Neurotransmitters, Drugs and Brain Function.
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ISBN: Hardback 0-471-97819-1 Paperback 0-471-98586-4 Electronic 0-470-84657-7

18 Alzheimer's Disease (AzD)

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Everybody suffers some intellectual and memory impairment with age. If it becomes very marked or occurs earlier in life (40+) it is known as dementia. Although it may be caused by alcoholism, cardiovascular disease such as multiple infarcts, and is often seen in the later stages of Parkinsonism, the most common cause is a neurodegenerative one, namely, Alzheimer's disease (AzD). In fact this is the primary and sole cause in over half the cases of dementia and is a contributory cause in a further quarter and the younger the patient, the more likely is the dementia to be of the Alzheimer type.

Alzheimer's disease generally presents itself as a relatively isolated failure of memory for recent events, particularly for names. Speech problems, disorientation with respect to time and place follow along with depression that can be interrupted by aggression. All aspects of higher brain function are then affected, memory loss becomes virtually total and movements very slow. Eventually the patient becomes almost totally incapacitated, doubly incontinent and bed-bound in which terrible state they may survive for 1–2 years. It is not surprising that its appearance is devastating not only to the patient but more particularly to family and friends. It can last from 3 to 20 years but 7 to 10 years is more common and while it may start in one as young as 20 it usually waits until well after 40. Some 10% or more of the population over 65 may suffer from it, a figure that more than doubles beyond 80 years. Also as life expectancy increases and the population becomes more aged the actual incidence will increase. The cost is thus becoming immense. In the United Kingdom alone, the annual cost to the health and social services of caring for people with AzD is estimated at over £2.0 billion (a hundred times more in the United States) but the total cost to society could be double that.

Despite its characteristic symptoms and even after the exclusion of other established causes, AzD can only be reliably diagnosed by neuropathology and microscopic examination of the brain. Indeed that is how it came by its name. In 1907, a German physician, Alois Alzheimer, described two distinct post-mortem changes in the brain of a woman patient who had died with an unusual mental illness. These were the now characteristically accepted markers of the disease, namely senile plaques and neurofibrillary tangles (Fig. 18.1).

PATHOLOGY

Microscopically the brains of AzD patients often show neuronal loss and some atrophy, much as in Down Syndrome, as well as widened sulci and narrowed gyri. Since,

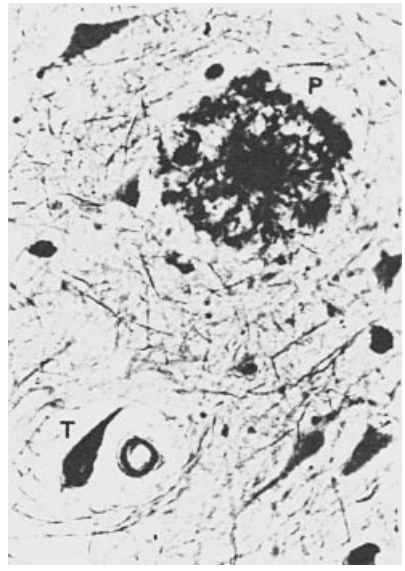

Figure 18.1 Typical tangle (T) and plaque (P) as visualised by silver impregnation in the cerebral cortex of a case of Alzheimer's disease. The extracellular plaque (10–50 μm diameter) consists of a central core of amyloid surrounded by glial processes and a number of neurites in a ring formation. The intracellular cytoplasmic tangle is composed of helical filaments in a paired format. (Reproduced with permission of Academic Press from Wischik and Crowther 1986)

however, such changes are not uncommon in elderly people (75+) these features can only really be considered indicators of the disease when found in younger patients. This does not apply to the plaques and tangles.

SENILE PLAQUES

Appropriate silver staining and immunohistochemical localisation of β-amyloid show these to be extracellular lesions which in their typical neurite form are roughly spherical in shape (10–50 μm diameter) with a central core of amyloid surrounded by glial processes and a rim of neurites. The amyloid can sometimes exist alone (compact plaque), when the neurites no longer react to silver staining or in a diffuse state (primary plaque) before neurites have formed. It is unclear whether the development of neuritic from diffuse plaques causes neurofibrillary pathology and neuronal dysfunction or results from those processes. Plaques are, however, indices of neuronal death, generally of large pyramidal cells. They are found mostly in the cerebral cortex, especially the hippocampus and frontal temporal area, and while most common in AzD brain they also occur in Down Syndrome and in pugilistic (brain damage) dementia and can even be found sparsely in the normal ageing brain.

NEUROFIBRILLARY TANGLES

These are intraneuronal cytoplasmic lesions found predominantly in large pyramidal cells, again, mostly within the hippocampus and frontal temperal cortex, and while they

can be seen in some other conditions, e.g. post-encephalatic Parkinsonism and Down Syndrome, they are generally considered to be more specific to AzD than the plaques. The tangles are composed of tau® protein, which normally promotes polymerisation of the microtubules that maintain cell structure, but for some reason has become hyperphosphorylated and deposited as helical filaments in a characteristic entwined paired format which disrupts neuron function. Hirano bodies, which are intraneuronal eosinophile inclusions, are also seen in AzD.

FORMATION OF β-AMYLOID AND ITS EFFECTS

Most cases of AzD show cerebrovascular amyloid deposits and the amyloid protein of senile plaques is the same as that found in blood vessels. It is referred to as β-amyloid protein and is part of a 695, 751 or 770 amino-acid amyloid precursor protein APP, which is a transmembrane protein and although its precise function is not clear, it is widely distributed and APP knock-out mice show reduced motor function. Normally so-called short 40 amino-acid-soluble derivatives of APP are produced by proteolytic cleavage of APP within the β (A4) amino-acid sequence but APP can also be cleaved

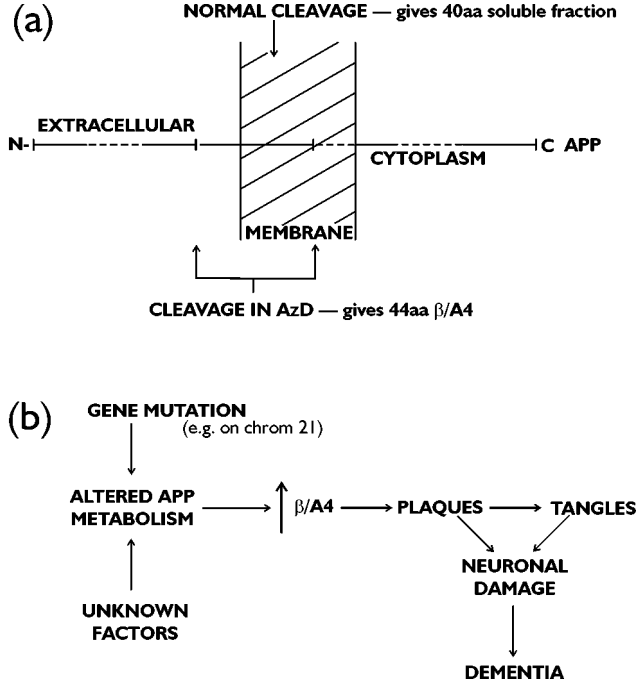

Figure 18.2 Production of senile plaque β/A4 amyloid protein. Amyloid β4 protein (β/A4) is part of a 695, 751 or 770 amino-acid amyloid precursor protein APP. This is a transmembrane protein which is normally cleared within the β/A4 amino acid sequence to give short 40 amino-acid soluble derivatives. It seems that under some circumstances as in Alzheimer's disease, APP is cleared either side of the β/A4 sequence to release the 42/43 amino acid β/A4 which aggregates into the amyloid fibrils of a senile plaque (a). (See also Fig. 18.5.) Some factors, e.g. gene mutation, must stimulate this abnormal clearage leading to the deposition of β/A4 amyloid protein as plaques and tangles and the death of neurons (b)

