Immediate and Sustained Improvements in Working Memory After Selective Stimulation of α7 Nicotinic Acetylcholine Receptors

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Background: Nicotine improves cognition in humans and animal models of neuropsychiatric disorders. Here, we sought to establish whether selective stimulation of the neuronal nicotinic α7 receptor could improve spatial working memory in nonhuman primates.

Methods: Beginning with an estimated dose range from rodent studies, the dose of the α7 agonist AZD0328 was titrated for a significant impact on working memory in rhesus macaques after acute administration. After training to stability on the spatial delayed response task, subjects were administered AZD0328 (1.6 ng/kg–.48 mg/kg; intramuscular) or vehicle 30 min before cognitive testing. AZD0328 (1 ng/kg–1.0 μg/kg; intramuscular) was then administered in a repeated, intermittent ascending dose regimen where each dose was given in two bouts for 4 days with a 1-week washout in between bouts, followed by 2-week washout.

Results: Acute AZD0328 improved cognitive performance when the dose was titrated down to .0016 and .00048 mg/kg from a cognitively impairing dose of .48 mg/kg. In a subgroup, sustained enhancement of working memory was evident for 1 month or more after acute treatment. Immediate and sustained cognitive enhancement was also found during and after repeated administration of AZD0328 at .001 mg/kg.

Conclusions: These findings demonstrate that extremely low doses of a nicotinic α7 agonist can have profound acute and long-lasting beneficial consequences for cognition, dependent upon the integrity of dorsolateral prefrontal cortex. Thus, the α7 receptor might have a fundamental role in the neural circuitry of working memory and in the synaptic plasticity upon which it might depend.

Key Words: Alzheimer's disease, nicotine, nonhuman primate, prefrontal cortex, schizophrenia, synaptic plasticity

Nicotine has wide-ranging effects on central nervous system function through activation of specific receptor subtypes in multiple neural systems. Stemming from the influence of these receptors on dopaminergic and glutamatergic transmission at cortical and subcortical levels, there is increasing focus on their role in mechanisms of cognition and addiction. The α7 neuronal nicotinic receptor has received particular attention for its involvement in smoking and schizophrenia. Patients with schizophrenia show a far higher incidence of smoking than the general population (1) and exhibit a deficit in sensory gating (poor P50 ratios) that is ameliorated by smoking (2,3). This P50 deficit seems to correlate with the abundance of the nicotinic acetylcholine α7 receptor ($nAChR_{\alpha7}$) in the brain (4) and has been mapped by linkage analysis to the chromosomal locus 15q13–q14, in which the α7 gene resides (5,6). Indeed, a polymorphism in this gene is associated with schizophrenia, the P50 deficit, and risk of smoking (7). These findings have prompted the supposition that smoking is an attempt by patients to self medicate, due to impoverished $nAChR_{\alpha7}$ signaling. The homomeric α7 receptor is a low-affinity subtype of nAChRs with the least sensitivity to nicotine for both activation and desensitization (8).

