The pharmacology of human working memory

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Abstract
Experimental studies conducted primarily on non-human primates have begun to address the anatomical and neurochemical correlates of working memory. There is an associated growing body of experimental literature investigating whether modulating key neurotransmitters can facilitate working memory in humans. This paper reviews evidence that acute modulation of dopamine in particular, but also noradrenaline, acetylcholine and serotonin may influence working-memory performance in humans. Differences in neurochemical specificity with regard to stages of working memory, type of working memory (spatial or non-spatial) and cortical effects are also discussed. This evidence has contributed to neuropharmacological understanding of working memory in humans. The important therapeutic consequences of a better understanding of facilitation of working memory is discussed in reference to schizophrenia, Parkinson’s disease and Alzheimer’s disease.

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Introduction
Working memory is the ability to maintain or hold temporary, active representations of information for further processing or recall (Baddeley, 1986; Just and Carpenter, 1992). The process is thought to have two components, short-term storage (generally in the order of seconds), and executive processes that operate on the stored material (Baddeley, 1992). Working memory is mediated by a widely distributed neural system in the human brain (Baddeley, 1986). Functional brain-imaging studies of humans have identified cortical regions that are involved in spatial and non-spatial working memory including occipital, temporal, parietal and prefrontal cortical areas (Friedman and Goldman-Rakic, 1994; Haxby et al., 1995; Petrides et al., 1993). Functional imaging studies also suggest that these regions maybe involved in different stages of working memory, with the occipitotemporal cortex involved in perceptual processing, and prefrontal cortex (PFC) involved with maintaining an active representation over the delay period (Courtney et al., 1997; Funahashi et al., 1993; Haxby et al., 1995; Miller et al., 1993; Shindy et al., 1994). Evidence also suggests that individual neurons show elevated persistent and tuned activity (memory fields) during components of working memory (Goldman-Rakic et al., 1989). These memory fields may be modulated by many neurochemical systems (Goldman-Rakic et al., 1990), including dopamine (Williams and Goldman-Rakic, 1993, 1995), serotonin (Jakab and Goldman-Rakic, 1998), noradrenaline (Arnsten and Goldman-Rakic, 1986), acetylcholine (Ragozzino, 2000), GABA (Rao et al., 2000) and glutamate (Seemans et al., 2001) in highly differentiated ways, suggesting that modulation of these neurochemical systems may affect different stages of working memory. There is a growing body of experimental literature focusing on the effects of acute modulation of these neurotransmitter systems on working-memory performance. To date, there has been no systematic review of the pharmacology of working memory.

This review is not intended to cover in detail the anatomical or cellular basis of working memory (for an anatomical review, see Baddeley, 1998; for a cellular review, see Goldman-Rakic, 1995, 1996), nor does it intend to focus in detail on the conceptual basis of working memory (for a review, see Baddeley and Hitch, 1974). The major aim of this review is to provide a broad overview of what is known about the pharmacology of human working memory from acute drug challenge studies in healthy human subjects. However, data from animal studies and clinical studies will be reviewed when literature on acute human challenges is limited, in order to compile a basic blueprint of the pharmacology of working memory, and to suggest a broad direction for future research in humans. This review will consider the broad construct of working memory, which encompasses both spatial and non-spatial domains. However, there are
possible differences in the neurochemical substrates that may subserve different domains, and the type of working memory used in between studies will therefore be highlighted.

The paper has three sections. The first and by far largest section of the review concentrates on a selection of neurotransmitter systems, which affect human working-memory performance when modulated by acute pharmacological agents. The most widely studied neurochemical system for working memory is the dopaminergic system, and the most commonly employed task paradigm is visuo-spatial working memory. These aspects will therefore form a majority of the review. However, there is also evidence to suggest that acute modulation of the noradrenergic, cholinergic and serotonergic systems can alter working-memory performance in humans. Animal studies suggest possible roles for the NMDA–glutamate system and GABA involvement, which will be discussed in the context of possible future directions for human studies. The second section will discuss directions for future research, with particular emphasis on the emerging importance of brain imaging in working-memory research. The final section of the review highlights the importance and possible clinical implications for individuals with schizophrenia, Parkinson’s disease and Alzheimer’s disease, of improving working-memory function with selective pharmacological agents.

The dopaminergic system

*D₁ vs. D₂ dopamine receptor agonists*

The most studied neurochemical system for working memory is the dopaminergic system. It has been widely reported that increasing dopamine levels in human subjects facilitates working-memory performance (Luciana et al., 1992, 1998; Luciana and Collins, 1997; Muller et al., 1998). However, the relative role of D₁ and D₂ dopamine receptors in modulating working memory is yet to be clarified in humans. Evidence suggesting that D₂ dopamine receptor agonists may facilitate working memory is generally based on studies using bromocriptine, a specific D₂ receptor agonist. The first of such studies was conducted by Luciana et al. (1992), who investigated the effect of an acute oral dose of 2.5 mg of bromocriptine on a sample of 8 young, healthy females, in their performance of a visuo-spatial delayed response task. The task involved presentation of a visual cue (a black dot) on a computer screen. The cue was removed for a delay of either 0 or 8 s. After the delay period, the subjects indicated the screen location of the cue with a fine-pointed light pen. The authors hypothesized that if bromocriptine influenced the working-memory component of the spatial task, bromocriptine administration would improve accuracy in the 8-s delay condition, and would have little effect in the 0-s delay condition. The authors observed a 44% improvement in the accuracy of identifying the cue location in the 8-s bromocriptine condition, compared to placebo. No improvement was seen in the 0-s delay condition following bromocriptine administration, and it was inferred that the D₂ receptor agonist might have facilitated spatial working-memory performance. However the small sample size of the study limited the generalizability of these results.

