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Targeting the Actions of Muscarinic Receptors on Dopamine Systems: New Strategies for Treating Neuropsychiatric Disorders

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Abstract

Cholinergic regulation of dopamine (DA) signaling has significant implications for numerous disorders, including schizophrenia, substance use disorders, and mood-related disorders. The activity of midbrain DA neurons and DA release patterns in terminal regions are tightly regulated by cholinergic neurons found in both the striatum and the hindbrain. These cholinergic neurons can modulate DA circuitry by activating numerous receptors, including muscarinic acetylcholine receptor (mAChR) subtypes. This review specifically focuses on the complex role of M2, M4, and M5 mAChR subtypes in regulating DA neuron activity and DA release and the potential clinical implications of targeting these mAChR subtypes.

CHOLINERGIC REGULATION OF DOPAMINE SIGNALING

Dopamine (DA) neuron activity and signaling play a crucial role in regulating brain circuits that control a wide range of behavioral outputs, including (but not limited to) motivation, motor control, reward processing, and cognition (1–3). Midbrain DA neurons can be subdivided broadly into two primary nuclei, the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA). DA neurons of the SNc project to the dorsal striatum (DS), while DA neurons of the VTA project to the nucleus accumbens (NAc) and cortical areas (4). Moreover, the DS and NAc can be further subdivided into anatomical regions with different cortical and thalamic inputs. For example, the lateral DS receives substantial inputs from the motor cortex and is heavily involved in motor learning, habitual behaviors, and action selection (5–9). In contrast, the medial DS receives inputs from somatosensory cortices and can play a key role in shaping goal-directed actions, compulsive behavior, and skill learning (10–12). Similarly, the NAc can be subdivided into core and shell regions with different projection patterns and inputs, which are implicated in motivated behavior, saliency, and reward processing (13–15). The ability of DA to regulate such a vast and diverse array of behavioral outputs is due, at least in part, to the fact that subpopulations of DA neurons are integrated into brain circuits that are involved in only a subset of these behavioral outcomes. Consistent with a critical role for DA in regulating these circuits, dysregulation of DA signaling is thought to play a crucial role in numerous disorders, including schizophrenia, depression, substance use disorders, and Parkinson's disease.

DA signaling is controlled by numerous neurotransmitters, including the neuromodulator acetylcholine (ACh). DA and ACh have been conceptualized as opposing neuromodulators where a balance is required for proper motor function (16, 17). For example, in Parkinson's disease, the death of DA neurons leads to a hypodopaminergic state that can be treated by boosting DA signaling with levodopa or tamping down cholinergic signaling with muscarinic receptor antagonists (18). This finding is consistent with the hypothesis that restoring the balance between ACh and DA signaling is critical to restoring normal motor function (19). As outlined below, new pharmacological and genetic tools have allowed detailed studies focused on understanding how the activation of different cholinergic receptor subtypes can regulate DA release. These studies have revealed a complex interaction between ACh and DA where different cholinergic receptors can regulate DA release in opposing directions depending on the receptor subtype activated and the brain region being assessed. In this review, we focus on the role of midbrain and striatal muscarinic acetylcholine receptors (mAChRs) in regulating DA neuron activity and release. We discuss how modulation of specific mAChR subtypes can provide robust modulation of DA signaling. We conclude by suggesting the therapeutic potential of targeting specific subtypes of mAChRs in the treatment of symptoms related to psychiatric conditions, including schizophrenia, depression, and substance use disorder (20, 21).

CHOLINERGIC SIGNALING IN THE MIDBRAIN

The midbrain, including the SNc and VTA, receives robust cholinergic inputs from the pedunculopontine nucleus (PPN) and laterodorsal tegmental nucleus (LDTg) (22). The LDTg is considered a primary cholinergic input to the VTA, with the caudal portion of PPN also innervating the VTA (23, 24), whereas the PPN is the primary cholinergic input into the SNc (25). Inactivation of the PPN and LDTg reduces phasic firing in VTA DA neurons, while activation facilitates burst firing (26–28), indicating that the PPN and LDTg can bidirectionally regulate DA neuron excitability.

Cholinergic innervation from the PPN and LDTg regulates neuronal activity in the midbrain by activating numerous receptor subtypes, including receptors belonging to either the nicotinic receptor or muscarinic receptor families (29). Nicotinic acetylcholine receptors (nAChRs) are

ionotropic receptors and can robustly regulate DA neuron activity, DA release, and reward-related behaviors, including nicotine reward (30; for a comprehensive overview of nicotinic receptor regulation of DA circuits, see 31–33). Here, we review how the activation of different mAChRs can influence DA signaling and DA-related behaviors. The mAChR family comprises five subtypes (M1–M5), all of which are G protein–coupled receptors. M1, M3, and M5 receptors canonically signal through activation of Gq-mediated signaling pathways, including Ca^{2+} mobilization and activation of phospholipase C. In contrast, M2 and M4 receptors typically signal through Gi-mediated pathways, resulting in the inhibition of cAMP accumulation. However, these receptors are complex and can regulate physiology and behavior through numerous noncanonical signaling pathways as well (34–36).

Of the five muscarinic receptor subtypes, M5 receptor messenger RNA is localized to SNc and VTA DA neurons (37–39), where it is the only mAChR subtype to have been detected. The expression of M5 receptors on DA neurons, combined with a relatively low expression level in other brain regions, highlights the potential for targeting M5 receptors to regulate DA neuron activity and DA release. In addition to M5 receptors, M2 and M4 receptors are also expressed in the midbrain on non-DA neurons. Both M2 and M4 receptors function as autoreceptors on LDTg and PPN terminals in the VTA (40, 41) (see **Figure 1**). Furthermore, M4 receptors are also expressed as heteroreceptors on the terminals of direct-pathway spiny projection neurons

