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Commentary

Trace amine-associated receptor 1 as a monoaminergic modulator in brain

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ABSTRACT

Brain monoaminergic systems play critical roles in mood, cognition, emotion, reward, learning and attention, and aberrance in brain monoaminergic activity is associated with a variety of neuropsychiatric disorders/diseases. The present commentary focuses on trace amine-associated receptor 1 (TAAR1) and its potential regulatory roles in brain monoaminergic systems. TAAR1 was discovered in 2001 and has been established to be a G-protein-coupled receptor signaling through the cAMP pathway. This receptor is activated by a broad spectrum of agonists, although there are notable species differences in ligand efficacy and potency. TAAR1 is expressed and widely distributed in brain monoaminergic systems and co-localized with the dopamine transporter in a subset of dopaminergic neurons in rhesus monkey and mouse brain substantia nigra. TAAR1 activation by the common biogenic amines, the trace amine β-phenylethylamine and methamphetamine alters the monoamine transporter function in both mouse and rhesus monkey brain synaptosomes, suggesting a modulatory role for this receptor in the presynaptic regulation of monoaminergic activity. However, little is known about other functional roles of TAAR1 in the brain. With a purpose to promote further studies on this receptor, we herein discuss the recent findings that provide insights into the functional significance and biological relevance of this receptor as a modulator in brain monoaminergic systems.

1. TAAR1 cloning and structural characterization

Dopamine, norepinephrine and serotonin, referred to as common biogenic amines, act as neurotransmitters and interact with specific presynaptic and postsynaptic receptors that belong to the rhodopsin superfamily of G-protein-coupled receptors (GPCRs). In addition to these amine neurotransmitters, trace amines, including β-phenylethylamine, tyramine, tryptamine, and octopamine are also found in the brain at trace levels. Although trace amines have been known to exist in mammalian brain for decades, little evidence supported their independent role in the brain, and hence they were generally considered to be by-products or false neurotransmitters. In 2001, Borowsky and colleagues cloned and characterized a new family of GPCRs now referred to as trace amine-associated receptors (TAARs) [1,2]. So far, more than 50 TAAR genes have been identified in mammals by genome scanning efforts, which include 9 genes in humans (including 3 pseudogenes), 9 in chimpanzees (including 6 pseudogenes), 19 in rats (including 2 pseudogenes), and 16 in mice (including 1 pseudogene) [2]. The discovery of this receptor family has rekindled an interest to investigate trace amines as neuromodulators or neurotransmitters in the brain, although most of these receptors still remain orphans.

TAAR1 has been found in different species [1–5], and in mammalians analyzed, TAAR1 is genetically encoded by a single exon. According to an analysis by Lindemann et al. [2], the TAAR1 gene and the genes for other TAAR members localize to a narrow region of 109 kb of human chromosome 6q23.1 (Fig. 1A), 192 kb of mouse chromosome 10A4, or 216 kb of rat chromosome 1p12 [2]. The human locus 6q23.1 has been reported to be genetically associated with schizophrenia and bipolar disorder [6,7], suggesting that TAAR genes may be implicated in polygenic neuropsychiatric disorders.

TAAR1 possesses several structural hallmarks characteristic of the rhodopsin/β-adrenergic receptor superfamily with 7 transmembrane domains, and the positions of its transmembrane domains reveal short N- and C-terminals. TAAR1 from non-human species displays different degrees of divergence in the amino acid sequence relative to human TAAR1 (Fig. 1B). In particular, the rhesus monkey TAAR1 shares a high structural similarity to human TAAR1 throughout each domain, whereas rat TAAR1 shows relatively high divergence (Fig. 1C), suggesting that the rhesus monkey TAAR1 may have a relatively high functional similarity and biological relevance to the human TAAR1.

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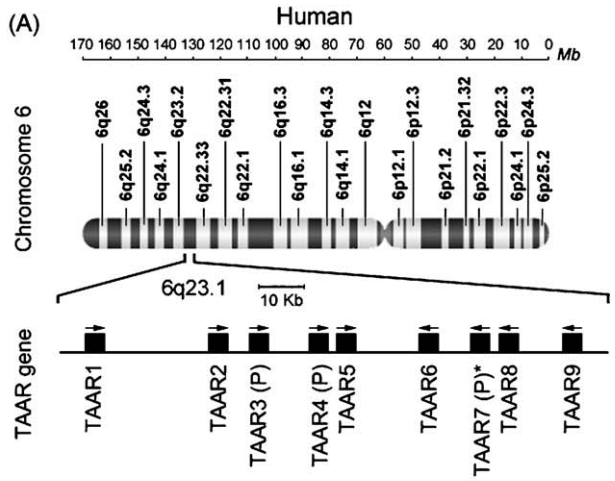
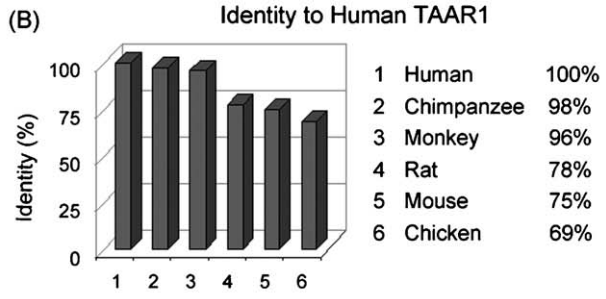
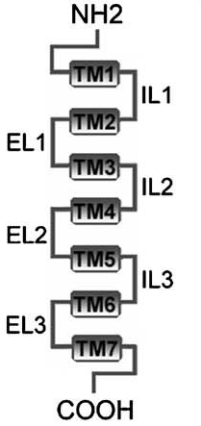




(C) Identity to Human TAAR1



Domain	Rhesus aa (I/T)	Rhesus (%)	Rat aa (I/T)	Rat (%)
N-tail	22/22	100	13/27	48
TM1	24/25	96	17/20	85
IL1	15/15	100	14/15	93
TM2	19/19	100	18/19	95
EL1	21/21	100	17/21	81
TM3	22/22	100	20/23	87
IL2	9/9	100	8/8	100
TM4	19/22	86	14/22	64
EL2	32/32	100	20/32	63
TM5	21/23	91	19/23	83
IL3	38/40	95	24/38	63
TM6	20/22	91	16/22	73
EL3	13/14	93	12/14	86
TM7	20/20	100	17/20	85
C-tail	30/32	94	24/28	86

Fig. 1. TAAR1: from gene to protein. (A) Chromosomal localization of TAAR genes in human. The TAAR1 gene along with the genes for other TAAR members is located on the chromosomal region of 6q23.1. The boxes indicate the positions of the genes but the width of the boxes is not representative of the length of the respective coding sequences. Arrows on top of the boxes indicate the reading orientation of the TAAR genes. (B) Analysis of amino acid sequences of TAAR1 from different species. The Identity (%) represents the percentage values defined as the result of the amino acids identical to human TAAR1 divided by the total amino acids numerically. Note the high Identity (%) among primates and the large divergence between primates and rodents. (C) Comparison of rhesus and rat TAAR1 to human TAAR1 by domains. The number of the amino acids identical to human TAAR1 (*I*) and the total amino acids (*T*) and the percentage values of *I*/*T* are shown.

