Trace Amine-Associated Receptor 1 Modulates Dopaminergic Activity

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ABSTRACT

The recent identification of the trace amine-associated receptor (TAAR)1 provides an opportunity to dissociate the effects of trace amines on the dopamine transporter from receptor-mediated effects. To separate both effects on a physiological level, a Taar1 knockout mouse line was generated. Taar1 knockout mice display increased sensitivity to amphetamine as revealed by enhanced amphetamine-triggered increases in locomotor activity and augmented striatal release of dopamine compared with wild-type animals. Under baseline conditions, locomotion and extracellular striatal dopamine levels were similar between Taar1 knockout and wild-type mice. Electrophysiological recordings revealed an elevated spontaneous firing rate of dopaminergic neurons in the ventral tegmental area of Taar1 knockout mice. The endogenous TAAR1 agonist p-tyramine specifically decreased the spike frequency of these neurons in wild-type but not in Taar1 knockout mice, consistent with the prominent expression of Taar1 in the ventral tegmental area. Taken together, the data reveal TAAR1 as regulator of dopaminergic neurotransmission.

Trace amines such as p-tyramine, β-phenylethylamine, octopamine, and tryptamine are endogenous amine compounds related to the classic neurotransmitters dopamine, serotonin, and noradrenaline by structure, metabolism, and tissue distribution (Philips, 1984). However, trace amines are found in the mammalian brain at concentrations approximately 1000-fold lower than catecholamines (Berry, 2004). Trace amines have been postulated to function as cotransmitters in classical neurotransmitter systems (Sávedra and Axelrod, 1976), as neurotransmitters in their own right (Sabelli et al., 1978) or as neuromodulators of catecholamines (for review, see Berry, 2004).

The identification of trace amine-specific receptors (Borowsky et al., 2001; Bunzow et al., 2001) and detailed characterization of the trace amine-associated receptor (TAAR) family in various species (Lindemann and Hoener, 2005; Lindemann et al., 2005) provided the basis to further elucidate the interplay between trace amines and catecholamines and to understand the physiological roles of trace amines at the molecular level. The TAAR family consists of three subgroups (TAAR1–4, TAAR5, and TAAR6–9) and is phylogenetically and functionally distinct from other G protein-coupled receptor families and from invertebrate octopamine or tyramine receptors (Lindemann and Hoener, 2005). With the exception of TAAR1 and TAAR4, none of the other TAARs are sensitive to one of the classical trace amines p-tyramine, β-phenylethylamine, octopamine, and tryptamine (Borowsky et al., 2001; Lindemann et al., 2005; Liberles and Buck, 2006). All TAARs except TAAR1 were recently detected in mouse olfactory sensory neurons, and mouse TAAR5, TAAR7, and TAAR3 have been shown to be activated by small-molecular-weight volatile amines, suggesting a potential role of TAARs as odorant receptors in rodents (Liberles and Buck, 2006). However, the endogenous ligands have not yet been identified for TAARs other than TAAR1.
and TAAR4. Taar1 is encoded by a single exon in all species analyzed, couples to the G protein Go,s, and it responds to p-tyramine and β-phenylethylamine, with an EC50 between 0.2 and 1.4 μM and with much lower sensitivity also to octopamine and tryptamine (Borowsky et al., 2001; Lindemann et al., 2005). In humans, all Taar genes are located in a narrow region of approximately 109 kb in the locus 6q23.1 (Lindemann et al., 2005), which has been genetically linked to schizophrenia and bipolar disorder (Cao et al., 1997; Cichon et al., 2001; Vladimirov et al., 2007).

The interest in trace amines and their target receptors is fueled by their proposed link to highly prevalent psychiatric disorders, most notably depression and schizophrenia (for reviews, see Branchek and Blackburn, 2003; Lindemann and Hoener, 2005). It has been reported that trace amines exhibit some amphetamine-like properties through inhibition of the dopamine transporter (Parker and Cubeddu, 1988; Berry, 2004; Sotnikova et al., 2004), but little is known about the effects of trace amines mediated directly by activation or inhibition of TAAR1. The Taar1 knockout mouse line allows to dissociate the specific contributions of dopamine transporter and of TAAR1 to the physiological effects of trace amines.

Materials and Methods

Animals

All animal experiments performed at F. Hoffmann-La Roche Ltd. (Basel, Switzerland) were performed in compliance with Swiss Federal and Cantonal laws on animal research and approved by the cantonal veterinary office.

