Article

Current Biology

Acetylcholine Mediates Behavioral and Neural Post-Error Control

Highlights

- Acetylcholine (ACh) is crucial for implementation of cognitive control
- ACh modulates post-error activity in visual brain areas recruited by the task
- Blocking muscarinic ACh receptors abolishes behavioral post-error adjustments
- Posterior medial frontal cortex controls cholinergic top-down modulations

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In Brief

Danielmeier et al. provide evidence that acetylcholine (ACh) mediates behavioral and neural adjustments after errors. These adaptive mechanisms are controlled by the medial frontal cortex. They are essential for optimizing future performance and, ideally, avoiding another error. The role of ACh in cognitive control has hitherto been neglected.

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Acetylcholine Mediates Behavioral and Neural Post-Error Control

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SUMMARY

Humans often commit errors when they are distracted by irrelevant information and no longer focus on what is relevant to the task at hand. Adjustments following errors are essential for optimizing goal achievement. The posterior medial frontal cortex (pMFC), a key area for monitoring errors, has been shown to trigger such post-error adjustments by modulating activity in visual cortical areas. However, the mechanisms by which pMFC controls sensory cortices are unknown. We provide evidence for a mechanism based on pMFC-induced recruitment of cholinergic projections to task-relevant sensory areas. Using fMRI in healthy volunteers, we found that error-related pMFC activity predicted subsequent adjustments in task-relevant visual brain areas. In particular, following an error, activity increased in those visual cortical areas involved in processing task-relevant stimulus features, whereas activity decreased in areas representing irrelevant, distracting features. Following treatment with the muscarinic acetylcholine receptor antagonist biperiden, activity in visual areas was no longer under control of error-related pMFC activity. This was paralleled by abolished post-error behavioral adjustments under biperiden. Our results reveal a prominent role of acetylcholine in cognitive control that has not been recognized thus far. Regaining optimal performance after errors critically depends on top-down control of perception driven by the pMFC and mediated by acetylcholine. This may explain the lack of adaptivity in conditions with reduced availability of cortical acetylcholine, such as Alzheimer's disease.

INTRODUCTION

An important reason for human error is the decline of selective attention to goal-relevant information. Thereby, distracting stimuli can elicit inappropriate reactions [1, 2]. For example, when you are driving, a message from your car's navigation system can divert your attention and lead to mistakes, which may accumulate and lead to severe consequences depending on how long it takes you to refocus your attention back to the road. While a range of remedial and adaptive mechanisms are recruited after errors [3, 4], refocusing selective attention to task-relevant input appears the best-suited means to avoid similar mistakes in the future and to resume correct task performance. In a previous study [5], we found specific top-down modulations in task-related visual brain areas following errors. These consisted of increased activity in task-relevant visual brain areas and decreased activity in task-irrelevant areas involved in encoding distracting stimulus features. Importantly, these post-error adjustments were predicted by the preceding error-related activity in posterior medial frontal cortex (pMFC). Although this suggests that pMFC triggers these adjustments in sensory areas, the mechanism via which pMFC might exert this top-down influence remains unknown.

Animal studies on attentional modulations (unrelated to errors) suggest a crucial role of acetylcholine (ACh) [6]. Based on these studies, we hypothesized that, beyond its general role in attention, acetylcholine could play a very specific role in the implementation of post-error adjustments of brain activity and behavior. Neuroanatomical studies in mammals suggest that the medial prefrontal cortex projects to the basal forebrain (BF) [7], which in turn sends cholinergic projections to visual cortical areas [8]. However, this suggestion requires careful corroboration in primates.

ACh acting on muscarinic ACh receptors (mAChRs) is crucial for attentional modulation of visual neurons [9, 10], and the modular organization of the BF might provide the architecture necessary for task-specific modulations of sensory cortical areas [6, 11–13]. We therefore tested the hypothesis that the pMFC controls post-error adjustments in task-related visual

