The trace amine-associated receptor 1 inhibits a hyperglutamatergic state induced by dopamine deficiency

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CONCLUSIONS:

- Dopamine dysfunction caused by genetic ablation of Nurr1 leads to a hyperglutamatergic state in striatum.
- This hyperglutamatergic state was normalized by a therapeutically used D_2 receptor agonist and by a selective TAAR1 agonist, acting via CB_1 receptors.
- We here identify a modulatory and potentially therapeutic role of TAAR1 on dysfunctional corticostriatal glutamate release.

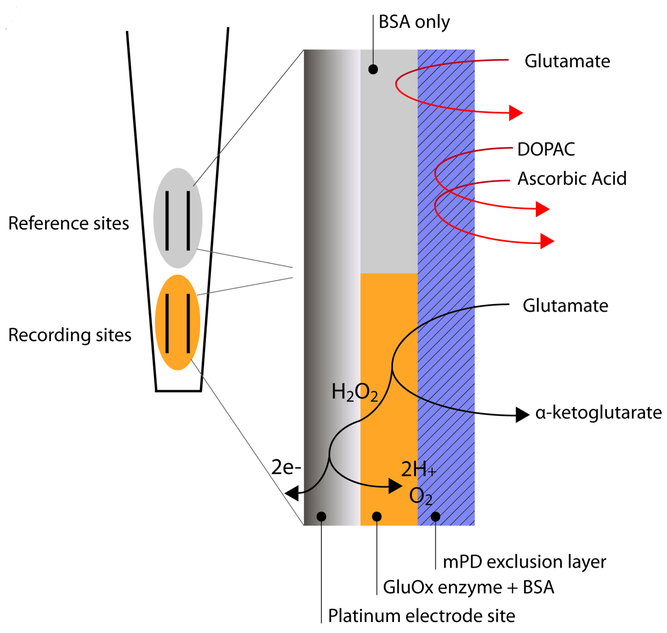
BACKGROUND:

The trace amine-associated receptor 1 (TAAR1) is expressed in the monoaminergic systems of the brain where it plays an important neuromodulatory role [1]. It has been proposed to interact with the dopamine D2 receptor (D_2R) and the dopamine transporter [1], and has been implicated in neuropathological disorders including schizophrenia, depression and Parkinson's disease (PD) [1]. PD is characterized by the progressive loss of dopaminergic neurons projecting from the substantia nigra pars compacta to the striatum, which results in altered dynamics of glutamate throughout the basal ganglia [2]. This imbalance may accelerate neuronal degeneration due to excitotoxic processes, and provides a possible therapeutic target [2]. However, due to rapid release and reuptake mechanisms, glutamatergic neurotransmission has been technically difficult to study *in vivo*. A recently developed enzyme-based microelectrode array (MEA) enables the detection of low glutamate levels on a subsecond time scale, and can be used for monitoring fast temporal fluctuations of glutamatergic neurotransmission in the intact brain of anaesthetized animals. Our aim was to assess the role of D_2R and TAAR1 in modulating striatal glutamate release in cNurr1^{DATCreER} knock-out (cNurr1 KO) mice, a genetic animal model of early PD exhibiting a progressive dopamine deficiency [3].

METHODS: The Fast Analytical Sensing Technology (FAST-16) system measures glutamate release, reuptake and recycling abilities.

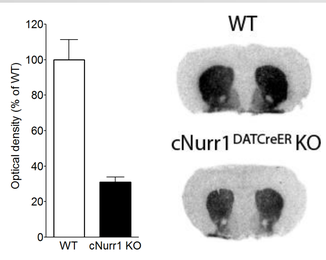
A. The MEA detects glutamate by enzymatic degradation

- Each MEA has four platinum sites (S2 model).
- Prior to experiments two platinum sites were coated with glutamate oxidase (GluOx), which cause enzymatic breakdown of glutamate into α-ketoglutarate and peroxide (H_2O_2), whereas the second pair of sites were coated with an inactive protein matrix (BSA) and used for self-referencing.
- All four sites were coated with a protective layer of metaPhenylene-diamine dihydrochloride (mPD) to block interferents such as dopamine, DOPAC and ascorbic acid.
- Responses recorded at the reference sites were subtracted from those generated at the recording sites, with the resulting signal representing glutamate measurements.
- A micropipette was aligned between the four sites and used for local delivery of 70 mM KCl and the compound of interest to stimulate depolarization and evoke glutamate release.

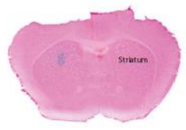


B. Generation of cNurr1 KO mice

Nurr1 was conditionally ablated by tamoxifen treatment of 5 weeks old Nurr1 floxed mice expressing the CreER^{T2} fusion protein under the dopamine transporter (DAT) gene promoter (cNurr1 KO) [3]. Littermates homozygous for the Nurr1 floxed allele but lacking a copy of the DATCreER^{T2} transgene were used as control animals (WT). Recordings were made in 6 months old mice. Autoradiographic detection of DAT in brain slices revealed a 67±3.8% reduction in cNurr1 KO compared to WT mice (*right*).



