Neuron, Vol. 35, 567–574, August 1, 2002, Copyright ©2002 by Cell Press

Cholinergic Modulation of Experience-Dependent Plasticity in Human Auditory Cortex

Christiane M. Thiel,[1,3,4] Karl J. Friston,[1] and Raymond J. Dolan[1,2]
[1]Wellcome Department of Imaging Neuroscience
Institute of Neurology
12 Queen Square
London WC1 3BG
[2]Royal Free Hospital School of Medicine
Pond Street
London NW3 2PF
United Kingdom
[3]Institute of Medicine
Research Centre Juelich
Leo-Brandt Str. 5
52425 Juelich
Germany

Summary

The factors that influence experience-dependent plasticity in the human brain are unknown. We used event-related functional magnetic resonance imaging (fMRI) and a pharmacological manipulation to measure cholinergic modulation of experience-dependent plasticity in human auditory cortex. In a differential aversive conditioning paradigm, subjects were presented with high (1600 Hz) and low tones (400 Hz), one of which was conditioned by pairing with an electrical shock. Prior to presentation, subjects were given either a placebo or an anticholinergic drug (0.4 mg iv scopolamine). Experience-dependent plasticity, expressed as a conditioning-specific enhanced BOLD response, was evident in auditory cortex in the placebo group, but not with scopolamine. This study provides in vivo evidence that experience-dependent plasticity, evident in hemodynamic changes in human auditory cortex, is modulated by acetylcholine.

Introduction

Primary sensory cortices show plasticity under a variety of circumstances. A well-characterized example is learning-related plasticity within auditory cortex evident in aversive conditioning paradigms (Edeline, 1999). 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). The neurobiological basis of aversive conditioning is well described (LeDoux, 1995) and provides a compelling model to study mechanisms of learning-related plasticity. Previous neuroimaging studies, using eye-blink and aversive conditioning paradigms, have provided evidence that experience-dependent changes occur in human auditory cortices (Molchan et al., 1994; Schreurs et al., 1997; Morris et al., 1998). These findings are in line with animal experiments demonstrating learning-related plasticity of primary and secondary auditory cortex receptive fields and maps in several paradigms (Diamond and Weinberger, 1986; Bakin and Weinberger, 1990; Edeline and Weinberger, 1993; Bakin et al., 1996; Condon and Weinberger, 1991; Kisley and Gerstein, 2001; Gonzalez-Lima and Scheich, 1986).

Animal evidence suggests that the expression of cortical sensory plasticity is facilitated by neuromodulators, particularly acetylcholine (ACh) (Rasmusson, 2000). Cholinergic cortical projections from nucleus basalis are implicated in modulating experience-dependent plasticity in auditory cortex (Weinberger, 1998), a brain region rich in muscarinic (m2) receptors (Zilles and Palomero-Gallagher, 2001). At present, there is no human data on pharmacological modulation of cortical plasticity. The motivation for the present study was to investigate cholinergic modulation of experience-dependent auditory cortex plasticity in the human brain. In the context of a neuroimaging experiment involving either positron emission tomography (PET) or functional magnetic resonance imaging (fMRI), we operationally define plasticity as experience-dependent changes in hemodynamic responses to one or more sensory stimuli (see also Morris et al., 1998) and provide evidence that reduction of cholinergic activity blocks this form of plasticity.

Results

We combined event-related fMRI with an anticholinergic drug challenge to block plastic changes during aversive auditory conditioning. A differential conditioning paradigm was used with two tones of different frequency as CS (400 or 1600 Hz). One of these tones was paired with an electric shock to the left leg (CS+) whereas the other was not (CS−). Subjects were randomly allocated to two groups and given either placebo or 0.4 mg scopolamine intravenously prior to conditioning. In both groups, half of the subjects received the low-frequency tone as CS+, whereas the other half received the high-frequency tone as CS+.

The paradigm consisted of three phases (see Figure 1). In a preconditioning phase, subjects were exposed to the two tones without shock to establish the location of auditory cortex responses to distinct tones. In a subsequent conditioning phase (depending on the group), the respective CS+ was paired in 50% of the trials with an electric shock coterminating with the tone (delay conditioning, see Experimental Procedures for details). A partial reinforcement schedule was used in order to facilitate assessment of responses to the CS+ in absence of the US. Finally, in an extinction phase, the two tones were again presented without shock. During all phases of the experiment, participants were asked to make a right index finger key press at the end of each tone. These response times were taken as an index of conditioning since galvanic skin responses cannot be recorded in subjects under scopolamine (Golding and Stott, 1997). Response time measures in conditioning paradigms have been previously used in our group in

[4]Correspondence: c.thiel@fz-juelich.de

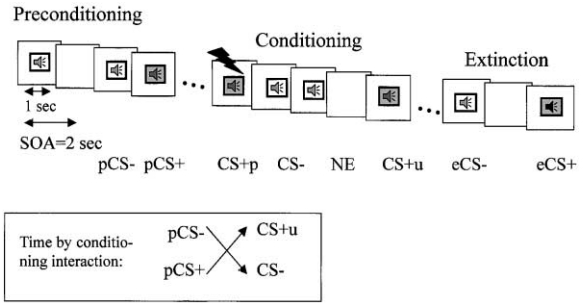

Figure 1. Illustration of Experimental Paradigm and Contrast for Investigation of Experience-Dependent Plasticity

We used a partial reinforcement schedule where only 50% of CS+ trials were paired with a shock (CS+p), and the other 50% were not paired (CS+u). Partial reinforcement schedules allowed us to assess evoked hemodynamic responses to the CS+ in the absence of the US. CS− is the conditioned stimulus that is never paired with shock during conditioning phase; eCS+ and eCS− are the conditioned stimuli during extinction phase. In half of the subjects, the low tone was the CS+ and the high tone was the CS−; in the other half, the low tone was the CS−, and the high tone was the CS+.

