Role of the Trace Amine Associated Receptors 5 (TAAR5) in Sensorimotor Functions

Daria Kalinina
Institute of Translational Biomedicine, Saint Petersburg State University
Maria Ptukha
Institute of Translational Biomedicine, Saint Petersburg State University
Anastasia Goriainova
Institute of Translational Biomedicine, Saint Petersburg State University
Natalia Merkulyeva
Institute of Translational Biomedicine, Saint Petersburg State University
Alena Kozlova
Institute of Translational Biomedicine, Saint Petersburg State University
Anna Volnova
Institute of Translational Biomedicine, Saint Petersburg State University
Raul Gainetdinov
Institute of Translational Biomedicine, Saint Petersburg State University
Pavel Musienko (p.musienko@spbu.ru)
Institute of Translational Biomedicine, Saint Petersburg State University

Research Article

Keywords: Monoamines , critical modulators, sensorimotor neural networks, TAAR5 , ECoG

Posted Date: April 30th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-468800/v1

Version of Record: A version of this preprint was published at Scientific Reports on November 29th, 2021.
See the published version at https://doi.org/10.1038/s41598-021-02289-w.

Role of the trace amine associated receptors 5 (TAAR5) in sensorimotor functions

D.S. Kalinina [1,2], M.A. Ptukha [1], A.V. Goriainova [1], N.S. Merkulyeva [1,3], A.A. Kozlova [1], A.B. Volnova [1], R. R. Gainetdinov [1], P.E. Musienko [1,3,*]

[1]Institute of Translational Biomedicine, Saint Petersburg State University, Saint Petersburg, 199034, Russia
[2]Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, Saint Petersburg, 194223, Russia
[3]Pavlov Institute of Physiology, Russian Academy of Sciences, Saint Petersburg, 199034, Russia

* Corresponding author: Dr. Musienko Pavel, Institute of Translational Biomedicine, Saint Petersburg State University, 199034 St. Petersburg, Russia p.musienko@spbu.ru

Abstract

Monoamines are critical modulators of the sensorimotor neural networks. Using trace amine associated receptor 5 (TAAR5) knockout mice that express beta-galactosidase marker, we observed TAAR5 expression in the medial vestibular nucleus and Purkinje cells in the cerebellum suggesting that TAAR5 might be involved in gaze stabilization, vestibular and motor control. Accordingly, in various behavioral tests TAAR5-KO mice demonstrated lower endurance but better coordination and balance compared to wild type mice. Furthermore, we found specific changes in striatal local field potentials and motor cortex ECoG such as a decrease in delta andan increase in theta oscillations of power spectra respectively. The data obtained suggest that TAAR5 plays a considerable role in the regulation of postural stability, muscle force, balance and motor coordination during active movements, likely via modulation of monoaminergic systems on different levels of sensorimotor control, including brainstem, cerebellum and forebrain.

Introduction

Trace amines (TAs) are present in vertebrate CNS at very low concentrations that are several hundred times lower than those of classical biogenic amines like dopamine and serotonin [1,2]. In 2001, two research groups[3,4] independently reported the cloning and identification of a novel family of mammalian aminergic G protein-coupled receptors (GPCRs), which were later called “trace amine-associated receptors (TAARs)”, acknowledging the fact that some members are unresponsive to TAs[5]. There are data indicating that TAs and TAARs are involved in neuromodulation, regulation of metabolism and have different effects on various systems, including the central nervous system[6–9]. However, until recently all TAARs, except TAAR1, were generally considered only as olfactory receptors involved in detection of innate odors.

To date, the role of TAs and their receptors (TAARs) in the control of sensorimotor functions is poorly understood. Several studies pointed out particular effects of TAs on spinal neural networks responsible for reflex and locomotor activity[10–14]. As for TAARs, the most studied one is TAAR1, but its participation in motor activity was investigated mainly in terms of interaction with drugs such as cocaine and amphetamine[15,16]. Administration of TAAR1 ligand induces rhythmic locomotor activity of the hind limb in a spinal animal[13], and TAAR1 mRNA was also found in structures involved in sensorimotor control: the medulla oblongata, cerebellum, hippocampus, basal ganglia, and spinal cord[3]. For several years the functions of other TAARs have been studied and several of them including TAAR5, can also be related to the sensorimotor control, particularly since the localization of TAAR5 was described in the spinal cord of newborn rats[13] as well as in other structures. Our recent study has shown distribution of TAAR5 in the hippocampus, amygdala and other limbic structures[17], along with an increase in total locomotor activity of the mice with a knockout of the gene encoding TAAR5 (TAAR5-KO mice). *In the present study, we evaluated TAAR5-KO mice in a set of behavioral, electrophysiological and histochemical experiments to investigate the role of TAAR5 receptors in various aspects of motor behavior.*

Results

Expression of TAAR5 in the cerebellum and vestibular nuclei

To address the physiological role of TAAR5 *in vivo*, a targeted mouse mutant was generated in which the bases from 1 to 320 of the Taar5 coding sequence were replaced by aLacZ-coding sequence [17] (**Fig. 1a**). Histochemical staining for LacZ allows to detect cells having TAAR5 in brain sections of KO animals.

We noted a discrete and specific LacZ labeling of some motor brain regions (**Fig. 1b-d**). A clear LacZ staining (marking cells expressing TAAR5) was found within the *cerebellum* (**Fig. 1b,c**) in all TAAR5-KO mice analyzed. Bright blue stained cells having large round soma profiles (382.93±127.1 μm^2) were documented, well corresponding to the Purkinje cells[18].

Then we investigated the distribution of LacZ in the brain region related to vestibular control. Densely packed large round or oval shaped soma cells were observed in vestibular complex (**Fig. 1b,d**), mainly medial vestibular nucleus (cross-sectional average area of 388.85±49.81 μm^2). Both shape and size of labeled cells correspond well to vestibular neurons[19]. We observed absence of LacZ staining at these areas in wild type animals (data not shown).