2. TAAR1 expression and distribution in brain monoaminergic systems

Before discovery of the TAAR family, specific binding sites for trace amines in the brain had been reported. The efforts to search for trace amine recognition sites in the brain can be tracked back in the literature to the early 1970s when Baldessarini and Vogt explored the uptake and subcellular distribution of β-phenylethylamine and tyramine [8]. Since that time, saturable and high-affinity binding sites for [^{3}H]β-phenylethylamine, [^{3}H]tyramine and [^{3}H]tryptamine have been found in the rodent brain [8]. The successful cloning and characterization of TAAR family members and the ability of TAAR1 to be activated by β-phenylethylamine and tyramine [1,3], which endogenously exist in the brain, suggest that trace amine binding sites in the brain may represent the location of TAARs. Indeed, several studies have shown that TAAR1 and its mRNA are widely expressed in the brain, especially in brain monoaminergic systems [1,9,10], suggesting that this receptor may play important roles in the brain.

2.1. Brain monoaminergic systems

Monoaminergic neurons synthesize, package and release monoamine neurotransmitters to transmit neuronal impulses between neurons. The regions that monoaminergic neurons originate from and project to in the brain form distinct monoaminergic systems that play important roles in the regulation of the motor, cognitive, affective, and arousal functions in the human brain [11–14]. There are three major systems defined by the three common biogenic amine neurotransmitters: the dopaminergic system, the noradrenergic system and the serotonergic system (Fig. 2).

The dopaminergic system consists of four major dopaminergic pathways, the mesolimbic pathway, the mesocortical pathway, the nigrostriatal pathway and the tuberoinfundibular pathway (Fig. 2A). The mesolimbic pathway originates in the ventral tegmental area and projects to the limbic system via the nucleus accumbens, the amygdala, the hippocampus and the olfactory tubercle. The mesocortical pathway also originates in the ventral tegmental area, but projects to the prefrontal cortex and surrounding structures. The nigrostriatal pathway starts from the substantia nigra and reaches the striatum (caudate and putamen). The tuberoinfundibular pathway links the arcuate nucleus of the mediobasal hypothalamus (the tuberal region) to the median eminence (the infundibular region). In the dopaminergic system, dopaminergic neurons synthesize, package and release dopamine, which is intimately involved in a variety of brain functions including cognition, motivation, reward, lactation and motor activity [15–19].

The noradrenergic (norepinephrine-containing) neurons in the brain originate mainly in the locus coeruleus and in the lateral tegmental area and broadly project to distant brain regions to form the noradrenergic system. The brain regions which receive innervation from noradrenergic neurons include the amygdala, cingulate gyrus, cingulum, hippocampus, hypothalamus, neocortex, spinal cord, striatum and thalamus (Fig. 2B). Noradrenergic neurons synthesize, store and release norepinephrine. In the central nervous system, norepinephrine is associated with different brain functions, including sleep, memory, learning, and emotions [20–22].

The central serotonergic (serotonin-containing) neurons originate from the raphe nuclei in the brainstem and extend throughout the central nervous system to form the serotonergic system. The major brain regions that receive innervation from serotonergic neurons include the thalamus, striatum, hypothalamus, nucleus accumbens, neocortex, cingulate gyrus, cingulum, hippocampus, amygdala, deep cerebellar nuclei, cerebellar cortex and spinal cord (Fig. 2C). In the central nervous system, serotonin mainly plays an inhibitory role that calms, soothes and generates feelings of general contentment and satiation [23,24]. This system has widespread and often profound implications in sleep, appetite, memory, learning, temperature regulation, mood, sexual behavior, endocrine regulation, aggression, anxiety and reward [25,26].

