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REVIEW

Pharmacological mechanisms and animal models of cognition

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Requirements for an effective animal model of cognition are discussed with special reference to the cholinergic hypothesis of Alzheimer's disease. It is argued, with reference to research on vasopressin and ACE inhibitors, that many putative animal models of cognition lack predictive clinical validity because they either confound the effects of cognitive and arousal processes, or fail to model a specific component of cognitive functioning. A survey of recent research on the cholinergic hypothesis illustrates how these weaknesses can be overcome. Studies involving scopolamine and basal forebrain excitatory amino acid lesion models of the cholinergic deficit in Alzheimer's disease have employed a delayed-matching-to-position test in rodents which, unlike passive avoidance, allows the effects of memory and attentional variables to be distinguished. In combination with recent human studies, these experiments suggest that the cholinergic system has a major role in executive control of attentional resources, and lead to the recommendation of a 'top down' strategy in the investigation of neurochemical processes and pharmacological mechanisms underlying cognition.

Keywords: Alzheimer's disease – Animal models – Attention – Cholinomimetics – Cognition – Memory – Neurochemical – Pharmacological mechanisms

INTRODUCTION

Animal tests of cognitive function have the potential to assist drug discovery both directly as screening procedures, and indirectly through their fundamental role in the investigation of the neurochemical bases of cognition. However, many putative tests of animal cognition cannot be expected to contribute to research via either of these routes because they simply miss their mark: they do not represent in a non-human species the kind of complex psychological processes which constitute human cognition. Consequently, these animal tests are inadequate tools in the investigation of pharmacological mechanisms, and their results lack predictive clinical validity. For example, Sarter *et al.* (1992a, b) recently identified 46 putative cognitive enhancers with cholinomimetic mechanisms. Each of these compounds has been claimed, on the basis of animal tests, to improve cognitive functioning. However, of the 20 that have been tested with human subjects, not one has provided evidence of clinical efficacy.

In the first part of this paper we will consider the distinction between cognitive and non-cognitive psychological processes such as arousal, with a view to identifying animal tests that are likely to have more predictive validity than some of those currently in common use. The discussion will focus on two "cautionary tales"; cases in which drugs (vasopressin and ACE inhibitors) were misidentified as cognition enhancers because they were tested using procedures that could not distinguish cognition enhancement from effects generated by an increase in arousal. The identification of tests of animal cognitive function is a necessary, but not a sufficient condition, for the development of effective pharmacological treatments for disorders such as Alzheimer's disease (AD). In addition, tests which are sensitive to specific cognitive processes are needed, and new pharmacological approaches must be explored. In the second section of the paper, the first of these challenges will be considered with reference to contemporary research on the cholinergic hypothesis, where progress is being made in the development of animal procedures that are differentially sensitive to deficits in memory and attention. Finally, we shall assess the prospect of preventative pharmacological treatment for AD.

COGNITION AND OTHER PSYCHOLOGICAL PROCESSES

The first and most obvious requirement of any test of animal cognition is that it measures a cognitive rather than a non-cognitive psychological process, and therefore in order to construct such a test, or to evaluate existing pro-

cedures, it is necessary to be able to distinguish cognitive from non-cognitive processes in both theory and practice. The concise Oxford English Dictionary defines "cognition" as the "faculty of knowing, perceiving, [and] conceiving, as opposed to emotion and volition". Contemporary psychologists use the term in a similar but more specific sense. They contrast cognitive processes with: sensory and motoric processes (those responsible for peripheral receipt of information from the environment and execution of behaviour); emotional and motivational or arousal processes; and, in some cases, associative or behavioural processes. In essence, it is believed that of all psychological processes—those which directly affect behaviour—only some, the cognitive processes, involve the active construction and manipulation of mental models or images of the environment. Consequently, in order to determine whether a test is sensitive to cognitive functioning in animals, it is necessary to find out whether the critical aspect of task performance requires the active formation and manipulation of internal representations. The details of how this may be achieved will depend on the nature of the task, and we will consider an example, involving a delayed conditional discrimination procedure, in some detail when discussing the cholinergic hypothesis below.

Thus, animal tests must allow the effects of drugs on cognitive processes to be distinguished from their effects on other psychological processes, especially sensory, motoric and non-specific arousal processes. In principle, compounds may improve task performance, not via their effects on cognitive processes, but by, for example, increasing an animal's perceptual acuity, affecting the precision of its control over limb movements, and/or modulating the intensity of its activity. In this way, drugs could improve performance in a manner analogous to that of spectacles, braces and cold showers. Such effects, as the analogy implies, may have therapeutic value, but they should be distinguished from effects on cognitive variables, in order to make animal models as predictive as possible. For example, if a drug is improving performance on the model task by increasing activity levels, then one might reasonably expect it to improve performance on other tasks in which a high response rate is optimal, and cause a decrement in performance on tasks in which slow responding is required. On the other hand, if the drug is improving performance on the model task because it is enhancing the efficiency of a particular cognitive process, then one could expect it to improve performance on all tasks involving that process, regardless of their response rate requirements. One might call the first kind of compound a "non-specific" or "Type I" cognition enhancer, and the second kind as a "specific" or "Type II" cognition enhancer. It does not matter how the distinction is marked, as long as it is not ignored. The risk of mistaking compounds which affect general activity levels (Type I cognition enhancers) for specific or Type II cognition enhancers is so great that we shall consider, in some detail, two cases in which an error may have been made.

Vasopressin

De Wied (1965) showed that peripherally administered pitressin or lysine-8-vasopressin reversed the facilitatory effect of neurohypophysectomy on shuttle box avoidance behaviour in rats, a finding which received support from several sources. The discovery that intracerebroventricular (i.c.v.) administration of vasopressin improved passive avoidance learning, and that it was 1000-fold more potent when administered i.c.v. than subcutaneously (s.c.), seemed to confirm that vasopressin played a role in memory processes through a central site of action (De Wied, 1976). However, in the early 1980s evidence began to emerge that vasopressin may have its effects via a peripheral alerting mechanism, rather than on central cognitive processes. For example, LeMoal *et al.* (1981) replicated De Wied's finding that peripherally administered arginine-vasopressin (AVP) prolonged the extinction of pole jumping avoidance in rats, but also showed that this effect was absent when the pressor effect of the compound was abolished. They made an elegant correlation of the direct effect of AVP on blood pressure with its effects on the extinction of avoidance behaviour. This raised the possibility that vasopressin facilitates performance through its peripheral effect on blood pressure, and further evidence in support of this hypothesis soon appeared. Sahgal *et al.* (1983) found that post trial i.c.v. administration of AVP improved the performance of some rats in a passive avoidance task, while impairing that of others, and argued that exogenous vasopressin may increase the rats' state of arousal. If an animal is in a state of low arousal before treatment, then an increase in arousal will facilitate performance, but if it is in an optimum or high arousal state, then the Yerkes-Dodson principle specifies that a further increase in arousal will impair performance.

These studies, and others (e.g. Koob *et al.*, 1981; Ettenberg *et al.*, 1983) undermine the hypothesis that vasopressin has a direct effect on cognitive function, and emphasise the care that should be taken to distinguish arousal modulation, from cognition enhancing, properties of compounds. Although it could have provided a salutary lesson, familiarity with the vasopressin case does not seem to have prevented a similar sequence of events from beginning to unfold with respect to angiotensin-converting enzyme (ACE) inhibitors.

