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Spatiotemporal specificity of H3 receptor signaling: from molecular basis to precise regulation of brain function

Yanrong Zheng[#], Zhuowen Fang[#] and Zhong Chen[*]

Zhejiang Key Laboratory of Neuropsychopharmacology, School of Pharmaceutical Sciences, The Fifth Affiliated Hospital of Zhejiang Chinese Medical University, Huzhou 313000, China

[#] Authors contributed equally: Yanrong Zheng, Zhuowen Fang

[*] Correspondence: chenzhong@zju.edu.cn (Chen Z)

Abstract

The histamine H3 receptor (H3R) is widely distributed throughout the brain, where it controls histamine release and influences other neurotransmitter systems. Therefore, it has been considered a promising drug target for conditions like narcolepsy, ADHD, schizophrenia, and obesity. Yet after decades of effort and many clinical trials, drugs that act on this receptor have produced only modest and inconsistent results. In this review, we argue that this gap lies in the spatiotemporal complexity of H3R functions. H3R signaling depends on multiple factors: which splice variant is expressed, whether the receptor is located pre- or postsynaptically, which brain region or circuit it operates in, whether the tissue is healthy or diseased, and how the ligand binds to it. We summarize recent advances in understanding H3R structure, biased signaling, optogenetic and photopharmacological tools, and strategies for delivering drugs to specific cell types. We also discuss emerging approaches that combine brain imaging with gene expression data to identify biomarkers for patient selection. Collectively, we propose that realizing the therapeutic potential of H3R will require a paradigm shift from systemic modulation to spatiotemporally precise intervention, grounded in the spatiotemporal complexity of H3R functions.

Keywords: Histamine, Histamine H3 receptor, CNS disorders, Spatiotemporal function, Biased signaling

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Introduction

Histamine was established as a central neurotransmitter in the late 1970s, mainly as a result of the early work by Schwartz and colleagues, who proved that a cluster of neurons within the tuberomammillary nucleus of the posterior hypothalamus expresses L-histidine decarboxylase (the histamine biosynthetic enzyme)[1–4]. On this basis, Arrang et al. reported in Nature in 1983 that histamine inhibits its own release in depolarized slices of rat cerebral cortex via a receptor pharmacologically separate from the known H1 and H2 subtypes, thereby defining a third histamine receptor, H3R[5]. Subsequent studies from the same group yielded the first pharmacologically useful tools, namely selective agonist (R)-α-methylhistamine and the antagonist thioperamide[6]. By 1990, the guanine nucleotide sensitivity of H3R signaling had positioned the receptor among the G protein-coupled receptors (GPCRs) in general, offering a molecular clue to its cloning[7].

The molecular identity of H3R was established in 1999, when Lovenberg and colleagues at the RW Johnson Pharmaceutical Research Institute identified the orphan receptor GPCR97 by screening expressed sequence tag databases for sequences homologous to adrenergic receptors and subsequently isolated its full-length cDNA from a human thalamic library[8]. The cloned receptor, designated *HRH3*, displayed substantial sequence similarity to the M2 muscarinic acetylcholine receptor, responded selectively to histamine by potently inhibiting adenylyl cyclase, and was insensitive to the other biogenic amines tested. This milestone triggered rapid expansion of H3R research across academia and the pharmaceutical industry. From 2001 onward, systematic characterization of alternative splice variants showed that the human HRH3 gene, located on chromosome 20 and comprising four exons, can generate at least 20 receptor isoforms[9]. Much of this structural diversity resides in the third intracellular loop (ICL3), where sequence variation can alter G-protein coupling and downstream signaling.

H3Rs are widely, but unevenly, distributed throughout the central nervous system[10,11]. The highest receptor densities occur in the striatal complex (caudate-putamen and nucleus accumbens), cerebral cortex (particularly the middle and deep layers), hippocampus (dentate gyrus, CA1, and CA3), posterior hypothalamus, globus pallidus, substantia nigra, olfactory bulb, and thalamus. Notably, H3R mRNA and receptor protein show partially divergent distributions, especially in the cortex and striatum, where binding sites extend well beyond the somata of H3R-expressing neurons. This anatomical mismatch indicates extensive anterograde transport of H3Rs to distal axon terminals[7,12].

This distribution underlies the classical view of H3R as both a somatodendritic and terminal autoreceptor in histaminergic neurons and an inhibitory heteroreceptor on non-histaminergic terminals. As an autoreceptor, H3R reduces neuronal firing at somatodendritic sites, inhibits vesicular histamine release at axon terminals, and suppresses histamine synthesis by decreasing L-histidine decarboxylase activity[11,13,14]. As a heteroreceptor, it restrains the presynaptic release of dopamine, norepinephrine, serotonin, glutamate, acetylcholine, and GABA in a region- and pathway-dependent manner[15]. H3R also exhibits substantial constitutive activity, $G_{i/o}$-mediated signaling in the absence of an exogenous agonist, in native brain tissue. This property is crucial when distinguishing the actions of inverse agonists from those of neutral antagonists. The ability of a single receptor to regulate such a broad transmitter repertoire made H3R an especially compelling therapeutic target.

Following its cloning, H3R attracted substantial investment from major pharmaceutical companies. Candidates that reached phase II

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Table 1. Summary of major H3R clinical compounds, outcomes, and possible spatiotemporal mismatch.

Compound	Company	Intended indication	Highest phase/outcome	Possible spatiotemporal mismatch source
ABT-288	Abbott	Cognitive impairment associated with schizophrenia	Phase II, insufficient clinical efficacy	Splice variant, pre- vs postsynaptic localization, brain region/circuit, disease state, biased signaling
GSK189254	GlaxoSmithKline	Alzheimer's disease	Phase II, insufficient clinical efficacy	
GSK239512	GlaxoSmithKline	Alzheimer's disease	Phase II, insufficient clinical efficacy	
MK-0249	Merck	Alzheimer's disease	Phase II, insufficient clinical efficacy	
MK-3134	Merck	Alzheimer's disease, dementia	Phase II, insufficient clinical efficacy	
PF-03654746	Pfizer	Attention-deficit/hyperactivity disorder (ADHD)	Phase II, insufficient clinical efficacy	
Pitolisant (BF2.649)	Bioprojet	Narcolepsy	Approved	

trials included ABT-288 (Abbott; cognitive impairment associated with schizophrenia), GSK189254 and GSK239512 (GlaxoSmithKline; Alzheimer's disease), MK-0249 and MK-3134 (Merck), and PF-03654746 (Pfizer; attention-deficit/hyperactivity disorder)[16–19]. Yet, with the notable exception of pitolisant (BF2.649; Bioprojet), which was approved for narcolepsy, these programs did not demonstrate sufficient efficacy in their intended central nervous system indications[14]. This recurrent translational failure contrasts sharply with the robust procognitive, neuroprotective, and antiseizure effects reported for H3R antagonists and inverse agonists across diverse preclinical models.

The mechanisms underlying this translational gap remain incompletely understood (Table 1). Species differences in receptor pharmacology and variability in pharmacokinetics may contribute, but neither explanation readily accounts for failures spanning structurally diverse compounds and mechanistically distinct disorders[20–22]. We argue that a deeper conceptual limitation has constrained the field: H3R has often been treated as a functionally uniform target, despite pronounced spatiotemporal heterogeneity in its signaling. As detailed below, H3R can couple to distinct G-protein families and recruit divergent effector pathways according to brain region, subcellular compartment (presynaptic terminal vs postsynaptic somatodendritic domain), neuronal identity, and physiological or pathological state. Systemic, nonselective H3R modulation may therefore engage opposing signaling programs across heterogeneous cell populations. Such pharmacology is poorly matched to the cell- and circuit-level precision required to treat complex central nervous system disorders[12,21,23,24].

Overall structural features and the diversity of downstream pathways of H3 receptors

Structural features

Structurally, H3R belongs to the class A GPCR family and shares the canonical seven-transmembrane (7TM) architecture. The receptor is highly conserved across different species, and the human full-length H3R comprises 445 amino acids. Within the histamine receptor family, H3R shares the highest sequence similarity with the H4 receptor.

Recent high-resolution cryo-EM studies have resolved the active-state H3R-G_i complexes at global resolutions of 2.6–3.0 Å in the presence of histamine or synthetic agonists (imetit, immepip, immethridine)[25–27], providing atomic-level insights into ligand recognition and G-protein coupling[25].