The $nAChR_{\alpha7}$ comprises a ligand-gated ion channel with calcium (Ca^{2+}) permeability comparable to that for the N-methyl-D-aspartate (NMDA) receptor. Moreover, $nAChR_{\alpha7}$ signaling potently raises intracellular free Ca^{2+} levels by virtue of the fact that Ca^{2+} influx through the receptor leads to activation of voltage-gated Ca^{2+} channels as well as Ca^{2+} release from the endoplasmic reticulum (9–11). Because mobilization of intracellular Ca^{2+} plays a critical role in synaptic plasticity and immediate early gene expression associated with learning and memory (12), it is essential to understand the involvement of $nAChR_{\alpha7}$ signaling in the cognitive deficits evidenced in neuropsychiatric disorders. Apart from its role in Ca^{2+} signaling, $nAChR_{\alpha7}$ is strategically located in neuronal circuitry associated with cognitive function. The messenger RNA expression for this receptor is evenly distributed over brain regions including frontal, temporal, and parietal cortices in human and nonhuman primates, and this expression has been shown to be elevated in hippocampus in Alzheimer's disease (13,14). In contrast, binding at $nAChR_{\alpha7}$ sites in cingulate cortex is considerably reduced in schizophrenia (15,16). In the hippocampus, immunogold labeling for the α7 subunit has revealed its widespread presence at nearly all synapses in CA1 stratum radiatum (17). This labeling includes pre- and postsynaptic sites at both γ-aminobutyric acid (GABA)ergic and glutamatergic synapses, suggesting that $nAChR_{\alpha7}$ might play a role in both excitatory and inhibitory circuit function, including regulation of transmitter release. Presynaptic $nAChR_{\alpha7}$ sites have been localized also to glutamatergic terminals in the ventral tegmental area (VTA), which might be the major mechanism by which stimulation leads to elevated dopamine release in ventral striatum and prefrontal cortex (PFC) (18,19). Similarly presynaptic facilitation of glutamate release has been demonstrated in PFC in vitro (20). Thus, α7 sites are uniquely targeted to key regions in cognition, including PFC, hippocampus, and VTA, and their density has been shown to be altered in neuropsychiatric conditions involving impaired cognition.

Taken together, the findings that $nAChR_{\alpha7}$ might substantially support glutamatergic, dopaminergic and GABAergic transmission in PFC has made this nicotinic receptor subtype a key target for the

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development of novel agents for treating cognitive deficits in schizophrenia. One such agent, the partial agonist AZD0328, increased dopamine neuron firing in VTA and stimulated prefrontal cortical dopamine release (21). Furthermore, AZD0328 enhanced acquisition of delayed reinforcement and improved performance of novel object recognition. On this basis, we investigated the dose-dependent ability of AZD0328 to enhance spatial working memory performance in nonhuman primates, as a translational measure of its potential efficacy for improving dorsolateral PFC function in schizophrenia.

Methods and Materials

Subjects

For the acute study, nine adult rhesus macaques (*Macaca mulatta*; from 6 to approximately 19 years of age) were trained to stability on the spatial delayed response task (see following text). For the multiple ascending dose study, eight adult rhesus macaques (8–17.5 years of age) were trained to stability before inclusion. Note, seven of the animals from the acute study were included in the multiple ascending dose study after a prolonged washout (≥5 weeks) and ascertaining stable baseline levels of performance on the delayed response task. Monkeys were tested 3–5 days/week and fed their standard chow and fruit immediately after cognitive testing. All animals were maintained and housed in accordance with federal as well as Yale/Veterans Administration institutional guidelines for the care and use of nonhuman primates.

Drug Administration

The selective neuronal nicotinic α7 agonist AZD0328 was obtained from AstraZeneca Pharmaceuticals (Wilmington, Delaware). The original design for the acute study consisted of a pseudorandomized administration schedule with either vehicle (phosphate buffer at pH 6.0) or one of three doses of AZD0328 (see following text) to be administered as an intramuscular (IM) injection 30 min before cognitive testing. The initial dose range was extrapolated from neuropharmacology studies in fimbria-fornix lesioned rats (ffx rats) and toxicology studies in nonhuman primates. In ffx rats, chronic administration of 1–3 mg/kg AZD0328 reversed deficits in delayed nonmatch to position (22), and acute administration of the same doses reversed deficits in hippocampal long-term potentiation (23). Pharmacokinetic (PK) studies in both rats and monkeys had suggested that doses .48, 1.6, and 4.8 mg/kg would provide similar plasma systemic exposure in primates to that found to reverse deficits in ffx rats in the aforementioned studies. However, initial observations in several monkeys indicated that doses in this range (1.6 and .48 mg/kg) were deleterious to cognition. Thus, a revised study design was formulated wherein doses were given at decreasing orders of magnitude down to .0000016 mg/kg. Due to time and logistical constraints, not all doses were given to all animals. This was due to persistent effects of the agonist on cognitive performance, which lasted for weeks in some animals (see Results). A minimum of 1-week washout was required between acute doses. Investigators testing the animals were blinded to drug treatments as far as possible.