ACE inhibitors

It is generally held that a study by Croog *et al.* (1986) on the effects of captopril (an ACE inhibitor), methyldopa and propranolol (a beta blocker) in hypertensive patients

showed that captopril, but not propranolol or methyldopa, had mood and cognition enhancing effects in patients during a 20 week treatment study. The authors concluded: "At 24 weeks, the captopril group was better off than the methyldopa group in terms of the measures of vitality, positive well-being, anxiety, and depression; physical symptoms and sexual dysfunction; cognitive function; work performance; and satisfaction with life" (Croog *et al.*, 1986, p. 1662). Subsequent animal studies apparently confirmed these findings, suggesting that captopril has cognition enhancing effects in rodents, and an anxiolytic action in rodents and marmosets (Costall *et al.*, 1990). In mice, some behavioural changes that have been interpreted as a cognition enhancing effect of the ACE inhibitors captopril and SQ29,852 were demonstrated in a black/white box habituation test. The apparatus used in this test consisted of a rectangular box, divided unequally on a 40/60 basis. The smaller compartment was painted black and dimly illuminated by a red lamp, and the larger one was painted white and brightly lit by a 60 W lamp. At the beginning of the test, each mouse was placed in the middle of the white area and allowed to escape into the black, dimly lit area through a small aperture at floor level in the middle of the dividing wall. On the first day, the escape latency was approximately 12 s, and on subsequent daily trials, the latency gradually decreased until, on the fifth day, the mice were taking approximately 4 s to leave the white compartment. Costall *et al.* (1990) found that the rate of decline in escape latency was accelerated by captopril (0.005-0.05 mg/kg, i.p., twice daily) and SQ29,852 (0.00005-0.0005 mg/kg, i.p., twice daily), and that while scopolamine (0.25 mg/kg, i.p.) caused an increase in escape latency in vehicle treated animals, this effect was not present in mice treated with both scopolamine and captopril.

These studies by Croog's and Costall's groups apparently provide converging human and animal evidence that ACE inhibitors have cognition enhancing properties, and therefore seem to warrant their development as cognition enhancing and anxiolytic agents. However, a closer reading of the clinical study by Croog *et al.* (1986) undermines these conclusions. The study was motivated in part by the non-compliance of many hypertensive patients in widely-prescribed propanolol and methyldopa treatments: "In treating patients with hypertension, physicians who are successful in controlling blood pressure may be unaware of the negative effect that antihypertensive drugs can have on the quality of life—on physical state, emotional well being, sexual and social functioning, and cognitive acuity—of their patients. Some patients perceive the use of antihypertensive medication to be more troubling than their seemingly symptomless disease, resulting in non-compliance and ineffectual long-term treatment" (Croog *et al.*, 1986, p. 1657). Given that methyldopa and propanolol have these side effects, it is likely that the captopril-treated subjects in the study of Croog *et al.* (1986) out-performed those treated with the other two drugs not because captopril enhances cognition and reduces anxiety, but because it has no effect on these variables, while methyldopa and propranolol depress cognition and increase anxiety. Captopril-treated subjects should have been compared, not only with those receiving methyldopa and propranolol, but also with untreated subjects, and/or with people receiving a different treatment for a different disorder, and/or with hypertensive patients receiving another compound that does not have detrimental effects on cognition.

Better controlled studies of ACE inhibitors, and other drugs for hypertension (Herrick *et al.*, 1989; Steiner *et al.*, 1990; Croog *et al.*, 1990), have recently been reviewed by Fletcher and Bulpitt (1991). In the main, these studies have compared the effects of enalapril (another ACE inhibitor) and captopril with those of propranolol and atenolol (a beta blocker with fewer side effects than propranolol), and/or with those of the calcium channel blockers verapamil or nifedipine. Steiner *et al.* (1990) confirmed that propranolol has an adverse effect on a number of indices of physical and psychological well-being, and showed that both enalapril and atenolol result in higher scores on these scales. Although these measures suggest that there is little difference between ACE inhibitors and beta blockers, they do not assess directly any effects that these compounds may have on cognitive processes. Several of the studies used a more direct test ("Trail-Making B"), which measures the speed and accuracy with which patients can join letters or numbers embedded in a mixed array. In their 1986 study, Croog *et al.* found that captopril-treated patients were significantly faster in this task than those given methyldopa, but no faster than those given propranolol. Furthermore, in their 1990 study Croog *et al.* observed a tendency for male patients treated with captopril to be slower than those receiving atenolol. Thus, when compared with a control drug, such as atenolol, it would appear that ACE inhibitors do not have a positive effect on cognitive processes. The results of the original Croog *et al.* (1986) study, mistaken for evidence that captopril is a cognition enhancer, show only that compared with methyldopa and propranolol it does not seem to have a detrimental effect on cognition.

The animal studies which apparently confirmed that ACE inhibitors have positive effects with respect to cognition and anxiety, do not, in fact, provide strong evidence contrary to the foregoing conclusion. In most of these studies (e.g. those involving marmoset threat and black/white box tests) the animals have been motivated to escape aversive stimulation. Consequently, they are likely to have been in states of high arousal, and, as in the case of vasopressin, ACE inhibitors may have improved their perform-

ance by reducing blood pressure, and therefore arousal, towards an optimal level. It is claimed that ACE inhibitors enhance cognition and reduce anxiety in a dose range below that at which they affect blood pressure, but since the cognition/anxiety range is also below that necessary to inhibit the enzyme in the brain, these data are difficult to interpret. The doses of captopril and SQ29,852 required to produce 50% inhibition of brain ACE are approximately 1.0 and 100 mg/kg, respectively (Hutson, 1992, personal communication).

The case of ACE inhibitors shows how the inadequacies of animal tests can be overlooked when their results are apparently consistent with human data. More specifically, it underlines the necessity for behavioural pharmacologists to check their conclusions against biochemical data; to ensure, inter alia, that what is claimed to be the effective dose of a centrally acting drug is, in fact, high enough to provide sufficient compound in the brain to inhibit the enzyme in the brain. For both vasopressin and ACE inhibitors, the mechanism of action of the compounds was unspecified and in the case of vasopressin it certainly included a non-specific arousal component. Thus, the first requirement of a model, to distinguish between the effects of drugs on cognitive and non-cognitive psychological processes, was not fulfilled.

COMPONENT COGNITIVE PROCESSES: THE CHOLINERGIC HYPOTHESIS

The expression "animal models of cognition" is ambiguous and therefore potentially misleading. Under one, false, interpretation, it implies that "cognition" is a single, homogeneous function or faculty that might be modelled in its entirety by a single procedure. In fact, as a glance at any contemporary psychology textbook indicates (e.g. Gleitman, 1986), the psychological processes which constitute human cognition are believed to be at least as numerous and diverse as the physiological and behavioural processes which constitute thermoregulation. Consequently, the second requirement of any animal model of cognition is that it model a specific component of cognition.

It would be easier to fulfil this requirement if one could refer to an agreed set or taxonomy of cognitive processes. Unfortunately, the general theories of cognition that could provide such a clear point of reference are still under development (e.g. Newell, 1990) and at present one finds within the literature on human cognition a potentially bewildering array of different methods of distinguishing cognitive processes one from another. However, current understanding of the cognitive system suggests that it has basic components responsible for attending, categorising, remembering, reasoning and planning respectively, and that each of these has important, semi-autonomous subcomponents. For example, "working memory", the system responsible for remembering task-specific information over relatively short periods of time, is distinguishable from "long-term memory" which retains task-general information over relatively extended periods of time. Furthermore, each of these has encoding, storage and retrieval subcomponents.

The vasopressin and ACE inhibitor studies discussed above not only confounded drug effects on cognition and arousal, but also fell short of the second requirement, in that they did not attempt to identify which component of the cognitive system was affected by the drugs, or how it was influenced. The role of the cholinergic system has been examined more thoroughly in this respect, and it is to this example that we now turn in search of a better example of how to identify animal models of cognition. Through our discussion we hope to indicate what animal tests have revealed about the role of acetylcholine (ACh) in cognitive processes, and how this research might influence future investigations of neurotransmitters and cognition. We shall also consider the relationship between animal and human tests of cognition; and attempt to distinguish tests that might be said to be "cognitive" from those in which performance can be attributed to the operation of simpler psychological processes.