The orthosteric binding pocket is formed by residues from TM3, TM5, TM6, and TM7, with additional contributions from extracellular loop 2 (ECL2). Notably, histamine binds to H3R in a reversed orientation relative to that observed in H1R and H2R: the imidazole ring is rotated by approximately 180° around the ligand's longitudinal axis (Fig. 1)[25–27]. In this pose, the primary amino group of the ethylamine chain forms a salt bridge with E206$^{5.46}$ on TM5, while the conserved D114$^{3.32}$ on TM3 interacts with the δ-nitrogen of the imidazole ring *via* hydrogen-bond and electrostatic contacts[25–27]. In H1R and H2R, the corresponding interaction is provided by F$^{6.52}$ and F$^{6.55}$ on TM6[25]. Mutagenesis of F398$^{7.39}$ or W402$^{7.43}$ in H3R reduces histamine affinity by more than 30-fold, whereas equivalent mutations in H1R/H2R have smaller effects[25]. A conserved

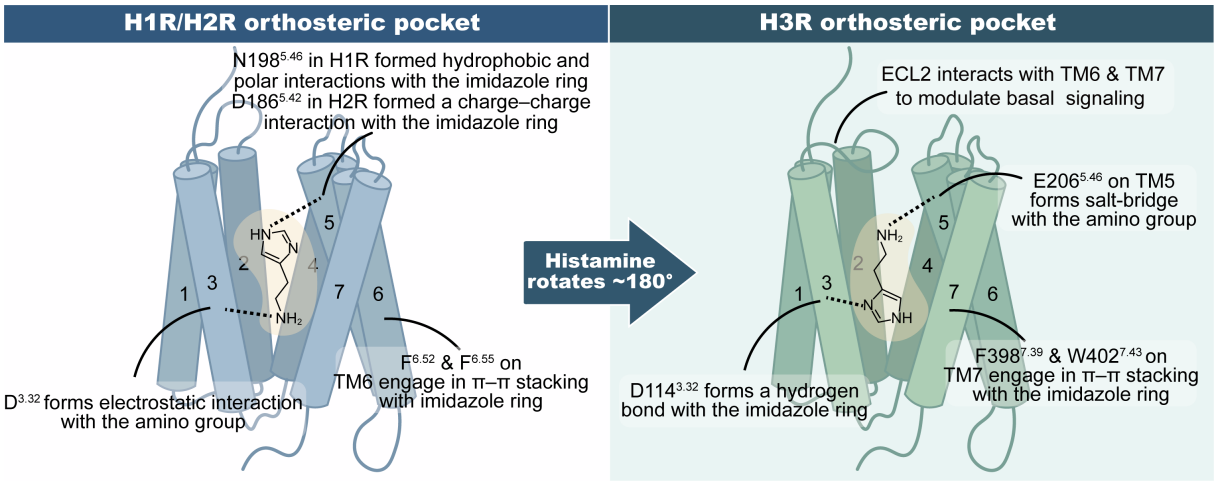

Fig. 1 Schematic diagram showing the structural differences in the orthosteric pocket of H1R/H2R/H3R.

$D^{3.32}$-Y/$V^{3.33}$-$Y^{6.51}$ motif is present across all histamine receptors and contributes to histamine recognition, but its specific contacts differ between subtypes[25]. In H3R, $D^{3.32}$ binds the imidazole ring rather than the amino group, and $Y^{6.51}$ forms hydrophobic interactions with the ligand[25,27]. ECL2 of H3R contains aromatic residues (e.g., F193 and Y194) that insert into the binding pocket and interact with TM6 and TM7. Mutagenesis of these residues reduces constitutive (agonist-independent) activity, indicating that ECL2 modulates basal signaling rather than directly participating in agonist binding[27].

H3R couples preferentially to $G_{i/o}$ proteins[25,26]. Structural comparisons with H2R-G_s and H1R-G_q complexes indicate that G-protein selectivity is associated with several features. The intracellular portions of TM5 and TM6 in H3R are shorter and less curved than those in H2R[25,26]. In H2R, an elongated TM5 bends toward TM6, creating additional contacts with the α5 helix of $G_{\alpha s}$; this feature is absent in H3R[26]. Moreover, the intracellular loops, particularly ICL2 and ICL3, contribute to the G-protein interface[25]. In H3R, residues $R^{34.54}$ (ICL2), F/$V^{6.25}$ (TM6), and $R^{6.29}$ (TM6) form polar and hydrophobic contacts with the α5 helix and the α4-β6 turn of $G_{\alpha i/o}$. Mutation of these residues reduces $G_{i/o}$ activation, whereas equivalent substitutions in H2R do not affect G_s coupling[25]. Meanwhile, the α5 helix of $G_{i/o}$ adopts an orientation in which its hook region is positioned closer to the conserved NPxxY motif and tilted toward TM2[25,28]; positioning differs from that observed in G_s-coupled complexes, where the α5 helix is shifted toward TM6[26]. At the H3R-G_i interface, polar interactions include salt bridges between receptor residues (e.g., $R^{34.54}$, $R^{6.29}$) and conserved acidic residues in the α5 helix of $G_{\alpha i}$ (e.g., D213, D216, D225)[25,26]. These contacts, together with the hydrophobic packing involving residues such as F/$V^{6.25}$, contribute to the stabilization of the complex and are consistent with the preferential coupling of H3R to $G_{i/o}$ over other G-protein subtypes[25].

Molecular dynamics simulations of the ternary histamine-H3R-G_i complex show that the active conformation, characterized by an outward displacement of the cytoplasmic end of TM6, is maintained over microsecond timescales only when both agonist and G_i protein are present. In the absence of G_i, histamine alone does not prevent TM6 inward rotation and the formation of an ionic lock between $R^{3.50}$ and $E^{6.30}$, which are associated with receptor inactivation[29].

Among the many features of H3R, $G_{i/o}$ coupling has long been taken as its primary signaling. Recent functional analyses now broaden this perspective, showing that H3R is able to recruit G_q and $G_{12/13}$ pathways[30,31]. Such an expanded repertoire of signals implies that H3R-induced cellular effects go well beyond simple G_i-related adenylyl cyclase suppression and involve complex, context-specific interactions of G subtypes and *β*-arrestin scaffolds. However, the precise structural determinant of H3R's capacity to couple to G_q and $G_{12/13}$ remains mostly unestablished, representing a critical gap in our current understanding of the H3R signaling network. Other than G proteins and *β*-arrestins, H3R may engage additional intracellular factors, thus raising its functional complexity[32].

Complementing these active-state structural insights, the crystal structure of H3R in complex with the non-imidazole antagonist PF-03654746, determined at 2.6 Å resolution, has provided an atomic-level framework for understanding how clinically relevant inverse agonists and antagonists stabilize the receptor in its inactive conformation[33]. The antagonist-bound H3R exhibits several hallmark features of the GPCR inactive state. For example, the $D^{3.49}$-$R^{3.50}$ salt bridge within the DRY motif is fully formed, whereas the $Y^{3.51}$ of this motif is substituted by $F133^{3.51}$ in H3R. TM6 shows only a modest outward displacement of 2.8 Å compared to the inactive H1R, in marked contrast to the 12 Å movement observed in active-state structures. The toggle switch $W371^{6.48}$ adopts a rotated conformation that stabilizes the extracellular half of TM6 in an expanded conformation, while the intracellular half is locked through hydrophobic interactions with $F367^{6.44}$ in the PIF motif.

PF-03654746 occupies a relatively shallow orthosteric pocket at the extracellular side, with an extended binding pocket formed by residues from TMs 2, 3, 6, 7, and ECL2. The antagonist establishes a network of interactions critical for its inhibitory activity. The carbonyl and N-ethyl-carboxamide moieties engage $E395^{7.36}$ and $Y91^{2.61}$ *via* hydrophobic and hydrogen-bonding contacts, respectively, and mutation of either residue substantially reduces or abolishes PF-03654746 inhibition. The 3-fluoro-phenyl moiety forms hydrophobic interactions with F193 (ECL2), a residue proposed to determine ligand specificity among aminergic receptors, while $Y374^{6.51}$ contributes additional hydrophobic contacts. Notably, the fluorine atom of the 3-fluoro-cyclobutane group engages a hydrogen bond with $C188^{45.50}$, and the pyrrolidine amine forms a salt bridge with the highly conserved $D114^{3.32}$ at the bottom of the pocket.

Unexpectedly, the H3R antagonist-bound structure also revealed a cholesterol molecule bound at an extrahelical site between TM1 and TM7. The β3-hydroxy head group of cholesterol forms a hydrogen bond with $E395^{7.36}$. This residue is the same one that participates in PF-03654746 recognition, suggesting a potential allosteric interplay between cholesterol binding and antagonist inhibition. Molecular dynamics simulations further demonstrated that cholesterol stabilizes the $W399^{7.40}$-$Y91^{2.61}$-PF-03654746 interaction network through π–π stacking, and mutation of $W399^{7.40}$ completely abolishes antagonist inhibition without affecting histamine activation. Cholesterol thus acts as a molecular 'glue' that stabilizes the TM1-TM7 interface and the $E395^{7.36}$-R27 (N-terminus) polar network, collectively locking H3R in an antagonist-favorable inactive conformation. In summary, the antagonist-bound H3R structure reveals that receptor inactivation is achieved through canonical inactive-state hallmarks, extensive antagonist contacts with both the orthosteric and extended binding pockets, and allosteric stabilization by cholesterol at the TM1-TM7 interface.

Splicing variants

Early transcriptomic and proteomic analyses identified 20 distinct mRNA transcripts of the human H3R gene[21,34,35], each potentially encoding a unique protein isoform. Among these, eight isoforms are functionally active, while the rest are considered nonfunctional, probably due to structural disruptions that impair receptor folding, membrane trafficking, or effector coupling. Notably, the existence of multiple non-functional isoforms does not preclude their biological relevance. These isoforms may play regulatory roles through heterodimerization with functional variants or through competition for translational machinery[36,37]. However, recent investigations into H3R splicing have narrowed this picture, identifying seven variant isoforms that all retained the ability to activate heterotrimeric G proteins[38]. Among the functionally validated isoforms, four have been characterized in detail: hH3R445 (full-length), hH3R415, hH3R365, and hH3R329. These numerical designations reflect differences in protein length caused by exon skipping or alternative splice-site usage. The main differences between these variants lie in the length of the third intracellular loop (ICL3) and/or the C-terminal tail, both of which, as mentioned earlier, are key regions for G-protein coupling and receptor desensitization. Alternative splicing in these regions gives rise to isoforms with distinct intracellular conformations, which in turn provide a structural basis for diversifying their functions.

