Nicotinic α7 receptors enhance NMDA cognitive circuits in dorsolateral prefrontal cortex

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The cognitive function of the highly evolved dorsolateral prefrontal cortex (dlPFC) is greatly influenced by arousal state, and is gravely afflicted in disorders such as schizophrenia, where there are genetic insults in α7 nicotinic acetylcholine receptors (α7-nAChRs). A recent behavioral study indicates that ACh depletion from dlPFC markedly impairs working memory [Croxson PL, Kyriazis DA, Baxter MG (2011) *Nat Neurosci* 14(12):1510–1512]; however, little is known about how α7-nAChRs influence dlPFC cognitive circuits. Goldman-Rakic [Goldman-Rakic (1995) *Neuron* 14(3):477–485] discovered the circuit basis for working memory, whereby dlPFC pyramidal cells excite each other through glutamatergic NMDA receptor synapses to generate persistent network firing in the absence of sensory stimulation. Here we explore α7-nAChR localization and actions in primate dlPFC and find that they are enriched in glutamate network synapses, where they are essential for dlPFC persistent firing, with permissive effects on NMDA receptor actions. Blockade of α7-nAChRs markedly reduced, whereas low-dose stimulation selectively enhanced, neuronal representations of visual space. These findings in dlPFC contrast with the primary visual cortex, where nAChR blockade had no effect on neuronal firing [Herrero JL, et al. (2008) *Nature* 454(7208):1110–1114]. We additionally show that α7-nAChR stimulation is needed for NMDA actions, suggesting that it is key for the engagement of dlPFC circuits. As ACh is released in cortex during waking but not during deep sleep, these findings may explain how ACh shapes differing mental states during wakefulness vs. sleep. The results also explain why genetic insults to α7-nAChR would profoundly disrupt cognitive experience in patients with schizophrenia.

Acetylcholine (ACh) acts through a variety of nicotinic and muscarinic receptors to modulate wakefulness (1–3) and orchestrate attention-related circuits in the brain (4). It is released during wakefulness and rapid eye movement (REM) sleep (1, 3), exciting the thalamus and cortex (2, 3, 5) and allowing conscious experience (6). Despite the established importance of ACh in cortical function, there is very little known about its effects at the cellular level in cognitively engaged circuits. One study of the primate primary visual cortex [V1 (7)] showed attentional modulation by muscarinic but not nicotinic receptors. However, there have been no physiological studies of cholinergic actions in higher association cortices in primates, even though behavioral data indicate that ACh is essential for the working memory (WM) functions of the dorsolateral prefrontal cortex (dlPFC) (8), a highly evolved brain region that subserves mental representation and executive function as well as the reactivation of long-term memories onto the "mental sketch pad" (9, 10).

Goldman-Rakic and colleagues discovered the cellular basis of the spatial WM functions of primate dlPFC (9). Lesions to the principal sulcal dlPFC in monkeys permanently impair spatial WM performance, whereas physiological recordings from this area have revealed "delay cells" that generate mental representations of visual space even when stimuli were no longer present in the environment (9). These delay cells can maintain information in temporary storage to guide prospective motor acts, thus integrating perception and action (11). These neurons maintain persistent firing throughout the delay period when information is held in WM, firing selectively for a "preferred direction" to create visuospatial representations (Fig. 1 *A*–*C*). Goldman-Rakic uncovered the cellular basis of these spatial WM functions and the circuitry underlying visuospatial representation (Fig. S1) (9): Neurons in dlPFC receive highly processed visuospatial information from the parietal association cortex, and layer III dlPFC pyramidal cell microcircuits excite each other to maintain persistent firing across the delay. Persistent firing also may involve reciprocal excitation with longer-range cortical–cortical circuits, for example, with the parietal association cortex (12). The spatial tuning of dlPFC delay cells is refined by GABAergic lateral inhibition from local basket and chandelier cells (Fig. S1). Recent studies have shown that the persistent firing of delay cells relies on glutamate NMDA receptors (NMDARs), including those with NR2B subunits, which are localized in the postsynaptic densities of glutamatergic synapses on spines in deep layer III (13). These pyramidal cells expand greatly in primate evolution (14), and are especially afflicted in schizophrenia (15, 16) and Alzheimer's disease [AD (17)].

A variety of higher cognitive disorders are associated with impaired dlPFC function and genetic insults to nicotinic α7 receptors (α7-nAChRs) and/or NMDAR signaling. There is extensive evidence linking genetic alterations of α7-nAChR to schizophrenia and attentional deficits (18, 19), including alterations at the transcription level (20). Recent data have also shown that α7-nAChR expression depends on neuregulin, another molecule linked to schizophrenia (21), and that smoking in schizophrenia may be a form of self-medication, normalizing expression of α7-nAChRs (22). More recent studies have linked α7-nAChRs to autism (23), attention deficit hyperactivity disorder [ADHD (24)], and AD (25), suggesting that a variety of dlPFC disorders are linked to alterations in α7-nAChR signaling. α7-nAChR agonists are currently under development as potential therapeutic treatments for these disorders, based in part on animal studies showing that systemic administration of α7-nAChR agonists can rescue WM deficits induced by NMDAR blockade (26, 27). However, the location and physiological roles of α7-nAChR in dlPFC circuits had not been known. The current study used electron microscopy and recordings from cognitively engaged monkeys to reveal α7-nAChR localization and actions in primate dlPFC. We report that α7-nAChRs are situated in the postsynaptic density of glutamatergic synapses in deep layer III of dlPFC, and that α7-nAChR stimulation is essential for the excitation of NMDAR-mediated WM circuits.

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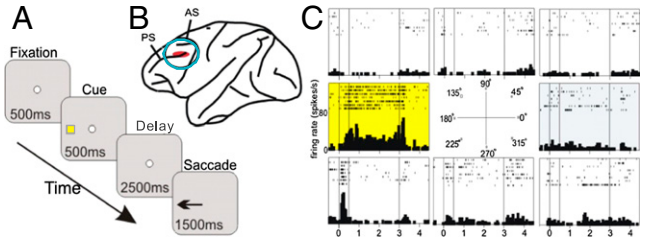

Fig. 1. Spatial working memory in primates. (*A*) The ODR spatial working memory task. (*B*) The recording locus in dlPFC. PS, principal sulcus; AS, arcuate sulcus. (*C*) A delay cell with spatially tuned, persistent firing during the delay period; the neuron's preferred direction is highlighted in yellow.