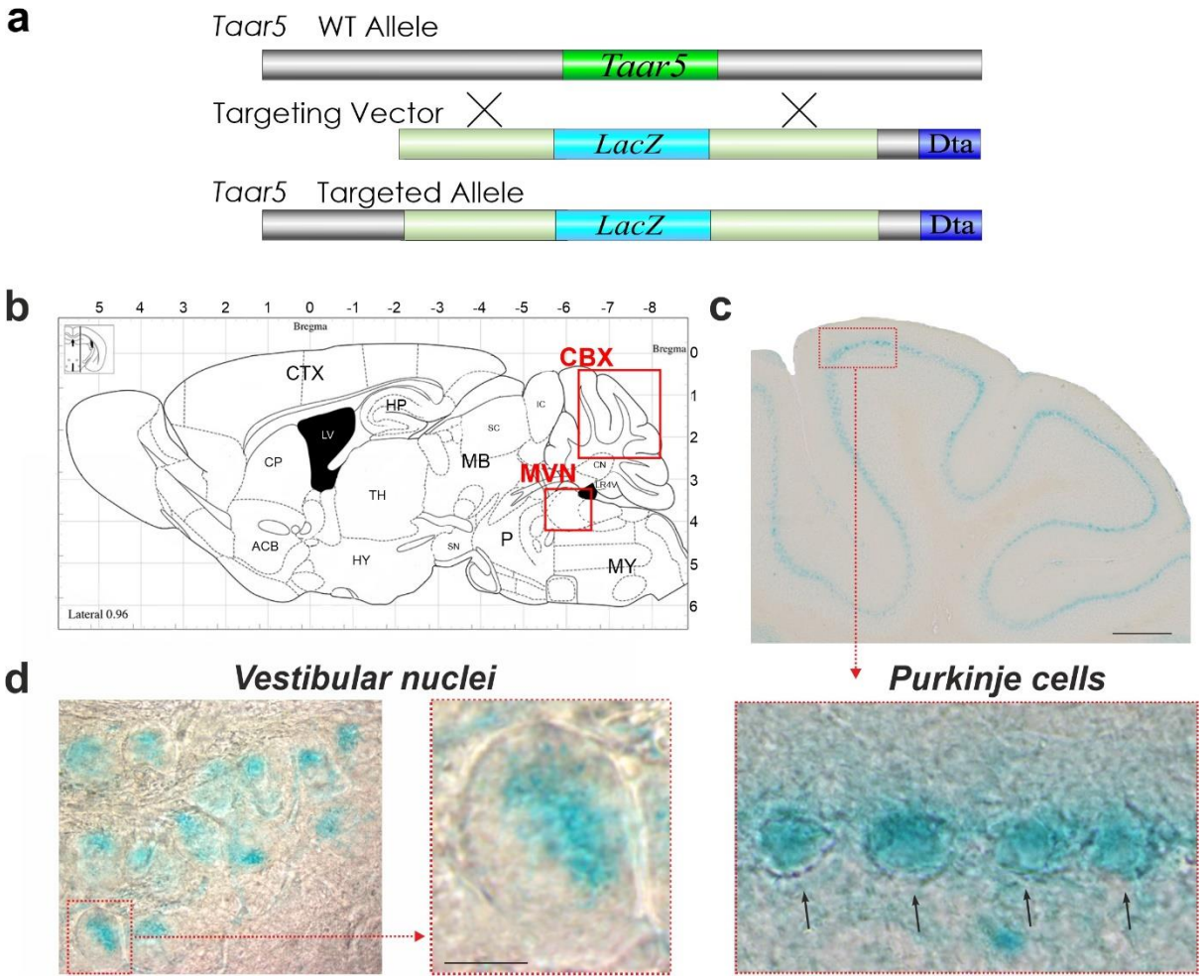

Fig. 1. *LacZ staining in the cerebellum of the TAAR5-KO mice.* ***a****. TAAR5-KO mice were generated using homologous recombination that produces TAAR5 gene inactivation through a replacement vector. The target gene is aligned with the targeting vector containing LacZ-coding sequence (see "Material and Methods" section);* ***b****. a total sagittal view of the mouse brain (modified from Paxinos and Franklin the Mouse Brain in Stereotaxic Coordinates, 2001*[49]*).* ***c****. LacZ stained neuronal profiles in the Purkinje cell layer of cerebellum (CBX). Dashed area - an enlarged view of the Purkinje cells. Calibration marker is 100 mkm.* ***d****. LacZ stained neuronal profiles in the medial vestibular nuclei (MVN). Dashed area - an enlarged view of the labeled cells. Calibration marker is 10 mkm. ACB – nucleus accumbens, CBX – cerebelum, CN - cerebellum nuclei, CP – caudate putamen, CTX - cortex, HP- hippocampus, HY - hypothalamus, IC-inferior colliculus, LR4V- lateral recess of 4th ventricle, LV – lateral ventricle, MB –midbrain, MVN - medial vestibular nuclei, MY – medulla, P - pons, SC - superior colliculus, SN -substantia nigra, TH – thalamus.*

Behavioral testing

Firstly, we used a Rotarod test allowing an assessment of motor coordination during task-required endurance. Knockout mice had a shorter time to fall compared to the WT (KO: 50.28 ± 2.036 s vs WT:76.85 ± 5.632 s, Mann-Whitney test, two-tailed, P=0.0025) possibly

reflecting less muscle strength or endurance (**Fig. 2a**). To verify this assumption, a few additional ladder tests were used: a Vertical ladder test allowing an assessment of the endurance, muscular strength and Irregular horizontal ladder test to assess complex motor coordination ability.

The Vertical ladder is aimed at assessing muscle strength. The group of knockout animals climbed slower than the wild type group (KO: 23.98 ± 2.756 s vs WT:17.01 ± 1.621 s, t-test, two-tailed, P=0.0392, t=2.181, df=24) (**Fig. 2b**). The total numbers of missteps did not differ. These results may indicate that TAAR5-KO mice are less hardy or have less muscle strength than the wild type.

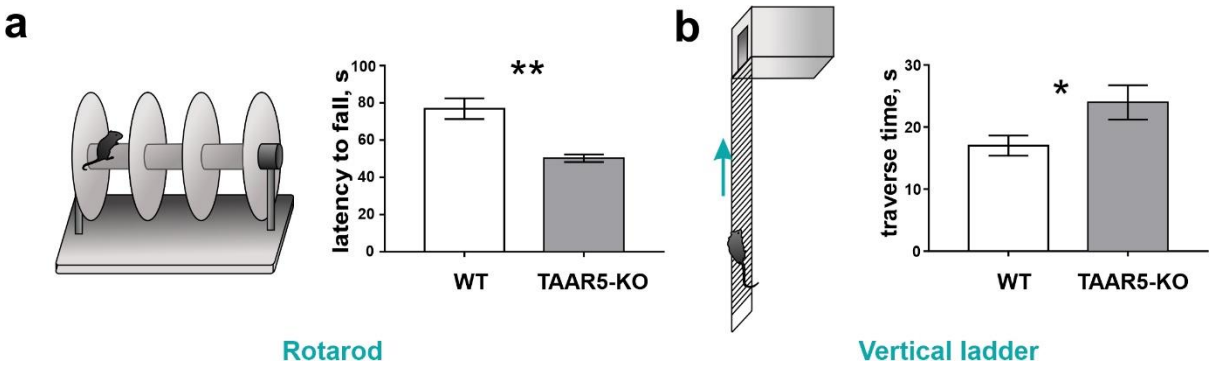

Fig. 2. *Behavioral testing of motor coordination, endurance and muscle strength in TAAR5-KO and WT mice.* ***a.*** *Latency to fall from the Rotarod.* ***b.*** *Total time to climb on the Vertical ladder. Values are presented as mean ± SEM, significance level *P < 0.05, ** - P < 0.01.*

To evaluate motor coordination and balance capacities we applied the Static rod and Vestibular challenge tests[20,21]. In the Static rod test (**Fig. 3a**) traverse time was significantly less for TAAR5-KO mice (KO: 5.16±0.59 s vs WT: 7.19±0.56 s, t-test, two-tailed, P=0.0451, t=2.260, df=11). In the Vestibular challenge test (**Fig. 3b**) both groups spent more time to traverse the beam after rotation, but TAAR5-KO mice were significantly quicker compared to WT (KO: 7.42±0.46 s vs WT: 11.68±1.74 s, t-test, two-tailed, P=0.0391, t=2.373, df=10). Thus, knockout mice are better at maintaining dynamic balance during locomotion on the beam.

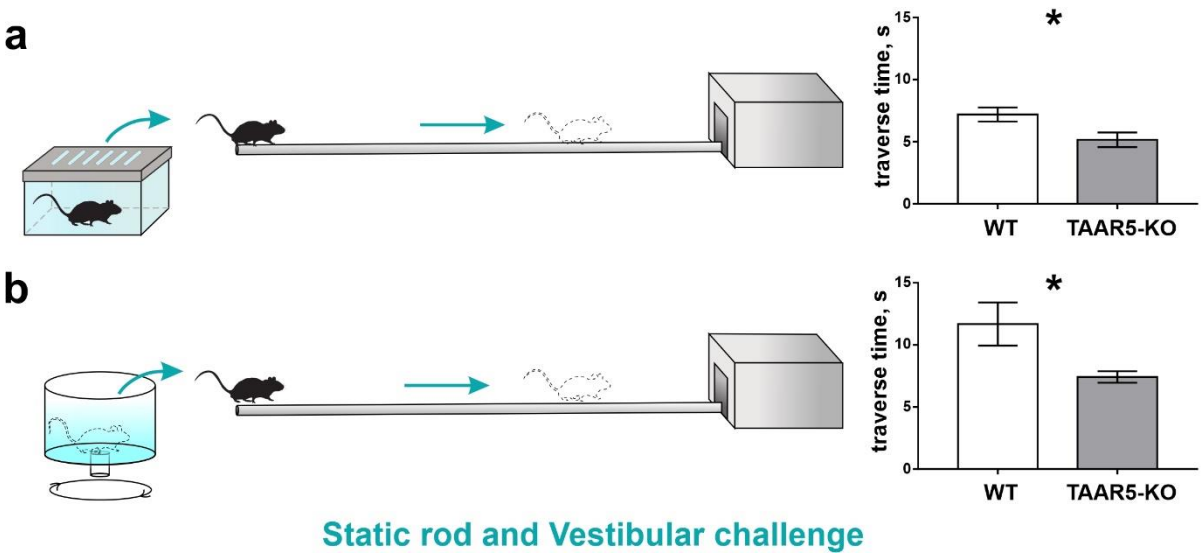

Fig. 3. *Behavioral testing of motor coordination on the rod, balance and vestibular control in TAAR5-KO and WT mice.* ***a.*** *Static rod test and traverse time for stepping on it.* ***b.*** *Vestibular challenge test and traverse time for stepping on it after 25 s rotation. Values are presented as mean ± SEM, significance level *P < 0.05.*

To assess the dynamic sensorimotor ability in changeable environment and complex interlimb coordination, required particular attention and concentration, an Irregular horizontal ladder test was used (**Fig. 4a,b**). Knockout mice had less missteps compared to WT animals (KO: 1.33±0.27% vs WT: 2.94±0.72%, Chi-square test, two-tailed, Chi-square=10.02, df=1, z=3.166, P=0.0015,). Missteps were unrelated to the limb (fore- or hindlimb, right or left limb). However, time to transfer the ladder (**Fig. 4b**) was approx. the same (KO: 17.86±1.25 vs WT: 16.15±1.75, t-test, n.s.). This may indicate better motor coordination in knockout mice.

