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**Keywords**
memory, humans, nicotine, receptor, allosteric, abstinence, potent, improves, episodic, model, cognitive, dysfunction, agonist, gsk1034702, m1

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ARTICLE

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# The potent $M_1$ receptor allosteric agonist GSK1034702 improves episodic memory in humans in the nicotine abstinence model of cognitive dysfunction

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**Abstract**

Episodic memory deficits are a core feature of neurodegenerative disorders. Muscarinic $M_1$ receptors play a critical role in modulating learning and memory and are highly expressed in the hippocampus. We examined the effect of GSK1034702, a potent $M_1$ receptor allosteric agonist, on cognitive function, and in particular episodic memory, in healthy smokers using the nicotine abstinence model of cognitive dysfunction. The study utilized a randomized, double-blind, placebo-controlled, cross-over design in which 20 male nicotine abstained smokers were tested following single doses of placebo, 4 and 8 mg GSK1034702. Compared to the baseline (nicotine on-state), nicotine abstinence showed statistical significance in reducing immediate ($p=0.019$) and delayed ($p=0.02$) recall. GSK1034702 (8 mg) significantly attenuated (i.e. improved) immediate recall ($p=0.014$) but not delayed recall. None of the other cognitive domains was modulated by either nicotine abstinence or GSK1034702. These findings suggest that stimulating $M_1$ receptor mediated neurotransmission in humans with GSK1034702 improves memory encoding potentially by modulating hippocampal function. Hence, selective $M_1$ receptor allosteric agonists may have therapeutic benefits in disorders of impaired learning including Alzheimer's disease.

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**Key words**: Alzheimer's disease, cognition, episodic memory, hippocampus, $M_1$ agonist, $M_1$ receptor.

## Introduction

Neurodegenerative disorders, including Alzheimer's disease (AD) and schizophrenia, are associated with impairment across a range of cognitive domains, although both disorders are characterized clinically by substantial impairment in episodic memory. This memory impairment underlies significant difficulties in activities of daily living including self-care and, consequently, there is an urgent need for therapies to improve memory in both AD and schizophrenia. In both disorders, the impairment in episodic memory reflects disruption to cholinergically modulated neurocognitive networks for memory that centre on the hippocampus (Hasselmo & Sarter, 2011); albeit through different disease specific pathological processes. Within these memory networks there is

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increasing evidence for impairment in muscarinic $M_1$ receptors and their associated signalling pathways. In AD, reductions in $M_1$ or $M_1/M_4$ receptors have been demonstrated in post-mortem autoradiography (Rodríguez-Puertas *et al.* 1997) and single photon emission computed tomography imaging (Pakrasi *et al.* 2007) studies. Although not all studies have been consistent and some report a compensatory increase in $M_1$ receptors (Overk *et al.* 2010), $M_1$ receptor signal transduction-related markers or function are reportedly decreased or impaired in AD (Ferrari-DiLeo *et al.* 1995; Potter *et al.* 2011; Tsang *et al.* 2006, 2007). Similarly, several post-mortem [$^3$H]pirenzepine-binding studies have demonstrated reductions in $M_1/M_4$ receptors in specific brain regions, including the hippocampus, in patients with schizophrenia (Crook *et al.* 2000, 2001; Dean *et al.* 2002; Deng & Huang, 2005). Hence, pharmacological modulation of muscarinic $M_1$ receptors may provide a therapeutic opportunity for amelioration of episodic memory impairment in both disorders.

Within neurocognitive networks for memory, post-synaptic $M_1$ receptors are predominantly expressed throughout the cortex and hippocampus (Levey *et al.* 1991). Activation of $M_1$ receptors increases ERK1/2 phosphorylation (Thiels & Klann, 2001), amplifies *N*-methyl-D-aspartate receptor mediated currents (Marino *et al.* 1998) and hippocampal long-term potentiation (LTP; Buchanan *et al.* 2010; Fernández de Sevilla *et al.* 2008), all of which are critical biochemical and cellular mediators of learning and memory (Malenka & Bear, 2004). Pre-clinical *in vivo* studies using selective $M_1$ receptor agonists have shown evidence for direct links between $M_1$ receptor activation and improved learning and memory (Brandeis *et al.* 1995; Fisher *et al.* 2002; Ruske & White, 1999; Vincent & Sepinwall, 1992; Watt *et al.* 2011).