Results

Electron Microscopic Localization. Two independent approaches, monoclonal antibodies and α-bungarotoxin (α-BTX), were used to visualize α7-nAChRs under the electron microscope in layer III of monkey dlPFC. Labeling predominated on spine membranes and could also be seen along dendritic shaft membranes and perisynaptic astrocytes; glutamatergic-like axons were rarely labeled, which included preterminal portions and exclusively asynaptic membranes of the varicosity (Fig. 2*A*). In dendritic spines, the postsynaptic density of glutamatergic-like, excitatory synapses was distinctly reactive (Fig. 2 *A–C*). Perisynaptic α7-nAChRs (i.e., localized within a 50-nm halo around the synaptic disk) were found flanking glutamatergic and nonglutamatergic synapses, including synapses converging on the same spine (synaptic triads; Fig. 2 *B–D*). Similar triads involved receptor-reactive spines receiving both a glutamatergic synapse and axon appositions from cholinergic terminals, which could also contain α7-nACh autoreceptors (Fig. 2*E*). We have recently identified NR2B-NMDARs within the postsynaptic density of spine glutamatergic synapses in deep layer III of monkey dlPFC (13), and thus α7-nAChRs are similarly positioned (summarized in Fig. 2*F*) to depolarize the spine synaptic membrane in dlPFC pyramidal cell circuits.

Physiological Actions of α7-nAChRs in Primate dlPFC. The physiological role of α7-nAChRs in WM was examined by combining single-neuron recordings from dlPFC (area 46; Fig. 1*B*) with iontophoretic drug application in monkeys performing a spatial oculomotor delayed response (ODR) task (Fig. 1*A*). The iontophoretic electrode delivers a minute amount of drug to influence nearby neurons but is inadequate to alter behavioral performance. In the ODR task, a brief cue occurs at one of eight locations ("cue"); the subject must remember this location over a delay (2.5 s; "delay") before moving its eyes to the remembered location to receive a reward ("response"). The spatial location changes randomly in each trial, requiring constant updating of WM. We recorded 90 neurons with persistent firing during the delay epoch (delay cells) that fired for the cell's preferred direction (e.g., Fig. 1*C*, yellow) but not for other locations, such as the opposite direction (nonpreferred direction in Fig. 1*C*, gray). The degree of spatial tuning was measured by d', the ability to distinguish preferred from nonpreferred spatial directions based on firing rate during the delay epoch. There were three distinct subtypes of delay cells, similar to those seen in previous studies of dlPFC (11). One prominent subset of delay cells, termed "memory delay cells," fired throughout the delay period to maintain information in short-term storage (52% of delay cells). There were also delay cells that began firing in the middle of the delay period and increased their firing in anticipation of the motor act (23% of delay cells), so-called preparatory set delay

