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Brain-Specific Overexpression of Trace Amine-Associated Receptor 1 Alters Monoaminergic Neurotransmission and Decreases Sensitivity to Amphetamine

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Trace amines (TAs) such as *β*-phenylethylamine, *p*-tyramine, or tryptamine are biogenic amines found in the brain at low concentrations that have been implicated in various neuropsychiatric disorders like schizophrenia, depression, or attention deficit hyperactivity disorder. TAs are ligands for the recently identified trace amine-associated receptor 1 (TAAR1), an important modulator of monoamine neurotransmission. Here, we sought to investigate the consequences of TAAR1 hypersignaling by generating a transgenic mouse line overexpressing *Taar1* specifically in neurons. *Taar1* transgenic mice did not show overt behavioral abnormalities under baseline conditions, despite augmented extracellular levels of dopamine and noradrenaline in the accumbens nucleus (Acb) and of serotonin in the medial prefrontal cortex. *In vitro*, this was correlated with an elevated spontaneous firing rate of monoaminergic neurons in the ventral tegmental area, dorsal raphe nucleus, and locus coeruleus as the result of ectopic TAAR1 expression. Furthermore, *Taar1* transgenic mice were hyposensitive to the psychostimulant effects of amphetamine, as it produced only a weak locomotor activation and failed to alter catecholamine release in the Acb. Attenuating TAAR1 activity with the selective partial agonist RO5073012 restored the stimulating effects of amphetamine on locomotion. Overall, these data show that *Taar1* brain overexpression causes hyposensitivity to amphetamine and alterations of monoaminergic neurotransmission. These observations confirm the modulatory role of TAAR1 on monoamine activity and suggest that *in vivo* the receptor is either constitutively active and/or tonically activated by ambient levels of endogenous agonist(s).
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INTRODUCTION

Dysregulation of monoamine neurotransmission occurs in numerous neuropsychiatric disorders, including schizophrenia, depression, attention deficit hyperactivity disorder, drug addiction, or Parkinson's disease. Recent evidence suggests that the trace amine-associated receptor 1 (TAAR1) is an important modulator of monoamine neurotransmission (Lindemann and Hoener, 2005; Wolinsky *et al*, 2007; Lindemann *et al*, 2008; Bradaia *et al*, 2009; Miller, 2011; Revel *et al*, 2011). As such, TAAR1 has become an attractive target for the treatment of neuropsychiatric disorders (Sotnikova *et al*, 2009; Revel *et al*, 2011).

TAAR1 is a seven-transmembrane domain receptor and a member of the TAAR family (Lindemann *et al*, 2005) that responds to various trace amines (TAs), endogenous biogenic amines related to classical monoamines, and present in the brain at low concentrations (Burchett and Hicks, 2006; Narang *et al*, 2011). TAs include *β*-phenylethylamine, *p*-tyramine, tryptamine, octopamine, and synephrine. TAs have been implicated in a wide range of neuropsychiatric disorders, including those associated with monoamine dysregulations (Burchett and Hicks, 2006; Berry, 2007; Grandy, 2007).

TAAR1 signals through the stimulatory G protein to elevate intracellular cAMP levels and stimulate inwardly rectifying K^+ channels (Borowsky *et al*, 2001; Bunzow *et al*,

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2001; Lindemann and Hoener, 2005; Grandy, 2007; Bradaia *et al*, 2009). In the mouse brain, TAAR1 is expressed throughout the limbic and monoaminergic systems, including the ventral tegmental area (VTA) and dorsal raphe nucleus (DRN) (Lindemann *et al*, 2008). The mice lacking *Taar1* (*Taar1*$^{-/-}$ mice) are hypersensitive to *d*-amphetamine, a potent psychostimulant (Wolinsky *et al*, 2007; Lindemann *et al*, 2008). Following an acute injection, they show exacerbated locomotor activity and elevated striatal release of dopamine (DA), noradrenaline (NA) and serotonin (5-hydroxytryptamine; 5-HT). *In vitro*, the spontaneous firing activity of DA and 5-HT neurons in the VTA and DRN, respectively, is markedly enhanced in mutants (Lindemann *et al*, 2008; Bradaia *et al*, 2009; Revel *et al*, 2011). This contrasts with the locus coeruleus (LC) where the firing rate of NA neurons does not differ between mutant and wild-type (WT) animals (Revel *et al*, 2011), consistent with the absence of detectable *Taar1* expression in this structure (Lindemann *et al*, 2005). Various studies have documented reciprocal interactions between TAAR1 and monoaminergic transporters such as the DA transporter (DAT) (Miller *et al*, 2005; Xie and Miller, 2007; reviewed in Miller, 2011), and monoaminergic receptors such as the D_2 and 5-HT_{1A} receptors (Wolinsky *et al*, 2007; Xie *et al*, 2007, 2008; Xie and Miller, 2008; Bradaia *et al*, 2009; Espinoza *et al*, 2011; Revel *et al*, 2011). Altogether, these data have suggested that in some brain regions, TAAR1 is an important modulator of monoaminergic activity.

Apart from TAs, TAAR1 responds to a wide variety of endogenous and synthetic compounds, including DA, amphetamine derivatives, and ergolines that all possess additional TAAR1-independent actions (Bunzow *et al*, 2001; Lindemann and Hoener, 2005; Grandy, 2007; Sotnikova *et al*, 2010). Such polypharmacology has made it challenging to understand the role and the biology of TAAR1. The recent discovery of a potent and selective TAAR1 antagonist (EPPTB, RO5212773) (Bradaia *et al*, 2009; Stalder *et al*, 2011) and an agonist (RO5166017) (Revel *et al*, 2011) is expected to facilitate the understanding of TAAR1 physiological functions and therapeutic potential. *In vitro*, use of these agents revealed that TAAR1 interacts functionally with the D_2 and 5-HT_{1A} receptors and modifies their pharmacological properties (Bradaia *et al*, 2009; Revel *et al*, 2011). *In vivo*, TAAR1 selective activation prevents both hyperdopaminergic- and hypoglutamatergic-induced hyperlocomotion in rodents, suggesting antipsychotic-like effects (Revel *et al*, 2011). Overall, these data show that modulation of monoaminergic neurotransmission by TAAR1 may impact on a broad variety of neurophysiological functions.

Electrophysiological recordings made *ex vivo* have suggested that TAAR1 is either constitutively active and/or tonically activated by ambient levels of endogenous agonist(s), presumably TAs (Lindemann *et al*, 2008; Bradaia *et al*, 2009; Revel *et al*, 2011). In order to confirm this finding and to further decipher the physiological roles of TAAR1, we created a genetically-modified mouse line in which *Taar1* is overexpressed in neurons. We sought to increase *Taar1* expression in the brain, including in structures that normally do not express the receptor, and reasoned that if this is sufficient to alter brain function, it would support the idea that *in vivo* TAAR1 is constitutively active and/or tonically activated by endogenous agonist(s). The mutant animals were then characterized by means of electrophysiological recordings, *in vivo* microdialysis measurements, and behavioural assessments.

MATERIALS AND METHODS

Compounds

All compounds were purchased from Sigma (Buchs, Switzerland) except for RO5073012, EPPTB (RO5212773), RO5166017, [^{3}H]RO5166017, and [^{3}H]RO5192022 that were synthesized at F. Hoffmann-La Roche (Basel, Switzerland).

Animals

All experiments performed at Roche were conducted in compliance with Swiss Federal and Cantonal laws on animal research and AAALAC regulations and received prior approval by the Cantonal Veterinary Office. All animals were housed under a 12 h light/12 h dark cycle (lights on at 0600 hours) at 22 ± 2°C, with *ad libitum* access to food and water. All recordings occurred in the first-half of the light phase, except where specified. Microdialysis studies were conducted at Mario Negri Institute (Milan, Italy) in conformity with the institutional guidelines, in compliance with national (D.L. n.116, G.U., suppl. 40, 18 Febbraio 1992, Circolare No. 8, G.U., 14 Luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996).