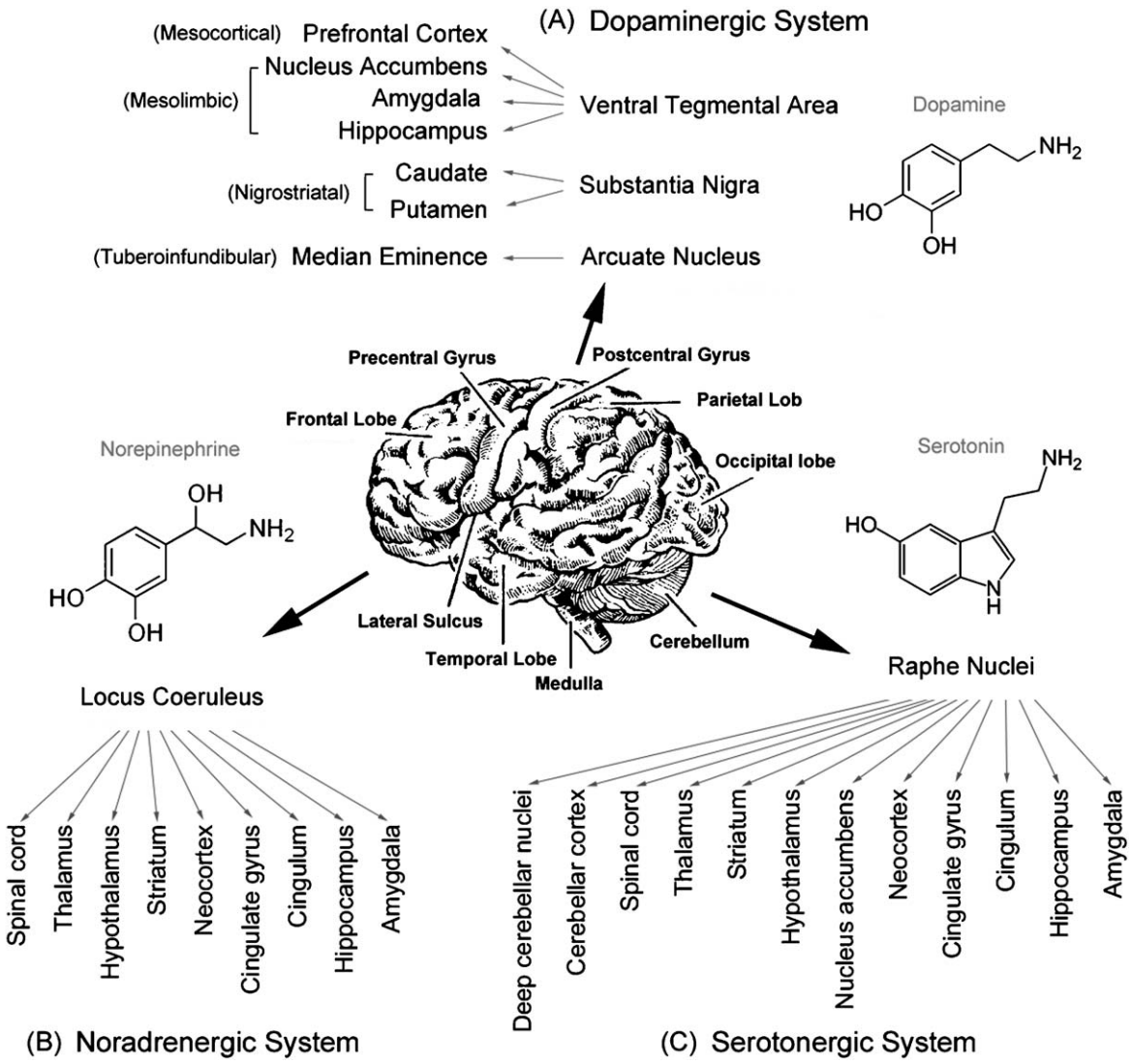


Fig. 2. Brain monoaminergic systems include the dopaminergic system, the noradrenergic system and the serotonergic system. (A) The dopaminergic system refers to the brain regions that dopaminergic neurons originate from and project to and include four major pathways. The mesocortical pathway runs from the ventral tegmental area to prefrontal cortex, the mesolimbic pathway runs from the ventral tegmental area to limbic nuclei (nucleus accumbens, amygdala, hippocampus), the nigrostriatal pathway runs from the substantia nigra to the striatum (caudate and putamen), and the tuberoinfundibular pathway runs from the arcuate nucleus in the mediobasal hypothalamus to the median eminence. (B) The noradrenergic system comprises the brain regions that the noradrenergic neurons reside in and extend to. Most of the noradrenergic neurons reside in the locus ceruleus and project to various distant brain areas. (C) The serotonergic system consists of the brain regions that the serotonergic neurons start from and project to. Most of the serotonergic neurons start from the raphe nuclei and project to various distant brain areas.

2.2. TAAR1 expression and distribution in the brain

TAAR1 mRNA expression in the mammalian brain was first reported by Borowsky et al. [1]. These investigators used reverse transcription (RT)-PCR to detect expression and to determine the distribution of TAAR1 mRNA in the human central nervous system. They reported that TAAR1 mRNA is expressed in discrete regions—low level expression (15–100 copies/ng cDNA) in the amygdala and trace levels (<15 copies/ng cDNA) in cerebellum, dorsal root ganglia, hippocampus, hypothalamus, pituitary and pontine reticular formation. These investigators also reported a widespread and unique distribution of TAAR1 mRNA in the mouse central nervous system by *in situ* hybridization histochemistry. Intense specific labeling was found in several mouse brain regions, including the mitral cell layer of the olfactory bulb, piriform cortex, arcuate nucleus, mesencephalic trigeminal nuclei, lateral reticular and hypoglossal nuclei, cerebellar Purkinje cells, and ventral horn of the spinal cord. Moderate labeling was evident in the frontal, entorhinal, and agranular cortices, ventral pallidum, thalamus, hippocampus, several hypothalamic nuclei, ambiguus, dorsal raphe, and gigantocellular reticular nuclei. Weaker staining was visible in the basal ganglia, amygdala, myelencephalon, and spinal cord dorsal horn. Notably, moderate expression of TAAR1 mRNA was detected in several monoaminergic cell groups including the substantia nigra, ventral tegmental area, locus coeruleus and dorsal raphe.

Recently, Xie et al. [9] used real-time RT-PCR to detect TAAR1 mRNA expression in selected regions of the rhesus monkey brain. The results showed that TAAR1 mRNA is widely expressed in the brain, including various monoaminergic regions: the dorsal and ventral caudate nucleus, putamen, substantia nigra, nucleus accumbens, ventral tegmental area, locus coeruleus, amygdala, and raphe nucleus. With the availability of a specific TAAR1 antibody directed against the third intracellular loop of human TAAR1, these investigators further used SDS-PAGE/Western blotting to detect TAAR1 protein expression in various regions of the rhesus monkey brain. The data showed that TAAR1 protein is detectable in the same brain regions that were positive in

expression of TAAR1 mRNA. In addition, immunohistochemical analysis revealed that the cellular distribution of TAAR1 in neurons is diffusely cytoplasmic within the perikaryon extending into the axon with occasional discernible membrane-associated expression. In both rhesus monkey and mouse brain substantia nigra, TAAR1 was demonstrated to be co-localized with the dopamine transporter in a subset of dopaminergic neurons, and meanwhile, neurons that only expressed TAAR1 or dopamine transporter were also visualized in the same area [9]. The expression of TAAR1 and dopamine transporter in the same dopaminergic neurons, along with the ability of dopamine to activate TAAR1 *in vitro*, suggests that TAAR1 may be directly related to dopamine transporter function and regulation. The close proximity of TAAR1-positive neurons with dopamine transporter-positive neurons suggests that TAAR1 may also indirectly regulate dopaminergic activity, including dopamine transporter function, through functional communication between neurons.

Lindemann et al. recently generated a transgenic mouse in which the entire TAAR1 coding sequence was replaced by a reporter gene consisting of LacZ fused to an N-terminal nuclear localization signal (NLS), and used an NLS-tagged version of the LacZ reporter to determine the expression and distribution of TAAR1 in the mouse brain [10]. They revealed a discrete and specific labeling of brain nuclei mainly in dopaminergic and serotonergic regions: the hypothalamus and preoptic area, ventral tegmental area, amygdala, dorsal raphe nucleus, nucleus of the solitary tract, and the parahippocampal region (rhinal cortices) and subiculum.