For the multiple ascending dose regimen, animals were required to be at their baseline performance before initiation. Here, AZD0328 (.000001–.001 mg/kg; IM) was administered in a repeated, intermittent ascending dose regimen according to the following protocol, where doses were escalated by an order of magnitude after each dose had been administered twice. Specifically, each dose was given in two bouts of 4 days with a 1-week washout between the first and second bout, followed by a washout of 2 weeks after the second bout. As in the acute study, animals were tested on the spatial delayed response task 30 min after injection. Investigators testing the animals were blind to administered doses as far as possible but were aware that the animals were on a rigid multiple dosing study.

PK Analysis

To support the behavioral pharmacological testing, the exposure to AZD0328 in rhesus macaques ($n = 5$ males + 2 females) after IM administrations at .048 and 1.7 mg/kg was investigated separately in two PK studies. Plasma samples were taken at .25, .5, 1, 1.5, 3, and 4 or 6 hours after injection. Plasma concentrations of AZD0328 were measured by a liquid chromatography/tandem mass spectrometry method (LC/MS/MS). Plasma samples were prepared by protein precipitation. The supernatant extracts were dried and injected on to a liquid chromatography mass spectrometer. The detection range for AZD0328 in plasma was 5–10,000 nmol/L. The PK parameters (e.g., $AUC_{[0-t]}$: area under the plasma concentration-time curve from time zero to t) were performed with noncompartmental analysis with WinNonlin software (V5.2) (Pharsight, Mountainview, California). Because these two studies suggest that PK properties of AZD0328 in this species is likely linear in the dose range of .048 and 1.7 mg/kg, the exposure to AZD0328 was estimated for any doses lower than .048 mg/kg on the basis of the observations from these two PK studies.

Cognitive Testing

Before initiation of these studies, animals were trained on a spatial delayed response task in a Wisconsin General Testing Apparatus. Details of the task have been previously described (24). In short, the monkey watches while the investigator baits one of two or more spatially distinct recessed wells with a preferred food treat, and the wells are covered with identical cardboard plaques. Next, an opaque screen is lowered for a variable delay (during which time the animal holds in mind the spatial location of the baited well), and once it is raised the animal is allowed to make a response to the correct well to achieve reward. On each test session, animals were required to perform 20 trials consisting of five variable delays (0,1, . . . 4) that were employed in a semirandom order for 4 trials each. Delays were multiples of a time constant (N) that was varied from 1 to 10 sec, dependent upon task difficulty. To achieve baseline stability for each animal, task difficulty was increased incrementally by increasing the length of the delays and/or increasing the number of spatially displaced wells from which the animal had to choose. Once animals reached a stable level of performance (65%–75% ± 2.5% correct over 20 consecutive test sessions), the number of wells and length of delays was then kept constant for the study. For both the acute and multiple ascending dose studies the animals performed the task over a range of 2–4 wells (mean = 2.6) with an average N = 5.22 sec and N = 5.25 sec, respectively. Two animals began scoring too high after the acute study and had their well number and/or N value increased, to reestablish baseline performance within the required range.

Data Analysis

For the acute study, mean and SEM for spatial delayed response performance were calculated for each animal across baseline, vehicle, and acute doses of AZD0328. One-way analysis of variance (ANOVA) was used across conditions to measure overall dose-dependent effects on spatial working memory performance. Because we had to unexpectedly test a wide range of doses and could not test all animals at all doses, we were not able to perform a repeated measures ANOVA and instead employed a Fisher's Least Significant Differ-

ence post hoc comparison for this analysis. Additionally, one-way ANOVA was used to assess sustained improvement in performance across and within four of these animals. For the multiple ascending dose study, one-way ANOVA was employed to determine whether there was a significant change in spatial working memory performance after the multiple ascending dose regimen compared with baseline/pretreatment. Regression analysis was used to assess performance trends across the multiple ascending dose regimen, including the final 2-week washout after the last bout of .001 mg/kg.

