

TAAR1 activation modulates monoaminergic neurotransmission, preventing hyperdopaminergic and hypoglutamatergic activity

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The trace amine-associated receptor 1 (TAAR1), activated by endogenous metabolites of amino acids like the trace amines p-tyramine and β -phenylethylamine, has proven to be an important modulator of the dopaminergic system and is considered a promising target for the treatment of neuropsychiatric disorders. To decipher the brain functions of TAAR1, a selective TAAR1 agonist, RO5166017, was engineered. RO5166017 showed high affinity and potent functional activity at mouse, rat, cynomolgus monkey, and human TAAR1 stably expressed in HEK293 cells as well as high selectivity vs. other targets. In mouse brain slices, RO5166017 inhibited the firing frequency of dopaminergic and serotonergic neurons in regions where *Taar1* is expressed (i.e., the ventral tegmental area and dorsal raphe nucleus, respectively). In contrast, RO5166017 did not change the firing frequency of noradrenergic neurons in the locus coeruleus, an area devoid of *Taar1* expression. Furthermore, modulation of TAAR1 activity altered the desensitization rate and agonist potency at 5-HT_{1A} receptors in the dorsal raphe, suggesting that TAAR1 modulates not only dopaminergic but also serotonergic neurotransmission. In WT but not *Taar1*^{-/-} mice, RO5166017 prevented stress-induced hyperthermia and blocked dopamine-dependent hyperlocomotion in cocaine-treated and dopamine transporter knock-out mice as well as hyperactivity induced by an NMDA antagonist. These results tie TAAR1 to the control of monoamine-driven behaviors and suggest anxiolytic- and antipsychotic-like properties for agonists such as RO5166017, opening treatment opportunities for psychiatric disorders.

drug discovery | serotonin | depression | schizophrenia | anxiety

In 2001, identification of the trace amine-associated receptor 1 (TAAR1) provided evidence for a direct biological effect of so-called trace amines (TAs) (1, 2), a subgroup of biogenic amines previously denoted as false neurotransmitters (3). TAs such as p-tyramine (pTyr), β -phenylethylamine (PEA), octopamine, and tryptamine are metabolites of amino acids with structural similarity to classical biogenic amines. Although they are only found at low concentrations in the brain, TAs have been implicated in a wide range of neuropathological disorders, including schizophrenia, major depression, anxiety states, Parkinson's disease, and attention deficit hyperactivity disorder (3, 4).

TAAR1, a member of the TAAR family (5, 6), is a G protein-coupled receptor (GPCR) that signals through G_s to elevate intracellular cAMP levels in response to TAs (6, 7). In vitro studies have shown a reciprocal regulation between TAAR1 and monoaminergic transporters, particularly the dopamine transporter (DAT) (8, 9). In the mouse brain, *Taar1* is expressed throughout the limbic and monoaminergic systems, including the ventral tegmental area (VTA) and dorsal raphe nucleus (DRN) (10). Mice lacking *Taar1* (*Taar1*^{-/-} mice) have no overt phenotype and appear similar to WT littermates in most neurological and behavioral tests (10–12). However, *Taar1*^{-/-} mice are hypersen-

sitive to the locomotor-stimulating effect of *d*-amphetamine and show elevated striatal release of dopamine (DA), noradrenaline (NA), and serotonin [5-hydroxytryptamine (5-HT)] after a *d*-amphetamine challenge (10, 12). Furthermore, the spontaneous firing rate of the VTA DA neurons is augmented in *Taar1*^{-/-} mice, and only in WT mice does pTyr decrease this firing rate (10). These observations suggest that TAAR1 is a negative modulator of monoaminergic neurotransmission.

Apart from the TAs, TAAR1 is activated by a range of endogenous molecules such as other biogenic amines (2, 5), thyroid hormone-derivative 3-iodothyronamine (T₁AM) (13, 14), and catechol-O-methyl transferase products (e.g., 3-methoxytyramine) (2, 15) or by synthetic substances such as amphetamine derivatives and ergolines (2, 12). However, all these ligands have TAAR1-independent effects through other targets, such as the monoaminergic transporters and receptors or the σ -receptors (3, 11, 13, 16). The lack of selective ligands has rendered identification of TAAR1 biological functions challenging. Recently, Bradaia et al. (17) described the first selective TAAR1 antagonist, *N*-(3-Ethoxyphenyl)-4-pyrrolidin-1-yl-3-trifluoromethyl-benzamide (EPPTB). Use of EPPTB revealed that TAAR1 tonically activates inwardly rectifying K⁺ channels in VTA DA neurons to reduce the basal firing activity (17). Importantly, EPPTB increased agonist potency at DA D₂ receptors while reducing their desensitization rate, strongly suggesting a functional link between TAAR1 and D₂ receptors.

Further elucidation of TAAR1 physiological functions and validation of its promising therapeutic potential require selective agonists. The ideal characteristics of such molecules are (i) high affinity and intrinsic activity (equal or superior to TAs), (ii) high selectivity for TAAR1 (in contrast to TAs), (iii) low metabolic turnover (in contrast to TAs), and (iv) favorable pharmacokinetic properties, such as a good brain penetration, to enable in vivo studies. Here, we report the identification of such a compound, RO5166017, which was used for the study of TAAR1 function in vitro and in vivo.