Therefore, one direct consequence of H3R splicing diversity is the potential for isoform-dependent biased signaling, leading to

preferential activation of certain downstream pathways over others. For example, hH3R445 and hH3R365 are the most abundantly expressed isoforms in the human brain, suggesting they represent the principal mediators of H3R signaling under normal conditions[34]. The hH3R445 and hH3R365 isoforms exhibit marked differences in regulatory dynamics, primarily attributable to their distinct GRK phosphorylation sites. Bioinformatic analysis reveals that hH3R365 lacks eight of the nine GRK2/3-targeted serine/threonine residues within ICL3 that are present in hH3R445, retaining only the C-terminal sites Ser358 and Ser359. This molecular difference translates into distinct desensitization patterns of these two isoforms. Following prolonged agonist exposure, hH3R445 undergoes nearly complete desensitization that recovers slowly, whereas hH3R365 displays only partial desensitization with faster resensitization. Paradoxically, agonist-induced loss of cell-surface receptors is more pronounced for hH3R365 than for hH3R445, indicating that desensitization and internalization are partially uncoupled processes. Furthermore, the two isoforms differ in inverse agonist responses, with A-331440 exhibiting efficacy only at hH3R365, suggesting biased inverse agonism[34]. Moreover, isoforms also impact the functional outcomes of ligands. For example, proxyfan, a protean agonist at the H3R, displays pronounced bias toward GSK3β phosphorylation, changes in cellular impedance, and ERK1/2 phosphorylation at the hH3R445, indicating that it preferentially promotes non-canonical signaling over the classical $G_{i/o}$-cAMP axis at this isoform[39]. In striking contrast, at the hH3R365 isoform, proxyfan fails to stimulate GSK3β phosphorylation and evoke calcium mobilization responses, despite robust calcium signals being elicited by reference agonists such as RaMH and imetit at this same isoform[39]. These data suggest that splice variation impacts ligand efficacy and signaling outcome, underscoring that functional characterization of H3R-targeting drugs with various isoform contexts. Crucially, while all functional H3R isoforms can activate G proteins, it remains unresolved whether they all respond to the same panel of ligands with identical affinity or potency, nor whether they exhibit uniform preferences for specific G_{α} subtypes (e.g., $G_{\alpha i/o}$ vs $G_{\alpha q/11}$).

The splicing-mediated functional diversification of H3R confers regulatory flexibility within the CNS, enabling precise modulation of multiple physiological processes. For example, the function of autoreceptors was supposed to be fulfilled by a short isoform, such as the hH3R413 receptor[40]. Notably, early studies also suggest the 20 H3R isoforms are not uniformly distributed across the human brain; rather, they exhibit distinct and spatially restricted expression profiles (see references for details)[21,41]. This regional heterogeneity suggests that individual isoforms may subserve area-specific functions, tailored to local neurochemical and circuitry demands. Nevertheless, it remains unclear whether these isoforms are preferentially enriched in particular cell types or synaptic compartments. From a technical standpoint, addressing this critical gap will require a shift from bulk transcriptomic profiling to full-length single-cell RNA sequencing approaches. Conventional short-read scRNA-seq methods, while powerful for cell-type classification, are inherently limited in their ability to unambiguously distinguish highly homologous splice variants due to their inability to capture full-length transcript coverage across variable exon-intron boundaries. To address these limitations, long-read scRNA-seq has emerged as a powerful approach that provides full-length transcript coverage at single-cell resolution[42,43]. Long-read technologies, such as PacBio Iso-seq and Oxford Nanopore sequencing, enable the direct sequencing of full-length RNA molecules, some exceeding 100,000 nucleotides in length[44,45]. This capability allows for more accurate characterization of isoform diversity, identification of novel splice variants, and quantification of percent spliced-in values. While long-read technologies offer significant advantages, they also face technical challenges, such as higher error rates and reduced throughput compared to short-read technologies[46,47]. To address these challenges, researchers have developed methods to integrate short- and long-read data, combining the strengths of both approaches. For instance, SCALPEL improves isoform quantification using paired long- and short-read scRNA-seq data, expanding the current scRNA-seq toolkit to explore post-transcriptional gene regulation across species, tissues, and technologies[48]. These emerging technologies may resolve the precise exon composition of each transcript, thereby enabling the unambiguous assignment of specific H3R isoforms to defined neuronal subtypes, glial populations, or synaptic compartments. Such cell-type-resolved isoform maps would be instrumental for guiding the development of isoform-selective ligands: if a particular isoform is preferentially enriched in a disease-relevant cell population (e.g., histaminergic neurons vs GABAergic interneurons), pharmacological targeting of that specific isoform could achieve cell-type-restricted modulation with potentially fewer off-target effects. Conversely, isoform-selective antagonists could be designed to spare isoforms expressed in non-target regions, thereby improving therapeutic windows for CNS disorders such as narcolepsy, schizophrenia, or Alzheimer's disease. Ultimately, integrating full-length single-cell transcriptomic datasets with high-resolution functional screening and structure-based drug design will be essential to unlock the translational potential of H3R splicing diversity, paving the way for next-generation precision therapeutics that exploit isoform-specific signaling fingerprints.

Other H3R interacting proteins

With regard to canonical G protein signaling, the physiological roles of H3R are strongly conditioned by physical and functional relations to a range of receptors, ion channels, and intracellular factors. The intracellular interaction pattern contributes, along with splicing, to an added level of complexity in its function.

In the striatum, H3R is co-expressed with dopamine D1 and D2 receptors (D1R and D2R) in medium spiny neurons (MSNs)[23,49]. Interestingly, H3R activation does not regulate the canonical cAMP/PKA/DARPP-32 pathway in either D1- or D2-MSNs *in vivo*, challenging the simplistic view of H3R as a conventional G_i-coupled receptor in this brain region. Instead, H3R engages distinct signaling modules depending on the MSN subtype: in D1-MSNs, it triggers a rapid and transient activation of the MAPK cascade (ERK-MSK1-rpS6) and a brief increase in GSK3β phosphorylation, whereas in D2-MSNs, it produces no MAPK response but induces a delayed decrease in GSK3β phosphorylation, yielding a biphasic pattern that reflects opposite temporal dynamics between the two cell types. As shown by co-immunoprecipitation and proximity ligation assays, H3R physically associates with both D1R and D2R. Notably, H3R co-activation antagonizes D1R-evoked MAPK signaling and completely abolishes D1R agonist-induced hyperlocomotion, while in D2-MSNs it counteracts D2R-mediated Akt-GSK3β signaling through a *β*-arrestin 2-PP2A-dependent, G protein-independent mechanism, without affecting cAMP levels, and similarly attenuates D2R-driven motor responses. This integrated model advanced understanding of how histaminergic and dopaminergic systems interact at the receptor and neural circuit levels, with potential implications for treating motor disorders such as Parkinson's disease.

Besides activating G proteins, H3R can also directly interact with other proteins. For example, it has been found that H3R antagonism confers neuroprotection against cerebral ischemia/reperfusion (I/R) injury by disrupting the physical association between H3R and

CLIC4, an interaction that is markedly enhanced under ischemic stress. Strikingly, the beneficial effects of H3R blockade persist in histamine-deficient (HDC$^{-/-}$) mice and are not reversed by antagonists of other histamine receptors, suggesting that these actions may arise from direct modulation of receptor-scaffold interactions. Once CLIC4 is released, it triggers dephosphorylation of the Akt/GSK-3β/mTOR pathway, which induces protective autophagy in neurons[32]. Similarly, H2R negatively regulates oligodendrocyte differentiation and remyelination through its stress-induced interaction with Axin2, a scaffold protein that promotes β-catenin degradation[50]. Under hypoxic-ischemic or demyelinating conditions, H2R-Axin2 binding stabilizes β-catenin *via* GSK3β inactivation, thereby activating the Wnt/β-catenin pathway and inhibiting oligodendrocyte maturation. In contrast, H2R antagonism or conditional deletion of H2R in differentiating oligodendrocytes disrupts this complex, accelerates oligodendrocyte differentiation, and promotes behavioral recovery after neonatal hypoxic-ischemic injury. Together, these findings position histamine receptors as stress-responsive signaling hubs that translate cellular injury into specific intracellular protein-complex rearrangements.

Constitutive activity

Beyond its canonical activation by endogenous histamine, H3R exhibits robust constitutive activity, which means it displays unequivocal basal signaling activity even without histamine. This property enables the receptor to autonomously modulate downstream effector pathways in a context-independent manner. Constitutive activity is supposed to serve as one of the molecular foundations for H3R's role as an autoreceptor, enabling tonic negative feedback regulation of histamine synthesis and release even in the absence of synaptic histamine. Moreover, the constitutive H3R signaling in the histamine-depleted striatum appears to actively destabilize corticostriatal circuitry through cell-type-specific signal transduction, providing a mechanistic framework for the repetitive behavioral phenotypes observed in histamine dysregulation-associated neuropsychiatric conditions such as Tourette syndrome[51]. In *HDC*$^{-/-}$ mice, striatal H3R expression is markedly upregulated, and the receptor's ligand-independent basal activity does not merely compensate for histamine loss, but actively reshapes the signaling landscape of both MSN subtypes[52,53]. Specifically, dMSNs exhibit elevated baseline MAPK phosphorylation, while iMSNs show reduced Akt phosphorylation, mirroring the effects of H3R agonism in wild-type animals. This constitutive H3R activity creates a state of pathological baseline bias in the striatal circuitry, rendering it hypersensitive to further challenge. Indeed, administration of an exogenous H3R agonist in *HDC*$^{-/-}$ mice triggers robust stereotypy, which is both necessary and sufficient to drive repetitive behavioral pathology.