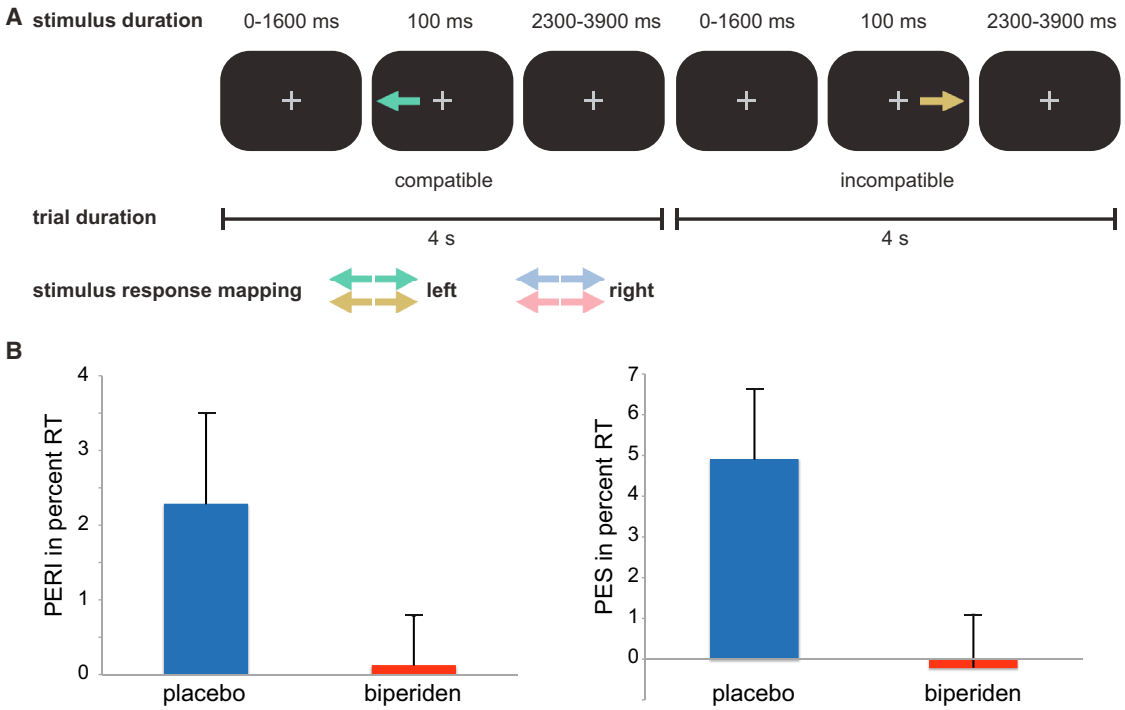

Figure 1. Task Sequence and Behavioral Results

(A) Modified Simon task. Colored arrows pointing right or left were presented on the corresponding side of the screen (arrow direction and presentation side were always congruent). Four different colors were used. Participants were asked to respond to the color (task-relevant) and ignore the arrow direction and presentation side (task-irrelevant). Examples of a compatible and an incompatible trial are shown. Total trial duration was 4 s, with an onset delay varying between 0 and 1.6 s.

(B) Behavioral post-error adjustments. Post-error reduction of interference (PERI) and post-error slowing (PES) are shown for placebo (blue) and biperiden (red) conditions as a percentage of the individual's mean reaction time (RT). Error bars represent SEM.

areas by modulating cholinergic inputs, likely originating from the BF. If blockade of cholinergic transmission at mAChRs attenuates behavioral and neural measures of post-error adjustments, this would be strong evidence for this candidate mechanism.

We scanned healthy volunteers with fMRI while they performed a modified Simon task (Figure 1A) under the influence of either placebo or the mAChR antagonist biperiden (0.04 mg/kg intravenous [i.v.]). Two sets of findings suggest that post-error adjustments in selective attention are indeed mediated by recruitment of cortical ACh. First, following errors, there was improved resolution of perceptual interference and slowing of motor responses. Both of these behavioral adjustments were blocked by biperiden. Second, error-related pMFC activation predicted the subsequent increases and decreases in visual cortical areas coding task-relevant and task-irrelevant stimulus features, respectively. Under biperiden, this relationship between pMFC activity and post-error modulation of visual cortical activity was abolished. These results are consistent with our suggested cholinergic mechanism underlying post-error adjustments.

RESULTS

Behavioral Results

General task performance (error rates, interference effects) did not differ between the biperiden and placebo sessions, but participants' correct responses were generally slower by about 33 ms in the biperiden condition (see Supplemental Behavioral Results). Blocking cholinergic transmission at mAChR abolished two key hallmarks of behavioral post-error adjustments (Figure 1B): task-specific post-error reduction of interference (PERI) and more general post-error slowing (PES) [3, 4, 14, 15]. Task-irrelevant and distracting stimulus features interfere with response selection, particularly when stimulus features are incompatible with the appropriate response, which is reflected in longer reaction times (RTs) on incompatible compared to compatible trials. PERI describes the finding that this interference effect is less pronounced on post-error trials as compared to post-correct trials, which is believed to reflect cognitive control processes related to improved interference resolution after errors [3, 16]. We calculated PERI as the difference in the interference effect between post-error and post-correct trials, normalized by each individual's mean RT per session (see Supplemental Experimental Procedures for details). Whereas significant PERI was found under placebo ($t_{23} = 1.87$, $p = 0.037$), it was absent under biperiden ($t_{23} = 0.17$, $p = 0.43$; difference between sessions, $t_{23} = 2.1$, $p = 0.024$; Figure 1B). The less task-specific PES is reflected in the prolongation of RTs on correct responses following an error [14]. PES was observed under placebo ($t_{23} = 2.83$, $p = 0.01$), but not after biperiden administration ($t_{23} = -0.17$, $p = 0.87$; difference between sessions, $t_{23} = 2.44$, $p = 0.023$, two-tailed).