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Results

Identification of RO5166017 and in Vitro Pharmacological Characterization. There seems to be considerable overlap between the pharmacophore space occupied by TAAR1 ligands and ligands of other biogenic amine receptors (2). Therefore, a medicinal chemistry program seeking to create TAAR1 ligands starting from adrenergic ligands as a source of lead compounds was started. One of the starting compounds was the amino-oxazoline S18616 (18), an α_{2A} -adrenergic receptor agonist, and an iterative series of structural modifications within this chemical class led to the discovery of RO5166017 [(S)-4-[(ethyl-phenyl-amino)-methyl]-4,5-dihydro-oxazol-2-ylamine] (Fig. 1).

RO5166017 exhibited high potency and efficacy at mouse, rat, cynomolgus monkey, and human TAAR1 stably expressed in HEK293 cells (Table 1). Maximal cAMP levels reached by RO5166017 stimulation (maximal efficacy) were in a range similar to that achieved by PEA stimulation (set as 100%) at human, rat, and monkey TAAR1 (81–95%) and somewhat lower at mouse TAAR1 (65%). Electrophysiological recordings in *Xenopus* oocytes as well as VTA and DRN slices resulted in EC_{50} and IC_{50} values for mouse TAAR1 (1.7–8 nM) comparable with those obtained by the cAMP assay in HEK293 cells (3.3 nM).

RO5166017 is highly selective for TAAR1, as evaluated from radioligand binding assays (Cerep) consisting of 123 target proteins (Tables S1 and S2). Whenever the binding of the specific reference labels was significantly inhibited by RO5166017 (10 μ M), further determination of the K_i revealed a low affinity (>100-fold weaker vs. mouse TAAR1). The only exceptions were the κ -opioid, adrenergic α_2 , and imidazoline I_1 receptors for which the selectivity ratios (K_i/K_i) were 79-, 64-, and 15-fold, respectively, in favor of mouse TAAR1. Finally, RO5166017 up to 30 μ M did not elicit cAMP production from mouse TAAR4, the only other TAAR family member activated by TAs (5).

Electrophysiological Characterization of RO5166017. The endogenous TAAR1 agonist pTyr inhibits the firing frequency of DA neurons in the VTA, where *Taar1* is expressed (10), whereas blockade of TAAR1 with EPPTB strongly increases their firing rate (17). Thus, we examined whether the synthetic TAAR1 agonist RO5166017 influences the firing of DA neurons in a manner similar to pTyr.

Application of RO5166017 (500 nM) inhibited the firing frequency of DA neurons in the VTA (Fig. 2A and B) as does pTyr (17). This effect was dose-dependent with an IC_{50} value of 1.73 nM, which is in agreement with the results obtained in other assays (Table 1). Similar to pTyr, RO5166017 reduced firing frequency by activating a K^+ -mediated outward current (Figs. S1 and S2A and B). Application of EPPTB (10 nM) completely reversed the RO5166017-induced effect and even reduced the current beyond the predrug baseline (Fig. S1), which is in line with previous data (17). Importantly, RO5166017 did not change the firing frequency or the membrane current in brain slices from *Taar1*^{-/-} mice (Fig. 2A and B and Fig. S1). These results show that RO5166017 activates TAAR1 in DA neurons to lower their firing activity similar to pTyr but with much higher potency.

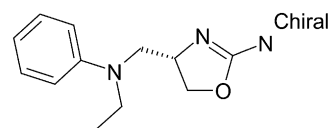


Fig. 1. Chemical structure of the selective TAAR1 agonist RO5166017.

Because *Taar1* is also expressed in the DRN (10), we asked whether RO5166017 and pTyr also affect the firing frequency of 5-HT neurons. Both compounds decreased the spike rate of DRN 5-HT neurons in WT mice (Fig. 2C and D and Fig. S3), with an IC_{50} value of 2.99 nM for RO5166017 (Table 1). As in the VTA, the inhibitory effects of pTyr and RO5166017 were blocked by EPPTB, which increased the firing frequency over the basal level. The outward current generated by RO5166017 in the DRN was also K^+ -mediated (Fig. S2C and D). Finally, neither RO5166017 (Fig. 2C and D) nor pTyr (Fig. S3) affected the firing rate of 5-HT neurons in *Taar1*^{-/-} mice, supporting the specificity of their effects in WT mice. Interestingly, the spontaneous spike rate in DRN slices from mutant mice was significantly increased compared with WT, suggesting that, similar to the VTA, TAAR1 in 5-HT neurons is either constitutively active or tonically activated by ambient levels of endogenous agonist(s).

In the locus coeruleus (LC), the absence of detectable *Taar1* expression (10) suggests that TAs have no direct effect on NA cells. To test this hypothesis, the electrical activity of NA neurons in this brain region was recorded. We found that neither RO5166017 (Fig. 2E and F) nor pTyr (Fig. S3) influenced the firing frequency. Furthermore, the spontaneous firing activity did not differ between slices from *Taar1*^{-/-} and WT mice (Fig. 2E and F and Fig. S3). This result further shows that the inhibition of neuronal activity by pTyr and RO5166017 observed in the VTA and DRN is specific for *Taar1*-expressing neurons, and it confirms the absence of functional TAAR1 in the mouse LC.