Two 4.7- and 1.7-kb genomic fragments located 5’ and 3’ from the Taar1 coding sequence were amplified from C57BL/6 genomic DNA by PCR with the oligonucleotides F3 and R1 and with the oligonucleotides F4 and R2, respectively (Fig. 1). The targeting vector was assembled from these genomic fragments, a LacZ coding sequence fused to a nuclear localization sequence (NLS; Kalderon et al., 1984), and a PgK-Neo6 (Galceran et al., 2000), and a diphtheria toxin cassette (Gabernet et al., 2005). The targeting vector was linearized with SacII and electroporated into C57BL/6 embryonic stem (ES) cells, and G-418 (Geneticin; Invitrogen, Paisley, UK)-resistant ES cell clones were selected as described previously (Gabernet et al., 2005). An ES cell clone carrying a homologous recombination event was

Fig. 1. Generation of Taar1 knockout mice. a, strategy for the targeted deletion of the Taar1 gene in mouse ES cells. The Taar1 coding sequence was replaced by a cDNA encoding for LacZ linked to a NLS sequence. The NLS-LacZ cDNA was fused in frame with the endogenous Taar1 start codon. S, SacI; E, EcoRV; and N, NsiI. The homologous recombination was confirmed by PCR on genomic DNA derived from Taar1+/− and Taar1−/− animals (b–e; PCR 1–4). b, PCR 1, PCR amplification of the whole Taar1 wild-type and the targeted Taar1 locus using oligonucleotides located 5’ and 3’ to the genomic fragments comprised in the targeting vector. The generated PCR fragments had the expected sizes (10.7 kb for wild-type allele and 13 kb for the targeted allele, respectively) and DNA sequence (data not shown). c, PCR 2, PCR using oligonucleotides surrounding the single Taar1 coding exon produced PCR fragments of correct sizes (2.7 kb for the wild-type allele and 5.1 kb for the targeted allele) and DNA sequence (data not shown). d, PCR 3, PCR amplification using oligonucleotides within the LacZ coding sequence and 5’ of the genomic arms comprised in the targeting vector resulted in a fragment of the expected size (7.3 kb) and DNA sequence (data not shown) only in the targeted allele. e, PCR 4, PCR amplification using oligonucleotides within the Neo6 coding sequence and 3’ of the genomic arms comprised in the targeting vector resulted in a fragment of the expected size (2.9 kb) and DNA sequence (data not shown) only in the targeted allele (Taar1−/−). f–h, analysis of whole brain cDNA from Taar1+/− and Taar1−/− animals for the presence of Gapdh (f), Taar1 (g), and LacZ (h) transcripts. Taar1 transcripts were detected only in Taar1−/− cDNA, and LacZ transcripts were found only in Taar1−/− cDNA. i, standard genotyping PCR with genomic DNA derived from Taar1+/−, Taar1−/−, and Taar1−/− animals of the F2 generation.
identified by PCR (data not shown), and it was used to generate chimeras according to standard protocols (Joyner, 1999). The recombinant allele was maintained in a pure C57BL/6 background (Charles River Laboratories, Les Oncins, France) in a specific-pathogen-free facility with a 12:12-h day/night cycle and ad libitum access to food and water.

The correct homologous recombination was further confirmed by PCR amplification of fragments PCR 1 to 3 (Fig. 1) from genomic DNA derived from tail biopsies of Taar1 m−/− animals and subsequent DNA sequence analysis (for details regarding PCR conditions and oligonucleotide sequences, see Supplemental Material). Standard genotyping was performed by PCR using oligonucleotides detecting the disrupted- and the intact Taar1 coding sequence, respectively (for details regarding PCR conditions and oligonucleotide sequences, see Supplemental Material).

The purity of the C57BL/6 genetic background of the knockout mouse line was confirmed by microsatellite analysis of Taar1 m−/− animals of the F1 generation using a total of 37 markers (for details regarding the panel of microsatellite markers and oligonucleotides used in the microsatellite analysis, see Supplemental Material). Genomic DNA of six individual animals was analyzed along with samples of C57BL/6, DBA2, and 129/SV mice (The Jackson Laboratory, Bar Harbor, ME).

Whole brain cDNA of 2-week-old and adult Taar1 m−/− and Taar1 m+/− mice was analyzed for the presence of transcripts encoding glycine/alanine-3-phosphate dehydrogenase (Gapdh), Taar1, and NLSLacZ by means of RT-PCR. Whole brain cDNAs were prepared essentially as described by Lindemann et al. (2005), and PCR was performed using the following oligonucleotides for the individual transcripts (for oligonucleotide sequences, see Supplemental Material): GAPDH, GAPDHU, and GAPDHD (452-bp PCR fragment), NLSLacZ, LacZ U1, and LacZ D1 (631-bp PCR fragment); and TAA1, mTAAR1 U1, and mTAAR1 D1 (936-bp PCR fragment).