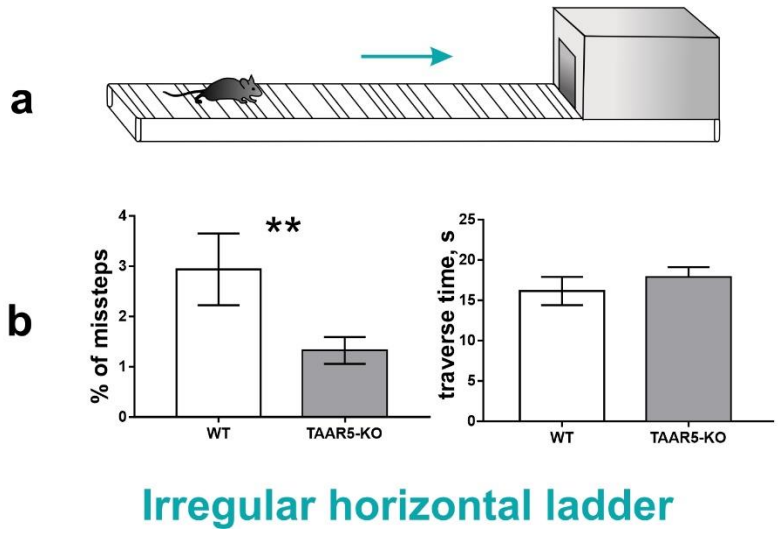

Fig. 4. *Behavioral testing of* complex interlimb coordination, required particular attention and concentration in *TAAR5-KO and WT mice.* ***a.*** *Illustration of the Irregular horizontal ladder test.* ***b.*** *The percent of the missteps and the traverse time. Missed rate of each animal was*

*expressed as percentage of total steps taken by all limbs and total time to transfer the ladder. Values are presented as mean ± SEM, significance level *P < 0.05, ** - P < 0.01.*

ECoG and striatal LFP power spectra of TAAR5-KO vs WT mice

Motor cortex ECoG and striatal LFP were recorded in awake freely moving TAAR5-KO and WT mice (**Fig. 5a,b,c**), then analyzed using the Fourier transform and compared. Power spectral density of the whole 0,9-20 Hz range was compared using two-way ANOVA.

Power spectral density of eM signal (**Fig. 5d**) was increased in TAAR5-KO mice compared to that of WT mice (KO: 0.754±0.109 vs WT: 0.565±0.098, two-way ANOVA, group factor F(1, 17209) = 233.9, P<0.0001). Sidak's post hoc multiple comparisons test showed significant differences in the range of 4,8-8,5 Hz (P<0.05), which corresponds to the theta band (**Fig. 5d**, grey area).

Analysis of striatal LFP showed significant differences between TAAR5-KO and WT (KO: 1.129±0.105 vs WT: 1.162±0.128, two-way ANOVA, group factor F(1, 17292) = 7.340, P=0.0068) (**Fig. 5e**). According to Sidak's post hoc test, significant differences were found in the 0,9-3,7 Hz range (P<0.05), which fits in the delta band range (**Fig. 5e**, grey area).

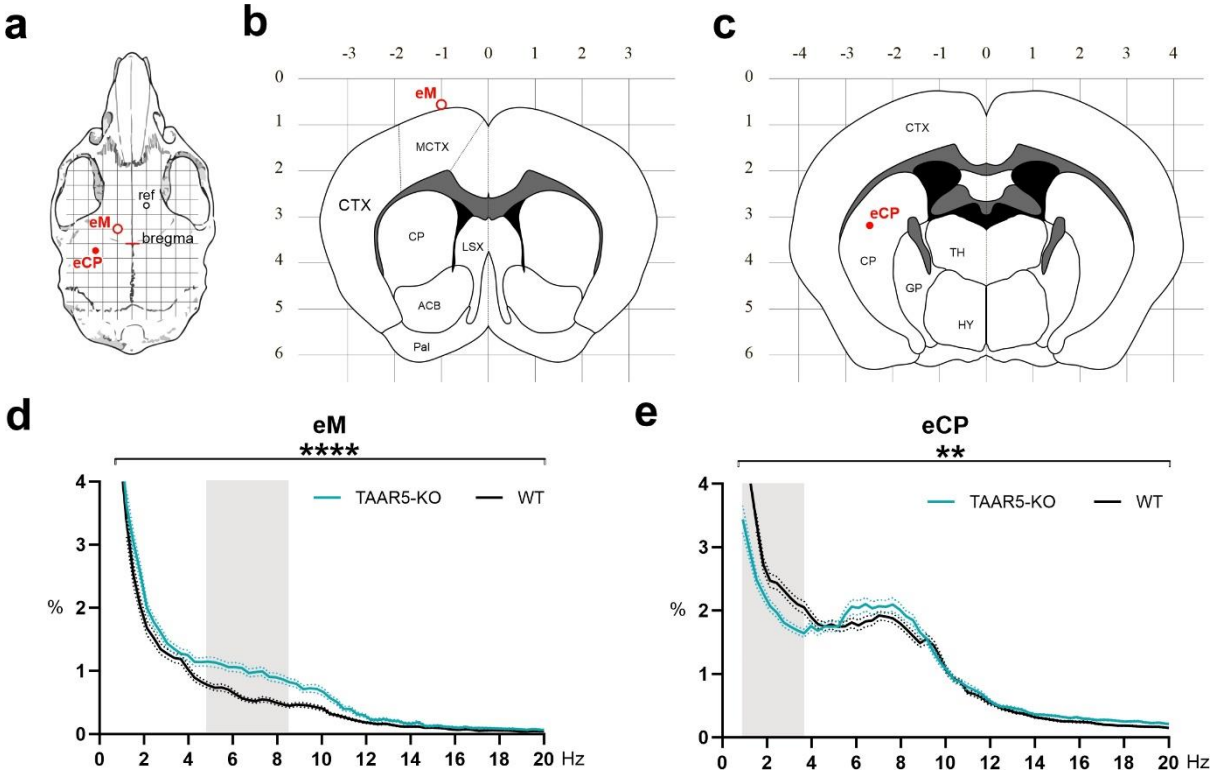

Fig. 5. *ECoG and striatal LFP.* ***a.*** *Visualization of electrode placement on the skull. Grid 1 mm.* ***b,c.*** *Visualization of eM (AP +1; ML -1) and eCP (AP -0.5; ML +2.5) electrode placements on sagittal slice 1.44 mm lateral to bregma (modified from Paxinos and Franklin the Mouse Brain in Stereotaxic Coordinates, 2001).* ***d.*** *Power spectral density of motor cortex (eM) electrocorticogram.* ***e.*** *Power spectral density of local field potentials in the striatum (eCP). X axis – signal frequency in Hz; Y axis – power spectral density in % of total density. Teal and black lines – power spectra of TAAR5-KO and WT mice respectively; dotted lines –*

*standard error of mean (SEM). **-P<0.01; ****-P<0.0001 two-way ANOVA of the whole 0,9-20 Hz range. Filled grey area – significant differences in Sidak's multiple comparisons post hoc test. eM (circle with red outline) – epidural screw above motor cortex; eCP (filled red circle) - intracerebral electrode in caudate putamen; ref – reference electrode (epidural screw), ACB – nucleus accumbens, CP – caudate putamen, CTX - cortex, GP – globus pallidus, HY - hypothalamus, LSX - Lateral septal complex, MCTX – motor cortex, Pal – pallidum, TH – thalamus.*

Discussion

Monoamines are critical modulators of neural networks of the brain and spinal cord that provide sensorimotor functions[22]. Trace amines are similar to classical monoamines (serotonin, dopamine, histamine, etc.) and have effect on their release and receptors[23]. However, the role of TAARs and especially TAAR5 in sensorimotor control is unknown. In this work, we investigated the sensorimotor functions in mice with a knockout of the gene encoding the receptor TAAR5.

Using LacZ labelling in TAAR5-KO mice we noted expression of the TAAR5 not only in the olfactory system[24], limbic brain areas processing olfactory information[17], and neurogenic zones[25] but also in the distinct motor centers of the brainstem and cerebellum. This motivated us to test sensorimotor capacities of TAAR5-KO mice in a variety of behavioral and electrophysiological tests. First, we observed significantly worse performance in the Vertical ladder (90^0) and the Rotarod test in TAAR5-KO mice, which can be related to a decrease in muscle strength or endurance. A likely reason could be alterations in the classical monoaminergic systems[17,25] and, in particular, histaminergic projections in the cerebellum of TAAR5-KO mice. This assumption can be supported by a recent study, which noted that histamine injection into the structures of the cerebellum in rats leads to an increase in motor coordination and endurance on the Rotarod[26].