TAAR1 Interacts with 5-HT_{1A} Autoreceptors in 5-HT Neurons of the DRN. In DA neurons of the VTA, TAAR1 interacts with D_2 autoreceptors to decrease D_2 agonist potency and promote D_2 desensitization (17). Having observed that TAAR1 influences the neuronal activity of 5-HT neurons, we asked whether TAAR1 in the DRN also interacts with 5-HT_{1A} autoreceptors. This subtype of 5-HT receptors is important for the modulation of mood, cognition, and motor behavior as well as for the response to some antidepressant and antipsychotic drugs (19–21).

Activating TAAR1 with RO5166017 resulted in a twofold increased potency of the 5-HT_{1A} partial agonist ipsapirone, whereas antagonizing TAAR1 activity by EPPTB was associated with a twofold decrease in ipsapirone potency (Fig. 3A). The influence of TAAR1 activity on the 5-HT_{1A} desensitization rate was then examined. Bath application of ipsapirone (10 μ M) to DRN slices of WT mice induced an outward current in 5-HT neurons that decreased during long activation (control) (Fig. 3B). Although preincubation of slices with RO5166017 did not significantly change the desensitization, it was clearly prevented by EPPTB. Application of the 5-HT_{1A} receptor antagonist WAY-100135 (1 μ M) blocked the

Table 1. Binding affinities and EC_{50}/IC_{50} values of RO5166017 at rodent and primate TAAR1

Parameter, assay, preparation	Mouse	Rat	Human	Cynomolgus monkey
K_i , binding, HEK293*	1.9 ± 1.2	2.7 ± 1.3	31 ± 4	24 ± 5
EC_{50} , cAMP, HEK293 [†]	3.3 ± 1.7 (65 ± 15%)	14 ± 10 (90 ± 17%)	55 ± 27 (95 ± 8%)	97 ± 53 (81 ± 1%)
EC_{50} , GIRK, <i>Xenopus</i> oocytes ^{‡§}	8.0 ± 1.2 (72 ± 2%)	n.d.	n.d.	n.d.
IC_{50} , patch clamp, VTA slices [§]	1.73	n.d.	n.d.	n.d.
IC_{50} , patch clamp, DRN slices [§]	2.99	n.d.	n.d.	n.d.

Values (in nM) represent the mean ± SEM from at least three independent experiments. Data in parentheses represent the maximal efficacy relative to PEA (EC_{50} and cAMP) or pTyr (EC_{50} and GIRK). n.d., not determined.

*Radioligand [³H]RO5166017 for mouse and rat TAAR1 and [³H]RO5192022 for human and cynomolgus monkey TAAR1.

[†]Upstate (Millipore) immunoassay for cAMP.

[‡]Current mediated by $K_{i,3.1}$ and $K_{i,3.2}$ coexpressed with TAAR1.

[§]Current at -50-mV holding potential.

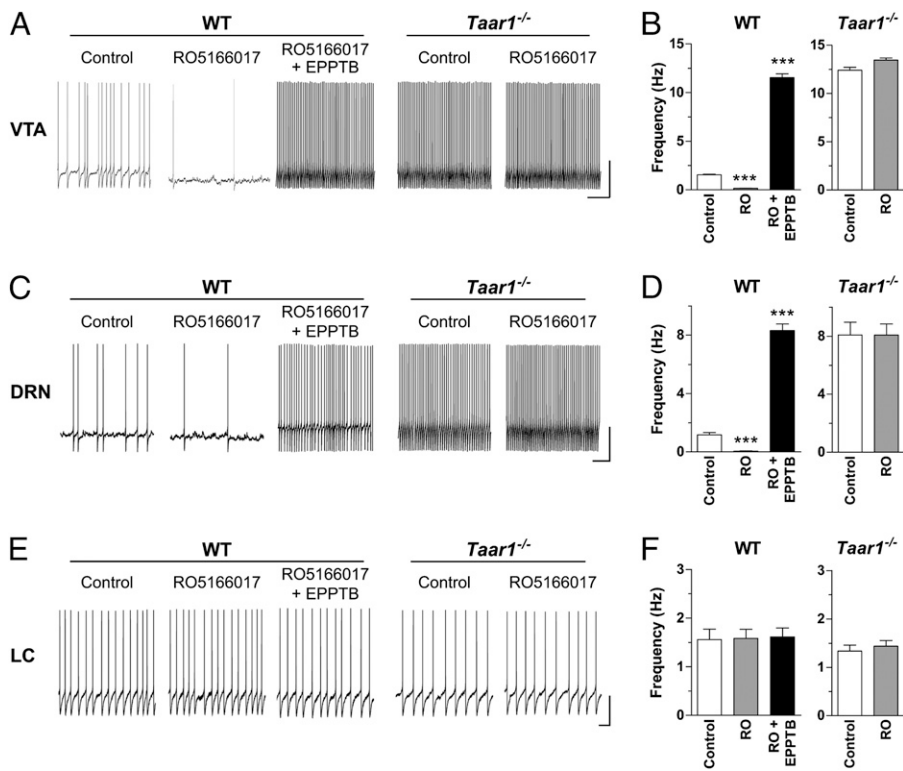


Fig. 2. RO5166017 inhibits the firing rate of DA and 5-HT neurons but not that of NA neurons. (A, C, and E) Representative recordings from brain slices of WT and *Taar1*^{-/-} mice. (Scale bar: VTA, 20 mV/s; DRN and LC, 30 mV/s.) (B, D, and F) Quantification bar graphs (mean \pm SEM; $n = 5$ neurons, recorded from three animals per condition). The firing frequency was assessed before (Control) and during application of RO5166017 (500 nM; RO) alone and in combination with EPPTB (10 nM). In WT mice, RO5166017 decreased firing of DA neurons in the VTA (A and B) and 5-HT neurons in the DRN (C and D), whereas application of EPPTB increased the firing rate above control levels. In brain slices of *Taar1*^{-/-} mice, the spontaneous firing frequencies of the DA and 5-HT neurons were increased compared with WT, and they were not affected by RO5166017. In the LC (E and F), the spontaneous firing rates of NA neurons in WT and *Taar1*^{-/-} mice were not significantly different, and RO5166017 had no effect in WT mice. *** $P < 0.001$ vs. the other two conditions.