Abbreviations: SOA, stimulus onset asynchrony; NE, nullevent; pCS+ and pCS−, the to-be-conditioned stimuli during the preconditioning phase; CS+, conditioned stimulus that is paired with shock during conditioning phase.

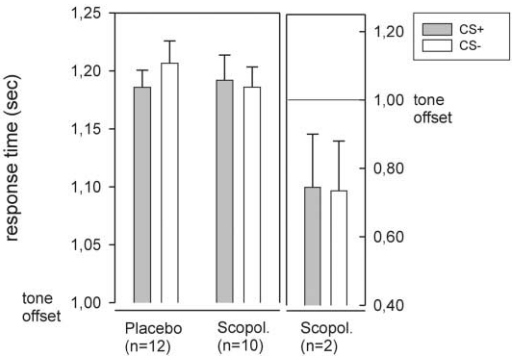

Figure 2. Behavioral Data

Mean response times and standard errors to CS+ (gray bars) and CS− (white bars) for placebo and scopolamine group. Two subjects were false alarming in the scopolamine group and are shown separately (right graph).

experiments where galvanic skin responses could not be measured. In this approach, conditioning is evident, as faster responses to the CS+ as compared to CS− (Critchely et al., 2002). After the experiment, subjects were debriefed regarding conditioning contingencies and the unpleasantness of the US (see Experimental Procedures for details). Note that in the following, all cerebral and behavioral responses analyzed use the unpaired CS+.

Mean response times to tones throughout the experiment were 1.19 ± 0.172 s for placebo subjects and 1.11 ± 0.509 s for scopolamine subjects. Mean response times were numerically lower in scopolamine subjects and more variable. This was accounted for by two subjects false alarming (i.e., making a response on average after 630 and 940 ms, when the actual task was to press the key following termination of the 1000 ms duration of the tone). In the placebo group, analysis of behavioral data showed reduced response times to the unpaired (i.e., without shock) CS+ compared to CS− during conditioning ($t[11] = -2.023$, $p = 0.034$, one tailed paired t test), confirming that the paradigm induced conditioning (see Figure 2). Subjects treated with scopolamine did not show a differential response to CS+ and CS− ($t[11] = 0.480$, $p = 0.320$ for whole group; $t[9] = -0.417$, $p = 0.687$ for group without subjects who were false alarming). To illustrate that false alarming did not influence differential responses to CS+ and CS−, data from the two subjects who were false alarming are presented separately (see Figure 2, right graph). The skin stimulation was rated as mildly unpleasant, and there was no difference in the rating between groups (Mann-Whitney U test, $Z = -0.569$, $p = 0.590$), suggesting that scopolamine did not change the perceived aversiveness of the US. Likewise, there was no group difference in awareness of contingencies (Mann-Whitney U test, $Z = -0.897$, $p = 0.410$). Awareness of contingencies correlated with the response time measure of conditioning (CS+/CS−) (Spearman correlation coefficient $r = -0.505$, $p = 0.012$).

To illustrate the effect of auditory stimulation in placebo and scopolamine subjects, fMRI activations to low and high tones versus baseline during preconditioning are shown in Figure 3. In both placebo and scopolamine groups, tones activated auditory cortex regions bilaterally. Note that the height of activation was not significantly different for scopolamine and placebo, even at a reduced threshold of $p < 0.01$, uncorrected (as tested for by a random effects analysis of the group by stimulation interaction). Concerning auditory cortex experience-dependent plasticity, we expected a conditioning-related enhancement of hemodynamic responses to low and high tone CS+, but not the respective high or low tone CS− in the placebo group. Furthermore, if the cholinergic innervation of the auditory cortex has a modulatory role in these plastic changes, reduced conditioning-related plasticity should be observed when cholinergic receptors are blocked with a systemic injection of scopolamine. These hypotheses were investigated with two planned comparisons.

First, we identified in both groups auditory regions showing conditioning-related plasticity. For this purpose, we used a contrast testing for a time (preconditioning versus conditioning) by conditioning (CS+ versus CS−) interaction (for illustration see Figure 1). This compares differential responses to unpaired CS+ relative to CS− during preconditioning and conditioning phases. Note that activations during extinction do not contribute to this analysis. Subsequently, a second analysis was performed to test for significant differences between placebo and scopolamine in these contrasts with a group (placebo versus scopolamine) by time (preconditioning versus conditioning) by conditioning (CS+ versus CS−) interaction. To restrict the analysis to auditory cortex regions responsive to tones and not to bias the analysis in favor of regions only responsive in the placebo group, both analyses were restricted to regions showing activations to tones in the scopolamine group (see Figure 3B).

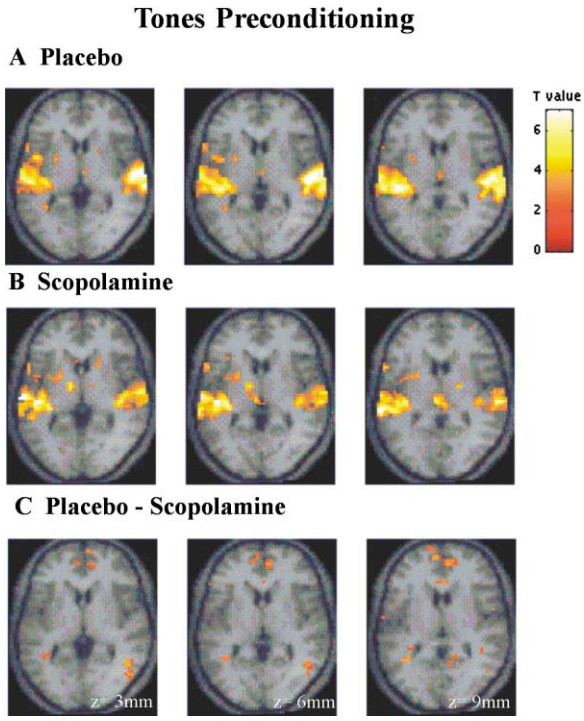

Figure 3. Tones Preconditioning

(A–C) Auditory cortical regions showing activations to low and high tones relative to a baseline, as identified by random effects analysis in (A) placebo and (B) scopolamine groups, respectively. In (C), the differences image (Placebo-Scopolamine) is shown. The image shows that activations in auditory cortex preconditioning are not significantly different between placebo and scopolamine subjects. All activations are thresholded at $p < 0.01$ (uncorrected for purposes of comparison) and are rendered on a transverse individual T1-weighted MRI.