**Behavioral Phenotyping**

**Locomotor Activity.** A computerized Digiscan 16 Animal Activity Monitoring System (Omnitec Electronics, Columbus, OH) was used to quantify spontaneous locomotor activity. Data were obtained simultaneously from eight Digiscan activity chambers placed in a soundproof room with a 12:12-h day/night cycle. All tests were performed during the light phase (6:00 AM–6:00 PM). Each activity monitor consisted of a Plexiglas box (20 × 20 × 30.5 cm) with sawdust bedding on the floor surrounded by invisible horizontal and vertical infrared sensor beams. The cages were connected to a Digiscan Analyzer linked to a PC constantly collecting the beam status information. With this system, different behavioral parameters could be measured, such as horizontal and vertical activity, total distance traveled, and stereotypies. The mice were tested via a pseudo-Latin squares design with at least a 10-day interval between two consecutive test sessions. Animals were habituated for 30 min. Vehicle (saline 0.9%) or d-amphetamine (1 and 2.5 mg/kg i.p.) was then administered to wild-type (n = 24) and Taar1 knockout (n = 24) mice. Locomotor activity was recorded during 30-min habituation and 90 min after treatment starting immediately after the mice were placed in the test compartment.

Mice were additionally assessed for body temperature, body weight, grip strength, and general motor coordination (rotated test; for protocols and data regarding this additional behavioral testing, see Supplemental Material).

**Statistics.** Behavioral observations were recorded as mean values ± S.E.M., and they were analyzed with an unpaired t-test. Locomotor activity data (total distance) were analyzed with a two-factor (genotype and dose) analysis of variance with repeated measures. Comparisons of dose effects in each genotype were undertaken with a repeated-measures analysis of variance, followed in significant cases by paired t-tests. A P value of 0.05 was accepted as statistically significant.

**In Vivo Microdialysis Assessment of Extracellular Biogenic Amine Neurotransmitter Levels**

Four-month-old male mice were used for these experiments.

**Surgery and Implantation of the Microdialysis Probe.** Forty-five minutes before anesthesia, mice received s.c. injections of 0.075 mg/kg buprenorphine. Mice were subsequently anesthetized with isoflurane and placed in a stereotaxic device equipped with dual manipulator arms and an anesthetic mask. Anesthesia was maintained with 0.8 to 1.2% isoflurane (v/v; support gas oxygen/air, 2:1). The head was shaved, and the skin was cut along the midline to expose the skull. A small bore hole was made in the skull to allow the stereotaxical insertion of the microdialysis probe (a vertical probe carrying a 2-mm polyacrylonitrile dialysis membrane; Brains Online, Groningen, The Netherlands) in the striatum (coordinates: A, 0.9 mm; L, −1.8 mm; and V, −4.6 mm). The probe was fixed using binary dental cement. Once the cement was firm, the wound was closed with silk thread for suture (Silkam; B. Braun Melsungen AG, Melsungen, Germany), the animal was removed from the stereotaxic instrument and returned to its cage. At the end of the surgery as well as 24 h later, mice were treated with meloxicam 1 mg/kg s.c. The body weight of an animal was measured before the surgery and in the following days to monitor its recovery from surgery.

**Microdialysis Experiments.** All microdialysis experiments were carried out 3 to 4 days after surgery in awake, freely moving mice. On the day of the experiment, the inlet of the implanted dialysis probe was connected to a microperfusion pump (CMA/Microdialysis, Solna, Sweden), and the outlet was connected to a fraction collector. The microdialysis probe was then perfused with Ringer’s solution (147 mM NaCl, 3 mM KCl, 1.2 mM CaCl2, and 1.2 mM MgCl2) at a constant flow rate of 1.5 μl/min, and dialysates were collected in 15-min aliquots in plastic vials containing 37.5 μl of 0.02 M acetic acid. Four samples of dialysates were collected before pharmacological treatment to determine the baseline levels of biogenic

**Drugs**

Drugs were purchased from Sigma Chemie (Buchs, Switzerland) at the highest purity available, and d-amphetamine was synthesized by F. Hoffmann-La Roche Ltd. Drugs were dissolved in 0.9% NaCl, and they were administered i.p. before the behavioral testing or the collection of microdialysis samples, as indicated for the individual experiments. In each experiment, drugs were administered following a pseudorandomized design over one to several treatment cycles, with a minimum period of 10 to 14 days between two cycles.

