

# The selective antagonist EPPTB reveals TAAR1-mediated regulatory mechanisms in dopaminergic neurons of the mesolimbic system

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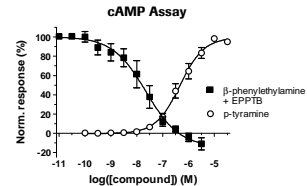
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## 1 – Introduction

Trace amine-associated receptor 1 (TAAR1) is a G protein-coupled receptor that is non-selectively activated by trace amines, endogenous metabolites of amino acids. TAAR1 is considered a promising drug target for the treatment of neuropsychiatric and neurodegenerative disorders. However, no selective agonists or antagonists to identify TAAR1-specific signaling mechanisms are available yet. Here we report the first selective TAAR1 antagonist, EPPTB, and characterize its physiological effects at dopamine neurons of the ventral tegmental area in mice.

## 2 – EPPTB, a specific antagonist at mouse TAAR1 decreases cAMP levels



**Inhibition of TAAR1-mediated increase in cAMP levels by EPPTB** (Ethoxy-phenyl-pyrrolidinyl-trifluoromethyl-benzamide) in transfected HEK293 cell.

Open circles, cAMP accumulation in response to increasing concentrations of p-tyramine ( $EC_{50} = 545 \pm 179$  nM). Data were normalized to the cAMP level obtained with 10  $\mu$ M  $\beta$ -phenylethylamine.

Filled squares, inhibition of  $\beta$ -phenylethylamine (1.5  $\mu$ M)-mediated cAMP accumulation by increasing concentrations of the antagonist EPPTB ( $IC_{50} = 27.5 \pm 9.4$  nM).

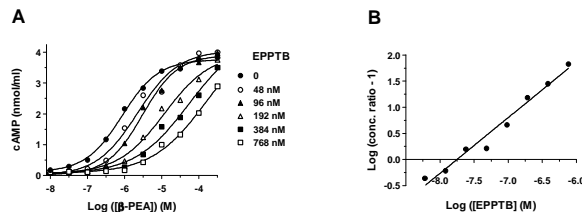
## 3 – Functional effects & binding affinities of p-tyramine & EPPTB at TAAR1

Compound	Parameter	Mouse	Rat	Human
p-tyramine	K <sub>i</sub> , binding, HEK293*	404 ± 129	70 ± 12	nd
	EC <sub>50</sub> , cAMP, HEK293**	545 ± 179	125 ± 36	1664 ± 135
	EC <sub>50</sub> , GIRK, oocytes <sup>5</sup>	167 ± 24	nd	nd
	EC <sub>50</sub> , patch-clamp, VTA slices <sup>5</sup>	305 ± 11	nd	nd
EPPTB	K <sub>i</sub> , binding, HEK293*	0.9 ± 0.1	942 ± 133	nd
	IC <sub>50</sub> , cAMP, HEK293**	22 ± 12	4540 ± 2050	7490 ± 2110
	IC <sub>50</sub> , GIRK, oocytes <sup>5</sup>	300 ± 150	nd	nd

**Comparison** of binding affinities and EC<sub>50</sub>/IC<sub>50</sub> values of p-tyramine and EPPTB at human and rodent TAAR1

\* radioligand [<sup>3</sup>H]-rac-2-(1,2,3,4-tetrahydro-1-naphthyl)-2-imidazole  
 \*\* Biotrak Enzyme Immunoassay for cAMP  
<sup>5</sup> current mediated by Kir3.1 and Kir3.2 co-expressed with TAAR1  
<sup>5</sup> current at -50 mV holding potential

## 4 – EPPTB is a competitive antagonist at mouse TAAR1



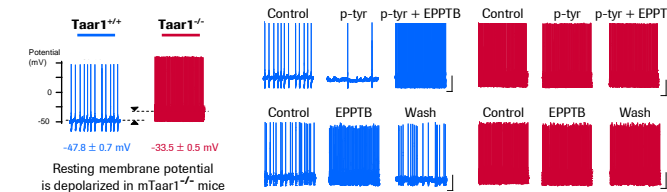
**Schild plot analysis of EPPTB in transfected HEK293**

A. Concentration-response curves for  $\beta$ -phenylethylamine ( $\beta$ -PEA) induced accumulation of cAMP under control conditions and in the presence of increasing concentrations of EPPTB.