A review of the literature in the last decade reveals that more research has been devoted to the study of ACh in cognitive processes than to that of any other neurotransmitter. This effort has, of course, been driven by the search for an effective treatment for Alzheimer's disease (AD). AD is characterised clinically by a progressive deterioration of cognitive functions, the most notable of which is the patients' difficulty in acquiring and retaining new information. The neuropathological and neurochemical dysfunctions in AD have been the focus of intensive study since the mid 1970s when three independent laboratories reported lower activity of the enzyme choline acetyltransferase (ChAT) in the cortex of patients dying with AD (Bowen *et al.*, 1976; Davis and Maloney, 1976; Perry *et al.*, 1977). ChAT is the biosynthetic enzyme for ACh and is specific marker for the integrity of cholinergic neurons. ACh in the cerebral cortex is principally derived from neurons of the basal forebrain which innervate widely the cortex and hippocampus.

The cortical depletion of ChAT is reported by some groups to parallel a loss of neurons in the basal forebrain (Whitehouse *et al.*, 1982) and there is general agreement that the severity of cognitive decline correlates with the degree of cholinergic loss and the severity of the neuropathological damage in the cortex (Mountjoy *et al.*, 1984). The aetiological relationships between the plaque and tangle formations, ChAT depletion and neuronal degeneration, however, remain controversial.

It is important to emphasize that AD frequently

involves degeneration in other forebrain neurochemical systems (Rossor, 1982). However, the cholinergic deficiency is the most common and the most severe neurotransmitter loss seen in AD. Combining this fact with the finding that in young, healthy volunteers scopolamine, a centrally acting muscarinic antagonist, produced short-term memory deficits which could be reversed by physostigmine (Drachmann 1977) was led to suggest that brain cholinergic systems are necessary for normal cognitive functioning.

Since these discoveries in the late 1970s, two broad approaches have been taken to the development of cholinomimetic replacement therapy for AD. One approach has focused on drugs that modulate directly the cholinergic system, and a second has sought to develop compounds that modulate other neurotransmitter systems in the brain and thus affect cholinergic function indirectly. Within the former approach, two classes of compounds have been developed: cholinesterase inhibitors, and muscarinic receptor agonists. However, in the course of the last decade both approaches have encountered pragmatic and theoretical problems.

Cholinomimetic replacement therapies

Cholinesterase inhibitors. The rationale for a cholinesterase inhibitor is based on the assumption that preventing the breakdown of ACh in the synapses enhances transmission at cholinergic receptors. Peters and Levin (1979) first reported that a cholinesterase inhibitor, physostigmine, in combination with lecithin (a precursor of ACh), improved the performance of patients with AD on a number of memory tests. However, the utility of physostigmine is limited by its poor bioavailability, adverse side-effect profile and short half life (Davis *et al.* 1983). Interest in the cholinesterase inhibitors increased when Summers *et al.* (1986) reported on the effects of oral 9-amino-1,2,3,4-tetrahydroaminoacridine (THA) in patients with AD. In a double-blind, placebo-controlled, cross-over study, they treated 17 patients for 3 weeks with THA, and the results were dramatic. THA improved performance on all four of the assessment tests, including those of short-term, or working, memory; the very kind of memory that deteriorates rapidly in the early stages of the disease. Recently, however, the U.S. Food and Drug Administration (FDA) (1991) have questioned the validity of these findings. They pointed out that there were several discrepancies between what Summers *et al.* (1986) reported and what had actually occurred. Specifically they stated that: (i) there was no documentation of randomly assigned treatment; (ii) blinding was not uniformly maintained; and (iii) one of the global rating scales was carried out retrospectively. However, it has been reported lately, that THA does have some beneficial effects in patients with AD (Eagger *et al.*, 1991), although they are much more modest than those claimed by Summers *et al.* (1986), and this year (1992) Warner Lambert have been granted a treatment "Investigational New Drug" (IND) licence by the FDA. Unfortunately, the long term use of THA results in liver damage thus limiting its therapeutic potential.

More recently a number of cholinesterase inhibitors have been discovered with longer duration of action and improved therapeutic/side-effect ratios. Eptastigmine, a carbamate derivative of physostigmine, is one such compound. It reverses scopolamine-induced performance deficits in rodent and primate tests (Dawson *et al.*, 1991b; Rupniak *et al.*, 1991a, b), and has a half life of 18 h (Freedman *et al.*, 1991a). In rats, doses of physostigmine that inhibit acetylcholinesterase activity by 30% render the animal incapable of performing a lever pressing task for access to food pellets. In contrast, doses of eptastigmine that result in 50% inhibition of acetylcholinesterase, and reverse the effects of scopolamine in behavioural tasks, have no detrimental effects on performance in the same task. This suggests that with an appropriate acetylcholinesterase inhibitor it may be possible, in humans, to achieve cognitive enhancement without adverse side-effects.

Muscarinic agonists. The second cholinomimetic approach has been to develop muscarinic receptor agonists, which stimulate the muscarinic receptors on the post-synaptic neuron. The first generation of these compounds is exemplified by RS-86. The results of clinical trials with RS-86 have proved difficult to interpret. They did not provide any evidence that RS-86 improves cognitive function, but it is not clear whether this is because RS-86 is genuinely ineffective, or because its many side-effects mask its remedial effects (Mouradian *et al.*, 1988).

The recent discovery that there are at least three muscarinic receptor subtypes (Bonner, 1989) has led to the suggestion that different receptor subtypes may mediate the peripheral side-effects and the cognition enhancing effects of full muscarinic receptor agonists such as RS-86 (Freedman *et al.*, 1989). Since post synaptic M_1 muscarinic receptors in the cortex and hippocampus appear to be relatively unaffected by AD, muscarinic receptor agonists that are selective for this receptor subtype have been sought. However, there is considerable evidence that this receptor subtype also mediates some of the side-effects induced by muscarinic agonists (e.g. Dawson *et al.*, 1991a).

It is clear that much time and effort has been devoted to the discovery of selective, high efficacy muscarinic receptor agonists. It is also becoming apparent that neurochemists are having considerable difficulty in developing receptor selective muscarinic agonists with an acceptable side-effect profile. However, functional selectivity can be achieved using partial agonists, acting at different sites with varying degrees of receptor reserve. This approach

has been adopted over the last 10 years by a number of laboratories, but since relatively little progress has been reported, it may be facing insurmountable problems.

Recently a novel series of low efficacy muscarinic receptor agonists has been discovered, one of which, L-689,660 (l-azabicyclo (2.2.2) octane, (3-(6-chloropyrazinly) malate, R-enantiomer) (Baker *et al.*, 1991), has been shown to have an agonist action at M_1 and M_3 receptors and an antagonist action at M_2 receptors (Hargreaves *et al.*, 1992; Freedman *et al.*, 1991b). Also, Fisher and colleagues have developed a putative M_1 selective ligand known as AF102B which has a lower intrinsic efficacy than L-689,660 (Fisher and Hanin, 1986; Fisher *et al.*, 1986). Both compounds have achieved limited success in a number of rodent and primate models in which performance is disrupted by the systemic administration of scopolamine (Dawson *et al.*, unpublished data), and L-689,660 reverses a scopolamine-induced deficit in a visuo-spatial memory task in primates (Rupniak *et al.*, 1991b). However, the doses of L-689,660 and AF102B that had effects on cognition also reduced the response rates of rats chain-pulling for food pellets on a random interval schedule, indicating that these compounds also have measurable effects on motor function. As a consequence L-689,660 and AF102B may encounter the same problems in the clinic as RS-86, that drug induced side-effects may preclude the testing of the compound at the doses required to influence cognitive processing. Although L-689,660 is a recent development, AF102B has been evaluated for a number of years (Fisher *et al.*, 1986). It is believed to be in development, but the paucity of information provided since the initial publications suggest that it is making slow progress to the clinic.