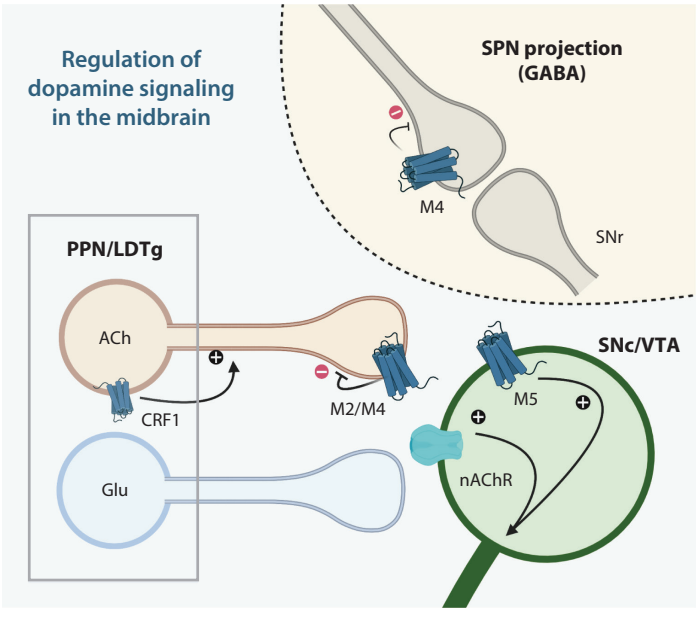

Figure 1

Muscarinic acetylcholine receptor expression in the midbrain and impact on dopamine circuit activity. M5 receptors are the only muscarinic receptor present on dopamine neurons, where they act to increase dopamine neuron excitability. M2/M4 receptor subtypes are expressed on cholinergic inputs to the midbrain, where they can inhibit ACh release and concurrent ACh-mediated changes in midbrain physiology. Finally, M4 receptors are expressed on SPN terminals and can inhibit GABA release and thereby regulate dopamine circuit activity outside of the basal ganglia. Abbreviations: ACh, acetylcholine; CRF1, corticotropin-releasing hormone receptor 1; GABA, γ-aminobutyric acid; Glu, glutamatergic; LDTg, laterodorsal tegmental nucleus; nAChR, nicotinic acetylcholine receptor; PPN, pedunculopontine nucleus; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; SPN, spiny projection neuron; VTA, ventral tegmental area. Figure adapted from images created with BioRender.com.

(SPNs) projecting into the substantia nigra pars reticulata (SNr), where they can reduce GABA release (25). As discussed below, these receptor populations can mediate distinct effects on DA circuit function.

REGULATION OF DA CIRCUITS BY M5 RECEPTORS IN THE MIDBRAIN

Cholinergic stimulation of midbrain nAChRs and mAChRs has been shown to increase DA neuron activity and promote burst firing, which increases DA levels in SNc and NAc (42, 43). In contrast, blockade of nAChRs and mAChRs in the VTA decreased phasic-evoked DA release in the NAc (44), demonstrating that modulation of cholinergic signaling can bidirectionally control DA release. Further studies using M5 knockout (KO) mice demonstrated that the M5 receptor was the primary receptor subtype mediating the effects seen with mAChR agonists (45, 46). The recent development of M5-selective pharmacological tools has further expanded our ability to study the impact of M5 receptor activation on DA signaling (47–49). Consistent with M5 KO mice results, M5-selective positive allosteric modulators (PAMs) can potentiate, and M5-selective antagonists can inhibit, the mAChR agonist-induced increases in midbrain DA neuron firing rates (50, 51). Taken together, results obtained using these genetic and pharmacological tools provide strong evidence that M5 receptors in the midbrain can robustly regulate DA neuron activity and release in downstream terminal regions, including the striatum and NAc.

REGULATION OF DA CIRCUITS BY M2/M4 RECEPTORS IN THE MIDBRAIN

The PPN and LDTg regulate striatal DA activity by releasing ACh and activating both mAChRs and nAChRs expressed on midbrain DA neurons of the SNc and VTA. M2 and M4 receptor subtypes are expressed on PPN and LDTg terminals in the SNc and VTA, which function as inhibitory presynaptic autoreceptors. Activation of these autoreceptors can result in reduced ACh release, thereby inhibiting ACh-induced activation of DA neurons (40, 52–54) (**Figure 1**). Interestingly, local injection of a mAChR agonist into the LDTg can also decrease the level of ACh in the VTA as measured by microdialysis (55), suggesting that mAChRs located on or near the LDTg can also influence ACh release. Furthermore, M4 KO, but not M2 KO, mice demonstrated an increased basal level of ACh in the VTA (41), suggesting that M4 receptors may play a more prominent role in regulating the activity of the LDTg. Consistent with this, mAChR antagonist–induced increases in midbrain ACh levels were absent in M4 KO but unchanged in M2 KO animals (41). Collectively these studies point to a critical role for M4 receptors in regulating midbrain ACh levels and concurrent ACh-induced changes in DA neuron activity. In addition to M4 receptor-mediated regulation of ACh release, M4 receptors are also expressed in the midbrain on GABAergic terminals of direct-pathway SPNs synapsing onto SNr neurons (see the SPN in **Figure 1**). Striatal DA release provides key regulation of these SPNs, which then release GABA onto SNr neurons. Accordingly, regulation of GABA release at the SPN-SNr synapse represents a downstream node at which DA circuit activity can be modulated. Activation of M4 receptors on these terminals can robustly reduce SPN-mediated GABA release in the SNr and locomotor activity (25). Collectively, these studies support the idea that M4 receptors in the midbrain can play key roles in regulating DA circuits through numerous mechanisms, including modulation of midbrain ACh and GABA release.

CHOLINERGIC REGULATION OF STRIATAL DA SIGNALING

While ACh-mediated regulation of DA neuron excitability in the midbrain plays a crucial role in regulating dopaminergic signaling, striatal ACh also plays a profound role in bidirectionally

modulating DA release. Cholinergic receptor mechanisms in the striatum can regulate local DA release independent of DA neuron activity, with significant behavioral implications (14). The primary source of ACh in the striatum is cholinergic interneurons (CINs) that are tonically active but can also be driven to burst or pause in response to behaviorally relevant stimuli (29, 56). In addition to CINs, the striatum also receives cholinergic inputs from the LDTg and PPN (57). While these hindbrain cholinergic inputs play a key role in regulating SPN activity and operant responding (58), they do not appear to robustly modulate DA release (59), indicating that CINs are the primary source of cholinergic regulation of local DA release in the striatum. Numerous systems tightly regulate CIN activity, including glutamatergic signaling (57–59), GABAergic signaling (60–63), and cholinergic signaling via mAChR autoreceptors (**Figure 2**). As outlined below, activation of striatal mAChRs can provide bidirectional control of DA release via numerous mechanisms that could be of potential utility in treating numerous psychiatric disorders.

