TAAR1 dependent and independent actions of the potential antipsychotic and dual TAAR1/5-HT\textsubscript{1A} receptor agonist SEP-383856

Marcus Saarinen\textsuperscript{1,}\textsuperscript{✉}, Ioannis Mantas\textsuperscript{1}, Ivana Flais\textsuperscript{1,2}, Richard Å gren\textsuperscript{3}, Kristoffer Sahlholm\textsuperscript{3,4}, Mark J. Millan\textsuperscript{5} and Per Svenningsson\textsuperscript{1,2,}\textsuperscript{✉}

© The Author(s) 2022

SEP-383856 (SEP-856) is a novel antipsychotic under clinical development. It displays a unique pattern of receptor interaction, with only weak (partial agonist) activity at dopamine D\textsubscript{2} receptors, yet more potent agonist activity at the trace amine associated receptor (TAAR1) and serotonin 5-HT\textsubscript{1A} receptor (5-HT\textsubscript{1A}). Nonetheless, these observations await independent confirmation and more detailed characterization of the in vitro and in vivo actions of SEP-856 at TAAR1 and 5-HT\textsubscript{1A} receptors would be instructive. Herein, we employed luminescence complementation technology in heterologous live cell systems, confocal microscopy, voltage clamp electrophysiology, behavioral readouts and TAAR1 knockout (KO) mice to study SEP-856 in further detail. We provide evidence for the ability of SEP-856 to activate TAAR1 at the surface plasma membrane, and show that this interaction results in Go\textsubscript{i} recruitment (pEC\textsubscript{50} = 6.08 ± 0.22; E\textsubscript{MAX} = 96.41% ± 15.26) and by extension, to G-protein inwardly rectifying potassium (GIRK) channel activation. Using TAAR1-KO mice, we find TAAR1 to be indispensable for SEP-856 control of body temperature, baseline locomotion reduction and for “antipsychotic-like” efficacy as characterized by a reversal of dizocilpine (MK-801) mediated disruption of pre-pulse inhibition. Conversely, the inhibition by SEP-856 of MK-801 induced locomotion was unaffected in TAAR1 KO mice. SEP-856 behaved as a low-potency, partial agonist at the 5-HT\textsubscript{1A} receptor, while it partially inhibited recruitment of D\textsubscript{2} receptor-coupled Go and GIRK by DA and acted as a weak partial agonist with low potency at the same receptor when applied alone. Our findings corroborate and extend previous observations on the molecular substrates engaged by this unique, dual TAAR1/5-HT\textsubscript{1A} receptor agonist and potential antipsychotic that could prove to have major advantages in the treatment of schizophrenia and other psychotic disorders.

Introductions

Schizophrenia and other psychotic disorders affect over 20 million people worldwide \textsuperscript{[1]}. Antipsychotic medications are the mainstay of pharmacological treatment for these debilitating mental afflictions. Pioneering work in the 1970s identified the dopamine D\textsubscript{2} receptor as a primary target of all first generation antipsychotics and was a pivotal advancement in neuropharmacology, even giving the D\textsubscript{2} receptor the temporary name “antipsychotic receptor” \textsuperscript{[2–4]}. Up until today, all FDA-approved antipsychotics act to some degree via the dopamine D\textsubscript{2} receptor. Unfortunately, centrally-acting dopamine D\textsubscript{2} receptor blockade can have serious side effects \textsuperscript{[5]}. For example, extrapyramidal symptoms and hyperprolacitinaemia are common side effects of first-generation antipsychotics (“neuroleptics”) acting as potent D\textsubscript{2} receptor antagonists \textsuperscript{[5, 6]}. Clozapine and “atypical” antipsychotics like olanzapine and quetiapine target a broader range of receptors, in particular 5-HT\textsubscript{2A} receptors which they more potently occupy than D\textsubscript{2} receptors \textsuperscript{[7]}. Some of these agents like aripiprazole and cariprazine behave as partial agonists at D\textsubscript{2} receptors \textsuperscript{[8, 9]}. The clinical effectiveness of these agents is however, still limited, in particular against negative and cognitive symptoms. Accordingly, there is substantial interest in mechanistically novel and therapeutically more effective classes of antipsychotic agents.

In this light, it is of considerable importance that, in 2019, Sunovion together with PsychoGenics published data on a behavioral phenotypic screen designed to detect non-dopaminergic antipsychotics \textsuperscript{[10]}. The compound named SEP-856 was proposed to act through 5-HT\textsubscript{1A} receptors and most interestingly, TAAR1. SEP-856 has subsequently been named Ulotaront and is being developed (Phase 3) by Sunovion who reported it to be clinically effective in the treatment of schizophrenia \textsuperscript{[11–13]}. Additionally, a TAAR1 partial agonist (Ralmitaront), developed by Hoffmann-La Roche is currently under investigation in a Phase II clinical trial for the treatment of schizophrenia (ClinicalTrials.gov ID: NCT03669640) with another separate Phase II trial prematurely terminated (ID: NCT04512066).

1Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden. 2Basal and Clinical Neuroscience, King’s College London, London, London, UK. 3Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden. 4Department of Integrative Medical Biology, Wallenberg Centre for Molecular Medicine, Umeå University, Umeå, Sweden. 5Neuroinflammation Therapeutic Area, Institut de Recherches Servier, Centre de Recherches de Croissy, Paris, France and Institute of Neuroscience and Psychology, College of Medicine, Vet and Life Sciences, Glasgow University, Scotland, Glasgow, UK. ✉email: marcus.saarinen@ki.se; per.svenningsson@ki.se

Received: 6 March 2022 Revised: 4 July 2022 Accepted: 30 July 2022 Published online: 13 September 2022
TAAR1 is a member of the G-protein coupled receptor (GPCR) superfamily, belonging to the class A (rhodopsin like) receptors [14]. As its name suggests, various aminergic compounds such as trace amines and several classes of psychoactive agents activate TAAR1 [15]. While occurring in several peripheral organs, TAAR1 is highly expressed in the brain [16, 17]. It is enriched in monoaminergic nuclei, such as the ventral tegmental area (VTA), substantia nigra pars compacta (SNc), dorsal raphe nucleus, and the nucleus of the solitary tract. It is also present in the prefrontal cortex, entorhinal cortex, hypothalamus, and amygdala [18, 19]. TAAR1-KO mice display an increase in the firing rate of VTA and dorsal raphe neurons, a phenomenon which can be replicated in wildtype (WT) mice via local application of the TAAR1 antagonist EPPTB [20]. This suggests that TAAR1 exerts a tonic, inhibitory effect on the activity of dopaminergic and serotonergic neurons. Furthermore, TAAR1-KO mice display behavioral hypersensitivity and more pronounced increases in extracellular levels of monoamines in projection regions such as the striatum upon exposure to psychostimulants [21, 22]. Conversely, agonist stimulation of TAAR1 blunts the actions of psychostimulants [23]. Collectively, these observations support the potential utility of TAAR1 agonists as antipsychotics.

The 5-HT1A receptor, also a Class A GPCR, is a brain-enriched member of the serotonergic receptor family [14, 17]. 5-HT1A receptors are expressed on both serotonin neurons and non-serotonin neurons. In serotonin neurons, the 5-HT1A receptor exerts an autoinhibitory effect, decreasing cell firing and 5-HT release [24, 25]. In addition, 5-HT1A receptors are highly expressed in several post-synaptic sites such as the cortex and hippocampal formation [26] where it regulates mood [27] and cognitive processes [28]. As mentioned above, many atypical antipsychotics antagonize 5-HT1A receptors, yet there is ample evidence that partial agonist actions at 5-HT1A receptors contribute to the functional profiles of many approved antipsychotics like aripiprazole, cariprazine, brexpiprazole, ziprasidone, and possibly lurasidone [29]. Taken together, partial 5-HT1A receptor agonism, at least when combined with antagonist or partial agonists properties at D₂ receptors, is considered a favourable feature for the treatment of schizophrenia [8, 30].

The purpose of this work was to further characterize the interaction of SEP-856 with TAAR1 and 5-HT₁A receptors and to further understand how this molecular signature influences its potential antipsychotic properties [21]. In particular, we utilized a novel, codon-optimized TAAR1 in Exp293F cells to efficiently study the G-protein coupling profile of this receptor along with another unexplored signaling pathway of SEP-856, TAAR1 mediated GIRK channel activation, using oocyte electrophysiology. We then used TAAR1 KO mice to study the in vivo actions of SEP-856 with various clinically relevant, behavioral models of antipsychotic-like activity. The data presented here provide compelling evidence that mainly TAAR1, and possibly 5-HT₁A receptors are involved in the action of SEP 856, underpinning a potentially novel mechanism of antipsychotic activity.

MATERIALS AND METHODS
HEK293T and Exp293F cells were maintained according to manufacturer’s conditions. G-protein recruitment was measured using a described system [31] in Exp293F cells. Oocytes were prepared and electrophysiological recordings done as previously described [32]. All animal experiments were approved by the Karolinska Institutet Animal Care and Use Committee according to Swedish guidelines in full compliance with European requirements. WT and TAAR1-KO [21, 22] mice were housed and behavioral experiments were performed as described in the Supplementary Methods. More information on all experimental methods included in this study including drug preparation, molecular biology, transfections, confocal imaging, flow cytometry, signaling assays and data analysis are available in detail in the Supplementary Methods.

RESULTS
In vitro work
TAAR1 pharmacology has been traditionally difficult due to very poor receptor expression and cell surface availability, even in heterologous overexpression systems. Furthermore, in vivo TAAR1 expression levels are low as determined by many online sequencing databases. To overcome this obstacle in order to elucidate the functions of SEP-856 on TAAR1, we generated two mammalian codon optimized constructs. One contained the first nine N-terminal amino acids of the β₂-adrenergic receptor (herein referred to as β-TA1), a strategy originally published by the Gainetdinov lab to increase cell surface expression [33], and one without (WT-TA1). Each construct contained triple hemagglutinin (HA) epitopes on the N-terminus with flexible linkers and C-terminal SmBiT tag (Supplementary Fig. 1). Receptor activation was assayed using the complementary luminescence (NanoBiT [31]) system between SmBiT-tagged TAAR1 and mini-G proteins fused to LGbT, whereby receptor activation induces G-protein recruitment and thus reconstitutes a functional NanoLuc enzyme, yielding luminescence in the presence of the NanoLuc substrate luciferin.