Collectively, the studies cited above provide evidence that TAAR1 is expressed and broadly distributed in the brain monoaminergic systems. Given the obvious importance of the monoaminergic systems in the brain, there continues to be a considerable interest in understanding the functional significance and biological relevance of TAAR1 in these systems. In this regard, amine compounds that activate this receptor *in vitro* have been identified. These ligands, which include endogenous monoamines and exogenous psychostimulant drugs of abuse, are functionally related to brain monoaminergic systems.

3. TAAR1 signaling and potential ligands

The cloning of TAAR1 from different species facilitated the investigation of TAAR1 signaling and the search for new specific ligands for the receptor. With the establishment of *in vitro* heterologous stable expression systems, TAAR1 signaling by its potential ligands has been extensively investigated. The initial studies that showed that TAAR1 was activated by trace amines *in vitro* support the proposal that TAAR1 is a trace amine receptor in the brain [1,5]. However, TAAR1 was not only activated by trace amines but also by other amine compounds and psychostimulant drugs *in vitro* [2–4,9,27,28], implying that TAAR1 may play a broader role in the brain than just serving as a receptor for the trace amines.

3.1. TAAR1 signaling through the cAMP pathway

In 2001, Borowsky and colleagues expressed human or rat TAAR1 in oocytes along with mRNA encoding the cAMP-responsive Cl channel, CFTR, to investigate TAAR1 signaling and search for its endogenous ligands. Their study demonstrated that tyramine, octopamine, dopamine and serotonin elicited inward currents in the transfected cells [1], indicating that the human and the rat TAAR1 are activated by these amine compounds via the cAMP signaling pathway. These investigators also expressed human TAAR1 in mammalian COS-7 cells for further investigation. They found that cAMP production in the transfected cells was initiated

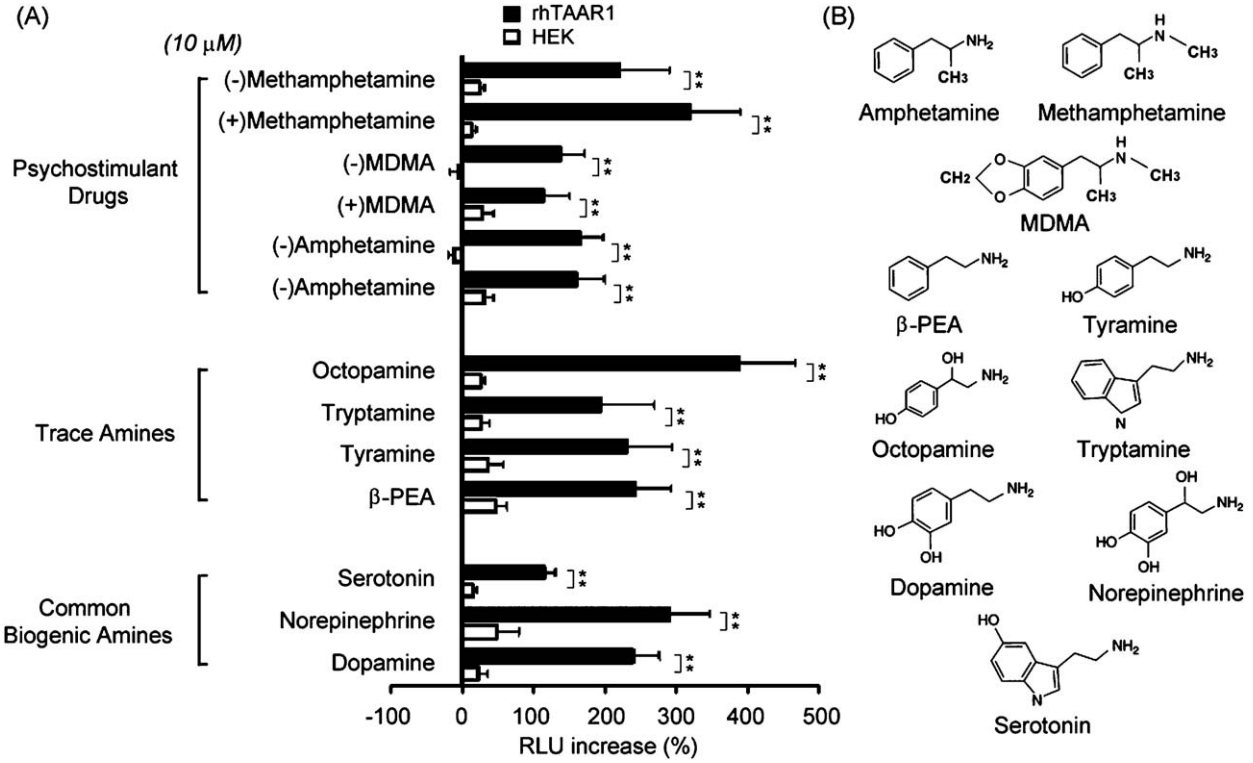


Fig. 3. Activation of rhesus monkey TAAR1 and the ligands. (A) Rhesus monkey TAAR1 (rhTAAR1) activation by different ligands. Reporter-transfected HEK293 cells with or without rhTAAR1 expression (labeled as rhTAAR1 and HEK, respectively) were treated with 10 μM of each drug for 18 h, and the cAMP production following drug treatment was determined by measurement of the expression level of CRE-Luc (a cAMP sensitive reporter). Data shown are values of mean ± SEM for three independent experiments performed in triplicate, $^{**}p < 0.01$. See [9] for details. (B) Structure of TAAR1 agonists.

most potently by β-phenylethylamine and tyramine, and more weakly by octopamine, tryptamine, dopamine, norepinephrine and serotonin. By testing candidate ligands in functional assays using HEK293 cells stably expressing rat TAAR1, Bunzow et al. found that rat TAAR1 was activated by a spectrum of ligands, and that its activation triggers accumulation of intracellular cAMP [3]. Inspired by these findings, Miller et al. cloned TAAR1 from the rhesus monkey and their laboratory demonstrated that rhesus monkey TAAR1 activation also led to cAMP accumulation in transfected cells, using CRE-luciferase reporter assays [4,9] (Fig. 3).

3.2. TAAR1 ligands and implications

The discovery of TAAR1 expression and distribution in the brain and in peripheral tissues evoked further research efforts to search for endogenous and exogenous ligands of the receptor [1]. Several lines of evidence indicated that TAAR1 is activated by a wide spectrum of compounds, including trace amines, common biogenic amines, thyronamines, and amphetamine-like psychostimulant drugs [1,3,4,9,27–30]. However, notable species differences exist in the selectivity of TAAR1 for these ligands [29,30], which may indicate that TAAR1 may play different roles in different species.

