Cholinergic modulation of learning and memory in the human brain as detected with functional neuroimaging

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Abstract

The advent of neuroimaging methods such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) has provided investigators with a tool to study neuronal processes involved in cognitive functions in humans. Recent years have seen an increasing amount of studies which mapped higher cognitive functions to specific brain regions. These studies have had a great impact on our understanding of neuroanatomical correlates of learning and memory in the living human brain. Recently, advances were made to go beyond the use of fMRI as a pure cognitive brain mapping device. One of these advances includes the use of psychopharmacological approaches in conjunction with neuroimaging. The paper will introduce the combination of neuroimaging and psychopharmacology as a tool to study neurochemical modulation of human brain function. A review of imaging studies using cholinergic challenges in the context of explicit and implicit learning and memory paradigms is provided which show that cholinergic neurotransmission modulates task-related activity in sensory and frontal cortical brain areas.

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1. Introduction

The clinical discovery that memory deficits in Alzheimer’s disease are concomitant with a loss of cholinergic markers (Perry et al., 1981) has sparked growing interest in the role that acetylcholine (ACh) plays in learning and memory. Psychopharmacological studies in human and animal subjects have shown concordantly that systemic cholinergic blockade results in deficits of attention, learning and memory (for review see Blokland, 1996 or Fibiger, 1991). Conversely, cholinesterase inhibitors often effectively reverse lesion and pharmacologically induced deficits. The behavioural results in humans are complemented by animal data showing that basal forebrain ACh modulates the responsiveness of cortical neurons (Krnjevic, Pumain, & Renaud, 1971; Kurosawa, Sato, & Sato, 1989), which is mediated by muscarinic receptors (Farkas, Korodi, & Toldi, 1996; Metherate, Cox, & Ashe, 1992; Sato, Hata, Masui, & Tsumoto, 1987). Experimental evidence further shows that cholinergic modulation seems to be specific in its effect for behaviourally relevant stimuli rather than enhancing neuronal responses globally (Ashe, McKenna, & Weinberger, 1989).

Cholinergic cell groups send widespread projections to the entire cortex. Two groups of cholinergic projection neurons are found: (i) the basal forebrain cholinergic neurons (including nucleus basalis, medial septum, and diagonal band of Broca) which innervate the cerebral cortex and hippocampus and (ii) the brain stem cholinergic neurons (including laterodorsal and pedunculopontine tegmental nuclei) which primarily innervate the thalamus. Additionally there are cholinergic interneurons in striatal areas (Cooper, Bloom, & Roth, 1996). Intracerebral injections and neurochemical lesions have enabled animal based research to manipulate cholinergic neurotransmission in circumscribed brain regions. In vivo microdialysis, single unit recordings and other methods are available to study effects of such cholinergic manipulations locally. In contrast, in the human brain, localisation of memory impairing or
promoting effects of cholinergic challenges was not achieved for many decades as human psychopharmacology was restricted to behavioural or electrophysiological measures with relatively low anatomical resolution (Knott, Harr, & Ilivitsky, 1997; Potter, Pickles, Roberts, & Rugg, 2000). With the advent of functional neuroimaging it became feasible to parallel animal research and localise cholinergic modulation of learning, memory, and related plastic changes in the living human brain.

2. Neuroimaging and psychopharmacology

Imaging the human brain during cognitive tasks is now possible using non-invasive methods. Most techniques, such as PET and fMRI are indirect measures of neuronal activity based upon changes in blood flow and blood oxygenation following neuronal activation. The coupling of neuronal activity to vascular changes is thus central to measurements in PET or fMRI. Neurovascular coupling has been investigated in detail and is reviewed elsewhere (e.g., Villringer & Dirnagl, 1995). In relation to psychopharmacological approaches it is important to note that a given drug might not only change neuronal activity but also global blood flow, local blood flow and/or neurovascular coupling. Many researchers are thus reluctant to use blood-flow based techniques to track drug effects—especially with fMRI, where the ratio of deoxygenated and oxygenated haemoglobin provides the basis for the BOLD (blood oxygenation level dependent) signal.