To examine the pharmacology of SEP-856, we began with interrogation of its effects on TAAR1. For all signaling experiments, we used the Exp293F cell line, which is grown in serum free media and thus avoids desensitization of the receptor via potential trace amines or serotonin present in animal serum. We first tested the ability of SEP-856 and the most potent suggested endogenous agonist, β-Phenethylamine (β-PEA), to recruit mini-G proteins through TAAR1 activation. Both compounds induced significant increases in Gα recruitment to WT-TA1 (Fig. 1A, Supplementary Fig. 2) and concentration-response curves for both compounds revealed similar agonist profiles (Fig. 1B). Additionally, no recruitment was observed with Gαq, Gα₁₂ (Fig. 1C). We attempted to measure β-Arrestin recruitment at TAAR1, however neither of our β-Arrestin constructs (with C or N terminal LGbT tags) indicated a successful agonist response when paired with the WT-TA1 construct (Supplementary Fig. 3B). Next, we tested several reported ligands of varying profiles to better rank agonists (Supplementary Fig. 4) to compare SEP-856 with, via Gαq coupling. β-PEA, p-Tyramine and 3-methoxytyramine (3-MT) all acted as full agonists whereas dopamine was a partial agonist and norepinephrine acted as a very weak partial agonist. Moreover, β-TA1 yielded an increased luminescent signal and response to norepinephrine (Supplementary Fig. 4D). These experiments indicate that SEP-856 acts as a potent full agonist on TAAR1, acting specifically via Gαq recruitment (pEC⁵₀: 6.08 ± 0.22, pMAX: 96.41% ± 15.26). In agreement with Dedic et al. [10], we find SEP-856 to be of slightly less potency than β-PEA (pEC⁵₀: 6.49 ± 0.23) on TAAR1, but of greater potency than p-Tyramine (pEC⁵₀: 5.65 ± 0.06, Supplementary Fig. 4E, F). Finally, to investigate an alternative aspect of TAAR1 downstream signaling, we expressed the codon optimized human TAAR1 construct in Xenopus laevis oocytes. This permitted assessment of the ability of SEP-856 to induce GIRK channel activation [20]. The reference agonist p-tyramine elicited GIRK current responses only in oocytes expressing exogenous Gαq (Fig. 1E). In the same oocytes, SEP-856 acted as a partial agonist (pEC⁵₀: 6.74 ± 0.43, pMAX: 67.15% ± 21.59 relative to p-tyramine) of human TAAR1 (Fig. 1D).

After establishing that SEP-856 acts as a potent agonist on TAAR1 via Gαq and having functional wildtype TAAR1 receptor expression, we sought to identify whether the construct is able to signal from the surface plasma membrane. This was important, as cell compartment specific signaling can give rise to various different outcomes as opposed to “standard” cell surface receptor signaling [34], which has been recently demonstrated for TAAR1 [35]. Likewise, the localization of TAAR1 has been difficult to pinpoint, with studies indicating intracellular, endoplasmic reticulum (ER) [33, 35], and more recently, ciliary localization in a thyroid...
cell line [36]. We reasoned that (1) since we observed rapid receptor activation by cell membrane- impermeable compounds such as dopamine and norepinephrine (2) TAAR1 is capable of GIRK channel activation, we should be able to detect TAAR1 at the cell surface plasma membrane. To address this issue, we first turned to confocal microscopy and expressed the WT-TA1 construct in HEK293T cells together with various cell organelle markers carrying a fluorescent mCherry tag (Fig. 2A). Antibody staining for the N terminal HA epitope tags on WT-TA1 revealed the receptor is mainly retained within the cell, consistent with earlier reports. We observed co-localization with several organelle markers, most evident in the endoplasmic reticulum (ER) and the Golgi apparatus (Fig. 2A). Next, we sought to expand on the pharmacology between SEP-856 and the 5-HT1A receptor since both SEP-856 binding and agonism was reportedly the greatest for the 5-HT1A receptor after agonism was induced using an EC80 concentration of serotonin in this study [10].

Unlike at TAAR1, SEP-856 proved to be a low-potency 5HT1A receptor partial agonist when compared with the endogenous agonist serotonin (Fig. 3A). Like serotonin, SEP-856 was able to induce recruitment of Gq, (EMAX: 36.67% ± 1.79), Ga3 (EMAX: 40.11% ± 0.70), and to a lesser extent, Ga12 (EMAX: 47.47% ± 4.98) (Fig. 3B and C, Supplementary Fig. 5). A similar promiscuous coupling profile of the 5-HT1A receptor is supported by several studies [37, 38]. However, the affinity for recruitment at all three G-protein subtypes was several orders of magnitude lower than that of serotonin. The potential for serotonin antagonism due to the partial agonist profile of SEP-856 on the 5-HT1A receptor was also evaluated using an EC80 concentration of serotonin in this study.
Fig. 2  Subcellular localization of WT-TA1. A Representative images captured using a laser scanning confocal microscope of HA-stained TAAR1 (first row) together with various subcellular localization markers tagged with the constitutively fluorescent protein mCherry (second row). Right: example of HA-stained 5-HT$_{1A}$ receptor together with the Golgi-surface plasma membrane protein Caveolin. Merged images of HA-stained receptor and localization marker are in the third row together with the nuclear marker DAPI. At least three different groups of cells were imaged for each condition. Yellow arrows indicate areas of notable co-localization between HA-stained receptor and the respective localization marker. Dashed lines indicate the cross section that was used to generate the profile plots shown below which graph the normalized intensity of the channel (Y axis, cyan: WT-TA1 and red: organelle marker) across the section (X axis). Cell surface receptor levels were measured using flow cytometry (B) with the staining intensity of surface labeled HA-tagged receptor shown in C. D depicts the mean fluorescence intensity of the surface stained receptors. Three separate batches of transfected cells for each receptor construct were combined, stained and analyzed as one.
SEP-856 was able to inhibit serotonin induced G\textsubscript{i} coupling to a modest extent (39.75% ± 8.93) at the highest concentration tested. We then performed \( \beta \)-arrestin recruitment assays to investigate any further potential signaling bias of SEP-856 (Fig. 3E). \( \beta \)-arrestin recruitment by SEP-856 revealed a similar partial agonist profile (EMAX:36.05% ± 6.30). To further validate the low-potency partial agonist profile of SEP-856 on the 5-HT\textsubscript{1A} receptor, we measured G\textsubscript{\( \beta \)\( \gamma \)} mediated GIRK activation using Xenopus laevis oocytes since 5-HT\textsubscript{1A} activation is known to robustly activate GIRK channels. Also in this assay system, SEP-856 was of lower potency and efficacy (EMAX: 55.03% ± 14.09) than serotonin (pEC\textsubscript{50} 8.62 ± 0.05, Fig. 3G, H), indicating that SEP-856 is able to induce GIRK channel mediated currents through the 5-HT\textsubscript{1A} receptor at high concentrations, presumably due to G\textsubscript{\( \alpha \)/o} coupling.