Systematic mutagenesis has identified the role of aromatic amino acids in ECL2 in modulating the constitutive activity of H3R and H4R[27]. These aromatic residues are essential for stabilizing the receptor's spontaneous active-state conformation. FSEC (Fluorescence Size-Exclusion Chromatography) and FSEC-TS (Thermostability assay) analyses have further suggested that increased constitutive activity correlates with reduced structural stability. This finding indicates that receptors with inherently lower conformational rigidity are more prone to spontaneously adopting an active conformation, thus exhibiting higher basal signal output. Conversely, stabilizing mutations that rigidify the receptor architecture tend to suppress constitutive activity[54]. Moreover, specific amino acid substitutions can selectively potentiate or dampen H3R constitutive signaling. For instance, introducing the C471R mutation enhances signal transduction capacity through conformational modulation[54]. Notably, the same mutation does not produce identical effects in other histamine receptor subtypes, underscoring that H3R constitutive activity is highly dependent on its unique conformational dynamics and structural context. Furthermore, evidence so far has not linked differential constitutive activity to specific H3R splice variants (e.g., hH3R445 vs hH3R365). There is no settled answer yet to the question of whether or not these isoforms differ in basal signaling.

Optogenetic and photopharmacological tools for the spatiotemporal study of H3 receptors

Optogenetics integrates genetic engineering with optical stimulation to achieve millisecond-precision neuronal control. This approach offers superior spatiotemporal resolution compared to traditional electrical stimulation or chemogenetic methods. It enables selective targeting of specific cell types and permits the co-expression of activators and inhibitors within the same neuron. The technique relies on light-to-electrical signal conversion. The 1971 discovery of bacteriorhodopsin, a light-activated proton pump, was limited by slow kinetics and weak currents, which precluded action potential generation in mammalian neurons[55]. Nevertheless, this finding laid the foundation for subsequent breakthroughs. In 2003, Karl Deisseroth's team identified the single-component, light-gated channels channelrhodopsins ChR1 and ChR2[56,57]. Their viral expression in neurons allowed for precise control over firing patterns and synaptic transmission. The term 'optogenetics' was formally introduced in 2004[58,59]. In recent years, optogenetic tools have also been applied to the spatiotemporal functional dissection of GPCRs, enabling pathway-selective and cell-type-specific modulation of receptor signaling *in vivo*.

OptoXRs (Fig. 2a) represent chimeric optogenetic systems made by joining the extracellular photosensitive domain of rhodopsin with the intracellular signal elements (cytoplasmic loops and C-terminus) of selected GPCRs. Such constructs make it possible to control spatiotemporally specific GPCR activity, without the drawbacks of whole-body pharmacology. Mechanistically, light causes isomerization of retinal inside the rhodopsin part, resulting in a conformational shift that spreads along the chimeric boundary to the GPCR intracellular regions. That shift resembles natural ligand-mediated activation, with recruitment of G proteins to start downstream effects (e.g., cAMP and MAPK pathways) at millisecond speeds and with micrometer-level localization. OptoXRs have been helpful in analyzing neural circuits, directing migration of particular cell types, and determining the behavioral roles of GPCR subtypes *in vivo*.

Building on this platform, an H3R-specific optogenetic tool, OptoH3R, has been constructed by fusing the photosensitive domain of rhodopsin with the intracellular signaling domains of the histamine H3 receptor. This chimeric protein enables light-controlled activation of downstream H3R pathways. Through two-by-two controls (vector plasmid vs OptoH3R construct, with and without light illumination), OptoH3R was validated to effectively activate the H3R downstream ERK pathway. Moreover, sustained light exposure induced OptoH3R internalization and its co-localization with endosomes, which can be reversed by the β-arrestin-selective inhibitor barbadin. Time-lapse fluorescence imaging revealed that the number of OptoH3R-positive puncta progressively increased over time following laser stimulation. These findings demonstrate that OptoH3R recruits β-arrestin signaling upon sustained optical

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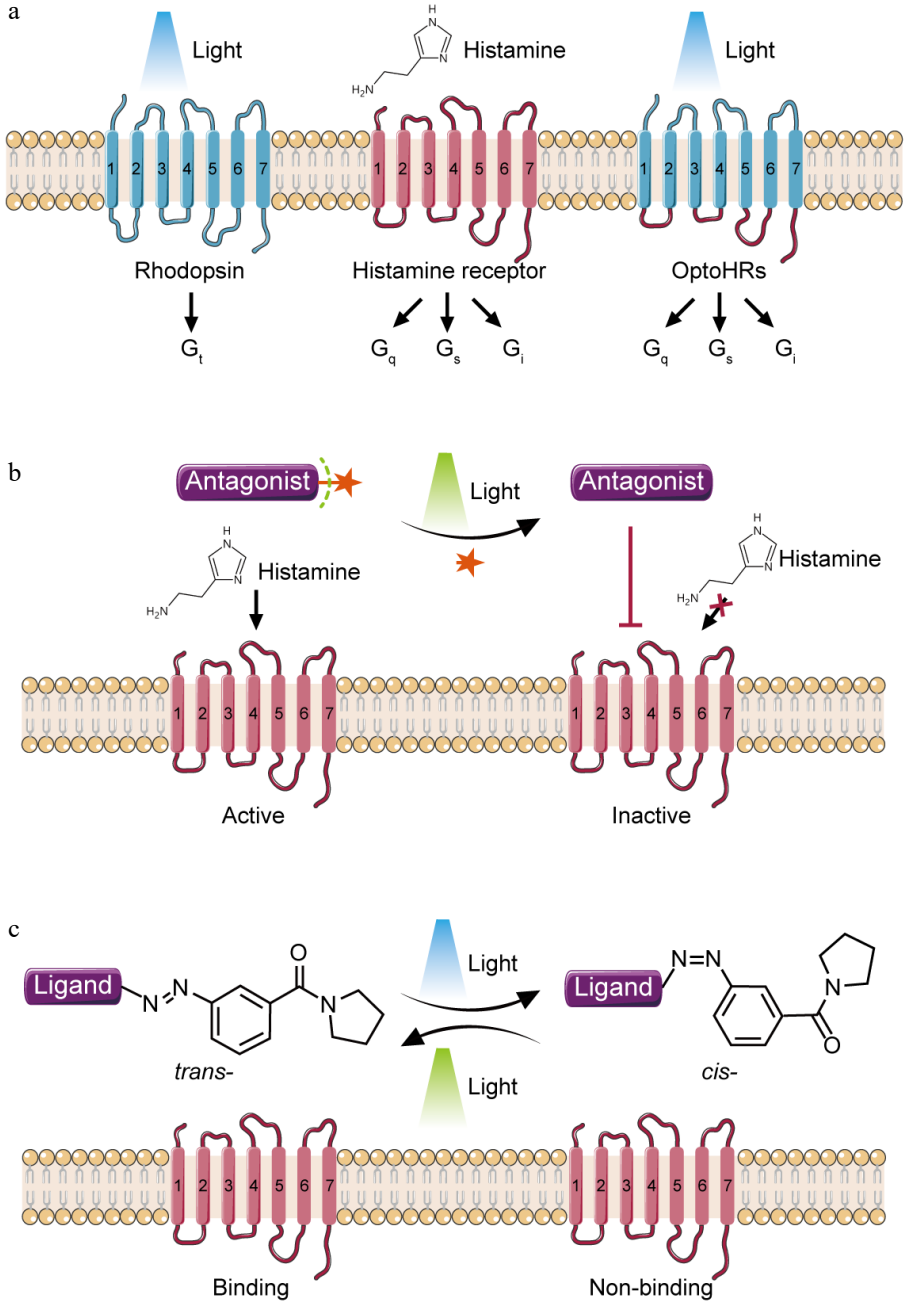

Fig. 2 Schematic diagram showing optogenetic tools for histamine receptor regulation. (a) OptoXRs; (b) Photocaging ligands; (c) Photoswitchable ligands.

stimulation, thereby recapitulating H3R desensitization dynamics. Together, these data suggest OptoH3R as a useful tool for dissecting both the acute signaling and long-term β-arrestin-related mechanisms of H3R with high spatiotemporal precision. Using this tool, a recent study revealed that postsynaptic H3R on ventral basal forebrain (vBF) cholinergic neurons, rather than presynaptic H3R on their axonal terminals, more efficiently suppresses hippocampal acetylcholine accumulation during fear memory retrieval, thereby effectively inhibiting contextual fear memory. Similarly, optogenetic manipulation of spatially restricted H3Rs has identified a critical role for postsynaptic H3Rs in the BNST GABAergic neurons in anxiety regulation, distinct from presynaptic H3Rs from upstream inputs. Collectively, these findings demonstrate that the OptoH3R tool uncovers the spatiotemporal specificity of H3R signaling, particularly highlighting postsynaptic H3R functions which were previously overlooked due to the limitations of pharmacological approaches.