Concerning the cholinergic system, it has been argued that pharmacological manipulations might influence neurovascular coupling (Tsukada et al., 1997). This argument is however based on evidence in monkeys where doses of scopolamine were 10 times higher than those used in human psychopharmacology and of critical relevance is the fact that no effects on rCBF were found at a lower dose. Furthermore, experimental evidence also suggests that psychopharmacological neuroimaging is viable even if the pharmacological challenge bears vascular effects. Gollub et al. (1998) were able to demonstrate that BOLD signal change in human visual cortex is unaffected by application of cocaine which induced a 14% decrease in global blood flow. Others have shown experimentally for specific drugs that the fMRI signal in primary sensory or motor areas is unaffected by drug challenge. For example, it was reported that activations in visual cortex are unchanged by nicotine administration (Jacobsen et al., 2002). If the drug had affected neurovascular coupling, changes should have been evident in all activated areas, including visual cortex. Indeed many cognitive neuroimaging studies with a pharmacological challenge have either demonstrated region specific drug effects or explicitly shown that the drugs’ action was not present in, for example, visual regions (Sperling et al., 2002; Thiel, Henson, Morris, Friston, & Dolan, 2001) thus arguing against global changes in blood flow or neurovascular coupling with the respective drug. This does, however, not exclude regionally specific vascular effects. In order to minimise both global and regional vascular effects of a drug, our paradigms always involve the analysis of differential effects. Comparing different stimulus types or conditions with each other, as compared to approaches which subtract a resting or fixation baseline from an activation condition, removes vascular confounds. Even if these are region specific or specific to activations per se (as shown for example for caffeine by Mulderink, Gittelman, Mesulam, & Parrish, 2002), they should equally influence both types of stimuli and thus subtract out in the direct comparison.

Compared to the benchmark technique of PET, the development and increasing use of fMRI offers several advantages in terms of higher temporal and spatial resolution and the feasibility of repeated testing within the same subjects. The latter is particularly advantageous as it allows to use within-subject designs where the volunteer acts as his or her own control. Further developments in fMRI, such as the use of event-related designs, have brought additional advantages, especially for the study of learning and memory. Such advantages include: (i) the possibility of randomly intermixing different trial types, such as for example previously seen and unseen stimuli in priming paradigms; (ii) investigating different stages of memory processes such as encoding, maintenance, and retrieval; and (iii) sorting events post hoc based on the subjects’ behaviour such as whether an event was subsequently remembered or not (Buckner et al., 1996; Josephs, Turner, & Friston, 1997; Rosen, Buckner, & Dale, 1998; Wagner, Koutstaal, & Schacter, 1999). Event-related designs thus provide a powerful context to investigate the role of cholinergic neurotransmission on different aspects of human learning and memory.

Combining neuroimaging with psychopharmacology basically involves the administration of a drug or respective placebo before volunteers undergo a cognitive task in PET or fMRI (see Fig. 1). A comparison between drug and placebo then reveals the drug’s action on task-related brain activity. Note that the findings of such studies identify biochemical modulation of brain activity that is induced by a specific task rather than excitation or inhibition of brain regions per se. The latter approach is also possible and excellently reviewed in Salmeron and Stein (2002). In principle, designs of psychopharmacological neuroimaging studies do not differ from conventional imaging experiments. For the above-mentioned reasons of vascular confounds it is, however, important that stimuli or activation conditions, such as a memory task are compared to a
Data analysis should include a within-group analysis showing task-related brain activity under drug and placebo, and finally a group by condition interaction showing areas with significant differences in task-related activity between the groups. Plotting activity changes in these areas will provide further information on the modulatory action of the drug on a given cognitive process. Imagine the design depicted in Fig. 1 was a conditioning fMRI study which involved two stimuli, A (CS+) and B (CS-). Under normal conditions, which are reflected in the placebo group, conditioning-related activity is evident in three brain regions, X, Y, and Z. With drug challenge, however, conditioning-related brain activity is smaller in region Y and absent in region Z. A group by condition interaction looking for differences in conditioning-related brain activity under drug and placebo yields significant differences in region Z only. As any interaction, this can be due to different reasons, and the plotted example illustrates that the reduction in BOLD signal in area Z is specific to A and thus a blockade of conditioning-related activity under drug. An absence of activations to both, A and B would also yield a group by condition interaction but argue for unspecific drug effects. It is thus important to further examine the BOLD signal in areas showing drug by condition interactions.