current induced by ipsapirone, which in the presence of EPPTB was even reduced below baseline. These observations not only show that TAAR1 and 5-HT_{1A} are functionally coupled but also suggest that a constitutive TAAR1 activity or tonic activation by ambient levels of endogenous agonist(s) is sufficient to drive such an interaction.

RO5166017 Exhibits Anxiolytic-Like Properties. To estimate whether RO5166017 can be used in vivo, its pharmacokinetic profile was

determined in C57BL/6 mice after single i.v. or oral bolus administration (Table S3). RO5166017 showed a moderate volume of distribution at steady state (3.14 L/kg), low binding to plasma proteins (75.9% free fraction), and a high brain to plasma concentration ratio (13:1). Overall, these pharmacokinetic properties are favorable and allow further investigations in vivo.

Monoamines are involved in the processing of anxious states. As TAAR1 modulates their activity, we examined if TAAR1 ac-

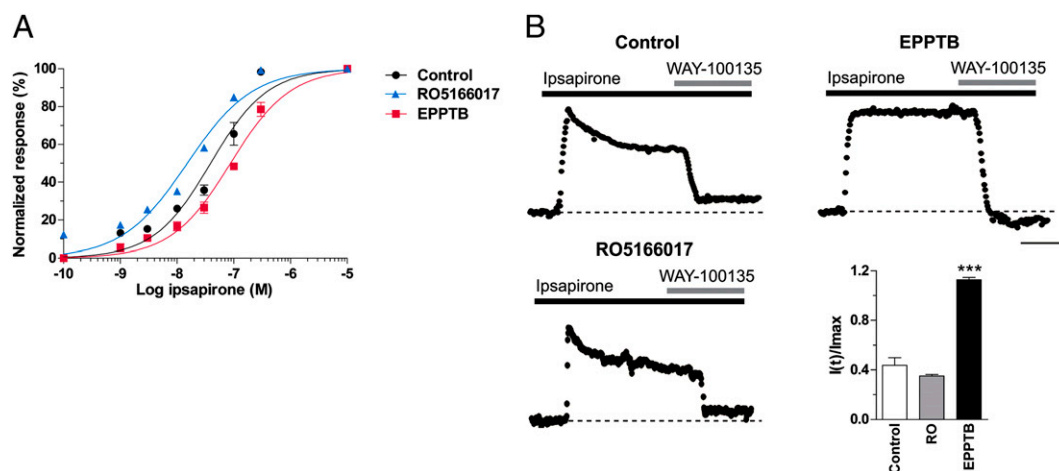


Fig. 3. TAAR1 activity modulates the 5-HT_{1A} receptor pharmacology in 5-HT neurons. (A) Dose–response relationships of the currents induced by the 5-HT_{1A} receptor agonist ipsapirone recorded from DRN 5-HT neurons in control slices and slices preincubated with EPPTB (10 nM) or RO5166017 (19 nM). Current amplitudes were normalized to the maximal current obtained with a saturating concentration of ipsapirone (10 μ M). pEC₅₀ values: control = 7.40 ± 0.04 (EC₅₀ = 39 nM); EPPTB = 7.05 ± 0.03 (EC₅₀ = 88 nM); RO5166017 = 7.82 ± 0.04 (EC₅₀ = 15 nM; $n = 5$). (B) Representative traces of ipsapirone (10 μ M)-induced currents in the absence (Control) and presence of EPPTB (10 nM) or RO5166017 (500 nM) followed by application of the 5-HT_{1A} receptor antagonist WAY-100135 (1 μ M). EPPTB prevented 5HT_{1A} receptor desensitization and reduced the holding current below the initial baseline (dotted line) when WAY-100135 was added. In contrast, RO5166017 did not significantly reduce 5-HT_{1A} receptor desensitization. (Scale bar: 15 pA per 5 min.) The bar graph quantitatively shows the degree of desensitization rate after continuous ipsapirone application for 15 min [$I(t)/I_{max}$, ratio of current amplitude after 15 min; I_{max} ; peak current amplitude]. Data represent the mean \pm SEM ($n = 5$). *** $P < 0.001$ vs. control.

