Brain-specific overexpression of TAAR1 causes hyposensitivity to amphetamine

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1 – Introduction

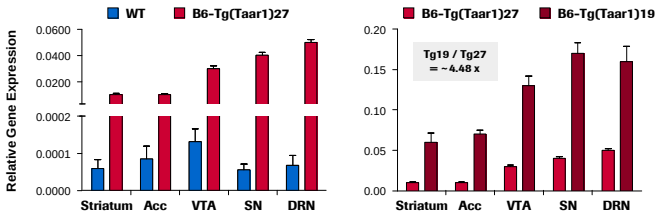
Trace amines, such as p-tyramine and beta-phenylethylamine, are metabolites of amino acids with structural similarity to classical biogenic amines that are found at low concentrations in the brain. For a long time, trace amines were believed to modulate neurotransmission by displacing classical biogenic amines from vesicular stores, or by acting on transporters in an amphetamine-like manner. It was not until the finding that trace amines bind to the trace amine-associated receptor 1 (TAAR1) [1], a member of a family of G-protein coupled receptors, that direct receptor-mediated mechanisms were also evoked. The interest in trace amines and their target receptor TAAR1 is fuelled by their proposed link to highly prevalent psychiatric disorders, most notably depression and schizophrenia [2].

To further explore the physiological roles of TAAR1, a mouse line with artificially elevated *Taar1* brain-expression was engineered. The mutants were investigated using behavioral (locomotor activity) recordings, in-vivo microdialysis measurements and electrophysiological studies.

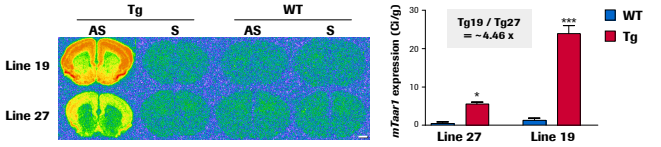
2 – Construction of *Taar1* transgenic mice

> **Thy-1.2 expression cassette was used to drive strong constitutive expression of *Taar1* in neurons of adult C57BL/6 mice**

> **Quantitative-PCR analysis from selected punched brain areas shows brain over-expression in the lines 19 and 27**



> **In situ hybridization indicates a general over-expression of *Taar1* in the brain of Taar1 transgenic mice**