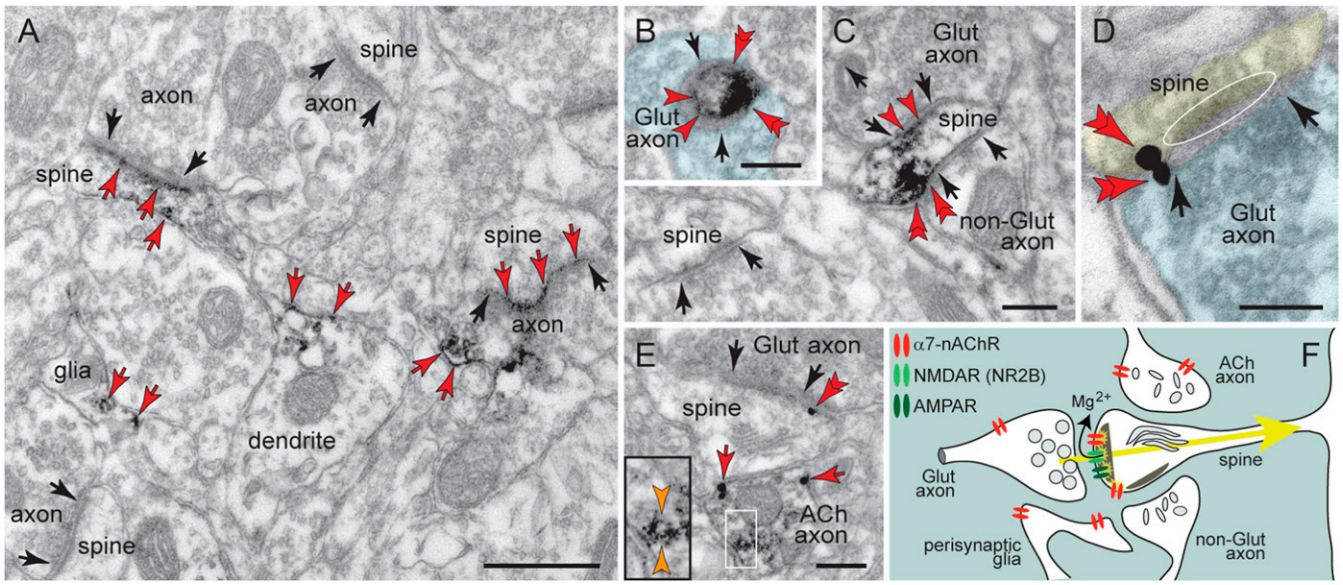

Fig. 2. Electron microscopic localization of α7-nAChR and colocalization with VAChT in dlPFC. (*A*) General pattern of α7-nAChR expression in layer III neuropil of monkey dlPFC; visualized with immunoperoxidase (red arrows). α7-nAChRs are most prevalent in dendritic spines, including localization within the postsynaptic density (compare with unlabeled spines). Labeling is also observed in dendritic shafts and astrocytic processes, and rarely can be detected in axons. (*B*) A tangential section through the synaptic disk reveals synaptic and perisynaptic α7-nAChRs at the postsynaptic membrane. (*C*) Synaptic and perisynaptic α7-nAChRs in an asymmetric, glutamatergic-like synapse as well as perisynaptically to a symmetric synapse onto the same spine (triad). An unlabeled axospinous synapse is shown for comparison. (*D*) α-BTX–gold labeling of perisynaptic α7-nAChRs; the white oval marks the postsynaptic density. (*E*) A spine receives a glutamatergic synapse and a cholinergic apposition in a triadic configuration. Gold-labeled α7-nAChRs are found both in the spine and the cholinergic axon (red arrows), identified with VAChT-immunoperoxidase at the secretory vesicle limiting membrane (orange arrowheads in *Inset*). Red arrowheads and double arrowheads point to synaptic and perisynaptic receptors, respectively; synapses are between the black arrows. [Scale bars, 0.5 μm (*A*), 200 nm (*B*, *C*, and *E*), 100 nm (*D*).] (*F*) Model of α7-nAChR actions in dendritic spines containing NR2B-NMDARs, AMPARs, and α7-nAChRs postsynaptically, and next to cholinergic input. Stimulation of α7-nAChRs depolarizes the spine postsynaptic membrane to relieve NMDAR Mg^{2+} block and permit NMDA actions.

cells (11). Finally, there were delay cells with a combined phenotype that fired throughout the delay period but ramped up in anticipation of the response, so-called ramp-up delay cells (25% of delay cells). The combined firing of these neurons provides the temporal integration needed to guide response in the absence of sensory stimulation (11). All three subtypes of delay cells were influenced by drug in a similar fashion, and thus were combined for these analyses.

Effect of blocking endogenous cholinergic actions at α7-nAChRs. The effect of blocking endogenous cholinergic actions at α7-nAChRs was assessed through iontophoretic application of two antagonists: a general nAChR antagonist, mecamylamine (Mec), and a selective α7-nAChR antagonist, methyllycaconitine (MLA). Both antagonists markedly decreased delay-related firing. Iontophoretic application of Mec (15–50 nA) produced a large reduction in neuronal firing for all task epochs in all directions (n = 10; Fig. S2; control vs. Mec, $P < 0.05$ for all task epochs, and for both preferred and nonpreferred directions). In contrast, iontophoretic application of MLA had more specific effects, reducing delay-related firing for the neurons' preferred direction without altering baseline firing or firing for the nonpreferred direction [n = 17; Fig. 3*A*; delay-related firing (spikes per s): control 19 ± 2, MLA 13 ± 2, $F_{1,16} = 31.218$, $P < 0.0005$; d': control 2.053 ± 0.273, MLA 0.980 ± 0.219, $F_{1,16} = 42.786$, $P < 0.0005$]. An example is shown in Fig. 3*B*, where application of MLA produced a marked, dose-related reduction in the spatially tuned delay-related firing ($F_{3,22} = 13.343$, $P < 0.0005$). Neuronal firing recovered to control levels once drug delivery stopped (control vs. recovery, $P = 0.972$). Importantly, MLA had differential effects on task epoch, with little effect on firing during cue presentation but greatly reduced firing during the delay period

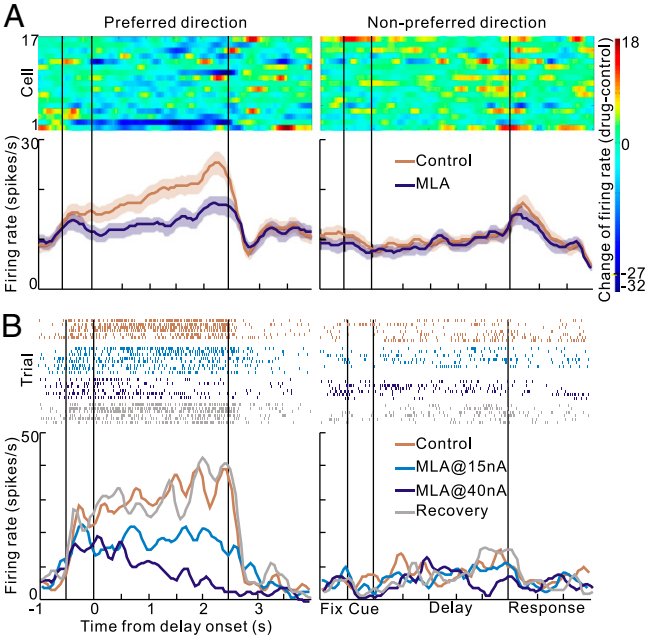

Fig. 3. Effects of α7-nAChR blockade on WM-related firing of dlPFC neurons. (*A*) The α7-nAChR antagonist MLA reduced WM-related firing of dlPFC neurons (n = 17). (*Upper*) The change in firing rate following MLA application for every neuron. Reduced firing is shown in blue (see scale bar on right). (*Lower*) The mean ± SEM firing rate for all neurons for the control (brown) vs. the MLA condition (blue; 5–50 nA; lowest dose that significantly decreased firing for each neuron). (*B*) Example of an individual neuron. Iontophoresis of MLA (15 or 40 nA; blue) decreased the neuron's delay-related firing in a dose-dependent manner. The neuron recovered firing once the drug was removed (gray).