fMRI Data

ACh Blockade Abolishes Functional Relationship between pMFC and Task-Relevant Visual Areas

fMRI data were analyzed using group independent component analysis applied to all data from all scanning sessions, followed by deconvolution of component time courses and single-trial amplitude estimation to disentangle brain activity on successive trials [1]. Our hypotheses were focused on the relationships between error-related responses in pMFC and post-error adjustments in visual brain areas processing task-relevant and -irrelevant features. Therefore, we first identified three independent components (ICs) that were located in the region of pMFC and showed significant error-related activity as in previous studies [5, 16, 17] (Table S1). Activity in the averaged pMFC ICs on error trials was increased compared to baseline in both conditions (both $p < 0.001$), but error-related pMFC activity under biperiden was reduced compared to placebo ($t_{23} = 2.141$, $p = 0.043$). Next, we identified task-relevant brain areas by selecting those ICs that overlapped with activations obtained from a separate color-localizer task. Replicating our previous findings [5], activity in these three task-relevant ICs was reduced on error trials, indicating a suboptimal activity level, but subsequently increased again after an error. Our hypothesis states that this post-error increase is driven by error-related pMFC responses recruiting basal forebrain cholinergic projections to the task-relevant visual areas. If this is the case, error-related pMFC responses will become uncorrelated with the post-error increase in color-coding visual ICs under biperiden. We tested this by means of a multiple regression model. The post-error increase in activity averaged across the three task-relevant ICs was the dependent variable; the error-related activity averaged across the three pMFC ICs, the session treatment (placebo or biperiden), and the interaction between these terms were the independent variables. The significant interaction of the pMFC error signal and treatment ($\beta = -0.64$, $t = -1.71$, $p = 0.047$) and follow-up correlations within each treatment condition indicate that under placebo, but not under anti-muscarinic medication, greater error-related pMFC activity predicted a stronger subsequent activity increase in task-relevant visual areas (placebo, $r = 0.46$, $p = 0.012$; biperiden, $r = -0.089$, $p = 0.66$; Figure 2). Multiple regression results for individual combinations of pMFC and task-relevant visual ICs further confirm this finding at the level of individual ICs (Table S2; Figure S1). That is, ACh seems to be crucial for pMFC-triggered top-down amplification of task-relevant brain areas after errors.

ACh Blockade Attenuates the Functional Relationship between pMFC and Task-Irrelevant Visual Brain Areas

We next sought to determine whether the same ACh dependency exists for the suppression of task-irrelevant visual areas following errors. Visual areas processing task-irrelevant features were identified as those six ICs in occipital cortex that did not overlap with activation from the color localizer. As observed previously [5], activity in task-irrelevant visual areas returned to baseline levels within one trial after an error. Therefore, the post-error decline was defined as the activity change between error and post-error trial. Multiple regression analysis on the mean post-error decline across the task-irrelevant visual ICs revealed a trend for an interaction of the mean pMFC error signal with treatment ($\beta = 0.41$, $t = 1.52$, $p = 0.068$). Thus, the correlation between the pMFC error signal and the post-error suppression of task-irrelevant visual activity tended to be stronger under placebo ($r = -0.51$, $p = 0.006$) than under biperiden ($r = -0.38$, $p = 0.033$; Figure 3). Although this difference is largely driven by a potential outlier (after outlier removal: $\beta = -0.05$, $t = -0.28$, $p = 0.61$), the results of multiple regression models performed on individual combinations of pMFC and task-irrelevant visual ICs demonstrated that the post-error decline in two of the six task-irrelevant ICs showed a significant interaction of the pMFC error signal with treatment (Figure S2; Table S2). In other words, stronger error signals in the pMFC predicted stronger post-error reduction of task-irrelevant activity in visual cortex. This relationship was substantially reduced under biperiden compared to placebo, but not completely abolished. The possibility that pMFC activity predicts activity changes in any brain region in an unspecific manner was ruled out by applying the same analyses to two control ICs (see Supplemental Analyses).