Beyond genetically encoded tools such as OptoXRs, chemical approaches, usually referred to as photopharmacology, employ externally applied light-sensitive molecules without modifying the target proteins[60]. Photopharmacology can be divided into two principal molecular strategies, namely photocaging and photoswitching. Photocaging involves covalently attaching a photoremovable protecting group to a biologically active molecule, rendering it inert until illumination triggers an irreversible bond cleavage ('uncaging') to release the active ligand with high temporal precision. This strategy has been successfully applied to a range of neurotransmitters (e.g., glutamate, GABA, dopamine, serotonin, oxytocin) and GPCR ligands, enabling pulse-like activation studies[61,62]. In contrast, photoswitching employs a photoresponsive moiety that

undergoes reversible trans-to-*cis* isomerization under different wavelengths, allowing the ligand to toggle repeatedly between active and inactive states. The azobenzene scaffold has so far emerged as the most versatile photoswitch moiety[63]. Therefore, photoswitchable GPCR ligands are typically designed with the incorporation of the azobenzene unit into a known template ligand via core or half-core substitution to maximize conformational change upon illumination[64,65].

For histamine research specifically, researchers have constructed a photocaged prodrug (Fig. 2b), VUF25549, by modifying the clinically used antihistamine desloratadine (a histamine H1R antagonist) with a BODIPY (boron-dipyrromethene) photoremovable protecting group[66]. This photocaged compound exhibits reduced affinity for H1R compared to the parent drug, displaying negligible antagonistic activity in the dark and thereby effectively avoiding nonspecific pharmacological interference. Upon illumination with 560 nm visible light, the BODIPY moiety undergoes photolytic uncaging, quantitatively releasing the active parent drug desloratadine and restoring its ability to suppress H1R signaling. For H3R, photoswitchable antagonists such as VUF14862 and VUF14738 have been developed (Fig. 2c), enabling light-induced receptor blockade or release with high spatiotemporal precision[67]. Employing a core-centered design strategy, researchers replaced the naphthalene core of a known H3R antagonist with an azobenzene photoswitchable moiety, yielding a series of ligands whose affinity for H3R could be modulated by light. Through systematic structure–activity relationship exploration, two key compounds emerged: VUF14738[68] and VUF14862[69]. Upon illumination at 360 nm, VUF14738 exhibits a 13.5-fold increase in H3R affinity, whereas VUF14862 has an 11.2-fold lower value. Each compound shows an unusually long thermal relaxation half-life (114 d for VUF14738 and 26 d for VUF14862 in buffered water) and is highly resistant to photobleaching, which makes it possible to analyze its photochemistry thoroughly by means of NMR, LC-MS, or UV-vis spectroscopy. In Xenopus oocytes co-expressing H3R and GIRK channels, a two-electrode voltage clamp electrophysiology assay showed that both compounds could regulate histamine-related GIRK currents in real time with light at 360 and 434 nm.

Recent advances in the functional spatial heterogeneity of H3 receptors

The broad distribution of H3R across brain regions, cell types, and subcellular compartments argues against a single, uniform signaling mechanism. Instead, H3R output is shaped by pathological state, subcellular localization, neuronal identity, circuit context, and G-protein coupling bias. These interacting dimensions can generate divergent and sometimes opposing effects from the same receptor. This layered view shifts the central question from whether H3R is present in a given region to which signaling state the receptor adopts in a defined cellular and temporal context (Fig. 3).

Yan et al. provided early evidence that disease state can remodel H3R signaling independently of its canonical histaminergic function. Cerebral ischemia/reperfusion increased H3R expression in cortical neurons, whereas pharmacological antagonism or genetic deletion of H3R reduced infarct volume[32]. Mechanistically, ischemia enhanced the interaction between H3R and chloride intracellular channel 4 (CLIC4). This complex sustained Akt/GSK-3β/mTOR/p70S6K signaling and suppressed protective autophagy, whereas H3R blockade disrupted the interaction and restored autophagic flux. Protection persisted in *HDC*$^{-/-}$ mice lacking endogenous histamine, confirming a histamine-independent mechanism[32]. Thus, under pathological conditions, H3R can function as a cell-death-promoting intracellular signaling node rather than solely as a presynaptic neuromodulatory receptor. The convergence of receptor-protein interaction, genetic deletion, and histamine-deficient models provides a causal chain linking pathological receptor remodeling to neuronal injury rather than merely associating H3R expression with ischemic damage.

Subcellular localization provides a second layer of specificity. Using OptoH3R to activate receptor signaling in defined compartments, Zhou et al. showed that H3R in ventral basal forebrain cholinergic neurons regulates contextual fear-memory retrieval[24]. Activation in cholinergic somata and dendrites reduced neuronal firing and suppressed memory retrieval, whereas activation at dorsal hippocampal terminals had no behavioral effect despite reducing local acetylcholine release[24]. Postsynaptic H3R signaling therefore has greater circuit-level gain than terminal signaling, demonstrating that receptor pools within the same neuronal population are not functionally equivalent. Because activation in both compartments can influence acetylcholine output, the critical distinction lies in the additional control of somatic excitability and its amplification across the broader cholinergic projection system.

H3R output is also determined by neuronal identity and receptor co-expression. In the striatum, H3R is expressed in both D1 receptor-expressing medium spiny neurons (D1-MSNs) and D2-MSNs but produces distinct downstream effects[49,70]. In D1-MSNs, H3R forms heteromers with D1R and redirects D1R signaling from G_s toward G_i-dependent MAPK/ERK1/2 activation[70]. H3R activation also increases inhibitory GSK3β Ser9 phosphorylation and opposes D1R-mediated locomotor activation. In D2-MSNs, the same agonist decreases GSK3β Ser9 phosphorylation without activating MAPK, revealing a cell-type-specific reversal[49,70]. In preclinical Huntington's disease models, targeting the D1R-H3R heteromer attenuated neurotoxic signaling and neuronal degeneration; its loss at advanced stages further suggests a stage-dependent therapeutic window[71]. Receptor heteromerization and MSN subtype therefore act together to determine pathway selection, illustrating how the molecular neighborhood of H3R can reshape both the magnitude and direction of its signal.

At the circuit level, internal state gates the behavioral relevance of H3R signaling. Lin et al. identified a tuberomammillary nucleus-to-bed nucleus of the stria terminalis (TMN-BNST) histaminergic projection involved in anxiety[72]. Activating this projection promoted anxiety-like behavior in naïve mice, whereas inhibiting it was anxiolytic only after acute stress. Postsynaptic H3Rs on BNST GABAergic neurons mediated this effect, while H3Rs on glutamatergic neurons and presynaptic heteroreceptors on upstream inputs were dispensable[72]. Thus, a defined H3R-dependent circuit can acquire different behavioral relevance according to brain state. Combining state-dependent circuit manipulation with cell-selective receptor mapping places H3R function within a defined projection, postsynaptic cell class, and behavioral state rather than attributing anxiety regulation to the receptor globally.

Biased coupling and the history of receptor activation together introduce a temporal dimension to H3R signaling. In a recent study, Zheng et al. identified H3R as selectively enriched in fat-responsive neurons of the paraventricular thalamus (PVT). Modulating H3R signaling in the PVT altered fat intake without affecting sugar consumption. Moreover, histamine boosted neuronal excitability *via* H3R-$G_{12/13}$-biased signaling[31]. In parallel, Zhao et al. showed that sustained histaminergic drive during sleep restriction instead induced *β*-arrestin-dependent H3R downregulation and reduced the excitability of fat-responsive PVT neurons. Restoring H3R expression

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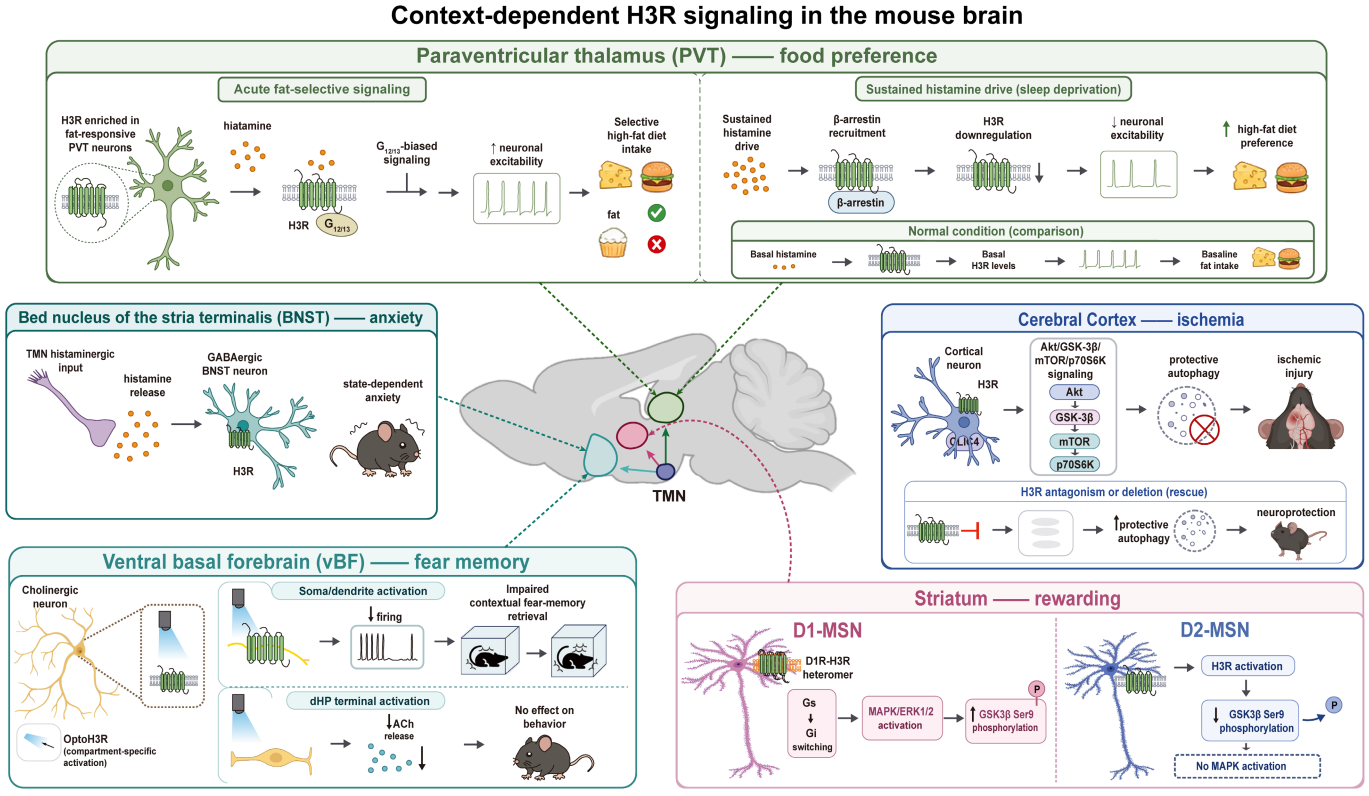