Despite their therapeutic potential, the magnitude of benefits to memory or cognitive function in humans produced by muscarinic agonists has been modest and often limited by peripheral $M_2$ and/or $M_3$ muscarinic receptor-related side-effects, such as severe gastrointestinal disturbance. The recent discovery that $M_1$ receptors possess an allosteric (or ectopic) site that is non-conserved across muscarinic acetylcholine receptor (mAChR) subtypes has provided the opportunity to develop $M_1$ receptor agonists with true receptor selectivity (Spalding *et al.* 2002) and therefore of great potential as memory enhancing agents in humans. In animals, the selective $M_1$ receptor allosteric agonist AC-260584 has been shown to improve learning and memory (Bradley *et al.* 2010; Vanover *et al.* 2008). However, it is unknown if similar pro-cognitive effects can be observed in humans due to the paucity of ligands available for probing muscarinic receptor subtypes, including the $M_1$ receptor.

GSK1034702 is a selective $M_1$ receptor allosteric agonist, being developed for the treatment of cognitive dysfunction in neurodegenerative disorders. It belongs to the series of novel *N*-substituted benzimidazolones recently described (Budzik *et al.* 2010; Huiban *et al.* 2011). *In vivo*, an isomer of GSK1034702 (i.e. compound 5; Budzik *et al.* 2010) enhanced cell firing in the CA1 region of the rat hippocampus and reversed the scopolamine-induced amnesia in the hippocampus dependent passive avoidance task of learning in a dose-related manner (Budzik *et al.* 2010). Using similar methods, GSK1034702 caused a concentration dependent increase of hippocampal CA1 neuronal firing rate, which persisted following repeated treatment (7 d) and reversed the scopolamine-induced amnesia in the passive avoidance task following both acute and sub-chronic (7 d at 6 mg/kg.d) treatment with challenge doses of 1, 3 or 10 mg/kg (see Supplementary Material and Supplementary Figs. S1 and S2).

The aim of this study was to investigate, for the first time, the acute effects of GSK1034702 on episodic memory as well as more general aspects of cognitive function in humans. The pro-cognitive effects of GSK1034702 were tested in healthy adult smokers who had been abstinent from nicotine (i.e. nicotine abstinence model of cognitive impairment). We used the nicotine abstinence model of cognitive impairment because: (*a*) synergistic interactions between muscarinic and nicotinic receptors at both a molecular and behavioural level have been reported (Ellis *et al.* 2006; Greenwood *et al.* 2009); (*b*) nicotine abstinence (for at least 12 h) in chronic smokers can reduce baseline cognitive function, including memory, as previously demonstrated (Myers *et al.* 2008).The primary hypothesis was that the $M_1$ allosteric agonist GSK1034702 would attenuate the nicotine abstinence-induced impairments in episodic memory. The effects of GSK1034702 on other aspects of cognitive function shown to be relevant to AD and schizophrenia were then investigated in exploratory analyses.

## Method

### *Subjects*

Twenty otherwise healthy male nicotine abstinent smokers [mean age = 32.7 yr; age range 19–54 and mean body mass index (BMI) = 24.9; BMI range 20.2–28.1] were recruited for this study. Subjects were included in the study if they smoked on average $\geqslant$10 cigarettes per day for at least 1 yr. All participants

had no history of psychiatric disorders (as assessed by the Mini-International Neuropsychiatric Interview), neurological disorders (including learning disorders), cardiovascular, liver, respiratory or gastrointestinal disorders and substance abuse, based on a physical examination and a clinical and psychiatric interview by a physician. Subjects were also free of any drugs of abuse, prescription medication and non-prescription medications including vitamins, herbal and dietary supplements. All participants gave written informed consent for participation in the study, which was approved by the Welwyn Clinical Pharmacology Ethics Committee, University of Hertfordshire, UK.

### *Design*

The study utilized a randomized, double-blind, placebo-controlled, cross-over design in which each participant was tested following three single dose treatment conditions (placebo, 4 mg GSK1034702 and 8 mg GSK1034702).

### *Procedure*

All subjects underwent screening 30 d before the study began at the GSK Clinical Unit Cambridge (CUC). In this screening session, the inclusion and exclusion criteria described above were applied and the subjects practised the cognitive assessments (although no data from these assessments were used in the study analysis). Subjects who satisfied screening were then randomized to placebo, 4 mg GSK1034702 or 8 mg GSK1034702. The three treatment sessions were separated by a minimum 1 wk washout period. Each study treatment session was given within a nicotine abstinence model. In this model, subjects were assessed across two study days (day 1 and day 2). On day 1, subjects were administered placebo at approximately 09:00 hours and, approximately 5 h later, baseline cognitive assessments and mood and craving questionnaire assessments were completed, while being allowed to smoke cigarettes *ad libitum* until midnight (i.e. nicotine 'on-state'). Subjects were housed at the CUC and were escorted by a staff member to a smoking area. On day 2, pre-drug cognitive testing and questionnaire assessments of mood and craving were performed in the nicotine 'abstinent state' at approximately 08:00 hours. On completion, subjects were dosed with either placebo or GSK1034702 and post-dose cognitive testing and mood/craving measurements were conducted between approximately 5 and 6 h post treatment to coincide with the time window corresponding to the highest exposure of GSK1034702 in plasma (2–6 h; $t_{max}$; 2–3 h). Cognitive and questionnaire assessments were performed approximately 14 h following nicotine abstinence. In addition to cognitive testing, electrophysiological recording was conducted between 3 and 4.5 h post treatment and these findings are reported elsewhere.