**Histochemistry on Tissue Sections**

Adult mice were transcerebrally perfused under terminal isoflurane anesthesia consecutively with phosphate-buffered saline (PBS) and fixative [2% (w/v) paraformaldehyde and 0.2% (w/v) glutaraldehyde in PBS]. Brains were postfixed for 4 h in fixative at 4°C, immersed overnight in 0.5 M sucrose in PBS at 4°C, embedded in OCT compound (Medite Medizintechnik, Nunningen, Switzerland) in Peel-A-Way tissue embedding molds (Polysciences, Warrington, PA), and frozen on liquid nitrogen. Tissue sections were cut on a cryostat (Leica Microsystems AG, Glattbrugg, Switzerland) at 10 to 50 μm, thaw-mounted on gelatin-coated glass slides (Fisher Scientific, Wohlen, Switzerland), air-dried at room temperature for 4 h, and processed immediately for LacZ staining.

For LacZ staining, tissue sections were washed five times for 10 min in PBS and then incubated for 16 to 24 h in LacZ-staining solution [1 mg/ml 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside, 5 mM KFe(CN)6, 5 mM K4Fe(CN)6, and 2 mM MgCl2 in PBS] at 37°C. The staining was stopped by washing the tissue sections five times for 10 min at room temperature in PBS. Tissue sections were dehydrated through an ascending ethanol series, equilibrated to xylene, coveredslipped with DePex (Serva, Heidelberg, Germany), and analyzed on an Axioplan I microscope equipped with an Axioskop digital camera system (Carl Zeiss AG, Feldbach, Switzerland).
amines and their metabolites. Mice were then treated intraperitoneally with 2.5 mg/kg d-amphetamine, and dialysate samples were collected for further 2.5 h. Dialysate samples were stored frozen at −80°C until analysis.

**Analysis of Microdialysis.** Dialysate samples were analyzed at Brains On-Line for monoamines and their metabolites. The concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin, 5-hydroxyindoleacetic acid (5-HIAA), and noradrenaline were measured by means of an HPLC equipped with an electrochemical detector essentially as described in van der Vegt et al. (2003). Concentrations of norepinephrine, dopamine, and serotonin were determined within the same samples by HPLC separation and electrochemical detection. Samples were split into two aliquots; one aliquot was used for the simultaneous analysis of norepinephrine and dopamine, the other was used for the analysis of serotonin.

**Norepinephrine and Dopamine.** Aliquots (20 μl) were injected onto the HPLC column by a refrigerated microsampler system, consisting of a syringe pump (model 402; Gilson, Villier Le Bel, France), a multicolonut injector (model 233 XL; Gilson), and a temperature regulator (model 832; Gilson). Chromatographic separation was performed on a reversed-phase 150-× 2.1-mm (3-μm) C18 Thermo BDO Hypersil column (Thermo Electron Corporation, Waltham, MA). The mobile phase (isocratic) consisted of a sodium acetate buffer (4.1 g/l sodium acetate with 2.5% (v/v) methanol, 150 mg/l Tiritrplex (EDTA), 150 mg/l 1-octanesulfonic acid, and 150 mg/l tetramethylammonium chloride (pH 4.1, adjusted with glacial acetic acid). The mobile phase was run through the system at a flow rate of 0.35 ml/min by an HPLC pump (model LC-10AD vp; Shimadzu, Kyoto, Japan).

Norepinephrine and dopamine were detected electrochemically using a potentios态度 (model Intro; Antec Leyden, Zoeterwoude, The Netherlands) fitted with a glassy carbon electrode set at +500 mV versus silver/silver chloride (Antec Leyden). Data were analyzed by Chromatography Data System software (class-vp; Shimadzu) software. Concentrations of monoamines were quantified by an external standard method.

**Serotonin.** Aliquots of dialysate (20 μl) were injected onto the HPLC column as described for norepinephrine and dopamine. Chromatographic separation was performed on a reversed-phase 100-× 2-mm (3-μm) C18 ODS Hypersil column (Phenomenex, Torrance, CA). The mobile phase (isocratic) consisted of a sodium acetate buffer (4.1 g/l Na acetate) with 4.5% (v/v) methanol, 500 mg/l Tiritrplex (EDTA), 50 mg/l 1-heptanesulfonic acid, and 30 μl/l tetraethylammonium chloride (pH 4.74 adjusted with glacial acetic acid). The mobile phase was run through the system at a flow rate of 0.4 ml/min by an HPLC pump (model LC-10AD vp; Shimadzu), serotonin was detected electrochemically using the same method as described for norepinephrine and dopamine.