In a subsequent study, using a larger sample of 66 young adults (aged 19–37 yr), Luciana and Collins (1997) provided additional evidence for a facilitating role of D₂ receptor agonists. However in this study the acute dose of 2.5 mg bromocriptine, which had facilitated performance in the earlier study (Luciana et al., 1992), did not appear to facilitate performance in the replication study. It was observed that performance accuracy was improved following administration of a smaller dose of bromocriptine (1.25 mg). More recently, Luciana et al. (1998), in a sample of 38 volunteers, again observed a facilitating effect of 1.25 mg of bromocriptine on spatial working memory when behavioural testing occurred between 3.5 and 5.5 h after drug administration. The authors suggested that the discrepancy between studies might be due to differences in the time of cognitive testing (Luciana and Collins, 1997). In the first study (Luciana et al., 1992), the delayed response task was administered between 2.5 and 3.5 h after drug administration, while in the subsequent studies (Luciana and Collins, 1997; Luciana et al., 1998) the delayed response task was administered between 3.5 and 5.5 h after drug administration. Luciana and colleagues argued that in the former study (which used a high dose of 2.5 mg and tested 1 h earlier), testing may have taken place while bromocriptine levels were ‘sub-maximal’, and the cognitive effects may be comparable to those for ‘maximal’ levels of 1.25 mg of bromocriptine in the latter studies.

This suggests that there may be an inverted U dose-related response of bromocriptine on spatial working-memory performance, with low doses facilitating performance and higher doses having no effect or an impairing effect. However, it should be noted that an apparent inverted U shape response may also be explained as the superimposition of an inverse dose-related sedative effect, in addition to the putative dose-related cognitive effects. Sedation has been widely associated with increased dopamine levels (Canales and Iversen, 2000; Schapira, 2000). Indeed, Luciana and Collins (1997) noted in their study that adverse effects of bromocriptine at ‘high’ levels (2.5 mg) resulted in a 50% withdrawal rate of subjects, and pointed out that this decreased the statistical power of analysis in the 2.5 mg...
group. Primate and non-primate studies have also described an inverted U dose-related response, with either insufficient or excessive dopamine receptor stimulation reported to be detrimental to working-memory function ( Arnsten and Goldman-Rakic, 1986; Cai and Arnsten, 1997; Goldman-Rakic, 1996; Murphy et al., 1996). It is likely that the complex effects of dopaminergic modulation of working memory is related to the disruption of tuned activity or memory fields that are regulated by an optimal level of functioning of neurochemical systems, including dopamine.

In contrast to the possible dose- and time-related effects of bromocriptine on working memory, Kimberg et al. (1997) observed performance responses following bromocriptine that appear to be dependent on the baseline working-memory capacity of the subject. In this study, a sample of 31 normal human subjects was divided into two groups, either high working-memory span or low working-memory span, on the basis of their scores on a verbal working-memory task. Each subject was tested on a variety of tasks, which were sensitive to prefrontal function (a card sorting task, associative memory task, context memory task, and a Stroop task), in addition to a spatial working-memory task similar to the delayed response task used by Luciana et al. (1992). In this study, an acute dose of 2.5 mg of bromocriptine showed an interesting pattern of effects on performance of these tasks. Subjects with a high- baseline working memory, as assessed by their performance on a verbal working-memory test, performed more poorly while under the influence of bromocriptine compared to placebo, while subjects with low-baseline working memory performed better following bromocriptine administration compared to placebo. While the spatial working-memory task was not significantly affected by baseline memory capacity when analysed separately, there was a trend similar to that observed with the other tasks. However this trend was not supported in a more recent study by the same authors (Kimberg et al., 2001). In this more recent study, the opposite effects to that observed in the former study was found, with subjects with higher baseline memory capacity actually showing improved performance following bromocriptine administration. The authors suggested that the contradictory results between the two studies might be explained by differences in the task and time of testing. However a major limitation of this study is the relatively small sample used, which might have affected the likelihood of finding small differences between groups.

While the studies on D_2 receptors and working memory are inconsistent, Muller et al. (1998) have shown evidence for D_1 but not D_2 receptor involvement in spatial working memory. In this study, bromocriptine (2.5 mg) failed to facilitate spatial working memory. They did however note evidence supporting a role for D_1 receptors in modulating working memory, as measured by a visuo-spatial delayed matching task designed to minimize motor control. The task involved presentation of a stimulus on a computer screen, and subjects were asked to memorize the location of the stimulus. After a delay of 2, 8 or 16 s, a second pattern was presented, which subjects identified as either being in the same location as the original stimulus, or having been moved slightly. Because no selective D_1 agonist was available for human research, a pharmacological subtraction design was applied. Thirty-two healthy young adults received either an acute oral dose of 0.1 mg pergolide (a combined D_1/D_2 receptor agonist) or 2.5 mg of bromocriptine (a D_2 receptor agonist). Dosages of pergolide and bromocriptine were thought to be comparable in terms of biological and therapeutic action. Consistent with Luciana et al. (1992), the main experimental task was performed between 2.5 and 3.5 h after drug intake. This time period was presumed to coincide with the period of peak plasma concentration of both drugs. The results indicated an improvement in spatial working memory at delays of 16 s in subjects treated with pergolide (D_1/D_2 receptor agonist), but not bromocriptine (D_2 receptor agonist). It was concluded that memory improvement after pergolide administration could be attributed to a modulatory effect of D_1 receptors.

