *et al.*[2] by showing that cleavage at this site occurs even in the absence of PS1.

N-terminally truncated Aβ peptides in amyloid plaques could be generated by post-production modification of the N terminus, rather than during cleavage by β-secretase. The pyrrolic modification of glutamate at residue 3 or 11 (ref. 12) of Aβ occurs after β-secretase cleavage. Aβ peptides that end at residue 42 may be relatively long-lived and so more susceptible to enzymes that cut at the N terminus, enhancing their heterogeneity by increasing their exposure to chemical modifications such as pyrrolation or oxidation.

Despite the technical difficulties in obtaining a full and accurate profile of all the Aβ species present in the amyloid plaques associated with various forms of Alzheimer's disease, we consider that amino-terminal truncation of Aβ is not unique to PS1-mutant familial Alzheimer's, and therefore question any proposed effect of PS1 mutations on β-secretase activity.

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Russo et al. *reply* — Gandy *et al.* compare our results[1] with their 1994 findings[2] that the amino-terminally truncated amyloid $Aβ_{11–42}$ was relatively abundant in two cases of familial Alzheimer's disease involving two distinct mutations in β-APP. However, four important differences should be borne in mind: the authors compare $Aβ_{11–42}$ with $Aβ_{1–42}$ and ignore $Aβ_{1–40}$, although both $Aβ_{1–40}$ and $Aβ_{1–42}$ are generated by β-secretase/BACE cleavage at residue Asp 1 (ref. 3); their data are not correlated with features related to disease severity, such as age at onset and duration; they did not examine brains with PS1 mutations (these were not known at that time); and their characterization was based on the use of size-exclusion chromatography and electrospray mass spectrometry to quantify formic-acid-extracted Aβ, whereas we used quantitative analysis of immunoprecipitated water-soluble Aβ on western blots, mass spectrometry to identify Aβ variants, and immunohistochemistry to reveal amino-truncated Aβ peptides in plaques.

Table 1 gives ratios of the amounts of full-length $Aβ_{1–40}$ and $Aβ_{1–42}$ to that of $Aβ_{11–40/42}$ species in brains of Alzheimer's patients. The results of Naslund *et al.*[2] give values that are consistently greater than unity even when all other N-terminally truncated Aβ species they detected in sporadic Alzheimer brains are added to $Aβ_{11–42}$. The main difference with our data is in sporadic rather than familial cases involving mutations in β-APP. The ratios between $Aβ_{1–40/42}$ and $Aβ_{11–40/42}$ that we found in PS1-mutant cases with early disease onset are much lower (less than unity) than those that Naslund *et al.* (and we) observed in familial Alzheimer's disease with β-APP mutations.

We proposed that increased formation of N-terminally truncated Aβ correlates with the early-onset phenotype of familial Alzeimer's disease linked to mutated PS1, and that a similar Aβ pattern may be shared by other malignant familial forms involving β-APP mutations[4,5]. We explained the over-representation of these truncated Aβ forms in PS1-mutant brains by postulating that some PS1 mutations directly or indirectly affect β-secretase cleavage. A variety of mechanisms that do not require a direct effect of PS1 on β-secretase might be involved.

Gandy *et al.* suggest that late progressive proteolysis of the Aβ amino terminus occurs after β-secretase cleavage, after which glutamate is cyclized at the N-terminal residues 3 and 11. This sequence of events seems unlikely, as N-terminally truncated and pyroglutamylated carboxy-terminal fragments of β-APP (created before γ-secretase cleavage), which are direct precursors of similarly modified Aβ peptides, are present in the brains of Alzheimer's patients[6]. The finding that N-terminally truncated C-terminal fragments of β-APP are more abundant in PS1-deficient neurons[7], quoted by Gandy *et al.* as evidence against our hypothesis, could also mean that PS1, either through its interaction with β-APP or its lack thereof, influences β-secretase cleavage. Some PS1 mutations may have the same effect on β-secretase cleavage as that produced by PS1 deficiency in a mouse knockout model.

We maintain that overrepresentation of N-terminally truncated Aβ forms may be characteristic of Alzheimer's disease that is associated with PS1 or other mutations and characterized by early onset and short duration, or other malignant phenotypic features. Whether PS1 mutations have an effect, be it direct or indirect, on β-secretase cleavage remains to be determined.

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Table 1 Ratios of $Aβ_{1–40/42}$ to $Aβ_{11–42}$ in brains from Alzheimer's disease patients

Disease type	*Naslund* et al.[2]	*Russo* et al.[1]
Sporadic	3.4 ($n = 10$)	1.4 ($n = 17$; 66–84 years)
APPK670M/N671L	1.7 ($n = 1$)	NA
APPV717I	1.2 ($n = 1$)	1.3 ($n = 2$; 59–63 years)
PS1 mutations (all)	NA	0.75 ($n = 11$; 40–81 years)
PS1 M139I and H163A	NA	0.3 ($n = 4$; 40–48 years)

Number of cases (n) and age at death are shown in parentheses. NA, not applicable.