REGULATION OF DA RELEASE BY STRIATAL mAChR AUTORECEPTORS

One of the most robust effects of striatal ACh on DA signaling is to promote DA release via activation of nAChRs located presynaptically at DA release sites (**Figure 2**). Activation of striatal

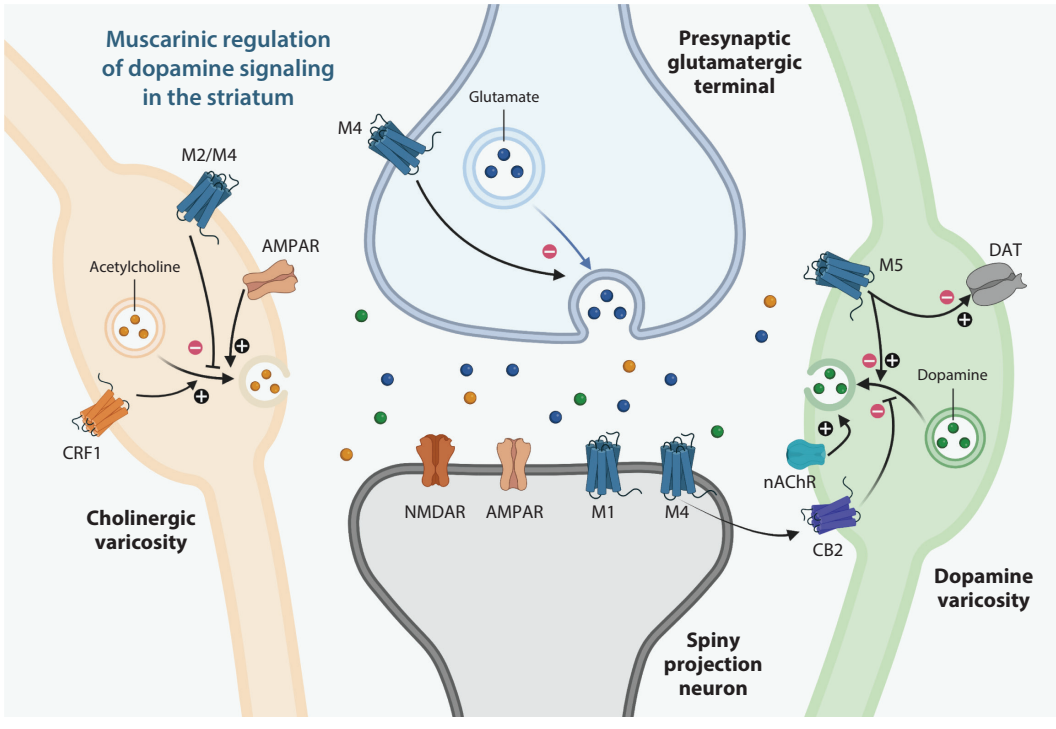

Figure 2

Muscarinic acetylcholine receptor expression in the striatum and impact on dopamine signaling. M2/M4 autoreceptors can inhibit acetylcholine release and concurrent acetylcholine-mediated regulation of DA release. M5 receptors found on DA terminals can directly regulate DA release, while M4 receptor subtypes expressed on spiny projection neurons can alter DA release via a CB2-dependent mechanism. Abbreviations: AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CB2, cannabinoid receptor 2; CRF1, corticotropin-releasing hormone receptor 1; DA, dopamine; DAT, dopamine transporter; nAChR, nicotinic acetylcholine receptor; NMDAR, *N*-methyl-D-aspartate receptor. Figure adapted from images created with BioRender.com.

nAChRs is sufficient to induce and potentiate the DA release that occurs with coincident excitation of DA terminals (60). However, when DA terminals are activated in a phasic, burst-like manner, nAChR activation reduces DA release, indicating that nAChRs act more as a DA filter than an enhancer or inhibitor of DA release (31). One of the best-studied mechanisms whereby mAChRs can regulate DA release is via activation of autoreceptors located presynaptically on CINs. Muscarinic agonists can inhibit evoked ACh release, an effect that is absent in M2/M4 KO mice and greatly reduced in M4 KO mice (61), indicating that M4 may be the primary autoreceptor subtype in the striatum. Application of mAChR agonists can induce a robust inhibition of DA release in both the DS and NAc that is sensitive to nAChR antagonists (62–64). Unlike nAChR antagonists, the application of mAChR antagonists has little to no effect on striatal DA release (60, 64), suggesting that mAChRs do not tonically regulate DA release in brain slice preparations. Elegant whole-cell experiments have observed M4 receptor–mediated spontaneous currents in SPNs that overexpress G protein–gated inwardly rectifying potassium (GIRK) channels (65), indicating that striatal ACh tone in brain slices is sufficient to provide at least some level of tonic mAChR activation. However, the application of M4-selective PAMs, which require ACh to induce receptor activation, does not affect either DA release or corticostriatal transmission in the absence of an exogenous agonist (62, 66). Interestingly, the ability of an M4-selective PAM to regulate spike timing–dependent plasticity was found to be absent with moderate repetitions of pulse pairing but was unmasked using the same stimulation paradigm when combined with either designer receptors exclusively activated by designer drugs (DREADD)-induced activation of CINs or inhibition of ACh cholinesterase (67). Furthermore, the application of a mAChR antagonist does not affect DA release evoked when CINs are stimulated by a single pulse, but it significantly increases DA release when CINs are optically activated in a phasic pattern (68). Collectively, these findings suggest that in striatal brain slices, where CIN firing rates are notably lower than those observed in vivo, levels of ACh release are sufficient to activate some mAChRs but are not sufficient to induce mAChR-mediated regulation of DA release. Accordingly, nAChR-mediated enhancement of DA release may prevail at lower ACh levels, while mAChR-mediated mechanisms may play a bigger role when ACh levels are more elevated. The mechanistic explanation for how tonic ACh release in brain slices is sufficient to activate robust nAChR- but not mAChR-mediated effects on DA release is not clear but may have to do with receptor expression levels or proximity of specific receptor populations from ACh release sites. Further studies are needed to further elucidate the relationship between ACh-induced, mAChR-mediated modulation of DA release and how it relates to CIN activity both in brain slices and in vivo.