3. Cholinergic modulation of learning and memory in the human brain

Even though the combination of psychopharmacology and neuroimaging offers many possibilities previously unavailable for human research, the approach is still limited in comparison to work in animals. It should be kept in mind when reading the following section, that pharmacological challenges in humans are always systemic and will affect every brain region containing the respective receptors. It is only by modulation of task-induced activity, that a pharmacological action can be localised. Note that areas identified by such group by condition interactions, do not necessarily represent primary areas of drug binding. Changes in brain activity may occur downstream from the initial site of pharmacological action by bottom up or top down modulation. This issue is amenable to further analysis by in vivo imaging of receptor binding using PET and radiolabelled drugs or studies of effective connectivity (Friston et al., 1997).

A further constraint in human research is the restricted range of available drugs licensed for use in humans. With respect to blockade of cholinergic function one is limited to the use of the muscarinic antagonist scopolamine. The nicotinic antagonist mecamylamine was applied in some studies, but is not available for experimental use in humans in several countries. Drugs to increase cholinergic neurotransmission on the other hand are more abundant and involve a variety of cholinesterase inhibitors, such as physostigmine. Nicotine which specifically stimulates nicotinic receptors is also easily available in different forms.

Despite these constraints, pharmacological neuroimaging studies make an important contribution to our understanding of cholinergic modulation of cognitive function in humans. In the following, PET and fMRI studies in healthy volunteers involving the acute application of a cholinergic drug in the context of a learning or memory paradigm are reviewed. For reasons of clarity the studies are classified into those using explicit memory paradigms and those using implicit memory paradigms. The terms explicit and implicit memory were originally introduced by Graf and Schacter (1985) and refer to the observation that memory retrieval can be
dependent (explicit) or independent (implicit) of explicit recollection. It was shown that in amnesia implicit memory functions involving classical conditioning, skill learning or priming are often preserved, suggesting neuronal differences in different processes or networks for implicit and explicit memory. The classification into explicit and implicit memory is also interesting from a pharmacological point of view. While cholinergic modulation of explicit memory has been shown behaviourally (Caine, Weingartner, Ludlow, Cudahy, & Wehry, 1981; Curran, Pooviboonsuk, Dalton, & Lader, 1991a; Frith, Richardson, Samuel, Crow, & McKenna, 1984; Nissen, Knopman, & Schacter, 1987; Rusted & Warburton, 1988), cholinergic effects on implicit learning are controversial and it has often been argued that implicit learning is not cholinergically modulated (Knopman, 1991).

### 3.1. Explicit memory

#### 3.1.1. Working memory

Brain mechanisms contributing to working memory have received a great deal of interest in the neuroimaging literature. The frontal cortex has been found to consistently activate under working memory conditions (see Fletcher & Henson, 2001 for review). Given the cholinergic contribution to explicit memory it is thus not surprising that one of the first neuroimaging studies involving a cholinergic manipulation used a working memory paradigm (Grasby et al., 1995). Subjects received either 0.4 mg scopolamine (s.c.) or placebo and were presented auditorily with short and long word lists which they were asked to remember and immediately recall. The difference between these two conditions was the greater involvement of memory processes in the long list condition. Memory-related brain activity was measured by PET and involved a comparison of rCBF in the long list condition with the short list condition. Behaviourally, the drug reduced the number of words recalled from the long list. Neuronally, it was found that blockade of cholinergic function with scopolamine attenuated memory-related rCBF in left and right prefrontal cortex and anterior cingulate cortex, suggesting that the memory-impairing action of scopolamine might be due to disturbed activity in these frontal brain regions. Authors were however not able to differentiate between encoding and retrieval due to the poor temporal resolution of PET rCBF imaging.

Effects of increased cholinergic neurotransmission on working memory have been studied in several experiments by Furey and colleagues using physostigmine (constant infusion 1 mg/h). In a first PET study (Furey et al., 1997) participants performed a visually presented working memory task for faces. They had to indicate by key press which of two test faces matched a previously presented face. Reaction times improved over scans under physostigmine, indicating improved recognition. Memory-related brain activity was measured by comparing rCBF under task performance with a resting baseline. The drug reduced memory-dependent activations in right inferior temporal cortex which extended into the cerebellum and right prefrontal cortex. The magnitude of rCBF reduction in prefrontal cortex correlated with decreased reaction times. Cholinergic effects were thus seen again in prefrontal cortex, the direction of these effects was however the same as that obtained with cholinergic blockade. Authors interpreted this somewhat counterintuitive result as an effect of reduced effort to perform the task under physostigmine. Increases in task difficulty or cognitive load are indeed often associated with increased frontal activations (e.g., Bullmore et al., 2003). From a behavioural point of view, the explanation of reduced prefrontal activations with reduced effort is thus reasonable. From a pharmacological point of view such explanation would however imply, that frontal effects are neurochemically unspecific since they should occur with any drug reducing the effort needed to perform the task. Furthermore, such explanation does not hold for effects of scopolamine, which should increase task effort but reduce frontal activations. Nor does it explain that a reduction of load-related frontal activity was found with scopolamine in a recent fMRI study (Bullmore et al., 2003).