### *Cognitive assessments*

Episodic memory was assessed using the International Shopping List Test (ISLT). The ISLT is a 12-word, three trial verbal list learning test. In this task, subjects were read a list of 12 words. Each word was a concrete noun and described an item of food commonly eaten. The experimenter asked the subjects: 'I am going to read to you a list of items I want you to get from the supermarket/store/market/shop etc.'. After the 12 words were read, the subjects were asked to recall as many of the words as they could. When they could recall no more words, the same list was read a second time with the words in the same order after the same instruction. This process was repeated three times. At the completion of the computerized battery, subjects were asked to recall as many of the items as they could from the shopping list following a delay of 20 min. This provides a measure of delayed recall. The primary performance measure from this test is the total number of words recalled across the three learning trials (total recall) and the total words recalled on the delayed recall trial.

In addition to memory, executive function was assessed using the Groton maze learning task, psychomotor function was assessed using the CogState detection task, visual attention was assessed using the CogState identification task and working memory was assessed using the one back working memory test. These tests have been described in detail elsewhere (Fredrickson *et al.* 2008; Thompson *et al.* 2011).

### *Mood and craving assessments*

Changes in mood were examined using the Visual Analogue Mood Scales (VAMS) (Bond & Lader, 1974). The VAMS consist of 16 bipolar scales, anchored at each end of a 100 mm line. Subjects placed a mark on each line that best described their current mood state. The scales were reduced to three subscales to assess alertness, contentedness and calmness. Nicotine withdrawal was assessed using the Minnesota Nicotine Withdrawal Scale – Revised (MNWS-R; Hughes & Hatsukami, 2005). The MNWS-R is a shorter version of the Minnesota Nicotine Withdrawal Scale, consisting of a self-report scale divided into seven items, each representing a specific state of the nicotine withdrawal

**Table 1.** Treatment-related adverse effects

| | Placebo, $N=21$ | 4 mg, $N=21$ | 8 mg, $N=22$ |
|---|---|---|---|
| Most frequent AEs | *n* (%) | *n* (%) | *n* (%) |
| Any AE | 7 (33) | 8 (38) | 14 (64) |
| Any AE related to investigational product | 2 (10) | 4 (19) | 12 (55) |
| Gastrointestinal | | | |
| Any event | 0 | 2 (10) | 4 (18) |
| Salivary hypersecretion | 0 | 1 (5) | 3 (14) |
| Abdominal pain | 0 | 1 (5) | 0 |
| Nausea | 0 | 0 | 1 (5) |
| Flatulence | 0 | 0 | 1 (5) |
| Non-gastrointestinal | | | |
| Any event | 0 | 2 (10) | 4 (18) |
| Headache | 3 (14) | 2 (10) | 3 (14) |
| Fatigue | 1 (5) | 2 (10) | 1 (5) |
| Hyperhidrosis | 0 | 0 | 3 (14) |
| Feeling of body temperature changes | 0 | 0 | 2 (9) |
| Lacrimation | 0 | 0 | 1 (5) |
| Flushing | 0 | 0 | 1 (5) |

AE, Adverse event.

syndrome (e.g. restlessness, desire to smoke, irritability).

### *Safety and tolerability*

The following safety and tolerability endpoints were monitored: adverse events (AEs); 12-lead electrocardiogram (ECG); Holter monitoring; vital signs (blood pressure, heart rate, respiration rate and body temperature); clinical laboratory evaluations (haematology, clinical chemistry, urinalysis and spirometry).

### *Pharmacokinetics*

Blood samples were collected at regular intervals for pharmacokinetic analysis.