Taar1 transgenic (Tg) C57BL/6J mice were generated at Roche by pronuclear injection (Ittner and Gotz, 2007), as described previously (Richards *et al*, 2003). The transgene consisted of the *Thy1.2* expression cassette (Richards *et al*, 2003) driving the expression of a fusion protein, which comprises an optimized Kozak sequence followed by an N-terminal influenza hemaglutinin viral leader sequence followed by the M1-FLAG epitope and a Met-Gly linker, and fused in frame to the mouse *Taar1* coding sequences (Borowsky *et al*, 2001; Lindemann *et al*, 2005). The murine *Taar1* transgene (B6-Tg(Taar1)2) was generated by PCR amplification from genomic DNA with the primers 5′-GCC TCGAGTCCACCATGAAGACGATCATCGCCCTGAGCTACA TCTTCTGCCTGGTGTTCGCCGACTACAAGGACGATGATG ACGCCATGGGCATGCATCTTTGCCACGCTATCAC-3′ and 5′-GCCTCGAGTTACAAAAATAGCTTAGACC-3′ and cloned using the introduced *Xho*I sites into the *Xho*I site of *Thy1.2* expression vector.

Behavioral Phenotyping

Mice were assessed for body temperature, body weight, grip strength, and general motor coordination (rotarod test) as described previously (Lindemann *et al*, 2008).

Electrophysiological Recordings

Electrophysiological recordings were performed as reported (Bradaia *et al*, 2005, 2009; Revel *et al*, 2011). Horizontal brain slices (250 μm) from adult (3–6 months of age) mice were prepared in cooled sucrose solution (in mM: sucrose 248, KCl 2, $MgSO_4$ 2, NaH_2PO_4 1.25, $NaHCO_3$ 26, D-glucose

11.1, and kynurenic acid 1). Slices were transferred into an incubation chamber containing artificial cerebrospinal fluid (aCSF; in mM: NaCl 119, KCl 2.5, $MgCl_2$ 1.3, $CaCl_2$ 2.5, NaH_2PO_4 1.0, $NaHCO_3$ 26.2, and D-glucose 11) at room temperature for at least 30 min. All solutions were continuously bubbled with 95% CO_2, 5% O_2. For experiments, the slices were transferred into a recording chamber containing ~2 ml of aCSF. The bath solution was continuously renewed at 1.5 ml/min. Experiments were performed at room temperature. Neurons were visualized using infrared differential interference contrast microscopy with a 60× objective. Patch pipettes were pulled from borosilicate glass capillaries with resistances of 2–4 MΩ. Cells with a series resistance larger than 20 MΩ were discarded. Data were obtained with an Axopatch 700B (Axon Instruments, Union City, CA, USA), filtered at 2 kHz, digitized at 10 kHz, and acquired and analyzed with pClamp10 (Axon Instruments).

Current-clamp was used to record spiking activity as described (Bradaia *et al*, 2009; Revel *et al*, 2011). The pipette solution contained (in mM): potassium gluconate 130, $MgCl_2$ 4, EGTA 1.1, HEPES 5, Na_2ATP 3.4, sodium creatine-phosphate 10, and Na_3GTP 0.1. In the VTA, DA cells were identified by their large hyperpolarization-activated current (I_h) evoked by hyperpolarizing pulses (from −50 to −120 mV) and an outward current in response to quinpirole (10 μM). GABAergic neurons were identified by the lack of I_h current signature. In the DRN, 5-HT cells were identified by their large I_h current in response to ipsapirone (100 nM). In the LC, NA neurons were identified by their response to UK14,304, followed by a block with yohimbine. All cells used for the statistical analysis displayed a stable firing activity for at least 30 min.

The miniature post-synaptic currents (mPSCs) were measured by whole-cell voltage-clamp as reported (Bradaia *et al*, 2005). The pipette solution contained (in mM): CsCl 130, HEPES 10, $MgCl_2$ 1, EGTA 10, Na_2ATP 2, Na_3GTP 0.3, and pH 7.3. Cells were held at −60 mV. For recording of the miniature excitatory post-synaptic currents (mEPSCs), slices were bathed in aCSF containing 0.5 μM tetrodotoxin and 10 μM bicuculline (control condition). For recording of the miniature inhibitory post-synaptic currents (mIPSCs), slices were bathed in aCSF containing 0.5 μM tetrodotoxin, 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and 30 μM (2*R*)-amino-5-phosphonopentanoic acid (AP5; control condition). The mPSCs were recorded successively under control conditions, then in presence of RO5166017 (500 nM), of RO5166017 with EPPTB (10 nM), and of RO5166017 with EPPTB plus either bicuculline (mEPSCs) or CNQX and AP5 (mIPSCs). For each condition, recording was made for 10 min starting 10 min after application of the solution. The mPSCs were analyzed using the Minianalysis program (Synaptosoft). The parameters for the detection of miniature events were modified from the default settings for GABAergic and glutamatergic events (threshold 15–20 pA). Parameters were kept consistent for a given cell. A segment of 10 min was recorded and analyzed for each experimental condition. The automatic mPSC detection was then performed for each segment and subsequently corrected manually for false positives and false negative events. The frequency of mPSCs, inter-event intervals, and mPSC amplitudes were then determined with Minianalysis.

In vivo Microdialysis

Microdialysis studies were performed as published (Calcagno and Invernizzi, 2010) and detailed in the Supplementary Methods available online. Briefly, microdialysis probes were surgically implanted into the mPFC or accumbens nucleus (Acb) of 3–5-months-old male WT and Tg mice. Following recovery, the probes were perfused with aCSF and consecutive 30-min samples of dialysate were collected from awake, freely moving mice. The animals were then treated intraperitoneally (i.p.) with *d*-amphetamine (2.5 mg/kg) and dialysate samples were collected for further 2.5 h. The dialysate samples were analyzed subsequently for DA, NA, and 5-HT concentrations by HPLC methods.

Measurement of Locomotor Activity

Locomotor activity (LMA) was assessed as the total distance (in cm) traveled by each animal as previously described (Lindemann *et al*, 2008; Revel *et al*, 2011). In the first experiment, adult *Taar1* Tg mice and WT littermates ($n = 23$–24/group) were tested using a pseudo-Latin squares design with 1 week between the two test sessions. The mice were placed into the activity monitor chamber for 30 min (habituation period), injected i.p. with saline (0.9% NaCl) or *d*-amphetamine (2.5 mg/kg in saline), and immediately returned to the chamber for recording of the LMA during 90 min. In the second experiment, *Taar1* Tg and WT mice ($n = 21$–24/group) were dosed orally (*per os*, p.o.) with either vehicle (H_2O + 0.3% Tween 80) or RO5073012 (10 mg/kg in vehicle) 30 min before *d*-amphetamine injection, at the beginning of the habituation period.

Pharmacological Studies

The cAMP and radioligand binding assays were performed as described (Revel *et al*, 2011), using [^{3}H]RO5166017 (mouse and rat TAAR1) or [^{3}H]RO5192022 (monkey and human TAAR1) as TAAR1 radioligands at a concentration equal to K_d. The selectivity of RO5073012 was determined from radioligand binding assays (CEREP, Paris, France; www.cerep.fr). Pharmacokinetics studies in male C57BL/6J mice and Wistar rats ($n = 2$/group) were made with standard protocols as published (Revel *et al*, 2011) and detailed in the Supplementary Methods available online.

Statistical Analyses

All data represent the mean ± SEM. LMA data were analyzed using the Student *t*-test, one- or two-way analysis of variance or a linear mixed-effect model, as appropriate, followed by Dunnett's or Tukey's *post-hoc* analyses. Electrophysiological data were analyzed with the Kolmogorov–Smirnoff test. Microdialysis data were analyzed with the Student's *t*-test or one-way analysis of variance with repeated measures followed by Tukey's *post-hoc* comparisons. A *P* value of 0.05 was accepted as statistically significant.

RESULTS

Generation of *Taar1* Transgenic Mice

To explore the physiological roles of TAAR1, a mouse line with elevated *Taar1* brain-expression was engineered. For this, a *Thy-1.2* expression cassette was used to drive strong constitutive expression of *Taar1* in neurons of adult C57BL/6J mice, as previously documented (Caroni, 1997; Luthi *et al*, 1997; Richards *et al*, 2003; Ittner and Gotz, 2007). Two lines of transgenic mice, B6-Tg(Taar1)19 and B6-Tg(Taar1)27, were created. Quantitative PCR analyses from selected punched brain areas (data not shown) and *in situ* hybridization (see Supplementary Figure S1 online) confirmed generalized brain overexpression of *Taar1* in both lines, contrasting with the absence of detectable expression in the WT littermates. Depending on brain structures, *Taar1* mRNA level was 3- to 4.5-fold higher in the brain of B6-Tg(Taar1)19 mice as compared with B6-Tg(Taar1)27 mice, presumably due to position and/or number of transgene insertion (Caroni, 1997; Ittner and Gotz, 2007). The mice with the least elevated *Taar1* expression (B6-Tg(Taar1)27 line) were selected for further investigations and subsequently designated as *Taar1* transgenic (Tg) mice.