Since PET studies are not able to investigate different stages of working memory such as encoding and retrieval related processes, authors subsequently performed an fMRI experiment to investigate different stages in the above working memory paradigm (Furey, Pietrini, & Haxby, 2000). This fMRI experiment yielded activity increases to faces under physostigmine in several extrastriate regions and the intraparietal sulcus which were bigger during encoding than retrieval. Again, reductions in prefrontal activity were found with physostigmine. But these were restricted to anterior dorsal prefrontal regions and not specifically related to individual subcomponents of working memory. The authors stress the cholinergic modulation of extrastriate regions and suggest that improved working memory under physostigmine is due to increased perceptual processing of task-relevant stimuli.

While increasing cholinergic neurotransmission with physostigmine will act on both nicotinic and muscarinic receptors, nicotine selectively binds to the former. The role of nicotinic neurotransmission on working memory in smokers and ex-smokers was examined by Ernst et al. (2001). A two back working memory task with visually presented letters was used and subjects received either 4 mg nicotine gum or placebo. rCBF was measured with PET and involved a comparison of the working memory condition (not dissociating encoding and retrieval) with a sensorimotor control task. Several differences were...
found between smokers and ex-smokers. Compared to placebo, smokers showed reductions of brain activity under nicotine during task performance while ex-smokers showed increases in prefrontal cortex and bilateral inferior parietal areas. The only effect common to both groups was a reduction of memory-related anterior cingulate cortex activity under nicotine. Since behavioural effects of nicotine were only evident in smokers it is not clear whether the drugs' differential effects in these two groups of subjects are due to altered sensitivity of cholinergic receptors in smokers and/or to the different behavioural outcome of nicotine administration. Nevertheless, the possibility of differential modulation of memory-related brain activity in smokers and non-smokers should be taken into account when using drugs affecting nicotinic cholinergic receptors.

3.1.2. Other explicit memory paradigms

A slightly different approach to localising the amnestic effects of cholinergic blockade was taken by Rosier et al. (1999). Subjects had to perform an abstract object recognition task where encoding and retrieval were separated by three days. Scopolamine (0.8 mg orally) was administered during encoding whereas PET measurements were performed during encoding testing three days later and involved a comparison between recognition and fixation. Scopolamine induced impairments in object recognition and decreased activity in left fusiform gyrus, which correlated with behavioural performance. Increases in activity were found in the thalamus and bilateral intraparietal cortex. The performance dependent decrease of fusiform cortex activity suggests that scopolamine exerts its main effect in an area that deals with processing and recognition of abstract objects. Even though the authors were not able to investigate scopolamine's effects on encoding, the advantage of their approach is to measure drug-related deficits without the presence of the drug during scanning.

The effects of scopolamine on encoding-related activity were investigated by Sperling et al. (2002) using fMRI and a face-name associative learning task. Prior to scanning, subjects received 0.4 mg scopolamine or placebo i.v. Scopolamine impaired face recognition postscanning. Encoding-related brain activity was isolated by comparing face-name association learning with fixation. Attenuation of encoding-related activity was evident in inferior prefrontal cortex, fusiform cortex, and hippocampus. The activation decreases in fusiform cortex are in accordance with the findings of Rosier et al. (1999) showing that one of the effects of scopolamine consists of attenuation of brain activity in areas associated with processing of task specific stimuli during encoding and retrieval. Apart from those “stimulus processing areas,” effects of cholinergic blockade were also evident in medial temporal regions such as hippocampus. Since these latter regions are the key areas that induce profound deficits of explicit memory when lesioned, further studies should focus on paradigms which specifically activate medial temporal regions.