### *Statistics*

The effects of abstinence on each cognitive task were examined using a mixed effect analysis of covariance (ANCOVA) model with subject as a random effect and period (i.e. day) as a fixed effect. Treatment comparisons for each cognitive task were examined using a mixed effect ANCOVA model, with subject as a random effect, period and treatment as fixed effects and baseline as a covariate. For all comparisons, the level of probability required for significance was set at 0.05. The significance level at which each of the comparisons was tested was at the 5% level. No adjustments for multiplication were performed because: (*a*) even though multiple outcome measures were used, performance on cognitive tests is highly correlated and therefore corrections that assume independence are too conservative; (*b*) the current study is one of the first of its kind and therefore we consider the outcomes to be hypothesis generating; (*c*) measures of effect size were computed for all comparisons and effects that were trivial in magnitude ($d<0.2$) were not interpreted irrespective of statistical significance. Confidence intervals (CI) are shown, where '0' represents no effect, as well as *p* values and Cohen's '*d*'.

## Results

### *Safety and tolerability*

GSK1034702 was well tolerated. The majority of AEs thought to be related to GSK1034702 were rated as mild severity, indicating no effect on routine activities of daily living. No subjects were withdrawn due to drug-related AEs. Although numbers in this study were small, muscarinic side-effects appeared to be dose-related with only gastrointestinal symptoms (salivary hypersecretion, abdominal pain and diarrhoea) reported at the 4 mg dose and non-gastrointestinal symptoms (headache, dizziness, lacrimation, flushing, hyperhidrosis and body temperature changes) appearing at the 8 mg dose. Gastrointestinal AEs were reported by 10% of subjects at the 4 mg dose and by 18% at the 8 mg dose (see Table 1).

No clinically significant abnormal ECG findings or Holter interpretations were reported during the study. No corrected QT (QTc) values >480 ms were reported although two subjects (one each in the 4 and 8 mg treatment groups) had QTcB changes from baseline, which were >30 to ⩽60 ms. Two subjects had vital signs of potential clinical importance without any symptoms. One subject had low diastolic blood pressure (44 mmHg) recorded during treatment with placebo and another had low diastolic blood pressure (44 mmHg) during treatment with 8 mg GSK1034702. Both subjects had low baseline diastolic blood pressure so these values were considered normal. No abnormal respiration or temperature changes were reported. The clinical laboratory findings showed no clinically significant abnormalities.

### *Cognitive function*

#### *The effect of nicotine abstinence on cognitive function*

Nicotine abstinence significantly reduced performance on the immediate ($d=0.63$; 95% CI −1.13 to −0.0.11; $p=0.02$) and delayed ($d=0.62$; 95% CI −1.14 to −0.0.11; $p=0.02$) recall trials of the ISLT compared to the nicotine on-state. No statistically significant effects of nicotine were observed for the detection ($d=0.16$), identification ($d=0.07$), one back working memory ($d=-0.07$) and Groton maze learning test ($d=0.16$).

#### *Effect of GSK1034702 on cognitive function in the nicotine abstinence model*

It was found that 8 mg GSK1034702 significantly improved immediate recall in the ISLT (i.e. number of correct responses; 95% CI 0.16–1.38; $p=0.014$) compared to placebo (Figs. 1 and 2), but had no effect on delayed recall. GSK1034702 (4 and 8 mg) had no significant effects on any of the other cognitive tasks (Fig. 2 and Table 2).

### *Mood and craving*

No significant differences were noted between the three treatments for the VAMS alertness, calmness and contentedness factors or withdrawal symptoms measured by the MNWS-R. However, subjects reported a greater desire or craving to smoke (95% CI 0.54–1.71; $p=0.0007$) and overall had greater withdrawal symptoms (i.e. MNWS-R total score; 95% CI 0.39–3.32; $p=0.0154$) under all treatment conditions in the nicotine 'abstinent state' compared to the nicotine 'on-state'.