Trace amines, which are derived from the metabolism of amino acids, have been known to exist in the brain for decades, but their functional significance is still not well understood. In mammals, these amines are generally present at very low levels [8] and there is little evidence that any synapse exclusively uses any of these amines for neurotransmission. Accordingly, these amines were thought of as false neurotransmitters in the brain. Nevertheless, aberrant levels of these amines were associated with various neuropsychiatric disorders/diseases, including depression, schizophrenia, migraine and attention-deficit hyperactivity disorder (ADHD) [8], suggesting that trace amines have a functional relevance in the brain. Since the initial observation that TAAR1 is activated by trace amines, further investigations across different laboratories have verified that trace amines are agonists for TAAR1 *in vitro* [4,9,29]. The overlap of TAAR1 distribution with trace amine binding sites in the monoaminergic system further suggests that TAAR1 may recognize endogenous brain trace amines and mediate their function. Although the levels of trace amines in the brain are low, it is possible that trace amines reach levels suitable for TAAR1 activation at very discrete locations such as synaptic clefts in the brain, particularly under certain pathological or pharmacological conditions [31].

The common biogenic amines are synthesized from precursor amino acids in specific neurons via enzymatic catalysis, and are stored in vesicles at axonal endings of monoaminergic neurons. In response to neuronal depolarization, these amine neurotransmitters are released into the synaptic clefts and interact with both presynaptic and postsynaptic receptors. In 2001, Borowsky and colleagues reported that both human and mouse TAAR1 are responsive to dopamine and serotonin in induction of cAMP production, though weaker relative to tyramine and β-phenylethylamine [1], and meanwhile, Bunzow and colleagues also reported similar results with rat TAAR1 [3]. Since the reported EC_{50} values of the common biogenic amines are much higher than those of trace amines [1,29], it is a current point of contention in the field whether TAAR1 is a functional receptor for the common biogenic amines. Studies using the CRE-luciferase assays demonstrated that rhesus monkey TAAR1 is similarly activated by trace amines and common biogenic amines, including dopamine, norepinephrine and serotonin, in terms of potency and efficacy [4,9,32]. Additional studies demonstrated that the common biogenic amines interact with TAAR1 to regulate monoamine transporter function in both rhesus monkey and mouse brain synaptosomes [32]. Together, these results provide evidence that rhesus and mouse TAAR1 is activated by the common biogenic amines. Since the common biogenic amines each have multiple specific receptors that have been well established in the brain, their ability to activate TAAR1 suggests that the classic monoamine neurotransmitters may play previously unrecognized roles mediated through TAAR1.

Amphetamine (1-methyl-2-phenethylamine) is a psychostimulant drug of abuse and can be prescribed to treat attention-deficit hyperactivity disorder, narcolepsy, obesity and related disorders. Amphetamine and its derivatives form a distinct group of psychostimulant drugs referred to as amphetamines, that share a common backbone structure (a phenyl ring connected to an amino group by a two-carbon side chain with a methyl group on carbon-1 of the side chain) and act by increasing extracellular levels of dopamine, norepinephrine and serotonin in the brain. Methamphetamine and 3,4-methylenedioxy-*N*-methylamphetamine (MDMA) are members of this group. Methamphetamine is a widely abused and highly addictive drug that increases wakefulness and physical activity and decreases appetite. It can also be used as a medication for attention-deficit hyperactivity disorder and obesity [33]. MDMA is an illicit stimulant/hallucinogen used, at least initially, to attain mental stimulation, emotional warmth, enhanced sensory perception, and increased physical energy. In the brain, a primary action of amphetamines is to elevate levels of extracellular monoamine neurotransmitters (dopamine, serotonin and norepinephrine) by promoting their release from nerve endings and via reverse transport (efflux) by monoamine transporters [34]. The distribution profile of TAAR1 in the brain and its ability to be activated by amphetamines *in vitro* suggest that TAAR1 is a receptor target for amphetamines in the brain and contributes to psychostimulant effects.

Another line of research demonstrated that some of thyronamines, a family of decarboxylated and deiodinated derivatives of the thyroid hormones thyroxine (T4) and 3,5,3′-triiodothyronine (T3), are potent agonists of TAAR1. 3-Iodothyronamine (T1AM), 3,5-diiodothyronamine (T_2AM), 3,5,3′-triiodothyronamine (T_3AM), as well as the deiodinated thyronamine (T_0AM) are effective in activation of both rat and mouse TAAR1 to induce cAMP accumulation in transfected cells *in vitro* [28]. Among these thyronamines, T_1AM is the most potent agonist. T_1AM has been found to be endogenously present in the brain and peripheral tissues. It is reported that T_1AM is implicated in regulation of body temperature and modulates monoamine transporter function in synaptosomal preparations [35,36]. However, the distribution and quantitation of T1AM in the brain remains unclear, and there is no direct evidence yet that the *in vivo* functional effects of T1AM on thermoregulation are mediated by TAAR1. Thus, it requires further studies to clarify whether thyronamines are endogenous TAAR1 agonists

3.3. Cellular localization of TAAR1

Confocal immunofluorescence imaging has been used to examine localization of an epitope-tagged rat TAAR1 and human dopamine D1 receptor stably expressed in HEK293 cells. Unlike the epitope-tagged D1 receptor, which was clearly distributed around the surface of the cell, TAAR1 seemed to be retained almost exclusively within the cytoplasm [3]. In 2005, Miller et al. expressed a chimera comprising rhesus monkey TAAR1 and EGFP (EGFP-rhTAAR1) in HEK293 cells and observed an apparent intracellular localization of EGFP-rhTAAR1 in the transfected cells [4]. Further analysis of the compartmental distribution and cell surface localization of rhesus monkey TAAR1 expressed in HEK293 cells showed that TAAR1 was membrane-attached, but not located on the cell surface [37]. Together, these findings point to the possibility that TAAR1 functions in an intracellular environment, though it is unclear whether the receptor resides primarily within

the cells or whether the cells lack some components for receptor trafficking to the plasma membrane. Such a profile of localization may be representative of TAAR1 localization in neurons in brain monoaminergic systems, but this has yet to be determined. Since monoaminergic neurons synthesize monoamines in the cytoplasm or uptake monoamines via transporters, and the monoamines are both TAAR1 agonists and monoamine transporter substrates, an intracellular localization of TAAR1 may be physiologically relevant. Consistent with this possibility, immunohistochemical staining of rhesus monkey brain neurons showed that the cellular distribution of TAAR1 is diffusely cytoplasmic within the perikaryon and extending into the axon [9].