Fig. 3 Schematic diagram showing recent advances in the functional heterogeneity of H3R. H3R signaling is context-dependent, shaped by pathological state (ischemia promotes pro-death CLIC4 complexes), subcellular compartment (somatic vs terminal pools in cholinergic neurons), cell type (D1- vs D2-MSN heteromers redirect G-protein bias), circuit state (TMN-BNST projection gated by stress), and activation history (acute G12/13 excitation vs chronic β-arrestin-mediated downregulation in PVT feeding neurons). These interacting dimensions can yield opposing behavioral outcomes from the same receptor. Thus, H3R should be viewed as a family of context-defined signaling states rather than a uniform target, explaining why global modulation has limited efficacy.

or inhibiting β-arrestin rescued the fat-preference phenotype[73]. Taken together, these findings support a model where acute H3R activation favors $G_{12/13}$-dependent excitation, whereas chronic stimulation promotes β-arrestin-mediated receptor adaptation. This contrast between rapid excitation and long-term receptor loss effectively links coupling bias to activation history and provides a mechanistic framework for how H3R signaling can switch its functions within the same thalamic feeding circuit.

Collectively, these findings organize H3R heterogeneity along interacting spatial and temporal dimensions. Brain region, cell identity, receptor partners, and subcellular compartment define the molecular context in which H3R operates, whereas pathological state, circuit activity, and ligand-exposure kinetics influence the pathway recruited. H3R is therefore better conceptualized as a family of context-defined signaling states than as a single target with a fixed signature. These levels are not independent: pathological or physiological state can alter receptor abundance and interacting proteins, while cellular location constrains the G proteins, effectors, and circuit consequences available to H3R.

This framework may help explain why systemic H3R modulation has shown limited clinical efficacy in central nervous system disorders: global drugs engage multiple receptor states, some with opposing functions. Mapping H3R signaling fingerprints across brain regions and cell types will therefore be essential for developing interventions with appropriate spatial and mechanistic precision. Accordingly, future drug development should prioritize signaling-state selectivity, receptor-complex targeting, or anatomically restricted delivery rather than relying exclusively on ligand affinity for H3R itself.

Spatiotemporal regulation of H3 receptor signaling

GPCR-biased signaling drug design of H3R

GPCR-biased signaling has emerged as a transformative paradigm in drug discovery, shifting the focus from simple agonism or antagonism to pathway-precise modulation (Fig. 4a)[74,75]. The central premise is to develop ligands that preferentially stabilize distinct receptor conformations, thereby selectively engaging either G-protein or β-arrestin cascades, which allows retention of desired therapeutic effects while circumventing off-target adverse events. The clinical value of biased ligands is well illustrated by opioid receptor examples. For the μ-opioid receptor (μOR), G-protein-biased agonists are designed to harness the G_i-mediated analgesic signaling while circumventing β-arrestin-driven respiratory suppression and constipation[76,77]. The most notable translational success is TRV130 (oliceridine), a G-protein-biased μOR agonist that has received clinical approval for pain management, marking the first time this strategy has reached patients[78]. Similarly, κ-opioid receptor (κOR) G-protein-biased agonists retain potent analgesia and antipruritic effects but significantly reduce aversion and sedation, which are typically associated with β-arrestin recruitment[79,80]. Despite these conceptual advances, precise spatiotemporal regulatory strategies specifically tailored to the H3R remain conspicuously underdeveloped. However, given that H3R is a typical GPCR that couples to multiple downstream effectors and exhibits signaling plasticity depending on cellular context and disease state, it is reasonable to apply the biased agonism framework to H3R-directed

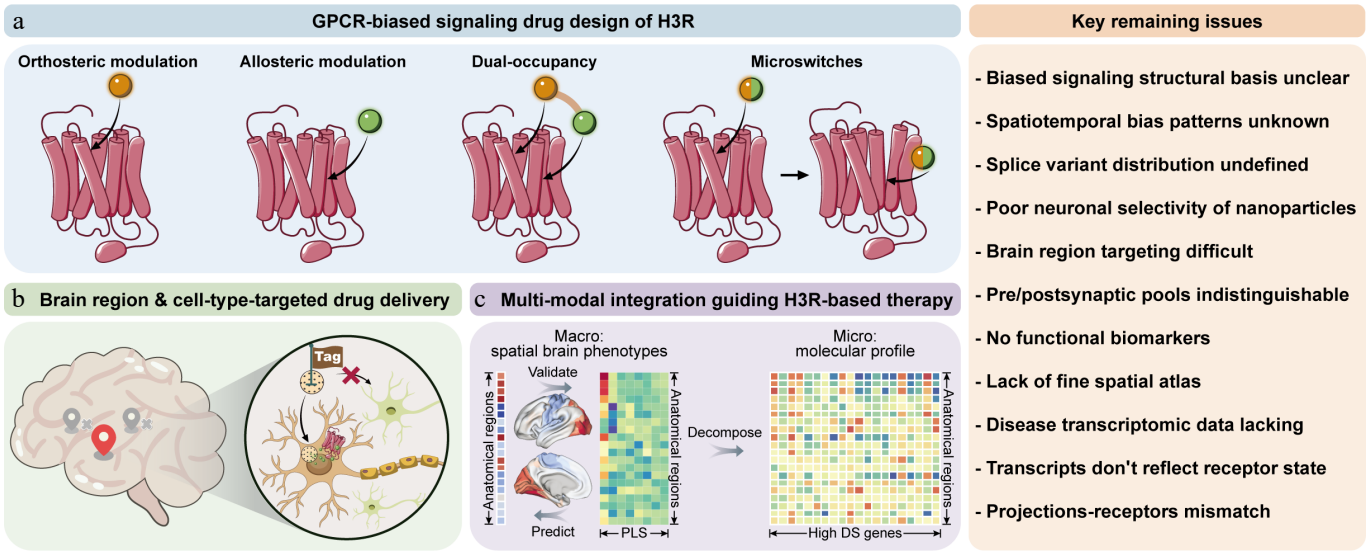

Fig. 4 Schematic diagram showing key remaining issues about spatiotemporal regulation of H3R. (a) GPCR-biased signaling drug design of H3R. (b) Brain region and cell-type-targeted drug delivery. (c) Multi-modal integration guiding H3R-based therapy.

drug discovery. Such H3R-biased ligands would be expected to preserve beneficial outcomes (e.g., targeted suppression of fat intake) without the side effects connected to non-selective pathway activation (e.g., drowsiness), which enlarges the therapeutic window and gives rise to ideas of spatiotemporal control of diverse or complex neurological states.

Biased ligands are developed through distinct operational workflows that integrate structural biology, medicinal chemistry, and functional screening. The most direct approach is biased agonism and biased antagonism, both of which operate through the orthosteric binding pocket. Generally, biased agonists can be identified by the biased activation of a desired downstream effector using high-throughput BRET or NanoBiT screening[81,82]. Following effector selection, rational or empirical medicinal chemistry is employed to optimize chemical substituents such that the ligand selectively stabilizes specific transmembrane helical rearrangements. This iterative design cycle is subsequently validated through structural determination (X-ray crystallography or cryo-EM) to resolve the active ligand-bound conformation, complemented by site-directed mutagenesis to pinpoint critical contact residues. Finally, the dose-response functional assays (e.g., cAMP accumulation for G_s/G_i pathways, or TANGO for arrestin recruitment) are needed to quantify the bias factor. For biased antagonists, the workflow is analogous but reversed in functional readout. Notably, the boundary between agonism and antagonism is not strictly fixed. For example, Carvedilol has previously been found to biasedly activate the β_2AR-arrestin pathway, but was later re-categorized through detailed pathway profiling as a biased antagonist that actively stabilizes an arrestin-permissive conformation while precluding G_s engagement[83,84].

Allosteric modulation represents a distinct strategy for achieving signaling bias. Unlike orthosteric ligands, which bind to the conserved endogenous ligand pocket, allosteric modulators engage topographically distinct sites on the receptor, typically on extracellular loops, transmembrane helical surfaces, or intracellular interfaces. By binding to these auxiliary sites, they induce conformational changes that fine-tune the receptor's response to orthosteric agonists, selectively enhancing or inhibiting specific downstream pathways without directly competing with the endogenous ligand. For example, the orthosteric binding pocket of CB1 is formed by residues from TM2, TM3, TM5, TM6, TM7, and the extracellular loops, accommodating endogenous cannabinoids and synthetic $\Delta^{[9]}$-tetrahydrocannabinol analogs[85]. In parallel, CB1 possesses a distinct allosteric pocket located on the extrahelical surface of the TM2-TM3-TM4 interface, where compounds such as the balanced ago-PAM ZCZ011 bind[86]. As a result, CB-05, a G_i-biased ago-allosteric modulator (ago-BAM), was developed. It has been shown that CB-05 produces robust analgesia without inducing catalepsy, hypothermia, or psychoactive effects typically associated with orthosteric CB1 agonists in mice[87].