Finally, to examine any influence of SEP-856 on dopamine D\textsubscript{2} receptor activity, we began by using the G-protein recruitment assay to measure the degree of G\textsubscript{i} interaction with the D\textsubscript{2} receptor after stimulation with SEP-856 compared with the endogenous agonist dopamine. The results revealed that SEP-856 stimulation resulted in very limited G\textsubscript{i} recruitment as opposed to dopamine (Fig. 4A). The concentration-response of SEP-856 implies it acts as a low potency partial agonist (EMAX: 9.14 ± 0.86, Fig. 4B) on the dopamine D\textsubscript{2} receptor. Next, we tested SEP-856’s potential as an antagonist. SEP-856 was able to partially reduce the degree of G\textsubscript{i} recruitment when co-administered with an EC\textsubscript{80} of dopamine compared to dopamine alone (Fig. 4C).
In vivo work

After examining SEP-856 effects in vitro we decided to probe its effects in vivo using WT and TAAR1-KO mice to pinpoint which behavioral parameters are TAAR1 dependent. It has been reported that selective TAAR1 agonists decrease core body temperature (CBT) [41]. Thus, to validate that SEP-856 exerts a functional physiological effect in vivo through TAAR1, we first tested if SEP-856 mediated a TAAR1 dependent effect on pre-pulse inhibition (PPI). RM-two way ANOVA showed a significant SEP-856 (10 mg/kg) effect at the highest pulse sound (12 dB) (Treatment: F(1, 34) = 6.109, p = 0.0186; Fig. 5C). Post-hoc analysis revealed a statistically significant increase in PPI with SEP-856 in WT mice (p = 0.0045), a phenomenon, which was abolished in TAAR1-KO mice (Fig. 5C). To consolidate the role of TAAR1 in this phenomenon, we proceeded to disrupt baseline PPI using the NMDA receptor antagonist MK-801 (0.4 mg/kg). RM-two way ANOVA showed a significant treatment effect at the highest pulse sound (12 dB) (Treatment: F(2, 42) = 35.36, p < 0.0001; Fig. 5D). Post-hoc test revealed a significant reversal of MK-801 induced PPI disruption in WT (p = 0.0114), but not TAAR1-KO mice with SEP-856 (10 mg/kg) at the highest intensity of pre-pulse (Fig. 5D). Next, we investigated whether SEP-856 was capable of decreasing MK-801 induced hyperactivity. MK-801 (0.4 mg/kg) treated mice displayed a significant increase in traveled distance, whereas pre-treatment with SEP-856 (10 mg/kg) was able to diminish this behavior, restoring it to the level observed in animals treated with vehicle (VEH) alone (Treatment: F(2, 24) = 32.5, p < 0.0001; Fig. 5E). Post-hoc analysis showed that SEP-856 was able to suppress MK-801 hyperactivity in both WT (p = 0.0015) and TAAR1-KO mice (p = 0.0007). No significant genotype or sex differences in the number of movements, grooming or rearing were found (Fig. 5F, G).
Effects of SEP-856 on PPI in both genotypes WT (agonists can inhibit dopaminergic cell signaling [42] presumably due to GIRK channel activation, we investigated whether SEP-856 (10 mg/kg, Supplementary Fig. 6A). RM-two way ANOVA showed a significant effect of amphetamine treatment to increase locomotion (Treatment: F(2,44) = 81, p < 0.0001). However, SEP-856 (10 mg/kg) failed to suppress d-amphetamine induced hyperactivity in both WT and TAAR1-KO mice. Conversely, modest potentiation of d-amphetamine induced locomotion was observed in TAAR1-KO mice compared with WT mice. Additionally, certain TAAR1 agonists have been shown to counteract hyperlocomotion induced by other hyperdopaminergic stimulants such as cocaine [42]. Therefore, we evaluated whether SEP-856 (10 mg/kg) pre-treatment could decrease cocaine (20 mg/kg) induced locomotion stimulation (Treatment: F(2,22) = 44, p < 0.0001). By analogy to our earlier observations using d-amphetamine, we did not detect any significant inhibition of cocaine-mediated hyperlocomotion with SEP-856 pre-treatment in either genotype (Supplementary Fig. 6B).

DISCUSSION
Main discussion
SEP-856 is attracting considerable interest owing to its clinically validated antipsychotic properties paired with a low affinity for the D2 receptor [10, 12]. Furthermore, it displays pronounced activity at TAAR1 [10, 43], while sharing the 5-HT1A partial agonism of several other antipsychotics [10]. However, in the initial study which introduced the compound, no studies in TAAR1 deficient mice were performed [10] and the G-protein coupling profile of SEP-856 at TAAR1 and SHT1A receptors were not explored. Herein, we demonstrate that the antipsychotic-like behavioral profile of SEP-856 involves TAAR1, although it is not fully dependent on this receptor. Further, employing a codon-optimized TAAR1 construct together with a SHT1A receptor construct, we also evaluated the G-protein coupling profiles and GIRK activation capacity of SEP-856 at both receptors in vitro.