3.4. Facilitation of TAAR1 signaling by monoamine transporters

In 2005, Miller et al. examined rhesus monkey TAAR1 activation by its agonists, β-phenylethylamine, amphetamine and MDMA, in the presence and absence of expression of the human dopamine transporter, and demonstrated a striking potentiation of TAAR1 signaling by the transporter in the transfected cells [4]. Follow-up studies provided evidence that co-expression of the human norepinephrine transporter or the human serotonin transporter also enhanced TAAR1 signaling in response to agonists which are also substrates for the transporter [9]. Since the potentiation of TAAR1 signaling is blocked by specific transporter inhibitors [9], it was concluded that the observed potentiation of TAAR1 signaling is transporter-dependent. These data provide further support for the intracellular localization of TAAR1 in the transfected cells. However, it is also possible that the transporters interact with TAAR1 to cause conformational change in the receptor to alter its sensitivity to the agonists.

3.5. Species difference in TAAR1 pharmacology

TAAR1 cloned from different species are differentially responsive to various drugs with regard to cAMP production *in vitro*. Wainscott et al. have compared the dose–response of human and rat TAAR1 to a series of β-phenylethylamine analogs, and reported that a number of agonist compounds had significantly different relative potencies for the human and rat TAAR1 [29]. Meanwhile, Reese et al. demonstrated that tyramine was a full agonist for the rodent (mouse and rat) TAAR1 but was a partial agonist at h-rChTAAR1 (human-rat chimera), whereas β-phenylethylamine was a potent full agonist at TAAR1 in each case. They also showed that methamphetamine was a full agonist at mouse TAAR1 and h-rChTAAR1, but a partial agonist at rat TAAR1 [30]. These findings suggest that TAAR1 displays species differences between human and rodent in its interaction with the ligands, which most likely result from the structural diversity. In contrast to the rodent, rhesus monkey is genetically, physiologically and behaviorally more similar to humans. Rhesus monkey TAAR1 shares a 96% similarity to human TAAR1 in the amino acid sequence. Though rhesus monkey and human TAAR1 have not been systematically compared for similarities or differences in terms of drug efficacy and potency, the cloned rhesus and human TAAR1 have been reported to be highly similar in their functional response to β-phenylethylamine in the CRE-luciferase assay [9].

4. Roles of TAAR1 in monoaminergic systems

The distribution of TAAR1 in brain monoaminergic nuclei along with its co-localization with the dopamine transporter in a subset of neurons in the substantia nigra [9] suggests that TAAR may act as a presynaptic modulator of monoaminergic activity in the brain. Accordingly, recent studies have investigated the role of TAAR1 in monoamine transporter regulation. Since monoamine autoreceptors are located presynaptically, their functional relationship with TAAR1 has also been investigated.

4.1. Monoamine transporters and their functional significance in brain monoaminergic systems

The common biogenic amines are released into the synaptic clefts and activate specific postsynaptic receptors to transmit neuronal impulses. To terminate neurotransmission, monoamine neurotransmitters are, in turn, cleared by active transport into monoaminergic neurons and/or glial cells by monoamine transporters [38–40], which is essential for the precise control of the duration and the intensity of monoaminergic neurotransmission. Monoamine transporters, including the dopamine transporter, norepinephrine transporter and serotonin transporter are members of a superfamily of Na^+/Cl^- dependent neurotransmitter transporters [38,41,42] that share genetic and structural similarity [41,43], and are located in dopaminergic neurons, noradrenergic neurons and serotonergic neurons, respectively. Although functional overlap exists between these transporters [44], they generally function by sequestering monoamines from specific nerve endings. Inability or dysfunction of monoamine transporters can cause aberrant levels of monoamines in the brain which are associated with a variety of neuropsychiatric disorders/diseases such as anxiety, depression, attention-deficit hyperactivity disorder, Parkinson's disease, schizophrenia, suicide and drug abuse/addiction [45–48]. Monoamine transporters are biological targets and/or conduits of psychostimulant drugs of abuse (cocaine and amphetamines) as well as therapeutic drugs used as medications for psychiatric disorders such as selective serotonin reuptake inhibitors (SSRIs) (fluoxetine, citalopram). Imaging of monoamine transporters *in vitro* or *in vivo* by PET or SPECT technologies has provided an important view of pathological and drug-induced modulation of monoamine transporters in neuropsychiatric disorders/diseases [49–53]. Accordingly, monoamine transporters play important physiological and pathological roles in brain monoaminergic systems.

4.2. β-Phenylethylamine regulates monoamine transporter function via interaction with TAAR1

β-Phenylethylamine has a heterogeneous distribution in the brain with highest concentrations in the mesolimbic and striatal structures. Owing to its similarity to amphetamine in structure and effects, it has been considered as an endogenous amphetamine, and numerous studies have reported its influence in the central nervous system, especially on catecholaminergic activity in the mammalian brain [54]. However, the mechanisms by which β-phenylethylamine exerts its effects are unclear. Xie and Miller [55] recently reported that β-phenylethylamine alters both uptake and efflux (inhibited uptake and induced efflux) functions of monoamine transporters, including the dopamine transporter, norepinephrine transporter and serotonin transporter, via interaction with TAAR1, in heterologous co-expression systems and in brain striatal and thalamic synaptosomes. This mediatory role of TAAR1 in the regulation of monoamine transporter function in brain synaptosomes provides the first direct evidence for a trace amine acting as a neuromodulator in the brain. β-Phenylethylamine also appears to be a substrate of the monoamine transporters [4], and therefore may compete with monoamine neurotransmitters for reuptake at the transport sites. However, the transport capacity of the transporters varies with the concentration of the substrates (greater transport at higher substrate concentration) [56], and the levels of β-phenylethylamine in the brain are very low. Accordingly, the competition of β-phenylethylamine against the monoamine neurotransmitters for reuptake may play a much more

limited role in modulating transporter function relative to the effects resulting from its interaction with TAAR1.

In addition to β-phenylethylamine, brain trace amines also include tyramine, tryptamine, and octopamine. Since these trace amines are all agonists at TAAR1 and are spatially and functionally related with the common biogenic amine neurotransmitters, the finding that β-phenylethylamine acts via TAAR1 to modulate monoamine transporter function may reveal a common and principal functional mechanism for the brain trace amines.