Secondly, evaluation of coordination and balance in the Static rod test showed that traverse time was less in TAAR5-KO mice and they have significantly better motor capacities after rotatory vestibular stimulation compared to WT mice. We used the rotatory vestibular stimulus with short duration and low frequency proposed by Tung[27] to stimulate hair cells in the semicircular canals of the peripheral vestibular labyrinth (predominately the horizontal semicircular canal). The vestibular primary afferent fibers arrive in the central nervous system in a nuclear complex, which forms the vestibular nuclei. It has been noted that they also receive other sensory modalities and especially proprioceptive input, and the main tract projecting to vestibular nuclei originates in the cerebellum[28]. The largest part of the vestibular complex is the medial vestibular nucleus (MVN)[29]; it is related to the vestibulo-ocular and vestibulospinal reflexes integrating inputs coming from vestibular and neck receptors and having motor output to the oculomotor nuclei and upper cervical cord[30]. Both play a key role in restoration of vestibular control after the vestibular perturbation test in the current study. Based on the results showing better motor coordination of TAAR5-KO mice in "Static rod test" and "Vestibular challenge", and beta-galactosidase labeling in the MVN, it can be suggested that TAAR5 may be involved in monoaminergic modulation that regulates balance and vestibular control. Dopaminergic projections have not been shown

morphologically but MVN contained meaningful amounts of dopamine[31] and D2 receptors have been detected mostly in the MVN[32]. It was suggested that histamine and dopamine modulate MVN excitability. Vestibular nuclei activity is under the control of tonic inhibition regulated by dopamine[30]. In particular, dopamine could be crucial in regulating the commissural pathways between vestibular nuclei and, in turn, static reflexes.

Furthermore, we found that TAAR5-KO mice have better complex motor coordination (a lower percentage of errors) during walking on the Irregular horizontal ladder, which requires spatial attention. These changes can be associated with the modulation of the serotonergic systems. Recent studies have shown improved motor skills in the balance beam test in mice with a knockout gene encoding 5-HT1d serotonin receptors[33]. These receptors are localized in the striatum [34], where a decrease in serotonin content was shown in TAAR5-KO mice lately[17].

Theta-rhythm, an increase in power density of which we observed in the cortex, is widely known to be associated with spatial attention and spatial memory as well as with specific types of locomotor activity deemed exploratory behavior[35–37]. Power of theta oscillations is thought to be modulated by serotoninergic projections: activation of 5-HT2c receptors and serotonin reuptake inhibition both lead to suppression of theta rhythm[38,39]. A significant decrease in tissue levels of serotonin observed in hippocampus of TAAR5-KO mice[17] may, therefore, be the cause for an increase in power in the theta range, since theta oscillations recorded above the motor cortex are almost certainly generated by the hippocampus[40].

We also observed a decrease in delta power spectral density in the striatum. Although delta oscillations can frequently be observed during sleep and under anesthesia, they are also known to correlate with several cognitive processes, such as motivation, attention and concentration[41,42], which are important for motor coordination and balance ability. Delta rhythm is known to occur during decision-making and may play a role in suppressing exploratory behavior[41,43]. Oscillations in the 0,5-4 Hz range are also considered to be a robust marker of dopamine depletion, particularly in basal ganglia, and correlate with motor dysfunction[44]. In case of TAAR5-KO mice, we have previously observed an increase in dopamine tissue levels in the striatum[25], which could lead to a decrease in power of delta-range oscillations, as well as to a change in sensorimotor control and improvement of motor coordination in these mice.

It is important to note that both the decrease in delta and the increase in theta oscillations may be indicative of higher exploratory drive in TAAR5-KO mice. Given that delta oscillations mainly occur when exploration is suppressed, and that theta rhythm, on the contrary, emerges during exploratory behavior, our data suggests increased locomotor/exploratory activity, which has been previously reported[17] and may play a critical role in complex motor function.

As has been pointed out earlier[17], we confirm here that TAAR5 (and likely TAARs in general) is not just ‘‘an olfactory receptor’’ involved in sensing innate odors[45] but is also involved in neuromodulation of sensorimotor controlin the brainstem, cerebellum and forebrain. Taking into account the specific effects in musculoskeletal systems as well as our previous finding of activation of adult neurogenesis[25], we consider the potential utility of future TAAR5-based agents as an approach to pharmacotherapy of neuromotor disorders.

Further detailed investigations are warranted to evaluate this possibility. Finally, given the close similarity between members of TAAR family, it would be important to evaluate the role of other TAARs in sensorimotor functions. Intriguingly, expression of other TAARs (TAAR1, TAAR8) has been previously reported in the mouse and human cerebellum[3,46].

Conclusion

To sum up, we showed that TAAR5-KO mice have a specific motor phenotype characterized by a decrease in muscle force, an improvement in balance and motor coordination that is related to the distribution of the TAAR5 in vestibular nuclei, cerebellum, as well as altered striatal delta- and cortical theta-oscillations. These results demonstrate participation of TAAR5 in the control of sensorimotor activity that can be due to the modulating effect of TAAR5 on the dopaminergic, serotonergic and potentially other aminergic systems, which carry out supraspinal regulation of neural networks involved in the control of postural and locomotor functions.

Methods

Animals

Behavioral testing and electrophysiological recordings were performed on male mice, while histochemical experiments were carried out on mice of both genders. The TAAR5-KO mice expressing beta-galactosidase mapping TAAR5 expression were generated by Deltagen Incorporation (San Mateo, CA, USA) and distributed by the NIH Knockout Mouse Project (KOMP). Breeding and genotyping of TAAR5-KO and wild type (WT) mice was performed as described[17]. All procedures involving animals and their care were approved by the Ethics Committee of St. Petersburg State University, St. Petersburg, Russia (approval number 131-03-4 and 131-03-5). All procedures were conducted under Russian legislation according to Good Laboratory Practice standards (directive # 267 from 19.06.2003 of the Ministry of Health of the Russian Federation) and in compliance with ARRIVE guidelines.

The mice were housed three to five per cage before the operating procedure and individually after the procedure. Standard lab conditions were maintained (12 h light/dark cycle, 21 ± 1°C and 40–70% humidity), with food and water provided *ad libitum*. All experiments were conducted during the light phase.

Immunohistochemistry

TAAR5 expression was analyzed by the use of the LacZ reporter gene inserted (**Fig. 1a**). All procedures for the LacZ labelling and visualization were documented previously[17,25]. In brief, under deep anesthesia (animals were pre-anesthetized with isoflurane and anesthetized with urethane at the dose of 2 g/kg), animals were sacrificed by decapitation. Thereafter, the brain was removed from the skull, rinsed in phosphate-buffered saline (PBS) 3 times and immediately fixed overnight in 4% paraformaldehyde (PFA). Frontal and sagittal

slices (40 mkm) were prepared using microtome Leica CM-3050S (Wetzlar, Germany). Both free-floating and attached slices were used for the histochemical evaluation of the LacZ. Slices were rinsed in 0.02% NP40 (Sigma, ab142227) and 2mM magnesium chloride in PBS, and thereafter incubated overnight in the staining solution contained 1 mg/ml X-gal (5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside, Sigma, B4252), 5mM potassium ferri- (K3Fe(CN)6, Sigma, 244023), 5mM ferro-cyanide (K4Fe(CN)6, Sigma, 455989) and 2mM MgCl2, at +37°C. Slices were cover-slipped with DPX (Sigma-Aldrich), and thereafter processed using Olympus BX51 microscope (Olympus Corporation, Japan). LacZ labeled cells were blue stained.

Behavioral testing

Rotarod (**Fig. 2a**) is used to assess motor coordination and balance, as well as endurance of the animal[21,47]. Stepwise speed increase from 10 rpm to 30 rpm (every 5 seconds by 5 rpm) was performed, after which a speed of 30 rpm was maintained *(Neurobotauпpics, Russia)*. The time of fall for each mouse was noted. The Rotarod test was performed on two groups of mice: TAAR5-KO (n = 7), WT (n = 5).

Vertical ladder (**Fig. 2b**) allows to evaluate muscle strength and endurance of the mouse and is a simpler modification of vertical grid test[48]. The ladder is 50 cm in height, and it has 90° degrees of inclination and metal rungs (3 mm diameter) with a distance of 1 cm between rungs. The mouse was placed on the first bottom rung and the time spent to climb as well as the missteps were noted. The test had 2 trials and the animals rested for 1 minute between trials. The Vertical ladder test was performed on two groups of mice: TAAR5-KO (n = 13), WT (n = 13).