**Slice Electrophysiology in the Ventral Tegmental Area**

Horizontal slices (250 μm in thickness cut with a VT1000 vibratome; Leica, Wetzlar, Germany) of the midbrain were prepared from Taar1 knockout and littermate wild-type mice. Slices were cooled in artificial cerebrospinal fluid containing 119 mM NaCl, 2.5 mM KCl, 1.3 mM MgCl2, 2.5 mM CaCl2, 1.0 mM NaH2PO4, 26.2 mM NaHCO3, and 11 mM glucose). Slices were continuously bubbled with a mixture of 95% O2 and 5% CO2 and transferred after 1 h to the recording chamber superfused with artificial cerebrospinal fluid (1.5 ml/min) at 32 to 34°C. The ventral tegmental area was identified as the region of the medial terminal nucleus of the accessory optic tract. Visualized whole-cell current-clamp recording techniques were used to measure the spontaneous firing rate and holding currents of neurons. All cells used for the statistical analysis displayed a stable firing activity for at least 30 min. The internal solution contained 140 mM potassium gluconate, 4 mM NaCl, 2 mM MgCl2, 1.1 mM EGTA, 5 mM HEPES, 2 mM Na2ATP, 5 mM sodium creatine phosphate, and 0.6 mM Na2GTP; the pH was adjusted to 7.3 with KOH. Data were obtained with an Axopatch 200B (Molecular Devices, Sunnyvale, CA), filtered at 2 kHz and digitized at 10 kHz, and acquired and analyzed with pClamp9 (Molecular Devices). Values are expressed as mean ± S.E.M. For statistical comparisons the Kolmogorov-Smirnov test was used. The level of significance was set at P = 0.05.

**Results**

**Generation of Taar1 Knockout Mice.** To address the physiological role of TAAR1 in vivo, a targeted mouse mutant [B6-Taar1tm1.1NLSLacZ/Bli] (subsequently designated Taar1−/−) was generated in which the entire Taar1 coding sequence was replaced by a reporter gene consisting of LacZ fused to an NLS (Fig. 1a). In the targeted allele, LacZ is expressed from the endogenous Taar1 promoter, thus providing a sensitive means to study TAAR1 tissue distribution. Homologous recombination in embryonic stem cells (data not shown) and homozygous mutants were diagnosed by PCR and sequence analysis of PCR-derived DNA fragments spanning the entire targeted and wild-type single exon-encoded Taar1 gene locus (Fig. 1, b–e). The gene replacement was further confirmed by means of RT-PCR on cDNA prepared from whole brains of 2-week-old and adult homozygous knockout mice (Taar1−/−) and wild-type siblings (Taar1+/+). As expected, transcripts encoding TAAR1 were detected only in wild-type but not in Taar1 knockout brain cDNA. In contrast, LacZ transcripts were amplified from Taar1 knockout but not from wild-type brain cDNA (Fig. 1, f–i).

To ensure a pure genetic background critical for behavioral analysis, the Taar1 knockout mouse line was generated using C57BL/6-derived ES cells, and it was subsequently maintained on a pure C57BL/6 background. The purity of the genetic background was confirmed by analyzing genomic DNA derived from the targeted ES clones and Taar1−/− animals at the F1 generation, respectively, with a panel of microsatellite markers (data not shown; for the selection of microsatellite markers and oligonucleotide sequences, see Supplemental Material).

**TAAR1 Is Expressed in Dopaminergic and Serotonergic Systems.** The TAAR1 expression pattern in the central nervous system was analyzed, making use of the LacZ reporter that was inserted into the Taar1 gene in frame with the endogenous start codon. Owing to the low Taar1 expression levels in mouse brain as revealed by our RT-PCR experiments (see above) and by previous reports (Borowsky et al., 2001; Bunzow et al., 2001), an NLS-tagged version of the LacZ reporter was chosen to improve sensitivity. Staining of serial brain sections of adult Taar1 knockout and wild-type brains revealed a discrete and specific labeling of nuclei mainly in dopaminergic and serotonergic brain areas; specifically in the hypothalamus and preoptic area (Fig. 2a, box i; and b), ventral tegmental area (Fig. 2a, box ii; and c), amygdala (Fig. 2a, box ii; and d), dorsal raphe nucleus (Fig. 2a, box iii; and e), nucleus of the solitary tract (Fig. 2a, box iv; and f), and in the parahippocampal region (rhinal cortices) and subiculum (Fig. 2g, arrows and arrowheads, respectively). In contrast to previous reports suggesting Taar1 mRNA localization in additional brain areas such as the olfactory bulb and cerebellar Purkinje cells (Borowsky et al., 2001), no LacZ expression was observed in these regions. However, it cannot be ruled out that the LacZ reporter might leave brain areas with particularly low Taar1 expression unrecognized due to the potentially lower sensitivity of the reporter compared with in situ hybridization. No LacZ staining was detected in...
Taar1 brain sections, and no obvious morphological alterations were found in Taar1−/− compared with Taar1+/+ brains (data not shown).