The comparison of WT and *Taar1* Tg mice did not reveal significant differences regarding their general state of health, viability, fertility, life span, nest building behavior, body weight, body temperature, and general motor functions and behavior (see Supplementary Figure S2 online). In females only, minor but statistically significant differences were seen for body weight (WT: 19.9 ± 0.3 g; Tg: 20.9 ± 0.2 g) and body temperature (WT: $37.3 \pm 0.1^{\circ}$C; Tg: $36.9 \pm 0.1^{\circ}$C). These observations show that under standard laboratory conditions neither ectopic nor elevated *Taar1* expression in the brain is sufficient to cause overt abnormalities.

Monoaminergic Neurons of *Taar1* Tg Mice have Increased Electrical Activity

In $Taar1^{-/-}$ mice, loss of functional TAAR1 causes an elevation of the spontaneous firing frequency in DA neurons of the VTA and in 5-HT neurons of the DRN, two regions where *Taar1* is normally expressed (Lindemann *et al*, 2008; Revel *et al*, 2011). Accordingly, we examined whether increased *Taar1* expression in Tg mice produces an opposite phenotype, ie lowering of the firing frequency. Surprisingly, the spontaneous firing rates of VTA DA neurons and DRN 5-HT neurons measured *ex vivo* were both augmented in Tg mice as compared with WT littermates (Figure 1a–d), similar to the situation observed in $Taar1^{-/-}$ mice. This increase was TAAR1-dependent, as bath application of either *p*-tyramine (see Supplementary Figure S3a–d online) or the specific TAAR1 agonist RO5166017 (Figure 1a–d) reversibly inhibited the high firing frequency of Tg mice.

The LC is an area devoid of detectable *Taar1* expression in the mouse (Lindemann *et al*, 2008). Accordingly, the firing activity of LC NA neurons is not altered by the lack of *Taar1* or by application of TAAR1 agonists (Revel *et al*, 2011). Here, we observed in Tg mice that ectopic expression of *Taar1* caused LC NA neurons to increase their firing activity (Figure 1e and f). As in the VTA and DRN, this augmentation was reversibly abolished by application of either *p*-tyramine (see Supplementary Figure S3e and f online) or RO5166017 (Figure 1e and f), supporting TAAR1-mediated effects. Altogether, these data show that the VTA, DRN, and LC monoaminergic neurons of *Taar1* Tg mice have increased spontaneous firing activity. Ectopically expressed TAAR1 is functional and sufficient to increase the firing rate of LC NA neurons and to confer sensitivity to TAAR1 agonists.

Increased Activity of VTA DA Neurons in *Taar1* Tg Mice Results from Reduced GABAergic Inhibitory Input

In the VTA, TAAR1 reduces the firing rate of DA neurons by activating K^+ channels (Bradaia *et al*, 2009). Accordingly, increased TAAR1 expression in DA neurons of *Taar1* Tg mice was expected to enhance K^+ channel activation and dampen the firing rate. Instead, DA neurons displayed elevated firing activity, suggesting that changes in electrical inputs over-stimulate their electrical activity. Within the VTA, a significant population of GABA neurons regulate DA neurons (Marinelli *et al*, 2006). Accordingly, we examined in mutant animals whether such GABA neurons have altered electrical activity due to the ectopic expression of *Taar1*. Patch-clamp recordings revealed a reduced spontaneous firing rate for these neurons in *Taar1* Tg mice (Figure 2a–d), suggesting decreased inhibitory inputs to VTA DA neurons. Application of the TAAR1 agonist RO5166017 further dampened the firing activity of VTA GABA neurons (Figure 2c and d), whereas subsequent application of the TAAR1 antagonist EPPTB completely reversed this effect and raised the firing rate to basal levels seen in control WT mice. This suggests that TAAR1 ectopically expressed in VTA GABA neurons is functional and either constitutively active or tonically activated by ambient levels of endogenous agonist(s). In the brain slices from WT animals, neither RO5166017 nor EPPTB affected the firing activity of VTA GABA neurons (Figure 2a and b), indicating that TAAR1 is normally absent from these neurons, or present at negligible levels.

Consistent with these recordings, we further observed that the frequency of mIPSCs was reduced in VTA DA neurons of *Taar1* Tg mice, as compared with WT mice (Figure 3a and b). Application of RO5166017 further decreased this frequency, whereas subsequent addition of EPPTB increased it above basal levels. In WT animals, mIPSC frequency was not affected by any of the TAAR1 ligands. Importantly, the amplitude of mIPSCs did not differ between WT and *Taar1* Tg mice and was not altered by TAAR1 ligands (Figure 3c), indicating that post-synaptic GABA receptors were unaffected. Thus, mIPSC frequency paralleled the firing rate of VTA GABA neurons (Figure 2d), supporting the role of these neurons in altering DA neuronal activity in *Taar1* Tg mice. These observations suggest that in mutant animals, ectopically expressed TAAR1 depresses the electrical activity of VTA GABA neurons. This presumably removes an inhibitory input to VTA DA neurons and contributes to elevate their firing activity under basal conditions.

Recording of the mEPSCs also showed that, similar to mIPSCs, mEPSCs frequency was reduced in *Taar1* Tg mice as compared with WT animals (Figure 3d and e). In the