Static rod and Vestibular challenge. In addition, we evaluated motor coordination and balance capacities of the mice by Static rod and Vestibular challenge tests[20,21]. A 100 cm long rod was placed 80 cm above the floor in such a way that one tip was fixed under the goal box ("fixed tip") and the other one was suspended ("suspended tip"). The fixed tip had a mark 10 cm distally from the target box ("finish point"). Mice were placed on a suspended tip with nose oriented towards the goal box and for Static rod test the traverse time (**Fig. 3a**) was registered (without any disturbance). To assess the impairment in vestibular control the Vestibular challenge test (**Fig. 3b**) was used[27]. The mouse was placed inside a plexiglass rotating box. A 3 Hz clockwise rotation lasting for 25 s was used to disturb the vestibular system. After such perturbations, the animal was placed on the rod and traverse time was examined (from the end of rotation to crossing the "finish point"). The Static rod and Vestibular challenge tests were performed on two groups of mice: TAAR5-KO (n = 7), WT (n = 6).

Irregular horizontal ladder (50 cm length) with irregularly located round rungs (**Fig. 4a**) was used to evaluate complex sensorimotor coordination, intricate combination of fore and hind limbs that requires particular attention and concentration. The test was repeated 3 times, but every trial had a different pattern of rung location. The correct steps and full missteps of all four limbs were counted. The Irregular horizontal ladder test was performed on two groups of mice: TAAR5-KO (n = 14), WT (n = 13).

All quantitative data obtained in the behavioral testing are presented as mean ± SE. Student's t-test was used for normally distributed data (Shapiro-Wilk normality test), for others - Manna-Whitney U-test.

Chronic electrophysiological recordings

For electrophysiological studies 11 animals were used: TAAR5-KO (n=5) and WT (n=6) mice.

Surgical procedure. Two types of electrodes were used: epidural screws for electrocorticogram (ECoG) recordings (0,5 mm in diameter; 1 mm in length; steel) and intracerebral electrodes for local field potential (LFP) recordings (50 μm in diameter; 1,2 mm/3,2 mm in length; tungsten wire in perfluoroalkoxy polymer isolation (795500, A-M Systems)). For each animal, three electrodes were implanted under general anesthesia (200 mg/kg Zoletil intraperitoneally; Xylazine 0.2mg/kg intramuscularly): epidural reference electrode (2 mm anterior to bregma and 1 mm lateral to midline); motor cortex (eM) epidural electrode (1 mm anterior to bregma and 1 mm lateral to midline); striatal (eCP) intracerebral electrode (3,2 mm in length, 0,5 mm posterior to bregma and 2,5 mm lateral to midline)[49] (**Fig. 5a,b**). Electrodes were placed using a micromanipulator in a stereotaxic frame. Electrodes were fixed on the skull with dental cement (Acrodent, JSC Ctoma, Ukraine). Three days after electrode implantation, 3 experiments were conducted on each animal no more frequently than every other day.

ECoG and LFP recordings. Experimental setting for electrophysiological recordings consisted of an amplifier (x1000 gain, USF-8; Beta Telecom), analog-to-digital converter L-791 (L-CARD,) and Bioactivity Recorder v5.44 software (D.A. Sibarov, Biotechnologies). During the recording process the animal was placed in a 20x20x25 cm plexiglas box, which along with the amplifier was located in an isolated grounded setting. Signal was band-pass filtered between 0.1 and 200 Hz, and digitized with 2500 samples per second per channel. Brain activity of TAAR5-KO and WT groups was recorded for 20 minutes in awake freely moving mice, 3 times for each animal on different days. These recordings were then used to compare spectral characteristics of TAAR5-KO and WT mice electrophysiological activity.

Data analysis. Using the Bioactivity Recorder software, 12 epochs without artifacts, 20 seconds in length, were picked out from each recording, then Fourier transform was performed using Clampfit 10.2.0.16 software (MDS Analytical Technologies). The resulting power spectra (frequency in Hz, power spectral density in mkV^2) of WT mice were compared to those of TAAR5-KO mice. The resulting data was in the 0,9-20 Hz range after data in the 0-0,9 range was excluded due to abundance of artifacts. The data was analyzed after normalization, which transforms all values from one data set into percentages of the sum of all power spectra values, which was necessary for standardization across the group. All data were tested for Gaussian distribution with the use of the Shapiro-Wilk normality test. All data were analyzed using two-way ANOVA (analysis of variance). Sidak's multiple comparisons test was performed in order to distinguish the most prominent differences. All statistical analyses were performed by using GraphPad Prism 8 (GraphPad Software, Inc., San Diego, CA, USA). All data is presented as mean ± standard error (SEM).

References:

1. Boulton, A. A. Letter: Amines and theories in psychiatry. *Lancet (London, England)* **2**, 52–53 (1974).
2. Berry, M. D. Mammalian central nervous system trace amines. Pharmacologic amphetamines, physiologic neuromodulators. *J. Neurochem.* **90**, 257–271 (2004).
3. Borowsky, B. *et al.* Trace amines: identification of a family of mammalian G protein-coupled receptors. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 8966–8971 (2001).
4. Bunzow, J. R. *et al.* Amphetamine, 3,4-methylenedioxymethamphetamine, lysergic acid diethylamide, and metabolites of the catecholamine neurotransmitters are agonists of a rat trace amine receptor. *Mol. Pharmacol.* **60**, 1181–1188 (2001).
5. Lindemann, L. *et al.* Trace amine-associated receptors form structurally and functionally distinct subfamilies of novel G protein-coupled receptors. *Genomics* **85**, 372–385 (2005).
6. Adriaenssens, A. *et al.* A Transcriptome-Led Exploration of Molecular Mechanisms Regulating Somatostatin-Producing D-Cells in the Gastric Epithelium. *Endocrinology* **156**, 3924–3936 (2015).
7. Berry, M. D., Gainetdinov, R. R., Hoener, M. C. & Shahid, M. Pharmacology of human trace amine-associated receptors: Therapeutic opportunities and challenges. *Pharmacol. Ther.* **180**, 161–180 (2017).
8. Mazzoli, R. & Pessione, E. The Neuro-endocrinological Role of Microbial Glutamate and GABA Signaling. *Front. Microbiol.* **7**, 1934 (2016).
9. Raab, S. *et al.* Incretin-like effects of small molecule trace amine-associated receptor 1 agonists. *Mol. Metab.* **5**, 47–56 (2016).
10. Ono, H., Ito, H. & Fukuda, H. 2-phenylethylamine and methamphetamine enhance the spinal monosynaptic reflex by releasing noradrenaline from the terminals of descending fibers. *Jpn. J. Pharmacol.* **55**, 359–366 (1991).
11. Reddy, S. V, Maderdrut, J. L. & Yaksh, T. L. Spinal cord pharmacology of adrenergic agonist-mediated antinociception. *J. Pharmacol. Exp. Ther.* **213**, 525–533 (1980).
12. Kitazawa, T., Saito, K. & Ohga, A. Effects of catecholamines on spinal motoneurones and spinal reflex discharges in the isolated spinal cord of the newborn rat. *Brain Res.* **351**, 31–36 (1985).
13. Gozal, E. *et al.* Anatomical and functional evidence for trace amines as unique modulators of locomotor function in the mammalian spinal cord . *Frontiers in Neural Circuits* vol. 8 134 (2014).
14. Hochman, S. Metabolic recruitment of spinal locomotion: intracellular neuromodulation by trace amines and their receptors. *Neural Regen. Res.* **10**, 1940–1942 (2015).
15. Lindemann, L. *et al.* Trace Amine-Associated Receptor 1 Modulates Dopaminergic Activity. *J. Pharmacol. Exp. Ther.* **324**, 948 LP – 956 (2008).
16. Liu, J. F., Thorn, D. A., Zhang, Y. & Li, J. X. Effects of Trace Amine-associated Receptor 1 Agonists on the Expression, Reconsolidation, and Extinction of Cocaine Reward Memory. *Int. J. Neuropsychopharmacol.* **19**, (2016).
17. Espinoza, S. *et al.* Trace Amine-Associated Receptor 5 Provides Olfactory Input Into Limbic Brain Areas and Modulates Emotional Behaviors and Serotonin Transmission. *Front. Mol. Neurosci.* **13**, 18 (2020).
18. McKay, B. E. & Turner, R. W. Physiological and morphological development of the rat cerebellar Purkinje cell. *Journal of Physiology* vol. 567 829–850 (2005).
19. Suárez, C. *et al.* Morphometric Analysis of the Vestibular Complex in the Rat. *Laryngoscope* **103**, 762???773 (1993).