However, the conclusion that pergolide was observed to facilitate working memory as a result of modulatory effects of D_1 receptors could be questioned, based on the potencies of the compound used. For a pharmacological subtraction technique to be successfully used in this study, pergolide and bromocriptine would be required to have similar affinities for D_2 receptors, while only pergolide should have an affinity for D_1 receptors. However, the ratio of D_1/D_2 receptor affinity for pergolide and bromocriptine has been reported to be comparable for the orally administered agonists (pergolide = 67 nM and bromocriptine = 60 nM) (De Keyser et al., 1995). Moreover, pergolide has been shown to have a greater affinity for D_2 receptors than bromocriptine (Clemens et al., 1993; Miyagi et al., 1996), and is up to 650 times more potent than bromocriptine at D_2 receptors (Zhang et al., 1995). It is therefore possible that the observed effects may have been due to D_2 receptor stimulation. However given that the inhibition of prolactin secretion (an indicator of D_2 receptor activity) was not significantly different between the two drug conditions in the study of Muller et al. (1998), it is likely that at least from a pharmacodynamic perspective, both pergolide and bromocriptine may have similar D_2 receptor efficacy. The D_1 receptor efficacy taken together with the known affinity of pergolide for D_1
receptors, and the observed effects on working memory, highlights the more prominent role for D1 receptors in working memory.

Unfortunately, the lack of an appropriate pharmacological tool, such as a potent D1 agonist (Muller et al., 1998), has limited the ability of researchers to directly investigate the effects of D1 receptor stimulation in humans to date. The evidence presented thus far indicates that D2 receptor stimulation may facilitate working-memory performance. There is also an indication of an inverted U dose-related response, with facilitation of working memory more often observed for lower plasma concentrations of D2 receptor agonists. However, it must be noted that the effects of dopamine agonists on working memory is dependent on a variety of factors including dose–response effects, time-spans for behavioural testing, difficulty of tasks, and baseline ability of individual subjects.

While evidence in non-human primates suggests that D1 receptors are predominantly involved in modulating working memory (Sawaguchi et al., 1990a,b; Williams and Goldman-Rakic, 1995), it is interesting that the studies in humans using bromocriptine indicate a possible role for D4 dopamine receptors. While D2 receptors are found in areas such as the PFC, they are 20-fold less abundant than D1 receptors (Lidow et al., 1991). The scarcity of D2 receptors in the PFC and other neocortical areas indicate that the effects of bromocriptine on working memory may not be a direct effect (Kimberg et al., 2001). Given the abundance of D4 receptors on layer V of the PFC (Goldman-Rakic et al., 1990), it has been suggested by Kimberg et al. (2001) that the downstream effects of bromocriptine (from areas rich in D4 receptors) through projections to the cortical areas (via layer V), may dominate the cortical effects of D2 receptor stimulation. However there is also evidence to suggest that there may be interactions between D2 and D1 receptors, such that modulating of the D2 receptors may affect D1 receptor function (Lidow and Goldman-Rakic, 1994; Lidow et al., 1998). Again, this indicates that D1 receptors may play a more prominent role in directly modulating working memory in humans, which may be highlighted further with the development of an appropriate D1 receptor agonist for use in humans.

Dopamine receptor antagonists

On the basis of evidence suggesting that dopamine receptor agonists may facilitate working memory, it would follow that dopamine receptor antagonists may impair working-memory performance. Unfortunately, there have been few studies conducted in healthy humans to examine the effect of acute doses of dopamine receptor antagonists on working-memory performance. Mehta et al. (1999), using a sample of 34 young healthy males, investigated the effect of the D2 dopamine antagonist sulpiride on spatial working memory. The authors reported that spatial working memory, as assessed by a sequence generation task, was impaired following both 200 and 400 mg doses of sulpiride, compared to placebo.

In the study by Luciana and Collins (1998), which investigated the effects of bromocriptine on completion of a visuo-spatial delayed response task, the effect of the D2 receptor antagonist haloperidol was also examined. Following a 3 mg oral dose of haloperidol, a decrease in performance was observed on the spatial working-memory task, as measured by a decrease in accuracy of identifying the location of the cue, compared to placebo. This decrement was observed at delays of 8 and 16 s, but not at a delay of 5 s (Luciana and Collins, 1998).

To our knowledge, there have been no other studies investigating the effects of haloperidol on working memory in normal human subjects. Negative effects of haloperidol on short-term memory have been reported for healthy elderly humans (Beuzen et al., 1999), although working memory was not tested. Fourteen subjects were given 3 mg of haloperidol once a day for 4 d and an acute effect was observed on day 1, with impairment in a series of memory tasks such as word recall and recognition (Beuzen et al., 1999).