either side of the β sequence to liberate the longer 42 (or 43) amino-acid-insoluble β-amyloid protein (Fig. 18.2). One possibility is that in AzD this process is excessive and the insoluble amyloid β protein (Aβ) aggregates to form the amyloid fibrils and core of the senile plaques. The protein may also stimulate the phosphorylation of tau and the production of neurofibrillary tangles. How it kills neurons is unclear. Suggestions include the production of free radicals, sensitisation to glutamate and increased Ca^{2+} influx. The last has been shown in *in vitro* studies but these tend to use concentrations in excess of those found in the brain and often with shorter and soluble synthetic forms of Aβ. Certainly the direct injection of β-amyloid or neurotic plaques into rat brain does not appear to kill neurons but continuous infusion of Aβ (1–40) into the cerebral ventricles of rats does lead to impairment of learning and memory (Nitta *et al*. 1997).

In fact no consistent correlation has been found between the appearance, distribution and number of amyloid plaques and either neuronal loss or the degree of dementia, although the latter correlates with the number of neurofibrillary tangles, which tend to precede plaques in appearance by some years. Also cortical amyloid deposits can be found in non-demented elderly patients. Thus the basic question appears to be: does the disease process, whatever that is, cause the development of AzD as well as the production of β-amyloid or is there production of β-amyloid, which then causes AzD? Consensus supports the latter but it is not proven.

AETIOLOGY

If β-amyloid deposition is responsible for AzD, it is important to know what causes its production. Since AzD is most common in the elderly and as β-amyloid is found in the normal aged brain, it is likely that AzD depends on some predisposing factor that increases amyloid production and which may strike early in life but is more likely to become apparent during ageing. There is strong evidence for a genetic component. Mutations of the APP gene on chromosome 21, the apolipoprotein E (ApoE) gene on chromosome 19, the presenilin 1 (Ps1) gene on chromosome 14 and presenilin 2 (Ps2) gene on chromosome 1 have all been implicated in AzD.

GENETIC MUTATION

A number of family mutations of the APP gene on chromosome 21 have been found, generally in early-onset AzD patients in different countries, all of which lead to increased β-amyloid production. Also chromosome 21 is abnormally trisomic in Down Syndrome and most Down sufferers develop AzD if they reach 40 years. In transgenic mice, expressing familial AzD mutations of APP, the overexpression of APP is accompanied by increased amyloid deposition but whether this is due to the mutation or overexpression of APP is uncertain. Also not all the animals show memory loss and that tends to precede the amyloid disposition.

One of the most likely risk factors for AzD is the patient's genotype for apolipoprotein E (ApoE), which is believed normally to be involved in neuronal repair and growth, but is also found in plaques and tangles. Three distinct forms of ApoE, E2, E3 and E4 are encoded on chromosome 19 but it is the ApoE, E4 allele that occurs at a much higher frequency in late-onset AzD patients (50%) compared with controls (16%) and binds to and possibly increases the formation of β-amyloid. Many early-onset cases

of familial AzD are associated with mutations in the genes for PS1 and PS2 on chromosomes 14 and 1 respectively. The precise physiological role of these 463 and 448 amino-acid transmembrane proteins is unclear but plasma and brain tissue from patients with PS mutations contain above-normal levels of the β-amyloid protein as do transgenic mice expressing PS mutations and cells transfected with mutant PS.

Thus all the above genetic mutations can lead to increased amyloid deposition and possibly AzD (see Smith 1998). Unfortunately familial AzD represents only the minority of cases and so other causes need to be considered.

HEAD INJURIES

It has been estimated that up to 15% of head injuries may lead to AzD with dementia being common among boxers (*dementia pugilistica*). Certainly such trauma is associated with diffuse amyloid deposits (not plaques) and a number of neurofibrillary tangles apparently identical to those in AzD.

ALUMINIUM

Reported positive associations between AzD and a high aluminium level in drinking water promoted that element as a risk factor for, or cause of, AzD. Since then aluminium in silicate form has been found in plaques and tangles and shown to impair the axonal transport of neurofilament. However, the occurrence of high brain levels of aluminium, either through environmental exposure or dialysis encephalopathy, is not associated with a greater incidence of AzD and the neurofibrillary changes it produces appear different from those of AzD. Currently while aluminium is accepted to be neurotoxic, it is thought to be a more likely cause of neurological impairments than AzD.

INFLAMMATION

The finding that patients treated with non-steroidal anti-inflammatory drugs (NSAIDs) like asprin were less likely to develop AzD stimulated the suggestion that AzD may have an inflammatory component and indeed NSAIDs have been shown to have a protective effect against AzD. It remains to be seen whether this is a true anti-inflammatory effect or whether the NSAIDs are protecting by reducing free radical production. Smoking appears to offer an accepted protection against AzD.

SUMMARY

Even if there is a link between the presence of tangles and plaques and the emergence of AzD, it is by no means certain how those markers could be responsible for all the symptoms. They do not seem to be sufficiently numerous or widely spread to disrupt brain function to the extent that eventually occurs in AzD, although their preferential location in the hippocampus and the known association of that area with memory processing could explain the loss of that faculty.

Since therapy for AzD, like that for the other major neurodegenerative disorder Parkinsonism, could depend on establishing to what extent its pathology is associated

with the loss of neurotransmitter function, it is important to consider NT changes in AzD.

NEUROTRANSMITTER CHANGES IN AzD

The NT most consistently implicated in AzD is ACh.

ACETYLCHOLINE

It is 20 years since a 50% reduction was noted in the level of choline acetyltransferase (ChAT), the enzyme responsible for ACh synthesis, in the frontal cortex and hippocampus of AzD patients (Bowen *et al.* 1976). This has since been confirmed by others (see Perry 1986). ACh itself was not easily measured at that time but a reduced synthesis of ACh from ^{14}C glucose was observed in brain tissue from AzD patients. There is in fact a significant correlation between the reduction in ChAT and both the increased number of plaques and tangles at death and the severity of mental impairment six months before death (Perry *et al.* 1978). ACh loss is not global, no change being found in the striatum or some parts of the cortex. Recently reduced ACh levels have been reported in CSF obtained by lumbar puncture, though it is surprising that it survived degradation (Tohgi *et al.* 1996).