Results

PK Analysis

Plasma concentrations of AZD0328 were measured in seven rhesus macaques after IM administration at doses of .048 mg/kg and 1.7 mg/kg. The time to reach maximal plasma exposure at both dose levels was .25 hours after injection. The exposure to AZD0328, as evaluated by the maximum plasma concentration reached, was 1990 ± 274 nM for 1.7 mg/kg and 75 ± 9 nM for .048 mg kg, respectively; the exposure, as evaluated by $AUC_{(0-t)}$, was 2934 ± 374 nM*h for 1.7 mg/kg and 68.6 ± 13.1 nM*h for .048 mg/kg, respectively. These results suggest that PK in this species is likely linear in the dose range of .048 to 1.7 mg/kg. Thus, the systemic exposure to AZD0328 in rhesus monkeys at any of the lower doses tested in the spatial delayed response task was estimated with an assumption of linearity extended to .0016–.00048 mg/kg (Table 1).

Cognitive Testing

Across the dose range tested, acute administration of AZD0328 produced enhancements or impairments of spatial working memory dependent upon the dose tested. One-way ANOVA across eight conditions (baseline, placebo, .48, .016, .0016 mg/kg, .00048, .000016, and .0000016 mg/kg AZD0328) revealed a main effect of treatment condition [$F(7,40) = 7.952$, $p < .0001$] with Fisher's Protected Least Significant Difference post hoc comparisons ($p < .05$), indicating that spatial working memory performance was significantly enhanced after doses of .0016 and .00048 mg/kg and, conversely, significantly impaired after acute administration of .48 and .000016 mg/kg compared with baseline and placebo conditions (Figure 1). Across conditions, performance of animals increased by approximately 9% correct trials at .0016 mg/kg and 11% correct trials at .00048 mg/kg. Delay-dependent consequences of the cognitively enhancing doses of AZD0328 (.0016 and .00048 mg/kg) are shown in Figure 2. Animals made a greater number of errors at long (3–4) N compared with short (0–1) N delays, consistent with previous findings. Notably, acute administration of either .0016 or .00048 mg/kg AZD0328 reduced the average number of errors made by the monkeys at both short and long delays compared with baseline.

A subset of individuals, including three monkeys of middle-age and one young adult monkey, displayed a period of sustained cognitive enhancement after 2–3 acute administrations of AZD0328. One-way ANOVA across this subset of animals for their original baseline scores (67.97 ± .93) and their scores during the enhanced performance period (≥1 month) after acute treatment with AZD0328 (77.07 ± 1.36) confirmed a statistically significant elevation of working memory performance [$F(1,6) = 40.60$; $p < .001$]. This finding was reconfirmed upon analyses of the data of each

Table 1. Pharmacokinetic Data for IM Administration of AZD0328

Dose (mg/kg)	Cmax (nM)	AUC (0–*t*) (nM *h)
1.7 (N = 4)[a]	1990 ± 274	2930 ± 374
.048 (N = 3)[a]	75 ± 9	68.6 ± 13.1
.0016[b]	1.84 ± .25	2.75 ± .37
.00048[b]	.641 ± .125	.767 ± .131

AUC, area under the curve; Cmax, maximum concentration reached; IM, intramuscular; N, number of animals.

[a]Exposures estimated on the basis of the observations at doses .048 and 1.7 mg/kg with a linear pharmacokinetic (PK) assumption.

[b]Values were based on observed plasma concentrations in rhesus monkeys after IM doses.