Typical antipsychotics such as haloperidol, which have D2 antagonistic properties, have been shown to impair working memory in schizophrenic patients, whereas atypical antipsychotics with less D2 antagonistic properties have been shown to improve working memory in schizophrenia (Honey et al., 1999). However, research investigating schizophrenic subjects has generally involved chronic administration of dopamine antagonists, and interpretation has been difficult as schizophrenic patients are generally regarded as having abnormalities in their dopaminergic systems (Goldman-Rakic, 1991). In addition, these antipsychotics also have other pharmacological properties, including serotonin receptor antagonism, which may independently influence working-memory functioning (see section on serotonin).

Interestingly, in primate studies an inverted U dose-related response of dopamine antagonists has been observed by Sawaguchi and Goldman-Rakic (1994); these authors also reported that injecting higher doses of the dopamine antagonist SCH-39166 (a selective D1 antagonist) into the dorsal PFC of rhesus monkeys was associated with greater impairments on an oculomotor delayed response task. However, Williams and Goldman-Rakic (1995) observed that lower concentrations of a D1 antagonist were associated with improved performance. It could be argued that this may be due to the fact that at
low doses, some antagonists may have partial agonistic effects (Clifford et al., 1998; Sprouse et al., 1998).

While challenge studies in humans with D$_2$ antagonists suggest that blocking this receptor may impair working memory, a recent study in primates by Castner et al. (2000), showed that D$_2$ antagonists may induce changes to the D$_1$ receptor signalling pathway supporting earlier receptor studies (Lidow and Goldman-Rackic, 1994; Lidow et al., 1998). In the study of Castner et al. (2000), impairments in both spatial and object working memory induced by the D$_2$ antagonist haloperidol were reversed by the selective D$_1$ receptor agonist ABT-431. This data further highlights the importance of D$_1$ receptors in modulating working memory and suggests that modulation of the D$_2$ receptor may indirectly modulate D$_1$ receptor function.

The noradrenergic systems

There is some evidence suggesting that modulation of noradrenaline may influence spatial working memory. Elliot et al. (1997) administered methylphenidate, a stimulant drug which increases synaptic concentration of both dopamine and noradrenaline by blocking their reuptake, to 28 young men. Following methylphenidate administration, the authors reported a significant improvement in performance of spatial working memory and planning tasks, but not attentional and fluency tasks (Elliot et al., 1997). Mehta et al. (2000) also studied spatial working-memory performance following methylphenidate administration. The task used in this study differed from the visuo-spatial delayed response tasks and delayed matching-to-sample task described earlier. In this study, 10 healthy male subjects were presented with red dots on a touch-sensitive computer screen. For each problem, the subject was instructed to systematically search through the array of red dots and the goal was to find blue tokens, which were obscured by the red dots. Once a token had been found behind a red dot, it was not used again to obscure a token. The task was to remember the location of dots that had been used to obscure the blue tokens. Methylphenidate was observed to have a greater effect on working memory in subjects with lower-baseline working-memory capacity. This suggests that the working-memory capacity of subjects influenced the effectiveness of methylphenidate on working-memory performance. These findings also support the studies on bromocriptine, which also indicated that changes in performance might be dependent on baseline working-memory capacity (Kimberg et al., 1997, 2001).

It has been reported that working-memory performance, as measured by a delayed matching-to-sample task, may be impaired by acute exposure to cold environments (Thomas et al., 1989). It has also been proposed that exposure to acute stress, such as cold, may disrupt the sustained release of the catecholamines, noradrenaline and dopamine (Bandaret and Lieberman, 1989). Shurtleff et al. (1994) investigated the effect on 8 male volunteers, of whole body exposure to 4 °C for 30 min, on a delayed matching-to-sample task. The task involved presentation of a matrix composed of red and green dots. The matrix was then removed from view for a delay period of 2, 8 or 16 s. Following the delay, two matrices were presented, of which one was identical to the original, and the subjects had to identify the original matrix. For a delay period of 16 s, exposure to cold impaired matching compared to placebo (22 °C). Shurtleff et al. (1994) also investigated the effect of administration of 150 mg/kg per body weight of l-tyrosine, a catecholamine precursor, 90 min before exposure to the cold. Subjects who were administered with l-tyrosine and were exposed to the cold did not significantly differ in performance to the placebo condition, and the authors concluded that the catecholamine precursor had a positive effect on working memory by protecting against the cold-induced memory deficits. l-tyrosine administered before exposure to the placebo condition did not influence performance, which suggests that l-tyrosine had an effect only in the cold environment.

While the effects of methylphenidate and l-tyrosine may be explained in terms of changes in dopamine levels, a possible role for noradrenaline in modulating working memory cannot be ruled out. Furthermore, Coull et al. (1995) reported that administration of the α-2 adrenoeceptor agonist clonidine, which effectively decreases noradrenaline in normal healthy humans, appeared to impair spatial working-memory performance. The authors also reported evidence of a dose-related effect, with 2.5 μg/kg producing a greater deficit in performance than 1.5 μg/kg.

The cholinergic systems

Cholinergic antagonists

The relationship between human memory and the cholinergic neurotransmitter system is well established in the literature. Early reports of the role of acetylcholine (ACh) in learning and memory (Drachman and Leavitt, 1974), and evidence of substantial reductions in neocortical cholinergic function in Alzheimer’s disease (Bartus et al., 1982; Nilsson et al., 1986; Perry et al., 1978), has provided evidence for a cholinergic hypothesis of memory. However the role of the cholinergic system in modulating human working memory is in its infancy.

