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| Journal: | *British Journal of Pharmacology* |
| Manuscript ID | 2022-BJP-0627-RP |
| Manuscript Type: | Research Article |
| Date Submitted by the Author: | 13-Jun-2022 |
| Complete List of Authors: | Manago', Francesca; Istituto Italiano di Tecnologia<br>Scheggia, Diego; Istituto Italiano di Tecnologia, Neuroscience and Brain Technologies<br>Pontillo, Maria; Bambino Gesu Pediatric Hospital<br>Mereu, Maddalena; Istituto Italiano di Tecnologia, Neuroscience and Brain Technologies<br>Mastrogiacomo, Rosa; Istituto Italiano di Tecnologia, Neuroscience and Brain Technologies<br>Udayan , Gayatri; Istituto Italiano di Tecnologia, Neuroscience and Brain Technologies<br>Valentini, Paola; Istituto Italiano di Tecnologia, Neuroscience and Brain Technologies<br>Tata, Maria Cristina; Istituto Italiano di Tecnologia, Neuroscience and Brain Technologies<br>Weinberger, Daniel; Lieber Institute for Brain Development<br>Weickert, Cynthia; Upstate University Hospital<br>Pompa, Pier Paolo; Istituto Italiano di Tecnologia, Neuroscience and Brain Technologies<br>De Luca, Maria Antonietta; University of Cagliari<br>Vicari, Stefano; Bambino Gesu Pediatric Hospital<br>Papaleo, Francesco; Istituto Italiano di Tecnologia, Neuroscience and Brain Technologies |
| Major area of pharmacology: | Neuropharmacology, Schizophrenia |
| Cross-cutting area: | Behavioural pharmacology, Microdialysis |
| Additional area(s): | Dopamine, In vivo |
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# Dopaminergic signaling and Behavioral Alterations by COMT-DTNBP1 Genetic Interaction and their Clinical Relevance

Francesca Managò[1*], Diego Scheggia[1*#], Maria Pontillo[2], Maddalena Mereu[1], Rosa Mastrogiacomo[1], Gayatri Udayan[3,4], Paola Valentini[3], Maria Cristina Tata[2], Daniel R. Weinberger[5], Cynthia S. Weickert[6], Pier Paolo Pompa[3], Maria A. De Luca[7], Stefano Vicari[2], Francesco Papaleo[1]

[1]*Genetics of Cognition laboratory, Neuroscience area, Istituto Italiano di Tecnologia, Genova, Italy.*
[2]*Department of Neuroscience, Bambino Gesù Children's Hospital, Piazza Sant'Onofrio 4, 00100 Rome, Italy.*
[3]*Nanobiointeractions&Nanodiagnostics, Istituto Italiano di Tecnologia, Genova, Italy.*
[4]*Department of Engineering for Innovation, University of Salento, Via per Monteroni, 73100 Lecce, Italy*
[5]*Lieber Institute for Brain Development, Johns Hopkins University Medical Campus, Baltimore, MD 21205, USA.*
[6]*Schizophrenia Research Laboratory, Neuroscience Research Australia, Sydney, Australia.*
[7]*Department of Biomedical Sciences, University of Cagliari, Cagliari, Italy.*
[#]*present address: Department of Pharmacological and Biomolecular Sciences, University of Milan, 20133, Milan, Italy.*

[*]These authors contributed equally to this work.

Correspondence: francesco.papaleo@iit.it



**Data Availability Statement.** The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

## Abstract

**Background and Purpose**. Cognitive and motor functions are modulated by dopaminergic signaling, which is shaped by several genetic factors. The biological effects of single genetic variants might differ depending on epistatic interactions that can be functionally multi-directional and non-linear.

**Experimental approach**. Behavioral and neurochemical assesments in genetically modified mice. Behaviroal assessments and genetic screening in human patietns with 22q11.2DS.

**Key Results**. Here, we confirm a genetic interaction between the COMT and DTNBP1 (dysbindin-1 or Dys1) genes that modulates cortical and striatal dopaminergic signaling in a manner not predictable by the effects of each single gene. In mice, COMT-by-Dys1 concomitant reduction leads to a hypoactive mesocortical and a hyperactive mesostriatal dopamine pathway, associated with specific cognitive and motor abnormalities. Like mice, in subjects with the 22q11.2 deletion syndrome (22q11.2DS, characterized by COMT hemideletion and dopamine alterations), COMT-by-Dys1 concomitant reduction was associated with analogous cognitive and motor disturbances. We then developed an easy and inexpensive colorimetric kit for the genetic screening of common COMT and Dys1 functional genetic variants for clinical application.