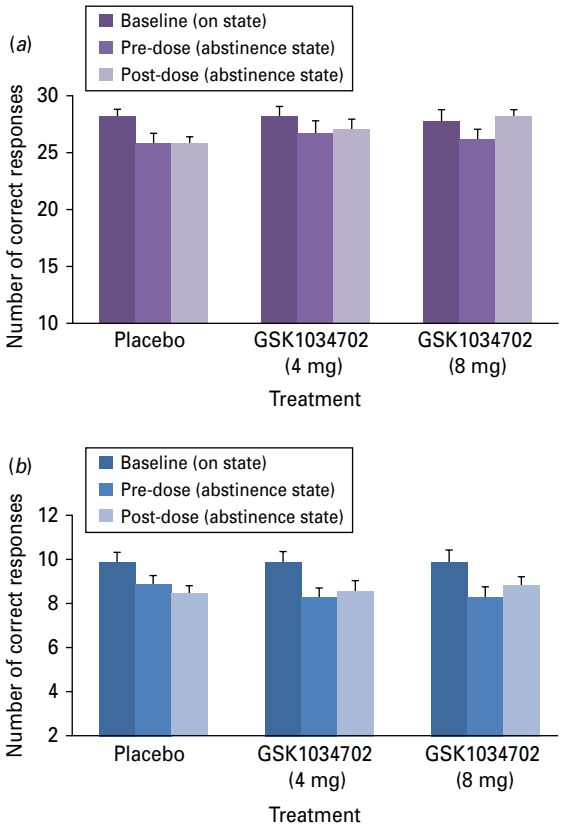


**Fig. 1.** International shopping list task. (*a*) Immediate recall; (*b*) delayed recall. *$p<0.05$ for difference between nicotine 'on-state' and nicotine 'abstinent state'. **$p<0.05$ for treatment difference in the nicotine 'abstinent state'. Data expressed as means ± S.E.M.

### *Pharmacokinetics*

Following oral administration of 4 and 8 mg GSK1034702, the mean $t_{max}$ was observed 2.3–3.5 h after dosing, with individual $t_{max}$ values ranging from 1 to 6 h after dosing.

## Discussion

The paucity of selective agonists and antagonists for cholinergic muscarinic receptor subtypes has been a major obstacle in elucidating the precise role of these receptors in modulating cognitive processes. In this study, we examined the effects of GSK1034702, a potent $M_1$ receptor allosteric agonist, on cognitive function in abstinent smokers. The key finding of the study was that the $M_1$ allosteric agonist GSK1034702 attenuated the abstinence-induced impairments in episodic memory (immediate recall) but had no effect on 'baseline' cognitive function.

Data that support the argument that the effect of GSK1034702 on episodic memory reflects selective

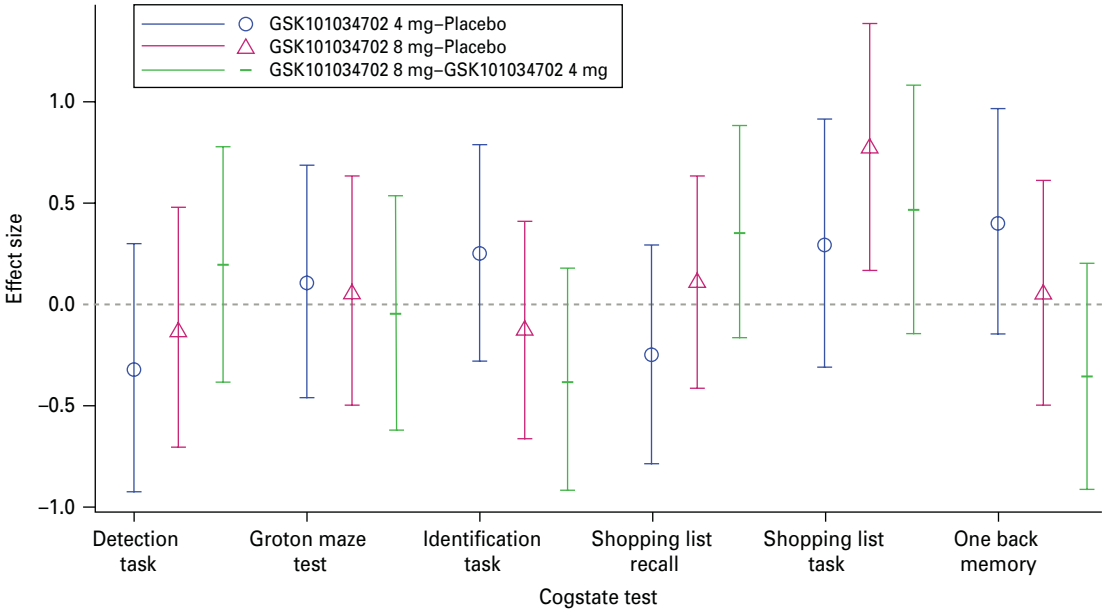


**Fig. 2.** Effect sizes for treatment-related differences (change from baseline) in task performance. Zero value indicates no change. Note: Shopping list recall (delayed recall), shopping list task (immediate recall).