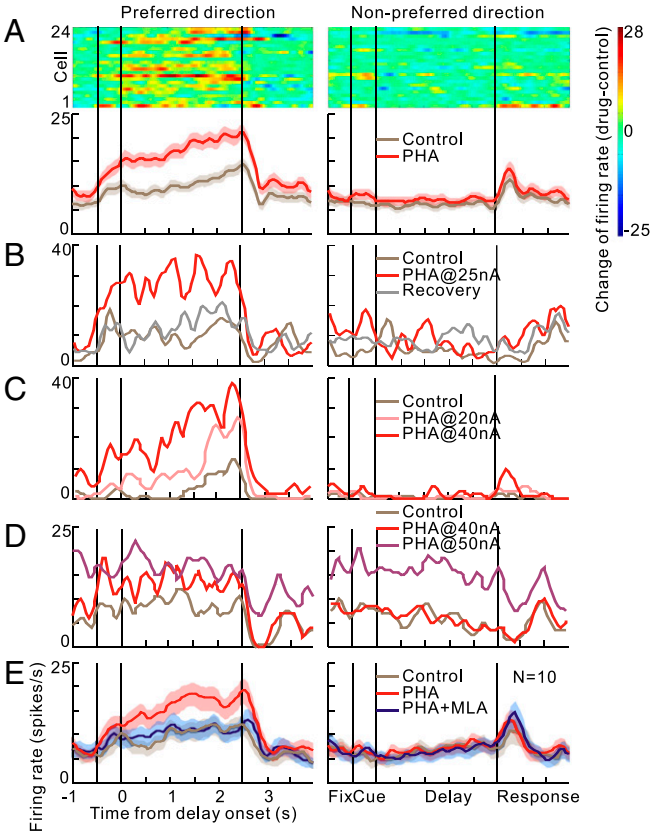

Fig. 4. Effects of α7-nAChR stimulation on WM-related firing of dlPFC neurons. (*A*) The α7-nAChR agonist PHA enhanced WM-related firing of dlPFC neurons (n = 24). (*Upper*) The change in firing rate following PHA application for every single neuron. Increased firing is shown in red (see scale bar on right). (*Lower*) The mean ± SEM firing rate for all neurons for the control (brown) vs. the PHA condition (red; 5–40 nA; highest dose that significantly increased firing and tuning for each neuron). (*B*) Example of an individual neuron. Iontophoresis of PHA (25 nA; red) significantly increased the neuron's delay-related firing; firing reduced to control levels following cessation of drug delivery (gray). (*C*) Example of a single neuron showing the dose-dependent effects of PHA (20–40 nA) on delay-related firing. (*D*) Inverted-U–shaped dose effects of PHA on a single neuron. Whereas PHA at 40 nA selectively increased firing for the neuron's preferred direction, application of PHA at 50 nA (purple) produced nonselective increases in firing for all directions. (*E*) The increase in delay-related firing induced by PHA (red) was reversed by coiontophoresis of the α7-nAChR antagonist MLA (blue; n = 10).

when spatial information is held in WM (Fig. 3 *A* and *B* and Fig. S3; change ratio of firing rate: delay −33 ± 5% vs. cue −21 ± 6%, $F_{1,16} = 4.897$, $P = 0.042$; significant drug x task epoch interaction: $F_{1,16} = 12.503$, $P = 0.003$). These data indicate that endogenous stimulation of nAChRs has essential excitatory influences on dlPFC neurons, and that nAChRs with α7 subunits are especially important for the spatially tuned, persistent firing underlying spatial WM.

Effect of stimulating α7-nAChRs. Next, we examined the effects of stimulating α7-nAChRs on primate dlPFC neurons using three highly selective α7-nAChR agonists: PHA543613 (PHA), DMAB-A, and TC1698 (TC). All three agonists produced significant enhancement in delay-related firing, with low doses having the most specific effects on delay-related firing. PHA (5–40 nA) consistently enhanced spatially tuned delay-related activity in all delay cells [n = 24; Fig. 4*A*; delay-related firing (spikes per s): control 10 ± 1, PHA 16 ± 2, $F_{1,23} = 64.377$, $P < 0.0005$; d': control 1.398 ± 0.152, PHA 3.016 ± 0.194, $F_{1,23} = 66.365$,

$P < 0.0005$]. Iontophoresis of PHA at 25 nA significantly increased the delay-related firing of a dlPFC delay cell for its preferred direction ($F_{2,25} = 32.061$, $P < 0.0005$; Fig. 4*B*), with firing recovering to baseline levels when drug was no longer applied (control vs. recovery: $P = 0.908$), and this was dose-dependent (Fig. 4*C*). Neurons with low delay-related firing under control conditions were most improved by PHA application, likely due to ceiling effects in neurons with more robust delay-related firing (Fig. S4; changes in delay-related firing followed an exponential regression: $y = 2.8301\ e^{-0.6603x}$, $R = 0.629$). Similar enhancing effects were observed with the two other α7-nAChR agonists, DMAB-A (Fig. S5*A*; $F_{1,3} = 8.067$, $P = 0.033$) and TC (Fig. S5*B*; $F_{1,6} = 33.214$, $P = 0.001$). In contrast to the specific enhancing effects of low-dose PHA, higher doses of PHA (30–50 nA, dependent on individual neuronal sensitivity) produced nonspecific increases in excitability for all spatial directions, eroding spatial tuning (Figs. 4*D* and 5*A*). The differences in drug sensitivity between neurons likely reflect variations in endogenous cholinergic stimulation. In summary, PHA had an inverted-U–shaped dose effect on the spatial tuning of dlPFC neurons, with lower doses enhancing tuning and higher doses eroding tuning (Fig. 5*A*). These physiological effects were replicated in the behavioral studies, where systemic administration of PHA produced an inverted-U dose–response on cognitive performance (Fig. 5*B*; $F_{4,33} = 6.381$, $P = 0.001$; post hoc comparisons: vehicle vs. 0.1 μg/kg: $P = 0.003$; vehicle vs. other doses: $P > 0.05$), and compare with previous behavioral studies of other α7-nAChR agonists (26, 28). Furthermore, the ability of PHA to enhance delay-related firing was reversed by coiontophoresis of MLA, consistent with α7-nAChR actions (Fig. 4*E*; PHA vs. PHA+MLA: $P < 0.0005$). This remarkable specificity of low-dose α7-nAChR agonists on dlPFC delay-related firing encourages their use as cognitive enhancers for PFC disorders.

Interactions between α7-nAChRs and NR2B-NMDAR neurotransmission. Finally, we examined interactions between α7-nAChRs and NR2B-NMDAR signaling in dlPFC. Previous research has shown that NR2B-NMDAR blockade markedly reduces spatially tuned delay-related firing (13), and these data were replicated in the current study, where low-dose iontophoresis of the NR2B-NMDAR selective antagonist Ro 25–6981 (Ro) reduced delay-related firing (a single-neuron example is shown in Fig. 6*A*; average neuronal response, $n = 12$, is shown in Fig. 6*B*; control vs. Ro: $P < 0.0005$).