Physical and Behavioral Properties of Taar1 Knockout Mice. The general health, physical state, and sensory functions of Taar1 knockout mice was examined according to a modified version of standard procedures used for behavioral phenotyping of genetically modified mice (Irwin, 1968; Hatcher et al., 2001). The comparison of all three genotypes (Taar1+/+, Taar1−/−, and Taar1−/−) did not reveal any significant differences regarding their general state of health, their viability, fertility, life span, and nest building behavior (data not shown) and their body weight and body temperature (see Supplemental Fig. S1). Regarding general motor functions and behavior, no differences between genotypes were observed when analyzing dexterity and motor coordination (see Supplemental Fig. S1), and there was no statistically significant difference in spontaneous locomotor activity, although Taar1−/− mice showed a trend to be slightly more active during the first 30 to 45 min of each recording session (Fig. 3).

Taar1 Knockout Mice Display Elevated Sensitivity to Amphetamine. The effect of d-amphetamine on the locomotor function of Taar1−/− and Taar1+/+ littermates was compared (Fig. 3). A dose of 1 mg/kg d-amphetamine caused a significant increase in locomotor activity in Taar1−/− animals ($P < 0.05$), but not in wild-type siblings, whereas no difference was observed in vehicle-treated animals (Fig. 3a). A higher dose of d-amphetamine (2.5 mg/kg i.p.) significantly increased locomotor activity also in wild-type animals ($P < 0.05$), but the effect was substantially stronger in Taar1−/− animals than in wild-type littermates ($P < 0.05$) (Fig. 3b).
The behavioral differences in response to d-amphetamine between Taar1−/− and wild-type animals were further analyzed by means of in vivo microdialysis in the striatum. The effect of d-amphetamine (2.5 mg/kg i.p.) on the extracellular levels of catecholamines in the striatum reached its maximum after 30 min in both genotypes, with peak levels of dopamine and norepinephrine in Taar1−/− mice 11- and 4.9-fold higher compared with baseline values, respectively (Fig. 4, a and c). The increased dopamine and norepinephrine releases were approximately 2.3-fold higher in Taar1−/− mice compared with wild-type siblings. No significant differences in basal levels of dopamine and norepinephrine were detected (Table 1).

The levels of the dopamine metabolite DOPAC were decreased in Taar1−/− compared with Taar1+/+ mice 45 min after d-amphetamine administration (Table 1) and returned close to basal levels after 135 min (Fig. 4b). There were no significant differences in the basal DOPAC levels between Taar1+/+ and Taar1−/− mice (Table 1). Serotonin levels remained unchanged after d-amphetamine application in wild-type animals (Table 1), but they increased by 2.5-fold compared with basal levels 30 min after administration in Taar1−/− mice (Fig. 4d). No significant differences were observed before as well as after d-amphetamine administration for the serotonin metabolite 5-HIAA in Taar1+/+ and Taar1−/− mice (Table 1).

The increased dopamine levels in Taar1−/− mice could derive from increased expression of the dopamine transporter. This can be excluded as radioligand binding with the dopamine transporter-specific radioligand [3H]GBR 12935 on brain sections of Taar1+/+ and Taar1−/− mice did not reveal significant differences in the expression levels in both genotypes (data not shown).