REGULATION OF DA RELEASE BY POSTSYNAPTIC M4 RECEPTORS

In addition to being expressed as autoreceptors on CINs, M4 receptors are also expressed postsynaptically on SPNs expressing the D1 subtype of the dopamine receptor (D1-SPNs) (69). This population of postsynaptic M4 receptors has been demonstrated to be critical modulators of DA-dependent behaviors such as psychostimulant-induced locomotion (70), as well as the antipsychotic-like behavioral efficacy of the muscarinic agonist xanomeline (71). Activation of mAChR receptors in the DS can induce a sustained inhibition of DA release that can be observed even in the presence of nAChR antagonists, indicating that this regulation is not mediated by autoreceptors. This inhibition was found to be mediated by M4 receptors on D1-SPNs via a cannabinoid type 2 (CB2) receptor–dependent mechanism (62). While M4 receptors are not canonically thought of as regulators of cannabinoid signaling, it has also been observed that M4-mediated long-term depression of glutamatergic inputs onto D1-SPNs is blocked by a CB1 antagonist (67). While more work is needed to determine the mechanisms whereby M4

activation can regulate endocannabinoid release and how endocannabinoid release can regulate DA release, these results suggest that CB2 receptors may be a novel target for regulating DA release and antipsychotic activity (72). While the administration of M4 PAMs has been demonstrated to reduce amphetamine-induced striatal DA release in vivo (73), the contribution of different M4 subpopulations to regulate spontaneous DA release in different striatal regions in vivo has yet to be determined. Future studies will be needed to further elucidate the roles for M4 autoreceptor– and heteroreceptor-mediated regulation of DA release to better understand how targeting these receptors could modulate DA signaling in numerous diseases, including schizophrenia, L-DOPA-induced dyskinesias, and substance use disorders.

REGULATION OF DA RELEASE BY STRIATAL M5 RECEPTORS

In addition to their robust effects on regulating DA release in the midbrain, M5 receptors expressed on DA terminals can also dynamically control striatal DA release. Activation of mAChRs in the NAc can increase DA and glutamate release independent of nAChRs, an effect that is absent in M5 KO, but not M2/M4 KO, mice (63). Interestingly, the M5-mediated increase in DA release is dependent on both agonist concentration and duration of agonist exposure. Low agonist concentrations induce a slow ramping increase in DA release that peaks at 15–20 min of continuous agonist exposure. However, continuous exposure to high agonist concentrations induces a similar magnitude of DA release enhancement that peaks within 5 min and then rapidly returns to baseline (74). The agonist Oxo-M is a hydrophilic compound (XLogP = −0.18), making it unlikely that the temporal nature of M5-mediated increases in DA release is due to drug exposure, as Oxo-M should rapidly equilibrate throughout brain slices. While the mechanisms underlying the time-dependent M5-mediated effects of DA release are not yet understood, it is possible that Oxo-M can modulate DA release through different mAChR subtypes that modulate DA release on different timescales. Consistent with this hypothesis, broad activation of mAChRs induced a slight decrease in DA release in the NAc of M5 KO mice (63). In addition, broad activation of mAChRs with high concentrations of Oxo-M has been observed to inhibit nAChR-independent DA release in the DS (62), an outcome that is reversed by an M4-selective antagonist that unmasks Oxo-M-mediated increases in DA release (75). Collectively, these findings could suggest that Oxo-M can induce both an enhancement of non-nAChR-mediated DA release through M5 receptors and a slower onset inhibition of DA release that is M4 receptor mediated. Future studies employing subtype-selective pharmacological tools and nonconstitutive KO mice could shed further light onto the seemingly opposing effects of M4 and M5 receptors on striatal DA release.

The mechanisms whereby M5 receptor activation modulates striatal DA release are still unclear. Since M5 receptors are expressed on DA terminals and canonically signal through Gq signaling pathways, it is possible that M5 could enhance DA release by mobilizing presynaptic Ca^{2+} signaling and concurrently modifying neurotransmitter release probability. In addition, M5 receptors can be rapidly recycled (76), and changes in M5 surface expression over time could also change the temporal aspects of how M5 receptors modulate DA release. M5 receptor activation in cultured neurons has been demonstrated to facilitate dopamine transporter (DAT) internalization via a protein kinase C (PKC)-dependent mechanism (77), indicating that M5 may also be able to regulate DA reuptake. Consistent with this hypothesis, presynaptic Gq-coupled receptors have also been demonstrated to cause biphasic changes in DAT transport that are capable of inducing either DAT insertion into the membrane via a D2 subtype of the dopamine receptor–dependent mechanism or PKC-mediated DAT internalization, causing increased striatal DA release following Gq-coupled receptor activation (78). Studies using the nonselective agonist Oxo-M have shown that M5 activation can induce an increase in DA release in striatal slices

(63, 74). However, selective potentiation of M5 using the M5-selective PAM VU0238429 unexpectedly inhibited DA release in the DS, an effect that was absent in M5 KO mice (51). While the reason for this discrepancy is still not clear, it is possible that this PAM may induce a form of signal bias that could alter M5 receptor recycling, PKC-dependent DAT trafficking, or presynaptic Ca^{2+} signaling that shifts the net result of M5 activation on DA release. It is also possible that the importance of these mechanisms may be different across striatal subregions. While the role of M5 receptor–mediated changes in striatal DA release is still not completely understood, it is possible that multiple mechanisms discussed above could lead to the kinetics of mAChR-mediated changes that are observed in DA release, as well as some of the regional specificity. Future studies are needed to shed light on the physiological importance of these different mechanisms, as well as shifting to look at the mechanisms and temporal changes in DA release that are induced by activation of striatal mAChRs by native ACh signaling.