Alternative compounds modulating the cholinergic system. Costall *et al.* (1989) have claimed that 5-hydroxytryptamine (5-HT) antagonists selective for the 5-HT_3 receptor modulate cognitive process in rodents and primates, and reverse the deficit induced by scopolamine in a food-reinforced alternation task in rats. More recently, Preston *et al.* (1992) have also claimed that a 5-HT_3 antagonist partially reversed the effects of scopolamine on a subset of cognitive tests in young human volunteers. Barnes *et al.* (1989) have shown that *in vitro* 5-HT_3 receptors mediate the inhibition of ACh release, and Tyers (1991) has suggested that 5-HT_3 antagonists may have their effect by increasing ACh release. However, in the absence of direct evidence that this is the case, and in view of the fact the doses administered in the *in vivo* tests (10.0 ng/kg i.p. in rats and mice; 1 and 10 ng/kg s.c. in marmosets) are unlikely to be sufficient to increase the release of the ACh centrally, this hypothesis requires further substantiation.

There are many new compounds under development that are putative modulators of the central cholinergic system (see Sarter *et al.*, 1992a, b for lists). Some of these may be sufficiently efficacious and free of side-effects to allow a thorough test of the hypothesis that improving cholinergic function will improve cognitive processes. However, as we shall outline below, it is only relatively recently that the role of the cholinergic system in cognitive processes has been uncovered. In the meantime, although there have been some encouraging clinical indications that cholinomimetic replacement therapy might be an effective treatment, the challenge is to develop a drug that can be given safely to elderly people suffering from the disease. This may be more difficult than anticipated, because ACh is not only widely utilised as a neurotransmitter in the CNS, but also plays a prominent role in the control of heart, lung and gut function. Administration of classical cholinesterase inhibitors and muscarinic agonists to healthy volunteers and AD patients is known to result in nausea, vomiting, high blood pressure, shallow breathing and many other adverse effects (Mouradian *et al.*, 1988). For these reasons it is essential to have animal models to identify drugs which enhance cognition at therapeutic doses devoid of major side-effects.

Animal models of the cholinergic deficit in Alzheimer's disease

There are several putative models of the cholinergic deficit in AD. These include the aged primate and rat models (Bartus *et al.*, 1978; Dunnett *et al.*, 1988; Rupniak *et al.*, 1991a). However, two models, the scopolamine model and the basal forebrain (nucleus basalis of Meynert: NBM) excitatory amino acid lesion model, have been researched extensively and have been the subject of some controversy (Fibiger, 1991; Sarter *et al.*, 1992a, b). As outlined above, scopolamine is a nonselective postsynaptic muscarinic receptor antagonist that blocks the stimulation of postsynaptic receptors by ACh. Drachmann (1977) has shown that scopolamine causes memory deficits when administered to normal, young volunteers; and the results of both passive avoidance and delayed-matching-to-position tests have provided evidence that scopolamine induces memory deficits in rats and monkeys (e.g. Bartus, 1978; Pontecorvo *et al.*, 1991; Murray *et al.*, 1991; Rupniak *et al.*, 1991a).

Passive avoidance is probably the most widely used test of long-term memory in rodents. In one version of this test, rats are placed in the bright compartment of a two compartment chamber, having been given a brief electric shock 24 h earlier, in the other, dark compartment. Under these circumstances, rats are usually reluctant to leave the bright chamber and enter the dark, but they tend to do so more readily if the NBM has been lesioned using quisqualic acid (Page *et al.*, 1991), or if the rats were treated

with scopolamine prior to being shocked on the previous day (e.g. deNoble, 1986; Dawson *et al.*, 1991).

Although apparently straightforward, and sensitive both to systemic scopolamine and excitatory amino acid lesions of the cholinergic basal forebrain, the passive avoidance test is unsatisfactory for two reasons. First, step-through latency data show marked between-subject, inter-experiment and inter-laboratory variability (Bammer, 1981; Shulz *et al.*, 1986). Second, when the shock-test interval is varied, rats do not always show the kind of delay-dependent effects that one would expect if the passive avoidance test assessed memory. Instead of declining smoothly with delay, step-through latency is sometimes longer with short and with long shock-test intervals than with intermediate delays (Kamin, 1956). Thus, under certain experimental conditions, the processes of learning and memory consolidation may not always follow the pattern that might be expected. In such cases the effects of compounds on the cognitive processes involved in this task should be considered with caution.

The delayed-matching-to-position (DMTP) test was developed by Dunnett (1985) and provides a powerful measure of working memory function. The procedure is conducted in an operant chamber, and each trial has three stages. In the first, sample stage, one of two levers enters the chamber, and the rat is required to press it. After the response, the lever is retracted and the second stage begins. In this, delay stage, the rat must go to the food tray and make "nose pokes" against the tray flap, for a variable period, until both levers are presented. To obtain a food reward in the final, choice stage, the rat must press the sample lever; the one that was presented and pressed during the first stage. In a variant of the DMTP test, the delayed-nonmatching-to-position (DNMTP) test, the rats are required at the choice stage to press the lever which was not presented at the sample stage. One of the advantages of these tasks is that they enable the investigator to check whether any deficits in performance are due to memory impairment or to sensory or motor deficits. If a drug causes deficits at long delays, but not at short delays, then this suggests that the animal can cope with the task demands and encode the sample information adequately, and implicates a memory-specific effect.

The sensitivity of the DMTP and DNMTP tests was first assessed using aged animals. Dunnett (1988) trained young (6 months), middle aged (15 months) and old (24 months) rats on both the DMTP and DNMTP tasks, and found that all of them performed well at short delays, and showed a decline in choice accuracy with longer delays. However, the rate of decline with delay was greater for old rats than for young and middle aged animals, and the pattern of performance of the old rats was comparable to that observed in aged monkeys (Bartus *et al.*, 1978) and humans (Sahakian *et al.*, 1988).

DMTP studies have challenged the view that scopolamine causes memory impairments, and have consequently cast doubt on the adequacy of the scopolamine model, and of the cholinergic hypothesis. The performance deficits following both 0.2 mg/kg (Dunnett, 1985; Dawson *et al.*, 1991) and 0.5 mg/kg of scopolamine (Dunnett, 1985) on DMTP were not delay-dependent. Instead of increasing with delay, the effect of scopolamine on percentage choice accuracy remained constant across sample-choice intervals, suggesting that scopolamine compromises initial processing or detection of features of the sample, but that, once encoded, their retention and retrieval is not affected (see also Pontecorvo *et al.* (1991) for a discussion of this effect).

However, these conclusions must be tempered by two considerations: (i) systemic administration of scopolamine affects all of the diverse cholinergic systems in the brain; and (ii) ceiling effects in DMTP and DNMTP may result in seemingly delay-dependent effects. First, it is apparent from a number of studies that discrete lesions of the cholinergic system produce both delay-dependent and delay-independent effects (discussed in detail below). Thus, systemic administration of scopolamine may affect the diverse systems equally, but the behavioural consequences of affecting one system may overshadow effects on another. Second, when DMTP or DNMTP are used and delay-dependent effects are claimed, the necessary delay by treatment interaction depends on the difference in choice accuracy between the treatment group and control group being greater at longer delays. The dependent measure in these studies is often "choice accuracy", with the number of correct choices expressed as a percentage of the number of trials attempted. However, it is not uncommon for the control groups to attain 95-100% correct at the 0 s. Thus, at the 0 s delay any difference between the control group and the treatment group may be masked by a "ceiling effect"; i.e. by the limits on performance imposed by the structure of the test (see Murray *et al.*, 1991 for an example). As we shall discuss later when considering human studies of DMTP, the results of this kind of procedure should be treated with caution when considering delay-dependent and delay-independent affects.