20. Brooks, S. P. & Dunnett, S. B. Tests to assess motor phenotype in mice: a user's guide. *Nat. Rev. Neurosci.* **10**, 519–529 (2009).
21. Deacon, R. M. J. Measuring motor coordination in mice. *J. Vis. Exp.* e2609 (2013) doi:10.3791/2609.
22. VanderHorst, V. G. J. M. & Ulfhake, B. The organization of the brainstem and spinal cord of the mouse: Relationships between monoaminergic, cholinergic, and spinal projection systems. *J. Chem. Neuroanat.* **31**, 2–36 (2006).
23. Gainetdinov, R. R., Hoener, M. C. & Berry, M. D. Trace Amines and Their Receptors. *Pharmacol. Rev.* **70**, 549 LP – 620 (2018).
24. Liberles, S. D. Trace amine-associated receptors: ligands, neural circuits, and behaviors. *Curr. Opin. Neurobiol.* **34**, 1–7 (2015).
25. Efimova, E. V *et al.* Increased dopamine transmission and adult neurogenesis in trace amine-associated receptor 5 (TAAR5) knockout mice. *Neuropharmacology* **182**, 108373 (2021).
26. Li, B., Zhu, J. N. & Wang, J. J. Histaminergic afferent system in the cerebellum: Structure and function. *Cerebellum and Ataxias* vol. 1 5 (2014).
27. Tung, V. W. K., Burton, T. J., Dababneh, E., Quail, S. L. & Camp, A. J. Behavioral assessment of the aging mouse vestibular system. *J. Vis. Exp.* (2014) doi:10.3791/51605.
28. Brodal, A. Vestibulocerebellar Input in the Cat: Anatomy. *Prog. Brain Res.* **37**, 315–327 (1972).
29. Romand, R. & Varela-Nieto, I. *Development of Auditory and Vestibular Systems*. (Academic Press, 2014). doi:10.1016/C2012-0-03648-4.
30. Vidal, P. P. *et al.* Chapter 28 - The Vestibular System. in (ed. Paxinos, G. B. T.-T. R. N. S. (Fourth E.) 805–864 (Academic Press, 2015). doi:https://doi.org/10.1016/B978-0-12-374245-2.00028-0.
31. Cransac, H., Cottet-Emard, J. M., Pequignot, J. M. & Peyrin, L. Monoamines (norepinephrine, dopamine, serotonin) in the rat medial vestibular nucleus: endogenous levels and turnover. *J. Neural Transm.* **103**, 391–392 (1996).
32. Yokoyama, C., Okamura, H., Nakajima, T., Taguchi, J.-I. & Ibata, Y. Autoradiographic distribution of [3H]YM-09151-2, a high-affinity and selective antagonist ligand for the dopamine D2 receptor group, in the rat brain and spinal cord. *J. Comp. Neurol.* **344**, 121–136 (1994).
33. Enjin, A. *et al.* Sensorimotor function is modulated by the serotonin receptor 1d, a novel marker for gamma motor neurons. *Mol. Cell. Neurosci.* **49**, 322–332 (2012).
34. Mengod, G., Cortés, R., Vilaró, M. T. & Hoyer, D. Distribution of 5-HT Receptors in the Central Nervous System. in *Handbook of Behavioral Neuroscience* vol. 21 123–138 (Elsevier, 2010).
35. Buzsaki, G. Theta oscillations in the hippocampus. *Neuron* **33**, 325–340 (2002).
36. McNaughton, N., Ruan, M. & Woodnorth, M. A. Restoring theta-like rythmicity in rats restores initial learning in the Morris water maze. *Hippocampus* **16**, 1102–1110 (2006).
37. Goutagny, R., Jackson, J. & Williams, S. Self-generated theta oscillations in the hippocampus. *Nat. Neurosci.* **12**, 1491–1493 (2009).
38. Kudina, T. A., Sudnitsyn, V. V, Kutyreva, E. V & Kichigina, V. F. The serotonin reuptake inhibitor fluoxetine suppresses theta oscillations in the electroencephalogram of the rabbit hippocampus. *Neurosci. Behav. Physiol.* **34**, 929–933 (2004).
39. Sörman, E., Wang, D., Hajos, M. & Kocsis, B. Control of hippocampal theta rhythm by serotonin: role of 5-HT2c receptors. *Neuropharmacology* **61**, 489–494 (2011).
40. Lalla, L., Rueda Orozco, P. E., Jurado-Parras, M.-T., Brovelli, A. & Robbe, D. Local

or Not Local: Investigating the Nature of Striatal Theta Oscillations in Behaving Rats. *eNeuro* **4**, ENEURO.0128-17.2017 (2017).
41. Harmony, T. The functional significance of delta oscillations in cognitive processing. *Front. Integr. Neurosci.* **7**, 83 (2013).
42. Knyazev, G. G. EEG delta oscillations as a correlate of basic homeostatic and motivational processes. *Neurosci. Biobehav. Rev.* **36**, 677–695 (2012).
43. Nácher, V., Ledberg, A., Deco, G. & Romo, R. Coherent delta-band oscillations between cortical areas correlate with decision making. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 15085–15090 (2013).
44. Whalen, X. T. C., Willard, A. M., Rubin, J. E. & Gittis, A. H. Delta oscillations are a robust biomarker of dopamine depletion severity and motor dysfunction in awake mice. *J. Neurophysiol.* **124**, 312–359 (2020).
45. Liberles, S. D. & Buck, L. B. A second class of chemosensory receptors in the olfactory epithelium. *Nature* **442**, 645–650 (2006).
46. Chiellini, G. *et al.* Distribution of exogenous [125I]-3-iodothyronamine in mouse in vivo: Relationship with trace amine-associated receptors. *J. Endocrinol.* **213**, 223–230 (2012).
47. Jones, B. J. & Roberts, D. J. The quantitative measurement of motor inco-ordination in naive mice using an accelerating rotarod. *J. Pharm. Pharmacol.* **20**, 302–304 (1968).
48. Kim, S. T., Son, H. J., Choi, J. H., Ji, I. J. & Hwang, O. Vertical grid test and modified horizontal grid test are sensitive methods for evaluating motor dysfunctions in the MPTP mouse model of Parkinson's disease. *Brain Res.* **1306**, 176–183 (2010).
49. Paxinos, G. & Franklin, K. B. J. *The Mouse Brain in Stereotaxic Coordinates*. (Academic Press, 2001).

Funding

This work was performed within project ID: 73025408 of the St. Petersburg State University, St. Petersburg, Russia (for D.K., A.G., N.M. and behavioral testing), supported by the state assignment of Ministry of Science and Higher Education of the Russian Federation AAAA-A18-118012290373-7 (for data analysis), by the Russian Science Foundation grant 19-75-30008 (for electrophysiological testing).

Author contributions statement

D.K., R.G. and P.M. conceived and designed the experiments; D.K. and A.G. obtained and analyzed the experimental behavioral data, A.V. and M.P. performed electrophysiological research, A.K. and N.M. provided the histochemical part. The manuscript was written by D.K., M. P and P.M. All authors revised and approved the final manuscript. P.M. supervised the study.