For receptors where the high conservation of the orthosteric pocket limits subtype or pathway selectivity, bitopic ligands offer a dual-occupancy strategy[88]. Unlike conventional ligands that engage only a single binding site, bitopic molecules comprise two distinct pharmacophores connected by a chemical linker, enabling simultaneous occupancy of both the orthosteric site and a topographically distinct secondary site on the same receptor. The design workflow typically begins with independent identification of an orthosteric pharmacophore and a fragment that binds to an adjacent allosteric or accessory pocket. The critical step involves systematic optimization of the linker, including variations in length, rigidity, attachment points, and spatial orientation, to ensure that both moieties can bind simultaneously without steric clashes. This dual engagement induces a unique, constrained receptor conformation that cannot be achieved by either fragment alone, thereby conferring enhanced selectivity and often a distinct signaling bias relative to the parent orthosteric ligand. C6 guano was developed as a bitopic ligand targeting the μOR[89]. The orthosteric pharmacophore of C6 guano is derived from a morphinan scaffold, which occupies the classical opioid-binding pocket. In parallel, the second moiety is designed to engage the sodium-ion allosteric pocket, which is known to modulate receptor conformation and G-protein coupling. By iterative optimization of the linker connecting these two components, the resulting bitopic ligand achieves high G-protein efficacy while exhibiting markedly reduced β-arrestin recruitment. Like oliceridine, this biased agent also exerts μ-opioid-receptor-dependent analgesia with fewer adverse effects such as respiratory depression and tolerance.

When the therapeutic goal requires modulation of receptor oligomers rather than individual protomers, the bivalent ligand strategy provides an alternative avenue. Unlike monovalent ligands that engage a single orthosteric site, bivalent molecules consist of

two pharmacophores connected by a linker, designed to bridge two spatially adjacent binding sites on a receptor homo- or heterodimer. The critical step involves conjugating two appropriate pharmacophores with a linker whose length, flexibility, and attachment geometry are systematically optimized to span the distance between the two orthosteric binding pockets of the respective protomers. For example, 6'-GNTI was designed as a bivalent ligand to simultaneously engage the orthosteric pockets of δOR and κOR within the heterodimeric complex. Through this dual occupancy, 6'-GNTI preferentially activates the δOR-κOR heterodimer over the individual monomeric receptors, inducing a G-protein signaling pattern that is distinct from that elicited by either receptor alone[90,91].

Finally, a structurally driven approach, namely microswitch and intracellular interface remodeling, directly depends on high-resolution static structures to achieve signaling bias[75]. Unlike ligand-centric strategies that focus on binding site occupancy, this approach begins with detailed Cryo-EM or X-ray crystallography data to pinpoint specific residue–residue interactions. If each residue's rotameric state is known for multiple activation configurations (e.g., inactive or active G-protein-bound, or active β-arrestin-bound forms), it is possible to plan ligand scaffold changes that lead to pathway selectivity. For example, FUB (MDMB-Fubinaca) binds to the orthosteric pocket of CB1. Upon FUB binding, the twin-toggle switch ($F200^{3.36}$/$W356^{6.48}$) undergoes conformational rearrangements accompanied by outward movements of TM3, TM6, and TM7, which together expand the intracellular cavity to accommodate the β-arrestin finger loop[92]. Notably, despite being a balanced agonist capable of activating both G_i and β-arrestin pathways, FUB exhibits distinct binding poses in the G_i- vs β-arrestin-coupled states, demonstrating that even subtle conformational changes at the transducer interface can propagate to the orthosteric binding pocket. This observation provides a structural basis for understanding how pathway selectivity may be achieved through rational ligand design.

Despite the conceptual appeal of these biased ligand strategies, their application to H3R remains confined to the orthosteric agonist domain. Systematic profiling of H3R agonists across multiple signaling endpoints has revealed that ligand-directed biased agonism is indeed a robust phenomenon at this receptor. For instance, proxyfan, a prototypical 'protean agonist' at H3R, and its congener iodoproxyfan both exhibit strong bias toward GSK3β phosphorylation and are markedly biased away from calcium signaling via the full-length hH3R445 isoform[39]. Imetit, another widely studied agonist, is heavily biased away from calcium signaling and shows preferential signaling through the shorter hH3R365 isoform. Notably, these bias patterns are isoform-dependent. hH3R365 is completely unable to stimulate GSK3β phosphorylation, supporting differential biased signaling *via* naturally co-expressed H3R splice variants. More recent studies have systematically evaluated ligand-directed biased agonism at the hH3R-445, -415, -365, and -329 isoforms across multiple $G_{\alpha i/o}$-mediated pathways (e.g., [^{35}S]GTPγS accumulation, cAMP inhibition, pERK1/2, pAKT) and β-arrestin2 recruitment. Quantitative bias analysis revealed that the direction and magnitude of ligand bias are both pathway- and isoform-dependent. For instance, at the full-length hH3R-445 isoform, proxifan and impentamine act as potent full agonists for cAMP inhibition yet behave as neutral antagonists for β-arrestin2 recruitment, demonstrating extreme bias toward the G protein pathway. Strikingly, at the ICL3-truncated hH3R-365 isoform, the bias is reversed. At this isoform, imetit and immepip exhibit high degrees of bias toward β-arrestin2 recruitment away from cAMP inhibition (45- and 48-fold, respectively). Global principal component analysis further revealed that β-arrestin2 recruitment and ERK1/2 phosphorylation are the two most highly correlated endpoints, whereas these are only weakly correlated with direct G protein binding, suggesting that β-arrestin2 recruitment may better reflect downstream H3R signaling in the CNS. Collectively, these findings demonstrate that H3R agonists display significantly altered bias patterns in a pathway- and isoform-dependent manner, underscoring the importance of isoform context in biased ligand design[21].

In contrast, biased ligands operating through allosteric, bitopic, or bivalent mechanisms remain virtually unexplored for H3R. To date, no allosteric modulator with documented signaling bias has been reported for H3R, although sodium ions in the allosteric site have been shown to influence orthosteric ligand binding of H1R[93]. Bitopic ligands targeting H3R have been proposed primarily in the context of dopamine D_2/D_3 receptor heterodimers, where bivalent molecules designed to bridge D_2R and H3R protomers have been synthesized and characterized[94]. However, these compounds have been evaluated mainly for binding affinity rather than biased signaling profiles, and the systematic optimization of linker geometry to achieve pathway selectivity has not yet been applied to H3R-directed bitopic design. Similarly, bivalent ligands targeting H3R homo- or heterodimers have been reported[95], but their functional characterization remains limited, and no evidence of signaling bias has been established. This stark contrast between the well-documented orthosteric biased agonism and the near-absence of other kinds of biased ligands for H3R underscores a significant gap in the current medicinal chemistry landscape. Bridging this gap will require integrated efforts combining structure-based design with high-throughput functional screening across multiple pathway endpoints and isoform contexts.

Despite its therapeutic promise in narcolepsy, ADHD, schizophrenia, and obesity, conventional H3R antagonists/inverse agonists (e.g., pitolisant) have yielded mixed clinical outcomes, possibly due to lack of pathway selectivity and the complex, context-dependent nature of H3R signaling. So far, the field faces several challenges to realize biased regulation of H3R. Although inactive and G_i-coupled structures are available, the conformational determinants that govern differential G protein vs β-arrestin engagement have not been systematically characterized, thus precluding rational biased ligand design. Moreover, H3R is expressed across multiple brain regions and cell types, with both pre- and postsynaptic localizations, and the functional outcomes of pathway-selective activation likely vary depending on neuronal context, disease state, and the local signaling environment. The spatiotemporal patterns of different biased signaling pathways and their associated physiological or pathological significance have yet to be systematically elucidated, which serves as a prerequisite for guiding function-targeted drug design. Furthermore, as a CNS target, H3R-directed compounds must exhibit favorable blood-brain barrier (BBB) permeability, metabolic stability, low off-target liability, and acceptable safety profiles, all of which narrow the chemical space available for introducing bias-conferring moieties and complicate the optimization of pathway-selective ligands. Together, these challenges highlight the need for integrated structural, pharmacological, and medicinal chemistry efforts to unlock the full therapeutic potential of biased H3R modulation.

Brain region and cell-type-targeted drug delivery

Emerging cell-specific targeting technologies offer new tools for realizing spatiotemporally precise modulation of H3R signaling (Fig. 4b). The most extensively explored strategy employs receptor-mediated transcytosis targeting peptides that bind to receptors overexpressed on brain capillary endothelial cells or specific neural cell types. For instance, RVG29, which targets nicotinic acetylcholine

receptors (nAchR) and GABA receptors expressed on neurons, has been used to functionalize nanoparticles for drug delivery in Alzheimer's disease[96]. Similarly, astrocyte-specific delivery can be achieved by modifying nanoparticles with adenosine ligands, which bind to adenosine receptors overexpressed on astrocytes and trigger receptor-mediated endocytosis, enabling cell-type-specific siRNA delivery to modulate astrocyte polarization following traumatic brain injury[97]. In addition to ligand-based strategies, physicochemical properties of nanocarriers can also confer inherent cell-type selectivity. For example, unmodified hydroxyl-terminated polyamidoamine (PAMAM) dendrimers possess intrinsic tropism for activated microglia and macrophages thanks to their small size (~4–5 nm) and nearly neutral surface charge, allowing them to cross the impaired BBB and preferentially accumulate in these cells without requiring additional targeting moieties[98].