Both of these points are neatly illustrated in a study by Dunnett *et al.* (1990). In this study various doses of scopolamine were administered to the hippocampus or prefrontal cortex of rats performing on a DNMTP task. Two doses of scopolamine, 4 and 12 μg/μl, were injected into the prefrontal cortex, and three doses, 4, 12 and 35 μg/μl, were injected into the hippocampus. Three injections of each dose were given in a counterbalanced order, and the series of prefrontal injections was completed before the hippocampal series began. The results showed that the 12 μg/μl dose administered into the prefrontal cortex disrupted performance at all delays, while, the 4 μg/μl dose had little

effect. The analysis of variance with factors of treatment and delay did not result in a treatment by delay interaction and as a consequence, the percent correct scores were collapsed across the delay factor and a *post hoc* Newman-Keuls test showed that the 12 μg/μl dose decreased the percent correct score relative to saline control scores. Thus, it appears that scopolamine injected into the prefrontal cortex resulted in a dose-dependent, but not a delay-dependent effect. In contrast, an analysis of variance of the results from the second series of injections into the hippocampus resulted in a significant treatment × delay interaction. This significant interaction permitted *post hoc* comparisons to be made between treatment levels at each of the delay intervals. These tests showed the dose of 35μg/μl significantly reduced the percent correct score to approximately 85% at the 0 s delay, and approximately 50-55% at delays from 4 s to 24 s. However, when the rats were given 12 μg/μl their performance differed from their saline control scores at all delays except 0 s.

These results imply that doses of scopolamine administered to the hippocampus result in dose *and* delay-dependent affects. However, as outlined above this conclusion is compromised in a number of respects. There may be an effect of order of injection route; injections of scopolamine into the prefrontal cortex may have provided the animals with an opportunity to learn to adapt their performance to the detrimental effects of scopolamine. It would also have been informative to have seen the effects of the 35 μg/μl dose injected into the prefrontal cortex, as a delay-dependent effect may have emerged. More importantly, however, is the fact that the saline control scores could not be greater than 100%. A difference between the treatment levels at the 0 s delay may have been masked by this upper limit on measured performance. While this study is suggestive of the differential effects of scopolamine when administered i.c.v., it also illustrates the difficult methodological problems that confront researchers in this area.

The effects of neurotoxic lesions on DMTP performance have also been interpreted as undermining the cholinergic hypothesis. These lesions, of the basal forebrain cholinergic system, have principally been made using ibotenic (IBO) and quisqualic (QUIS) excitatory amino acids. When used at the appropriate concentrations, these neurotoxins destroy cells at the site of injection while sparing fibres of passage.

Etherington *et al.* (1987) began undermining the cholinergic hypothesis with a report on the effects of ibotenic, quisqualic and surgical fimbria-fornix (FF) lesions on DMTP and DNMTP performance in the nine-hole box task, in which instead of pressing a lever at the choice stage, the rat must poke its nose into one of two holes, depending on which was illuminated at the beginning of the trial. FF lesions reduce cholinergic input to the hippocampus, but unlike excitatory amino acid lesions to the NBM, not to the frontal cortex. Etherington *et al.* (1987) found that while FF lesions induced delay-dependent deficits on both DMTP and DNMTP tests, IBO and QUIS lesions did not. IBO lesioned rats showed deficits at all delays, and the performance of those with QUIS lesions was, remarkably, indistinguishable from that of sham-operated controls. Moreover, the reduction in ChAT levels in the frontal cortex was 51% for the QUIS group and only 17% for the IBO group.

These results suggested that large reductions in cholinergic activity in the frontal cortex do not induce memory deficits as was earlier assumed. Apparently confirming this, Page *et al.* (1991) showed that lesions of the NBM with alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) deplete neocortical ChAT by more than 70% and yet do not affect acquisition or retention in a water maze task. These results have led one reviewer to conclude that "In the absence of adequate functional definition, programs aimed at developing cholinergic pharmacotherapies for the cognitive deficits in AD are based more on faith than on established facts" (Fibiger, 1991, p. 223). Similarly, Dunnett *et al.* (1991), in a review of the effects of IBO and QUIS lesions on water maze, passive avoidance, DMTP and serial 5-choice discrimination tests, concluded that "In view of the extensive cortical ChAT depletions in all these studies, these observations rule out an essential role of the NBM-cortical cholinergic neurons in learning (including reference memory) *per se*" (Dunnett *et al.*, 1991, p. 496). Thus the weight of evidence from lesion studies and results with scopolamine suggest that cholinergic systems are not involved in memory *per se*. However, a study by Heise *et al.* (1976) showing that scopolamine affects attention, and a study by Kirk *et al.* (1988) showing that low doses of scopolamine affect discrimination, but not the rate of forgetting in a delayed conditional discrimination task, suggest that cholinergic systems may play an important role in modulating attention, and possibly other components of the cognitive system.

Acetylcholine and attention

The role of the cholinergic system in attentional processes in humans has been widely studied. For example, Dunne and Hartley (1986) claimed that scopolamine selectively impaired performance on an attentional task in humans that required active allocation of attentional capacity. In contrast, scopolamine enhanced performance on a task which involved "automatic processing". Sahakian *et al.* (1989) found that nicotine (a cholinergic receptor agonist) improved performance of patients with probable AD on information processing and attentional tasks. However, whether AD primarily affects attentional processes is a

subject of controversy. Sahakian *et al.* (1990) found that in a subset of patients with early to moderate AD, attentional processes were spared relative to mnemonic processes. By contrast, Struat-Hamilton *et al.* (1988) found convincing evidence of attentional dysfunction in patients with AD. These differences may be attributable to divergent methodology or implicit models of attentional function. Nevertheless, it is clear that if the cholinergic system regulates attention, rather than memory, and if AD is characterised by memorial, rather than attentional impairments, then the cholinergic hypothesis would be an unsatisfactory account of AD. However, research by Baddeley and his colleagues suggests that the primary deficit in AD may, after all, be attentional.

Morris and Baddeley (1988) have suggested that a deficit in controlling the central executive component of working memory may be at the root of AD. The central executive is one of three principal components of the working memory system, a theoretical construct designed to replace that of short-term memory (Baddeley and Hitch, 1974). The central executive is served by two slave systems, the visuo-spatial sketch pad and the phonological loop. As their names imply, the former is responsible for maintaining and processsing visual and spatial information, and the latter processes speech-based information. The central executive is an attentional control system that has access to long-term memory and coordinates information from the slave systems.

Baddeley *et al.* (1986) provided evidence that when AD patients with mild to moderate dementia, are required to coordinate information and allocate attention to two tasks simultaneously, their performance is poor relative to that of age-matched controls. Their subjects performed a pursuit tracking task, designed to occupy the visuo-spatial sketch pad, in combination with a digit span task, involving the phonological loop. The difficulty of both tasks was adjusted so that, when each task was undertaken in isolation, the performance of AD patients and controls was equal. However, when the tasks were undertaken simultaneously, the performance of AD patients was significantly impaired, implicating dysfunction of the central executive. Furthermore, when the subjects were retested over a 12-month period, both single and dual task performance was sustained in controls, but the dual task performance of AD patients deteriorated in a manner not entirely attributable to any decline in single task performance (Baddeley *et al.*, 1991). These results are consistent with the hypothesis that the deterioration is in central executive control, in attentional switching, rather than in the memorial slave systems themselves.