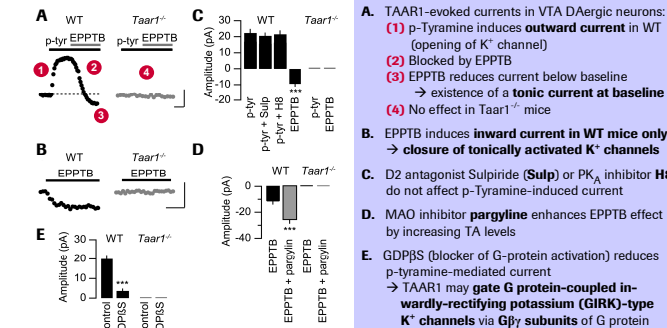
B. Schild plot derived from the concentration-response curves. Concentration ratio is the EC<sub>50</sub> for  $\beta$ -PEA in the presence of EPPTB divided by the EC<sub>50</sub> for  $\beta$ -PEA under control conditions. The slope of the line fitted to the concentration ratios is 1.065, which suggests that EPPTB is a competitive antagonist.

## 5 – TAAR1 activation inhibits firing of VTA dopaminergic neurons via GIRKs

TAAR1 inhibits spontaneous electrical activity in VTA dopaminergic neurons



TAAR1 tonically activates inwardly rectifying K<sup>+</sup> channels



A. TAAR1-evoked currents in VTA DAergic neurons:

- (1) p-Tyramine induces outward current in WT (opening of K<sup>+</sup> channel)
- (2) Blocked by EPPTB
- (3) EPPTB reduces current below baseline → existence of a tonic current at baseline
- (4) No effect in Taar1<sup>-/-</sup> mice

B. EPPTB induces inward current in WT mice only → closure of tonically activated K<sup>+</sup> channels

C. D2 antagonist Sulpiride (Sulp) or PK<sub>A</sub> inhibitor H8 do not affect p-Tyramine-induced current

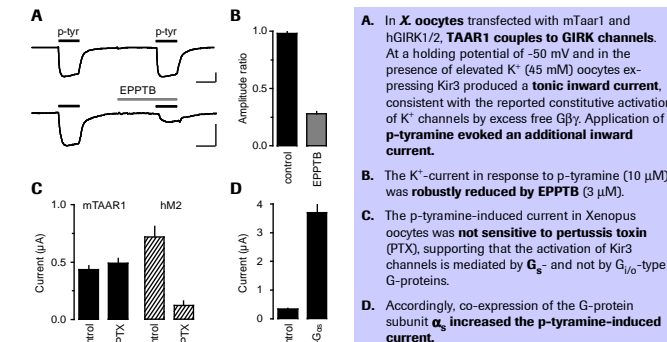
D. MAO inhibitor pargyline enhances EPPTB effect by increasing TA levels

E. GDPβS (blocker of G-protein activation) reduces p-tyramine-mediated current

→ TAAR1 may gate G protein-coupled inwardly-rectifying potassium (GIRK)-type K<sup>+</sup> channels via Gβγ subunits of G protein

## 6 – TAAR1 couples to Kir3 channels in Xenopus oocytes

TAAR1 activates Kir3 channels in X. oocytes via PTX-insensitive G-protein



A. In X. oocytes transfected with mTaar1 and hGIRK1/2, TAAR1 couples to GIRK channels.

At a holding potential of -50 mV and in the presence of elevated K<sup>+</sup> (45 mM) oocytes expressing Kir3 produced a tonic inward current, consistent with the reported constitutive activation of K<sup>+</sup> channels by excess free Gβγ. Application of p-tyramine evoked an additional inward current.