Figures

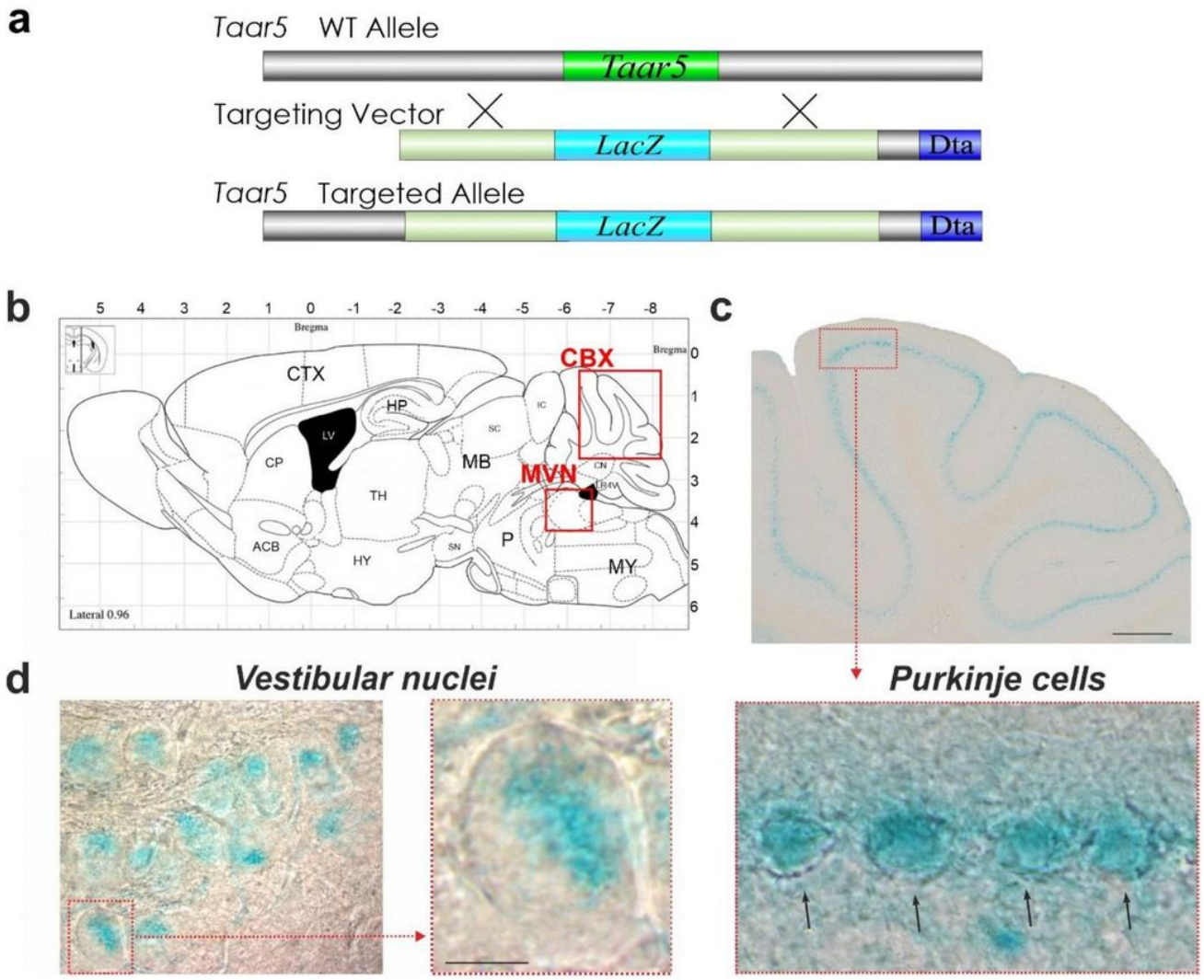

Figure 1

LacZ staining in the cerebellum of the TAAR5-KO mice. a. TAAR5-KO mice were generated using homologous recombination that produces TAAR5 gene inactivation through a replacement vector. The target gene is aligned with the targeting vector containing LacZcoding sequence (see “Material and Methods” section); b. a total sagittal view of the mouse brain (modified from Paxinos and Franklin the Mouse Brain in Stereotaxic Coordinates, 200149). c. LacZ stained neuronal profiles in the Purkinje cell layer of cerebellum (CBX). Dashed area - an enlarged view of the Purkinje cells. Calibration marker is 100 mkm. d. LacZ stained neuronal profiles in the medial vestibular nuclei (MVN). Dashed area - an enlarged view of the labeled cells. Calibration marker is 10 mkm. ACB – nucleus accumbens, CBX – cerebelum, CN - cerebellum nuclei, CP – caudate putamen, CTX - cortex, HPhippocampus, HY - hypothalamus, IC-inferior colliculus, LR4V- lateral recess of 4th ventricle, LV – lateral ventricle, MB –midbrain, MVN - medial vestibular nuclei, MY – medulla, P - pons, SC - superior colliculus, SN -substantia nigra, TH – thalamus.

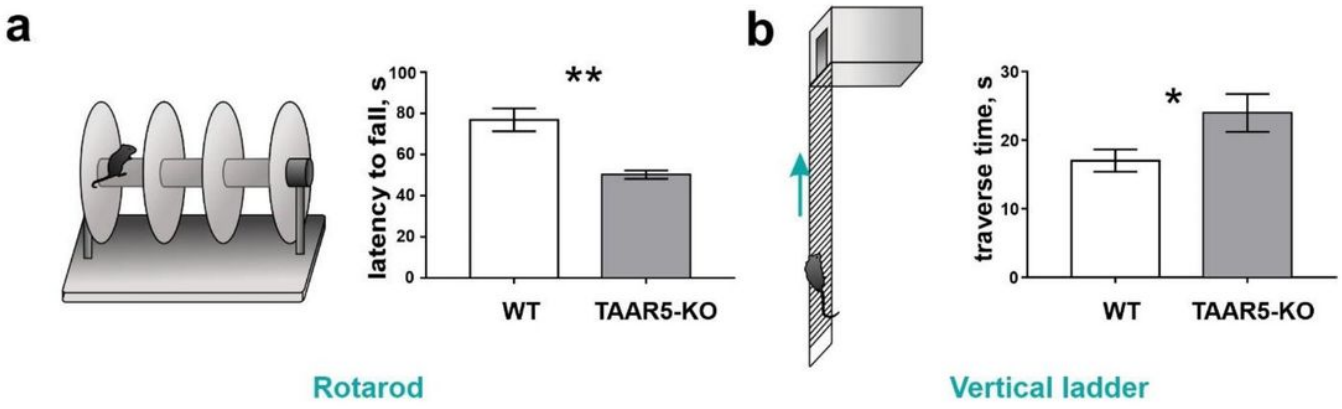

Figure 2

Behavioral testing of motor coordination, endurance and muscle strength in TAAR5- KO and WT mice. a. Latency to fall from the Rotarod. b. Total time to climb on the Vertical ladder. Values are presented as mean ± SEM, significance level *P < 0.05, ** - P < 0.01.

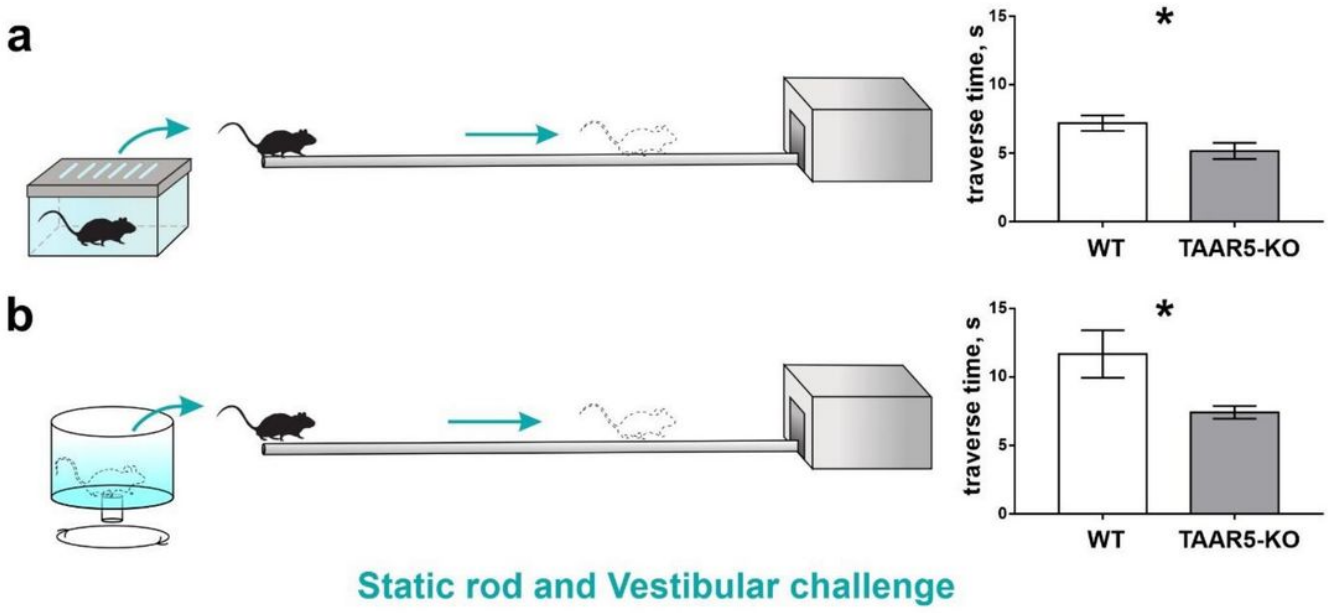

Figure 3

Behavioral testing of motor coordination on the rod, balance and vestibular control in TAAR5-KO and WT mice. a. Static rod test and traverse time for stepping on it. b. Vestibular challenge test and traverse time for stepping on it after 25 s rotation. Values are presented as mean ± SEM, significance level *P < 0.05.