Early evidence for a cholinergic modulation of working memory in normal human subjects derives from phar-
macological strategies using selective antagonists, particularly for the muscarinic receptors. Rasmussen and Dudar (1979) reported that oral administration of scopolamine impaired performance on a spatial working-memory task, which involved drawing a previously presented maze. In general, subjects were observed to draw extra turns in the maze while under the influence of scopolamine. The authors noted that a non-spatial working-memory task (digit memory task) was also impaired, but the impairment was less than that seen for the spatial working-memory task. However, Mewaldt and Ghoneim (1979), using a digit memory task, found an impairment in numeric working memory following administration of $8 \mu g/kg$ scopolamine in a healthy human sample. Similarly, Duka et al. (1996), in an independent group design study using 36 healthy subjects, also observed deficits in a numeric working-memory task following administration of two doses of scopolamine (0.5 and 1 mg). This study also indicated that there might be a dose-related relationship between cholinergic muscarinic receptor antagonism and working-memory impairments. A dose–response relationship was supported by Robbins et al. (1997), who investigated the effects of three doses of scopolamine (200, 400 and 600 $\mu g$) on a delayed matching-to-sample visuo-spatial working-memory task. The authors reported that in a sample of 24 male volunteers, all 3 doses impaired performance compared to placebo. The authors also observed a dose-related effect, with larger doses associated with greater decreases in accuracy and greater latency in the matching of the sample.

However, it appears that, like the dopaminergic system, the effect of scopolamine on working memory may also be sensitive to the behavioural task used. In a study by Kopelman and Corn (1988), scopolamine had no significant effect on span tests or a measure of verbal short-term forgetting, which they classed as more passive types of working memory. However, cholinergic blockade appeared to produce impairment in a visuo-spatial short-term forgetting task, in addition to impairing performance on a distracter task used in a verbal memory test (Kopelman and Corn, 1988). This data suggests that the decrease in cholinergic function affected tasks with greater processing requirements.

A study by Rusted and Warburton (1988) also reported that administration of scopolamine impaired spatial working memory, but did not have an effect on a working-memory task for objects. Twenty healthy young adults completed a series of non-verbal working-memory tasks, and it was found that a subcutaneous injection of 0.6 mg/ml of scopolamine significantly impaired spatial working memory, while working memory for abstract shapes was not impaired. The addition of a concurrent articulation task used to load the working-memory articulatory loop, led the authors to interpret their results as indicative of an effect of scopolamine at the level of the working-memory central executive mechanism (readers interested in the theoretical component of working memory are directed to Baddeley and Hitch, 1974).

Rusted (1988), observed similar results in a study that employed a larger dosage, administered orally (1.2 mg). In this study a semantic working-memory task was employed, and scopolamine significantly reduced the number of words recalled. A more recent study by Rusted et al. (1991) again observed scopolamine to impair working-memory performance at the level of the central executive system.

While blocking muscarinic receptors have been shown to impair various working-memory processes, it has also been shown that this impairment can be reversed by globally increasing synaptic acetylcholine levels. Ebert et al. (1998), employed a sample of 10 healthy male volunteers, and observed that following 0.6 mg of subcutaneously injected scopolamine, performance was impaired in both a spatial working-memory task (recognition of location of windows in a house front) and numeric memory task (yes/no matching of numbers to a previously presented set). The authors observed that administration of a single dose of 0.5, 1 and 2 mg of phystostigmine (a cholinesterase inhibitor which increases acetylcholine levels) caused dose-dependent short-term reversal of these working-memory decrements.

Recently we have shown the nicotinic receptor antagonist mecamylamine (20 mg), to induce a delay-dependent impairment of visual recognition memory of objects, with maximum impairments found after a 12 s delay (Thompson et al., 2000). This study suggests the manipulation of the nicotinic receptor system may also modulate working-memory processes.

Taken together, these findings suggest that cholinergic processes, particularly the cholinergic muscarinic but also the nicotinic receptor system, may modulate working memory. The available data suggests that the types of working memory most affected by muscarinic receptor antagonism are spatial and numeric working memory, while nicotinic receptor antagonism has been shown to impair object recognition working memory. However one must keep in mind that not all forms of working memory have been examined (particularly with the nicotinic receptor system) and it is possible that these systems could modulate a wider range of working-memory processes.

**Cholinergic agonists**

Studies investigating the effects of globally increasing
cholinergic function have generally focused on the acetylcholinesterase inhibitor physostigmine, which inhibits metabolism of acetylcholine and effectively increases acetylcholine levels. Furey et al. (1997) conducted one of the first studies to investigate working memory and acute increases in cholinergic transmission in healthy volunteers. In this study, working memory for faces was examined. The task involved a 4-s presentation of a face, which subjects were instructed to remember. After a delay of 6 s, two test faces appeared simultaneously, side by side, and the subjects had to identify the original face. In a sample of 13 healthy human volunteers, the efficiency of identifying the correct face (as measured by reaction time) was observed to improve following infusion of 1 mg/h of physostigmine. The control group (n = 8), which did not significantly differ in age, education or gender distribution to the experimental group, received a saline infusion and showed no change in reaction time (Furey et al., 1997). Furey et al. (2000a,b), replicated these results in two more recent studies. Again, subjects who received physostigmine showed improved working-memory performance for faces.