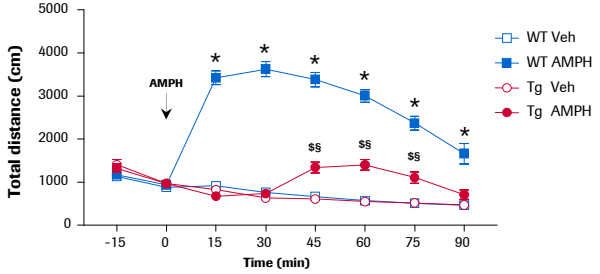


Methods

Generation of Taar1-overexpressing mice: Transgenic C57BL/6 mice were generated by pronuclear injection of a transgene consisting of the *Thy1.2* expression cassette [3] driving the expression of a fusion protein with 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 *Taar1* coding sequence [4]. The murine Taar1 transgene was generated by PCR from genomic DNA with the oligos 5´-GCCTCGAGTCCACCATGAAGACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTGTTCGCCGACTACAAGGACGATGATGACGCCATGGGCATGCATCTTTGCCACGCTATCAC-3´ and 5´-GCCTCGAGTTACAAAAATAGCTTAGACC-3´ and cloned using the introduced *Xho*I sites into the *Xho*I site of *Thy1.2* expression vector. Two lines of Taar1 transgenic mice, **B6-Tg(Taar1)19** and **B6-Tg(Taar1) 27**, were selected. Their general health, viability, fertility, life span, body weight, body temperature, general motor functions and behavior are normal.
Quantitative PCR: Total-RNA was extracted from the **striatum**, accumbens nucleus (**Acc**), ventral tegmental area (**VTA**), substantia nigra (**SN**) and dorsal raphe nucleus (**DRN**) using the miRNeasy Mini kit (Qiagen), and treated with DNAse I. Reverse transcription and polymerase chain reaction were performed in triplicates with 2 ng total-RNA and TaqMan ABI probes as previously reported [5]. A relative quantification method based on GAPDH expression was used. Data are mean ± SEM (n=4/group).
In-situ hybridization: Radioactive in situ hybridization was performed as described previously [6]. Twenty µm-thick coronal brain sections cut with a cryostat were hybridized overnight at 54°C with ^{35}S-UTP-labeled antisense and sense riboprobes for mouse Taar1 (bases 200-999 from GenBank accession no. NM_053205), treated with RNase A, washed (SSC 0.1×, 60°C), dehydrated, exposed to a Fujifilm imaging plate (BAS-SR2025) along with ^{14}C radioactive standards, and scanned (Fujifilm BAS-5000). Calibrated optical density bilateral measurements of mRNA levels in the dorsal striatum were performed on 2 consecutive sections using ImageJ (NIH). For each animal (n=4/group), the signal measured with the sense probe was subtracted to that measured with the antisense probe. Scale: 1 mm. *, P < 0.05 vs WT (line 27); ***, P < 0.001 vs WT (line 19) and vs Tg line 27 (one-way ANOVA, followed by Tukey-Kramer's test).

3 –TAAR1 transgenic mice are hyposensitive to amphetamine

> **Upon d-amphetamine challenge (2.5 mg / kg, ip), TAAR1 Tg mice do not show increased locomotion compared to WT littermates**

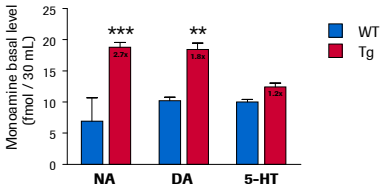


Method

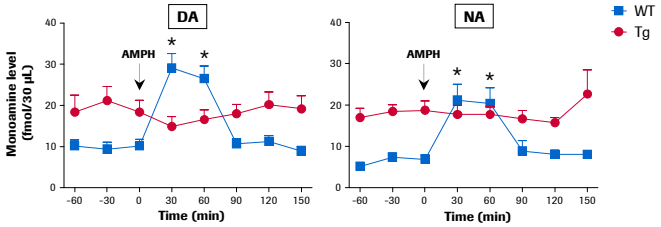
A computerized Digiscan 16 Animal Activity Monitoring System (Omnitech Electronics), consisting of a Plexiglas box (20 x 20 x 30.5 cm) with sawdust bedding on the floor, surrounded by invisible infrared sensor beams, was used to quantify locomotor activity. Taar1 transgenic mice (B6-Tg(Taar1)27) and wildtype littermates were habituated to the system for 30 min, then injected with vehicle (saline 0.9%) or d-amphetamine (AMPH, 2.5 mg/kg i.p.) . Locomotor activity was recorded for 90 min after treatment. n = 23-24 mice per group. *, P < 0.05 versus WT Veh; §, P < 0.05 versus Tg AMPH; §, P < 0.05 versus Tg WT AMPH (2-way ANOVA, followed by Bonferroni's test).

4 –TAAR1 transgenic mice have abnormal monoamine levels

> **Under basal conditions, TAAR1 transgenic mice have increased extracellular levels of NA and DA in the accumbens nucleus**



> **d-Amphetamine injection (2.5 mg / kg, ip) does not change extracellular DA nor NA in the accumbens nucleus of TAAR1 transgenic mice**