activation of the muscarinic $M_1$ receptor stem from the pharmacology of GSK1034702. When tested at human recombinant $M_1$ receptors in the fluorometric imaging plate reader (FLIPR) assay, which measures $Ca^{2+}$ mobilization, an isomer of GSK1034702 (i.e. compound 5) showed potent $M_1$ agonist activity ($pEC_{50} = 8.1$) and good selectivity against all four receptor human muscarinic receptor subtypes (>100 fold selective for $hM_1$ over $hM_2$-$M_5$; Budzik *et al.* 2010). Similarly, GSK1034702 was a potent agonist at $hM_1$ receptors ($pEC_{50} = 8.1$) and displayed at least 100-fold selectivity over $hM_{2–5}$ receptors. GSK1034702 also demonstrated partial agonist activity (intrinsic activity ~0.6) at rat, marmoset and human native tissue $M_1$ receptors in a guanosine 5′-*O*-[$\gamma$-thio]triphosphate ([$^{35}$S]GTP$\gamma$S) binding assay as previously described (Salah-Uddin *et al.* 2008). The agonist activity of GSK1034702 was maintained in post-mortem human cortex from controls and AD patients, as measured using [$^{35}$S]GTP$\gamma$S binding ($pEC_{50}$ values of 7.0 in control tissue and 7.5 and 7.0 in mild and severe disease tissue samples, respectively).

Site-directed mutagenesis of key amino acid residues on $hM_1$ receptors and the FLIPR assay were also used to establish whether GSK1034702 activates $hM_1$ receptors through a site distinct to that of the orthosteric site agonist acetylcholine (ACh). The potency of ACh to activate the $hM_1$ receptor mutated at the orthosteric site ($Tyr^{381}Ala$; Y381A) was 1000-fold lower than its potency to activate the wild-type receptor. In contrast, the potency of GSK1034702 was not significantly affected by this point mutation ($pEC_{50}$ values of 7.5 and 7.7, respectively). Another mutation in the orthosteric binding site, $Asn^{382}Ala$ (N382A), also reduced the potency of Ach (>30-fold), as compared to wild-type but did not significantly affect that of GSK1034702 ($pEC_{50}$ values of 7.2 and 7.7, respectively). In contrast, a mutation in transmembrane domain 2 ($Phe^{77}Ile$; F77I) significantly reduced the potency of GSK1034702 ($pEC_{50}$ values of 6.8 *vs.* 7.7) without altering the potency of Ach (see Supplementary Material and Supplementary Table S1). These data suggest that GSK1034702 functions through a site on the $hM_1$ receptor that is distinct to that of ACh.

Overnight nicotine abstinence was associated with a selective impairment in episodic memory (immediate and delayed recall) as measured with the CogState ISLT. No impairments were observed on other cognitive tasks probing attention, working memory and executive function. The failure to impair other cognitive domains may be related to the study sample (i.e. smokers who smoked ⩾10 cigarettes per day for at least 12 months). Previous studies have reported abstinence-induced impairments in other domains, including attention, working memory and executive function, in heavier smokers (i.e. smokers who smoked ⩾15 cigarettes per day for 12 months; Myers *et al.* 2008). While the present study comprised lighter smokers, they reported greater desire or craving to smoke and overall reported more withdrawal

**Table 2.** Treatment-related differences in cognitive performance in the nicotine 'abstinent state'

| Comparison | Effect size (Cohen's *d*) | LS mean test | LS mean reference | 95% CI | *p* value | S.D. |
|---|---|---|---|---|---|---|
| Detection task: speed of performance | | | | | | |
| 4 mg – PBO | −0.32 | 2.46 | 2.46 | −0.93 to 0.30 | 0.31 | 0.03 |
| 8 mg – PBO | −0.12 | 2.46 | 2.46 | −0.71 to 0.47 | 0.68 | |
| 8 mg – 4 mg | 0.19 | 2.46 | 2.45 | −0.38 to 0.77 | 0.50 | |
| Groton maze learning test: total error rate | | | | | | |
| 4 mg – PBO | 0.11 | 37.5 | 36.34 | −0.46 to 0.68 | 0.69 | 10.45 |
| 8 mg – PBO | 0.06 | 37.0 | 36.34 | −0.50 to 0.63 | 0.82 | |
| 8 mg – 4 mg | −0.05 | 37.0 | 37.49 | −0.61 to 0.52 | 0.87 | |
| Identification task: speed of performance | | | | | | |
| 4 mg – PBO | 0.25 | 2.65 | 2.64 | −0.28 to 0.78 | 0.34 | 0.04 |
| 8 mg – PBO | −0.12 | 2.64 | 2.64 | −0.66 to 0.41 | 0.64 | |
| 8 mg – 4 mg | −0.37 | 2.64 | 2.65 | −0.91 to 0.17 | 0.17 | |
| International shopping list recall (delayed recall): number of correct responses | | | | | | |
| 4 mg – PBO | −0.24 | 8.29 | 8.73 | −0.78 to 0.29 | 0.36 | 1.80 |
| 8 mg – PBO | 0.11 | 8.93 | 8.73 | −0.41 to 0.63 | 0.67 | |
| 8 mg – 4 mg | 0.35 | 8.93 | 8.29 | −0.16 to 0.87 | 0.17 | |
| International Shopping List Task (immediate recall): number of correct responses | | | | | | |
| 4 mg – PBO | 0.30 | 26.75 | 25.85 | −0.32 to 0.92 | 0.33 | 2.97 |
| 8 mg – PBO | 0.77 | 28.14 | 25.85 | 0.16–1.38 | 0.01 | |
| 8 mg – 4 mg | 0.47 | 28.14 | 26.75 | −0.14 to 1.08 | 0.13 | |
| One back memory task: accuracy of performance | | | | | | |
| 4 mg – PBO | 0.40 | 1.46 | 1.42 | −0.15 to 0.97 | 0.15 | 0.11 |
| 8 mg – PBO | 0.05 | 1.42 | 1.42 | −0.50 to 0.61 | 0.85 | |
| 8 mg – 4 mg | −0.35 | 1.42 | 1.46 | −0.91 to 0.20 | 0.21 | |