The generation of a successful assay system to investigate TAAR1 activation and Ga protein dependent TAAR1 signaling was pivotal for the current work. Indeed, it has been suggested that TAAR1 displays G-protein coupling promiscuity, with signaling via multiple Ga-protein couplings reported [35, 44]. Here, using our novel assay system, we show that SEP-856 displays full agonist properties in TAAR1 coupling to Gaq, but does not induce recruitment of Gαi1, Gαq, and Gα12/13. Prominent Gαi coupling paired...
with a pEC50 of 6.49 for β-PEA matches well with potencies described by Lindemann et al. [45] (pEC50: 6.52), Navarro et al. [44] (pEC50: 6.79) and Borowski et al. [46] (pEC50: 6.49), indicating the assay is accurate and sensitive enough to allow conclusions about TAAR1 pharmacology to be drawn. Furthermore, the rank-order of potencies in this assay closely matches those reported in previous reports, with β-PEA and p-Tyramine ranking as high potency agonists compared with dopamine and norepinephrine. Importantly, we were able to utilize the same hTAAR1 construct (minus a SmBit tag) in Xenopus oocytes for electrophysiological studies. Our results show that SEP-856 is able to induce TAAR1 dependent GIRK channel activation [20]. Curiously, SEP-856 appears to act as a partial agonist towards GIRK channel activation despite acting as a full agonist in Gq, recruitment assays and cAMP accumulation assays [10]. This unique feature is likely to play a role in its in vivo effects but further studies are required to elucidate the functional consequences of cAMP/PKA activation combined with ion channel mediated inhibition.

The cellular localization of a receptor is understood to play a role in the outcome of its signaling event [47]. Importantly, unlike most GPCRs, TAAR1 is mainly localized intracellularly [33, 48]. Our own data corroborates this finding, with the most abundant colocalization of TAAR1 observed together with the ER resident protein Calrectulin. Additionally, TAAR1 colocalization with the Golgi marker BGAL4-T1 but not the trans-Golgi marker TGN38 supports the limited forward trafficking of TAAR1 to the surface plasma membrane. Nevertheless, we observed TAAR1 activation by cell membrane-impermeable compounds such as dopamine and norepinephrine. Therefore, we showed that WT-TA1 (TAAR1 lacking N-glycosylation sites or additional surface localization boosters) can be detected on the cell surface plasma membrane in low yields using flow cytometry. This finding is supported by another recent study [36], which described plasma membrane localization of TAAR1 in a thyroid cell line. Collectively this data suggests that SEP-856 can signal through surface plasma membrane TAAR1 coupled to Gq.

Our behavioral studies uncovered two distinct physiological responses to SEP-856 which help explain its TAAR1 agonist properties in vivo. First, we showed that SEP-856, similar to other TAAR1 agonists, decreases core body temperature presumably reflecting TAAR1 expression in the neurons of the mediod optic nucleus [41]. Next, we demonstrated that SEP-856 exerts a strong inhibitory effect on baseline locomotion in WT, but not TAAR1-KO mice. Considering that TAAR1 is expressed in both dopamine and glutamate neurons [22, 41], we questioned if SEP-856 effects on hyperdopaminergic (d-amphetamine, cocaine) and hypoglutamatergic (MK-801) drug induced hyperlocomotion are mediated by TAAR1. Interestingly, SEP-856 hinders MK-801 induced hyperactivity regardless of the genotype but fails to counteract the effects of dopamine-releasing agents (amphetamine and cocaine) on locomotion. Finally, we demonstrate that the ability of SEP-856 to increase sensorimotor gating (PPi) is reliant on TAAR1. Dedic et al. [10] showed modulation of PPI by SEP-856 at doses of 3, 10 or 30 mg/kg, which is in agreement with our data at 10 mg/kg. Furthermore, we extend on this behavioral finding by showing that SEP-856 pre-treatment counters MK-801 induced baseline PPI disruption exclusively in WT but not TAAR1-KO mice. This model, even if still with limitations, is more relevant to putative antipsychotic properties than baseline PPI. Additionally, the selective TAAR1 agonist o-phenylidiotyramine was recently shown to increase PPI in WT but not in TAAR1-KO mice [41] which supports the notion of agonism at this receptor directly influencing sensorimotor gating. Pre-pulse inhibition remains one of the primary behavioral tools to study potential antipsychotic like effects of compounds. Therefore our data underpins the relevance of TAAR1 agonism to antipsychotic-like properties, at least in regards to a reinforcement of the ability to filter out irrelevant information.

5-HT1A receptors are targeted by numerous psychoactive molecules such as psychedelics (LSD, psilocin) anxiolytic agents (Buspirone) female hypoactive sexual desire disorder medications (Flibanserin) and antidepressants (Vilazodone). The physiological actions of these 5-HT1A ligands are likely partially dependent on selective targeting of 5-HT1A in different brain locations as a result of differential G-protein coupling profiles [49]. Even though 5-HT1A is typically described as Gi/o-coupled, promiscuous Ga-protein coupling has been shown with coupling to both Ga and Gq proteins [37, 38]. Here, we report that SEP-856 behaves as a low-potency partial agonist for 5-HT1A able to recruit Ga and Gq equally well, and provide evidence for Gq recruitment. This relatively poor G-protein recruitment induced via SEP-856 was also seen with β-arrestin recruitment, without any obvious signal bias between the two observed when comparing the response to serotonin. Additionally, we show that despite low-potency partial agonism, SEP-856 can act as a weak antagonist on the 5-HT1A receptor. Moreover, SEP-856’s 5-HT1A partial agonism is not only observable with Ga and β-Arrestin coupling but also in Gβγ derived GIRK activation. Interestingly, the pEC50 fold difference between serotonin and SEP-856 in these assays varied (1.25 × 5-HT vs SEP-856, Gq, and 1.70 × 5-HT vs SEP-856, GIRK) indicative of a potentially lower efficacy of SEP-856 to activate GIRK channels than to recruit Ga proteins.