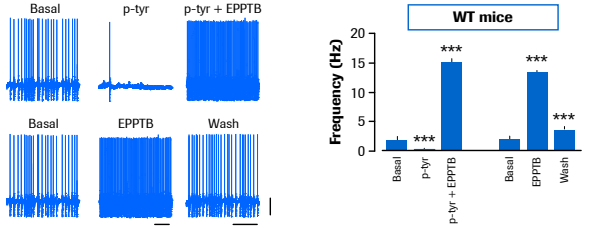


Method

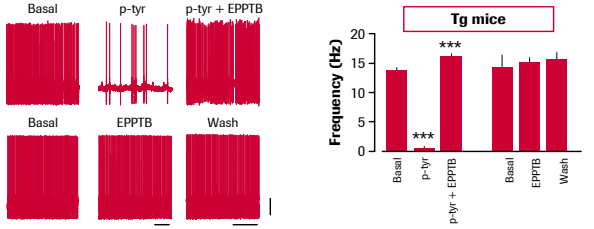
Extracellular noradrenaline (**NA**), dopamine (**DA**) and serotonin (**5-HT**) were measured by microdialysis coupled to HPLC in conscious animals as described in [7,8]. Adult male mice (B6-Tg(Taar1)27 or wt) were anesthetized with equithesin and surgically implanted with a vertical microdialysis probe in the nucleus accumbens (AP 1.2 mm, ML -1.8 mm, DV -4.9 mm with respect to bregma with a 20° angle to the sagittal plane) about 24 h before probe perfusion. Thirty-min fractions were collected for 90 min before and 150 min after 2.5 mg/kg i.p. d-amphetamine or vehicle (0.9% NaCl). ***, P < 0.0001; **, P < 0.02 vs. WT (Student's t-test). *, P < 0.05 vs time 0 in WT (repeated-measures ANOVA, followed by Tukey-Kramer's test). No statistical effect of time is seen in the Tg mice. Mean ± SEM. N = 7 / group.

5 – TAAR1 transgenic mice have increased firing of VTA dopaminergic neurons

> **In WT mice, spontaneous electrical activity in VTA DAergic neurons is inhibited by p-tyramine and augmented by a specific TAAR1 antagonist**



> **Tg mice show high spontaneous electrical activity in VTA DAergic neurons, which is inhibited by p-tyramine**



Method

Visual whole-cell current-clamp recordings techniques were used to measure spontaneous spike activity of VTA dopaminergic neurons in midbrain horizontal slices (250 µm) from adult mice (B6-Tg(Taar1)27 or wt), as described in reference [9]. Dopaminergic cells were identified by large I_h current and an outward current in response to the D2 agonist quinpirole (10 µM). **EPPTB**: Ethoxy-phenyl-pyrrolidinyl-trifluoromethyl-benzamide (K_i: 0.9 ± 0.1 nM ; EC_{50}: 22 ± 12 nM; HEK293 cells stably transfected with mouse Taar1). **p-tyr**: p-tyramine (trace amine; non-selective TAAR1 agonist). Scales: 20 pA / 10 s. ***, P < 0.0001 (n = 10; Kolmogorov-Smirnov's test).

6 – Summary

Taar1 transgenic mice show:

- Generalized brain-specific **over-expression** of *Taar1*
- **Delayed and reduced locomotor activity** response to **d-amphetamine challenge** (opposite to the *Taar1*$^{-/-}$ mice [9])
- **Elevated extracellular catecholamine levels** under basal conditions in the accumbens nucleus
- **No change of extracellular dopamine and noradrenaline** concentrations in the accumbens nucleus following **d-amphetamine** challenge
- **Increased spontaneous electrical activity** of the VTA dopaminergic neurons

> These data contrast with those obtained in the *Taar1*$^{-/-}$ mice, which are hypersensitive to d-amphetamine, and further illustrate the close interaction between Taar1 and the monoaminergic system.

References:

[1] Borowsky et al. (2001) Proc Natl Acad Sci USA 98: 8966; **[2]** Lindemann & Hoener (2005) Trends Pharmacol Sci 26: 274; **[3]** Richards et al. (2003) J Neurosci 23: 8989; **[4]** Bunzow et al. (2001) Mol Parmacol 60: 1181; **[5]** Ozmen et al. (2009) Neurodegen Dis 6: 29; **[6]** Revel et al. (2007) Proc Natl Acad Sci U S A. 2007 104):13816; **[7]** Calcagno et al 2009 Int J Neuropsychopharmacol. 12: 793; **[8]** Renoldi and Invernizzi 2006 J Neurosci Res 84: 961; **[9]** Lindemann et al. (2008) J Pharm Exp Ther 324: 948.