Neuronal Correlates of Post-Error Slowing

In addition to these precise, task-specific adjustments, we also found that PES, a more general behavioral adjustment, was absent under biperiden. Therefore, we next investigated whether biperiden also affects the relationship between PES and the brain areas mediating it. The mechanisms underlying PES are still the subject of debate, but it has been suggested that PES reflects general motor inhibition, which could be part of an orienting response elicited by the error [3, 4]. PES is associated with a brain network comprising the pre-supplementary motor area (pre-SMA), right posterior inferior frontal cortex (IFC), and subthalamic nucleus (STN) [5, 18–20], which has been linked to motor slowing and an increased motor threshold [21]. In the present dataset, we identified IC34 as the component that most closely matched the previously described right hemispheric network associated with PES. IC34 covered the pre-SMA and lateral IFC in addition to lateral temporal areas of the right hemisphere. Activity during error trials in this IC correlated with PES in the placebo session ($r = 0.65$, $p = 0.001$; two-tailed), but not in the biperiden session ($r = -0.24$, $p = 0.252$; Figure 4). There was a significant interaction between the error-related activity in this component and drug, indicating a significant difference between these two correlations ($\beta = -0.28$, $t = -3.00$, $p = 0.004$).

DISCUSSION

The present study demonstrates the importance of ACh for goal-directed behavior in humans. In particular, it reveals a previously unknown cholinergic mechanism for adjustments that allow resumption and optimization of task performance after an error has been committed. Notably, this cholinergic mechanism seems to be specific for the improvement of selective attention after errors, but not for the maintenance of attention in general, since the Simon interference effect, another measure of cognitive control, was not affected by biperiden (see Supplemental Behavioral Results).

Our results show that both neural and behavioral post-error adjustments are dependent on ACh. The behavioral post-error effects PERI and PES were not detectable under biperiden administration. Furthermore, we show that increased selective attention after errors, particularly evident from the increasing activity in task-relevant brain areas and decreasing activity in

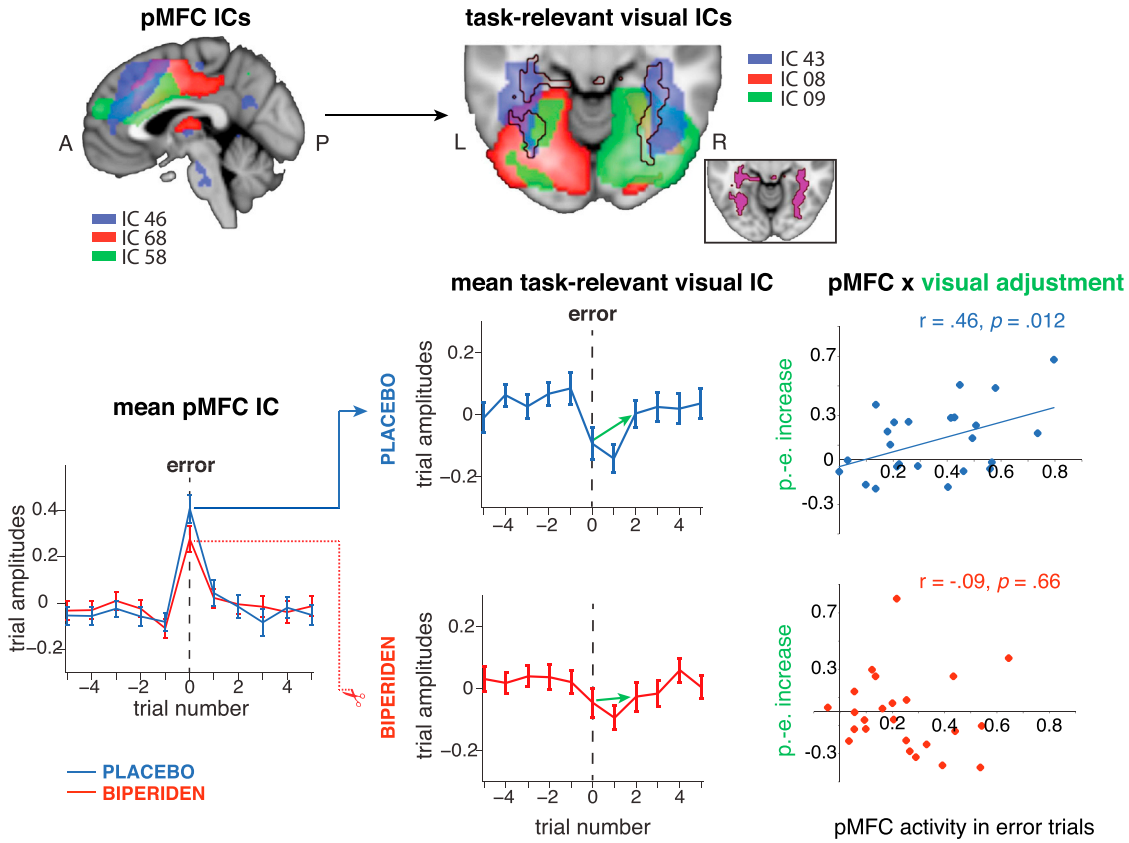

Figure 2. Error-Related Activity in Posterior Medial Frontal Cortex Components Predicts the Strength of the Subsequent Activity Increase in Task-Relevant Visual Areas