Our results from both Ga recruitment and GIRK activation assays indicate a lower potency of SEP-856 at the 5-HT1A receptor than Dedic et al. [10] (pEC50: 5.64 cAMP inhibition vs. 4.76 Gq, recruitment, 4.49 β-arrestin recruitment and 5.08 GIRK activation). Likewise, while our TAAR1 G-protein recruitment assay data largely agrees with existing observations for β-PEA potency on the receptor, the described potency of SEP-856 here (pEC50: 6.08) is slightly lower than described by Dedic et al. (pEC50: 6.85) and another cAMP accumulation assay published more recently [50]. These differences can potentially be explained due to the differences in functional assays employed here, as second messenger assays are typically very sensitive and are prone to signal amplification. Different signaling assays are known to elicit different responses from ligands which may be a consequence of ligand bias or a limitation of the assay itself [51]. Likewise, the degree of G-protein recruitment required for second messenger generation and saturation is not well described. However, the profile of SEP-856 as a 5-HT1A receptor low-potency partial agonist is validated and expanded on in this study. It is unclear to what extent SEP-856 acts in vivo through 5-HT1A receptors in view of the relatively low to modest potencies described here. The ability of SEP-856 to suppress MK-801 induced hyperactivity, even following TAAR1 deletion, indicates a possible involvement of 5-HT1A receptors in SEP-856 mediated antipsychotic-like efficacy. This is supported by the dense expression of 5-HT1A receptor on cortical and hippocampal neurons [52] and the effect of 5-HT1A receptor antagonists on hypoglutamatergic states [53, 54]. Likewise, we find that the potent 5-HT1A antagonist NAN-190 (Fig. 3D) is able to counteract MK-801 induced hyperactivity in both genotypes (Supplementary Fig. 7). Furthermore, TAAR1 activation is known to increase potencies of 5-HT1A targeting drugs in 5-HT neurons, which may reflect an increased response in vivo that is not presently investigated in vitro [42]. Low potency partial agonism at the 5-HT1A receptor is a feature of several antipsychotics such as aripiprazole and therefore cannot be ignored in the functional profile of SEP-856 [9, 29]. Also, a recent in silico perspective utilizing molecular dynamics simulations supports functions at both TAAR1 and the 5-HT1A receptor [55].

Finally, in agreement with the initial SEP-856 study, we show that this ligand exhibits partial D2 receptor agonism and antagonism in vitro, albeit with very low potency and efficacy, making this property unlikely to play any meaningful role in vivo. Also, SEP-856 maintains its weak D2 receptor partial agonist properties with regards to Gβγ dependent GIRK activation, in line
with the previously described low efficacy of this drug at the D₂ receptor. In vivo, SEP-856 has not been previously evaluated using the d-amphetamine-induced hyperlocomotion in WT and TAAR1-KO mice [56], a well-established model for evaluating D₂ antagonists and antipsychotics. Similarly, other TAAR1 agonists have been reported to display inhibition of cocaine mediated hyperactivity [23, 42] and L-DOPA-induced motor sensitization [53]. However, it is unclear if all TAAR1 agonists are capable of suppressing hyperdopaminergic psychostimulant-mediated locomotion. For example, ROS073012, a TAAR1 partial agonist with low intrinsic efficacy, failed to reduce d-amphetamine hyperlocomotion in WT mice [57]. In the same study, it is reported that d-amphetamine fails to trigger hyperlocomotion in transgenic TAAR1-overexpressing mice. However, ROS073012 restored the sensitivity of TAAR1-overexpressing mice to d-amphetamine [57]. Additionally, TAAR1-KO mice display an enhanced sensitivity to monoamine releasing agents [19, 21, 22, 58]. Therefore, it was important to assess the effectiveness of SEP-856 using both the d-amphetamine and cocaine behavioral challenges to not only exclude any potential classical D₂ antagonism but also expand on the limited data with TAAR1 agonists using hyperdopaminergic stimulants. In our study, we demonstrate that SEP-856, despite acting as a full TAAR1 agonist, does not reduce d-amphetamine or cocaine induced hyperlocomotion in either WT or TAAR1-KO mice. Conversely, we noticed a small potentiation of amphetamine hyperlocomotion in TAAR1-KO mice with SEP-856 treatment. TAAR1-KO mice appear to sensitize faster to amphetamine than WT mice [59] and it is possible SEP-856 accelerates this process through as of yet, an unknown mechanism. Nonetheless, the inability of SEP-856 to inhibit d-amphetamine effects in vivo supports a non-potent D₂ antagonistic profile of the drug.

Limitations and future outlook. While our study overcomes several challenges of TAAR1 pharmacology, several others justify further studies. Since TAAR1 appears to be largely intracellular and trace amines have been shown to readily cross cellular membranes [60], it will be important to study signaling at these locations separately. ER TAAR1 signaling may be exclusive to Go₁₃ as has been recently suggested for amphetamine [35] and further work is needed to separate SEP-856 signaling outcomes in different cellular compartments. Additionally, while we have explored the molecular pharmacology of SEP-856 on the 5-HT₁ₐ receptor in detail here, our analysis did not conclusively address the role 5-HT₁ₐ receptor-dependent effects of SEP-856 on behavior. Future investigations would ideally involve 5-HT₁ₐ receptor-KO mice since 5-HT₁ₐ receptor antagonists themselves alter relevant behavioral readouts (Supplementary Fig. 7), making 5-HT₁ₐ receptor pharmacological intervention of SEP-856 studies difficult to interpret. In their recent review [11], Dedic et al. also point to the possible contribution of other serotonin receptors (namely 5-HT₂ and 5-HT₄) to the clinical efficacy of SEP-856 which showed modest to poor potencies for the compound. These receptors were not explored in this paper but warrant additional studies.