Since ACh is mostly synthesised in nerve terminals, the reduction in cortical ChAT must reflect a loss of cholinergic nerve terminals and as there are few cholinergic neurons in the cortex, these must be the endings of axons that come from cholinergic neurons in the subcortical nucleus basalis (Fig. 6.7). In fact there is a dramatic loss ($<70\%$) of such neurons in AzD, especially in younger patients, although there is some evidence that the loss of cortical ChAT is greater than the cell loss and that degeneration starts in the cortical terminals and proceeds retrogradely to the cell bodies. Plaques and tangles are also found in the nucleus basalis but lesion of it does not induce their formation in the cortex and their cortical location does not just coincide with cholinergic innervation.

No overall reduction in cholinergic muscarinic receptors was found but recent studies with relatively specific ligands show a loss of presynaptic M_2 receptors, in keeping with the loss of terminals, but no reduction in postsynaptic M_1 receptors. Some acetylcholinesterase is found in plaques.

ACh AND β-AMYLOID

Low concentrations of solubilised β-albumin inhibit ACh release in slices from rat hippocampus and cortex areas which show degeneration in AzD, but not in slices from the striatum which is unaffected. While not totally specific to ACh, since some inhibition of NA and DA and potentiation of glutamate release have been reported, this effect is achieved at concentrations of Aβ below those generally neurotoxic. Since β-amyloid can inhibit choline uptake it is also possible (see Auld, Kar and Quiron 1998) that in order to obtain sufficient choline for ACh synthesis and the continued function of cholinergic neurons, a breakdown of membrane phosphatidyl choline is required leading to cell death (so-called autocannibalism). β-amyloid can also reduce the secondary effects of M_1 receptor activation such as GTPase activity

and IP_3 production. To what extent these events can occur *in vivo*, let alone with insoluble β-amyloid, which forms the plaques, is not clear but soluble β-amyloid itself is also increased significantly in AzD brain and when infused into the ventricles of rats reduces ChAT activity.

MONOAMINES

Of course, cholinergic neurons are not the only ones with axon terminals in the cortex and if their degeneration does originate in the cortex then other afferants and their neurons could also be affected. This contention is supported by reported reductions in the number of NA neurons in the locus coeruleus, and 5-HT neurons in dorsal raphé but these are less marked (approximately 50%) than the loss of cholinergic neurons. Accompanying reductions in cortical NA and 5-HT are also seen but are again lower than those for ChAT but 5-HT_2 receptors are reduced (43%).

SOMATOSTATIN

Among a number of peptides studied it is only the reduction of somatostatin in the temporal, parietal and frontal cortices that correlates with the severity of dementia in AzD, although corticotrophin-releasing factor is lower. Reductions in somatostatin do not generally parallel those of ChAT, its concentration being almost normal in the hippocampus and nucleus basalis, where ChAT levels are lowest and there is no evidence that it is localised in cholinergic neurons.

GLUTAMATE

Despite the loss of cortical pyramidal cells no reduction in glutamate levels has been found generally in AzD, except in parts of the hippocampus where the density of glutamate nerve terminals is very high. Here the NT pool could form a sufficiently major part of the total tissue content that any reduction in that measure would indeed reflect a loss in NT glutamate. A reduction in the sodium-dependent D-[^{3}H] aspartate binding, which is presumed to label glutamate nerve terminals, has been shown for some (e.g. temporal) but not all areas of the cortex or the hippocampus despite the widespread loss of neurons. This picture is also complicated by the binding of aspartate to glial cells that multiply to occupy lost neuronal space. Although there are some reports of a reduction in the number of glutamate NMDA receptors in the hippocampus, this has not been found generally in the cortex.

Some of the symptoms of AzD are similar to those seen in patients with cortico-cortical disconnection, i.e. a loss of cortical association fibres running from one part of the cortex to another. These include difficulties in recognising known objects through sensory inputs such as touch or smell (*agnosia*), producing or understanding spoken or written words (*aphasia*), and initiating or performing familiar movements (*apraxia*). All these impairments show not only a loss of memory but also an inability to link (associate) the activity of different cortical functions and areas. Since the fibres that normally link the areas arise from glutamate-releasing pyramidal cells, their degeneration would implicate some loss of glutamate.

ANIMAL TESTS OF MEMORY FUNCTION

Since these test an animal's ability to initially perform and then repeat a simple behavioural task, the animal can be said to have learnt some function, which means they have remembered or memorised certain actions. They are tests of memory only in so far as memory is an essential part of learning which may be defined as the process by which an experience is somehow incorporated into the brain so that it can be retrieved. Animal tests are, of course, very basic but human memory can be much more complex since we can memorise occurences, events and impressions that do not actually require active accquisition or learning. Also animal tests, whether induced by drugs or used to test for a drug effect, can be influenced by the drug's possible modification of attention, arousal and motor function. Length of memory can be evaluated by varying the time between the initial learning and the subsequent tests.

The tests generally involve some form of maze but the simplest is the passive avoidance test. In this the animal learns that in a certain environment it will be punished with an electric shock for some particular action, like stepping onto a special part of the floor of the test chamber. The test of memory is how long the rat avoids (remains passive to) making the movement that will initiate the shock. Of course, drugs that reduce the animal's anxiety also modify the response. Using a maze in its simplest T shape, the animal is placed at the base of the vertical arm and a food reward at the end of one of the horizontal arms. Clearly the animal has to learn which arm contains the reward. Memory is assessed by the time taken for a food-deprived animal to reach the reward and the number of false arm entries. This simple system can be made more complex by introducing many more arms and branches but the principle is the same.

In a radial maze a number of arms of equal length radiate from a central point, where the animal is placed. Initially food is placed at the end of each arm and the rat is expected to learn that fact by exploring and entering each arm. The test of memory is to see whether on re-exposure to the maze the rat remembers only to enter an arm not previously visited and so still containing food.

The Morris (1984) water maze is a large circular glass tank (1.5 m diameter) filled with opaque (e.g. dye-treated) water to a depth of some 50 cm. A small platform, large enough to take the rat, is placed in the water with its top about 1.5 cm below the surface, so it cannot be seen. When placed in the water the rat finds and escapes to the platform, the position of which is apparently learnt by reference to peripheral visual markers. Memory is demonstrated by the rat's ability to swim to the platform when put back into the water at various points and measured by the time or the length of path taken to do so (Fig. 18.3).

NEUROTRANSMITTERS IN MEMORY PROCESSING AND APPROPRIATE SYNAPTIC FUNCTION

Since AzD is characterised by an impairment of memory, which is a normal brain function, then a consideration of which NTs and brain circuitry are implicated in the laying down and retrieval of memory may provide an indication of not only which NTs we should expect to be affected in AzD but also which need to be manipulated to therapeutic advantage. Again, most evidence points to ACh and glutamate.