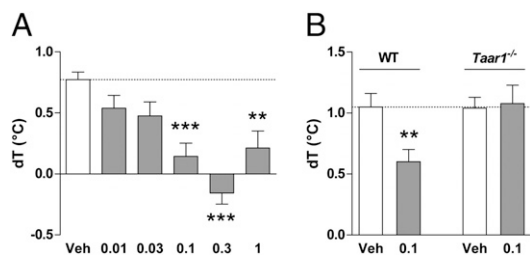


Fig. 4. RO5166017 reverses stress-induced hyperthermia (SIH). (A) In NMRI mice, RO5166017 significantly reversed the SIH (dT) at doses 0.1 and 0.3 mg/kg without affecting basal T_b (Fig. S4). At 1 mg/kg, the decrease of dT was attributed to a nonspecific effect on T_b (Fig. S4A). The diagram combines the data of two experiments. $***P < 0.001$, $**P < 0.01$ vs. Veh ($n = 8$ –16 per group). (B) RO5166017 (0.1 mg/kg) failed to decrease dT in *Taar1*^{-/-} C57BL/6 mice in contrast to their WT littermates. In both genotypes, basal T_b was not affected (Fig. S4A). $**P < 0.01$ vs. WT/Veh ($n = 8$ –10 per group). Numbers on the x axes are oral doses of RO5166017 in mg/kg. Veh, vehicle. All data represent the mean \pm SEM.

tivation by RO5166017 influences anxious states. The stress-induced hyperthermia (SIH) paradigm, a model that reflects the activation of the autonomic nervous system by measuring body temperature (T_b) in response to mild stress, was used. This model is considered robust and reproducible, with good clinical predictive validity (22, 23). In the SIH paradigm, the measure for anxiety is the increase in temperature (dT) over a 15-min time window in response to the mild stress of measuring rectal temperature.

RO5166017 given orally dose-dependently prevented the SIH in Naval Medical Research Institute (NMRI) mice (Fig. 4A), a standard, easy to handle, outbred mouse line that enables re-

liable rectal temperature reading. Although ineffective at low concentrations, RO5166017 significantly reversed SIH at doses 0.1 and 0.3 mg/kg (Fig. 4A) without decreasing basal T_b (T1) (Fig. S4). Mice treated with a higher dose (1 mg/kg) experienced a decrease in basal T_b by $\sim 1^\circ\text{C}$ compared with those with vehicle only (Fig. S4A). Importantly, RO5166017 (0.1 mg/kg) significantly reversed the SIH in WT but not *Taar1*^{-/-} C57BL/6 mice (Fig. 4B) without alteration of basal T_b (Fig. S4A). These data suggest that RO5166017 exhibits TAAR1-mediated anxiolytic-like properties at doses 0.1–0.3 mg/kg.

RO5166017 Inhibits Psychostimulant- and Genetically-Induced Hyperlocomotion. *Taar1*^{-/-} mice are hypersensitive to the effects of amphetamine, with enhanced locomotor activity (LMA) and increased striatal release of DA, 5-HT, and NA compared with WT (10, 12), suggesting that TAAR1 is an important modulator of the monoaminergic systems. Because most psychostimulant drugs interact with monoaminergic neurons to elevate extracellular monoamine concentration (24), we investigated how the TAAR1 agonist RO5166017 alters their effects in vivo. For this, the effects of RO5166017 were examined on psychostimulant-induced hyperlocomotion. Mice injected with cocaine, a nonselective competitive inhibitor of monoamine transporters, displayed elevated LMA compared with vehicle-treated mice (Fig. 5A and Fig. S5A). RO5166017 given orally prevented this effect in a dose-dependent manner, similar to the atypical antipsychotic olanzapine (Fig. S5B). RO5166017 alone had little or no effect on LMA. In *Taar1*^{-/-} mice submitted to the same paradigm, cocaine elevated LMA to a similar extent as in WT mice, but only in WT mice, RO5166017 prevented the cocaine-induced hyperlocomotion (Fig. 5B). Interestingly, RO5166017 also inhibited stereotypies induced by cocaine in WT mice (Fig. S6A) similar to olanzapine (Fig. S6B), and this effect was lost in *Taar1*^{-/-} mice (Fig. S6C). This suggests that, in mice, RO5166017 blocks the psychostimulant effects of cocaine through TAAR1.

The mutant mouse line that lacks the *Slc6a3* gene encoding the DAT (DAT^{-/-} mice) represents a model of persistent hyperdopaminergia and related behavioral abnormalities used to evaluate compounds on endophenotypes of DA-related psychiatric disorders (24). As previously shown (25, 26), saline-treated DAT^{-/-} mice are spontaneously hyperactive in a novel environment. Treatment with RO5166017 i.p. dose-dependently suppressed hyperlocomotion in DAT^{-/-} mice (Fig. 5C) similar to classical (haloperidol) and atypical (olanzapine and clozapine) antipsychotics (27). This finding further indicates that at least this effect of TAAR1 activation on DA-related function is independent of DAT. In WT mice, the reduction of LMA after introduction in the novel environment was reduced at 0.5 mg/kg but not at the other doses tested (Fig. S5C). Importantly, the effect