BEHAVIORAL AND THERAPEUTIC IMPLICATIONS

The ability of mAChRs in the midbrain and striatum to regulate DA activity has significant implications for drug- and mood-related behaviors in rodents and humans (20, 79). Mice lacking the M5 receptor show decreases in the reinforcing effects of cocaine and morphine but not for food (80, 81). Consistent with this, systemic administration of the M5 negative allosteric modulator (NAM) ML375 decreased self-administration of cocaine, opiates, and alcohol, including the ability of drug-associated cues to reinstate responding (37, 82, 83). Furthermore, the administration of a short-acting M5 NAM (VU6008667) has also been demonstrated to decrease oxycodone self-administration without affecting oxycodone antinociception (84). M5 receptors, particularly in the VTA, are also implicated in regulation of mood-related behaviors in rats (20). While the effects of M5 NAMs on drug self-administration can provide robust behavioral effects across multiple substances, we still do not know how different M5 receptor subpopulations contribute to these responses. Future studies using local infusions of M5-selective drugs will be needed to elucidate the role of M5 receptors expressed on DA neurons in the midbrain and M5 receptors expressed on DA terminals in the striatum and NAc in altering self-administration. Interestingly, infusion of a M5 NAM into the VTA attenuates the prodepressive behavioral responses, including impairments in motivated behavior induced by increased cholinergic tone in the VTA (85, 86), indicating that modulation of M5 in the midbrain is sufficient to alter depression-related responses. The ability of M5-selective drugs to robustly modulate drug- and mood-related behavioral responses, combined with the restricted expression pattern of M5 receptors, makes this receptor an exciting target for treating DA-related disorders.

M4 receptors are also implicated in drug- and mood-related behaviors in rodents, as administration of M4 PAM decreases cocaine self-administration and shifts behavior away from cocaine taking to a liquid food reward (87). Moreover, M4 PAM administration can attenuate cocaine-induced hyperactivity, and reinstatement, as measured by the conditioned place preference test (88). Interestingly, M4 receptors on CINs and M4 receptors on SPNs seem to mediate opposing functions regarding natural rewards and the rewarding aspects of drugs of abuse (89).

In addition, activation of M4 receptors can also induce robust antipsychotic-like behavioral effects (90) that are mediated primarily via M4 receptors expressed on SPNs (71), an effect that is likely in part mediated by M4-mediated regulation of DA release (62, 73). Numerous ongoing clinical trials are currently testing mAChR-based therapies as a novel strategy for treating schizophrenia. These clinical efforts are primarily built off a seminal study demonstrating that the mAChR agonist xanomeline can improve outcome measures related to the positive, negative, and cognitive symptoms in schizophrenics (91). However, a clinical limitation of mAChR agonists

is their peripheral side effects, including gastrointestinal discomfort, bradycardia, and increased sweating and secretions (92). To counteract the peripheral effects of mAChR agonists, therapeutic drugs have been developed that incorporate a peripherally restricted muscarinic antagonist (93). This strategy has been shown to have a reduced side effect profile associated with peripheral stimulation of mAChRs (94). In addition, M4-selective PAMs are being tested in the clinic as a strategy to maintain antipsychotic efficacy with reduced side effect liability (95). Ongoing and future clinical studies will yield important insights into the ability of mAChR-based therapies to treat schizophrenia and could open the door to exploring these targets as potential therapeutics for a range of DA-related disorders, including substance use, depression, and schizophrenia.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Salamone JD, Correa M. 2012. The mysterious motivational functions of mesolimbic dopamine. *Neuron* 76:470–85
2. Howe MW, Dombeck DA. 2016. Rapid signalling in distinct dopaminergic axons during locomotion and reward. *Nature* 535:505–10
3. Ott T, Nieder A. 2019. Dopamine and cognitive control in prefrontal cortex. *Trends Cogn. Sci.* 23:213–34
4. Poulin JF, Caronia G, Hofer C, Cui Q, Helm B, et al. 2018. Mapping projections of molecularly defined dopamine neuron subtypes using intersectional genetic approaches. *Nat. Neurosci.* 21:1260–71
5. Parent A, Hazrati LN. 1995. Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. *Brain Res. Brain Res. Rev.* 20:91–127
6. Graybiel AM. 2008. Habits, rituals, and the evaluative brain. *Annu. Rev. Neurosci.* 31:359–87
7. O'Hare JK, Ade KK, Sukharnikova T, Van Hooser SD, Palmeri ML, et al. 2016. Pathway-specific striatal substrates for habitual behavior. *Neuron* 89:472–79
8. Jurado-Parras MT, Safaie M, Sarno S, Louis J, Karoutchi C, et al. 2020. The dorsal striatum energizes motor routines. *Curr. Biol.* 30:4362–72.e6
9. Markowitz JE, Gillis WF, Jay M, Wood J, Harris RW, et al. 2023. Spontaneous behaviour is structured by reinforcement without explicit reward. *Nature* 614:108–17
10. Kupferschmidt DA, Juczewski K, Cui G, Johnson KA, Lovinger DM. 2017. Parallel, but dissociable, processing in discrete corticostriatal inputs encodes skill learning. *Neuron* 96:476–89.e5
11. Seiler JL, Cosme CV, Sherathiya VN, Schaid MD, Bianco JM, et al. 2022. Dopamine signaling in the dorsomedial striatum promotes compulsive behavior. *Curr. Biol.* 32:1175–88.e5
12. Yin HH, Ostlund SB, Knowlton BJ, Balleine BW. 2005. The role of the dorsomedial striatum in instrumental conditioning. *Eur. J. Neurosci.* 22:513–23
13. Patriarchi T, Cho JR, Merten K, Howe MW, Marley A, et al. 2018. Ultrafast neuronal imaging of dopamine dynamics with designed genetically encoded sensors. *Science* 360:eaat4422
14. Mohebi A, Pettibone JR, Hamid AA, Wong JT, Vinson LT, et al. 2019. Dissociable dopamine dynamics for learning and motivation. *Nature* 570:65–70
15. Kutlu MG, Zachry JE, Melugin PR, Cajigas SA, Chevee MF, et al. 2021. Dopamine release in the nucleus accumbens core signals perceived saliency. *Curr. Biol.* 31:4748–61.e8
16. Bordia T, Perez XA, Heiss J, Zhang D, Quik M. 2016. Optogenetic activation of striatal cholinergic interneurons regulates L-dopa-induced dyskinesias. *Neurobiol. Dis.* 91:47–58