However, most data from studies with AD patients suggest, at first sight, that the deficit is primarily indeed one of memory, i.e. that information can be encoded, but not retained over time (Hart *et al.*, 1987; Sahakian *et al.*, 1988). On closer scrutiny however, evidence is emerging that patients with AD perform less well than age-matched controls in a delayed-matching-to-sample (DMTS) test because they discriminate the stimuli in the sample stage less well than the age-matched controls. In an analysis of DMTS data obtained from patients with AD, Money *et al.* (1992) found that *log d*, a measure of discriminability which is analogous to the *d'* of signal detection theory, is significantly reduced by AD. In contrast, although the AD patients performed less well than the controls at all sample-choice delays, the rate of forgetting (*log b*) did not differ between groups. Money *et al.* (1992) acknowledge the differences between their results and those obtained by Sahakian *et al.* (1988), who found, in a similar test, that AD patients performed as well as the controls at the shortest delay, but that their performance, unlike that of controls, declined with increasing delay. In the study of Money *et al.* (1992), the performance of the control group did decline with increasing delay.

Thus in the experiment by Sahakian *et al.* (1988) the task requirements for the control and patient groups may not be equivalent. The absence of a delay-dependent effect in the control group suggests that the memory load for this group was relatively light, and that they were performing at an optimal level at all delays. If this were the case, one would expect the controls to have out-performed the AD patients at the short delay, but such superiority on the part of the controls may have been masked by a ceiling effect. Thus, the studies by Baddeley's and Money's groups may have revealed group differences because they afforded better control for task difficulty. It is interesting to note that when rats are treated with systemically administered scopolamine or given non-specific lesions of the NBM, their performance on DMTS tasks is similar to that of the AD patients in the study by Money *et al.* (1992). In all three cases, the *difference* between the performance of control and experimental groups is not delay-dependent. If a memory trace were declining more rapidly in experimental subjects then one would expect their deficiency with respect to control subjects to increase with delay.

Taken together, these results suggest that when the cholinergic system is compromised, deficits in stimulus encoding or attention result. Furthermore, recent support for this hypothesis has come from rat studies using the nine-hole box paradigm described above. Everitt (1992; unpublished results) found that when rats are given lesions of the NBM with the excitatory amino acid AMPA, which results in a more specific lesion of the cholinergic system than was previously obtainable, attentional rather than memory deficits are found.

If we accept, on the basis of the findings of Baddeley *et al.* (1986), that attentional deficits are primary in AD, then it implies not only that a cholinergic hypothesis continues to be viable, but also that animal models of cognition have

played a significant role in sustaining its vigour through adaptation and refinement. Without the work of Dunnett, Robbins and others using the DMTP rat model, Baddeley's research might have led us off the cholinergic track. Their animal model of cognition has functioned proactively; not merely reflecting, but revealing in advance of human studies, what may be a fundamental feature of the Alzheimer's deficit.

In the last two decades research into the neurochemical processes underlying cognition has been driven by the need to identify a treatment for a disease that affects an ever-growing elderly population. The primary approach has been "bottom up"; an attempt has been made to identify the neurochemical basis of the disorder and thereby its psychological characteristics. Since this approach has made relatively little progress it may be appropriate to explore more fully the alternative "top-down" approach represented, almost exclusively by Baddeley and his coworkers. This may be a depressing conclusion for the behavioural pharmacologist, but it is clear that careful consideration must be given to identifying the specific nature of the deficit in AD. If this can be achieved, then animal models that truly reflect cognitive rather than non-cognitive processes can be developed. Only in this way will we obtain the tools necessary to identify the contributions of various neurochemical systems.

Amyloid protein and Alzheimer's disease

In the meantime, recent developments suggest that future approaches to the treatment of AD will be of a preventative, rather than a remedial, nature, and we shall consider these briefly before closing. When Alois Alzheimer looked down his microscope at the brains of his patients he noted that a peculiar substance had been deposited through the entire cortex. This substance appears to play a major role in developing "senile plaques", the core of which consists of amyloid beta protein. Although Alzheimer identified these plaques, they have been neglected in research on the pathology of AD until relatively recently. This is probably because amyloid deposits are not found exclusively in patients with AD; they also develop as a result of brain injury, and accumulate in other organs as a consequence of various diseases and genetic disorders (e.g. Allsop *et al.*, 1989).

The role of amyloid deposition in AD has recently attained prominence following a report by Goate *et al.* (1991) that some cases of familial AD could be linked to an amyloid precursor protein (APP) gene mutation (see Hardy and Allsop, 1991 for a review). Hardy and Allsop have suggested that drugs may be developed to inhibit the formation of amyloid or to prevent the neurotoxicity that may result from abnormal fragments of APP. The search for such a drug, difficult under any circumstances, is likely to be further obstructed by the lack of a rodent model of abnormal APP processing. Rodents do not spontaneously develop amyloid deposits (Selkoe, 1991), and attempts to produce mice with a potential for amyloid deposition through genetic engineering have been less than successful (see *Nature*, **356**, p.23, 1992; *Science*, **255**, p.1200, 1992). Primates undergo an age-related beta-amlyoidosis, but they may not be a practical species with which to screen compounds.

CONCLUSIONS

The final general requirement for an animal model of cognition is that it seek to model a cognitive process that is not unique to humans. This may seem blindingly obvious, but it is worth stating explicitly for two reasons: first, since we have stressed throughout this paper that subtle reasoning and empirical procedures are necessary to construct animal models of cognition, we should make it clear that we do not regard these as sufficient. There are likely to be certain human cognitive processes which have no counterparts in other species, and therefore no amount of knowledge and skill could be expected to yield appropriate animal models. The processes most likely to be unique to humans are any which are intrinsically linguistic; i.e. dedicated to interpreting and producing language. Second, while the requirement may be obvious, some of its implications are not so easy to elucidate. It not only renders unlikely animal models of cognitive processes such as lexical search, but also of such apparently non-linguistic processes as those responsible for remembering episodes from the past. For example, intuitively it is highly likely that non-human animals have "episodic memory"; that they are capable of summoning up a mental image of what happened to them yesterday morning, with an accompanying sense that those events occurred at a specified time in the recent past. However, it is not clear that we can get beyond this intuition to produce a test of episodic memory function for animals, because animals cannot tell us what they are remembering. In the absence of such verbal report, we can assess whether the events of yesterday morning have affected an animal, but not whether they are remembered by the animal as the events of yesterday morning. Cases such as that of episodic memory are likely to be the exception rather than the rule. However, we have tried to illustrate in this paper, that with skill and ingenuity in the development of animal models of cognition, considerable progress can be made in determining the pharmacological mechanisms controlling cognitive processes.

REFERENCES

Allsop D, Haga SI, Haga C, Ikeda SI, Mann DMA, Ishii T (1989) Senila plaques in Down's syndrome brains show a close relationship with cell bodies of neurons. *Neuropathology and Applied Neurobiology*, **15**, 531-542.

Baddeley AD (1992) Working memory. *Science*, **255**, 556-559.

Baddeley AD and Hitch GJ (1974) Working memory. In: *Recent Advances in Learning and Motivation*, Vol. III (Ed. G Bower), pp. 47-90. Academic Press, New York.

Baddeley AD, Logie R, Bressi S, Della Sala S and Spinnler H (1986) Dementia and working memory. *Quarterly Journal of Experimental Psychology*, **38a**, 603-618.

Baddeley AD, Bressi S, Della Sala S, Logie R and Spinnler H (1991) The decline of working memory in Alzheimer's disease. *Brain*, **114**, 2521-2542.

Baker R, Street LJ, Reeve AJ and Saunders J (1991) Synthesis of azabicyclic pyrazine derivatives as muscarinic agonists and the preparation of a chloropyrazine analogue with functional selectivity at subtypes of muscarinic receptor. *Journal of the Chemistry Society, Chemical Communication*, 760-762.

Bammer G (1982) Pharmacological investigations of neurotransmitter involvement in passive avoidance responding: a review and some new results. *Neuroscience and Biobehavioural Reviews*, **6**, 247-296.