In the placebo group, experience-dependent plasticity was evident in a region of right (and to a lesser extent, left) auditory cortex when low tones were used as CS+. When high tones were used as CS+, experience-dependent plasticity was observed in left auditory cortex (experience-dependent plasticity in right auditory cortex was only evident at a lower threshold, i.e., $p = 0.014$, uncorrected) (Figure 4A). The opposite contrast (i.e., increases to CS− and decreases to CS+) did not yield any significant activity in auditory regions (as identified by random effects analysis, $p < 0.001$, uncorrected). No significant experience-dependent plasticity, even at a lower threshold ($p < 0.01$, uncorrected), was found in auditory cortex after scopolamine for either the low- or high-tone CS+ (Figure 4B).

To confirm that experience-dependent plasticity observed in the placebo group was significantly impaired with scopolamine, direct comparisons were carried out (Figure 4C). A group × time × conditioning interaction for the low-tone CS+ group was evident in the same right auditory region that showed experience-dependent plasticity under placebo. For the high-tone CS+ group, a group × time × conditioning interaction was found in right auditory cortex, slightly more posterior and lateral to the activations found for the low-tone CS+ group. Additionally, for this high-tone CS+ group, the same left auditory region that expressed experience-dependent plasticity under placebo showed a group by plasticity interaction at a reduced threshold ($p < 0.005$, uncorrected). Thus, the anticholinergic drug scopolamine blocked experience-dependent auditory cortex plasticity as expressed in experience-dependent changes in hemodynamic responses.

Figure 4D further illustrates the pattern of the group × time × conditioning interaction in right auditory cortex for the low- and high-tone groups, respectively. Both plots are derived from the maximally active voxel identified in right auditory cortex by the group × time × conditioning interaction (as shown in Figure 4C) and illustrate percent signal change during preconditioning, conditioning, and extinction phases in all groups. Irrespective of whether low- or high-frequency tones were used as CS+ in subjects receiving placebo, there was an increase in right auditory cortex activation to the CS+ when preconditioning was compared with conditioning and a decrease in activation (i.e., adaptation) for the CS−. In contrast, subjects receiving scopolamine showed either adaptation (i.e., a reduction of activations between preconditioning and conditioning phases) of auditory cortex activations for both CS+ and CS− (low-tone group) or a reduction of activations to CS+ and increased activations to the CS− (high-tone group). Note that this pattern was also found when the two volunteers with false alarms were excluded.

To assess drug-induced sedation as a contributory factor to the observed plasticity, an additional analysis was performed where we correlated subjective sedation with the magnitude of experience-dependent plasticity in auditory cortex in scopolamine treated subjects. There was no correlation between measures of sedation and plasticity in auditory cortex in the low-tone CS+ group ($r = 0.557$, $p = 0.251$ Pearson correlation coefficient) or high-tone CS+ group ($r = -0.579$, $p = 0.228$ Pearson correlation coefficient). An additional concern is the possibility of drug effects on global perfusion; consequently, to investigate whether the drugs might have interfered with global cerebral blood flow (gCBF), we compared the estimated global BOLD signal across the brain between the two drug groups. No differences in this global estimate were evident (independent t test $t[22] = 0.780$, $p = 0.444$). Finally, we evaluated whether autonomic side effects of the drug administration might have influenced conditioning. Subjective ratings of the most frequently reported autonomic side effect (i.e., a dry mouth) was correlated with conditioning. We found no relationship between those two measures ($r = 0.170$, $p = 0.439$, Spearman correlation coefficient).

Discussion

Using a conditioning paradigm, the study provides evidence that reduction of cholinergic neurotransmission blocks experience-dependent plasticity in human auditory cortex. In considering these findings, there are a number of issues that require discussion in so far as they present potential confounds.

One important consideration is the possibility that drugs such as scopolamine might affect global and/or regional cerebral blood flow (gCBF/rCBF). Tsukada and