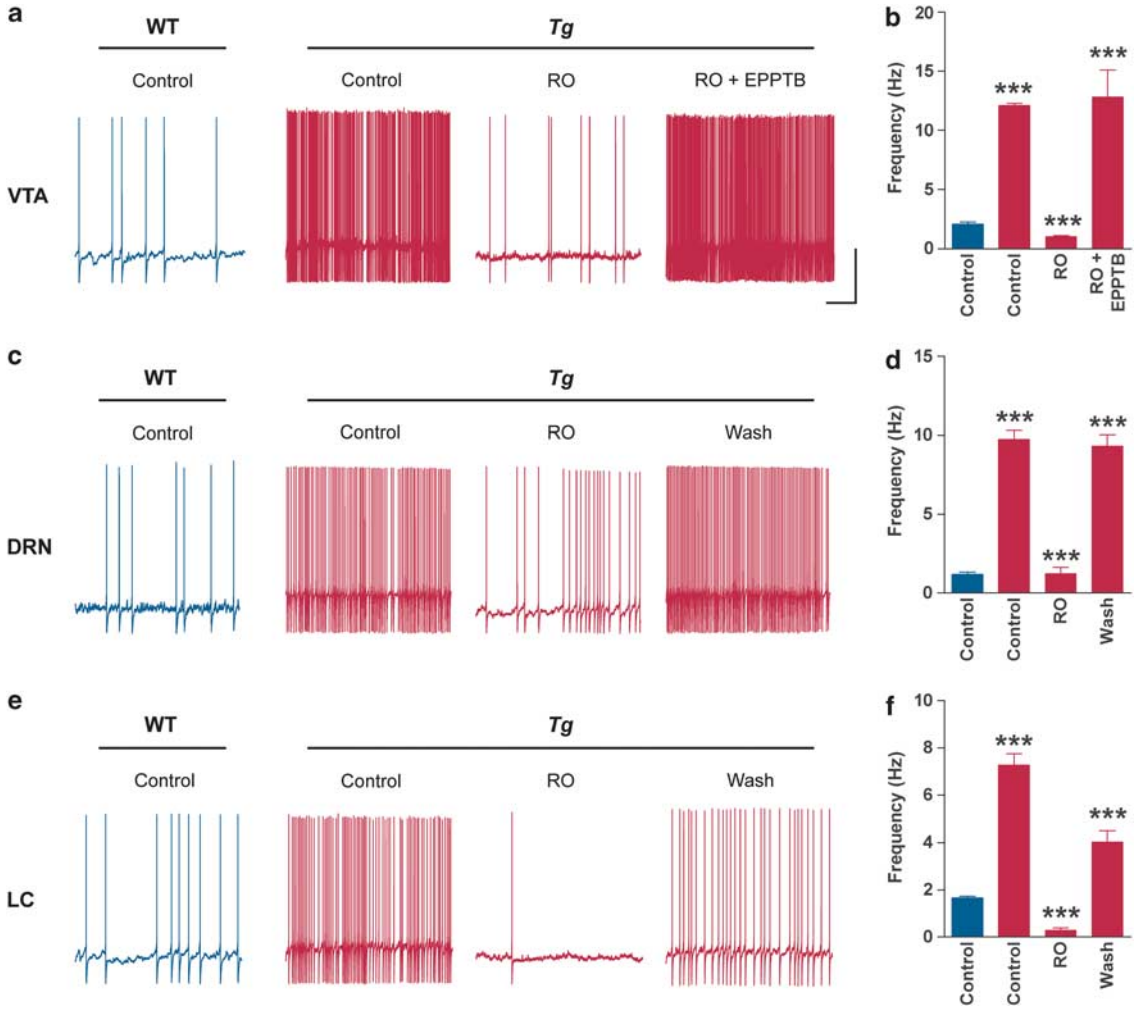

Figure 1 The spontaneous firing rate of dopaminergic, serotonergic, and noradrenergic neurons is increased in *Taar1* transgenic mice. (a) Representative current-clamp recordings from dopaminergic neurons of the ventral tegmental area (VTA), (c) serotonergic neurons of the dorsal raphe nucleus (DRN), and (e) noradrenergic neurons of the locus coeruleus (LC) in brain slices from either *Taar1* transgenic mice (Tg) or wild-type littermates (WT), together with respective quantification bar graphs (b, d, and f) (blue bars, WT; red bars, *Taar1* Tg mice). Under basal conditions (Control), spontaneous firing frequencies were higher in *Taar1* Tg mice as compared with WT animals. Application of the TAAR1 agonist RO5166017 (500 nM; RO) dampened the firing rates, an effect that was reversed either by co-application of the TAAR1 antagonist EPPTB (10 nM; RO + EPPTB) in the VTA (a and b) or upon washout (Wash) in the DRN and LC (c–f). Bar = 20 mV/1 s. Data represent the mean ± SEM ($n = 5$ neurons from three animals per condition). ***$P < 0.001$ *vs* preceding condition (Kolmogoroff–Smirnov test).

mutant animals, it was further reduced by RO5166017, and subsequent application of EPPTB blocked this effect. Interestingly, RO5166017 also decreased mEPSC frequency in WT mice, an effect reversed by addition of EPPTB. This reveals that in WT animals, TAAR1 is normally present in excitatory neurons connecting to VTA DA neurons, and that its activation reduces excitatory inputs to DA neurons. The mean amplitude of the mEPSC was altered neither by the genotype nor by the treatment (Figure 3f), showing that post-synaptic receptors were unaffected. Taken together, these experiments indicate that in WT animals, TAAR1 normally modulates excitatory inputs to VTA DA neurons, whereas in *Taar1* Tg animals it alters both excitatory and inhibitory DA inputs. In mutant animals, the weakening of inhibitory inputs from the GABA neurons appears to predominate over the reduction of excitatory inputs and the potentially enhanced K^+ channels activation in DA neurons, causing the VTA DA neurons to fire at a higher rate.

Taar1 Tg Mice have Elevated Basal Release of Monoamines

Monoaminergic neurons in the VTA, DRN, and LC project widely to corticolimbic brain structures where they regulate a variety of critical functions. Because the spontaneous firing activities of VTA DA, DRN 5-HT, and LC NA neurons are increased in *Taar1* Tg mice, we examined whether DA, 5-HT, and NA release was augmented concurrently. Using *in vivo* microdialysis, we observed that basal NA and DA extracellular levels were significantly elevated in the Acb of *Taar1* Tg mice (Figure 4a). NA and DA basal levels in Tg mice represented 272 and 180% of the basal values in WT, respectively. Extracellular 5-HT level increased non-significantly in the Acb (Figure 4a), but raised significantly in the medial prefrontal cortex (mPFC) where it reached 190% of the basal values measured in WT littermates (Figure 4b). By contrast, the extracellular level of glutamate in the mPFC was unaffected (Figure 4b). These data show that in *Taar1*

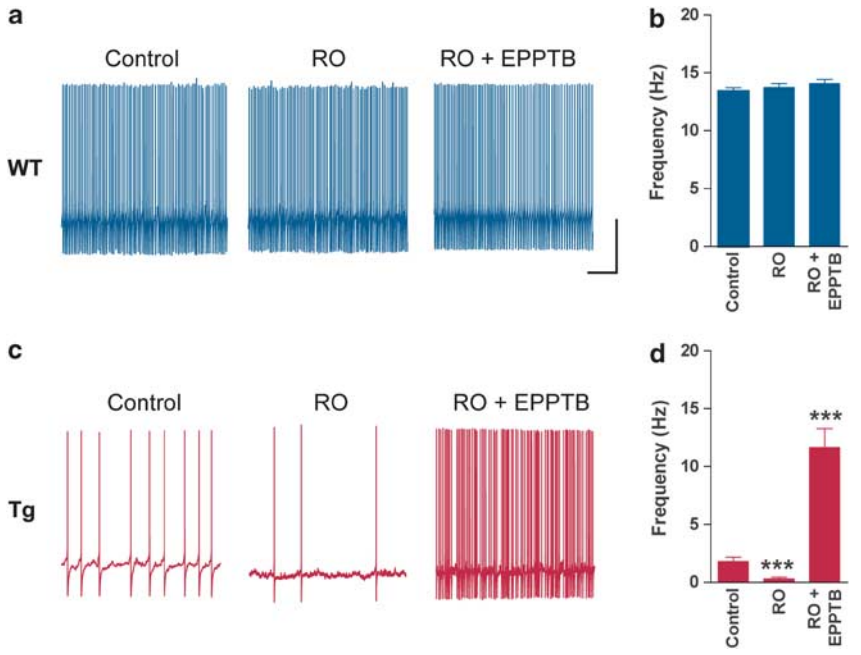

Figure 2 The spontaneous firing rate of ventral tegmental GABAergic neurons is reduced in *Taar1* transgenic mice. (a–d) Current-clamp recordings of GABA neurons in the ventral tegmental area (VTA) of (a and b) wild-type animals (WT) and (c and d) *Taar1* transgenic mice (*Tg*). Shown are representative traces (a and c) and corresponding quantification graphs (b and d). In WT, neither the TAAR1 agonist RO5166017 (500 nM; RO) nor the TAAR1 antagonist EPPTB (10 nM; RO + EPPTB) affected the spontaneous firing frequency (a and b). In *Taar1* Tg mice, the spontaneous firing rate was lower than in WT animals (c and d), and was further reduced by application of RO5166017. Co-application of EPPTB blocked this inhibition and raised the firing rate above control levels. Bars = 20 mV/1 s. Data represent the mean ± SEM ($n = 5$ neurons from three animals per condition). ***$P < 0.001$ *vs* preceding condition (Kolmogoroff–Smirnov test).

Tg mice, the elevated spontaneous firing activity of monoaminergic nuclei translates into higher monoamine release, an effect that may be enhanced by an increase of the TAAR1-promoted DA and NA efflux through DAT and NET, respectively (Xie and Miller, 2007, 2008, 2009; Xie *et al*, 2008; Miller, 2011). This contrasts with *Taar1*$^{-/-}$ mice, where the elevated spontaneous firing rate of VTA DA and DRN 5-HT neurons does not convert into higher striatal release of monoamines under basal conditions (Lindemann *et al*, 2008; Bradaia *et al*, 2009; Revel *et al*, 2011). Furthermore, these observations support the idea that *in vivo* TAAR1 is either constitutively active and/or tonically activated by endogenous agonist(s), presumably TAs and possibly also DA, a weak TAAR1 partial agonist (Borowsky *et al*, 2001; Lindemann *et al*, 2005), with increased levels in *Taar1* Tg mice.