Sagittal view of posterior medial frontal cortex independent components (pMFC ICs): blue = IC46, red = IC68, green = IC58, A = anterior, P = posterior. Task-relevant visual ICs: blue = IC43, red = IC08, green = IC09, L = left, R = right, black outline and violet areas in inset = activity from the color localizer task. Below, mean activity of the averaged pMFC (left) and task-relevant visual (middle) ICs are shown for errors (±5 trials) under placebo (blue) and biperiden (red; note that the color coding of time course data is independent of the colors used to visualize the ICs). Note that baseline activity here reflects brain activity during successfully solved trials. The green arrow indicates the post-error activity increase (p.-e. increase) in task-relevant visual areas, which is part of the correlation (right panel). p.-e. increase was steeper with placebo than with biperiden ($p = 0.03$). All y axis units are z scores. Error bars represent SEM. Right: scatterplots for individual pMFC activities on error trials (x axes) and individual post-error increases over two trials (y axes) in task-relevant visual areas, shown separately for placebo (blue) and biperiden (red). The correlations demonstrate that pMFC activity in error trials predicts the subsequent activity increase in task-relevant visual areas under placebo, but not under biperiden. This relationship is indicated by the blue arrow and by the dotted line and scissors, respectively, in the left panel. See also Figure S1.

task-irrelevant visual areas, is mediated by cortical ACh. This top-down modulation seems to be triggered by error-related activations in the pMFC. There are at least two potential explanations for the observed effects: biperiden could have exerted its effects either within the pMFC or in the visual cortex. Although error-related pMFC activity was reduced under biperiden compared to placebo ($t_{23} = 2.141$, $p = 0.043$), there was a clear error-related activity peak in the pMFC under biperiden. Moreover, the behavioral error rate was not altered under biperiden, as would be expected if pMFC activity had been compromised considerably under ACh blockade. Therefore, the observed effects most likely resulted from an ACh receptor blockade in visual cortices. This is in line with animal studies reporting connections from pMFC neurons to the BF [7], which in turn sends cholinergic projections to visual brain areas [8], where mAChRs appear crucial for the modulation of neuronal excitability [8, 9]. The cholinergic cortical projections from the BF are organized in a modular fashion, such that different subregions of the nucleus basalis of Meynert each preferentially target distinct cortical areas [12, 13, 22]. This organization potentially enables task-specific modulations of cortical areas. Recent optogenetic work in mice demonstrated that activation of BF cholinergic neurons enhances neuronal activity in visual cortex and improves visual discrimination on a trial-by-trial basis [23]. Cortical ACh release amplifies glutamate-evoked responses via a muscarinic mechanism [24], and activation of mAChRs has been shown to be crucial for the effect of attention on excitability of visual cortical neurons [8, 9]. However, our results demonstrate that the role of ACh extends beyond sustaining attention to the task at hand. Our data show that phasic top-down modulations of ACh transmission are crucial to rapid, flexible adjustments of cortical activity in response to an error. Specifically, our findings show that this cholinergic influence is tailored to current task demands: it enables enhancement of relevant visual information

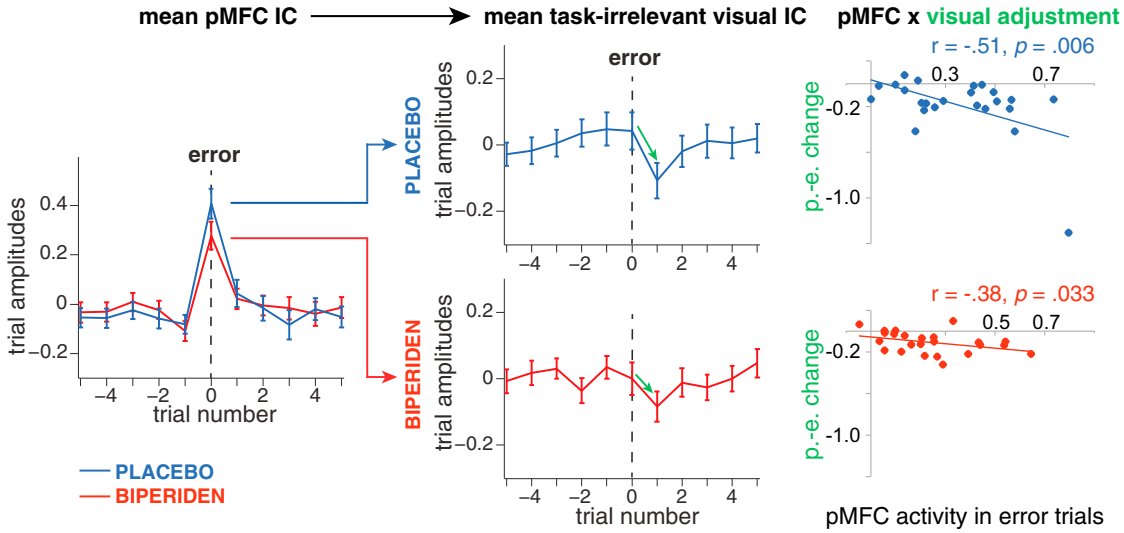

Figure 3. Error-Related Activity in pMFC ICs Predicts the Strength of the Subsequent Activity Decrease in Task-Irrelevant Visual Areas