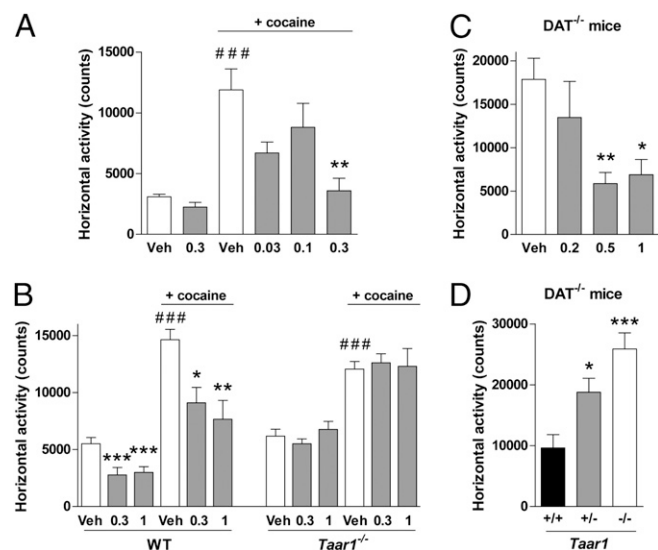


Fig. 5. RO5166017 blocks hyperlocomotion induced by cocaine or lack of DAT. (A) RO5166017 administered orally dose-dependently reduced cocaine (15 mg/kg i.p.)-induced hyperlocomotion. $###P < 0.001$ vs. saline/Veh; $**P < 0.01$ vs. cocaine/Veh. Cocaine/RO5166017 (0.3 mg/kg) does not differ from groups without cocaine ($n = 8$ per group). (B) RO5166017 failed to antagonize cocaine (20 mg/kg i.p.)-induced hyperlocomotion in *Taar1*^{-/-} mice but not in WT littermates. $###P < 0.001$ vs. saline groups; $***P < 0.001$, $**P < 0.01$, and $*P < 0.05$ vs. Veh ($n = 10$ per group). (C) RO5166017 given i.p. dose-dependently reduced hyperlocomotion in spontaneously hyperactive dopamine transporter knockout (DAT^{-/-}) mice. $**P < 0.01$, $*P < 0.05$ vs. Veh ($n = 7$ –8 per group). (D) RO5166017 (0.5 mg/kg i.p.) failed to inhibit hyperlocomotion in DAT^{-/-} mice deficient for *Taar1* (^{-/-}) and had reduced effects in DAT^{-/-}/*Taar1*^{+/-} mice (^{+/-}). ^{+/-}, *Taar1* WT. $***P < 0.001$, $*P < 0.05$ vs. (^{+/-}) ($n = 12$ –22 per group). Numbers on the x axes are oral doses of RO5166017 in mg/kg. Veh, vehicle. Data represent the mean \pm SEM.

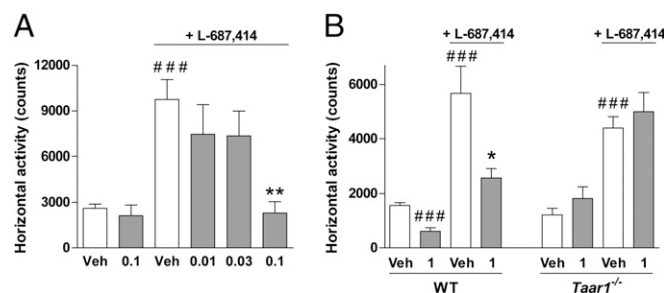


Fig. 6. RO5166017 dose-dependently blocks L-687,414-induced hyperlocomotion. (A) In NMRI mice, RO5166017 administered orally dose-dependently blocked hyperlocomotion triggered by L-687,414 (50 mg/kg s.c.). $###P < 0.001$ vs. saline/Veh; $**P < 0.01$ vs. L-687,414/Veh. L-687,414/RO5166017 (0.1 mg/kg) does not differ from groups without L-687,414 ($n = 7$ –8 per group). (B) In *Taar1*^{-/-} C57BL/6 mice but not in WT littermates, RO5166017 failed to antagonize hyperlocomotion triggered by L-687,414 (75 mg/kg s.c.). $###P < 0.001$ vs. saline/Veh; $*P < 0.05$ vs. L-687,414/Veh ($n = 6$ –8 per group). Numbers on the x axes are oral doses of RO5166017 in mg/kg. Veh, vehicle. Data represent the mean \pm SEM.

of RO5166017 (0.5 mg/kg, i.p.) was reduced in $DAT^{-/-}/Taar1^{+/-}$ mice and was completely absent in double mutant $DAT^{-/-}/Taar1^{-/-}$ mice (Fig. 5D). Thus, the inhibitory action of RO5166017 on DA-dependent hyperactivity, at least at this dose, is fully mediated by TAAR1.

Finally, preclinical and clinical evidence suggest that hypofunction of glutamatergic NMDA receptors plays an important role in the pathophysiology of schizophrenia (28). Hyperlocomotion induced by (3R,4R)-3-amino-1-hydroxy-4-methyl-2-pyrrolidinone (L-687,414), a well-characterized selective and brain-penetrating glycine site NMDA receptor antagonist, has been recently described as a method to identify antipsychotic-like action of drugs (29) in relation to the glutamatergic hypothesis theory of schizophrenia. In this paradigm, RO5166017 dose-dependently reversed hyperlocomotion induced by L-687,414 (Fig. 6A and Fig. S5D). This effect was similar to known antipsychotic drugs (29), including olanzapine (Fig. S5E), and it was not seen in $Taar1^{-/-}$ mice (Fig. 6B). These observations further show that selective TAAR1 activation by RO5166017 produces antipsychotic-like effects.

Discussion

To date, all studies of TAAR1 function have relied on nonselective agonists such as TAs or thyronamine derivatives (7). Selective and potent TAAR1 compounds were not available to elucidate the physiological role of the receptor and verify the promising therapeutic hypotheses. Here, we report on RO5166017, a TAAR1 agonist with high affinity, efficacy, and selectivity as well as favorable pharmacokinetic properties. RO5166017 was used in vitro and in vivo to show that selective stimulation of TAAR1 affects firing activity of DA and 5-HT neurons and triggers anxiolytic- and antipsychotic-like effects in mice.