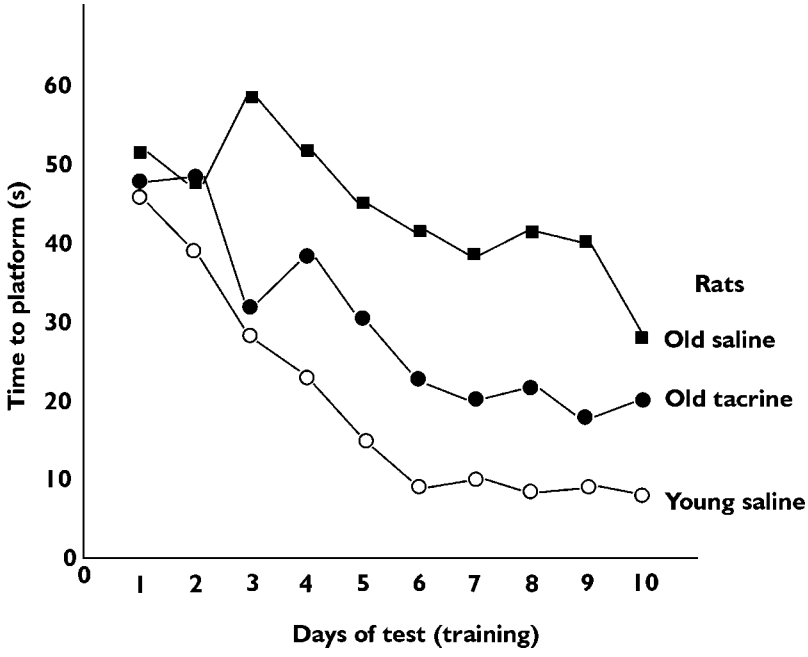

Figure 18.3 Acquisition of the water maze task as an index of learning and memory. In this example rats were trained daily to find a platform just submerged below water in a circular (150 cm) glass tank painted black and the time taken to reach it recorded. Young (4-month) saline-treated rats (o) quickly learnt and from day 6 consistently swam to the platform in less than 10 s. By contrast, aged (22-month) rats (■) took significantly longer to acquire the task and by day 10 were still taking about 30 s. The anticholinesterase drug tacrine (30 mg kg^{-1} daily.ip) given to such aged rats (●) significantly reduced the time to acquire (6 days) and perform the task (20 s). Adapted from Yavich *et al.* (1996)

ACETYLCHOLINE

ACh is implicated in memory for two main reasons:

(1) It has been known for many years that antimuscarinic drugs like hyoscine, which enter the brain, cause amnesia when used clinically, e.g. pre-operatively, to reduce bronchial secretions. In experimental studies in both humans and animals they disrupt both the acquisition and the performance of learned behaviour. Anti-cholinestrase drugs have the opposite effect. It is by no means certain, however, that the memory defects induced by antimuscarinics are identical to those seen in AzD.
(2) Exocytoxic lesions of the nucleus basalis with excitatory amino acids such as quisqualic and ibotenic reduce cortical ChAT activity and impair memory performance in animals. Unfortunately although quisqualate is more effective than ibotenic in reducing ChAT, it has less effect on memory (acquisition of passive avoidance), suggesting some additional effect of ibotenic acid not concerned with the ACh. Nevertheless, the memory defect induced by ibotenic acid is similar to that obtained with hyoscine and can be reversed, as studies in the rat water maze test show, by implanting fibroblasts with high ChAT activity in the cortex to secrete ACh. Anticholinesterase inhibition and foetal brain grafts containing cholinergic

neurons have also been shown to partially reverse the effects of lesions of the nucleus basalis.

So if ACh is involved in memory function, what does it do? Any attempt to answer that question has to follow some consideration of how memory is thought to be processed. Many neuroscientists believe that memory is achieved by changes in the strength of synaptic connections (activation) between neurons and that increases in such synaptic activity somehow reinforce the pattern of neuronal activity during the memorising of an event so that it can be more easily restored later. One form of such plasticity is long-term potentiation (LTP), which has been mostly studied in the hippocampus where, as in other areas associated with memory, there is the appropriate complex synaptic morphology.

That the hippocampus is important for memory is generally accepted. This is not because it is a site of major degeneration in AzD, that finding can only be used to account for the memory loss if memory is known to be dependent on the hippocampus, but because lesions of that region are known to impair memory. Case reports in the medical literature are rightly mistrusted but few people have felt inclined to disregard the evidence presented by one 27-year-old male mechanic who underwent bilateral hippocampal removal for intractable epilepsy in Montreal in 1953. While that condition was improved the operation has not been repeated because memory loss was almost total, so while he appeared to behave reasonably normally (and still does), he cannot remember where he lives, what he has just eaten or the person he met a few minutes previously.

Long-term potentiation can be defined as the increased effectiveness (potentiation) of synaptic transmission which may last for hours (possibly days) and is triggered experimentally by a brief burst of high-frequency stimulation of presynaptic inputs so that the response to any following input is much greater than normal. It was first demonstrated *in vivo* (Bliss and Loma 1973) but much studied *in vitro*. There is considerable debate as to whether the potentiation is of pre- or postsynaptic origin, or both, and while neurons can discharge spontaneously at an appropriate tetanic frequency (e.g. 200 Hz) it is not known how this may arise in normal neurophysiological processing. So to what extent LTP is essential to the memory process is unclear but there is no disputing the fact that despite all the evidence for the involvement of ACh in memory, antimuscarinic drugs do not affect LTP. ACh does, however, have the ability to partially depolarise neurons by reducing K^+ efflux (Chapter 6) and so make them more likely to fire repetitively to an incoming impulse. On the other hand, LTP is blocked by glutamate NMDA antagonists.

GLUTAMATE

As outlined above, changes in glutamate levels and function in AzD are much less clear-cut than for ACh, despite the fact that the lost pyramidal cells presumably use glutamate as a NT. On the other hand, glutamate, unlike ACh, does appear to be essential for LTP and if that is important in the memory process then so is glutamate. LTP is increased by NMDA agonists as well as being blocked by NMDA antagonists, which also decrease learning in animals. Such drugs have not been risked in patients but phencyclidine, which has been used as an anaesthetic (and drug of abuse), is known to cause amnesia and has been found to directly block NMDA receptor channels.

OTHER NTs

Just as there is less degeneration of monoamine than cholinergic neurons in AzD, so they have less influence on memory function. Generally in both animals and humans, increases in NA activity (α_1 agonists, α_2 antagonists) improve cognition although both positive and negative effects have been reported with α_2 agonists and cardiovascular effects cannot be ruled out in all these studies. 5-HT_3 antagonists such as zacopride and ondansetron have been shown to produce some improvement in cognitive performance in animal and human studies. Removal of the posterior pituitary in rats shortens retention of a conditioned avoidance response, an effect which can be overcome by the administration of vasopressin. Variable but generally weak positive effects on cognition have been seen with this peptide in humans. Opioids tend to impair and their antagonists improve memory in animals (see McGough, Introlni-Collison and Castellano 1993).