Several behavioral readouts have been developed to screen for, and study antipsychotic like actions of compounds. In this paper, we have used two of the arguably most established assays, sensorimotor gating (PPI) and inhibition of hyperlocomotion induced by psychostimulants. Since SEP-856 is able to diminish MK-801 hyperactivity (this paper) and PCP hyperactivity [10], but not d-amphetamine or cocaine induced hyperactivity, the effectiveness of SEP-856 as an antipsychotic justifies further behavioral studies which may also aid in classifying the behavioral phenotype of novel, non-dopaminergic antipsychotics. Interestingly, atypical antipsychotics with 5-HT₂ receptor antagonism such as clozapine appear to display greater antagonism of hyperactivity/stereotypy exerted by PCP than d-amphetamine [61–63].

In conclusion, while certain other (still to be unveiled) mechanisms of action of SEP-856 may be involved in its clinical efficacy in schizophrenia, the present study sheds further light on the nature of its molecular interactions with TAAR1 and 5-HT₂ receptor sites, and supports their likely collective roles in the expression of its antipsychotic and other functional actions. In view of the originality and therapeutic effectiveness of SEP-856, which may represent true progress in the management of schizophrenia and other psychiatric disorders, additional studies are warranted to further clarify exactly how it exerts its actions.

REFERENCES

1. Charlson FJ, Ferrari AJ, Santomauro DF, Diminic S, Stockings E, Scott JG, et al. Global Epidemiology and Burden of Schizophrenia: Findings From the Global Burden of Disease Study 2016. Schizophr Bull. 2018;44:1195–203.
2. Madras BK. History of the Discovery of the Antipsychotic Dopamine D₂ Receptor: A Basis for the Dopamine Hypothesis of Schizophrenia. J Hist Neurosci. 2013;22:62–78.
3. Millan MJ, Goodwin GM, Meyer-Lindenberg A, Ove Ogen S. Learning from the past and looking to the future: Emerging perspectives for improving the treatment of psychiatric disorders. Eur Neuropsychopharmacol J Eur Coll Neuropsychopharmacol. 2015;25:599–656.
4. Meltzer HY. Update on typical and atypical antipsychotic drugs. Annu Rev Med. 2013;64:393–406.
5. Stroup TS, Gray N. Management of common adverse effects of antipsychotic medications. World Psychiatry. 2018;17:341–56.
6. Muench J, Hamer AM. Adverse effects of antipsychotic medications. Am Fam Physician. 2010;81:617–22.
7. Meltzer YH. New Trends in the Treatment of Schizophrenia. CNS Neurol Disord Drug Targets. 2017;16:900–6.
8. Jordan S, Koprvica V, Chen R, Tottori K, Kikuchi T, Altar CA. The antipsychotic antiparkinson is a potent, partial agonist at the human 5-HT₁A receptor. Eur J Pharm. 2002;441:137–40.
9. Shapiro DA, Renock J, Cioffi M, Chiodo LA, Liu L-X, Sibley DR, et al. Aripiprazole, A Novel Atypical Antipsychotic Drug with a Unique and Robust Pharmacology. Neuropsychopharmacology. 2003;28:1400–11.
10. Dedic N, Jones PG, Hopkins SC, Lew R, Shao L, Campbell JE, et al. SEP-363856, a Novel Psychotropic Agent with a Unique, Non-D₂ Receptor Mechanism of Action. J Pharm Exp Ther. 2019;371:1–14.
11. Dedic N, Dworak H, Zeni C, Rutigliano G, Howes OD. Therapeutic Potential of TAAR1 Agonists in Schizophrenia: Evidence from Preclinical Models and Clinical Studies. Int J Mol Sci. 2021;22:13185.
12. Koblan KS, Rent J, Hopkins SC, Krystal JH, Cheng H, Goldman R, et al. A Non-D₂-Receptor-Binding Drug for the Treatment of Schizophrenia. N. Engl J Med. 2020;382:1497–506.
13. Correll CU, Koblan KS, Hopkins SC, Li Y, Dworak H, Goldman R, et al. Safety and effectiveness of olotaran (SEP-363856) in schizophrenic patients: results of a 6-month, open-label extension study. Npj Schizophr. 2021;7:63.
14. Pándy-Szekerés G, Esguerra M, Hauser AS, Caroli J, Munk C, Pilger S, et al. The G protein database, Gprobdb. Nucl. Acids Res. 2021;50: D518–D525.
15. Gainedotin RB, Hoener MC, Berry MD. Trace amines and their receptors. Pharm Rev. 2018;70:549–620.
16. Dodd S, F. Carvalho A, Puri BK, Maes M, Bortolasci CC, Morris G, et al. Trace Amine-Associated Receptor 1 (TAAR1): A new drug target for psychiatry? Neurosc Biobehav Rev. 2021;120:537–41.
17. Mathias U, Linn F, HB M, Cecilia L, Per O, Adil M, et al. Tissue-based map of the human proteome. Science. 2015;347:1260419.
18. Espinoza S, Lignani G, Caffino L, Maggi S, Sukhanov I, Leo D, et al. TAAR1 Modulates Cortical Glutamate NMDA Receptor Function. Neuropsychopharmacology. 2015;40:2217–27.
19. Lindemann L, Meyer CA, Jeanneau K, Bradaia A, Ozmen L, Bluethmann H, et al. Trace amine-associated receptor 1 modulates dopaminergic activity. J Pharm Exp Ther. 2008;324:948–56.
20. Bradaia A, Trube G, Stalder H, Norcross RD, Ozmen L, Wettstein JG, et al. The selective agonist EPPTB reveals TAAR1-mediated regulatory mechanisms in dopaminergic neurons of the mesolimnic system. Proc Natl Acad Sci. 2009;106:20081–6.
21. Di Cara B, Maggio R, Aloisi G, Rivet J-M, Lundius EG, Yoshitake T, et al. Genetic Deletion of Trace Amine 1 Receptors Reveals Their Role in Auto-Inhibiting the Actions of Ecstasy (MDMA). J Neurosci. 2011;31:16928–40.
22. Mantas I, Vallianatou T, Yang Y, Shariatgorji M, Kalomoiri M, Fridjonsdottir E, et al. TAAR1-Dependent and -Independent Actions of Tyramine in Interaction With
23. Revel FG, Moreau J-L, Pouzet B, Mory R, Bradaia A, Buchy D, et al. A new perspective for schizophrenia: TAAR1 agonists reveal antipsychotic- and antidepressant-like activity, improve cognition and control body weight. Mol Psychiatry. 2013;18:543–56.