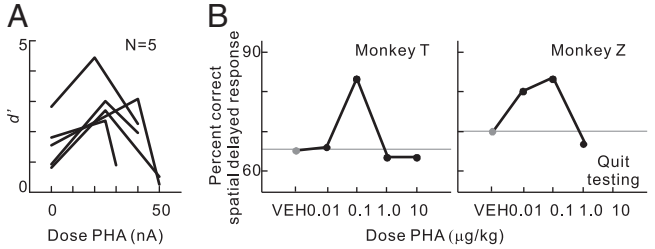

Fig. 5. Inverted-U dose-dependency curve of α7-nAChR agonist PHA. (*A*) PHA produced an inverted-U dose effect on spatial tuning of delay neurons in dlPFC whereby low doses increased and high doses decreased spatial tuning as measured by d'. Please note that individual neurons ($n = 5$) present varying sensitivities to PHA (20–50 nA), due to differences in the level of endogenous ACh or to varying distances between the neuron and the iontophoretic pipette. However, all showed inverted-U dose–response curves. (*B*) Similar to the electrophysiology study, PHA produced an inverted-U dose–response curve on performance in the spatial WM task in monkeys, with low doses improving and higher doses having no effect or impairing performance. Examples of dose–response curves for two individual monkeys are shown. Overall, the 0.1 μg/kg dose consistently improved performance, whereas other doses were not significantly different from vehicle control. A similar inverted-U dose–response has been seen in cognitive assessments of other α7-nAChR agonists in monkeys.

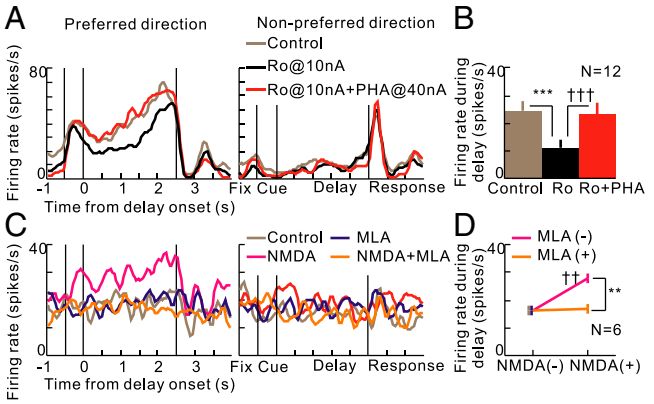

Fig. 6. Interaction between α7-nAChRs and NMDARs mediates WM-related firing of dlPFC neurons. (*A* and *B*) Iontophoretic application of PHA (red) reversed the reduction in delay-related firing produced by application of the NR2B-NMDAR blocker Ro (black). (*A*) Single-neuron example. (*B*) Average response of all dlPFC neurons ($n = 12$, mean ± SEM). ***$P < 0.0005$; $^{\dagger\dagger\dagger}P < 0.0005$. (C and D) Stimulation of α7-nAChRs is required for NR2B-NMDAR–dependent neuronal-related firing. (*C*) An example of a single neuron showing that iontophoresis of NMDA by itself (magenta) increased delay-related firing in a neuron with weak delay-related firing under control conditions. In contrast, iontophoresis of NMDA following MLA blockade of α7-nAChR (orange) was no longer effective in exciting dlPFC delay neurons. (*D*) Average firing rate of six dlPFC neurons during the delay period for the neurons' preferred direction (mean ± SEM). Data analysis showed a significant interaction between NMDA and MLA, whereby NMDA increased firing when applied alone but had no effect during α7-nAChR blockade. **$P < 0.005$; $^{\dagger\dagger}P < 0.005$.

This reduction in firing was reversed by coiontophoresis of PHA (Fig. 6 *A* and *B*; Ro vs. Ro+PHA: $P < 0.0005$). Similar protective effects were observed if PHA was coapplied with Ro from the beginning and then removed to reveal the loss of firing with Ro alone (Fig. S6; $n = 7$; control vs. PHA+Ro: $P = 0.619$; PHA+Ro vs. Ro: $P = 0.002$). These results, in conjunction with the ultrastructural data, are consistent with spatial and functional interaction between the two receptors. The observed localization of postsynaptic α7-nAChRs in the axospinous synapse (summarized in Fig. 2*F*) suggests that α7-nAChRs may provide a permissive depolarization to facilitate NMDAR transmission, similar to AMPARs. To test this hypothesis, we examined whether iontophoresis of NMDA becomes ineffective under conditions of α7-nAChR blockade. Indeed, iontophoresis of NMDA alone significantly increased delay-related firing (a single-neuron example is shown in Fig. 6*C*; average neuronal response, $n = 6$, is shown in Fig. 6*D*; control vs. NMDA: $P < 0.005$) but failed to increase firing when coapplied following α7-nAChR blockade with MLA (Fig. 6 *C* and *D*; control vs. NMDA+MLA: $P > 0.6$; significant NMDA x MLA interaction: $P < 0.005$).

Discussion

The current study reveals that α7-nAChRs are positioned in the synapses of layer III dlPFC pyramidal cell circuits in primates, and that endogenous stimulation of these receptors has an essential role in the WM-related firing in NMDAR-mediated circuits. In particular, the data establish that endogenous ACh stimulation of α7-nAChRs plays a remarkably specific role in enhancing persistent neuronal firing in the absence of sensory stimulation, and explain why depletion of ACh from primate dlPFC would have such devastating effects on spatial WM, producing deficits comparable to ablation of the cortex itself (8). Conversely, the data illuminate why acute cigarette inhalation can enhance WM performance and dlPFC the blood-oxygen-level-dependent activity in humans (29), and why α7-nAChR

agonists can improve the performance of a variety of memory and attention tasks that depend on PFC circuits (30–33). The study identifies the cellular basis for these behavioral actions, showing that stimulation of α7-nAChRs produces an inverted-U dose–response both for spatially tuned neuronal firing patterns and for behavioral performance, enhancing NMDAR-mediated neuronal representations of visual space.