In *Taar1* Tg mice, enhanced extracellular levels of monoamines under basal conditions may trigger desensitization of the receptors and transporters. We examined this possibility and found no genotype difference in the expression (see Supplementary Figure S4 online) or density (see Supplementary Figure S5 online) of D_1 or D_2-like DA receptors, as measured by quantitative PCR or autoradiographic analysis, respectively. Similarly, the gene expression of the 5-HT and NA transporters did not differ between genotypes, and although the expression of the gene encoding the DAT increased slightly in *Taar1* Tg mice (see Supplementary Figure S4 online), the autoradiographic density of the DAT remained comparable to that in WT mice (see Supplementary Figure S5 online). Moreover, there were no significant gene expression differences for tyrosine hydroxylase in the VTA and tryptophan hydroxylase 2 in the DRN, the rate-limiting enzymes for the synthesis of DA and 5-HT, respectively (see Supplementary Figure S5 online). Thus, enhanced monoaminergic neurotransmission in *Taar1* Tg mice did not appear to alter significantly either the synthesis of monoamines or the amount of receptors and transporters.

Taar1 Tg Mice are Hyposensitive to the Effects of Amphetamine

Considering that the monoaminergic systems in *Taar1* Tg mice exhibit increased activity under basal conditions, we examined whether a behavioral output such as LMA is increased concomitantly. Spontaneous LMA did not differ significantly from that of WT mice (Figure 5a and b), which is similar to *Taar1*$^{-/-}$ mice whose spontaneous LMA level is unaltered (Lindemann *et al*, 2008). Such knockout mice, however, are hypersensitive to *d*-amphetamine and display a greater LMA response as compared with WT littermates (Lindemann *et al*, 2008). Here, we observed that *Taar1* Tg mice reacted to *d*-amphetamine in an opposite manner and showed a reduced LMA response in comparison to WT littermates (Figure 5a and b). The onset of the LMA response was delayed by approximately 30 min, lasted approximately half the time of the WT response (Figure 5a), and its amplitude was only 34% of that in WT littermates (Figure 5b). These observations indicate that *Taar1* Tg mice are hyposensitive to *d*-amphetamine.

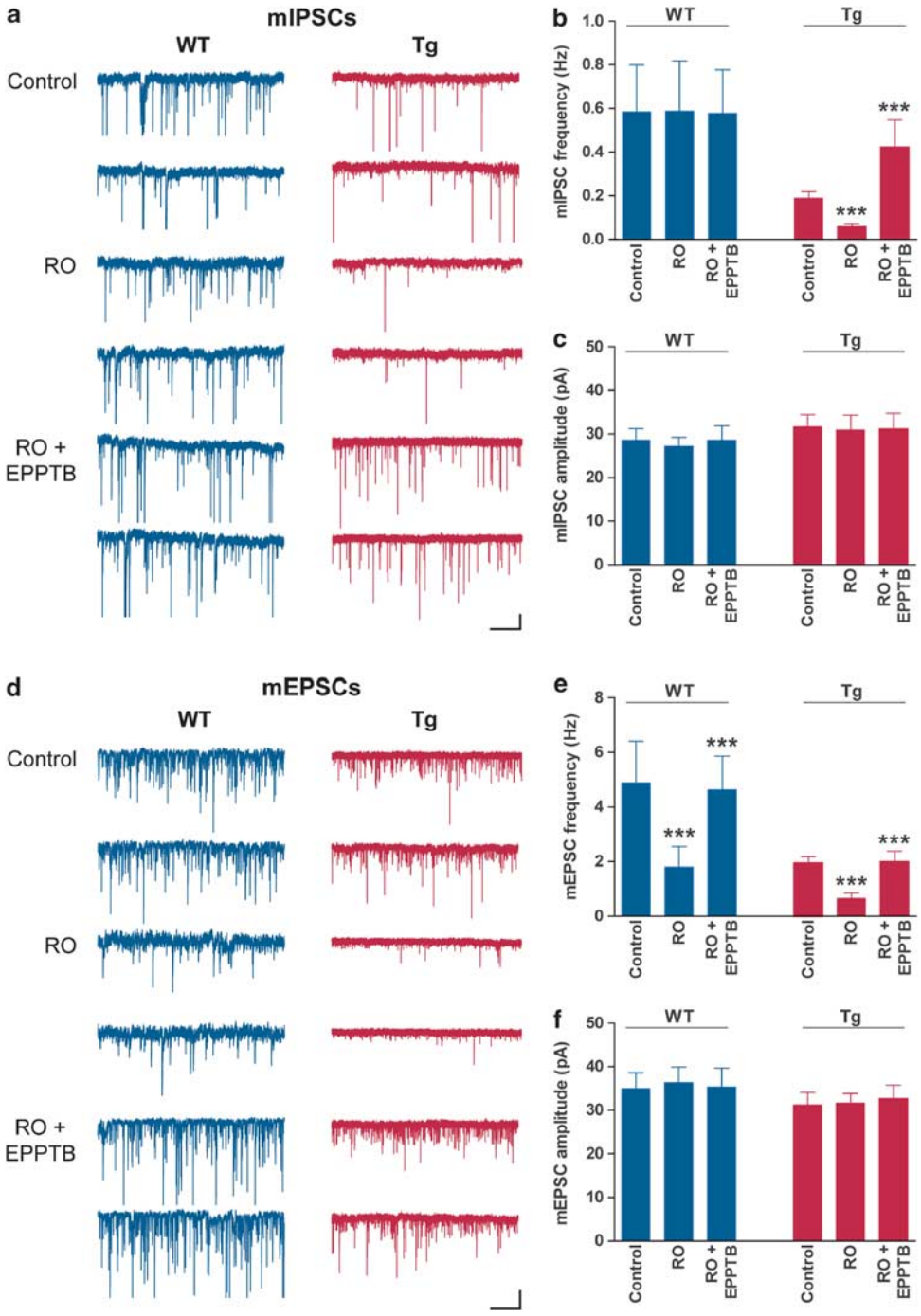

Figure 3 Excitatory and inhibitory inputs to ventral tegmental dopaminergic neurons are altered in *Taar1* transgenic mice. Miniature inhibitory (a–c) or excitatory (d–f) post-synaptic currents (mIPSCs or mEPSCs, respectively) were recorded in the ventral tegmental dopaminergic neurons of WT and *Taar1* Tg mice. Shown are representative consecutive traces (a and d) and quantifications of the post-synaptic current frequencies (b and e) and amplitudes (c and f). In *Taar1* Tg mice, mIPSC and mEPSC frequencies were reduced as compared with those in WT mice, and were further decreased by application of RO5166017 (500 nM; RO). Co-application of EPPTB (10 nM; RO + EPPTB) reversed the effect of RO5166017 and even raised mIPSC frequency above control levels. In WT mice, mEPSC but not mIPSC frequency was significantly reduced by RO5166017, which was fully reversed by co-application of EPPTB. The amplitudes of the mIPSCs and mEPSCs did not differ between WT and *Taar1* Tg mice, and were not affected by any treatments. Bars = 10 pA/5 s. Data represent the mean ± SEM ($n = 5$ neurons from three animals per condition). ***$P < 0.001$ *vs* preceding condition (Kolmogoroff–Smimov test).

Amphetamine Sensitivity is Restored by the TAAR1 Partial Agonist RO5073012

To determine whether such hyposensitivity is fully mediated by TAAR1, we examined whether pharmacological reduction of TAAR1 activity restores the response to *d*-amphetamine. Unfortunately, the specific TAAR1 antagonist EPPTB (Bradaia *et al*, 2009) could not be used owing to its poor pharmacokinetic properties, in particular its rapid clearance. To compensate for this deficiency, we developed the TAAR1

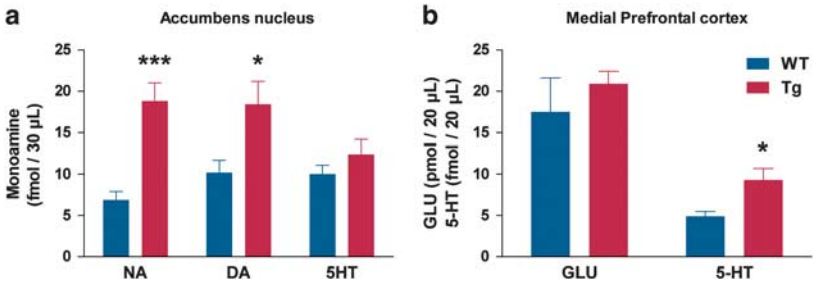

Figure 4 Basal monoamine levels are augmented in *Taar1* transgenic mice. (a) The basal extracellular concentrations of noradrenaline (NA), dopamine (DA) and serotonin (5-HT) in the accumbens nucleus and (b) of glutamate (GLU) and 5-HT in the medial prefrontal cortex were measured by means of *in vivo* microdialysis in wild-type (WT) or *Taar1* transgenic (Tg) mice. The basal levels of NA and DA in the accumbens nucleus and that of 5-HT in the medial prefrontal cortex were significantly higher in *Taar1* Tg mice as compared with WT. Data represent the mean ± SEM ($n = 7$–8). $^{*}P < 0.05$, $^{***}P < 0.001$ *vs* WT (Student's *t*-test).