3.1.3. Summary explicit memory

Cholinergic modulation of explicit learning and memory occurs on two levels. First, modulation was evident in “stimulus processing” brain areas, such as the fusiform cortex or other extrastriate areas. Second, effects were also found in a more “learning related” network including the prefrontal cortex and hippocampus. Cholinergic effects on frontal cortical brain activity suggest that intact cholinergic neurotransmission in this brain region might critically contribute to memory performance. The exact mechanism of such modulation is however not easily explained by the available data since activity reductions were observed with both cholinergic stimulation and cholinergic blockade. Further work is needed to resolve these discrepancies. The pattern of cholinergic modulation in extrastriate regions on the other hand nicely demonstrates activity decreases with cholinergic blockade (scopolamine) and activity increases with cholinergic stimulation (physostigmine). Such effects indicate that cholinergic neurotransmission might increase the efficacy of processing task relevant stimuli in explicit learning paradigms. Further studies will need to determine whether these extrastriate effects contribute to cholinergic modulation of frontal cortical activity or whether frontal cortical activations are independent of extrastriate drug effects.

3.2. Implicit memory

Pharmacological neuroimaging studies are often designed from a behavioural point of view and intend to localise amnesic or memory-promoting effects of pharmacological agents (e.g., Furey et al., 2000; Grasby et al., 1995; Sperling et al., 2002). The paradigms used during imaging are thus sensitive to drug induced behavioural impairments but might induce widespread brain activations, especially when compared to baseline, which are not necessarily truly memory-related. Our approach therefore uses relatively simple paradigms, such as priming and conditioning where learning has reliable neuronal correlates which are well described by prior neuroimaging work and restricted to specific brain areas. Implicit learning paradigms such as conditioning also bear the advantage that they can be implemented in animal experiments and thus provide the possibility for complementary research in animals and humans.

3.2.1. Repetition priming

Priming describes a behavioural phenomenon where prior exposure to a stimulus facilitates or biases its subsequent processing. One potential neuronal signature
for this form of learning was established in monkey and termed “response suppression,” a decrement in response to repeated stimuli in neurons that fire to initial presentation (Desimone, 1996). In humans, analogous decreases in haemodynamic response following stimulus repetition (i.e., “repetition suppression”) in brain areas such as extrastriate and frontal cortices have been repeatedly demonstrated with neuroimaging methods (Buckner, Koutstaal, Schacter, & Rosen, 2000; Henson, Shallice, & Dolan, 2000; Schacter & Buckner, 1998). It has been suggested that decreased BOLD activity with stimulus repetition is due to a sharpening of cortical representations leading to faster behavioural responses (Wiggs & Martin, 1998). Even though the link between behavioural, neuronal, and BOLD responses is probably more complex (see Henson & Rugg, 2003 for further discussion), the important point for our purposes was that repetition suppression is reliably observed in extrastriate cortices and concurrent with the behavioural phenomenon of priming. Repetition suppression thus provides a useful platform from which pharmacological modulation of implicit learning can be studied with neuroimaging techniques.

Using event-related fMRI, we investigated cholinergic modulation of neuronal and behavioural indices of priming with two tasks, a word stem completion and a face repetition paradigm (Thiel, Henson, & Dolan, 2002c; Thiel et al., 2001). In both task, volunteers were given either placebo or scopolamine (0.4 mg i.v.) prior to study. The experimental question of interest was whether scopolamine would modulate ‘repetition suppression’ in extrastriate and frontal regions.

Word stem completion tasks are used in several priming studies. They involve the presentation of a list of words with an incidental learning instruction. After a short interval, a list of three letter word stems is provided and subjects are asked to complete each stem with the first word that comes to mind. The measure of priming is the number of stems completed with words from the previously presented list. In our experiment, volunteers studied the word list prior to scanning. This was followed by a test phase inside the scanner where subjects were presented with a completion task for the stems of the words presented in the study phase (“old word stems”) randomly intermixed with stems of non-presented words (“new word stems”). Scopolamine reduced behavioural measures of priming (see Fig. 2A, left graph). Since scopolamine impaired the behavioural expression of repetition priming, we next asked whether these effects are expressed in modulatory influences on the neuronal index of priming, i.e., repetition suppression. We therefore identified brain regions showing significant repetition suppression under placebo and drug. Second, we compared the magnitude of these reductions by contrasting the placebo and drug group, i.e., we tested for a group (placebo and drug) by repetition interaction. In the placebo group, repetition-related decreases were evident in several brain areas, including left extrastriate cortex, left inferior frontal cortex, and left middle frontal cortex, regions previously shown to manifest ‘repetition suppression’ effects (e.g., Buckner et al., 2000). The comparison of repetition-related effects under placebo and drug revealed a significant interaction in these same regions including left extrastriate, left middle frontal, and to a lesser extent, left inferior frontal cortex. In other words, ‘repetition suppression’ was impaired in the presence of scopolamine. This drug-by-repetition interaction reflected an absence of ‘repetition suppression’ following scopolamine which is shown for left extrastriate cortex in Fig. 2A (see Thiel et al., 2001 for further discussion).