4.3. Presynaptic receptor balancing between TAAR1 and monoamine autoreceptors

The ability of TAAR1 to regulate monoamine transporter function in response to β-phenylethylamine [55] and the co-localization of TAAR1 with the dopamine transporter in neurons in the substantia nigra [9] together suggest that TAAR1 is a presynaptic modulator in monoaminergic systems, and raises the question of whether TAAR1 activity could be influenced by presynaptic monoamine autoreceptors that are also located presynaptically. Monoamine autoreceptors provide feedback regulation of monoamine neurotransmitter release (exocytosis). The D2 dopamine autoreceptor located in dopaminergic neurons modulates dopamine synthesis [57] and release [58], the 5-$HT_{1A/1B}$ autoreceptors located in serotoninergic neurons regulate serotonin release [59,60], and the adrenergic $\alpha_{2A/2B}$ autoreceptors contribute to regulation of norepinephrine release [61,62]. Intriguingly, the D2 autoreceptor is also implicated in the regulation of dopamine transporter activity [63,64], suggesting that the monoamine neurotransmitters may also alter monoamine transporter function via interaction with monoamine autoreceptors in the brain. Recently, Wolinsky et al. reported a large increase of striatal high-affinity D2 receptors in TAAR1 knockout mice [65], suggesting that TAAR1 function is physiologically related to monoamine autoreceptors.

D2s (short isoform) activation by dopamine, α_{2A} activation by norepinephrine, and 5-HT_{1B} activation by serotonin enhance the uptake of [^{3}H]dopamine, [^{3}H]norepinephrine, and [^{3}H]serotonin, respectively. Conversely, TAAR1 activation by these common biogenic amines inhibits the uptake of these [^{3}H]monoamines in transfected cells [32], suggesting that common biogenic amines can regulate monoamine transporter function via interaction with both TAAR1 and the monoamine autoreceptors. In rhesus monkey and wild-type mouse synaptosomes, dopamine, norepinephrine and serotonin significantly inhibit the uptake of [^{3}H]dopamine, [^{3}H]norepinephrine or [^{3}H]serotonin and induce their efflux, respectively, in the presence but not in the absence of specific monoamine autoreceptor inhibitors [32], suggesting that TAAR1 activity in response to the common biogenic amines is retarded by the monoamine autoreceptors that are simultaneously activated. Since TAAR1 and the monoamine autoreceptors are both responsive to the common biogenic amines but have opposite effects in transporter modulation, a concept of presynaptic receptor balancing established by these receptors was introduced by Xie et al. as a presynaptic regulatory mechanism [32].

Although no direct evidence has yet been obtained for the role of TAAR1 in neurotransmitter release (exocytosis), it is possible that the cross-talk between the receptor signaling of TAAR1 and the monoamine autoreceptors is also influential in release regulation. Accordingly, the implication of TAAR1 in presynaptic monoaminergic regulation challenges traditional feedback mechanisms. Notably, the trace amines, including β-phenylethylamine, tyramine, tryptamine and octopamine, are also TAAR1 agonists but differ from the common biogenic amines in that they lack interaction with the monoamine autoreceptors [9,32]. It is therefore most likely that selective interaction of trace amines with TAAR1 biases the receptor balancing between TAAR1 and the monoamine autoreceptors in their response to the common biogenic amines, and consequently modifies the effects of common biogenic amines in transporter regulation. Trace amines are heterogeneously distributed in mammalian brain tissues, their distribution spatially parallels the origins and terminal projection areas of monoaminergic neurons, and they are synthesized and released along with the common biogenic amines [8]. Accordingly, this may suggest a novel mechanism by which trace amines exert their effects in brain monoaminergic systems.

4.4. TAAR1 is a target of amphetamine-like psychostimulants

In 2001, Bunzow et al. reported for the first time that TAAR1 is activated by amphetamine and its derivatives *in vitro* [3], which was confirmed and extended by several follow-up studies across different laboratories [4,9,33,34]. Recent studies revealed an enhanced sensitivity of TAAR1 knockout mice to amphetamine, and an amphetamine-induced increase in the release of dopamine and norepinephrine in the TAAR1 knockout mouse striatum [10,65]. The data may suggest a role for TAAR1 in mediating the effects of amphetamine in the brain. However, since adaptive changes such as a dramatic increase of high affinity D2 receptors occur in these knockout mice [65], the enhanced sensitivity of TAAR1 knockout mice to amphetamine may be caused by adaptive changes and therefore may not be a direct consequence of the TAAR1 deficit.

In contrast, in heterologous expression systems, methamphetamine activation of TAAR1 dramatically inhibits uptake and induces efflux of [^{3}H]dopamine through the dopamine transporter [66], which provides direct evidence that methamphetamine targets TAAR1 and regulates transporter function via interaction with TAAR1. Methamphetamine is a widely abused and highly addictive psychostimulant and neurotoxic drug that dramatically interferes with cognition (memory and attention) and emotion (euphoria, surge in productivity, increase in self-esteem and impulsion) [67,68]. In the brain, a primary action of methamphetamine is to elevate the levels of extracellular monoamine neurotransmitters, especially dopamine, via interfering with their reuptake and promoting their release at the nerve endings [34,69,70], which is thought to account for the mechanism by which methamphetamine exerts its psychostimulant effects. Monoamine transporters, especially the dopamine transporter, are biological targets and conduits by which methamphetamine causes effects on transport of the monoamine neurotransmitters [71,72]. In addition to its ability to compete with monoamines for uptake at the transport sites [34,73], methamphetamine induces non-competitive effects on the transporters that are associated with phosphorylation cascades, such as down regulation of the transporters and transport reversal [74,75] to affect neurotransmitter transport, for which the mechanisms are largely unclear. The finding that methamphetamine regulates dopamine transporter function via interaction with TAAR1 suggests that TAAR1 is a mediator of non-competitive effects caused by methamphetamine in the brain.

Recently, Xie and Miller [76] compared the effects of methamphetamine on the uptake and efflux of [^{3}H]dopamine in brain striatal synaptosomes generated from rhesus monkeys, wild type and TAAR1 knockout mice. The results revealed that methamphetamine caused a TAAR1-dependent inhibition of [^{3}H]dopamine uptake and induction of [^{3}H]dopamine efflux via the dopamine transporter. These data provide direct evidence that methamphetamine exerts non-competitive effects on the dopamine transporter via its interaction with TAAR1 in the brain. Accordingly, it is reasonable to speculate that new drugs selectively targeting TAAR1, if developed, may be effective

modulators of the dopamine transporter and act distinctly from drugs which directly bind to monoamine transporters. In this regard, these initial findings may provide an impetus for the development of novel therapeutic drugs as medication of methamphetamine addiction which is a serious social and medical problem.