**TAAR1 Activity Decreases the Spontaneous Firing Rate of Dopaminergic Neurons in the Ventral Tegmental Area.** The differences in dopaminergic neurotransmission between Taar1+/+ and Taar1−/− littermates (~1.5–2 months of age) were further investigated by electrophysiolog-
electrical recordings in dopaminergic neurons in the ventral tegmental area. Dopaminergic neurons were identified by their large hyperpolarization-activated cation currents (Vacher et al., 2006). The mean spike frequency recorded under current-clamp conditions in Taar1\textsuperscript{+/+} (n = 22) and in Taar1\textsuperscript{−/−} mice (n = 25) was 2.3 ± 0.8 and 17.2 ± 1.2 Hz (P < 0.0001; Fig. 5a), respectively, thereby revealing a significantly higher firing rate in Taar1\textsuperscript{−/−} neurons compared with wild-type neurons. The data suggest that in wild-type mice TAAR1 tonically decreases the firing rate of dopaminergic neurons. It was further observed that the resting membrane potential in dopaminergic neurons in Taar1\textsuperscript{−/−} mice (−33.5 ± 0.5 mV; n = 26) was depolarized compared with Taar1\textsuperscript{+/+} animals (−47.8 ± 0.7 mV; n = 22). The depolarized resting membrane potential in Taar1\textsuperscript{−/−} mice could be either cause or consequence of the increased firing rate. Next, it was tested whether application of the TAAR1 agonist p-tyramine decreases the spontaneous firing rate of dopaminergic neurons in the ventral tegmental area of wild-type mice. Bath application of 10 \mu M p-tyramine caused a significant decrease in the spike frequency in Taar1\textsuperscript{+/+} (control, F = 2.1 ± 0.3 Hz; p-tyramine, F = 0.63 ± 0.04 Hz; n = 19, P < 0.0001) but not in the Taar1\textsuperscript{−/−} mice [control, F = 16.73 ± 1.15 Hz; p-tyramine, F = 16.57 ± 1.35 Hz; n = 15; P < 0.05 (Fig. 5b)]. A hyperpolarization of the resting membrane potential from −46.2 ± 0.6 to −55.6 ± 1.2 mV (n = 19) after p-tyramine application in wild-type but not in Taar1\textsuperscript{−/−} littermates was also observed. Both the decrease in spike frequency and the hyperpolarization after application of p-tyramine have been reported previously for wild-type dopaminergic neurons in the ventral tegmental area (Geracitano et al., 2004). Together with the depolarization recorded in Taar1\textsuperscript{−/−} mice, this finding suggests that TAAR1 is either constitutively active or tonically activated by an endogenous ligand. These observations provide evidence that TAAR1 negatively modulates the spontaneous firing of dopaminergic neurons in the ventral tegmental area.

### Discussion

The Taar1 knockout mouse mutant reported here is based on a pure C57BL/6 genetic background with a complete deletion of the Taar1 coding sequence. In the mouse mutant, the NLS\textsubscript{LacZ} cDNA is expressed from the endogenous Taar1 locus, thus providing a reporter for the TAAR1 tissue distribution.

The Taar1 knockout displays a hypersensitivity toward the psychostimulant d-amphetamine as revealed by behavioral and neurochemical observations. The hyperlocomotion induced by a single amphetamine challenge was substantially stronger in Taar1\textsuperscript{−/−} animals compared with wild-type siblings with maximum levels reached at 45 min after injection (Fig. 3). In vivo microdialysis revealed that the amphetamine-triggered release of dopamine, norepinephrine, and serotonin reached approximately 2.5-fold higher levels in the Taar1\textsuperscript{−/−} than in the Taar1\textsuperscript{+/+} animals, with a maximum at 30 min after amphetamine application (Fig. 4, a–d). For these findings, any strong bias originating from a potential baseline phenotype of the knockout can be excluded. The physical, neurological, and behavioral phenotypes (see Supplemental Fig. S1) including basal locomotor activity (Fig. 3) and the baseline extracellular concentrations of all neurotransmitters studied (Fig. 4, a, c, and d) are indistinguishable between Taar1\textsuperscript{−/−} and Taar1\textsuperscript{+/+} animals. The effects of amphetamine on locomotor activity and extracellular dopamine and norepinephrine levels in Taar1\textsuperscript{−/−} and Taar1\textsuperscript{+/+} mice seen here are comparable to observations reported by Wolinsky et al. (2007) with another TAAR1 knockout mouse line.
However, whereas for our Taar1 knockout mouse line the locomotor activity responses were significantly different between Taar1\(^{-/-}\) and Taar1\(^{+/+}\) littermates using 1.0 and 2.5 mg/kg d-amphetamine in several experiments, Wolinsky et al. (2007) observed significantly different amphetamine responses only for a dose of 1 mg/kg, but not for 2.5 mg/kg. This discrepancy could be due to the different genetic background of the two Taar1 knockout mouse lines: whereas the Taar1 knockout described here comprises a congenic C57BL/6 background, the Taar1 knockout reported by Wolinsky et al. (2007) comprises a mixed 129Sv\(\times\)C57BL/6 genetic background. The effects seen with amphetamine are most likely composed of two elements. First, amphetamine triggers the release of dopamine and norepinephrine from synaptic vesicles (King and Ellinwood, 1992), which in turn produces elevated locomotor activity. Second, a modulation of catecholamine neurotransmission through direct activation of TAAR1 by trace amines has been suggested (Berry, 2004; Geracitano et al., 2004), consistent with Taar1 expression in brain areas with predominantly dopaminergic and serotonergic neurotransmission (Fig. 2) (Borowsky et al., 2001). The latter mechanism is further supported by the different firing pattern observed in dopaminergic neurons of Taar1\(^{+/+}\) and Taar1\(^{-/-}\) animals (Fig. 5).