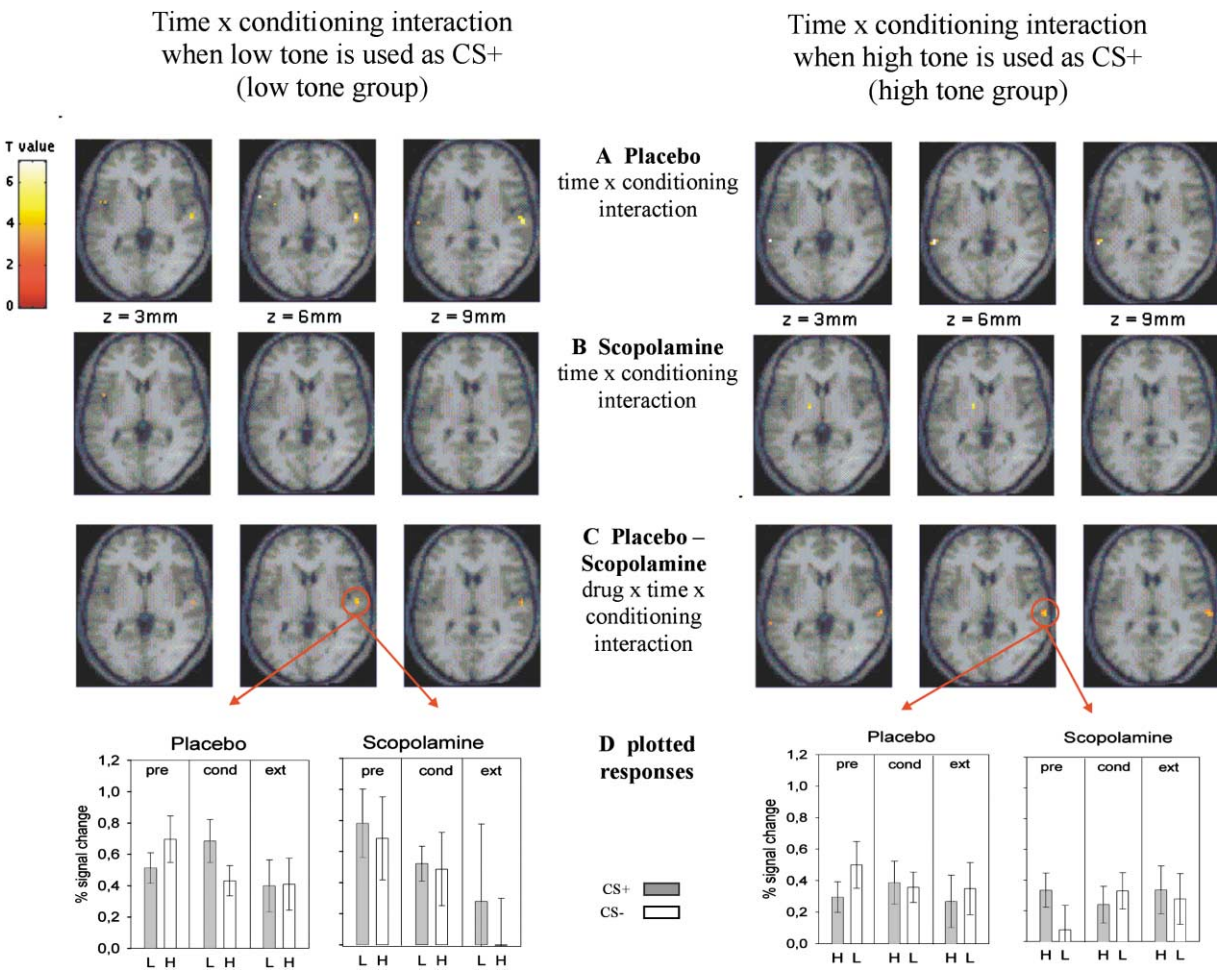

Figure 4. Experience-Dependent Plasticity in Auditory Cortex

(A) Auditory regions showing a time (preconditioning versus conditioning) × conditioning (unpaired CS+ versus CS−) interaction in the placebo group when a low tone (n = 6; left) or high tone (n = 6; right) CS+ was used. Experience-dependent plasticity was evident bilaterally in auditory cortex (x = 54, y = −15, z = 6, Z = 3.50; x = −57, y = −9, z = 6, Z = 3.47) for the low-tone group and left auditory cortex (x = −63, y = −42, z = 6, Z = 4.71) for the high-tone group. For the high-tone group, a right auditory cortex activation was only observed at a reduced threshold (x = 66, y = −27, z = 6, Z = 2.39, p = 0.009).

(B) No significant time × conditioning interaction in auditory cortex was found with scopolamine for either the low- (n = 6; left) or the high- (n = 6; right) tone group.

(C) Auditory regions showing a group (placebo versus scopolamine) × time × conditioning interaction. Significant differences indicating a higher time × conditioning interaction in placebo compared to scopolamine subjects was found for low- and high-tone groups in the right auditory cortex (low: x = 57, y = −15, z = 6, Z = 3.31; high: x = 66, y = −27, z = 6, Z = 3.65). Additionally, for the high-tone group, a left auditory region showed a group × time × conditioning interaction at a reduced threshold (x = −63, y = −39, z = 0, Z = 2.55; p = 0.005). All activations (thresholded at $p < 0.01$, uncorrected for purposes of illustration) were identified by random effects analysis.

(D) Plots of percent signal change. Mean and SEM as a function of group, time, and conditioning. The plots are derived from the most significant voxel in right auditory cortex, showing a group × time × conditioning interaction as identified by a random effects analysis as shown in (C). Voxel: x = 57, y = −15, z = 6 for the group where the low tone was used as CS+ and voxel: x = 66, y = −27, z =6 for the group where the high tone was used as CS+. Note the increase in right auditory cortex activation to the CS+ (gray symbols) when comparing preconditioning with the conditioning phases and the decrease in activation for the CS− (white symbols) in the placebo group while scopolamine subjects showed decreases in activations for the CS+.

Abbreviations: L, low tone; H, high tone; pre, preconditioning; cond, conditioning; ext, extinction.

colleagues (1997) have shown in monkeys that rCBF to somatosensory stimulation is abolished by scopolamine. The doses shown to yield this effect were, however, ten times higher than the dose used here, and of critical relevance is the fact that no effects on rCBF were found at a lower dose. To account for possible differences in gCBF between the groups in our data, global scaling was used in the data analysis. Furthermore, we found no evidence for a difference when we compared the estimated global BOLD signal between the groups, which makes significant changes in gCBF unlikely. Finally, it should be noted that pharmacological effects mediated through neurovascular coupling cannot explain the differential responses to CS+ relative to CS−.

A potential confound when investigating drug effects in humans is sedation, which has been reported frequently after drugs such as scopolamine. We have assessed sedation with visual analog scales and corre-

lated these measures with auditory cortex plasticity. We found no relationship between plasticity and sedation in scopolamine subjects. In addition, it needs to be acknowledged that false positives in imaging data can be obtained due to movement artifacts. We do not think that the reported differential activations are due to movement, since movement parameters were included as confounds in the analysis (see the Experimental Procedures section).