THERAPY

Therapy should be aimed at either

(a) The manipulation of NTs known to be affected by the neurodegeneration or
(b) The attenuation and possible reversal of any cause of AzD such as amyloid production and deposition

It must be made clear from the outset, however, that there is currently no way of stopping the progression of (i.e. curing) the disease and that the most that can be achieved at present is some restoration of memory function in the early stages.

MANIPULATION OF NEUROTRANSMITTERS

AUGMENTING CHOLINERGIC FUNCTION

Since ACh appears to be important in memory processing and as its concentration is significantly reduced in appropriate brain areas in AzD then augmenting its action should at least improve memory function. ACh activity may be increased by

(1) Enhancing its synthesis (and presumed release) through giving the precursor choline
(2) Stopping its degradation by cholinesterase with anticholinesterase drugs
(3) Reproducing its action with appropriate agonists — (a) muscarinic, (b) nicotinic

Approaches (1) and (2) depend on sufficient cholinergic function remaining to make its supplementation feasible, while all three methods suffer from the fact that not only does ACh have other central effects (e.g. in striatum), but also numerous peripheral parasympathomimetic ones, such as increased bronchial and gastric secretion or reduced heart rate.

Increased synthesis

This requires the provision of the precursor choline which is often given as lecithin (phosphatidyl choline), a natural source of choline found in many foods such as eggs and fish. Large doses (9–10 g) have to be given, probably to overcome the body's

natural ability to restrict plasma choline levels, and the fact that only a very small percentage is converted to ACh. Brain penetration is modest but uptake into cholinergic nerve terminals is through a sodium-dependent high-affinity system that is normally adequately supplied and possibly saturated with choline. In any case, ACh can only be synthesised from choline in cholinergic nerve terminals, many of which will have degenerated, and just increasing the activity of those remaining may not be adequate. Whether choline could reverse the choline leakage and resulting autocannibalism (see above) of cholinergic neurons is not known.

Reduced degradation

ACh is metabolised extraneuronally by the enzyme acetylcholinesterase, to reform precursor choline and acetate. Blocking its activity with various anticholinesterases has been widely investigated and some improvement in memory noted. Such studies have invariably used reversible inhibition because of the toxicity associated with long-term irreversible inhibition of the enzyme. Physostigmine was the pilot drug. It is known to improve memory in animals and some small effects have been seen in humans (reduces number of mistakes in word-recall tests rather than number of words recalled), but it really needs to be given intravenously and has a very short half-life (30 min).

The limited effectiveness of physostigmine did, however, encourage the development of longer-acting orally effective anticholinesterases such as tacrine (tetrahydroaminoacrydine), velnacrine and donepezil.

Clinical evaluation of anticholinesterases and other drugs in AzD

The newer anticholinesterases have all been subject to large and often multicentred trials. These take various forms but generally include an initial assessment of disease severity over a few weeks while on placebo alone, then a drug-dose evaluation before the chosen drug dose(s) is compared directly with placebo for some weeks in two groups. Confirmation of any drug effect is usually obtained by finishing with all patients on placebo. Although performed double-blind generally, only patients that respond in the early evaluation period enter the final drug trial and those with severe AzD are excluded altogether. Results from a simpler Phase III drug study showing some efficacy for donepezil are shown in Fig. 18.4 (Rogers *et al.* 1998).

The evaluation of drug effectiveness in AzD is not without its difficulties. There is a need to record changes in both cognitive function and general performance. Two primary measures are the Alzheimer's Disease Assessment Scale of cognition (ADAS-cog) and the Clinician's Global Impression of Change (CGIC). The former measures such things as memory, language, orientation, reason and praxis, on a 0–70 scale range. The higher the score, the more severe the condition, and as most patients normally decline at the acquisition rate of 5–10 extra points a year, any reduction of 4 or more points is considered a drug effect. The CGIC scale, as its name implies, is a more global measure of patient function not only in cognition but also in general behaviour and daily living obtained by the clinician interviewing both patients and carers. On a 7-point scale, improvement is represented by 1, worsening by 7 and no change by 4. Other measures include the patient's own evaluation of quality of life (QoL) noting their general feelings and ability to eat, sleep and relate to others. Generally improvements in

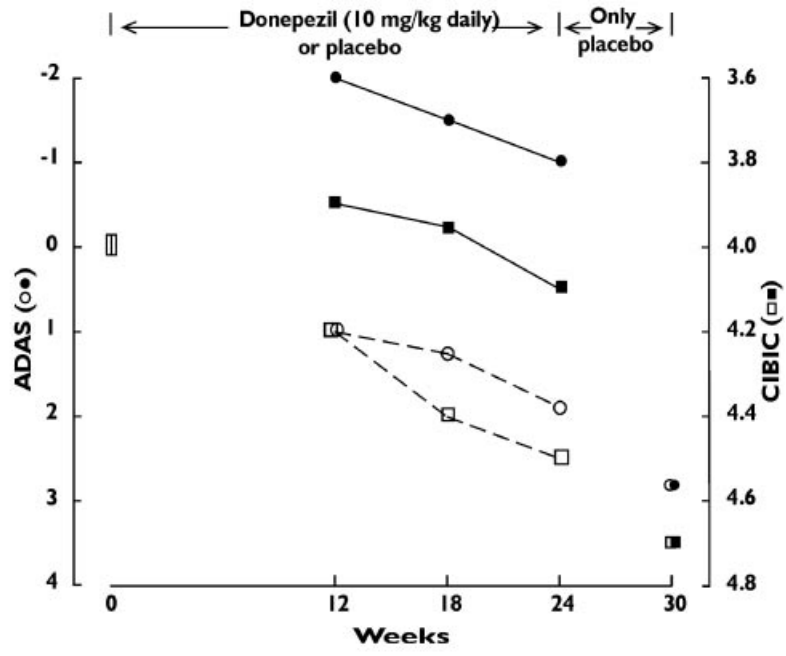

Figure 18.4 Clinical assessment of the efficacy of the anticholinesterase drug, donepezil, in Alzheimer's disease. Summary redrawing of some of the results of a large double-blind placebo-controlled trial by Rogers *et al*. (1998) © Lippincott Williams & Wilkins in which the effect of donepezil ($10\,mg\,day^{-1}$) was tested on men and women over 50 years with uncomplicated Alzheimer's disease ($N = 157$) compared with placebo ($n = 162$), using a number of measures including the Alzheimer's Disease Association Assessment Scale of cognition (ADAS-cog) and a more global assessment (CIBIC plus) equivalent to the Clinician's Interview-based Impression of Change scale based on clinical, patient and family assessment of cognition and behaviour. The results show that donezepil has had a significant ($p = 0.009$–0.0001) beneficial effect by both the ADAS (●—●) and CIBIC (■–■) assessments, when compared with placebo (○–○ and □–□) from 12th to 24th week. Two further features, characteristic of such therapy, are also apparent; (i) the drug has a greater effect on cognition (ADAS) than on overall state of health (CIBIC) and (ii) it does not retard the progress of the disorder (no difference between drug and placebo groups 6 weeks after cessation of drug). ABAS scores range from 0 to 70 (min–max symptoms) with patients normally deteriorating at a rate of 7–11 extra points per year so that any reduction in that rate constitutes an improvement. The CIBIC scale scores 1–7, with 1 = marked improvement, 4 = no change and 7 = worsening

cognition (ADAS-cog scores) are more easily achieved than in the overall quality of life (CGIC) (see Fig. 18.4) and is a useful reminder that AzD is not just a loss of memory.