The application of p-tyramine causes a significant decrease in the firing rate and a concomitant hyperpolarization of the membrane potential in wild-type but not in Taar1\(^{-/-}\) dopaminergic neurons, which is well in line with reports from Geracitano et al. (2004). In contrast, in the Taar1\(^{-/-}\) mice the spontaneous firing rate of dopaminergic neurons is 8.6-fold higher, and the resting membrane potential is depolarized compared with Taar1\(^{+/+}\) mice (Fig. 5). This suggests that TAAR1 is tonically active under physiological conditions, either due to the presence of an ambient ligand or because of constitutive receptor activity. It has been proposed that trace amines stimulate, in an amphetamine-like manner, a transporter-mediated efflux of dopamine from the dendrites of dopaminergic neurons (Raiteri et al., 1978). The increased spontaneous firing rate observed in Taar1\(^{-/-}\) dopaminergic neurons does not measurably increase basal levels of extracellular dopamine in the striatum, as shown in our microdialysis experiments (Fig. 4). It is conceivable that in Taar1\(^{-/-}\) mice, the increased firing rate and the associated increase in dopamine release is offset by the reduced release of dopamine due to lack of TAAR1 activity. It has been proposed that TAAR1 activity causes an indirect activation of D\(_2\) dopamine autoreceptors by increasing the efflux of newly synthesized dopamine (Geracitano et al., 2004). This could explain the hyperpolarization observed after p-tyramine application, because D\(_2\) autoreceptors are positively coupled to G protein-coupled inwardly rectifying potassium channels (Kir3) that hyperpolarize the membrane (Schmitz et al., 2003). One possible mechanism underlying a TAAR1-mediated activation of D\(_2\) receptors could involve a formation of heterodimers of TAAR1 with D\(_2\) receptors. Such a positive modulation of D\(_2\) dopamine receptors through formation of heterodimers has been reported for the somatostatin receptor 5 (Rocheville et al., 2000). However, it is equally possible that TAAR1 causes a hyperpolarization by a direct coupling to K\(^+\) channels via G\(\beta\gamma\), as described for a variety of other G protein-coupled receptors (Mark and Herlitze, 2000). It is noteworthy that dopaminergic neurons in the ventral tegmental area also receive excitatory input from glutamatergic afferents originating in the prefrontal cortex and inhibitory input from local GABAergic neurons (Yamaguchi et al., 2007). Alternatively, tonically active TAAR1 might elicit a net inhibitory effect on D\(_2\) receptors in wild-type animals (Wolinsky et al., 2007); the absence of TAAR1 in the knockout mice would consequently result in a D\(_2\) disinhibition, which in turn would be reflected in the observed supersensitivity to psycho-stimulants such as amphetamine. TAAR1 may by any of the discussed mechanisms modulate the neurotransmitter release at glutamatergic or GABAergic terminals, respectively, and thereby control the firing rate and the resting membrane potential of dopaminergic neurons.

Dopaminergic neurons projecting to the striatum originate from the substantia nigra and ventral tegmental area in the midbrain area where dopamine is produced. According to the LacZ signals, TAAR1-positive cell bodies are indeed located in the ventral tegmental area (Fig. 2, a and c) and the amygdala (Fig. 2, a and d), confirming an anatomical overlap between TAAR1 and dopaminergic brain structures. The ventral tegmental area plays important roles in the context of drug addiction and withdrawal (Aston-Jones and Harris, 2004), and the amygdala is essential for attention, memory, emotions, and fear (Nathan et al., 2004; Yaniv et al., 2004). Staining was furthermore detected in parahippocampal regions (rhinal cortices) and the subiculum (Fig. 2g), which both play an essential role in memory processes (Eichenbaum, 2000; de Curtis and Pare, 2004), and in the medial preoptic area that is involved in the modulation of sleep (McGinty et al., 2004). Thus, Taar1 has been almost exclusively detected in brain areas associated with mood, attention, memory, fear, and addiction, which is in agreement with the proposed roles of trace amines in the context of conditions involving emotional and behavioral dysfunctions (Lindemann and Hoener, 2005).

In summary, a close interaction between TAAR1 and the dopaminergic system has been demonstrated. TAAR1, either constitutively active or stimulated by agonists negatively modulates the firing rate of dopaminergic neurons and the release of dopamine in response to the psycho-stimulant amphetamine.

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References


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