Very similar results for scopolamine were obtained in the face priming paradigm (Thiel et al., 2002c). Volunteers were presented in a study phase outside the scanner with a subset of famous and unfamiliar faces and asked to make fame judgments. This was followed by a test phase inside the scanner where subjects were presented with the whole set of faces, containing randomly intermixed famous and unfamiliar faces that were either presented in the study phase or were not. Participants were asked to make fame judgements. The mean correct reaction times for first versus second presentation of famous and unfamiliar faces provided the behavioural index of repetition priming in this paradigm. Scopolamine impaired priming of famous faces (see Fig. 2B, left graph). The placebo group showed repetition suppression to famous faces in right fusiform cortex. Scopolamine impaired this repetition suppression (see Fig. 2B, right graph). Consequently, both paradigms underline a role of ACh in repetition priming. Cholinergic modulation is expressed as an attenuation of ‘repetition suppression’ in the same brain areas associated with repetition effects in the placebo group and in previous studies using the respective paradigm (Buckner et al., 2000; Henson et al., 2000).

Our neuroimaging evidence is in contrast to most of the prior behavioural (Knopman, 1991) or animal electrophysiological (Miller & Desimone, 1993) data which could not find evidence for cholinergic modulation of repetition effects. Two potential differences between our and prior studies might explain the discrepant findings. First, we would like to suggest that cholinergic deficits in priming paradigms are only found with higher doses of scopolamine. The only other study showing an impairment of priming (Vitiello et al., 1997) used a similar dose of scopolamine as we did (i.e., 0.5 mg i.v.) while other studies (e.g., Knopman, 1991; Schifano & Curran, 1994) used effectively lower doses (between 0.3 and 0.6 mg i.m.). Note that Schifano and Curran (1994), who used two doses of scopolamine found a tendency towards attenuated repetition priming at the higher drug dose. Another critical difference between priming studies
pertains to the delay between study and test phase which was ≈40 min in our studies. Since repetition suppression can be sensitive to lag (Henson et al., 2000) it may be that weaker repetition suppression with longer lags is more sensitive to drug influences. Indeed, Nissen et al. (1987) found that word fragment completion was impaired by scopolamine when there was a 60 min delay between study and test phase but not when the delay was 5 min as usually used in word stem completion paradigms (although the authors attributed these lag effects to an influence of explicit memory). We would thus like to suggest that cholinergic modulation of implicit learning in priming paradigms is only seen with longer lags between study and test phase.