Potential Cellular Actions in dlPFC. There are several possible subcellular sites for α7-nAChR actions. The highly specific enhancement of delay-related firing for the neuron's preferred direction that follows low-dose agonist application likely involves stimulation of postsynaptic α7-nAChRs in spines and direct facilitation of NMDAR glutamate transmission in the recurrent excitatory circuits that drive WM. Stimulation of presynaptic α7-nAChRs may also contribute, for example, by magnifying endogenous glutamate release patterns, and nonspecific increases in glutamate release may account for generalized increase in neuronal firing with high doses of α7-nAChR agonists. In rat PFC, nAChRs increase glutamate, ACh, and monoamine release, including glutamate from thalamic terminals, although some of these actions may involve other nAChR subtypes (34, 35). α7-nAChRs are also found in rodent synapses (36), suggesting that the receptor could have similar actions across species. However, most physiological studies in rodent PFC have observed presynaptic nicotinic actions (35, 37–39). It may be that spine α7-nAChRs are not prevalent or only have subtle effects on neuronal physiology in rodents, as α7-nAChRs are much more prominent in primate than rodent brain (40). Thus, these important actions may be magnified in primate evolution. It should be noted that other nicotinic (38, 39, 41–43) and muscarinic (44) cholinergic receptors also have important influences on PFC function in rodents, and it is likely that these receptors also influence dlPFC function in primates.

α7-nAChR Interactions with NMDAR in dlPFC. The study reveals an important physiological interaction between α7-nAChRs and NR2B-NMDARs in primate dlPFC, consistent with previous behavioral studies (26, 27), suggesting that α7-nAChRs have a permissive role upon NMDAR actions in dlPFC circuits. These permissive actions could occur in concert with, or in place of, the traditional AMPAR role in depolarizing NMDARs (45), as suggested by Berg and colleagues (36), and by ultrastructural findings in rodent cortex (46). The cholinergic system provides essential excitatory input for activating thalamic and cortical neurons during alert waking and during REM sleep (1, 2, 5, 47, 48), and dlPFC is especially sensitive to changes in arousal state (49). As ACh is an important modulator of arousal state, α7-nAChRs may provide an alerting mechanism by which ACh can bring NMDAR higher cortical circuits "online" to produce a conscious cognitive state, whereas the absence of its release, such as occurs in deep sleep, may render dlPFC NMDAR synapses "silent." Such a permissive role for α7-nAChRs on NR2B-NMDAR opening may be particularly important in dlPFC, where higher cognitive function is markedly influenced by the state of arousal (50). In contrast, α7-nAChRs have little effect on neuronal firing in primate V1 (7), a cortical region that processes visual information even in the anesthetized state.

Relevance to the Etiology and Treatment of Cognitive Disorders. α7-nAChR agonists are in development for the treatment of a variety of cognitive disorders, and the findings of this study support this approach. Selective α7-nAChR agonists may provide a mechanism to improve cognition without the addictive properties of nicotine that may arise from β2-containing nAChRs (51). Our findings emphasize that appropriate dosage will be key to the success of developing cognitive enhancers for humans, given the inverted-U dose–response curves in neuronal firing and behavioral performance (28). The loss of beneficial effects with increasing dose may not be due to receptor desensitization or side effects (as may occur with still-higher doses), but instead arises from nonspecific effects on neuronal excitability. Thus, successful cognitive enhancement in humans will require subtle manipulations that enhance mental representations and respect the sensitivity and specificity of dlPFC circuitry.

A number of mental disorders involve deficits in dlPFC function. Schizophrenia is characterized by grave impairments in WM (52) that are associated with reduced dlPFC activity and thought disorder (53). Reductions in dlPFC activity are also associated with dysregulated behavior and attention in mania (54) and ADHD (55), especially in the right hemisphere, whereas dorsomedial PFC hypoactivity is associated with social deficits in both schizophrenia and autism (56). PFC deficits are also a component of dementias such as AD, where there is early accumulation of β-amyloid and phosphorylated tau early in the disease, including tangles in layer III pyramidal cells (17). All of these disorders involve genetic alterations (24, 57–60) or molecular interactions (25, 61) of/with α7-nAChR signaling, including evidence of reduced receptor expression in PFC of patients with schizophrenia (22) or autism spectrum disorders (60). Genetic alterations of α7-nAChRs in schizophrenia are particularly well established (18). High rates of smoking in schizophrenia may be an attempt to normalize the firing pattern of the dlPFC circuits most afflicted in schizophrenia, and recent data indicate that smoking may help to normalize α7-nAChR expression in these patients (22). The current data suggest that smoking may help to bring PFC NMDAR-driven circuits online, and that selective α7-nAChR agonists may ameliorate key deficits in schizophrenia. As PFC circuits also provide top-down regulation of emotion, stimulation of α7-nAChRs in PFC may also contribute to the mood-stabilizing effects of nicotine in individuals with posttraumatic stress disorder (62) and depression (63). The current study provides a molecular and cellular basis for these therapeutic effects, demonstrating a key role for α7-nAChRs in strengthening primate PFC cognitive function.

Materials and Methods

All experiments were performed in accordance with National Institutes of Health guidelines, and were approved by the Yale Institutional Animal Care and Use Committee. Further details can be found in *SI Materials and Methods*.

Physiology and Iontophoresis. Details of the recording and iontophoresis procedures have been described previously (13). Two-way ANOVA was used to examine the spatially tuned delay-related activity. One-way or two-way ANOVA was used to test drug effects.

Electron Microscopic Localization. Immunoelectron microscopy and α-bungarotoxin were used to label α7-nAChRs and to colabel for vesicular ACh transporter (VAChT) in layers II/III of monkey dlPFC. All single- and double-labeling procedures, including controls, and electron microscopy processing have been described previously (64).

Behavioral Studies. The drug effects on spatial working memory performance were assessed using a manual delayed-response task in a Wisconsin General Testing Apparatus as described previously (65).