However, specific targeting of cholinergic receptors has not supported previous studies that have shown improvements in working memory with global enhancement of cholinergic function. Park et al. (2000) investigated the effects of the indirect cholinergic agonist nicotine on spatial working memory. The authors reported that smokers performed significantly worse after smoking a cigarette, compared to baseline. However, there are methodological weaknesses in this study that may explain the negative findings. First, the apparent cognitive effects reported in this study may be explained on the basis that subjects were asked to abstain from smoking for 24 h prior to testing. After abstaining for 24 h, subjects are in a nicotine-deficient state. If nicotine is then administered, it could be argued that this amounts to a serotonin re-uptake inhibitor and releasing agent, which effectively increases serotonin levels, on the visuo-spatial delayed response task used by same authors when examining the effects of dopamine manipulation. It was observed that at delays exceeding 4000 ms, fenfluramine appeared to impair working-memory performance. The authors suggested that serotonin may have constrained spatial working memory through an inhibitory effect on dopamine, based on evidence suggesting serotonin and dopamine have opposing roles with respect to emotional and motor behaviours. However, this interaction effect was not explicitly tested.

A further explanation for the findings reported by Park et al. (2000) is the complexity of the nicotine effect. In addition to its effect as a non-selective cholinergic agonist, nicotine also causes the release of dopamine in the basal ganglia and nucleus accumbens (Pidoplichko et al., 1997). A study in rats indicated that working-memory deficits induced by nicotinic antagonists might be reversed by administration of the dopamine agonist quinpirole (Levin and Rose, 1995), suggesting modulation of dopaminergic transmission by the cholinergic system. Therefore, in studies using smokers who have abstained for a period of time, it is probable that there is a reduction in dopaminergic function, as a result of a hypo-nicoticnic state, and this may explain the working-memory deficit observed by Park et al. (2000). Such a hypo-dopaminergic state has also been demonstrated with chronic nicotine treatment, leading to down-regulation of D3 receptor binding (Janson et al., 1992). Therefore, although nicotine is a non-selective cholinergic agonist, the effects of nicotine on working memory cannot be attributed purely to cholinergic function and may be mediated at least in part by dopaminergic processes.

To conclude, there is surprisingly limited research into the effects of cholinergic modulation on working-memory performance in healthy humans. The research by Furey et al. (2000a,b) does suggest that increasing cholinergic function improves working-memory performance. However, these studies are limited to working memory of faces, and further research is required to examine the role of the cholinergic system in other form of spatial and non-spatial working memory. Overall, the evidence presented indicates that although the working-memory task examined appears to be highly important when considering the effect of cholinergic function on working memory, decreases in cholinergic function are associated with impaired performance, while increases in function appear to improve performance.

**Serotonin as an inhibitory modulator**

The putative role of serotonin in learning and memory is amongst the least understood of the monoaminergic neurotransmitters (Gold and Zornetzer, 1983; Luciana et al., 1998; Ogren, 1985). Luciana et al. (1998) investigated the effect of administering a 60 mg dose of fenfluramine, a serotonin re-uptake inhibitor and releasing agent, which effectively increases serotonin levels, on the visuo-spatial delayed response task used by same authors when examining the effects of dopamine manipulation. It was observed that at delays exceeding 4000 ms, fenfluramine appeared to impair working-memory performance. The authors suggested that serotonin may have constrained spatial working memory through an inhibitory effect on dopamine, based on evidence suggesting serotonin and dopamine have opposing roles with respect to emotional and motor behaviours. However, this interaction effect was not explicitly tested.

There is also evidence suggesting that serotonin may modulate the cholinergic system and therefore have an indirect effect on cognition (for a review, see Steckler and...
In a study by Little et al. (1995), healthy human subjects were infused with 0.08 mg/kg of m-CPP, a combined serotonin agonist/antagonist. It was observed that the infusion of m-CPP augmented deficits in word recall, word recognition and objects naming, which had earlier been induced by administration of scopolamine. Although working memory was not tested in this study, studies in rodents have indicated an interaction between the serotoninergic and cholinergic systems in working-memory functions (Miura et al., 1993; Ohno and Watanabe, 1997; Richter-Levin and Segal, 1989). Richter-Levin and Segal (1989) conducted an example of such a study. In this study, a reduction of serotonin synthesis, following the administration of the specific inhibitor of tryptophane hydroxylase ρ-chlorophenylalanine (PCPA), was observed to exaggerate a spatial working-memory deficit (as measured by performance on a spatial water maze), which was induced by blockade of cholinergic transmission, using atropine.

At present, there is insufficient empirical evidence to permit any conclusions about the role of serotonin in working-memory functions. However, the evidence suggests that future research investigate whether serotonin may interact with the dopaminergic and cholinergic systems in modulating working memory in humans.

Other neurochemical systems

Evidence suggests that individual neurons show elevated persistent and tuned activity (memory fields) during working memory that may be modulated by many neurochemicals (see review by Goldman-Rakic, 1995). Little is known about the role of inhibitory mechanisms in the regulation of working memory, but since the majority of interneurons use the inhibitory neurotransmitter GABA, it has been suggested that GABA may be important for working-memory functions (Goldman-Rakic, 1995). Recent evidence from a study (Rao et al., 2000), using two rhesus monkeys has implicated GABA\(_{\rho}\) in working-memory functioning. Rao et al. (2000) also investigated the effect of iontophoresed bicuculline methiodide (BMI), which effectively blocks GABA\(_{\rho}\) on neurones of the PFC in monkeys performing an oculomotor delayed response task. It was reported that BMI caused disinhibition of the neurons and resulted in a loss of spatial tuning, that is, regulation of spatial working memory in the PFC.

