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Cholinergic capacity mediates prefrontal engagement during challenges to attention: evidence from imaging genetics

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ABSTRACT

In rodent studies, elevated cholinergic neurotransmission in right prefrontal cortex (PFC) is essential for maintaining attentional performance, especially in challenging conditions. Apparently paralleling the rises in acetylcholine seen in rodent studies, fMRI studies in humans reveal right PFC activation at or near Brodmann's areas 9 (BA 9) increases in response to elevated attentional demand. In the present study, we leveraged human genetic variability in the cholinergic system to test the hypothesis that the cholinergic system contributes to the BA 9 response to attentional demand. Specifically, we scanned (BOLD fMRI) participants with a polymorphism of the choline transporter gene that is thought to limit choline transport capacity (Ile89Val variant of the choline transporter gene *SLC5A7*, rs1013940) and matched controls while they completed a task previously used to demonstrate demand-related increases in right PFC cholinergic transmission in rats and right PFC activation in humans. As hypothesized, we found that although controls showed the typical pattern of robust BA 9 responses to increased attentional demand, Ile89Val participants did not. Further, pattern analysis of activation within this region significantly predicted participant genotype. Additional exploratory pattern classification analyses suggested that Ile89Val participants differentially recruited orbitofrontal cortex and parahippocampal gyrus to maintain attentional performance to the level of controls. These results contribute to a growing body of translational research clarifying the role of cholinergic signaling in human attention and functional neural measures, and begin to outline the risk and resiliency factors associated with potentially suboptimal cholinergic function with implications for disorders characterized by cholinergic dysregulation.

Introduction

Cholinergic projections from basal forebrain to prefrontal cortex (PFC) are necessary for attentional performance (Hasselmo and Sarter, 2011), and abnormalities in the cholinergic system are implicated in the attentional deficits associated with neurodegenerative and psychiatric disorders (Counts and Mufson, 2005; Mesulam, 2004; Mufson et al., 2000; Sarter et al., 2012, 2014a; Xie and Guo, 2004). However, little is known about how non-pathologic variation of endogenous cholinergic signaling influences attention and modulates PFC function in humans. The present study used an imaging genetics approach in healthy adults to address this gap in our knowledge. Specifically, we examined how cortical activation is affected by a common coding variant (minor allele frequency = 8–11%) in the presynaptic choline transporter (*SLC5A7*), Ile89Val, previously shown to reduce choline transporter function (Okuda et al., 2002). We demonstrate that compared to controls, Ile89Val carriers exhibit reduced activation in right PFC in response to attentional demands.

The rodent version of the attention task used in the present study (the Sustained Attention Task with a distractor condition; Gill et al., 2000; McGaughy and Sarter, 1995) has been instrumental in documenting the role of cholinergic modulation of the frontoparietal cortex in attentional performance, especially under challenging conditions (Broussard et al., 2009; St Peters et al., 2011). Performance in the standard, no-distractor (SAT) condition induces increases in acetylcholine (ACh) release in right medial PFC relative to no-task baseline, and ACh release is further increased during the distractor (dSAT) condition, in which signal detection is made more difficult by a flashing background (St Peters et al., 2011). The critical contributions of elevated PFC cholinergic activity to performance appear to be largely right-lateralized (Apparsundaram et al., 2005; Martinez and Sarter, 2004), and the mechanisms by which cholinergic inputs to right PFC stabilize performance under challenging conditions are a topic of intense research interest (reviewed in Hasselmo and Sarter, 2011; Sarter et al., 2014b). Although the cholinergic system has traditionally been described as a diffuse neuromodulator, more recent work demonstrates that cholinergic inputs are capable of modulating highly specific cortical circuitry in

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right PFC to enhance cue detection mechanisms, facilitate the filtering of distractors, and modify sensitivity and biases (Hasselmo, 1995; Hasselmo and McGaughy, 2004; Hasselmo and Sarter, 2011; Sarter and Bruno, 1997; St Peters et al., 2011).

The present study used a parallel version of the dSAT previously developed and validated for human use (Demeter et al., 2008; see Lustig et al., 2013 for discussion of psychometric properties and areas of cross-species correspondence and discrepancy in behavioral effects). (Fig. 1). Functional imaging studies of the dSAT show that challenges to attention increase right-lateralized PFC activation in humans, paralleling the ACh increases seen in rodents. For example, an arterial spin labeling study employing long task blocks revealed that relative to fixation baseline, SAT performance increased perfusion at or near right Brodmann area 9 (BA 9) near middle frontal gyrus, and perfusion in this region was further increased during the distractor condition (Demeter et al., 2011). A recent BOLD event-related design study replicated these findings with peak activation found in right inferior frontal gyrus (IFG) also near BA 9 (Berry et al., in preparation). There is some variation in the exact location of peak distractor-related activation, as might be expected from the different samples, designs, and imaging modalities, but the findings converge to suggest that neural activity in the right PFC, and specifically right BA 9 in the region along middle and inferior frontal gyrus, plays an important role in the brain's response to attentional challenge (see also Kim et al., 2006 for converging evidence from a different sustained attention task).

Inline Supplementary Fig. S1 can be found online at http://dx.doi.org/10.1016/j.neuroimage.2014.12.036.