17. Moehle MS, Conn PJ. 2019. Roles of the M_4 acetylcholine receptor in the basal ganglia and the treatment of movement disorders. *Mov. Disord.* 34:1089–99
18. Duvoisin RC. 1967. Cholinergic-anticholinergic antagonism in parkinsonism. *Arch. Neurol.* 17:124–36
19. Barbeau A. 1962. The pathogenesis of Parkinson's disease: a new hypothesis. *Can. Med. Assoc. J.* 87:802–7
20. Nunes EJ, Kebede N, Bagdas D, Addy NA. 2022. Cholinergic and dopaminergic-mediated motivated behavior in healthy states and in substance use and mood disorders. *J. Exp. Anal. Behav.* 117:404–19
21. Foster DJ, Bryant ZK, Conn PJ. 2021. Targeting muscarinic receptors to treat schizophrenia. *Behav. Brain Res.* 405:113201
22. Wang HL, Morales M. 2009. Pedunculopontine and laterodorsal tegmental nuclei contain distinct populations of cholinergic, glutamatergic and GABAergic neurons in the rat. *Eur. J. Neurosci.* 29:340–58
23. Holmstrand EC, Sesack SR. 2011. Projections from the rat pedunculopontine and laterodorsal tegmental nuclei to the anterior thalamus and ventral tegmental area arise from largely separate populations of neurons. *Brain Struct. Funct.* 216:331–45
24. Maskos U. 2010. Role of endogenous acetylcholine in the control of the dopaminergic system via nicotinic receptors. *J. Neurochem.* 114:641–46
25. Moehle MS, Pancani T, Byun N, Yohn SE, Wilson GH 3rd, et al. 2017. Cholinergic projections to the substantia nigra pars reticulata inhibit dopamine modulation of basal ganglia through the M_4 muscarinic receptor. *Neuron* 96:1358–72.e4
26. Lodge DJ, Grace AA. 2006. The laterodorsal tegmentum is essential for burst firing of ventral tegmental area dopamine neurons. *PNAS* 103:5167–72
27. Pan WX, Hyland BI. 2005. Pedunculopontine tegmental nucleus controls conditioned responses of midbrain dopamine neurons in behaving rats. *J. Neurosci.* 25:4725–32
28. Floresco SB, West AR, Ash B, Moore H, Grace AA. 2003. Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. *Nat. Neurosci.* 6:968–73
29. Zhang YF, Reynolds JNJ, Cragg SJ. 2018. Pauses in cholinergic interneuron activity are driven by excitatory input and delayed rectification, with dopamine modulation. *Neuron* 98:918–25.e3
30. Xiao C, Zhou CY, Jiang JH, Yin C. 2020. Neural circuits and nicotinic acetylcholine receptors mediate the cholinergic regulation of midbrain dopaminergic neurons and nicotine dependence. *Acta Pharmacol. Sin.* 41:1–9
31. Exley R, Cragg SJ. 2008. Presynaptic nicotinic receptors: a dynamic and diverse cholinergic filter of striatal dopamine neurotransmission. *Br. J. Pharmacol.* 153(Suppl. 1):S283–97
32. Faure P, Tolu S, Valverde S, Naude J. 2014. Role of nicotinic acetylcholine receptors in regulating dopamine neuron activity. *Neuroscience* 282:86–100
33. Picciotto MR, Kenny PJ. 2021. Mechanisms of nicotine addiction. *Cold Spring Harb. Perspect. Med.* 11:a039610
34. Foster DJ, Conn PJ. 2017. Allosteric modulation of GPCRs: new insights and potential utility for treatment of schizophrenia and other CNS disorders. *Neuron* 94:431–46
35. Scarpa M, Molloy C, Jenkins L, Strellis B, Budgett RF, et al. 2021. Biased M1 muscarinic receptor mutant mice show accelerated progression of prion neurodegenerative disease. *PNAS* 118:e2107389118
36. van der Westhuizen ET, Choy KHC, Valant C, McKenzie-Nickson S, Bradley SJ, et al. 2020. Fine tuning muscarinic acetylcholine receptor signaling through allostery and bias. *Front. Pharmacol.* 11:606656
37. Gould RW, Gunter BW, Bubser M, Matthews RT, Teal LB, et al. 2019. Acute negative allosteric modulation of M_5 muscarinic acetylcholine receptors inhibits oxycodone self-administration and cue-induced reactivity with no effect on antinociception. *ACS Chem. Neurosci.* 10:3740–50
38. Vilaro MT, Palacios JM, Mengod G. 1990. Localization of m5 muscarinic receptor mRNA in rat brain examined by in situ hybridization histochemistry. *Neurosci. Lett.* 114:154–59
39. Weiner DM, Levey AI, Brann MR. 1990. Expression of muscarinic acetylcholine and dopamine receptor mRNAs in rat basal ganglia. *PNAS* 87:7050–54
40. Garzon M, Pickel VM. 2016. Electron microscopic localization of M2-muscarinic receptors in cholinergic and noncholinergic neurons of the laterodorsal tegmental and pedunculopontine nuclei of the rat mesopontine tegmentum. *J. Comp. Neurol.* 524:3084–103