RO5166017 Is a High-Affinity, Potent, Selective, and Bioavailable TAAR1 Agonist.

RO5166017 exhibits high binding affinity for TAAR1 and high potency to stimulate cAMP production, particularly at rodent TAAR1. Compared with pTyr, RO5166017 exhibited 200-fold higher affinity ($K_i = 1.9$ vs. 404 nM) and potency to activate cAMP production ($EC_{50} = 3.3$ vs. 545 nM) at mouse TAAR1 (17), whereas T_1AM and its derivative *o*-phenyl-3-iodotyramine show EC_{50} values of 112 and 35 nM, respectively (30). In *Xenopus* oocytes coexpressing mouse TAAR1 with human $K_{ir}3$ channels, RO5166017 evoked an inward current similar to pTyr but with a 21-fold lower IC_{50} (8 vs. 167 nM) (17). Finally, RO5166017 also reduced the firing frequency of VTA DA neurons by activating an outward K^+ current similar to pTyr but with 179-fold higher potency (1.7 vs. 305 nM) (17). Such electrophysiological effects are presumably of postsynaptic nature, although presynaptic action cannot be excluded.

In contrast to the TAAR1 agonists currently available, RO5166017 is selective against a large panel of targets, including the monoaminergic receptors and transporters. An exception is the imidazoline receptor I_1 , against which the selectivity ratio is only 15-fold. However, I_1 imidazoline binding sites are unlikely to mediate the effects of RO5166017. RO5166017 did not affect NA neurons in the LC where harmaline, a putative endogenous imidazoline binding sites ligand, increases firing activity (31). In contrast, it reduced the firing of DA neurons in the VTA, where no I_1 imidazoline binding sites were seen (31). Importantly, RO5166017 had no effect in $Taar1^{-/-}$ mice, both in electrophysiological and behavioral studies.

Finally, a critical advantage of RO5166017 over TAs and other TAAR1 ligands comes from its pharmacokinetic properties. TAs, due to rapid degradation by monoamine oxidases (7), have a very short half-life in the order of 30 s in the brain and 5 min in plasma (32, 33). In contrast, RO5166017 is stable, with a half-life of several hours in plasma. Furthermore, it shows good bioavailability and high brain penetration, enabling peripheral administration for in vivo studies, including oral dosing.

TAAR1 Modulates 5-HT Activity. Previous studies indicated that TAAR1 modulates dopaminergic activity, presumably through functional interaction with DAT and the D_2 receptor (8–10, 12, 17). We now show that both RO5166017 and pTyr reduce the

firing frequency of 5-HT neurons in the DRN, strongly suggesting that TAAR1 also modulates the serotonergic system. In the VTA, TAAR1 alters neuronal firing through activation of G protein-coupled inwardly-rectifying potassium (GIRK) channels (17), and the mechanism is likely to be similar in the DRN 5-HT neurons. In addition, it was observed that TAAR1 influences the functioning of 5-HT $_{1A}$ autoreceptors in the DRN. Their desensitization upon ipsapirone application is TAAR1-dependent, as coapplication of EPPTB prevents 5-HT $_{1A}$ desensitization. This observation is reminiscent of the VTA, where D_2 desensitization after quinpirole application was abolished by the TAAR1 antagonist (17). Promotion of monoamine autoreceptor desensitization, thus, emerges as an important function of TAAR1. This may occur either by direct interaction or by favoring interactions with the GPCR desensitization machinery.

Finally, agonist potency at 5-HT $_{1A}$ increased with TAAR1 activation, whereas it decreased with TAAR1 blockade. Interestingly, this situation is reversed compared with the D_2 receptors, at which agonist potency is promoted by TAAR1 blockade (17). Thus, although TAAR1 activation decreases firing activity in both DA and 5-HT neurons, it modulates the pharmacology of monoamine autoreceptors according to the cellular environment. This is likely to have critical impacts on the functioning of the dopaminergic and serotonergic systems. Interestingly, 5-HT $_{1A}$ autoreceptors in the DRN show greater sensitivity to 5-HT compared with postsynaptic 5-HT $_{1A}$ heteroreceptors located in the corticolimbic structures (19, 21). It is tempting to hypothesize that TAAR1 contributes to this difference of sensitivity, which is important in the management of psychiatric diseases like schizophrenia, anxiety, and depression (21).

The 5-HT $_{1A}$ autoreceptor is considered a therapeutic target for some neuropsychiatric disorders (20, 21, 34). It is believed to delay the therapeutic action of antidepressants that increase 5-HT levels, and only after the progressive desensitization of 5-HT $_{1A}$ can therapeutic effects occur (20, 34). Because TAAR1 seems necessary for the desensitization of 5-HT $_{1A}$ autoreceptors, adequate activation of TAAR1 may be critical for the therapeutic action of antidepressants. Depression is indeed associated with insufficiency of brain TAs, notably PEA (35), and coadministration of PEA or its precursor phenylalanine with the MAOI selegiline improves mood in patients with major depression, including those that are treatment-resistant (35, 36). Thus, association of specific TAAR1 agonists such as RO5166017 to classical antidepressant treatments might accelerate and improve therapeutic efficacy.

Selective Activation of TAAR1 Shows Anxiolytic- and Antipsychotic-Like Actions.