However, the translation of these novel cell-specific targeting approaches to H3R regulation still presents many difficulties. H3R is predominantly expressed on neurons, yet currently available nanoparticle delivery techniques recognize glial cells far more reliably than they do individual neuronal subtypes. Selecting surface markers suitable for the identification of particular neuronal classes is a hard problem since most receptors used by neurons are not unique to one type and may be shared among several cell types. Even with substantial progress in nanoparticle engineering for improved BBB penetration, obtaining region-specific brain delivery is still uncertain. Most systemically administered nanoparticles accumulate broadly at sites of BBB disruption (e.g., injury or tumor) rather than in specific anatomical regions such as the hypothalamus, cortex, or striatum. Even within a given brain region, H3R can reside on both pre- and postsynaptic compartments, yet no known targeting method identifies their subcellular localizations. Nevertheless, despite these obstacles, pursuing H3R-targeted delivery remains a worthwhile endeavor since emerging technologies, including spatially resolved single-cell transcriptomics[99,100] and nanobody platforms[101,102], may provide novel approaches to overcome these limitations. Table 2 summarizes the current evidence gap between these extrapolated strategies and H3R-specific development across the approaches.

Multi-modal integration guiding H3R-based therapy

An essential prerequisite for translating H3R-directed strategies into actual clinical practice is the identification of reliable biomarkers that tell us whether a given behavioral impairment results from H3R dysfunction. Without this kind of biomarker, even highly complex spatiotemporal treatments would lack a rational patient-selection framework, risking ineffective treatment or misinterpretation of trial outcomes. However, identifying such biomarkers for H3R brings special difficulties. Unlike peripheral GPCRs, whose activity can often be inferred from circulating hormones or metabolites, H3R is a central membrane receptor whose functional state is not readily reflected by peripheral readouts. Furthermore, direct examination of tissue is seldom feasible in individuals suffering from neuropsychiatric illness.

Recent advances in multi-modal integration, combining neuroimaging, electroencephalography, fluid biomarkers, and computational modeling, may help to solve some problems. Figure 4c provides a schematic overview of this integrative framework. Functional magnetic resonance imaging (fMRI) gives access to macroscopic network dynamics in a non-invasive fashion, but lacks sufficient resolution to identify exactly which neurons or molecular entities are responsible for any given connectivity defect. On the other hand, single-cell sequencing at the microscopic level yields anatomical and molecular detail without functional interpretation. An integrative analysis of fMRI and single-cell data is now being pursued as an effective cross-scale method, which may yield biomarkers tying macroscopic dysfunction to specific cellular substrates.

Table 2. Summary of spatiotemporal targeting strategies: extrapolated evidence vs corresponding H3R development.

	Extrapolated evidence (other GPCRs/CNS delivery systems)		Corresponding H3R development	
	Strategy/tool	Source of evidence	Strategy/tool	Status
Optogenetic and photopharmacological tools	OptoXR chimeric platform	General GPCR optogenetic platform (rhodopsin-GPCR chimera)	OptoH3R	Direct H3R evidence: validated *in vitro* and *in vivo* (ERK activation, β-arrestin internalization, fear memory, and anxiety circuits)
	Photocaged ligand	VUF25549, H1R antagonist desloratadine modified with BODIPY cage	N/A	Not yet developed for H3R
	Azobenzene photoswitch design strategy	General GPCR photopharmacology approach	VUF14862/ VUF14738	Direct H3R evidence: validated by GIRK current electrophysiology
GPCR-biased signaling drug design	G-protein-biased agonist, Oliceridine (TRV130)	μ-opioid receptor, clinically approved	N/A	Not yet developed for H3R
	κOR G-protein-biased agonist	κ-opioid receptor, preclinical	N/A	Not yet developed for H3R
	G_i-biased ago-allosteric modulator, CB-05	CB1 receptor, preclinical	N/A	Not yet developed for H3R
	bitopic ligand, C6 guano	μ-opioid receptor, preclinical	N/A	Not yet developed for H3R
	bivalent ligand, 6'-GNTI	δOR-κOR heterodimer, preclinical	N/A	Not yet developed for H3R
GPCR-biased signaling drug design	Microswitch/intracellular interface remodeling, FUB example	CB1 receptor structural data (Cryo-EM/X-ray)	N/A	Inactive- and G_i-coupled H3R structures are available, but conformational determinants of G-protein vs β-arrestin bias have not been characterized
Brain region and cell-type-targeted drug delivery	RVG29-functionalized nanoparticles	nAChR/GABA receptor targeting, Alzheimer's disease model	N/A	Not yet developed for H3R
	Adenosine-ligand nanoparticles	Adenosine receptor on astrocytes, TBI model	N/A	Not yet developed for H3R
	PAMAM dendrimers	Intrinsic tropism for microglia/macrophages (non-receptor-mediated)	N/A	Not yet developed for H3R

Several analytical frameworks have been developed to facilitate this integration. The Allen Human Brain Atlas (AHBA) pioneered the field of imaging-transcriptomics by registering postmortem microarray-based gene expression data to structural MRI coordinates[103], enabling the identification of spatial expression patterns that covary with imaging-derived phenotypes. Subsequent refinements have incorporated single-cell RNA sequencing (scRNA-seq) data to provide cellular context for these genetic associations, either *via* enrichment tests using predefined cell-type marker lists or through bulk deconvolution algorithms that estimate cell-type abundances from the AHBA's bulk tissue data. These approaches have linked macroscale imaging phenotypes to broad cell classes and have incorporated spatial null models to account for the inherent spatial autocorrelation of brain tissue. More recently, single-cell scoring tools such as Seurat (AddModuleScore)[104], UCell[105], and VISION[106] have enabled direct scoring of individual cells based on imaging-transcriptomics-derived gene sets, allowing researchers to move beyond bulk tissue estimates and examine cellular heterogeneity at single-cell resolution. Together, these frameworks have begun to reveal how molecularly defined cell subpopulations contribute to circuit-level functional organization and disease vulnerability.

Despite these advances, translating such integrative frameworks to H3R biology still faces notable challenges. H3R may exist in multiple splice variants that differ in their signaling properties, tissue distribution, and likely their biological functions. However, the spatial localization of these variants and their contributions to central nervous system disorders remain largely unknown, and clarifying these isoform-specific features is a prerequisite for developing meaningful biomarkers. Moreover, the current imaging-transcriptomics paradigm, which mostly relies on the Allen Human Brain Atlas (AHBA), is constrained by its spatial resolution[107,108]. The AHBA is based on microarrays and samples multiple anatomical regions of the postmortem human brain, with several sampling sites per region. However, its resolution is at the millimeter level, which is far from reaching single-cell or subcellular resolution. Furthermore, there is a general lack of sequencing data from patients with neuropsychiatric disorders. Given that disease states are associated with profound changes in cell-type composition, activation states, and transcriptional programs, predictive models built solely on healthy reference data are likely to misestimate the cellular contributions relevant to disease. Finally, even with improved spatial resolution and disease-relevant reference data, transcript levels alone cannot inform us about functional receptor states, including conformational changes, signaling bias, or pre- vs post-synaptic localization. This underscores the need for complementary approaches, including H3R-specific PET tracers and activity-dependent readouts, to bridge the gap between transcriptional capacity and actual receptor function.

Conclusions and perspectives

The histamine H3 receptor has long defied the expectations raised by its broad CNS expression and functional regulation of histamine release. Despite three decades of intensive study, systemically administered H3R ligands have yielded modest and inconsistent clinical outcomes, which possibly lies in the spatiotemporal expression and functional complexity. Conventional pharmacology may be fundamentally mismatched to this complex biology. A key issue currently facing H3R-based drug development is the need to systematically characterize H3R expression and function across different spatial contexts, including different brain regions, cell types, and subcellular localizations. Further progress will also require coordinated efforts on biased signaling regulation, cell-type- and region-specific delivery, and functional biomarkers to guide patient selection.

Other than the three therapeutic axes discussed above, several frontier directions merit further investigation. One such direction is the longstanding 'projection-receptor mismatch' in the histaminergic system[109]. Intriguingly, this mismatch appears to be particularly pronounced for postsynaptic H3Rs. Constitutive activity has been proposed as one possible mechanism behind this phenomenon. However, this mechanism fails somewhat to explain the complexity found in practice, since constitutive signaling tends to generate a homogeneous downstream tone (either activating or inhibiting). Moreover, H3R has a much broader array of downstream responses than H1R or H2R[30], suggesting that its own metabolites or native ligands might also be able to affect H3R. Whether or not these alternate ligands help to shape the functional heterogeneity of H3R is an issue of considerable interest requiring careful analysis.

Ethical statements

Not applicable.

Author contributions

The authors confirm their contributions to this work as follows: conceptualization: Zheng Y; visualization: Zheng Y, Fang Z; investigation: Zheng Y, Fang Z; funding acquisition: Zheng Y; supervision: Chen Z; writing – original draft: Zheng Y, Fang Z; writing – review and editing: Zheng Y, Fang Z, Chen Z. All authors reviewed the results and approved the final version of the manuscript.

Data availability

Data sharing is not applicable to this review as no datasets were generated or analyzed.

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Conflict of interest

The authors have no conflicts of interest to declare.

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