41. Tzavara ET, Bymaster FP, Davis RJ, Wade MR, Perry KW, et al. 2004. M_4 muscarinic receptors regulate the dynamics of cholinergic and dopaminergic neurotransmission: relevance to the pathophysiology and treatment of related CNS pathologies. *FASEB J.* 18:1410–12
42. Forster GL, Blaha CD. 2000. Laterodorsal tegmental stimulation elicits dopamine efflux in the rat nucleus accumbens by activation of acetylcholine and glutamate receptors in the ventral tegmental area. *Eur. J. Neurosci.* 12:3596–604
43. Miller AD, Blaha CD. 2005. Midbrain muscarinic receptor mechanisms underlying regulation of mesoaccumbens and nigrostriatal dopaminergic transmission in the rat. *Eur. J. Neurosci.* 21:1837–46
44. Solecki W, Wickham RJ, Behrens S, Wang J, Zwerling B, et al. 2013. Differential role of ventral tegmental area acetylcholine and N-methyl-D-aspartate receptors in cocaine-seeking. *Neuropharmacology* 75:9–18
45. Forster GL, Yeomans JS, Takeuchi J, Blaha CD. 2002. M5 muscarinic receptors are required for prolonged accumbal dopamine release after electrical stimulation of the pons in mice. *J. Neurosci.* 22:RC190
46. Steidl S, Miller AD, Blaha CD, Yeomans JS. 2011. M_5 muscarinic receptors mediate striatal dopamine activation by ventral tegmental morphine and pedunculopontine stimulation in mice. *PLOS ONE* 6:e27538
47. Bender AM, Garrison AT, Lindsley CW. 2019. The muscarinic acetylcholine receptor M_5: therapeutic implications and allosteric modulation. *ACS Chem. Neurosci.* 10:1025–34
48. Gentry PR, Kokubo M, Bridges TM, Cho HP, Smith E, et al. 2014. Discovery, synthesis and characterization of a highly muscarinic acetylcholine receptor (mAChR)-selective M_5-orthosteric antagonist, VU0488130 (ML381): a novel molecular probe. *ChemMedChem* 9:1677–82
49. Gentry PR, Kokubo M, Bridges TM, Kett NR, Harp JM, et al. 2013. Discovery of the first M_5-selective and CNS penetrant negative allosteric modulator (NAM) of a muscarinic acetylcholine receptor: (*S*)-9b-(4-chlorophenyl)-1-(3,4-difluorobenzoyl)-2,3-dihydro-1*H*-imidazo[2,1-*a*]isoindol-5(9b*H*)-one (ML375). *J. Med. Chem.* 56:9351–55
50. Garrison AT, Orsi DL, Capstick RA, Whomble D, Li J, et al. 2022. Development of VU6019650: a potent, highly selective, and systemically active orthosteric antagonist of the M_5 muscarinic acetylcholine receptor for the treatment of opioid use disorder. *J. Med. Chem.* 65:6273–86
51. Foster DJ, Gentry PR, Lizardi-Ortiz JE, Bridges TM, Wood MR, et al. 2014. M_5 receptor activation produces opposing physiological outcomes in dopamine neurons depending on the receptor's location. *J. Neurosci.* 34:3253–62
52. Baghdoyan HA, Lydic R, Fleegal MA. 1998. M2 muscarinic autoreceptors modulate acetylcholine release in the medial pontine reticular formation. *J. Pharmacol. Exp. Ther.* 286:1446–52
53. Coleman CG, Lydic R, Baghdoyan HA. 2004. M2 muscarinic receptors in pontine reticular formation of C57BL/6J mouse contribute to rapid eye movement sleep generation. *Neuroscience* 126:821–30
54. Roth MT, Fleegal MA, Lydic R, Baghdoyan HA. 1996. Pontine acetylcholine release is regulated by muscarinic autoreceptors. *NeuroReport* 7:3069–72
55. Mark GP, Shabani S, Dobbs LK, Hansen ST. 2011. Cholinergic modulation of mesolimbic dopamine function and reward. *Physiol. Behav.* 104:76–81
56. Doig NM, Magill PJ, Apicella P, Bolam JP, Sharott A. 2014. Cortical and thalamic excitation mediate the multiphasic responses of striatal cholinergic interneurons to motivationally salient stimuli. *J. Neurosci.* 34:3101–17
57. Dautan D, Huerta-Ocampo I, Witten IB, Deisseroth K, Bolam JP, et al. 2014. A major external source of cholinergic innervation of the striatum and nucleus accumbens originates in the brainstem. *J. Neurosci.* 34:4509–18
58. Dautan D, Huerta-Ocampo I, Gut NK, Valencia M, Kondabolu K, et al. 2020. Cholinergic midbrain afferents modulate striatal circuits and shape encoding of action strategies. *Nat. Commun.* 11:1739
59. Brimblecombe KR, Threlfell S, Dautan D, Kosillo P, Mena-Segovia J, Cragg SJ. 2018. Targeted activation of cholinergic interneurons accounts for the modulation of dopamine by striatal nicotinic receptors. *eNeuro* 5:ENEURO.0397-17.2018
60. Threlfell S, Lalic T, Platt NJ, Jennings KA, Deisseroth K, Cragg SJ. 2012. Striatal dopamine release is triggered by synchronized activity in cholinergic interneurons. *Neuron* 75:58–64

61. Zhang W, Basile AS, Gomeza J, Volpicelli LA, Levey AI, Wess J. 2002. Characterization of central inhibitory muscarinic autoreceptors by the use of muscarinic acetylcholine receptor knock-out mice. *J. Neurosci.* 22:1709–17
62. Foster DJ, Wilson JM, Remke DH, Mahmood MS, Uddin MJ, et al. 2016. Antipsychotic-like effects of M_4 positive allosteric modulators are mediated by CB_2 receptor-dependent inhibition of dopamine release. *Neuron* 91:1244–52
63. Shin JH, Adrover MF, Wess J, Alvarez VA. 2015. Muscarinic regulation of dopamine and glutamate transmission in the nucleus accumbens. *PNAS* 112:8124–29
64. Threlfell S, Clements MA, Khodai T, Pienaar IS, Exley R, et al. 2010. Striatal muscarinic receptors promote activity dependence of dopamine transmission via distinct receptor subtypes on cholinergic interneurons in ventral versus dorsal striatum. *J. Neurosci.* 30:3398–408
65. Mamaligas AA, Ford CP. 2016. Spontaneous synaptic activation of muscarinic receptors by striatal cholinergic neuron firing. *Neuron* 91:574–86
66. Pancani T, Bolarinwa C, Smith Y, Lindsley CW, Conn PJ, Xiang Z. 2014. M4 mAChR-mediated modulation of glutamatergic transmission at corticostriatal synapses. *ACS Chem. Neurosci.* 5:318–24
67. Shen W, Plotkin JL, Francardo V, Ko WK, Xie Z, et al. 2015. M4 muscarinic receptor signaling ameliorates striatal plasticity deficits in models of L-DOPA-induced dyskinesia. *Neuron* 88:762–73
68. Cachope R, Mateo Y, Mathur BN, Irving J, Wang HL, et al. 2012. Selective activation of cholinergic interneurons enhances accumbal phasic dopamine release: setting the tone for reward processing. *Cell Rep.* 2:33–41
69. Bernard V, Normand E, Bloch B. 1992. Phenotypical characterization of the rat striatal neurons expressing muscarinic receptor genes. *J. Neurosci.* 12:3591–600
70. Jeon J, Dencker D, Wortwein G, Woldbye DP, Cui Y, et al. 2010. A subpopulation of neuronal M_4 muscarinic acetylcholine receptors plays a critical role in modulating dopamine-dependent behaviors. *J. Neurosci.* 30:2396–405
71. Dencker D, Wortwein G, Weikop P, Jeon J, Thomsen M, et al. 2011. Involvement of a subpopulation of neuronal M_4 muscarinic acetylcholine receptors in the antipsychotic-like effects of the M_1/M_4 preferring muscarinic receptor agonist xanomeline. *J. Neurosci.* 31:5905–8
72. Ferranti AS, Foster DJ. 2022. Cannabinoid type-2 receptors: an emerging target for regulating schizophrenia-relevant brain circuits. *Front. Neurosci.* 16:925792
73. Byun NE, Grannan M, Bubser M, Barry RL, Thompson A, et al. 2014. Antipsychotic drug-like effects of the selective M_4 muscarinic acetylcholine receptor positive allosteric modulator VU0152100. *Neuropsychopharmacology* 39:1578–93
74. Razidlo JA, Fausner SML, Ingebretson AE, Wang LC, Petersen CM, et al. 2022. Chronic loss of muscarinic M_5 receptor function manifests disparate impairments in exploratory behavior in male and female mice despite common dopamine regulation. *J. Neurosci.* 42:6917–30
75. Moehle MS, Bender AM, Dickerson JW, Foster DJ, Qi A, et al. 2021. Discovery of the first selective M_4 muscarinic acetylcholine receptor antagonists with in vivo antiparkinsonian and antidystonic efficacy. *ACS Pharmacol. Transl. Sci.* 4:1306–21
76. Bendor J, Lizardi-Ortiz JE, Westphalen RI, Brandstetter M, Hemmings HC Jr., et al. 2010. AGAP1/AP-3-dependent endocytic recycling of M_5 muscarinic receptors promotes dopamine release. *EMBO J.* 29:2813–26
77. Underhill SM, Amara SG. 2021. Acetylcholine receptor stimulation activates protein kinase C mediated internalization of the dopamine transporter. *Front. Cell Neurosci.* 15:662216
78. Kearney PJ, Bolden NC, Kahuno E, Conklin TL, Martin GE, et al. 2023. Presynaptic Gq-coupled receptors drive biphasic dopamine transporter trafficking that modulates dopamine clearance and motor function. *J. Biol. Chem.* 299:102900
79. Yohn SE, Weiden PJ, Felder CC, Stahl SM. 2022. Muscarinic acetylcholine receptors for psychotic disorders: bench-side to clinic. *Trends Pharmacol. Sci.* 43:1098–112
80. Basile AS, Fedorova I, Zapata A, Liu X, Shippenberg T, et al. 2002. Deletion of the M_5 muscarinic acetylcholine receptor attenuates morphine reinforcement and withdrawal but not morphine analgesia. *PNAS* 99:11452–57