A variety of molecules activate TAAR1 in vitro (2, 14), but their administration to animals results in an array of responses from which TAAR1-mediated effects are challenging to distinguish. PEA produces amphetamine-like hyperactivity, although not in $DAT^{-/-}$ mice (26). T_1AM decreases T_b dramatically to produce torpor-like states (14), but WT and $Taar1^{-/-}$ mice are affected equally, suggesting TAAR1-independent mechanisms (13). Thus, RO5166017 was designed to examine in vivo the effects of TAAR1 selective activation. In the mouse, dosing of RO5166017 alone did not trigger any obvious behavioral or physiological manifestations. RO5166017 did not produce hyperlocomotion like PEA but rather, an occasional and minor decrease of LMA. At high dose, it reduced T_b by $\sim 1^\circ C$ in the SIH test but clearly did not cause torpor episodes like T_1AM . Instead, we found that RO5166017 displays psychoactive properties in mice. Selective TAAR1 activation reduced anxiety in the SIH paradigm and fully prevented psychostimulant-induced and persistent hyperdopaminergic-related hyperactivity similar to the antipsychotic drug olanzapine (27). Importantly, these effects were not observed in $Taar1^{-/-}$ mice, showing that the anxiolytic- and antipsychotic-like properties of RO5166017 result from selective activation of TAAR1. Furthermore, the fact that RO5166017 suppressed hyperactivity of mice lacking DAT strongly suggests that DAT is not required for this effect of TAAR1 on DA-related functions. Potent activity of RO5166017 in pharmacological or genetic models of hyperdopaminergia indicates potential activity of TAAR1 agonists in conditions that

may result from abnormally enhanced dopaminergic transmission such as schizophrenia.

Conclusion

TAAR1 is a promising therapeutic target for the treatment of neuropsychiatric disorders, but the diversity and polypharmacology of the agonists available have rendered dissection of its physiological functions challenging. Here, we report on RO5166017, a synthetic selective and *in vivo* active TAAR1 agonist. *In vitro*, RO5166017 inhibited the firing frequency of VTA DA and DRN 5-HT neurons. Furthermore, modulation of TAAR1 activity altered the pharmacology of DRN 5-HT_{1A} receptors, demonstrating that TAAR1 modulates not only DA but also 5-HT neurotransmission. *In vivo*, although silent by itself, RO5166017 prevented psychostimulant-induced hyperlocomotion, inhibited novelty-driven hyperactivity of DAT^{-/-} mice, and prevented SIH in WT but not *Taar1*^{-/-} mice. These results link TAAR1 to the control of monoaminergic-driven behaviors and underline the antipsychotic and anxiolytic potential of TAAR1 agonists.

Materials and Methods

Details on animals, membrane preparation, radioligand binding, cAMP assay, pharmacokinetic measurements, stress-induced hyperthermia, and statistical analysis are provided in *SI Materials and Methods*. Electrophysiological recordings were made as in ref. 17 and are detailed in *SI Materials and Methods*.

Compounds. All compounds were purchased from Sigma except for cocaine, *d*-amphetamine, olanzapine, L-687,414, EPPTB (RO5212773), RO5166017, [³H]RO5166017, and [³H]RO5192022 ([³H](S)-4-(2,4-Difluoro-phenyl)-4,5-dihydro-oxazol-2-ylamine), which were synthesized at Roche.

Measurement of Locomotor Activity and Stereotypies. For psychostimulant studies, recordings were as reported (10). LMA was measured as the number of horizontal beam breaks (horizontal activity) cumulated over 30 min. Stereotypy time was assessed as the total time that stereotypic behaviors (repetitive beam breaks with intervals less than 1 s) were monitored over 30 min. For cocaine studies, C57BL/6 mice (*n* = 8 per group) were treated per os (p.o.) with vehicle (H₂O + 0.3% tween 80) or RO5166017 (0.03–3 mg/kg in vehicle), placed into the activity monitor chamber for 30 min (habituation period), injected i.p. with saline (0.9% NaCl + 0.3% tween 80) or cocaine (15 mg/kg in saline), and returned to the recording chamber for immediate monitoring of behavior (recording period). The same paradigm was used in *Taar1*^{-/-} mice (*n* = 10 per group) with RO5166017 (0.3–1 mg/kg p.o.) and cocaine (20 mg/kg i.p.) using a repeated measures design with at least 10 d between two sessions. For L-687,414 studies, NMRI mice (*n* = 8 per group) were dosed p.o. with vehicle or RO5166017 (0.01–1 mg/kg in vehicle) 15 min before receiving saline or L-687,414 (50 mg/kg in saline) s.c. The habituation period was 15 min. For *Taar1*^{-/-} mice, animals (*n* = 8 per group) were dosed with RO5166017 (0.3 mg/kg p.o.), were placed into the recording chamber for 45 min, received saline or L-687,414 (75 mg/kg) s.c., and were returned to the recording chamber for 15 min (thus, 60-min habituation) before LMA was recorded.

For DAT^{-/-}, WT, and double mutant (DAT^{-/-}/*Taar1*^{-/-}) mice, LMA was measured as reported (26). DAT^{-/-} mice were placed into activity monitor chambers for 30 min to fully manifest their novelty-driven hyperactivity; then, they were treated with saline or RO5166017 (0.2–1 mg/kg i.p.), and horizontal activity was monitored for 90 min. Nonhabituated WT mice were treated before placement into the monitoring chambers, and LMA was recorded for 90 min.

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