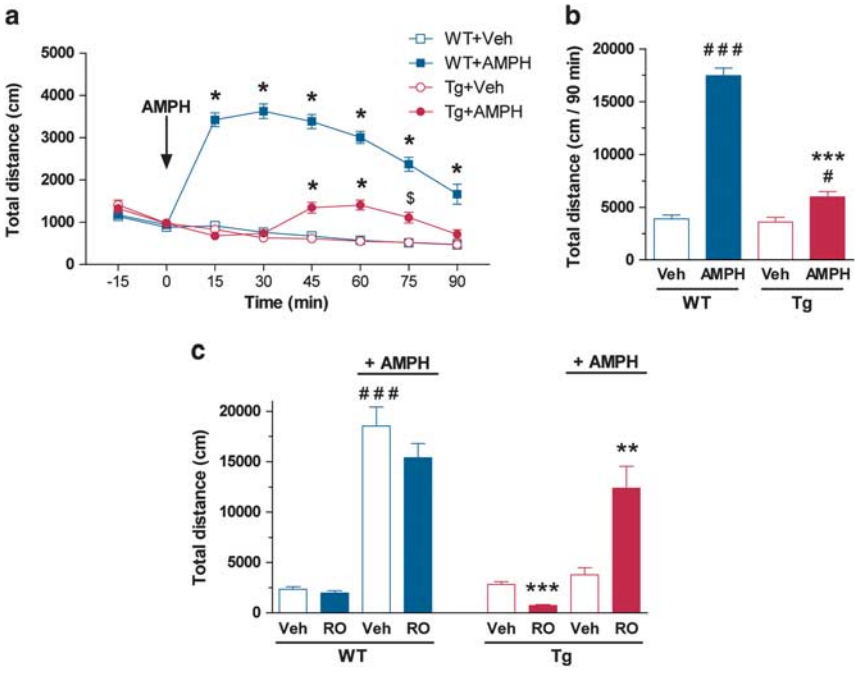

Figure 5 *Taar1* transgenic mice show reduced sensitivity to the locomotor stimulating effects of *d*-amphetamine, which is restored by the TAAR1 partial agonist RO5073012. (a) *Taar1* transgenic mice (Tg) exhibited a reduced and delayed response to *d*-amphetamine (2.5 mg/kg, i.p. AMPH) as compared with wild-type littermates (WT), whereas WT and Tg mice treated with vehicle (Veh) showed similar levels of locomotor activity. The arrow indicates the time of injection. $^{*}P < 0.001$, $^{\$}P < 0.01$ *vs* all other groups (linear mixed-effect model, followed by Tukey's tests). (b) Locomotor activity cumulated for the 90 min following AMPH treatment. $^{###}P < 0.001$, $^{\#}P < 0.05$ *vs* WT/Veh; $^{***}P < 0.001$ *vs* WT/AMPH (two-way analysis of variance: effect of genotype, $F_{(1,90)} = 121.5$, $P < 0.001$; effect of AMPH, $F_{(1,90)} = 222.6$, $P < 0.001$; Interaction, $F_{(1,90)} = 109.8$, $P < 0.001$; followed by Tukey's *post-hoc* test; $n = 23$–24/group). (c) RO5073012 (10 mg/kg, p.o.) did not significantly affect the locomotor activity of WT mice treated with saline or AMPH. In Tg mice, however, RO restored sensitivity to AMPH while depressing the locomotor activity of saline-treated animals. $^{###}P < 0.001$ *vs* WT/saline/Veh; $^{***}P < 0.001$ *vs* Tg/saline/Veh; $^{**}P < 0.001$ *vs* Tg/AMPH/Veh (Mixed-effects model. Significant interaction of genotype, RO and AMPH, $F_{(1,127)} = 33.14$, $P < 0.001$. WT mice only: significant effect of AMPH: $F_{(1,60)} = 178.71$, $P < 0.001$ ($^{###}$). No significant effect of RO: $F_{(1,60)} = 0.91$, $P = 0.345$. Tg mice only: significant interaction of RO with AMPH: $F_{(1,67)} = 48.67$, $P < 0.001$. Tg mice with saline: effect of RO, $F_{(1,22)} = 80.7$, $^{***}P < 0.001$. Tg mice with AMPH: Effect of RO, $F_{(1,22)} = 8.93$, $^{**}P < 0.01$. $n = 21$–24/group. Data represent the mean ± SEM.

partial agonist RO5073012 ((4-chloro-phenyl)-(3H-imidazol-4-ylmethyl)-isopropyl-amine; see Supplementary Figure S6 online) identified in a medicinal chemistry program directed towards TAAR1-selective compounds (Revel *et al*, 2011). RO5073012 possesses high affinity at mouse, rat, cynomolgus monkey, and human TAAR1 stably expressed in HEK293 cells (Table 1), and potently activates cAMP production in all species, but with very partial efficacy (24–43%) as compared with β-phenylethylamine. Furthermore, RO5073012 shows high selectivity for TAAR1, as evaluated from radioligand binding assays consisting of 98 target proteins (see Supplementary Table S1 online; ⩾150-fold selectivity of mouse TAAR1 K_i *vs* target K_i). Thus, RO5073012 is a highly potent and selective TAAR1 agonist with low intrinsic efficacy, and, as such, is well suited for investigations aiming at reducing TAAR1 activity in mice.

Table 1 Binding Affinities and EC_{50} Values of RO5073012 at Rodent and Primate TAAR1

Parameter, assay, preparation	Mouse	Rat	Human	Monkey
K_i, binding, HEK293 cells[a]	3.2 ± 1.1	1.1 ± 0.2	5.8 ± 1.9	0.5 ± 0.3
EC_{50}, cAMP, HEK293 cells[b]	23 ± 21 (26 ± 5%)	25 ± 24 (24 ± 8%)	25 ± 18 (34 ± 4%)	8.8 ± 0.1 (43 ± 1%)

Values are given as nM and represent the mean ± SEM obtained from at least three independent experiments. Values in parentheses represent the maximal efficacy relative to β-phenylethylamine.
[a]Radioligand [^{3}H]RO5166017 for mouse and rat TAAR1, [^{3}H]RO5192022 for human and cynomolgus monkey TAAR1.
[b]Upstate (Millipore) immunoassay for cAMP.

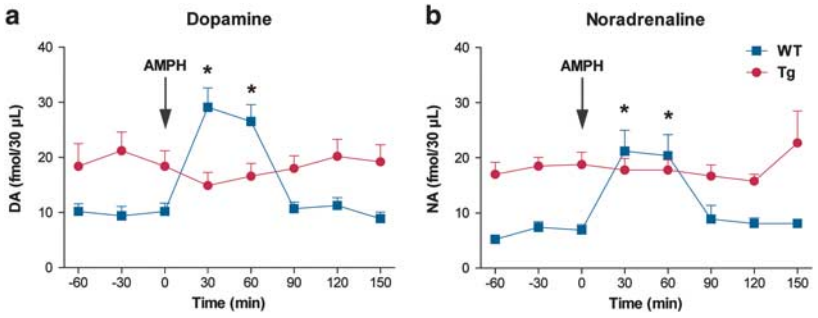

Figure 6 *d*-Amphetamine does not alter the release of dopamine and noradrenaline in the accumbens nucleus of *Taar1* transgenic mice. The extracellular concentrations of (a) dopamine and (b) noradrenaline were monitored in conscious animals by *in vivo* microdialysis before and after injection of *d*-amphetamine (2.5 mg/kg, i.p. AMPH). AMPH triggered a transient release of dopamine and noradrenaline in wild-type mice (WT) but not in *Taar1* transgenic mice (Tg) where monoamine extracellular concentrations remained at constantly elevated levels. Arrows indicate the time of injection. Data represent the mean ± SEM ($n = 6$–8/group). *$P < 0.05$ *vs* time 0 in WT (one-way analysis of variance with repeated measures, followed by Tukey's *post-hoc* test).