3.2.2. Conditioning

Since ACh seemed to modulate neuronal correlates of priming we asked whether these findings could be extended to other implicit learning situations. Aversive conditioning is a form of associative learning in which a previously neutral stimulus, such as a tone (conditioned stimulus, CS), acquires significance through its prediction of a future aversive event, such as an electric shock (unconditioned stimulus, US). Brain systems involved in aversive conditioning are well described (LeDoux, 1995). Conditioning paradigms thus provide a compelling model to study mechanisms of learning-related plasticity. In the context of a neuroimaging experiment, we operationally define plasticity in a broad sense as experience-dependent changes in haemodynamic responses to relevant sensory stimuli. Prior neuroimaging studies, using eye-blink and aversive conditioning paradigms, have provided evidence that learning-related plasticity occurs in human auditory cortices (Molchan, Sunderland, McIntosh, Herscovitch, & Schreurs, 1994; Morris, Friston, & Dolan, 1998; Schreurs et al., 1997).
Animal data suggest that cholinergic cortical projections are important for modulating such learning-related plasticity (Weinberger, 1997). We thus designed a psychopharmacological event-related fMRI study to address cholinergic modulation of experience dependent changes in the human brain which was very much based on animal experiments. We used a differential conditioning paradigm with partial reinforcement where BOLD activity to an unpaired CS+ can be contrasted with activations to a CS−. Since both stimuli are physically identical, differential activity to the CS+ must be due to its acquired significance during conditioning. Conditioned stimuli were high (1600 Hz) and low tones (400 Hz), one of which was paired with an electrical shock (Thiel, Friston, & Dolan, 2002b). Prior to scanning, subjects were given either placebo or 0.4 mg i.v. scopolamine. Conditioning-related enhancement of haemodynamic responses to the CS+ but not the respective CS− was evident in the placebo group. Under scopolamine, the enhancement of BOLD activity to the CS+ was blocked, suggesting that cholinergic receptors are involved in these conditioning-related responses (Fig. 3, left graph). The findings provide in vivo evidence that conditioning-related plasticity in human auditory cortex is attenuated by blockade of cholinergic (muscarinic) neurotransmission. They are supported by a wealth of animal literature and nicely illustrate that psychopharmacological approaches in neuroimaging are able to extend findings based on animal research to the human brain.

Studying modulatory effects of drugs on learning-related plasticity in humans is of significance for the study of mechanisms of recovery and treatment effects in patients with neurological damage. But rather than showing a cholinergic blockade of learning-related changes, a critical experiment to conduct from a clinical point of view would be one aiming to increase learning-related plasticity. Indeed, there is behavioural evidence showing recovery promoting actions of cholinergic treatment in aphasia (Berthier, Hinojosa, Martin Md, & Fernandez, 2003). We therefore conducted a follow-up study using the same differential conditioning paradigm as above but a physostigmine infusion to enhance ACh and possibly conditioning-related activity (Thiel et al., 2002a). Data in the placebo group showed again enhanced BOLD response to the CS+ in auditory cortex, indicating learning-related changes (Fig. 3, right graph). In contrast to our expectations however, the physostigmine group did not show any differential activation to CS+ vs. CS−. This absence of conditioning-related activations was however different from that seen previously with scopolamine (see left graph) and due to an increase of activations to the CS−. Note that activations to the CS+ were not different between drug and placebo, i.e., there was also an increase of activation to the CS+ under physostigmine (but this was similar to the increase observed with the irrelevant CS−).

It has been shown that pairing a tone with direct iontophoretic application of ACh in place of the US produces conditioning specific changes in receptive fields in auditory cortex which can be blocked with atropine (see Weinberger, 1995 for review). Even though the relationship between receptive field analysis in animals and BOLD signal change in humans has not been established, we would like to use this evidence from animal data to speculate on the neuronal mechanisms of cholinergic modulation observed with neuroimaging in our conditioning experiments. Imagine that under normal conditions the pairing of the CS+ with a shock would induce a release of ACh which would contribute

![Fig. 3. Effects of scopolamine and physostigmine on auditory cortex during conditioning. Plots of percent signal change (mean and SEM) of two voxels in auditory cortex illustrating cholinergic modulation of conditioning-related activity under scopolamine, physostigmine, and their respective placebo control (for full data see Thiel, Bentley, & Dolan, 2002a and 2002b). Left graph: Effects of cholinergic blockade with scopolamine in a right auditory cortex voxel showing significant group by conditioning effects (x = 57, y = −15, z = 6). Right graph: Effects of cholinergic enhancement with physostigmine. Activity in a left auditory cortex voxel showing a group by conditioning interaction in the follow up study (x = −63, y = −18, z = 9). Note that in comparison with placebo, scopolamine reduced activations to the CS+ whereas physostigmine increased activations to the CS−. CS, conditioned stimulus.](image-url)
to development of conditioning-related plasticity. This experience-dependent enhancement of responses requires a temporal coincidence between neuronal depolarisation produced by ACh and neuronal excitation produced by the sensory stimulus (Hars, Maho, Edeline, & Hennevin, 1993). With scopolamine, any release of ACh upon stimulation is ineffective due to blockade of muscarinic ACh receptors resulting in a lack of conditioning-related response increase. This is reflected in similar activations to the CS− and CS+ in our first study (Thiel et al., 2002b). The pharmacological action of physostigmine on the other hand is an increase in ambient ACh levels, and a prolongation of cholinergic action upon stimulation. Such mechanism could be beneficial in increasing the action of ACh when released in response to a CS+; but it might also interfere with the precise timing necessary for conditioning-related plasticity and result in a temporal overlap of still increased cholinergic activity with a following irrelevant stimulus (i.e., CS−). Temporal coincidence of the CS− with a still elevated cholinergic activity might then result in similar enhancements of neuronal activation to both the CS− and CS+ which was observed in this follow-up study (Thiel et al., 2002a). We therefore suggest, that cholinergic blockade reduces activations to relevant stimuli while cholinergic stimulation with physostigmine results in inordinate activations to irrelevant stimuli. Both mechanisms interfere with learning-related plasticity, one by decreasing the “signal” and the other by increasing “noise.”