PBO, Placebo.

Data show treatment related change from pre-dose baseline. Summary statistics are reported [effect size, least square (LS) mean, overall between-subject S.D., 95% confidence intervals (CI) and *p* value].

symptoms following nicotine abstinence suggesting that the overnight nicotine abstinence induced the desired physiological effect. Alternatively, it is possible that the discrepancy between the current study and the study by Myers *et al.* (2008) could be explained by other factors, such as sample size and the type of cognitive tasks. The latter study had a slightly larger sample size ($n=25$) and included different cognitive tasks to probe attention (continuous performance task), working memory (2-back) and executive function (arithmetic test). It is possible that these tasks were more demanding and hence more susceptible to abstinence induced impairment.

Animal studies have provided insights into the precise role of $M_1$ receptors on cognitive function. Studies using muscarinic $M_1$ receptor knockout mice have suggested that $M_1$ receptors are not essential for memory formation or initial stability of memory in the hippocampus. For example, Miyakawa *et al.* (2001) did not observe global impairments in hippocampus-dependent cognitive tasks probing spatial reference memory (Morris water maze) or fear learning (contextual fear conditioning). Slight impairments were noted in auditory-cued fear conditioning and working memory measured using the eight-arm radial maze, but these were thought to be caused by the hyperactivity phenotype observed in the $M_1$ receptor knockout mice (Miyakawa *et al.* 2001). A subsequent study also reported no global impairments in hippocampus-dependent cognitive tasks but, rather, selective deficits on tasks requiring interaction between the hippocampus and cortex (Anagnostaras *et al.* 2003). Specifically, $M_1$ receptor mutant mice showed impairments in non-matching to sample tasks probing working memory and consolidation (win-shift radial arm and social discrimination learning), while having no effects or improving performance on matching to sample tasks probing learning (i.e. contextual fear conditioning and Morris water maze). These findings have been interpreted in the context of network models describing cholinergic function in memory processing (Buzsaki, 1989; Hasselmo, 1999). According to these models, cholinergic signals function as a switch between inflow (i.e. encoding) and outflow (recall)

modes of the hippocampus with $M_1$ receptor stimulation hypothesized to bias processing away from the hippocampus and to the cortex (Anagnostaras *et al.* 2003). The authors argued that this model may explain the deficit in cortex dependent working memory and consolidation of remote memories because the loss of $M_1$ receptors in the mutant mice would bias processing away from the cortex (Anagnostaras *et al.* 2003). They also reasoned that the model could explain the enhancement in acquisition of contextual memories in the $M_1$ mutant mice because processing bias would be shifted to the hippocampus processing contextual memory without interference from previously established cortical traces (Hasselmo, 1999).