In vivo, RO5073012 displayed favorable pharmacokinetic properties (see Supplementary Table S2 online). In WT mice, RO5073012 used at a dose of 10 mg/kg (p.o.) did not affect the LMA of control mice, nor did it significantly prevent *d*-amphetamine from increasing LMA (Figure 5c, left panel). By contrast, RO5073012 significantly reduced basal LMA in *Taar1* Tg mice (Figure 5c, right panel). Furthermore, *Taar1* Tg mice treated with RO5073012 exhibited hyperlocomotion in response to *d*-amphetamine, in contrast to the mice that received vehicle only. This shows that in Tg mice, selective reduction of TAAR1 activity by RO5073012 restores the sensitivity to *d*-amphetamine.

Amphetamine does not Alter Monoamine Release in *Taar1* Tg Mice

To understand why *d*-amphetamine fails to trigger hyperlocomotion in *Taar1* Tg mice, we examined how it affects the release of DA and NA. Following a *d*-amphetamine challenge, the extracellular levels of DA and NA increased transiently, but markedly, in the Acb of WT mice (Figure 6). By contrast, *d*-amphetamine did not alter DA and NA extracellular concentrations in the Acb of *Taar1* Tg mice, which remained at constant levels throughout the experiment (Figure 6). This response diverges from that of *Taar1*$^{-/-}$ mice, which show exacerbated striatal release of DA and NA in response to *d*-amphetamine (Lindemann *et al*, 2008). The release of 5-HT in the Acb of *Taar1* Tg mice and WT did not differ, as both genotypes maintained constant levels following *d*-amphetamine injection (data not shown). Altogether, these data show that *Taar1* Tg mice are hyporesponsive to *d*-amphetamine at least partly because of their inability to increase limbic DA and NA release from basal levels in response to the psychostimulant.

DISCUSSION

Identified in 2001, TAAR1 has rapidly emerged as an important regulator of monoaminergic activity and, as such, a potential target for the treatment of mental disorders like schizophrenia and depression (Berry, 2007; Miller, 2011; Revel *et al*, 2011). A key finding has been that mutant mice lacking the *Taar1* gene are hypersensitive to the psychostimulant *d*-amphetamine, as evidenced by increased LMA response and striatal release of monoamines following an acute challenge (Wolinsky *et al*, 2007; Lindemann *et al*, 2008). To complement these observations, we generated a line of transgenic mice that overexpress *Taar1* in the brain. In contrast to *Taar1*$^{-/-}$ mice, we reveal that such animals are hyposensitive to *d*-amphetamine.

Ectopically Expressed TAAR1 is Functional and Tonically Activated

In the Tg mice reported here, expression of *Taar1* was controlled by the *Thy-1.2* expression cassette. Such genetic construction is well characterized and enables strong, constitutive expression of the transgene specifically in the brain (Caroni, 1997; Luthi *et al*, 1997; Richards *et al*,

2003). The onset of expression occurs approximately 6–7 days after birth, which avoids interference with early brain development and enables examination of transgene effects only during the late development and adulthood. Expression is then stable (ie constitutive) throughout adulthood. In the brain, *Thy-1.2*-driven expression is restricted to neurons, and outside the central nervous system, weak expression has been reported in the lung only (Caroni, 1997). The strong context sensitivity of the expression cassette results in expression patterns that depend on the site of insertion. Accordingly, we generated two lines of mice that had comparable spatial expression patterns but distinct degrees of expression levels, with higher levels of *Taar1* expression observed in the B6-Tg(Taar1)19 mice.

The *Taar1* Tg mice comprise some limits that should be considered. Overexpression is not limited to the sole neuron population normally expressing *Taar1* in WT. Accordingly, part of the effects observed in Tg mice may be due to the ectopic expression of the receptor and not solely to higher expression levels. Ectopically expressed TAAR1 appears to be functional, as exemplified by the NA neurons of the LC and the GABA neurons of the VTA that in Tg mice gain sensitivity to TAAR1 ligands. Importantly, the higher spontaneous firing rate of NA neurons in the LC, a structure that does not seem to express functional TAAR1 in WT animals (Lindemann *et al*, 2008; Revel *et al*, 2011), and the higher firing rate of VTA GABA neurons in presence of the TAAR1 antagonist EPPTB are two observations that support the possibility that TAAR1 is constitutively active and/or that endogenous agonist(s) are present to activate the receptor.

As seen from radioactive *in situ* hybridization, we observed that *Taar1* mRNA levels in the brain are under detection levels in WT mice, showing that, at least in the brain *Taar1* is expressed at low levels. This is in agreement with similar attempts in rats (Bunzow *et al*, 2001; Grandy, 2007), and justifies the use of an indirect method based on a reporter gene to map *Taar1* expression (Lindemann *et al*, 2008). However, these data contrast with an earlier report of *Taar1* expression in several brain regions, including the VTA, substantia nigra, DRN, and LC using non-radioactive *in situ* hybridization in rat (Borowsky *et al*, 2001). These data also contrast with findings that TAAR1 interacts functionally with the NA transporter in thalamic synaptosomes, suggesting that *Taar1* is expressed by the NA cells of the LC (Xie *et al*, 2008). Such discrepancies may result from species and/or methodological differences (eg, radioactive *vs* non-radioactive detection) and illustrate the challenges of detecting *Taar1* expression reliably. It will be important that future experiments demonstrating *Taar1* expression contain proper controls, such as knock-out animals as a negative control. Along this line, the *Taar1* Tg mice also represent a suitable positive control, as we were able to detect *Taar1* mRNA using both quantitative PCR and *in situ* hybridization. This observation comes in support and validation of the absence of *Taar1* detection in WT animals. Similarly, the strong and generalized expression of *Taar1* in neurons of the Tg mice may prove useful in a variety of biochemical experiments (eg, western blotting), as a large amount of the receptor can be obtained easily as compared with WT animals.

Increased Monoaminergic Neurotransmission in *Taar1* Tg Mice does not Translate into Overt Behavioral Abnormalities

Despite the generalized brain overexpression of *Taar1*, the Tg mice did not show overt behavioral abnormalities under basal conditions and appeared similar to WT animals, although we do not exclude that physical functions that have not been examined (eg, olfaction, social behaviors) might be altered. This is in line with the *Taar1*$^{-/-}$ mice, which do not show an overt phenotype under basal conditions (Wolinsky *et al*, 2007; Lindemann *et al*, 2008) as well as with carnivores, where *Taar1* is a pseudogene and naturally non-functional (Vallender *et al*, 2010). Interestingly, this apparent behavioral normality of *Taar1* Tg mice contrasts with the elevated activity of the monoaminergic system under basal conditions, as observed in the electrophysiological recordings and microdialysis experiments. In particular, increased basal monoaminergic neurotransmission did not translate into significantly higher spontaneous LMA. A possible explanation could be that increased monoaminergic activity *in vivo* augments the levels of endogenous TAs, which may in turn exert inhibitory feedback. Alternatively, ectopic expression of TAAR1 downstream of the striatal monoaminergic receptors may exert compensatory actions.

Although the elevated spontaneous firing frequency of VTA DA and DRN 5HT neurons is reminiscent of the phenotype observed in *Taar1*$^{-/-}$ mice (Lindemann *et al*, 2008), it has distinct origins. In the *Taar1* Tg mice, we showed in the VTA that this high firing rate results from altered network activity, mainly because inhibitory GABA neurons are made less active by the ectopically expressed TAAR1. Similar effects may occur in inhibitory neurons upstream of DRN 5-HT and LC NA neurons, also resulting in increased firing frequency. Application of TAAR1 agonists, either RO5166017 or *p*-tyramine, reduced the firing frequency of monoaminergic neurons to, or even below WT control levels, which we believe reflects the direct inhibitory effect of TAAR1 activation on cell firing (Bradaia *et al*, 2009). Along this line, we observed that the TAAR1 agonists induced a hyperpolarization of the membrane potential in current-clamp recordings and an outward current in voltage-clamp recordings, while subsequent application of EPPTB elicited opposite effects (data not shown), consistent with earlier studies (Geracitano *et al*, 2004; Lindemann *et al*, 2008; Bradaia *et al*, 2009; Revel *et al*, 2011). These effects presumably reflect the TAAR1-mediated activation of inwardly rectifying K^+ channels characterized previously (Bradaia *et al*, 2009).