Mean activity of the averaged pMFC (left) and task-irrelevant visual (middle) ICs are shown for errors (±5 trials) under placebo (blue) and biperiden (red). All y axis units are z scores. Error bars represent SEM. The green arrow indicates the post-error activity decline (p.-e. change) in task-irrelevant visual areas, which is part of the correlation (right). Correlations are depicted between pMFC activity in error trials and the subsequent activity change in task-irrelevant visual areas for the placebo (blue) and biperiden (red) session separately. See also Figure S2.

and suppression of irrelevant, potentially distracting visual input when an increase in selective attention is needed, e.g., after performance errors have occurred.

In contrast to these facilitatory effects of ACh in perceptual areas, layer V pyramidal neurons are in fact transiently hyperpolarized by ACh, a process which may occur via an M1-dependent increase in SK-type calcium conductance [25, 26]. This distinct modulation of layer V neurons may underlie our finding that PES was abolished under biperiden. Presumably, error-related increases in cortical ACh transiently suppress layer V motor neurons, thereby reducing corticospinal excitability, which has been suggested to cause PES [3]. Since on the one hand PES might be the result of an increased motor threshold, i.e., reduced corticospinal excitability, and on the other hand ACh also acts on layer V pyramidal neurons potentially influencing corticospinal excitability, it seems likely that biperiden influences the relationship between activity in the pre-SMA-IFC-STN network and PES.

The results of the present study emphasize the significance in performance monitoring and cognitive control of the BF, a brain structure that has so far been neglected within these topics. Future studies should investigate the function of the BF directly and in more detail in the context of cognitive control. Our finding that muscarinic action of ACh on sensory cortices mediates top-down attentional control may be also important for the understanding of impairments in Alzheimer's disease (AD). AD is associated with a decline in cholinergic neurons in the BF and thus a relative deficit of cortical ACh. Among other cognitive functions, selective attention is impaired in AD patients [27, 28]. Our findings suggest that treatment with ACh esterase inhibitors, increasing the availability of ACh in sensory and association cortices, should improve modulations of selective attention and the ability to adapt behavior after errors. Indeed, improvements in selective attention with this treatment have been observed [29]. Thus, it is conceivable that the positive therapeutic effect on daily life activities may result in part from a partial restoration of the ability of AD patients to flexibly adjust behavior to acute performance problems.

EXPERIMENTAL PROCEDURES

Participants

Thirty male participants took part in the study. Four participants were excluded from further analyses because of poor task performance (three participants had more than 10% missed responses; one participant had an unusually high percentage of errors [49%] in the placebo condition). Another participant was excluded due to structural brain abnormalities, and one participant quit the experiment due to dizziness. Thus, the final sample consisted of 24 participants (one left-handed, mean age 26.04 years, range 21–33 years) with normal or corrected-to-normal vision. Since color blindness was an exclusion criterion for the study, all participants were tested for color vision deficiencies beforehand. All participants gave written informed consent to the procedure, which had been approved by the local ethics committee of the Medical Faculty of the University of Cologne. The study was performed in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Drug Administration

Participants were scanned twice in two different sessions. In one session, participants received biperiden (Akineton; 0.04 mg/kg i.v., range 2.04–3.44 mg), an antagonist at the mAChR (M1; note that we cannot rule out that the observed effects in our study are mediated by mAChRs other than M1 class: although usually considered M1 specific, biperiden also binds to M2 receptors, albeit significantly less). In the other session, participants received a placebo (saline, i.v.). We based our decision to study muscarinic signaling on previous findings that attentional modulation in the macaque visual cortex depends on mAChR rather than nicotinic AChR [9]. Moreover, in healthy human participants, the application of the nicotinic antagonist mecamylamine alone has no effect on various cognitive functions including attention, whereas muscarinic antagonism alone or the combination of both results in impairments [30]. We preferred biperiden over other muscarinic drugs, e.g. atropine or scopolamine, because the latter have strong effects on M3 receptors [31] located on the iris dilator and tear gland [32]. In contrast, biperiden binds much less to M3 than to M1 receptors and is thus less likely to induce ocular side effects potentially impairing