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1. Hobson JA (1992) Sleep and dreaming: Induction and mediation of REM sleep by cholinergic mechanisms. *Curr Opin Neurobiol* 2(6):759–763.
2. McCormick DA, Bal T (1997) Sleep and arousal: Thalamocortical mechanisms. *Annu Rev Neurosci* 20:185–215.
3. Steriade M (2004) Acetylcholine systems and rhythmic activities during the waking–sleep cycle. *Prog Brain Res* 145:179–196.
4. Parikh V, Sarter M (2008) Cholinergic mediation of attention: Contributions of phasic and tonic increases in prefrontal cholinergic activity. *Ann N Y Acad Sci* 1129:225–235.
5. Lee SH, Dan Y (2012) Neuromodulation of brain states. *Neuron* 76(1):209–222.
6. Perry E, Walker M, Grace J, Perry R (1999) Acetylcholine in mind: A neurotransmitter correlate of consciousness? *Trends Neurosci* 22(6):273–280.
7. Herrero JL, et al. (2008) Acetylcholine contributes through muscarinic receptors to attentional modulation in V1. *Nature* 454(7208):1110–1114.
8. Croxson PL, Kyriazis DA, Baxter MG (2011) Cholinergic modulation of a specific memory function of prefrontal cortex. *Nat Neurosci* 14(12):1510–1512.
9. Goldman-Rakic PS (1995) Cellular basis of working memory. *Neuron* 14(3):477–485.
10. Fuster JM (2008) *The Prefrontal Cortex* (Academic, San Diego), 4th Ed.
11. Quintana J, Fuster JM (1999) From perception to action: Temporal integrative functions of prefrontal and parietal neurons. *Cereb Cortex* 9(3):213–221.
12. Chafee MV, Goldman-Rakic PS (1998) Matching patterns of activity in primate prefrontal area 8a and parietal area 7ip neurons during a spatial working memory task. *J Neurophysiol* 79(6):2919–2940.
13. Wang M, et al. (2013) NMDA receptors subserve persistent neuronal firing during working memory in dorsolateral prefrontal cortex. *Neuron* 77(4):736–749.
14. Elston GN, et al. (2006) Specializations of the granular prefrontal cortex of primates: Implications for cognitive processing. *Anat Rec A Discov Mol Cell Evol Biol* 288(1): 26–35.
15. Selemon LD, Goldman-Rakic PS (1999) The reduced neuropil hypothesis: A circuit based model of schizophrenia. *Biol Psychiatry* 45(1):17–25.
16. Glantz LA, Lewis DA (2000) Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Arch Gen Psychiatry* 57(1):65–73.
17. Bussière T, et al. (2003) Progressive degeneration of nonphosphorylated neurofilament protein-enriched pyramidal neurons predicts cognitive impairment in Alzheimer's disease: Stereologic analysis of prefrontal cortex area 9. *J Comp Neurol* 463(3):281–302.
18. Martin LF, Freedman R (2007) Schizophrenia and the alpha7 nicotinic acetylcholine receptor. *Int Rev Neurobiol* 78:225–246.
19. Hajós M, Rogers BN (2010) Targeting alpha7 nicotinic acetylcholine receptors in the treatment of schizophrenia. *Curr Pharm Des* 16(5):538–554.
20. Severance EG, Yolken RH (2008) Novel alpha7 nicotinic receptor isoforms and deficient cholinergic transcription in schizophrenia. *Genes Brain Behav* 7(1):37–45.
21. Mathew SV, et al. (2007) Alpha7 nicotinic acetylcholine receptor mRNA expression and binding in postmortem human brain are associated with genetic variation in neuregulin 1. *Hum Mol Genet* 16(23):2921–2932.
22. Mexal S, et al. (2010) Differential regulation of alpha7 nicotinic receptor gene (CHRNA7) expression in schizophrenic smokers. *J Mol Neurosci* 40(1-2):185–195.
23. Deutsch SI, Urbano MR, Burket JA, Herndon AL, Winebarger EE (2011) Pharmacotherapeutic implications of the association between genomic instability at chromosome 15q13.3 and autism spectrum disorders. *Clin Neuropharmacol* 34(6):203–205.
24. Williams NM, et al. (2012) Genome-wide analysis of copy number variants in attention deficit hyperactivity disorder: The role of rare variants and duplications at 15q13.3. *Am J Psychiatry* 169(2):195–204.
25. Parri HR, Hernandez CM, Dineley KT (2011) Research update: Alpha7 nicotinic acetylcholine receptor mechanisms in Alzheimer's disease. *Biochem Pharmacol* 82(8): 931–942.
26. Buccafusco JJ, Terry AV, Jr. (2009) A reversible model of the cognitive impairment associated with schizophrenia in monkeys: Potential therapeutic effects of two nicotinic acetylcholine receptor agonists. *Biochem Pharmacol* 78(7):852–862.
27. Cannon CEPV, et al. (2013) The nicotinic α7 receptor agonist GTS-21 improves cognitive performance in ketamine impaired rhesus monkeys. *Neuropharmacology* 64: 191–196.
28. Castner SA, et al. (2011) Immediate and sustained improvements in working memory after selective stimulation of α7 nicotinic acetylcholine receptors. *Biol Psychiatry* 69(1): 12–18.
29. Xu J, et al. (2006) Effects of acute smoking on brain activity vary with abstinence in smokers performing the N-Back task: A preliminary study. *Psychiatry Res* 148(2-3): 103–109.
30. Levin ED (2012) α7-Nicotinic receptors and cognition. *Curr Drug Targets* 13(5): 602–606.
31. Young JW, et al. (2007) Impaired attention is central to the cognitive deficits observed in alpha 7 deficient mice. *Eur Neuropsychopharmacol* 17(2):145–155.
32. Thomsen MS, El-Sayed M, Mikkelsen JD (2011) Differential immediate and sustained memory enhancing effects of alpha7 nicotinic receptor agonists and allosteric modulators in rats. *PLoS One* 6(11):e27014.
33. Freedman R, et al. (2008) Initial phase 2 trial of a nicotinic agonist in schizophrenia. *Am J Psychiatry* 165(8):1040–1047.
34. Rao TS, Correa LD, Adams P, Santori EM, Sacaan AI (2003) Pharmacological characterization of dopamine, norepinephrine and serotonin release in the rat prefrontal cortex by neuronal nicotinic acetylcholine receptor agonists. *Brain Res* 990(1-2): 203–208.