The findings observed in $M_1$ mutant mice are however inconsistent with pre-clinical studies that have examined hippocampal activity and nature of cognitive improvement following *in vivo* administration of selective $M_1$ receptor agonists. For example, the $M_1$ receptor allosteric agonist and isomer of GSK1034702 (i.e. compound 5 in the *N*-substituted benzimidazolone series; Budzik *et al.* 2010) was shown to enhance cell firing in the CA1 region of the hippocampus. Similarly, we have shown that GSK1034702 caused a significant increase of hippocampal CA1 neuronal firing rate, which persisted following repeated treatment (7 d). In support of the latter studies showing modulation of hippocampal activity, the selective $M_1$ receptor allosteric agonist, AC-260584, has been shown to improve spatial memory in a hippocampus-dependent Morris water maze task (Vanover *et al.* 2008) and visual recognition memory in the novel object recognition memory task of working memory (Bradley *et al.* 2010) that is reliant on the integrity of both the cortex (perirhinal cortex) and hippocampus. Similarly, compound 5 described above (Budzik *et al.* 2010) and GSK1034702 reversed the scopolamine-induced amnesia in the hippocampus dependent passive avoidance task of learning. The findings of the current study showing improvements in hippocampal dependent encoding of episodic memory with GSK1034702 in humans are consistent with the latter studies in animals. Overall, both pre-clinical and clinical studies using selective $M_1$ receptor agonists suggest that $M_1$ receptors are important for hippocampus dependent memory formation. These findings do not support the model proposed by Anagnostaras *et al.* (2003) based on their work with $M_1$ receptor mutant mice, suggesting $M_1$ receptor stimulation would bias processing away from the hippocampus and to the cortex (Anagnostaras *et al.* 2003).

Cholinergic innervation of the hippocampus and muscarinic $M_1$ receptors is critical for the encoding of episodic memories (Hasselmo & Sarter, 2011). In rodents, local infusion of scopolamine into the hippocampus has been shown to impair encoding of spatial information (Blokland *et al.* 1992; Rogers & Kesner, 2003). In humans, muscarinic receptor antagonists such as scopolamine have been shown to impair encoding of stimuli for subsequent free recall and cued recall (Atri *et al.* 2004; Ellis *et al.* 2006; Ghoneim & Mewaldt, 1975, 1977; Petersen, 1977). Herein, we report that selectively activating $M_1$ receptors with GSK1034702 improved acquisition or encoding of new memories as shown by an improvement in learning in the immediate recall task. This is consistent with pre-clinical studies in rodents showing improvements in learning and memory with the selective $M_1$ receptor allosteric agonist, AC-260584 (Bradley *et al.* 2010; Vanover *et al.* 2008). Septohippocampal cholinergic pathways have been shown to excite hippocampal pyramidal neurons (for a review, see Nicoll, 1985) and this pathway plays a critical role in memory functions, including encoding of episodic memories (for reviews, see Blokland, 1996; Hasselmo & Sarter, 2011). Muscarinic receptor activation has also been shown to enhance hippocampal pyramidal cell spiking response to afferent input (Cole & Nicoll, 1984; Madison & Nicoll, 1984) and physiologically released ACh from cholinergic neurons has been shown to enhance LTP of excitatory synaptic transmission in the hippocampus through postsynaptic $M_1$ mAChR activation (Buchanan *et al.* 2010; Shinoe *et al.* 2005). Similar enhancement of hippocampal cell firing rate and LTP has also been reported with the selective allosteric $M_1$ receptor agonists (Buchanan *et al.* 2010; Budzik *et al.* 2010). Furthermore, ACh release in the hippocampus has been shown to correlate with improved spatial learning performance in rats (Fadda *et al.* 2000). Together, these findings suggest that the improvements in encoding and learning following $M_1$ receptor stimulation may be a consequence of selective enhancement of the responsiveness of the hippocampus to afferent input for encoding and subsequent consolidation via plasticity mechanisms including induction of LTP.

The effects of GSK1034702 were specific to episodic memory encoding, which was impaired following nicotine abstinence. In contrast, GSK1034702 had no effects on 'baseline' or 'unimpaired' cognitive function (i.e. cognitive processes that were not impaired by nicotine abstinence, including attention, working memory and executive function). It is possible that GSK1034702 may have attenuated these latter cognitive processes if they were also 'impaired'. Indeed, previous studies have shown robust modulation of

attention and working memory following muscarinic receptor antagonism with scopolamine (Ellis & Nathan, 2006; Hasselmo & Sarter, 2011), suggesting that $M_1$ receptor agonists could have positive effects on attention and working memory. Further studies are required to determine if GSK1034702 could attenuate impaired attention, working memory and executive function in other models of cognitive dysfunction (i.e. scopolamine or sleep deprivation) or patients with AD or schizophrenia.

Episodic memory deficits are the hallmark of AD and these deficits are more related to learning (encoding and storage) of information rather than retrieval. In this study we provide evidence showing improvements in episodic memory in an experimental model of cognitive dysfunction. These findings are encouraging and suggest that selective $M_1$ agonists may have efficacy in the treatment of disorders associated with impaired learning and this warrants further investigation.

## Note

For supplementary material accompanying this paper, visit http://dx.doi.org/10.1017/S1461145712000752

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## Statement of Interest

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