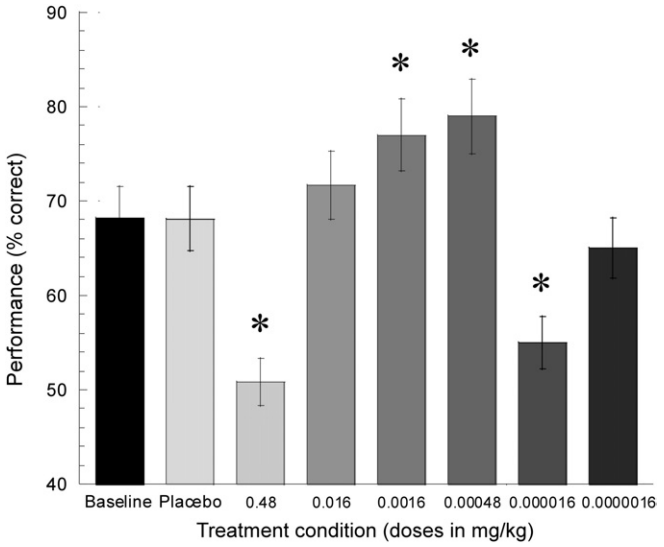

Figure 1. Group data for the effects of acute administration of AZD0328 (.0000016–.48 mg/kg; intramuscular) on spatial delayed response performance. The mid-range doses of .0016–.00048 mg/kg were found to be cognitively enhancing, whereas impairments in performance were observed after administration of either low (.000016 mg/kg) or high (.48 mg/kg) doses. *Significant difference compared with baseline and placebo; $p < .05$.

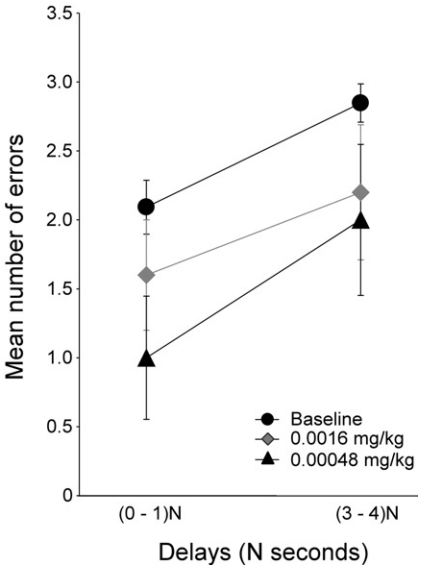

Figure 2. The mean number of errors is shown for short (0–1) time constant (N) and long (3–4) N delays across three conditions: baseline (solid circle), .0016 mg/kg AZD0328 (gray diamond), and .00048 mg/kg AZD0328 (solid triangle). The time constant, N, varied from 1 to 10 sec, dependent upon the level of difficulty of the individual on the task. Acute administration of either dose of AZD0328 improved performance at both long and short delays compared with baseline.

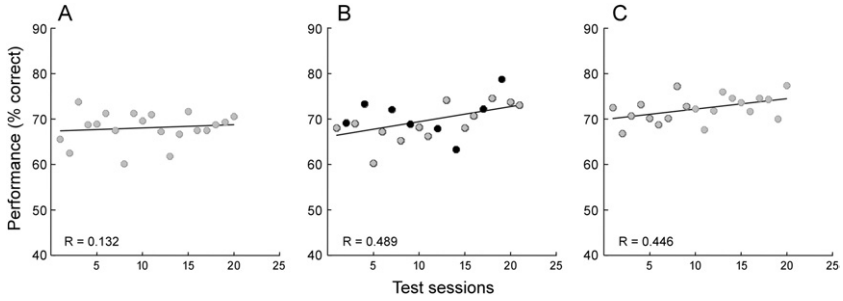

Figure 3. Group data for performance on the spatial delayed response task across baseline **(A)**; repeated, intermittent escalated dosing with AZD0328 **(B)**; and the first 2 months after treatment **(C)**. Significant cognitive enhancement was observed across the escalating dose regimen ($F = 5.96$; $p = .025$) compared with baseline performance. Moreover, modest yet significant improvement was carried over into the second month after cessation of treatment ($F = 4.48$; $p = .049$) **(C)**. For the multiple ascending regimen, drug treatment epochs are shown as black circles and pretreatment/washouts are shown as gray circles **(B)**.

individual: [1: $F(1,45) = 29.79$, $p < .001$; 2: $F(1,47) = 13.07$, $p < .001$; 3: $F(1,31) = 10.78$, $p = .003$; and 4: $F(1,32) = 7.10$, $p = .012$]. The sustained enhancement in spatial working memory across this group corresponded to an absolute increase of approximately 10% more correct responses on average.