The parallels between the rodent and human findings, as well as homologies between rat and human PFC (see discussion by Brown and Bowman, 2002) invite the hypothesis that cholinergic neurotransmission contributes to the increased right PFC activation during attentional challenges seen in humans measured using fMRI. Here we test this hypothesis by examining how performance and cortical activation are affected by genetic variation in the high-affinity choline transporter (CHT, Ile89Val variant (*SLC5A7* rs1013940)). CHT transports choline from the extracellular space into presynaptic terminals, a key rate-limiting step in the synthesis of ACh (Simon et al., 1976; Yamamura and Snyder, 1972). Expression of the Ile89Val variant of the CHT gene *SLC5A7 in vitro* reduces the rate of choline transport by approximately 40-60% compared to the major allele (Okuda et al., 2002). The Ile89Val variant is present in approximately 8% of Caucasians (English et al., 2009), raising the possibility that this genetic variant may have significant population effects on cortical function and attentional performance.

Mice with a heterozygous deletion of the CHT gene show normal basal ACh release but a reduced cholinergic response to both task-induced attentional demands and direct basal forebrain stimulation (Paolone et al., 2013; Parikh et al., 2013). Somewhat surprisingly in light of the extensive previous evidence indicating the necessity of basal forebrain cholinergic modulation of prefrontal circuitry for attentional performance (see discussion above), CHT +/- animals had relatively preserved performance (Parikh et al., 2013). In additional analyses, Paolone et al. (2013) found that these animals had higher cortical density of $\alpha4\beta2^*$ nicotinic ACh receptors (nAChRs) and that their performance was more vulnerable to the detrimental effects of the nAChR antagonist mecamylamine, suggesting an increase in nAChRs as a possible compensatory mechanism.

Here we tested the hypothesis that in humans, Ile89Val is accompanied by diminished enhancement of right BA 9 activation during distractor challenge. To preview our results, this hypothesis was supported, and additional exploratory analyses suggested an alternative or compensatory pathway involved in maintaining performance in response to distractor challenge for the Ile89Val group. These findings represent an important step in establishing a link between altered endogenous cholinergic capacity and human functional neural measures associated with cognitive control. The close correspondence between rodent and human tasks and the coordinated genetic approach allows the results of this research to have strong translational potential for better understanding the neurobiological mechanisms underlying attentional control during distractor challenge and the contribution of cholinergic signaling to PFC activation in BOLD fMRI studies.

Methods

Participants

13 Ile89Val heterozygotes and 13 controls homozygous for the dominant allele participated in the fMRI study. Participants were matched

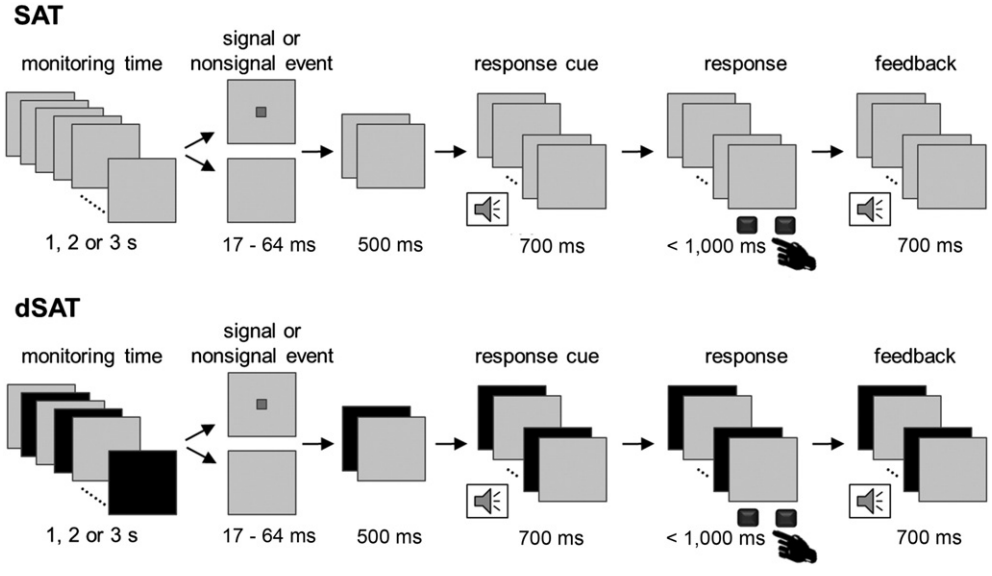

Fig. 1. Sustained Attention Task (SAT). Each trial consisted of a variable duration monitoring interval followed by the presentation of a signal or nonsignal event. The signal was a gray square on a silver background and varied in duration. Signal and nonsignal events were pseudorandomized and occurred with equal frequency. Participants were cued to respond by a low frequency buzzer. Participants responded via buttonpress using one index finger for signal trials and the other index finger for nonsignal trials (left-right key assignment counterbalanced across participants). Correct responses were followed by a high frequency feedback tone; incorrect responses and omissions did not result in feedback. The distractor condition, dSAT, increased the attentional control demands of the task by adding a global, continuous visual distractor. During dSAT trials, the screen flashed from gray to black at 10 Hz. SAT, dSAT, and fixation (not pictured) trials were pseudorandomly intermixed.