Barnes JM, Barnes NM, Costall B, Naylor RJ and Tyers MB (1989) 5-HT_3 receptors mediate inhibition of acetylcholine release in cortical tissue. *Nature*, **338**, 762-763.

Bartus RT (1978) Evidence for a direct cholinergic involvement in scopolamine-induced amnesia in monkey: effects of concurrent administration of physostigmine and methylphenidate with scopolamine. *Pharmacology Biochemistry and Behavior*, **9**, 833-836.

Bartus RT and Johnstone HR (1976) Short term memory in rhesus monkeys: disruption from the anti-cholinergic scopolamine. *Pharmacology Biochemistry and Behavior*, **5**, 39-46.

Bartus RT, Fleming DL and Johnstone HR (1978) Aging in the rhesus monkey: debilitating effects on short term memory. *Journal of Gerontology*, **33**, 858-871.

Bonner T (1989) New subtypes of muscarinic acetylcholine receptors. *Trends in Pharmacological Sciences*, **10** (Supplement), Subtypes of Muscarinic Receptors IV.

Bowen DM, Smith CB, White P and Davidson AN (1976) Neurotransmitter-related enzymes and indices of hypoxia in senile dementia and other abiotrophies. *Brain*, **99**, 459-496.

Costall B, Jones BJ, Kelly ME, Naylor RJ and Tomkins DM (1989) Exploration of mice in a black and white test box: validation as a model of anxiety. *Pharmacology Biochemistry and Behavior*, **32**, 777-785.

Costall B, Domeney AM, Gerrard PA, Horovitz P, Kelly ME, Naylor RJ and Tomkins DM (1990) Effects of captopril and SQ29,852 on anxiety-related behaviours in rodent and marmoset. *Pharmacology Biochemistry and Behavior*, **36**, 13-20.

Croog SH, Levine S, Testa MA, Brown B, Bulpitt CJ, Jenkins CD, Klerman GL and Williams GH (1986) The effects of antihypertensive therapy on the quality of life. *New England Journal of Medicine*, **314**, 1657-1664.

Croog SH, Kong W and Levine S (1990) Hypertensive black men and women. Quality of life effects of antihypertensive medications. *Archives of Internal Medicine*, **150**, 1733-1741.

Davis P and Maloney AJ (1976) Selective loss of central cholinergic neurons in Alzheimer's disease. *The Lancet*, **2**, 1403.

Davis KL, Mohs RC, Rosen WG, Levi MI and Horvath TB (1983) Memory enhancement with oral physostigmine in Alzheimer's disease. *New England Journal of Medicine*, **308**, 701.

Dawson GR, Johnstone S, Bayley P and Iversen SD (1991a) The effects of a novel muscarinic agonist, L-689,660 in the mouse tail-flick test of antinociception. *British Journal Pharmacology*, 110P.

Dawson GR, Bentley G, Draper F, Rycroft W, Iversen SD and Pagella PG (1991b) The behavioral effects of heptyl physostigmine a new cholinesterase inhibitor, in tests of long-term and working memory in rodents. *Pharmacology Biochemistry and Behavior*, **39**, 865-871.

deNoble VJ (1986) Vinpocetine enhances retrieval of a step-through passive avoidance response in rats. *Pharmacology Biochemistry and Behaviour*, **26**, 183-186.

De Wied D (1965) The influence of posterior and intermediate lobe of the pituitary and putuitary peptides on the maintenance of a conditioned avoidance response in rats. *International Journal of Neuropharmacology*, **4**, 157-167.

De Wied D (1976) Behavioural effects of intraventricularly administered vasopressin and vasopressin fragments. *Life Sciences*, **19**, 685-690.

De Wied D (1977) Peptides and behaviour. *Life Sciences*, **20**, 195-204.

Drachman D (1977) Memory and cognitive function in man: does the cholinergic system have a specific role. *Neurology*, **27**, 783-790.

Dunne MP and Hartley LR (1986) Scopolamine and the control of attention in humans. *Psychopharmacology*, **89**, 94-97.

Dunnett SB (1985) Comparative effects of cholinergic drugs and lesions of the nucleus basalis or fimbria-fornix on delayed matching in rats. *Psychopharmacology*, **87**, 357-363.

Dunnett SB, Evenden JL and Iversen SD (1988) Delay-dependent short-term memory deficits in aged rats. *Psychopharmacology*, **96**, 174-189.

Dunnett SB, Wareham AT and Torres EM (1990) Cholinergic blockade in prefrontal cortex and hippocampus disrupts short-term memory in rats. *NeuroReport*, **1**, 61-64.

Dunnett SB, Everitt BJ, Robbins TW (1991) The basal forebrain-cortical cholinergic system: interpreting the functional consequences of excitotoxic lesions. *Trends in Neurosciences*, **14**, 494-504.

Eagger SA, Levy R and Sahakian BJ (1991) Tacrine in Alzheimer's disease. *The Lancet*, **337**, 989-992.

Etherington R, Mittleman G and Robbins TW (1987) Comparative effects of nucleus basalis and fimbria-fornix lesions on delayed matching and alternation tasks. *Neuroscience Research Communications*, **1**, 135-143.

Ettenberg A, LeMoal M, Koob GF and Bloom FE (1983) Vasopressin potentiation in the performance of a learned appetitive task: reversal by a pressor antagonist analog of vasopressin. *Pharmacology Biochemistry and Behavior*, **18**, 645-647.

Everitt BJ (1992) Excitotoxic lesions of the basal forebrain cholinergic neurons: effects on learning, memory and attention. *Paper presented at the European Brain and Behaviour Society Workshop on Neurodegenerative Diseases: Animal Models and Clinical Perspectives*, Huddinge, Sweden.

Fibiger HC (1991) Cholinergic mechanisms in learning, memory and dementia: a review of recent evidence. *Trends in Neurosciences*, **14**, 220-223.

Fisher A and Hanin I (1986) Potential animal models for senile dementia of Alzheimer's type with particular emphasis on AF64-A induced cholinotoxicity. *Annual Review of Pharmacology and Toxicology*, **26**, 161-181.

Fisher A, Heldman E, Brandies R, Pittel Z, Dachir S, Levy A and Karton I (1986) AF102B; a novel putative M_1 agonist reverses AF64-A induced cognitive impairments in rats. *Society for Neuroscience Abstracts*, **12**, 702.

Fletcher AE and Bulpitt CF (1991) Quality of life on ACE inhibitors. A review of the results of recent trials in hypertension. In: *Current Advances in ACE Inhibition* (Ed. GA MacGregor and

PS Sever), pp 286-288. Churchill Livingstone, UK.

Food and Drugs Administration U.S. (1991) An interim report on the effects of tacrine as a treatment for Alzheimer's dementia. *New England Journal of Medicine*, **324**, 349-352.

Freedman SB, Harley EA and Patel S (1989) Direct measurement of muscarinic agents in the central nervous system of mice using *ex vivo* binding. *European Journal of Pharmacology*, **174**, 253-260.

Freedman SB, Iversen LL, Rugarli PL and Harley EA (1991a) Heptylphysostigmine: a potent inhibitor of acetylcholinesterase with long duration of action. *Paper presented at the Second International Springfield Symposium on Advances in Alzheimer Therapy*, May 3-5, 1991.

Freedman SB, Patel S, Marwood R, McKnight AT, Newberry N, Hurley CJ, Hill RG and Hargreaves RJ (1991b) The pharmacology *in vivo* of L-689,660 a functionally selective M_1/M_3 muscarinic agonist. *British Journal of Pharmacology*, **104**, 457P.

Gleitman H (1991) *Psychology*, 3rd Edn. Norton, New York.

Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, Giuffa L, Haynes A, Irving N, James L, Mant R, Newton P, Rooke K, Roques P, Talbot C, Pericak-Vance M, Roses A, Williamson R, Rossor M, Owen M and Hardy J (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature*, **349**, 704-707.

Hargreaves RJ, Knight AT, Scholey K, Newberry N, Street L, Hutson P, Semark J, Harley EA, Patel S and Freedman SB (1992) L-689,660 a novel cholinomimetic with functional selectivity for M_1 and M_3 receptors. *British Journal of Pharmacology*, in press.

Hardy J and Allsop D (1991) Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends in Pharmacological Sciences*, **12**, 383-388.

Hart RP, Kwentus JA, Taylor JR and Harkins SW (1987) Rate of forgetting in dementia and depression. *Journal of Consulting and Clinical Psychology*, **55**, 101-105.

Heise GA, Connor R and Martin RA (1976) Effects of scopolamine on variable intertrial interval spatial alternation and memory in the rat. *Psychopharmacology*, **49**, 131-137.

Herrick AL, Waller PC, Berkin KE, Pringle SD, Callender JS, Robertson MP, Findlay JG, Murray GD, Reid JL, Lorimer AR, Weir RJ, Carmichall HA, Robertson JIS, Ball SG and Innes GT (1989) Comparison of enalapril and atenolol in mild to moderate hypertension. *American Journal of Medicine*, **86**, 421-426.

Kamin LJ (1956) The effects of termination of the CS and avoidance of the US on avoidance learning. *Journal of Comparative and Physiological Psychology*, **49**, 420-424.

Kirk RC, White GK and McNaughton N (1988) Low dose scopolamine affects discriminability, but not rate of forgetting in delayed conditional discrimination. *Psychopharmacology*, **96**, 541-546.

Koob GF, LeMoal M, Gaffori O, Manning M, Sawyer WH, Rivier J and Bloom FE (1981) Arginine vasopressin and a vasopressin antagonist peptide: opposite effect on extinction of active avoidance in rats. *Regulatory Peptides*, **2**, 153-163.

LeMoal M, Koob GF, Koda LY, Bloom FE, Manning M, Sawyer WH and Rivier J (1981) Vasopressor receptor antagonist prevents behavioural effects of vasopressin. *Nature*, **291**, 491-493.

Money EA, Kirk RC and McNaughton N (1992) Alzheimer's dementia produces a loss of discrimination but no increase in rate of memory decay in delayed matching to sample. *Neuropsychologia*, **30**, 133-143.

Morris RG, Baddeley AD (1988) Primary and working memory functioning in Alzheimer's-type dementia. *Journal of Clinical and Experimental Neuropsychology*, **10**, 279-296.

Mountjoy CQ, Rossor MN, Iversen LL and Roth M (1984) Correlation of cortical cholinergic and GABA deficits with quantitative neuropathological findings in senile dementia. *Brain*, **107**, 507-518.

Mouradian MM, Mohr E, Williams JA and Chase TN (1988) No response to high-dose muscarinic agonist therapy in Alzheimer's disease. *Neurology*, **38**, 606-608.

Murray TK, Cross AJ and Green AR (1991) Reversal by tetrahydroaminoacridine of scopolamine-induced memory and performance deficits in rats. *Psychopharmacology*, **105**, 134-136.

Newell A (1990) *Unified Theories of Cognition*. Harvard University Press, Cambridge, MA.

Page KJ, Everitt BJ, Robbins TW, Marston HM and Wilkinson LS (1991) Dissociable effects on the spatial maze and passive avoidance acquisition and retention following AMAP- and ibotenic acid-induced excitotoxic lesions of the basal forebrain in rats: differential dependence on cholinergic neuronal loss. *Neuroscience*, **43**, 457-472.

Perry EK, Perry RH, Blessed G and Tomlinson BE (1977) Necropsy evidence of central cholinergic deficits in senile dementia. *The Lancet*, **1**, 189.

Peters BH and Levin HS (1979) Effects of physostigmine and lecithin on memory in Alzheimer's disease. *Annals of Neurology*, **6**, 219-221.

Pontecorvo MJ, Clissold DB, White MF and Ferkany JW (1991) *N*-methyl-D-aspartate antagonists and working memory performance: comparison with the effects of scopolamine, propranolol, diazepam and phenylisopropyladenosine. *Behavioural Neuroscience*, **105**, 521-535.

Preston GC, Millson DS, Ceuppens PR and Warburton DM (1992) Effects of 5-HT_3 receptor antagonist GR68755 on scopolamine-induced cognitive deficit in healthy subjects. *British Journal of Clinical Pharmacology*, **33**, 546P.

Rossor MN (1982) Dementia. *The Lancet*, **2**, 1200-1204.

Rupniak NMJ, Samson NA, Tye SJ, Field MJ and Iversen SD (1991a) Evidence against a specific effect of cholinergic drugs on spatial memory in primates. *Behavioural Brain Research*, **43**, 1-6.

Rupniak NMJ, Tye SJ and Iversen SD (1991b) Cholinomimetic activity of RS86, AF102B and L-689,660. *Society for Neuroscience Abstracts*, Part I, 700, 274.10(p).

Sahakian BJ, Morris RG, Evenden JL, Heald A, Levy R, Philpot M and Robbins TW (1988) A comparative study of visuospatial memory and learning in Alzheimer type dementia and Parkinson's disease. *Brain*, **111**, 695-718.

Sahakian B, Jones G, Levy R, Gray J and Warburton D (1989) The effects of nicotine on attention, information processing and short-term memory in patients with dementia of the Alzheimer type. *British Journal of Psychiatry*, **154**, 797-800.

Sahakian BJ, Dowes JJ, Eagger S, Evenden JL, Levy R, Philpott MP, Roberts AC and Robbins TW (1990) Sparing of attentional relative to mnemonic function in a subgroup of patients with dementia of the Alzheimer type. *Neuropsychologia*, **28**, 1197-1213.

Sahgal A, Wright C, Edwardson JA and Keith AB (1983) Corticotrophin releasing factor is more potent than some corticotrophin-related peptides in affecting passive avoidance behaviour in rats. *Neuroscience Letters*, **36**, 81-86.

Sarter M, Hagan J and Dudchenko P (1992a) Behavioural

screening for cognition enhancers: from indiscriminate to valid testing. Part I. *Psychopharmacology*, **107**, 144-159.

Sarter M, Hagan J and Dudchenko P (1992b) Behavioural screening for cognition enhancers: from indiscriminate to valid testing. Part II. *Psychopharmacology*, in press.

Selkoe DJ (1991) Amyloid protein and Alzheimer's disease. *Scientific American*, **265**, 40-47.

Shulz D, Chernichovsky D and Allweis A (1986) A novel method for quantifying passive avoidance behaviour based on exponential distribution of step-through latencies. *Pharmacology Biochemistry and Behavior*, **25**, 979-983.

Steiner SS, Friedhoff AJ, Wilson BL, Wecker JR and Santo JP (1990) Antihypertensive therapy quality of life: a comparison of atenolol, captopril, enalapril and propranolol. *Journal of Human Hypertension*, **4**, 217-255.

Struat-Hamilton IA, Rabbit PMA and Huddy A (1988) The role of attention in the visuo-spatial memory of patients suffering from dementia of the Alzheimer type. *Comparative Gerontology*, **2**, 129-134.

Summers WK, Majovski LV, March GM, Tachiki K and Kling A (1986) Oral tetrahydroaminoacridine in long-term treatment of senile dementia, Alzheimer type. *New England Journal of Medicine*, **315**, 1241-1245.

Tyers M (1991) 5-HT_3 receptor antagonists. *Drug Design and Delivery*, **7**, 175-181.

Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT and DeLong MR (1982) Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science*, **215**, 1237-1239.

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