35. Parikh V, Ji J, Decker MW, Sarter M (2010) Prefrontal beta2 subunit-containing and alpha7 nicotinic acetylcholine receptors differentially control glutamatergic and cholinergic signaling. *J Neurosci* 30(9):3518–3530.
36. Berg DK, Conroy WG, Liu Z, Zago WM (2006) Nicotinic signal transduction machinery. *J Mol Neurosci* 30(1-2):149–152.
37. Konradsson-Geuken A, et al. (2009) Second-by-second analysis of alpha 7 nicotine receptor regulation of glutamate release in the prefrontal cortex of awake rats. *Synapse* 63(12):1069–1082.
38. Couey JJ, et al. (2007) Distributed network actions by nicotine increase the threshold for spike-timing-dependent plasticity in prefrontal cortex. *Neuron* 54(1):73–87.
39. Mansvelder HD, Mertz M, Role LW (2009) Nicotinic modulation of synaptic transmission and plasticity in cortico-limbic circuits. *Semin Cell Dev Biol* 20(4):432–440.
40. Han ZY, et al. (2003) Localization of [^{3}H]nicotine, [^{3}H]cytisine, [^{3}H]epibatidine, and [^{125}I]alpha-bungarotoxin binding sites in the brain of *Macaca mulatta*. *J Comp Neurol* 461(1):49–60.
41. Tian MK, Bailey CD, De Biasi M, Picciotto MR, Lambe EK (2011) Plasticity of prefrontal attention circuitry: Upregulated muscarinic excitability in response to decreased nicotinic signaling following deletion of α5 or β2 subunits. *J Neurosci* 31(45): 16458–16463.
42. Guillem K, et al. (2011) Nicotinic acetylcholine receptor β2 subunits in the medial prefrontal cortex control attention. *Science* 333(6044):888–891.
43. Poorthuis RB, et al. (2013) Layer-specific modulation of the prefrontal cortex by nicotinic acetylcholine receptors. *Cereb Cortex* 23(1):148–161.
44. Santini E, Sepulveda-Orengo M, Porter JT (2012) Muscarinic receptors modulate the intrinsic excitability of infralimbic neurons and consolidation of fear extinction. *Neuropsychopharmacology* 37(9):2047–2056.
45. Isaac JT, Nicoll RA, Malenka RC (1995) Evidence for silent synapses: Implications for the expression of LTP. *Neuron* 15(2):427–434.
46. Levy RB, Aoki C (2002) Alpha7 nicotinic acetylcholine receptors occur at postsynaptic densities of AMPA receptor-positive and -negative excitatory synapses in rat sensory cortex. *J Neurosci* 22(12):5001–5015.
47. Buzsàki G, Gage FH (1989) The cholinergic nucleus basalis: A key structure in neocortical arousal. *EXS* 57:159–171.
48. Hasselmo ME, Sarter M (2011) Modes and models of forebrain cholinergic neuromodulation of cognition. *Neuropsychopharmacology* 36(1):52–73.
49. Muzur A, Pace-Schott EF, Hobson JA (2002) The prefrontal cortex in sleep. *Trends Cogn Sci* 6(11):475–481.
50. Arnsten AF, Wang MJ, Paspalas CD (2012) Neuromodulation of thought: Flexibilities and vulnerabilities in prefrontal cortical network synapses. *Neuron* 76(1):223–239.
51. Picciotto MR, et al. (1998) Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature* 391(6663):173–177.
52. Barch DM, Ceaser A (2012) Cognition in schizophrenia: Core psychological and neural mechanisms. *Trends Cogn Sci* 16(1):27–34.
53. Perlstein WM, Carter CS, Noll DC, Cohen JD (2001) Relation of prefrontal cortex dysfunction to working memory and symptoms in schizophrenia. *Am J Psychiatry* 158(7):1105–1113.
54. Blumberg HP, et al. (1999) Rostral and orbital prefrontal cortex dysfunction in the manic state of bipolar disorder. *Am J Psychiatry* 156(12):1986–1988.
55. Rubia K, Smith AB, Brammer MJ, Toone B, Taylor E (2005) Abnormal brain activation during inhibition and error detection in medication-naive adolescents with ADHD. *Am J Psychiatry* 162(6):1067–1075.
56. Sugranyes G, Kyriakopoulos M, Corrigall R, Taylor E, Frangou S (2011) Autism spectrum disorders and schizophrenia: Meta-analysis of the neural correlates of social cognition. *PLoS One* 6(10):e25322.
57. Leonard S, Freedman R (2006) Genetics of chromosome 15q13-q14 in schizophrenia. *Biol Psychiatry* 60(2):115–122.
58. Hong CJ, Lai IC, Liou LL, Tsai SJ (2004) Association study of the human partially duplicated alpha7 nicotinic acetylcholine receptor genetic variant with bipolar disorder. *Neurosci Lett* 355(1-2):69–72.
59. Ancín I, et al. (2010) Evidence for association of the non-duplicated region of CHRNA7 gene with bipolar disorder but not with schizophrenia. *Psychiatr Genet* 20(6): 289–297.
60. Yasui DH, et al. (2011) 15q11.2-13.3 chromatin analysis reveals epigenetic regulation of CHRNA7 with deficiencies in Rett and autism brain. *Hum Mol Genet* 20(22): 4311–4323.
61. Snyder EM, et al. (2005) Regulation of NMDA receptor trafficking by amyloid-beta. *Nat Neurosci* 8(8):1051–1058.
62. Rasmusson AM, Picciotto MR, Krishnan-Sarin S (2006) Smoking as a complex but critical covariate in neurobiological studies of posttraumatic stress disorders: A review. *J Psychopharmacol* 20(5):693–707.
63. Mineur YS, Picciotto MR (2010) Nicotine receptors and depression: Revisiting and revising the cholinergic hypothesis. *Trends Pharmacol Sci* 31(12):580–586.
64. Paspalas CD, Wang M, Arnsten AF (2013) Constellation of HCN channels and cAMP regulating proteins in dendritic spines of the primate prefrontal cortex: Potential substrate for working memory deficits in schizophrenia. *Cereb Cortex* 23(7): 1643–1654.
65. Gamo NJ, Wang M, Arnsten AFT (2010) Methylphenidate and atomoxetine improve prefrontal cortical function via noradrenergic alpha-2 and dopaminergic D1 receptor stimulation. *J Am Acad Child Adolesc Psychiatry* 49:1011–1023.