Response times to the CS served as measure of conditioning. They have previously been shown to indicate conditioning through decreased time to respond to the CS+ and can be used in paradigms were electrodermal responses cannot be measured (e.g., after scopolamine administration) (Critchely et al., 2002). Here, response time data is included to demonstrate that the paradigm was valid to obtain conditioning (in the placebo group). We do not infer any casual relationship between these behavioral measures and plasticity, as measured with fMRI. The aim of this study was to investigate the effects of scopolamine on experience-dependent plasticity in auditory cortex, regardless of its behavioral correlates. Indeed, the behavioral expression of learning is not necessary for auditory cortex plasticity since the latter also develops in differential conditioning paradigms where the two-tone discrimination task is too difficult for behavioral learning to take place (Edeline and Weinberger, 1993).

Since our main interest was the detection of auditory cortex plasticity, we used a paradigm that was optimized for the detection of a differential effect on auditory cortex responses as measured with fMRI (Josephs and Henson, 1999). This resulted in a paradigm with short intertrial intervals (2 s in the case where there were no null events between two succeeding stimuli) and a tone-shock interval of 1 s. Additionally, for reasons of analysis, a partial reinforcement schedule was applied that allows sampling of hemodynamic responses to nonpaired CS+ stimuli and so avoids the confound of a response to a US in paired presentations. Even though these requirements were suboptimal for conditioning to occur, response times and BOLD contrast in auditory cortex showed differential responses to CS+ and CS−.

In animals, frequency-responsive receptive fields in primary auditory cortex are retuned during aversive conditioning such that receptive fields are shifted toward the frequency of the CS with reduced responses to the previous best frequency (Bakin and Weinberger, 1990). The increased BOLD responses in auditory cortex to the CS+ and decreases to the CS− in placebo subjects thus parallel the cellular responses reported in animals. The findings are also in line with two previous PET studies using eyeblink conditioning where increased rCBF was evident in primary auditory cortex when conditioning was compared with pseudoconditioning (Molchan et al., 1994; Schreurs et al., 1997). Interestingly, the plastic changes were more prominent in the auditory cortex contralateral to the side of US stimulation, a finding that we also observed (i.e., right auditory cortex plasticity with US applied to left leg for low-tone CS+).

Although the contrasts we used did not investigate auditory cortex responses during extinction, the plots in Figure 4D suggest, particularly for the group where a low tone was used as CS+, that plastic changes seen in human auditory cortex in the placebo groups might be subject to extinction. Decreases in auditory cortex activity during extinction have also been described in eyeblink conditioning in humans (Molchan et al., 1994; Schreurs et al., 1997). In animals, it has been shown that receptive field plasticity in secondary auditory cortex is subject to extinction (Diamond and Weinberger, 1986), while conditioned onset responses in auditory association cortex were found to be resistant to extinction trials (Quirk et al. 1997).

It is widely reported that increases in cortical ACh release occur when animals are presented with behaviorally relevant, aversive stimuli (Acquas et al., 1996; Thiel et al., 2000). A striking recent observation is that auditory cortex plasticity can be induced by cholinergic manipulations alone. Thus, auditory cortex receptive field and map plasticity can be induced by pairing a tone with stimulation of nucleus basalis (Bakin and Weinberger, 1996; Miasnikov et al., 2001; Kilgard and Merzenich, 1998; Hars et al., 1993) or an application of acetylcholine (Metherate and Weinberger, 1989). Additionally, plasticity induced by pairing nucleus basalis stimulation with a tone is blocked by systemic or cortical atropine, data that reinforce the view that muscarinic ACh-receptor activation is crucial to this form of plasticity (Bakin and Weinberger, 1996; Miasnikov et al., 2001). The blockade of plasticity under scopolamine is not due to increased or decreased auditory cortex responsivity under anticholinergic challenge. This is evident in the plots in Figure 4D that show that plastic changes are impaired under scopolamine in the low-tone group where hemodynamic responses to tones preconditioning are numerically higher under drug and in the high-tone group where responses to tones preconditioning are numerically lower under drug, thus arguing against baseline differences in affecting plasticity. The finding that blockade of auditory cortex cholinergic activity with the muscarinic antagonist scopolamine significantly impaired auditory cortex plasticity thus makes a new link from animal to human data.

The pattern of auditory cortex activity in the scopolamine group showed activity decreases to the CS+ from the preconditioning to the conditioning phase, which resembles the decreased hemodynamic responses to CS− from the preconditioning to the conditioning phase in the placebo group. Decrements in neuronal discharges in the auditory cortex are normally found in animals in habituation paradigms where a tone of a given frequency is presented repeatedly (Condon and Weinberger, 1991). Habituation is a form of nonassociative learning that occurs to repeated presentation of innoxious stimuli. The activity decreases in the scopolamine group to the CS+ might be interpreted as an interference with the process through which the CS+ acquires predictive value for the aversive US. Furthermore, the observation that BOLD response decreases are expressed under scopolamine might suggest that cholinergic modulation does not interfere with habituation-related changes in auditory cortex neuronal response. Further experiments are needed to test this hypothesis.

We are aware that although there are striking similarities between learning-related receptive field or map plasticity in animals and plasticity seen with neuroimaging methods in humans, there is no direct evidence that

these changes are engendered by the same mechanisms. Apart from differences in the underlying techniques used to measure plasticity (e.g., neuronal versus hemodynamic responses), animal studies mostly compare receptive fields before and after training has taken place. Our paradigm compared experience-induced changes before and during training. Nevertheless, the fact that both measures of plasticity are sensitive to cholinergic manipulations suggest at least some commonality.

Even though the present study focused on cholinergic modulation of plasticity in auditory cortex, which is particularly rich in muscarinic receptors, there is evidence that cholinergic modulation of cerebral responses is not restricted to the auditory cortex and also evident in other brain areas. For example, in a prior pharmacological fMRI study we have shown a modulation of extrastriate and frontal cortical areas in a repetition-priming paradigm under scopolamine (Thiel et al., 2001).