Repeated, intermittent escalating treatment with AZD0328 via a multiple ascending dose design resulted in significant and sustained enhancement of spatial working memory performance. Group data for the effects of repeated, intermittent dosing with AZD0328 (.000001–.001 mg/kg; IM) on spatial delayed response performance are shown in Figure 3. The group showed significant cognitive enhancement across the escalating dose regimen ($F = 5.96$; $p = .025$) (Panel B) compared with their pretreatment/baseline performance (Panel A). Modest yet significant improvement carried over into the second month after cessation of treatment across the group ($F = 4.48$; $p = .049$) (Panel C). For the multiple ascending regimen, drug treatment epochs are shown in black, and pretreatment and washouts are shown in gray with each data point being collapsed across the group (N = 8; note that data for one animal after the .0001-mg/kg dose is not included, because this animal received an additional bout of this dose). Regression lines are shown for each condition to illustrate the overall change in performance during the actual treatment regimen. We next examined whether animals showed sustained cognitive enhancement because of completing the multiple ascending dose regimen of AZD0328. One-way ANOVA revealed that the animals showed sustained and persistent cognitive enhancement after the multiple ascending dose regimen (72.31 ± .66) compared with their baseline/pretreatment levels of performance [68.07 ± .78; $F(1,38) = 17.35$; $p < .001$], and this improvement persisted into the second month after the final dose of AZD0328 across the group. Moreover, enhanced performance in three of these individuals extended out to more than 6 months after treatment (data not shown). Finally, we examined whether the positive regression during the ascending dose regimen was largely due to the effects at the highest dose of AZD0328 (.001 mg/kg), whether there were any summative effects of the two bouts at this dose, and how consistent such effects were across individuals. Interestingly, the first bout of AZD0328 at .001 mg/kg produced an increase in performance in most animals, whereas after the second bout, a consistent increase in performance was observed across all individuals tested (Figure 4).

Discussion

This study demonstrated that the dose range for AZD0328 that was predicted to be effective in enhancing spatial working memory on the basis of early preclinical indications from cognitive studies in rodents was excessive in the primate, even at the lowest dose in that range of .48 mg/kg. Only when the dose was titrated down three orders of magnitude was cognitive enhancement observed within a discrete range from .00048 to .0016 mg/kg. The improvement in performance constituted an absolute increase in performance of some 11% above placebo scores or a relative increase of 16% in comparison with baseline and placebo scores as the best estimate of the normal level of performance of the animals. Although modest, this improvement should be considered substantial, because this group of young adult and middle-aged animals was previously trained to a highly stable level of performance and they were not tested in a deficit state. Moreover, a subgroup of four animals showed a significant sustained enhancement of performance for several weeks after receiving an acute dose of AZD0328. In concordance with this evidence for cognitive enhancement, we also saw an improvement in working memory performance across the multiple ascending dose regimen of administration that was particularly evident by the second dose of .001 mg/kg. Again, a modest sustained enhancement was also evident for several weeks after this regimen. Although we could not perform a repeated measures ANOVA on the acute study data, the results of the Fisher's post hoc comparisons showed a good concordance with the efficacy of the multiple dosing at .001 mg/kg in the same animals. Thus, this $nAChR_{\alpha 7}$ partial agonist seems to produce both acute and sustained cognitive enhancement at unexpectedly low doses on the basis of the efficacious exposures initially found in rodent models and estimated receptor occupancy. We and others have previously demonstrated efficacy at such low doses (and presumed ex-