24. Lesch, K-P, Waider J. Serotonin in the Modulation of Neural Plasticity and Networks: Implications for Neurodevelopmental Disorders. Neuron. 2012;76:175–91.

25. Deneris ES, Wyler SC. Serotonergic transcriptional networks and potential importance to mental health. Nat Neurosci. 2012;15:519–27.

26. Marazziti D, Marracci S, Paleogo L, Rotondo A, Mazzanti C, Nardi L, et al. Localization and gene expression of serotonin1A (SHT1A) receptors in human brain postmortem. Brain Res. 1996;658:55–59.

27. Samuels BA, Mendez-David I, Faye C, David SA, Pierz KA, Gardier AM, et al. Serotonin 1A and Serotonin 4 Receptors: Essential Mediators of the Neurogenic and Behavioral Actions of Antidepressants. Neurosci. 2014;22:26–45.

28. Ogren SO, Eriksson TM, Elvander-Tott E, D’Addario C, Ekstrom JC, Svenssonsson P, et al. The role of 5-HT1A receptors in learning and memory. Behav Brain Res. 2008;195:54–77.

29. Newman-Tancredi A. The importance of 5-HT1A receptor agonism in anti-psychotic drug action: rationale and perspectives. Curr Opin Investig Drugs. 2010;11:802–12.

30. Bantick RA, Deakin JFW, Grasby PM. The 5-HT1A receptor in schizophrenia: a promising target for novel atypical neuroleptics? J Psychopharmacol. 2001;15:37–46.

31. Wan G, Okasah N, Inoue A, Nehmé R, Carpenter B, Tate CG, et al. Mini G protein probes for active G protein-coupled receptors (GPCRs) in live cells. J Biol Chem. 2018;293:7466–73.

32. Sahlholm K, Barchad-Avitzur O, Marcellino D, Gómez-Soler M, Fuxe K, Ciruela F, et al. Probing GPCRs in mammalian brain: Receptor autoradiography and in situ hybridization studies of new ligands and newly identified receptors. Histochem J. 2012;31:37–40.

33. Barak LS, Salahpour A, Zhang X, Masri B, Sotnikova TD, Ramsey AJ, et al. Pharmacological Characterization of Membrane-Expressed Human Trace Amine-Associated Receptor 1 (TAAR1) by a Bioluminescence Resonance Energy Transfer cAMP Biosensor. Mol Pharm. 2008;7:584–95.

34. Jong Y-J, Harmon SK, O’Malley KL. GPCR signalling from within the cell. Br J Pharm. 2018;175:4026–35.

35. Underhill SM, Hulihen PD, Chen J, Fenoll-Ferrer C, Rizzo MA, Ingram SL, et al. Amphetamine signal through intracellular TAAR1 receptors coupled to Ga13 and Ga5 in discrete subcellular domains. Mol Psychiatry. 2021;26:1208–23.

36. Qatao M, Venugopalan V, Al-Hashimi A, Rehders M, Hein Z, et al. Trace Amine-Associated Receptor 1 Trafficking to Cilia of Thyroid Epithelial Cells. Cells. 2021;10:1518.

37. Inoue A, Raimondi L, Fadji FMN, Singh G, Kishi T, Uwamizu A, et al. Illuminating G protein-coupled receptors. Neurpsychopharmacology. 2021;47:2319–2329.

ACKNOWLEDGEMENTS

The authors would like to thank V.Glaros for his help and expertise regarding flow cytometry experiments and data analysis. Likewise, the authors would like to thank lila Koliar for the Expil293F cell line.

AUTHOR CONTRIBUTIONS

MS, IM, PS designed the experiments. MS performed in vitro experiments, IM, IF performed in vivo experiments and RA conducted the oocyte electrophysiology studies. MS, IM, RA performed data analysis. MS, IM, RA, KS, JMM, PS wrote the manuscript.
Open access funding provided by Karolinska Institute.

COMPETING INTERESTS
MJM is a consultant for PsychoGenics who were involved in the initial discovery and experimental characterization of SEP-856. At that time, he was not involved with the Company. He has no other interests to declare. All other authors do not have anything to disclose. This study was supported by grants from Karolinska Institutet (to MS, PS), Vetenskapsrådet (to PS, MS, IM, IF, number 2019-01422), Konung Gustaf Vs och Drottning Victorias Frimurarestiftelse, John and Lucille van Geest Foundation (to PS) Åhlénstiftelsen (to KS, number mB3 h18), Stiftelsen Lars Hiertas Minne (to KS and RÅ grant numbers FO2013-0609 and FO2020-0289), and Magnus Bergvalls stiftelse (to KS and RÅ, grant numbers 2018-02980 and 2020-04055). KS is currently a fellow at the Wallenberg Center for Molecular Medicine at Umeå University. PS and RÅ are funded by Region Stockholm.

ADDITIONAL INFORMATION
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41386-022-01421-2.

Correspondence and requests for materials should be addressed to Marcus Saarinen or Per Svenningsson.

© The Author(s) 2022