Conclusion

Our study has addressed cholinergic modulation of experience-dependent plasticity in humans and provides evidence that the neurotransmitter acetylcholine is important for such cortical plasticity. The broader implication of this study is that it demonstrates that neurochemical manipulation of plasticity can be investigated in the human brain. In this regard, such an approach has significance for the study of mechanisms of recovery and treatment effects in patients with neurological damage.

Experimental Procedures

Subjects

Twenty-nine right-handed participants (16 male, 13 female; age range: 18–36 years) with no history of medical or psychiatric disease gave informed consent to participate in the study. A clinical evaluation was first carried out to ensure that subjects had no conditions contraindicative for scopolamine administration. Only young (below 40 years of age), healthy (without medical problems and not on any kind of medication apart from contraceptives) volunteers were included in the study. Ethics approval was obtained from the ethics committee of the local hospital. Five participants were excluded from analysis due to technical failure during sound or shock delivery. The effective group size was n = 12 for placebo (8 male, 4 female; mean age 25.41 ± 1.38) and n = 12 for scopolamine (6 male, 6 female; mean age 26.08 ± 1.79). Both groups were divided into two subgroups (n = 6), depending on whether the low or high tone was used as CS+.

Drugs

A double-blind drug administration technique was used. Subjects received either an intravenous (iv) injection of 0.4 mg scopolamine (fixed dose) or saline 90 min before the start of the experiment. Scopolamine was administered intravenously since oral scopolamine has variable absorption, poor bioavailability, and is behaviorally less effective (Putcha et al., 1989). A dose of 0.4 mg of scopolamine was chosen because intravenous injections in this dose range have been reported to affect a variety of cognitive functions (Vitiello et al., 1997). Scopolamine was administered 90 min before scanning since prior studies have shown that cognitive effects of parenteral scopolamine peak from 90 to 150 min after administration (Safer and Allen, 1971; Sannita et al., 1987; Curran et al., 1991; Ebert et al., 1998), a time window in which scopolamine binds preferentially to cerebral ACh receptors (as opposed to cerebellar and thalamic receptors, Frey et al., 1992). Prior to scanning, subjective drug effects such as sedation were assessed with visual analog scales (Bond and Lader, 1974). Additionally, pulse rate was measured and other bodily symptoms specific to scopolamine (e.g., dry mouth) were rated on scales. Throughout the experiment, volunteers were checked for adverse side effects, and physostigmine was kept in place in case of adverse reactions to the drug.

Experimental Paradigm

The paradigm consisted of a preconditioning phase, a conditioning phase, and an extinction phase. Two neutral tones (pure sine tones 400 and 1600 Hz) with a duration of 1 s were used as CS. Stimuli (including one third "null events" [NE], Josephs and Henson, 1999) were delivered in a randomized order every 2 s via headphones. Subjects were informed that they would receive skin stimulation during the experiment but that this was irrelevant to the task. They were asked to make a right index finger key press at the end of each tone. During the preconditioning and extinction phases the low (400 Hz) and high (1600Hz) tones were delivered 25 times each. During conditioning, the tones were delivered 50 times each, and one of them (CS+) was paired in 50% of trials with an electric shock to the left leg that coterminated with the tone (stimulation parameters: constant current, square wave pulses; duration, 0.25 ms intensity range, 6–12 mA, set by subject prior to scanning). The assignment of the low or high tone to either CS+ or CS− was randomized across placebo and scopolamine subjects. The use of a differential conditioning paradigm enabled us to ensure that increased neuronal responses to the CS+ were not due to generally increased excitability, attention, or motivational changes during the conditioning phase by comparing activations to the CS+ with those to the CS−. We used a 50% partial reinforcement strategy (i.e., only half of the presentations of the CS+ were paired with the US) to allow us to assess evoked hemodynamic responses to the CS+ in the absence of the US. Partial reinforcement schedules have been successfully used in previous fMRI studies to analyze responses to the CS+ in absence of the US (Buchel et al., 1998). In all analyses, only responses to the CS+ unpaired were compared with the CS−. After the end of the experiment, subjects rated the skin stimulation on a scale from zero to six (not unpleasant = 0, very mildly unpleasant = 1, mildly unpleasant = 2, moderately unpleasant = 3, moderately severely unpleasant = 4, severely unpleasant = 5, extremely severely unpleasant = 6). Additionally, they were asked to rate the pleasantness of the two tones. Awareness of the contingencies was evaluated with a semistructured interview. First, they were asked to tell when during the scanning session (i.e., beginning, middle, end, or throughout) they received the aversive skin stimulation and how many they received. Second, they were asked whether there was any relationship between the tones and the stimulation. If they did not notice any relationship, they were told that one of the tones was paired with the shock and should guess which of the tones it was. The answers were scored on a scale from zero to three (3 = contingency reported even without being asked, i.e., before or after the first question; 2 = contingency reported when asked, i.e., after the second question; 1 = no contingency reported but guessed right when asked to guess; 0 = no contingency reported and guessed wrong when asked to guess).

Imaging and Image Processing

A VISION MRI system (Siemens, Erlangen, Germany) operating at 2T was used to obtain T2*-weighted echoplanar (EPI) images with blood oxygenation level dependent (BOLD) contrast. 250 volumes of 30 2 mm thick axial slices were acquired sequentially every 3.5 mm, leaving a 1.5 mm gap between slices (repetition time = 2.5 s, echo time = 40 ms). The first five volumes were discarded to allow for T1 equilibration effects. All volumes were realigned spatially to the first volume, and the timeseries for voxels within each slice realigned temporally to acquisition of the middle slice. Resulting volumes were normalized to a standard EPI template based on the MNI reference brain (Ashburner and Friston, 1999) in Talairach space (Talairach and Tournoux, 1988) and resampled to $3 \times 3 \times 3$ mm^3 voxels. The normalized images were smoothed with an isotropic Gaussian kernel of 8 mm full-width half-maximum to accommodate intersubject anatomical variability.