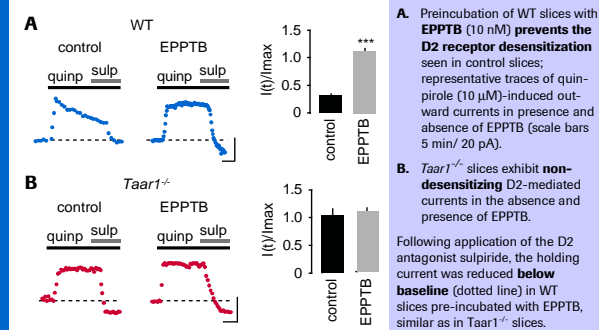
B. The K<sup>+</sup>-current in response to p-tyramine (10  $\mu$ M) was robustly reduced by EPPTB (3  $\mu$ M).

C. The p-tyramine-induced current in Xenopus oocytes was not sensitive to pertussis toxin (PTX), supporting that the activation of Kir3 channels is mediated by G<sub>s</sub>- and not by G<sub>i/o</sub>-type G-proteins.

D. Accordingly, co-expression of the G-protein subunit  $\alpha_s$  increased the p-tyramine-induced current.

## 7 – TAAR1 activity modulates D2 receptor desensitization rate

D2 receptors are constitutively activated or tonically active in absence of TAAR1 activity



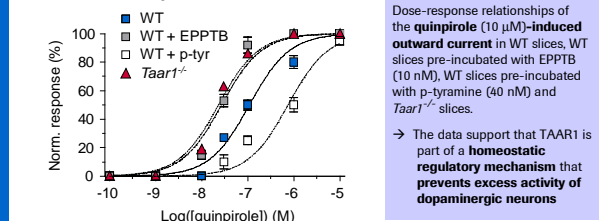
A. Preincubation of WT slices with EPPTB (10 nM) prevents the D2 receptor desensitization seen in control slices; representative traces of quinpirole (10  $\mu$ M)-induced outward currents in presence and absence of EPPTB (scale bars 5 min/ 20 pA).

B. Taar1<sup>-/-</sup> slices exhibit non-desensitizing D2-mediated currents in the absence and presence of EPPTB.

Following application of the D2 antagonist sulpiride, the holding current was reduced below baseline (dotted line) in WT slices pre-incubated with EPPTB, similar as in Taar1<sup>-/-</sup> slices.

## 8 – Increased potency of dopamine at D2 receptors in Taar1-/-

Lack of TAAR1 activity results in ~4-fold increase in agonist potency at the D2 receptor



Dose-response relationships of the quinpirole (10  $\mu$ M)-induced outward current in WT slices, WT slices pre-incubated with EPPTB (10 nM), WT slices pre-incubated with p-tyramine (40 nM) and Taar1<sup>-/-</sup> slices.

→ The data support that TAAR1 is part of a homeostatic regulatory mechanism that prevents excess activity of dopaminergic neurons

## 9 – Summary

- EPPTB is a potent & selective mouse TAAR1 antagonist (inverse agonist)
  - TAAR1 activation inhibits the electrical activity of dopaminergic neurons in the VTA by activation of GIRK
  - TAAR1 is able to activate both, adenylate-cyclase and GIRK, whereas most other G<sub>s</sub>-coupled receptors do not activate GIRK
  - TAAR1 may exhibit constitutive activity or tonic activation by endogenous agonist(s)
  - TAAR1 activity modulates D2 receptor desensitization rate (TAAR1 antagonist EPPTB inhibits desensitization in dopaminergic neurons of the VTA)
  - TAAR1 activity downmodulates D2 agonist potency in dopaminergic neurons (maybe part of a homeostatic feedback mechanism)
- These data provide evidence for a close interaction between TAAR1 and the dopaminergic system. TAAR1 - either constitutively active or stimulated by agonists - negatively modulates the firing rate of dopaminergic neurons and the release of dopamine.

References: [1] Borowsky et al. (2001) Proc Natl Acad Sci USA 98: 8966; [2] Lindemann & Hoener (2005) Trends Pharmacol Sci 26: 274; [3] Lindemann et al. (2008) J Pharm Exp Ther 324: 948