Taar1 Tg Mice are Hyposensitive to Amphetamine

Whereas *Taar1* Tg mice did not show overt behavioral abnormalities under basal conditions, a clear phenotype emerged upon acute amphetamine stimulation. Tg mice exhibited a blunted LMA response, correlated by a lack of catecholamine discharge in the Acb. Several possibilities may account for these observations.

Amphetamines are agonists at TAAR1 (Bunzow *et al*, 2001; Miller, 2011). Accordingly, in addition to its regular

effects in WT mice, *d*-amphetamine presumably activates exogenous TAAR1 in a range of structures, including monoaminergic components, the net result being that TAAR1 overexpression protects from *d*-amphetamine psychostimulant effects. Along this line, limiting TAAR1 activation by the partial agonist RO5073012 restored the ability of *d*-amphetamine to trigger LMA in *Taar1* Tg mice, whereas it decreased amphetamine-induced LMA non-significantly in WT mice.

Monoamine neuron firing and basal release are elevated in *Taar1* Tg mice, so that the pools of synaptic vesicles may be limited (Rizzoli and Betz, 2005). In this context, an additional strong stimulation may not be able to trigger massive monoamine release. Interestingly, *Taar1* Tg mice show a normal LMA response to cocaine (data not shown), a psychostimulant drug with a different mode-of-action (Torres *et al*, 2003). Cocaine increases monoamine synaptic concentrations essentially by competitive inhibition of their transporters, whereas amphetamines promote the release of monoamines into the extracellular space. As a result, amphetamine elevates extracellular DA levels 2–3 times more than cocaine at doses that elicit similar levels of LMA (Ramsey *et al*, 2008). Although the effects of amphetamine may be limited by the constitutive overactivity of monoaminergic neurons, the ability of cocaine to block monoamine transporters appears to remain intact, enabling monoamine concentration to rise above basal levels. This supports the hypothesis that *d*-amphetamine produces little psychostimulant effects in *Taar1* Tg mice because monoamine vesicular stores are depleted.

Finally, TAAR1 represses DA reuptake, favors DA efflux and possibly also promotes DAT internalization *in vitro* (Miller *et al*, 2005; Xie *et al*, 2007; Miller, 2011). In Tg mice, TAAR1 activation by amphetamine may be enhanced, causing a greater reduction of DA uptake. Considering that DAT blockade attenuates the ability of amphetamine to enhance extracellular DA in the Acb (Baumann *et al*, 1994), such reduction of DA uptake may also contribute to moderate the effect of amphetamine.

There exist a few other cases of mice hyposensitive to amphetamine. Mice heterozygous for the phosphate-activated glutaminase 1 (GLS1) gene (*GLS1 het* mice) do not respond to the behavioral stimulating effects of amphetamine, and similar to the *Taar1* Tg animals reported here, are devoid of behavioral abnormalities at baseline (Gaisler-Salomon *et al*, 2009). However, in contrast to *Taar1* Tg mice, *GLS1 het* mice show normal striatal DA release at baseline and blunted, but present amphetamine-induced increase in DA efflux. In the aphakia (*ak*) mice deficient in Pitx3, a transcription factor enriched in DA neurons, selective loss of DA neurons occurs in the substantia nigra while sparing the VTA (Hwang *et al*, 2003; Nunes *et al*, 2003; van den Munckhof *et al*, 2003). Such animals are hyperactive, and amphetamine does not increase, but rather suppresses, baseline LMA (Ardayfio *et al*, 2010). Finally, the β-arrestin 2 knock-out mice also show a strongly reduced responsiveness to amphetamine (Beaulieu *et al*, 2005), the profile and amplitude of which resembles that seen in *Taar1* Tg mice. This suggests the possibility that TAAR1 not only signals via $G\alpha_S$/cAMP but might also directly or indirectly interfere with the β-arrestin 2/Akt/GSK3 pathway.

In WT Animals, VTA DA Neurons Receive TAAR1-Expressing Excitatory Inputs

In *Taar1* Tg mice, examination of mIPSCs and mEPSCs in the DA neurons of the VTA confirmed that ectopically expressed TAAR1 alters both the inhibitory and excitatory inputs of these neurons, including those from VTA GABA neurons. In WT mice, however, these recordings revealed valuable information on the localization of TAAR1 endogenous expression. Application of the TAAR1 agonist RO5166017 did not alter mIPSC frequency, indicating that TAAR1 is not present in the pre-synaptic compartment of inhibitory inputs to DA neurons. Along this line, RO5166017 did not alter the firing frequency of VTA GABA neurons in WT mice. By contrast, the agonist inhibited mEPSC frequency, revealing that TAAR1 is present in the pre-synaptic compartment of excitatory inputs to DA neurons. This is consistent with, and participates to the reduction of firing of VTA DA neurons seen upon TAAR1 activation with agonists in WT animals (Revel *et al*, 2011). Whether such expression of *Taar1* in excitatory inputs to DA neurons occurs locally in the VTA or in neurons located distantly remains to be determined. The VTA contains a small population of glutamatergic neurons (Yamaguchi *et al*, 2007; Nair-Roberts *et al*, 2008), which establish local synapses on DA and on non-DA neurons (Dobi *et al*, 2010), in addition to distant connections with neurons in the Acb and prefrontal cortex (Yamaguchi *et al*, 2011). Such glutamatergic neurons, identified by detection of the mRNA encoding the vesicular glutamate transporter 2 (VGluT2), contain two subpopulations distinguished by the absence or presence of tyrosine hydroxylase (TH), and termed VGluT2-only and VGluT2-TH neurons, respectively (Yamaguchi *et al*, 2011). The VGluT2-only neurons form the major subpopulation and are present throughout the A10 region, whereas the VGluT2-TH neurons are fewer and reside only in the medial portion of the A10 region. Preliminary data indicate that, in the VTA, *Taar1* is expressed almost exclusively in tyrosine hydroxylase-expressing cells (data not shown), making it unlikely that TAAR1 is expressed in the glutamatergic neurons of this region.

Alternatively, the VTA receives excitatory inputs from multiple structures in the cortex, basal forebrain, and brainstem (Sesack and Carr, 2002; Geisler *et al*, 2007; Sesack and Grace, 2010). A fraction of these projections excite DA neurons directly, mainly through glutamate or acetylcholine release. These inputs to the VTA appear essential for the induction of the bursting activity of DA neurons and for the effects of drugs of abuse (Tzschentke, 2001; Harris and Aston-Jones, 2003; Sesack and Grace, 2010). Accordingly, expression of TAAR1 in these circuits may modulate the occurrence of burst events in VTA DA neurons and alter the effects of drugs of abuse. Deciphering the origin of the *Taar1*-expressing excitatory neurons that project to VTA DA neurons will require extensive investigations, like tracing experiments in $Taar1^{-/-}$ mice expressing the β-galactosidase reporter (Lindemann *et al*, 2008). Candidates may reside in the amygdala or the DRN, structures that express *Taar1* and project to the VTA.

Conclusion

TAAR1 emerges as a key element modulating monoamine systems and thus represents a promising therapeutic target for a variety of neuropsychiatric disorders. Fine dissection of TAAR1 expression, functions, and characterization of its therapeutic potential have proved to be challenging due to a conjunction of several parameters, such as low expression levels, the absence of specific anti-TAAR1 antibodies, the polypharmacology of its endogenous ligands (TAs), or the absence of specific ligands. After providing *Taar1*$^{-/-}$ mice (Lindemann *et al*, 2008) and more recently specific TAAR1 antagonist and agonist compounds (Bradaia *et al*, 2009; Revel *et al*, 2011), we created and characterized a transgenic mouse line overexpressing *Taar1* in central nervous system neurons. Under baseline condition, these mice lack overt abnormalities but show constitutive hyperactivity of monoamine neurons. This suggests that TAAR1 is tonically active *in vivo*, either due to constitutive activity or continued action of endogenous agonist(s). By contrast, stimulation with *d*-amphetamine did not trigger typical psychostimulant effects in *Taar1* Tg mice, and failed to increase monoaminergic release from basal levels. Overall, these data confirm the modulatory role of TAAR1 on monoaminergic activity and indicate that the *Taar1* Tg mice may represent a valuable tool for further investigations of TAAR1 biology.

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DISCLOSURE

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)