The idea that physostigmine might increase irrelevant signals in healthy volunteers seems in contrast to data by Furey et al. (2000) who suggest that cholinergic stimulation improves stimulus processing. First note, that the study by Furey et al. (2000) did not employ task-irrelevant stimuli so that no conclusions can be drawn about possible increases of processing of irrelevant stimuli with cholinergic stimulation. Second, the beneficial effect of physostigmine on task-relevant stimuli in the Furey study, which we could not find in our experiment might be linked to the fact that their task was cognitively more demanding (working memory for faces vs. key press to a tone) and that cholinergic stimulation may be especially beneficial in such situations.

3.2.3. Summary implicit memory

This section on implicit memory presented four of our own studies on cholinergic modulation of implicit learning. In both learning paradigms neuronal correlates are well defined and involve differential experience-dependent effects to otherwise similar stimuli. Repetition-related changes were evident as experience-dependent response decreases whereas conditioning-related changes were evident as experience-dependent response increases. It was shown that cholinergic blockade interfered with both of these experience-dependent changes. The aim to enhance experience-dependent responses with administration of physostigmine failed, which could reflect that cholinergic stimulation in healthy volunteers effectively overstimulates an otherwise perfectly balanced cholinergic system. It needs to be investigated in future studies, whether effects of cholinergic stimulation are beneficial in states of reduced cholinergic activity, such as Alzheimer’s disease. Indeed there is in vitro evidence for differential effects of cholinesterase inhibitors on ACh release in normal brains and brains of Alzheimer patients (Nordberg, Nilsson-Hakansson, Adem, Lai, & Winblad, 1989).

4. Summary and future perspectives

I have presented several studies which tried to localise cholinergic modulation of learning-related brain activity. The results obtained are diverse and depend on the paradigm used. Nevertheless, if one has to come to an integration of cholinergic neuroimaging studies two common findings should be stressed. First, in several experiments, cholinergic modulation was seen in frontal cortical areas, which are known to activate in neuroimaging studies of learning and memory, suggesting that memory impairing or promoting effects of cholinergic drugs in these paradigms are be closely linked to modulation of frontal cortical activity. Second, in several paradigms cholinergic modulation was also demonstrated in areas that are involved in processing the task relevant stimuli such as fusiform cortex, extrastriate regions or auditory cortex. The finding that cholinergic neurotransmission modulates activity in such areas would suggest a cholinergic role in stimulus processing and attentional function which is also supported by behavioural and neurochemical evidence in animals (Blokland, 1996; Sarter & Bruno, 1997). Such finding does not preclude a cholinergic role in learning and memory, it just underlines that experimental designs are needed were learning and stimulus processing are unconfounded.

It is still early days for pharmacological neuroimaging studies and more experiments are clearly required to obtain further information on the role of ACh in cognitive function. Such experiments should compare: (i) the effects of the same drug in different paradigms (especially those of learning and memory vs. attention) and (ii) the effects of different cholinergic drugs within one paradigm. Further insights into the role of ACh on integrated activity in the human brain during learning and memory are expected from new approaches, which investigate interactions between brain regions, rather than activity within one region (Buchel & Friston, 2001). Such measures of effective connectivity, which assess the influence that one brain regions exerts over another offer the possibility to gauge changes in functional strength of
a putative anatomical connection. In relation to psychopharmacological imaging, the pharmacological manipulation of such connection strengths (e.g., Coull, Buchel, Friston, & Frith, 1999) will offer new and exciting insights into neurochemical modulation of connected networks in the human brain.

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References