The initial enthusiasm for tacrine and velnacrine, which are the anticholinesterases most studied clinically, has been tempered by the fact that not all patients respond. Most show the peripheral parasympathomimetic effects of cholinesterase inhibition, e.g. dyspepsia and diarrhoea, as well as nausea and vomiting, and about half of the patients develop hepatotoxicity with elevated levels of plasma alanine transaminase. While some peripheral effects can be attenuated with antimuscarinics that do not enter the brain, these add further side-effects and the drop-out rate from such trials is high ($<75\%$) in most long-term studies. Donepezil appears to show less hepatotoxicity but its long-term value remains to be determined.

Generally, anticholinesterases produce some improvement in cognitive function and the quality of life in the early stages of AzD but that needs to be balanced against side-effects.

Some of the cognitive improvements with tacrine, which is chemically related to amidopyridine, may be due to blockage of K^+ channels.

Use of agonists

Muscarinic

Since most postynaptic cholinergic receptors in the brain are muscarinic and as they do not appear to be reduced in AzD, despite some degeneration of pyramidal neurons, the use of muscarinic agonists could be worth while. Early studies with bethanecol, arecoline and oxotremorine, mixed M_1 and M_2 agonists, showed little benefit and newer drugs have not been much better. Peripheral cholinergic effects are a problem and central infusion, which has been tried with bethanecol to no great effect, is hardly a practical proposition. There is, however, a realisation that more appropriate drug or drug combinations could be developed and tried. Thus, theoretically anyway, the requirement is for a specific M_1 agonist that readily crosses the blood–brain barrier. Avoiding M_2 receptor stimulation will also mean no activation of presynaptic auto-receptors and counterproductive inhibition of ACh release, and fewer peripheral effects. These latter could also be avoided with an M_1 antagonist that does not cross the blood–brain barrier. Even then successful therapy may be negated by a requirement for ACh to be released physiologically from appropriate neurons.

Nicotinic

Despite the paucity of nicotinic receptors in the brain there is considerable evidence that AzD is less common among smokers. Whether this is due to the action of inhaled nicotine is uncertain, but nicotine is known to stimulate presynaptic receptors on cholinergic nerve terminals which, unlike the muscarinic ones, result in increased ACh release.

MODULATING GLUTAMATE FUNCTION

If long-term potentiation (LTP) is important in memory function and as it can be blocked by glutamate NMDA antagonists (see above) then increasing NMDA activity is of putative value in AzD. In reality this presents a problem because overstimulation of the receptor could not only increase neuronal function up to convulsive level but even cause neurotoxicity. Briefly, NMDA applied to rat cortex causes retrograde degeneration of cholinergic neurons in nucleus basalis while NMDA antagonists prevent anoxic destruction of cultured hippocampal neurons and brain damage caused by cerebral vascular occlusion in rodents. The ischaemia the latter produces causes such an excessive neuronal discharge and release of glutamate that the intense activation of NMDA receptors produces a prolonged neuronal depolarisation, Ca^{2+} entry and cell death. Possibly a weak partial NMDA agonist, or a drug acting at one of the NMDA receptor subsites (see Chapter 10) like that for glycine, may be of some value. Whether glutamate-induced neuronal death, which is enhanced by β-amyloid, plays any part in the actiology of AzD is uncertain but controlling glutamate activity so that it can be

increased enough to facilitate memory processes without undue excitation of neurons will be difficult.

GABA

Although there is no neuropathological evidence to implicate GABA in AzD it is known that agonists at the benzodiazepine receptor site not only augment GABA function but also cause amnesia. So it is possible that an inverse agonist, or perhaps even an antagonist, for the benzodiazepine receptor could have the opposite effect and improve memory. In humans, one antagonist, the β carboline derivative ZK93426, showed some improvement in learning and memory tests. It also improves acquisition in animal-learning tests and counteracts the impairment caused by scopolamine, as does the β-carboline inverse agonist DMCM. The fear of inducing anxiety or even convulsions with inverse benzodiazepine agonists has prompted the evaluation of partial inverse agonists (see Abe, Takeyama and Yoshimura 1998).

OTHER NTs

There have been few attempts to manipulate the monoamines in AzD and those using selegiline, the MOA_B inhibitor, have shown little effect although the 5-HT_3 antagonist, ondansetron, may give a slight improvement.

Despite the clear loss of somatostatin in AzD a synthetic analogue L-363586 had no beneficial effect on memory loss.

ATTENUATION OF DEGENERATION

Even if NT manipulation had provided an effective therapy in AzD it would still be important to stop the progression of degeneration and the disease process itself. The failure of the NT approach makes it even more vital.

β-AMYLOID

While the precise cause of AzD remains unknown, the evidence implicating β-amyloid is such as to justify attempts to reduce its involvement. The activity of β-amyloid might be reduced by:

(a) stopping its production by reducing the phosphorylation and proteolysis of APP
(b) increasing its breakdown
(c) counteracting its toxic effects through plaque formation

APP is normally cleaved within the Aβ sequence by an unidentified protease, so-called α-secretase, so that most of the extracellular APP is released in a soluble form into the extracellular fluid (see Checler 1995). When β-amyloid is formed another protease (β) splits APP so that the complete Aβ sequence persists at the extracellular end of the remaining membrane and intracellular APP chain. This is then cleaved by anaother protease (γ-secretase) to release the β-amyloid (Fig. 18.5). Potentiation of α- or blockage of β- and γ-secretase could reduce the production of Aβ which becomes insoluble and is precipitated (see Hardy 1997).