Another system that may be important in working-memory function is the NMDA receptor complex. Although no research on modulation of glutamate–NMDA receptor complex and working memory appears to have been conducted in either human or non-human primates, research conducted in rodents indicates that the glutamate system may also be important for working-memory function. For example, blockade of NMDA receptors located at the dorsomedial PFC in rats has been reported to impair spatial working memory (Aura and Riekkinen, 1999). Selective and competitive NMDA receptor antagonists, which block NMDA receptor activity, have been reported to increase the number of errors in working memory (Gutnikov and Rawlins, 1996; Ohno et al., 1992, 1993; Pontecorvo et al., 1991; Puma et al., 1998; Puma and Bizot, 1998). On the other hand, spermidine, an agonist of the polyamine site on the NMDA receptor/channel complex, has been reported to reduce scopolamine-induced errors in working memory, as assessed by the three-panel runway task (Kishi et al., 1998). However, the NMDA agonist did not influence working memory when injected alone (Kishi et al., 1998). This again suggests that interactions between neurotransmitter systems may be important for working-memory function.

Future research

The research reviewed in this paper gives an insight into the pharmacology of human working memory. The current authors suggest that there are three major areas in which future research could expand. The first concerns the heterogeneity of working-memory tasks employed. Specific task sensitivity to pharmacological manipulation is not uncommon in memory research. A recent investigation of secondary episodic memory pharmacology showed that the effect that anti-cholinergic (scopolamine), anti-dopaminergic (haloperidol) and/or GABA modulators (benzodiazepines) had on memory performance depended on the exact task used, although all tasks were designed to examine ‘secondary episodic memory processes’ (Rammasayer et al., 2000). One of the limitations of the research completed to date, is the diverse range of tasks employed to examine working memory. The contentious issue of whether there are different anatomical substrates for different types of information held in working memory was alluded to in the Introduction to this review. It is possible that spatial and non-spatial working memory have different anatomical substrates, which may also result in differences in the pharmacology of the processes. However, perhaps a more important concern is the ‘within-type’ difference between tasks, as there appears to be little consistency between researchers as to what, for example, a spatial working-memory task should consist of. Therefore, it is difficult to compare differences between performance effects of a specific neurotransmitter system on spatial and non-spatial working-memory tasks when the differences between the within-type tasks are high. Development of
well-standardized tasks appears an important step for future research. It may be interesting to look at the effects of a specific drug on a number of tasks defined as testing one type of working memory, such as ‘numeric working memory’ or ‘spatial working memory’ and see how and if the results differ significantly. Further, using the same task to examine numerous neurotransmitter systems may also aid in comparing the effects of different neurotransmitter systems on working-memory performance.

Secondly, it is suggested that brain imaging may play a large role in the future research on neurochemical modulation of working memory. Overall, the evidence presented in this review indicates that acute modulation of specific neurotransmitter systems can influence human working-memory performance, but our knowledge of the cortical basis of these effects is indirect. However, with the use of brain imaging techniques it is possible to examine the direct cortical effects of pharmacological manipulation. In addition it is possible that brain-imaging techniques, like functional magnetic resonance imaging (fMRI), positron emission topography (PET) and various electrophysiological techniques, may be useful if identifying the cortical effects of pharmacological modulation on the various stages of working memory, including early encoding and the holding or maintenance component.

There has been limited research thus far which has employed brain-imaging technology and acute pharmacological manipulation. Indeed, the work of Furey et al. (1997, 2000a,b) who have used PET and fMRI imaging to investigate the effect of cholinergic enhancement on working memory appears to be paving the way for the new direction of research into working-memory pharmacology. Furey et al. (1997) observed that in subjects receiving physostigmine, there was a decrease in rCBF to the right PFC regions during working-memory tasks, that did not occur at rest. The decrease in rCBF to this area was also observed to significantly correlate with an improvement in working-memory function (as assessed by reaction time). In their more extensive replication study, Furey et al. (2000a) again observed a correlation between improved performance and a decrease in rCBF to the right PFC activity. Similarly, decreases in the left temporal cortex, anterior cingulate and left hippocampus rCBF were observed. This study also observed a correlation between improved performance and an increase in rCBF in the medial occipital cortex.

Based on this evidence, Furey et al. (2000b) suggested that cholinergic enhancement of working-memory performance appears to be the result of increases in neuronal activity in regions associated in early perceptual processing, and decreases in activity in regions associated with memory maintenance. This hypotheses was supported in a more recent functional magnetic resonance imaging (fMRI) study which investigated the cortical responses of 7 healthy subjects to different sub-components of the working-memory task, following administration of physostigmine (Furey et al., 2000b). As expected, enhancement of visual processing in the ventral occipital cortex during encoding, and decreased activity in the anterior PFC during maintenance of information, was observed. The authors concluded that enhancement of cholinergic activity improves working memory by focussing perceptual processing in extrastriate visual cortices, particularly during encoding. It is suggested that by producing a more robust visual percept during encoding, working-memory maintenance is simplified and less effort is required by the PFC to maintain the information.