Statistical Analysis of Images

Imaging data were analyzed with Statistical Parametric Mapping software (SPM99, Wellcome Department of Cognitive Neurology,

London) (Friston et al., 1995), employing a random effects analysis. Data were globally scaled to 100 across scans and high-pass-filtered at 1/32 Hz. The hemodynamic response to stimulus onset for each event type was modeled by a canonical synthetic hemodynamic response function (HRF) and its first-order temporal derivative. Nine event types were modeled, comprising of eight effects of interest and missed responses as effect of no interest. Effects of interest were the two frequency tones during preconditioning (pCS+ and pCS−), one of which was going to be paired in 50% of trials with a shock during conditioning (CS+ paired, CS+ unpaired, and CS−). The CS− was randomly divided into two event types to parallel the CS+. The last two event types were the two tones during extinction (eCS+ and eCS−). Head movement parameters were included as confounds, which regress out residual, movement-related variance. Linear contrasts of parameter estimates for the canonical HRF for each subject were taken to a second level analysis to generate statistical parametric maps (SPMs) of the t statistic. We restricted our analysis to three contrasts. (1) A contrast identifying voxels with significant responses to presentation of tones during the preconditioning phase [pCS+ + pCS−] (Figure 3). (2) A contrast investigating experience-dependent plasticity using a time (preconditioning versus conditioning) × conditioning (CS+ versus CS−) interaction [CS+ − pCS+] − [CS− − pCS−], separately for placebo and scopolamine subjects in the low- and high-tone groups (Figures 4A and 4B). (3) A contrast comparing the effects of placebo and scopolamine in the low- and high-tone groups on experience-dependent plasticity by a group × time × conditioning interaction (Placebo[CS+ − pCS+] − [CS− − pCS−]) − (Scopolamine [CS+ − pCS+] − [CS− − pCS−]) (Figure 4C). For this latter analysis, percent signal change for the most significant voxel was plotted for illustration (Figure 4D). Such plots are used to illustrate the pattern of the interaction investigated with a specific contrast. Contrasts 2 and 3 were masked with the main effect of tones during preconditioning. To exclude the possibility that a mask collapsed over both groups' preferentially identified regions showing plasticity in the placebo group due to higher preconditioning activations to tones, the scopolamine group data (thresholded at $p < 0.01$, uncorrected) was used as a mask within which to identify plastic changes in auditory cortex.

Acknowledgments

We thank the radiographers for help with fMRI scanning; medical colleagues, especially John Morris, for help with injections; and Philip Allen for construction of the skin stimulator. Thanks to Richard Frackowiak for his helpful comments on an earlier version of this manuscript. This work was supported by program grants from the Wellcome Trust to R.J.D. and K.J.F. and a research grant from the German Research Foundation (DFG) to C.M.T.

Received: September 27, 2001
Revised: May 9, 2002

References

Acquas, E., Wilson, C., and Fibiger, H.C. (1996). Conditioned and unconditioned stimuli increase frontal cortical and hippocampal acetylcholine release: effects of novelty, habituation and fear. J. Neurosci. *16*, 3089–3096.

Ashburner, J., and Friston, K. (1999). Nonlinear spatial normalization using basis functions. Hum. Brain Mapp. *7*, 254–266.

Bakin, J.S., and Weinberger, N.M. (1990). Classical conditioning induces CS-specific receptive field plasticity in the auditory cortex of the guinea pig. Brain Res. *536*, 271–286.

Bakin, J.S., and Weinberger, N.M. (1996). Induction of a physiological memory in the cerebral cortex by stimulation of the nucleus basalis. Proc. Natl. Acad. Sci. USA *93*, 11219–11224.

Bakin, J.S., South, D.A., and Weinberger, N.M. (1996). Induction of receptive field plasticity in the auditory cortex of the guinea pig during instrumental avoidance conditioning. Behav. Neurosci. *110*, 905–913.

Bond, A., and Lader, M. (1974). The use of analogue scales in rating subjective feelings. Br. J. Med. Psychol. *47*, 211–218.

Buchel, C., Morris, J., Dolan, R.J., and Friston, K.J. (1998). Brain systems mediating aversive conditioning: an event-related fMRI study. Neuron *20*, 947–957.

Condon, C.D., and Weinberger, N.M. (1991). Habituation produces frequency-specific plasticity of receptive fields in the auditory cortex. Behav. Neurosci. *105*, 416–430.

Curran, H.V., Schifano, F., and Lader, M.H. (1991). Models for memory dysfunction? A comparison of the effects of scopolamine and lorazepam on memory, psychomotor performance and mood. Psychopharmacology (Berl.) *103*, 83–90.

Critchely, H.D., Mathias, C.J., and Dolan, R.J. (2002). Fear-conditioning in humans: the influence of awareness and autonomic arousal on functional neuroanatomy. Neuron *33*, 653–663.

Diamond, D.M., and Weinberger, N.M. (1986). Classical conditioning rapidly induces specific changes in frequency receptive fields of single neurons in secondary and ventral ectosylvian auditory cortical fields. Brain Res. *372*, 357–360.

Ebert, U., Siepmann, M., Oertel, R., Wesnes, K.A., and Kirch, W. (1998). Pharmacokinetics and pharmacodynamics of scopolamine after subcutaneous administration. J. Clin. Pharmacol. *38*, 720–726.

Edeline, J.M. (1999). Learning-induced physiological plasticity in the thalamo-cortical sensory systems: a critical evaluation of receptive field plasticity, map changes and their potential mechanisms. Prog. Neurobiol. *57*, 165–224.

Edeline, J.M., and Weinberger, N.M. (1993). Receptive field plasticity in the auditory cortex during frequency discrimination training: selective retuning independent of task difficulty. Behav. Neurosci. *107*, 82–103.