81. Thomsen M, Woldbye DP, Wortwein G, Fink-Jensen A, Wess J, Caine SB. 2005. Reduced cocaine self-administration in muscarinic M_5 acetylcholine receptor-deficient mice. *J. Neurosci.* 25:8141–49
82. Berizzi AE, Perry CJ, Shackleford DM, Lindsley CW, Jones CK, et al. 2018. Muscarinic M_5 receptors modulate ethanol seeking in rats. *Neuropsychopharmacology* 43:1510–17
83. Gunter BW, Gould RW, Bubser M, McGowan KM, Lindsley CW, Jones CK. 2018. Selective inhibition of M5 muscarinic acetylcholine receptors attenuates cocaine self-administration in rats. *Addict. Biol.* 23:1106–16
84. Teal LB, Bubser M, Duncan E, Gould RW, Lindsley CW, Jones CK. 2023. Selective M_5 muscarinic acetylcholine receptor negative allosteric modulator VU6008667 blocks acquisition of opioid self-administration. *Neuropharmacology* 227:109424
85. Nunes EJ, Rupprecht LE, Foster DJ, Lindsley CW, Conn PJ, Addy NA. 2020. Examining the role of muscarinic M5 receptors in VTA cholinergic modulation of depressive-like and anxiety-related behaviors in rats. *Neuropharmacology* 171:108089
86. Nunes EJ, Kebede N, Haight JL, Foster DJ, Lindsley C, et al. 2023. Ventral tegmental area M5 muscarinic receptors mediate effort-choice responding and nucleus accumbens dopamine in a sex-specific manner. *J. Pharmacol. Exp. Ther.* 385:146–56
87. Thomsen M, Crittenden JR, Lindsley CW, Graybiel AM. 2022. Effects of acute and repeated administration of the selective M_4 PAM VU0152099 on cocaine versus food choice in male rats. *Addict. Biol.* 27:e13145
88. Dall C, Weikop P, Dencker D, Molander AC, Wortwein G, et al. 2017. Muscarinic receptor M_4 positive allosteric modulators attenuate central effects of cocaine. *Drug Alcohol Depend.* 176:154–61
89. Klawonn AM, Wilhelms DB, Lindstrom SH, Singh AK, Jaarola M, et al. 2018. Muscarinic M4 receptors on cholinergic and dopamine D1 receptor-expressing neurons have opposing functionality for positive reinforcement and influence impulsivity. *Front. Mol. Neurosci.* 11:139
90. Shannon HE, Rasmussen K, Bymaster FP, Hart JC, Peters SC, et al. 2000. Xanomeline, an M_1/M_4 preferring muscarinic cholinergic receptor agonist, produces antipsychotic-like activity in rats and mice. *Schizophr. Res.* 42:249–59
91. Shekhar A, Potter WZ, Lightfoot J, Lienemann J, Dube S, et al. 2008. Selective muscarinic receptor agonist xanomeline as a novel treatment approach for schizophrenia. *Am. J. Psychiatry* 165:1033–39
92. Broadley KJ, Kelly DR. 2001. Muscarinic receptor agonists and antagonists. *Molecules* 6:142–93
93. Brannan SK, Sawchak S, Miller AC, Lieberman JA, Paul SM, Breier A. 2021. Muscarinic cholinergic receptor agonist and peripheral antagonist for schizophrenia. *N. Engl. J. Med.* 384:717–26
94. Correll CU, Angelov AS, Miller AC, Weiden PJ, Brannan SK. 2022. Safety and tolerability of KarXT (xanomeline-trospium) in a phase 2, randomized, double-blind, placebo-controlled study in patients with schizophrenia. *Schizophrenia* 8:109
95. Krystal JH, Kane JM, Correll CU, Walling DP, Leoni M, et al. 2022. Emraclidine, a novel positive allosteric modulator of cholinergic M4 receptors, for the treatment of schizophrenia: a two-part, randomised, double-blind, placebo-controlled, phase 1b trial. *Lancet* 400:2210–20