The mediatory role of TAAR1 in methamphetamine regulation of dopamine transporter function is supported by the finding that the [^{3}H]dopamine efflux caused by methamphetamine interaction with TAAR1 is dependent on PKC-driven phosphorylation [66,76], which is generally a downstream event in receptor signaling. PKC-dependent phosphorylation plays a key role in the functional regulation of the dopamine transporter by methamphetamine. Methamphetamine increases striatal PKC activity [77], which in turn leads to down-regulation of dopamine transporter function [78]. Both methamphetamine-induced phosphorylation and down-regulation of dopamine transporter function were prevented by PKC inhibition in striatal synaptosomes [72]. Furthermore, PKC-driven phosphorylation is also implicated in dopamine transporter internalization [72,79]. Accordingly, the association of the TAAR1-mediated methamphetamine effects in induction of [^{3}H]dopamine efflux with PKC-driven phosphorylation [66,76] suggests that methamphetamine may interact with TAAR1 to trigger the PKC phosphorylation pathway and consequently affect dopamine transporter function.

4.5. A perspective for the roles of TAAR1 in the monoaminergic systems

The co-localization of TAAR1 with the dopamine transporter in neurons of the substantia nigra and the involvement of TAAR1 signaling in monoamine transporter regulation along with monoamine autoreceptors support the hypothesis that TAAR1 is a presynaptic modulator of monoaminergic regulation. Based on the findings discussed above, a putative schematic model for receptor modulation of monoamine transporter function is depicted in Fig. 4. Monoamine neurotransmitters are released into the synaptic cleft and then are cleared by monoamine transporters. The transporter function is regulated by TAAR1 and monoamine autoreceptors. Common biogenic amines interact with both TAAR1 and monoamine autoreceptors to affect transporter function. The opposite effects of TAAR1 and monoamine autoreceptors in response to common biogenic amines may provide a presynaptic balance in the control of transporter function. Trace amines and amphetamine-like psychostimulant drugs bias this balance due to their selective interaction with TAAR1. Autoreceptor inhibitors block autoreceptor signaling and therefore indirectly reinforce TAAR1 activity and bias this balance.

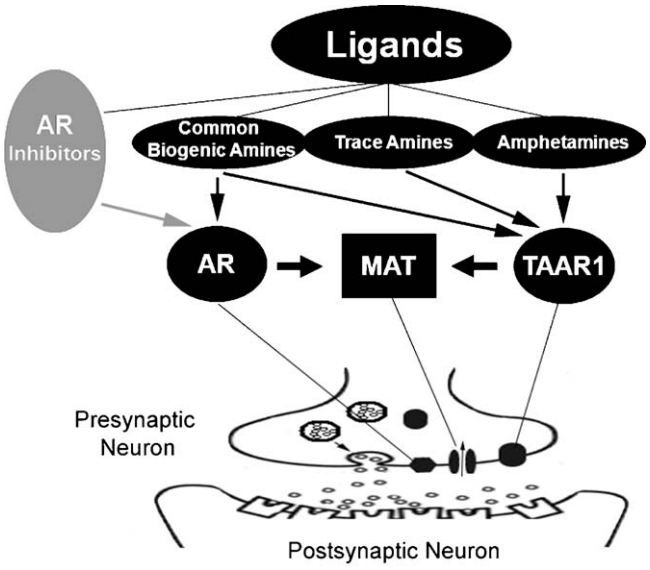


Fig. 4. Schematic model for monoamine transporter regulation by receptors. TAAR1 and monoamine autoreceptors (AR) are located in presynaptic monoaminergic neurons and are implicated in monoamine transporter (MAT) regulation. Common biogenic amines regulate monoamine transporter function by interaction with both TAAR1 and the specific autoreceptors, but trace amines and psychostimulant amphetamines interact only with TAAR1. Monoamine autoreceptor inhibitors block the autoreceptor signaling and consequently silence its influence on the monoamine transporter.

However, this model does not exclude the possibility that TAAR1 is also located in the postsynaptic neurons where it may regulate postsynaptic function in the monoaminergic systems. In monoaminergic neurotransmission, monoamine neurotransmitters are released into synaptic clefts to interact with specific postsynaptic receptors to transmit the neuronal impulses between neurons. If TAAR1 also resides on the postsynaptic membrane, it certainly adds complexity to the neurotransmission process or its regulation. Another interesting issue is the cellular localization of TAAR1 in neurons. The intracellular localization of TAAR1 in the transfected cells suggests that this receptor may also reside within neurons. Since the common biogenic amines interact with both TAAR1 and the specific monoamine autoreceptors in regulation of monoamine transporter function, it is predictable that, if TAAR1 is intracellularly localized in the neurons, its signaling in response to the common biogenic amines should lag behind the signaling of the monoamine autoreceptors that are on the membrane surface. If this is the case, the transporter regulation by TAAR1 and the monoamine autoreceptors is not synchronous. This raises a research interest in the functional relevance of such timing of receptor signaling. On the other hand, if TAAR1 is intracellularly localized, it would be interesting to clarify how this receptor functions in the neurons that do not express monoamine transporters.

5. Concluding remarks

TAAR1 is a member of the TAAR receptor family and was initially established to be functionally associated with trace amines [1,3]. It is a G-protein-coupled receptor that signals through the cAMP signaling pathway. Its distribution in brain monoaminergic systems, its co-localization with the dopamine transporter in dopaminergic neurons, its activation by monoamines that are neurotransmitters or neuromodulators in monoaminergic systems, its targeting by psychostimulant amphetamines that primarily influence monoaminergic activity and its modulatory roles in monoamine transporter regulation together reveal that it is a monoaminergic modulator in the brain. TAAR1 activation by trace amines and β-phenylethylamine regulation of monoamine transporter function via interaction with TAAR1 suggest that it is a trace amine receptor that mediates the effects of the trace amines in the brain. Trace amines are widely distributed in brain monoaminergic systems and their aberrances are associated with various neuropsychiatric disorders/diseases. The establishment of TAAR1 as a trace amine receptor resurrects the question of the functional significance of the trace amines in the brain. Meanwhile, the implication of TAAR1 in the activity of the common biogenic amines and its balancing in signaling and function with the monoamine autoreceptors provide a new insight into presynaptic feedback regulatory mechanisms. The association of TAAR1 with the psychostimulant amphetamines suggests that TAAR1 may play a vital role in psychostimulant action and may reveal a new potential pharmacological target for the treatment of psychostimulant abuse.

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