Frey, K.A., Koeppe, R.A., Mulholland, G.K., Jewett, D., Hichwa, R., Ehrenkaufer, R.L., Carey, J.E., Wieland, D.M., Kuhl, D.E., and Agranoff, B.W. (1992). In vivo muscarinic cholinergic receptor imaging in human brain with [11C]scopolamine and positron emission tomography. J. Cereb. Blood Flow Metab. *12*, 147–154.

Friston, K.J., Holmes, A.P., Worsley, K.J., Poline, J.-P., Frith, C.D., and Frackowiak, R.S. (1995). Statistical parametric maps in functional imaging: a general linear approach. Hum. Brain Mapp. *2*, 189–210.

Golding, J.F., and Stott, J.R. (1997). Comparison of the effects of a selective muscarinic receptor antagonist and hyoscine (scopolamine) on motion sickness, skin conductance and heart rate. Br. J. Clin. Pharmacol. *43*, 633–637.

Gonzalez-Lima, F., and Scheich, H. (1986). Neural substrates for tone-conditioned bradycardia demonstrated with 2-deoxyglucose. II. Auditory cortex plasticity. Behav. Brain Res. *20*, 281–293.

Hars, B., Maho, C., Edeline, J.M., and Hennevin, E. (1993). Basal forebrain stimulation facilitates tone-evoked responses in the auditory cortex of awake rat. Neuroscience *56*, 61–74.

Josephs, O., and Henson, R.N. (1999). Event-related functional magnetic resonance imaging: modelling, inference and optimization. Philos. Trans. R. Soc. Lond. B Biol. Sci. *354*, 1215–1228.

Kilgard, M.P., and Merzenich, M.M. (1998). Cortical map reorganization enabled by nucleus basalis activity. Science *279*, 1714–1718.

Kisley, M.A., and Gerstein, G.L. (2001). Daily variation and appetitive conditioning-induced plasticity of auditory cortex receptive fields. Eur. J. Neurosci. *13*, 1993–2003.

LeDoux, J.E. (1995). Emotion: clues from the brain. Annu. Rev. Psychol. *46*, 209–235.

Metherate, R., and Weinberger, N.M. (1989). Acetylcholine produces stimulus-specific receptive field alterations in cat auditory cortex. Brain Res. *480*, 372–377.

Miasnikov, A.A., McLin, D., and Weinberger, N.M. (2001). Muscarinic dependence of nucleus basalis induced conditioned receptive field plasticity. Neuroreport *12*, 1537–1542.

Molchan, S.E., Sunderland, T., McIntosh, A.R., Herscovitch, P., and Schreurs, B.G. (1994). A functional anatomical study of associative learning in humans. Proc. Natl. Acad. Sci. USA *91*, 8122–8126.

Morris, J.S., Friston, K.J., and Dolan, R.J. (1998). Experience-dependent modulation of tonotopic neural responses in human auditory cortex. Proc. R. Soc. Lond. B Biol. Sci. *265*, 649–657.

Putcha, L., Cintron, N.M., Tsui, J., Vanderploeg, J.M., and Kramer, W.G. (1989). Pharmacokinetics and oral bioavailability of scopolamine in normal subjects. Pharm. Res. *6*, 481–485.

Quirk, G.J., Armony, J.L., and LeDoux, J.E. (1997). Fear conditioning enhances different temporal components of tone-evoked spike trains in auditory cortex and lateral amygdala. Neuron *19*, 613–624.

Rasmusson, D.D. (2000). The role of acetylcholine in cortical synaptic plasticity. Behav. Brain Res. *115*, 205–218.

Safer, D.J., and Allen, R.P. (1971). The central effects of scopolamine in man. Biol. Psychiatry *3*, 347–355.

Sannita, W.G., Fioretto, M., Maggi, L., and Rosadini, G. (1987). Effects of scopolamine parenteral administration on the electroretinogram, visual evoked potentials, and quantitative electroencephalogram of healthy volunteers. Doc. Ophthalmol. *67*, 379–388.

Schreurs, B.G., McIntosh, A.R., Bahro, M., Herscovitch, P., Sunderland, T., and Molchan, S.E. (1997). Lateralization and behavioral correlation of changes in regional cerebral blood flow with classical conditioning of the human eyeblink response. J. Neurophysiol. *77*, 2153–2163.

Talairach, J., and Tournoux, P. (1988). Co-Planar Stereotaxic Atlas of the Human Brain. (Stuttgart: George Thieme Verlag).

Thiel, C.M., Muller, C.P., Huston, J.P., and Schwarting, R.K. (2000). Auditory noise can prevent increased extracellular acetylcholine levels in the hippocampus in response to aversive stimulation. Brain Res. *882*, 112–119.

Thiel, C.M., Henson, R.N.A., Morris, J.S., Friston, K.J., and Dolan, R.J. (2001). Pharmacological modulation of behavioural and neuronal correlates of repetition priming. J. Neurosci. *21*, 6846–6852.

Tsukada, H., Kakiuchi, T., Ando, I., Shizuno, H., Nakanishi, S., and Ouchi, Y. (1997). Regulation of cerebral blood flow response to somatosensory stimulation through the cholinergic system: a positron emission tomography study in unanesthetized monkeys. Brain Res. *749*, 10–17.

Vitiello, B., Martin, A., Hill, J., Mack, C., Molchan, S., Martinez, R., Murphy, D.L., and Sunderland, T. (1997). Cognitive and behavioral effects of cholinergic, dopaminergic, and serotonergic blockade in humans. Neuropsychopharmacology *16*, 15–24.

Weinberger, N.M. (1998). Physiological memory in primary auditory cortex: characteristics and mechanisms. Neurobiol. Learn. Mem. *70*, 226–251.

Zilles, K., and Palomero-Gallagher, N. (2001). Cyto-, myelo-, and receptor architectonics of the human parietal cortex. Neuroimage *14*, 8–20.