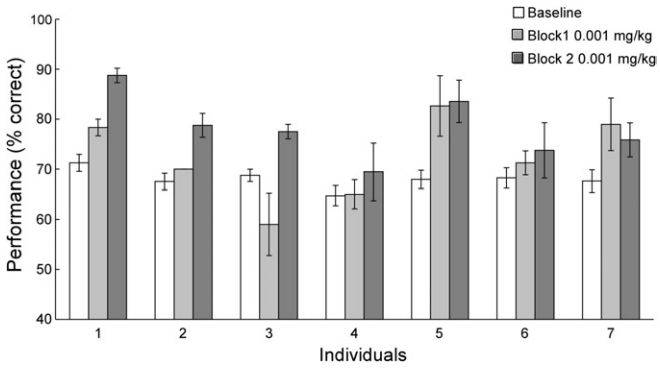

Figure 4. Delayed response data (mean ± SEM % correct performance) for each of the seven monkeys at baseline (light gray) and for both the first (gray) and second (dark gray) bouts of AZD0328 at the dose of .001 mg/kg in the multiple ascending dose regimen. Although the first bout produced improvement in some of the animals, the second bout produced a consistent enhancement in cognitive performance in all seven subjects.

posures), produced by D1 agonists in animals with a dopamine deficiency (24–26). We showed that repeated exposure to the D1 agonist ABT-431 at very low doses could produce a long-lasting improvement in working memory in aged animals and a similarly long-lasting restoration in this cognitive performance in young adult animals treated with chronic haloperidol. This raises the possibility that both D1 and α7 signaling might have highly sensitive mechanisms of action in common, including those involved in synaptic plasticity and neuroadaptive processes. Although the doses that elicited enhanced spatial working memory performance in monkeys are very low, extrapolation of PK measurements made at higher doses suggests that plasma exposures were in the range of human α7 receptor binding affinity ($K_i = 3$ nmol/L) (21). The present findings prompted additional preclinical rodent studies that revealed significant effects of AZD0328 on dopamine neuron firing, prefrontal dopamine levels, novel object recognition, and acquisition of a delayed reinforcement operant task at doses only fractionally above .001 mg/kg (21). It has recently been reported that another $nAChR_{\alpha7}$ agonist, EVP-6124, showed signs of efficacy in producing improvements in symptoms in conjunction with significantly larger P300 and mismatch negativity components in electroencephalographic recordings at a similar dose/exposure (27). In addition to cognitive enhancement, we also found evidence that this α7 agonist could induce acute cognitive deficits at both a high and an ultra-low dose. This apparent inverted-U function in relation to dose has previously been reported in studies of the behavioral effects of nicotine and for both cognitive performance and prefrontal dopamine release for AZD0328 and the partial agonist MEM3454 (21,28,29). Although the impairments observed at the high dose might be attributable to significant 5-HT^3 blockade (21), those observed at the low dose of just .000016 mg/kg require additional investigations to seek an explanation. However, we did observe one of the lowest group performance scores at the second dose of .0001 mg/kg during the multiple dosing regimen (Figure 3B), suggesting that there might be an alternative mechanism operating in the low-dose range of AZD0328. One possibility is that this dose can induce a significant level of receptor desensitization or internalization while being insufficient to produce significant ion channel opening. Taken together, the aforementioned evidence suggests that $nAChR_{\alpha7}$ stimulation taps into particularly sensitive mechanisms for learning and memory that are involved in the process of working memory.