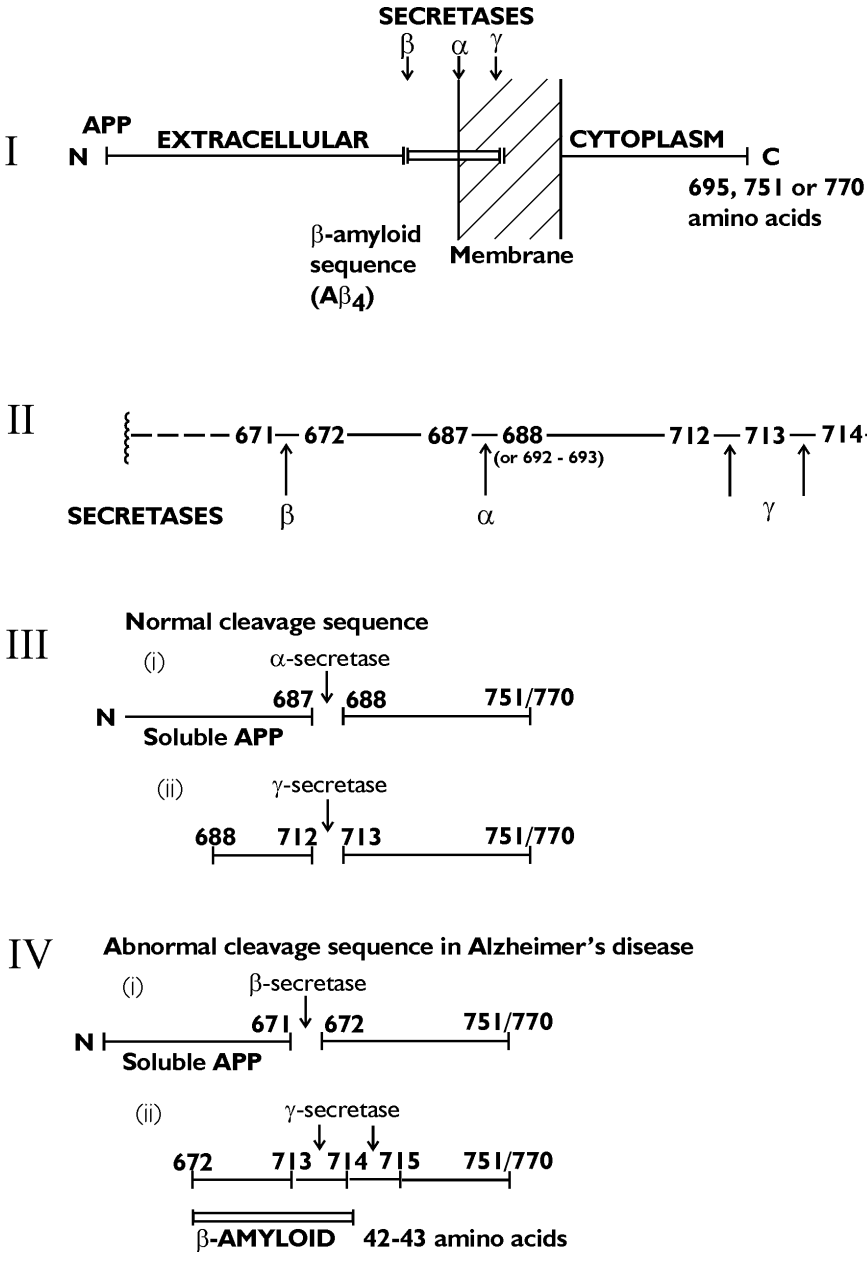

Figure 18.5 Schematic representation of possible cleavage sites of APP by α, *β* and γ-secretase and the production of *β*-amyloid protein. (I) This shows the disposition of APP molecules in 695, 751 and 770 amino-acid chain lengths. Much of it is extracellular. The *β*-amyloid ($A\beta_4$) sequence is partly extracellular and partly in the membrane. (II) An enlargement of the *β*-amyloid sequence. (III) Normal cleavage of APP by α-secretase occurs in the centre of $A\beta_4$ sequence to release the extracellular APP while the remaining membrane and intracellular chain is broken down by γ-secretase to give two short proteins that are quickly broken down. (IV) In Alzheimer's disease *β* rather than α-secretase activity splits off the extracellular APP to leave the full $A\beta_4$ sequence remaining attached to the residual membrane and intracellular chain. 42/43 amino acid *β*-amyloid sequence is then split off by γ-secretase activity

These must be worthwhile objectives and the recent identification by a number of research groups (see Skovronsky and Lee 2000 for description and details) of β-secretase as the membrane-bound aspartyl protease (BACE), β-site APP cleaving enzyme, paves the way for developing possible chemical inhibitors of its activity for experimental and clinical evaluation, although that remains for the future.

NEUROTROPHIC FACTORS

Whether or not the production of β-amyloid can be curtailed, it would be desirable to either replace the damaged neurons or encourage the remaining functional ones to ramify further and exhibit more influence. The former, which requires tissue or cell line grafts, is currently not feasible and barely investigated experimentally but there is much interest in the possible use of neurotrophic proteins (neurotrophins) that encourage neuronal growth and differentiation.

A number of these have been isolated and identified but the first to be discovered (see Levi-Montalcini 1987), and the most studied, is nerve growth factor (NGF) which, despite its name, is not universally effective on all neurons. In the periphery it is mainly released in tissues containing sympathetic nerves that take it up and transport it retrogradely to the cell body where it acts. In the brain, however, it has more influence on cholinergic than noradrenergic or other neurons so that NGF protein and MRNA expression is highest in cholinergic innervated areas of the brain such as the hippocampus and cortex while its binding sites (receptors) are mainly in subcortical regions with cholinergic neurons like the nucleus basalis. In fact injection of NGF into the latter's projection areas like the hippocampus and cortex result in its uptake and transport back to the nucleus. So it may be assumed that normally the cortically produced NGF is transported back to cholinergic subcortical neurons where it exerts its trophic action. Certainly NGF increases ChAT production when added to cultured cholinergic neurons and its intraventricular infusion in rats and primates prevents the loss of ChAT activity in and degeneration of, cholinergic neurons caused by transection of the septal hippocampal cholinergic pathway, or ibotenic acid injection into the nucleus basalis. Intraventricular NGF has also been shown to improve learning and memory in aged rats and those with lesions to cholinergic pathways. So if NGF is so important for the growth and function of the cholinergic neurons, that appear so vulnerable in AzD, can they be restored and AzD controlled by administering NGF? Before that question can be answered some practical problems have to be overcome, namely how to obtain and administer it.

If immune reactions are to be avoided then recombinant human factor should be used and that cannot be produced in large quantities. In any case, it is a large protein that will have to be injected directly into the brain. Even if these problems can be overcome the spread and intensity of any NGF effect has to be restricted so that excessive neuritic growth and inappropriate increases in synaptic connections do not occur.

In addition to these problems there is no evidence of reduced NGF in AzD although levels and receptor number are lower in the nucleus basalis. In fact the levels of NGF were found to be increased in the cortex and hippocampus (Scott *et al.* 1995) and while this could just be due to fewer cholinergic fibres to transport it away from the cortex it does suggest its synthesis is normal and possibly even increased. At least it throws doubt on the value of augmenting NGF as a therapy for AzD.