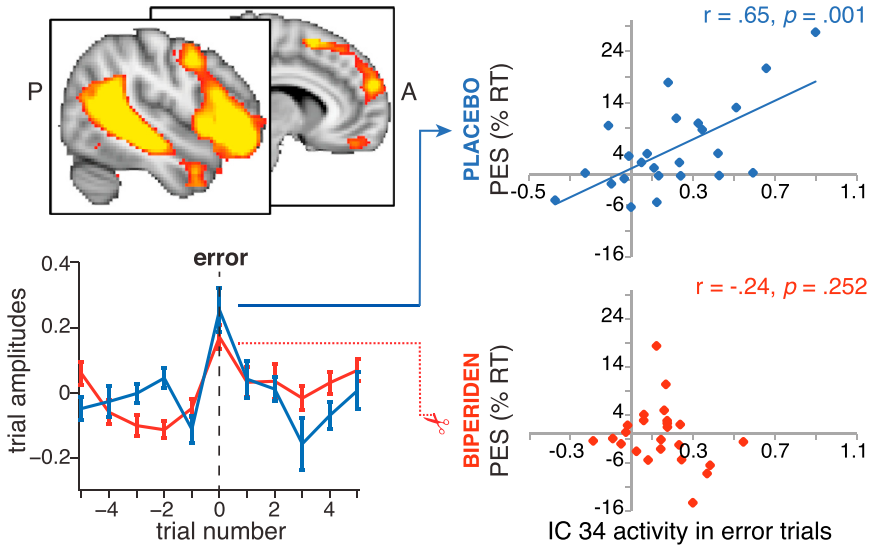

Figure 4. Correlation between Right Hemispheric Network and Post-Error Slowing Is Modulated by Biperiden

Error-related activity in right-hemispheric component (IC34) overlapping with brain regions previously associated with post-error slowing (PES) and motor inhibition predicts individual PES in the placebo condition (blue), but not with biperiden (red). The sagittal slices at x = 8 and x = 50 show the extension of the component overlapping with the pre-SMA and right inferior frontal cortex. Left: mean activity (z scores) of the component activity is shown for errors (±5 trials). Error bars represent SEM. Right: scatterplots depict a significant correlation between error-related component activity and PES expressed as a percentage of the individual's mean reaction time (RT) in the placebo condition, and no correlation in the biperiden condition. P = posterior, A = anterior.

stimulus perception. According to data acquired in rats, biperiden has excellent bioavailability, with a brain-to-blood unbound concentration ratio varying from 30 to 75 depending on the brain region [33]. The elimination half-life is biphasic; the first stage is reached after 1.5 hr and the terminal stage after 24 hr [34]. In view of this pharmacokinetic profile, participants received the injection right before the fMRI scan. The study was conducted with a double-blind randomized crossover design. The two sessions occurred at least 8 days apart to assure complete washout of the drug before the next session.

Study Procedure

Prior to the study, participants were informed about the pharmacological properties of biperiden, its typical clinical use, and its potential adverse effects. After providing informed consent, participants practiced 24 trials of the Simon task and two blocks of the color localizer (see below). Participants were then asked to complete a visual analog scale (VAS) on subjective feelings [35], and heart rate and blood pressure were measured. Immediately prior to scanning, participants received either the drug biperiden or a placebo. The order of drug and placebo session was counterbalanced across participants. During MRI scanning, participants always completed the Simon task first (duration 38.5 min) and the localizer task thereafter (duration 8.5 min) to ensure that the maximum of drug activity occurred while participants performed the Simon task. After participants left the scanner, heart rate and blood pressure were measured again, and participants completed the VAS a second time. To check for psychomotor retardation, they performed the trail-making task (version B) and 60 trials of a simple forced-choice reaction time task, in which they were asked to respond as quickly and accurately as possible to a symbolic square button presented to the left or right of a central fixation cross by pressing a corresponding response button (see [36] for details). Thereafter, participants were released. At the end of the second session, they were debriefed about the purpose of the study and informed about the order of drug and placebo session. One participant was excluded from the analysis of the forced-choice reaction time task due to technical issues. There were no differences in reaction times and error rates between drug and placebo session in this task; however, participants took longer to complete the trail-making task in the biperiden session compared to the placebo session (Table S3).