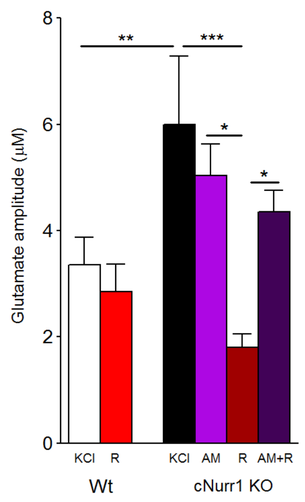
E. MEA placement was validated by local infusion of methylene blue followed by nuclear fast red staining



ABBREVIATIONS: AA: ascorbic acid; AM: AM251; BSA: bovine serum albumin; DA: dopamine; GluOx: glutamate oxidase; MEA: microelectrode array; mPD: 1,3-phenylenediamine; PD: Parkinson's disease; R: ropinirole; RO: RO5166017; TAAR1: trace amine-associated receptor 1.

RESULTS:

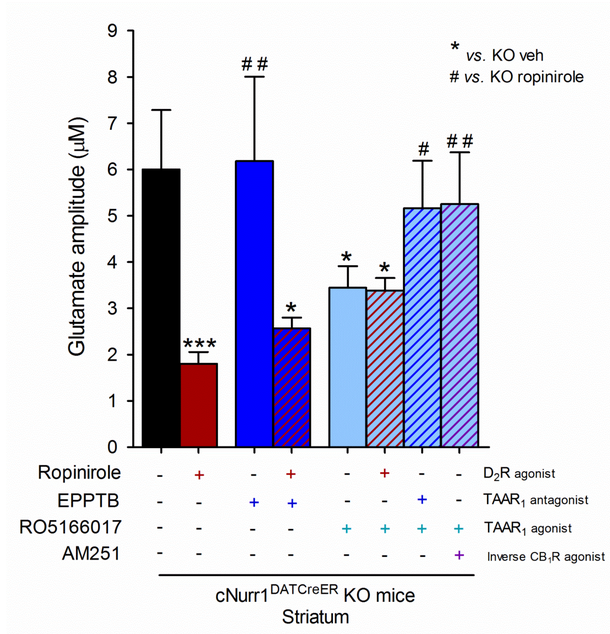
Evoked corticostriatal glutamate release was enhanced under dopamine deficiency and attenuated by activation of D_2R and TAAR1



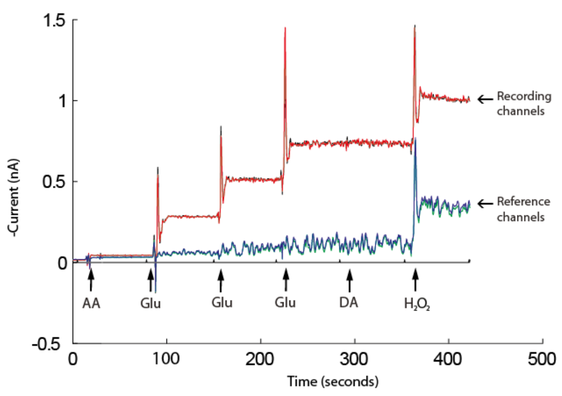
- Evoked glutamate concentrations were significantly increased in the striatum of cNurr1 KO mice compared to WT mice.
- The enhanced glutamate release in cNurr1 KO mice was inhibited by local administration of D_2R agonist ropinirole (100 μM) (R).
- Blockade of CB_1R by AM251 (AM) did not affect striatal glutamate release, but attenuated ropinirole's effect (AM+R).
- This supports that D_2R attenuate dysfunctional glutamate release via a mechanism involving CB_1R, possibly via retrograde endocannabinoid signaling.

- The TAAR1 antagonist EPPTB (10 nM) did not affect corticostriatal glutamate release or the effect of ropinirole.
- The TAAR1 agonist RO5166017 (500 nM) inhibited enhanced glutamate release.
- Blockade of CB_1R by AM251 attenuated the effect of RO5166017.
- No effects were seen in WT animals.
- This suggests that local TAAR1 agonism, via CB_1R, mediates a rapid attenuating effect on the hyperglutamatergic state in experimental PD.

Statistics: One-way ANOVA followed by Fisher's LSD *post hoc* test, *p<0.05; **p<0.01; ***p<0.001

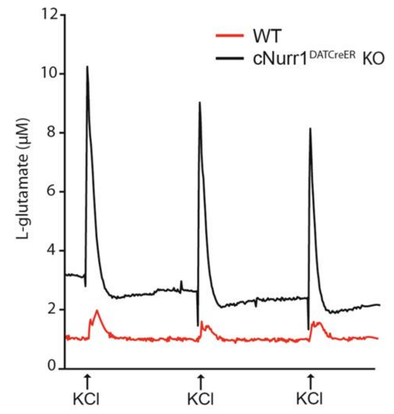


C. *In vitro* calibration of MEA



The electrode sites coated with GluOx responded to glutamate and H_2O_2, but not to the interferents ascorbic acid (AA) and dopamine (DA), which were repelled by the mPD coating.
The electrode sites with no GluOx coating responded only to molecules not repelled by mPD and were used for self-referencing.

D. *In vivo* recording



The arrows indicate KCl ejections which were made at 1 minute intervals. Ejections were followed by increased concentration of glutamate at the recording channels. During analysis the signal recorded at the reference channels was subtracted from that of the recording channels to remove background noise.

OBJECTIVES: To assess the roles of D_2R and TAAR1 in modulating striatal glutamate release in cNurr1^{DATCreER} mice, a genetic animal model of early Parkinsonism exhibiting a progressive pathology of the midbrain dopaminergic system.

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NO POTENTIAL CONFLICT OF INTEREST.

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