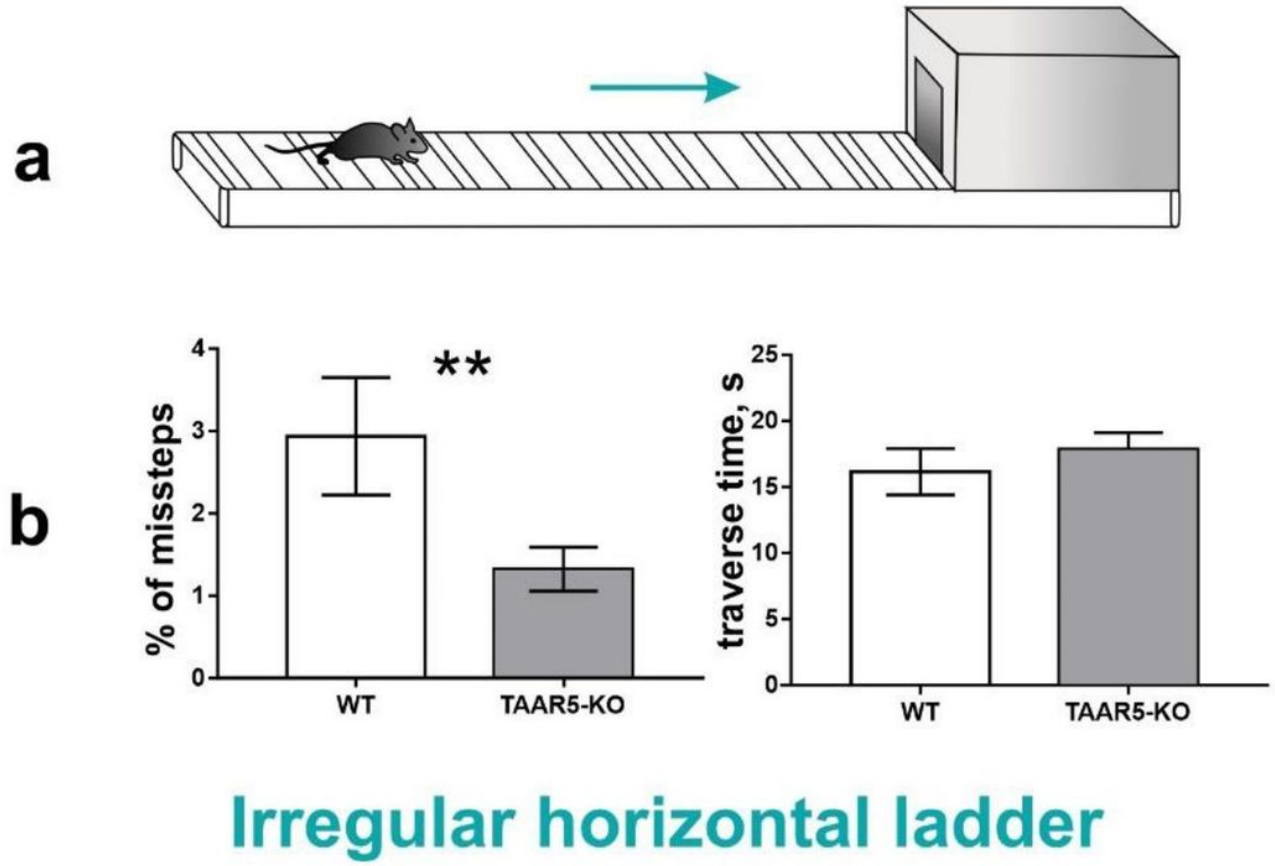

Figure 4

Behavioral testing of complex interlimb coordination, required particular attention and concentration in TAAR5-KO and WT mice. a. Illustration of the Irregular horizontal ladder test. b. The percent of the missteps and the traverse time. Missed rate of each animal was expressed as percentage of total steps taken by all limbs and total time to transfer the ladder. Values are presented as mean ± SEM, significance level *P < 0.05, ** - P < 0.01.

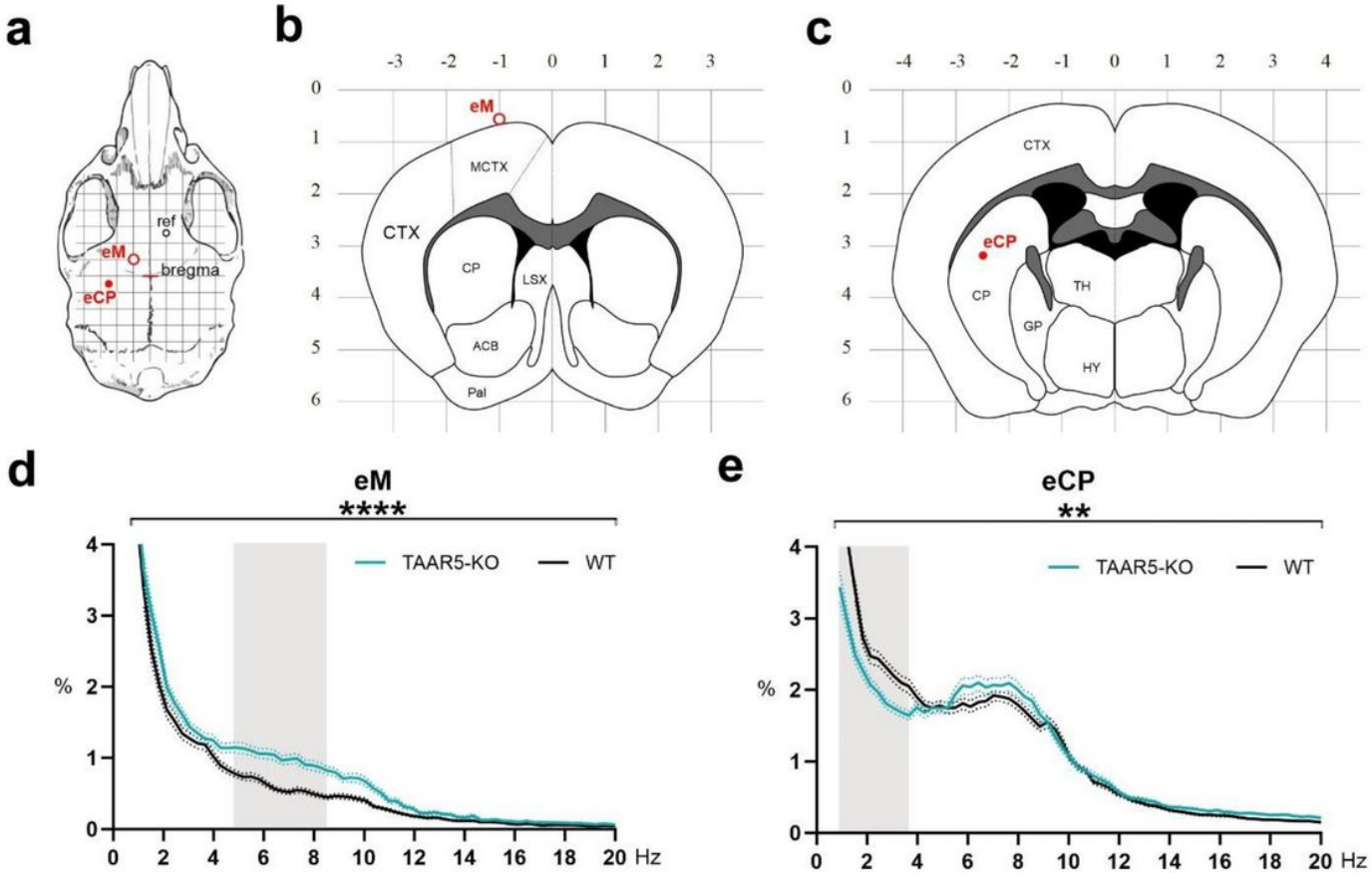

Figure 5

ECoG and striatal LFP. a. Visualization of electrode placement on the skull. Grid 1 mm. b,c. Visualization of eM (AP +1; ML -1) and eCP (AP -0.5; ML +2.5) electrode placements on sagittal slice 1.44 mm lateral to bregma (modified from Paxinos and Franklin the Mouse Brain in Stereotaxic Coordinates, 2001). d. Power spectral density of motor cortex (eM) electrocorticogram. e. Power spectral density of local field potentials in the striatum (eCP). X axis – signal frequency in Hz; Y axis – power spectral density in % of total density. Teal and black lines – power spectra of TAAR5-KO and WT mice respectively; dotted lines – standard error of mean (SEM). **-P<0.01; ****-P<0.0001 two-way ANOVA of the whole 0,9-20 Hz range. Filled grey area – significant differences in Sidak's multiple comparisons post hoc test. eM (circle with red outline) – epidural screw above motor cortex; eCP (filled red circle) - intracerebral electrode in caudate putamen; ref – reference electrode (epidural screw), ACB – nucleus accumbens, CP – caudate putamen, CTX - cortex, GP – globus pallidus, HY - hypothalamus, LSX - Lateral septal complex, MCTX – motor cortex, Pal – pallidum, TH – thalamus.