Task

Participants performed a modified Simon task [37] in which colored arrows were presented on a black background on the left or right side of the screen (6° of visual angle from screen center, stimulus size 1.5° of visual angle). A fixation cross was constantly displayed in the center of the screen. Four different isoluminant colors were used as stimuli (chosen from [38]). Two colors were mapped to a right-hand response button, and the other two to a left-hand response button. Participants were instructed to react to the color of arrows and ignore the presentation side. Thus, response side could be either congruent or incongruent with the side of stimulus presentation (50% incongruent trials). Arrow direction was always congruent with presentation side, i.e., arrows presented on the left side of the screen pointed to the left and vice versa, to avoid introducing another level of (in)congruency. Trials were pseudo-randomized to avoid direct repetitions of the same color and to counterbalance transitions of congruent and incongruent trials. Trial duration was 4 s, with different onset delays at the beginning of each trial (0, 400, 800, 1,200, or 1,600 ms) to improve temporal sampling of the hemodynamic response. This resulted in an oversampling of the actual image acquisition time of 2 s by a factor of 5. Following this onset delay, an arrow was presented for 100 ms, and the fixation cross alone was then shown until the total trial duration reached 4 s. Participants were instructed to react as quickly as possible. The task consisted of 504 stimulus trials and 72 pseudo-randomly interspersed null events during which only the fixation cross was presented (duration 4 s). For every correct response with an RT of less than 500 ms, participants received 4 points. For every correct response with longer RTs, participants received 1 point. They did not gain any points for incorrect responses. At the beginning of every third to fourth null event, participants received performance feedback. The number of gained points was displayed on the screen together with a virtual "high score" for comparison. The virtual "high score" was calculated online as 75% of the maximally possible number of points after that trial. Participants were self-motivated to beat the "high score," which was possible only if reactions were both fast and accurate.

In addition to the Simon task described above, fMRI data were acquired during a color localizer task in order to identify color-processing brain areas (see Supplemental Experimental Procedures).

fMRI Data Analyses

MRI image acquisition parameters and the standard fMRI analysis of the localizer task are described in the Supplemental Experimental Procedures. For the fMRI data of the Simon task, we used a different analysis approach. Because the delay and duration of the hemodynamic response renders it difficult to analyze the temporal dynamics of successive trials with standard fMRI analysis procedures, we employed a deconvolution/single-trial amplitude estimation approach described by Eichele et al. [1]. This method draws upon a group independent component analysis (ICA; see Supplemental Experimental Procedures for details).

Statistical Analyses

To test our hypothesis that error-related pMFC activity predicts subsequent post-error adjustments in task-relevant (color-encoding) visual areas and that this top-down modulation is conveyed by ACh, we used a regression model to explain post-error changes of activity in task-relevant visual cortex

as a function of error-related pMFC activity, session treatment (placebo or biperiden), and the interaction between pMFC activity and treatment. Specifically, we write $p.e.m. = \beta_0 + \beta_1 e + \beta_2 t + \beta_3 (e \times t)$, where *p.e.m.* refers to the post-error modulation of the activity in the visual ICs, *e* is the error signal (error-related activity) in the pMFC, and *t* is an indicator variable for session treatment (placebo = 0; biperiden = 1). Our primary interest is the sign and significance of the interaction term, β_3, which we predict to be negative, indicating that the relationship between pMFC error activity and post-error modulation is reduced in biperiden. Because of our directed hypothesis, p values are reported for one-tailed tests unless noted otherwise.

In our main analysis, *e* is defined as the activity on the error trial, averaged across the three pMFC ICs, and *p.e.m.* is defined as the post-error activity change, averaged across the three task-relevant visual ICs. To match the timing found in our previous study, we calculated post-error activity change as the slope between error trial and the error +2 trial [5]. In follow-up analyses, we fit the same model to the error signals and post-error modulations of individual ICs. That is, we fit nine separate regression models based on all combinations of the three pMFC ICs and three task-relevant visual ICs.

The same regression model and procedure were used to test our hypothesis that pMFC activity predicts subsequent post-error adjustments in task-irrelevant visual areas and that this relationship is affected by biperiden. In the primary analysis, *p.e.m.* is defined as the post-error activity change averaged across the six task-irrelevant visual ICs and is calculated as the slope between error trial and the error +1 trial to match the faster recovery of task-irrelevant ICs, as seen in our previous study [5]. As in the case of task-relevant ICs, we performed follow-up tests using the activity in individual ICs, resulting in 18 separate regression models (3 pMFC ICs × 6 task-irrelevant ICs).

Our next hypothesis stated that post-error slowing (PES) is associated with activity in a right-hemispheric network consisting of pre-SMA, lateral IFC, and STN, and that this relationship might be influenced by biperiden. We identified IC34 as a component that covered the abovementioned brain areas in addition to lateral temporal areas of the right hemisphere. In a separate model, we tested whether error-related activity in IC34 is correlated with PES and whether this relationship is modulated by biperiden. In this analysis, *p.e.m.* is defined as the behavioral PES effect, calculated as described above, and *e* is the error signal (error-related activity) in IC34.

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures, four tables, Supplemental Experimental Procedures, Supplemental Behavioral Results, and Supplemental Analyses and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.04.022.

AUTHOR CONTRIBUTIONS

C.D., G.J., and M.U. designed the experiment. C.D. and O.A.O. collected the data. C.D., E.A.A., and T.E. analyzed the data. All authors wrote the manuscript.

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