Nevertheless NGF from mouse mandibular gland has been infused into the right lateral ventricle of two patients (67 and 57 years) for three months at a rate of 75 μg/h. The younger showed no change in memory performance; the older some improvement after one month, which ceased after the infusion was stopped. Both patients had various reversible side-effects such as back pain and weight loss.

OTHER DRUG THERAPY

In the face of the failure of rational approaches in the treatment of AzD it is perhaps not surprising that there have been many less rational ones. These include the use of vasodilators and nootropics. The former, such as hydergine, a mixture of ergot alkaloids, are intended to increase cerebral blood flow and neuronal metabolism despite some reduction in blood pressure, while the latter, like piracetam, are metabolic stimulants that increase cerebral metabolism and ATP production. Neither are of proven value in AzD.

Although there is no evidence that the neuronal degeneration of AzD results, as in cardiovascular ischaemia, from the excitotoxicity of increased intracellular Ca^{2+}, some calcium channel blockers have been tried in AzD. They have had little effect but surprisingly a pyrrolidone derivative nefiracetam, which opens L-type voltage-sensitive calcium channels (VSCCs) reduces both scopolamine- and β-amyloid-induced impairments of learning and memory in rats (Yamada *et al*. 1999). This effect can be overcome by VSCC antagonists, but nefiracetam has not been tried in humans.

SUMMARY

Clearly there is a long way to go in the treatment of AzD particularly as even the most active of the generally ineffective therapies discussed above have only addressed the early symptoms of memory loss. In such a progressive and debilitating disease that is only a small step. Finding a NT malfunction is obviously a long way from providing an effective treatment, let alone a cure. That is more likely to come from attempts to reduce neuronal degeneration (see Selkoe 1999).

REFERENCES AND FURTHER READING

Abe, K, Takeyama, C and Yoshimura, K (1998) Effect of S-8510, a novel benzodiazepine inverse agonist, on basal forebrain lesion-induced dysfunction in rats. *Eur. J. Pharmacol.* **347**: 145–152.

Allen, NRP and Burns, A (1995) The treatment of Alzheimer's disease. *J. Psychopharm.* **9**: 43–49.

Auld, DS, Kar, S and Quiron, R (1998) β-amyloid peptides as direct cholinergic neuromodulations: a missing link? *Trends Neurosci.* **21**: 43–49.

Bliss, TVP and Loma, T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetised rabbit following stimulation of the perforant path. *J. Physiol.* **232**: 331–356.

Bowen, DM, Smith, CB, White, P and Davison, AN (1976) Neurotransmitter-related enzymes and indices of hypoxia in senile dementia and other abiotrophies. *Brain* **99**: 454–466.

Checler, F (1995) Processing of the β-amyloid precursor protein and its regulation in Alzheimer's disease. *J. Neurochem.* **65**: 1431–1444.

Davis, RE, Emmerling, MR, Jaen, JC, Moos, WH and Spiegel, K (1993) Therapeutic intervention in dementia. *Crit. Rev. Neurobiol.* **7**: 41–83.

Hardy, J (1997) Amyloid, the presenilins and Alzheimer's disease. *Trends Neurosci.* **20**: 154–159.

Levi-Montalcini, R (1987) The nerve growth factor 35 years later. *Science* **237**: 1154–1162.

Maragos, WF, Greenamyre, JT, Penny Jr, JB and Young, AB (1987) Glutamate dysfunction in Alzheimer's disease: an hypothesis. *Trends Neurosci.* **10**: 65–68.

McGough, JL, Introlni-Collison, IB and Castellano, C (1993) Involvement of opioid peptides in learning and memory. In *Opioids II. Handbook of Experimental Pharmacology*, Springer-Verlag, New York, pp. 429–448.

Morris, RGM (1984) Development of a water-maze procedure for studying spatial learning in the rat. *J. Neurosci. Meth.* **11**: 47–60.

Neve, RL and Robakis, NK (1998) Amyloid disease: a re-examination of the amyloid hypothesis. *Trends Neurosci.* **21**: 15–19.

Nitta, A, Fukuta, T, Hasegawa, T and Nabeshima, T (1997) Continuous infusion of β-amyloid proteins into cerebral ventricle induces learning impairment and neuronal and morphological degeneration. *Jpn J. Pharmacol.* **73**: 51–57.

Perry, EK (1986) The cholinergic hypothesis — ten years on. *Brit. Med. Bull.* **42**: 63–69.

Perry, EK, Tomlinson, BE, Blessed, G, Bergmann, K, Gibson, PH and Perry, RH (1978) Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *Brit. Med. J.* **2**: 1457–1459.

Rogaeu, EI, Sherrington, R, Rogaevo, EA, Levesque, G, Ikeda, M, Liang, Y, Chl, H, Lin, C, Holman, K, Tsuda, T, Mar, L, Sorbi, S, Nacmias, R, Placentini, S, Amaduco, L, Chumakov, L, Cohen, D, Lannfelt, L, Fraser, PE, Rommens, JM and St George-Hyslop, PH (1995) Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome I related to Alzheimer's disease type 3 gene. *Nature* **376**: 775–778.

Rogers, SL, Farlow, MR, Doody, RS, Mohs, R, Friedhoff, LT and the Donepezil Study Group (1998) A 24-week, double blind, placebo-controlled trial of donepezil in patients with Alzheimer's disease. *Neurology* **50**: 136–145.

Scott, SA, Mufson, EJ, Weingartner, JA, Skou, KA and Crutcher, KA (1995) Nerve growth factor in Alzheimer's disease: Increased levels throughout the brain coupled with decline in nucleus basalis. *J. Neurosci.* **15**: 6213–6221.

Selkoe, DJ (1999) Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature* **399** (Suppl. 6738): A23–A31.

Skovronsky, DM and Lee, VM-Y (2000) β-secretase revealed: starting gate for race to novel therapies for Alzheimer's disease. *Trends Pharmacol. Sci.* **21**: 161–163.

Smith, MA (1998) Alzheimer's disease. *Int. Rev. Neurobiol.* **42**: 1–44.

Tohgi, H, Abe, T, Kimura, M, Saheki, M and Takahashi, S (1996) Cerebrospinal fluid acetylcholine and choline in vascular dementia of Binswanger and multiple small infarct types as compared with Alzheimer-type dementia. *J. Neural Trans.* **103**: 1211–1220.

Wischik, CM and Crowther, RA (1986) Subunit structure of the Alzheimer tangle. *Brit. Med. Bull.* **42**: 51–56.

Yamada, K, Tanaka, T, Mamiya, T, Shiotani, T, Kameyama, T and Nabeshima, T (1999) Improvement by nefiracetam of β-amyloid — (1-42) — induced learning and memory impairments in rats. *Brit. J. Pharmacol.* **126**: 235–244.

Yavich, L, Sirvio, J, Haapalinna, A, Puumala, T, Koivisto, E, Heinonen, E and Reikkinen, PJ (1996) The systemic administration of tacrine or selegiline facilitate spatial learning in aged Fisher 344 rats. *J. Neural Trans.* **103**: 619–626.