Given the prominent projections of basal forebrain/septal cholinergic projections to hippocampus and PFC and the abundance of $nAChR_{\alpha7}$ in these regions, it is not surprising that the cholinergic system has a widespread influence on attention and cognition (30–33). Nicotinic acetylcholine receptor signaling is now recognized as playing a prominent role in attentional and cognitive processes (34,35), and considering the association of $nAChR_{\alpha7}$ with sensory-gating deficits in schizophrenia discussed earlier, elevation of signaling through this particular receptor subtype might be invaluable for improving prefrontal function in patients. As discussed in the preceding text, there are multiple mechanisms by which $nAChR_{\alpha7}$ stimulation might contribute to cognitive function. Of these, the potential contribution to the induction and maintenance of the persistent neuronal activity integral to working memory might be of the highest importance. The generation of cation influx by $nAChR_{\alpha7}$ activation, particularly its Ca^{2+} component, might have a profound influence on the activity of voltage-gated calcium and ion channels including NMDA currents as well as the synchronized depolarization of neurons into "upstates" that might form the basis of persistent activity in the brain (36–38). This has yet to be directly determined for a selective $nAChR_{\alpha7}$ agonist. The significance of this mechanism was indicated by a recent report showing that an $nAChR_{\alpha7}$ agonist protects against cognitive deficits in the nonhuman primate induced by the noncompetitive NMDA antagonist ketamine (39). Alternatively, potentiation of GABA signaling in local circuits might also be critical for the γ synchronization that might underlie attentional and working memory processes as well as the local circuit inhibition that constrains and shapes information flow and maintenance (40–43). Allosteric modulation of $nAChR_{\alpha7}$ has been reported to selectively depolarize hippocampal interneurons and promote GABA transmission (44). Likewise, the partial agonist SSR180711 was shown to induce large GABA-mediated postsynaptic currents in hippocampal neurons and to augment the amplitude of inhibitory postsynaptic currents evoked in CA1 pyramidal cells (45). Coupled with the finding that a $GABA_{A\alpha2/3}$ agonist tended to improve working memory and frontal γ synchrony in a recent pilot study in patients with schizophrenia (46), $nAChR_{\alpha7}$-mediated support for GABA transmission in prefrontal circuitry might play a role in its ability to enhance cognition.

In conjunction with these potential mechanisms for the acute beneficial effects of AZD0328, we provide evidence here for induction of a persistent enhancement of working memory that corroborates evidence from other $nAChR_{\alpha7}$ agonist studies. As discussed earlier, $nAChR_{\alpha7}$ has a key role in neuronal Ca^{2+} signaling that is associated with long-term changes in synaptic efficacy and functional connectivity. Calcium signaling involving activation of Ca^{2+}/calmodulin-dependent protein kinase II, mitogen activated protein kinase, extracellular signal-regulated kinase 1/2, and cyclic AMP response element binding protein (CREB) has an established role in dendritic arborization, spine formation, and axodendritic synaptogenesis, both in development and adulthood (47–51). Activation of these cell signaling pathways by a novel α7 agonist has recently been shown to correlate with improvements across a broad range of attentional and cognitive mechanisms, including delayed matching-to-sample in the monkey, mouse models of working memory, short-term recognition memory, and consolidation of long-term memory, as well as normalization of sensory gating deficits induced by the $nAChR_{\alpha7}$ antagonist methyllycaconitine (52). Along with evidence for augmentation of long-term potentiation (45), these findings strongly suggest a prominent role for $nAChR_{\alpha7}$ in regulating synaptic efficacy and promoting the reorganization of functional circuitry associated with critical mechanisms in attention and cognition. Thus, loss of the $nAChR_{\alpha7}$-component of Ca^{2+} signaling during development/adulthood, including CREB and Ca^{2+}/calmodulin-dependent protein kinase II activation, might contribute to the loss of neuropil and spinodendritic atrophy observed in dorsolateral PFC in schizophrenia (52–55). Notably, the active phosphorylated form of CREB has been found to be considerably depleted in frontal cortex in schizophrenia (56).

In conclusion, $nAChR_{\alpha7}$ seems to be a highly relevant target for treating cognitive deficits in disorders such as schizophrenia and Alzheimer's disease not just by immediate facilitation of excitatory and inhibitory elements in critical circuitry but also by enabling long-term changes in the same circuitry that serve to normalize and improve cognition. As found in this case for AZD0328, the influence of an agonist at remarkably low doses is sufficient to impact cognition via modulation of a ligand-gated ion channel that can induce persistent changes in the neural circuitry underlying working memory.

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