Recently the cortical effects of working memory have also been examined for the dopaminergic system. Kimberg et al. (2001), using fMRI, observed that following bromocriptine administration, there were reductions in task-related brain activity in the parietal and occipital cortex during the maintenance component of a two-back working-memory task. These results support the study of Mehta et al. (2000) who also found that methylphenidate (which increases dopamine and noradrenaline levels) induced task-related reductions in blood flow in the PFC and parietal cortex which correlated with improvements in spatial working-memory performance.

Taken together these brain-imaging studies indicate that the manipulation of dopamine may modify brain activity during the maintenance component of working memory, while cholinergic manipulation may have more of an effect on the encoding component of working memory. Clearly, further research is required to investigate the effects of selective neurochemical modulation on different stages of working memory, but these studies highlight the importance of brain imaging as a tool to examine the effects of pharmacological manipulation on specific cortical areas, and on the different sub-components of working-memory processes.

Finally, future research on working memory in humans will be aided by the development of new and more selective agents which can be used to target specific receptor systems and a wider range of neurochemical systems. This will no doubt shed light into the pharmacology of working memory.

Clinical implications

Working-memory deficits have been reported in patients with clinical disorders such as schizophrenia (Goldman-Rakic, 1991), Parkinson’s disease (Sahakian et al., 1993) and Alzheimer’s disease (Levy et al., 1994; Sano et al., 1993). Dysfunction of the dopaminergic system has been
implicated in schizophrenia, and brain imaging studies of schizophrenic patients have suggested that the PFC may be particularly critical (Andreasen, 1988). Moreover, PET studies have observed decreased numbers of prefrontal dopamine D_{1} receptors in schizophrenic patients (Okubo et al., 1997). Schizophrenic subjects have been frequently observed to be impaired in tasks thought to rely on working-memory processes, such as the Wisconsin Card Sorting Test (Kolb and Wishaw, 1983).

Individuals with Parkinson's disease have also been observed to have abnormal dopaminergic functions, and are reported to exhibit a number of 'prefrontal' dysfunctions including deficits in delayed response spatial memory tasks (Freedman and Oscar-Berman, 1986). Although poverty of behaviour is a common symptom observed in Parkinson's disease, recent evidence suggests that cognitive impairments are not necessarily explained by this symptom (Antal et al., 1998). It has been reported that while medicated Parkinson's disease patients with severe clinical symptoms are impaired in spatial, verbal and visual working memory, medicated patients with mild clinical symptoms appear to be impaired only in spatial working memory (Owen et al., 1997). A recent review of cognitive dysfunction in Parkinson's disease suggests that
spatial working memory, along with attentional set-shifting, appear to be selectively impaired in the early stages of the disease (Antal et al., 1998). Similarly, patients with Alzheimer’s disease (a disorder characterized in part by a degeneration of cholinergic neurons) are reported to also have working-memory deficits (Iversen, 1998). Physostigmine, which effectively increases acetylcholine levels, has been observed to improve working memory in patients with Alzheimer’s disease (Levy et al., 1994; Sano et al., 1993).

The evidence that working memory is modulated by a number of neurochemical systems including dopaminergic and cholinergic systems in humans suggests that targeting these systems with specific agents may potentially be beneficial for patients with working-memory deficits as a result of their illness.

Summary
There is evidence suggesting that human working memory may be modulated by the dopaminergic system. Stimulation of D<sub>1</sub> dopamine receptors appears to facilitate spatial working-memory performance in humans. However, it must be noted that the majority of evidence supporting this contention has come from one laboratory and other groups have reported conflicting evidence. A possible contributor to the conflicting evidence is the apparent lack of consistency in the behavioural tests used between studies, dose-related effects, task difficulty and baseline differences in working-memory capacity between subjects. While studies on the role of D<sub>2</sub> receptors in human working memory is inconclusive, evidence supports an involvement of D<sub>1</sub> receptors.

In addition to dopamine, other neurotransmitters such as acetylcholine, noradrenaline and serotonin may also modulate human working memory. Cholinergic stimulation has been observed to enhance working-memory performance, while blocking cholinergic receptors has been reported to impair working-memory performance. Increasing levels of noradrenaline has also been reported to enhance working-memory performance. There is insufficient evidence to conclude whether serotonin plays a role in working memory, although the evidence to date indicates that any effect is most likely inhibitory, perhaps through modulation of other neurotransmitter systems, such as the dopaminergic and cholinergic systems. This has yet to be explicitly tested. While other systems such as GABA and glutamate may also play some role in modulating working memory, this has also not been investigated in humans.

There are still many unanswered questions regarding the neurochemical basis of working memory, especially in relation to the possible role of serotonin, GABA, and glutamate, and possible interactions between neurochemical systems. However, with the development of more selective drugs for testing in humans, we may be able to better understand the pharmacology of working memory. With the use of brain-imaging techniques, further information regarding the direct cortical effects of pharmacological manipulation, and the specific stage of working memory affected by a pharmacological agent, may also be obtained. Understanding which neurotransmitters can be modulated to enhance working memory may have treatment implications for schizophrenia, Parkinson’s disease and Alzheimer’s disease, and may ultimately play a role in specific treatments.

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