**Conclusions & Implications**. These findings illustrate a predicted epistatic interaction of two dopamine-related genes and their functional effects, supporting the need to address genetic interaction mechanisms at the base of complex behavioral traits.

## Introduction

Dopamine is involved in the neuromodulation of cognitive and motor processes through a widespread network system in which the functional interaction between the prefrontal cortex (PFC) and striatal brain regions plays major roles [1-3]. Alterations in the dopaminergic regulatory system are consistently reported in several psychiatric, neurodevelopmental, and neurodegenerative disorders [4, 5]. In agreement, many genes relevant to dopaminergic signaling have been reported to contribute in cognitive and motor alterations of these pathologies as well as in the responses to their treatments [6-8]. However, genetic associations to complex behaviors and human illnesses often fail to recognize the critical role of epistasis [9, 10]. Notably, gene-gene interactions underlying the functional basis of behavior might be synergistic, but also nonlinear and multidirectional.

We have previously explored non-linear genetic interactions between dopamine-related genetic variants in the context of mouse and human working memory [11, 12]. In particular, the relative advantage of reduced catechol-O-methyltransferase (COMT) activity on enhanced PFC-mediated working memory could be paradoxically and inversely modulated by genetic variations reducing Dys1 [12], a gene involved in D2 membrane trafficking [13, 14]. Individual differences in COMT enzymatic activity have been linked to changes in cortical dopamine and related cognitive functions in humans and rodents [15-20]. Similarly, individual genetic variations in Dys1 can affect dopamine signaling through D2/D3 receptors mechanisms and related cognitive and motor processes in humans and mice [11, 13, 14, 21, 22]. However, despite knowledge of COMT and Dys1 single genes effects in the dopaminergic system, the effects of a COMT-Dys1 epistatic interaction on brain dopaminergic signaling remains unexplored. Moreover, to what extent this genetic interaction might affect cognitive domains other than working memory or even other dopamine-dependent behaviors, is unknown.

Here, we studied the selective contribution of functional genetic variations resulting in reduced COMT in the context of reduced Dys1 expression on brain dopaminergic signaling as well as on other cognitive, social and motor functions in mice. We then explored if this epistatic interaction would have a clinical relevance in patients with the 22q11.2 hemideletion syndrome (22q11.2DS),

which involves deletion of the COMT gene on the affected chromosome. Indeed, patients with 22q11.2DS are characterized by cognitive disabilities and increased risk of psychiatric disorders [23], have altered dopamine metabolites in peripheral blood [24, 25], as well as dopamine alteration at the striatal level [26]. Unexpectedly, and in contradiction to what have been suggested by single genes effects, we found that a concomitant genetic reduction of both COMT and Dys1 resulted in increased D2 receptor levels in the PFC, but reduced in the striatum. Similarly, the concomitant genetic reduction of both COMT and Dys1 reduced amphetamine-induced dopamine release in the PFC, but increased release in the striatum. This was paralleled in mice by a supersensitivity to the locomotor effects triggered by amphetamine, selective cognitive deficits in measures of recency memory and attentional set-shifting abilities, and sensorimotor gating deficits. Similarly, in patients with 22q11.2DS, the carriers of genetic variants further reducing COMT expression and reducing Dys1 expression showed the worst cognitive and motor phenotypes. Functional genetic variants are commonly represented in human population; thus we also validated a simple and cheap colorimetric genetic screening for the COMT and Dys1 functional genetic variants. These findings may have general implications to pathologies linked with altered dopaminergic signaling (e.g. schizophrenia, ADHD, Parkinson's, autism) as well as for therapies targeting the dopaminergic system (e.g. antipsychotics and psychostimulants).

## Methods

**Mice**. All procedures were approved by the Italian Ministry of Health (permits n. 230/2009-B, 107/2015-PR and 749/2017-PR) and local Animal Use Committee and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the European Community Council Directives. Routine veterinary care and animals' maintenance was provided by dedicated and trained personnel. All mice used were littermates and bred by double heterozygous mating (♀dys$^{+/-}$ COMT$^{+/-}$ × ♂dys$^{+/-}$ COMT$^{+/-}$). Mice were identified by PCR analysis of tail DNA. Mice were group-housed (2–4/cage) in a climate-controlled animal facility (22 ± 2 °C), maintained on a 12-h light/dark cycle and given free access to food and water, except during the ID/ED Operon Task. Testing was conducted in male mice, 3–7-months old, during the light phase. Experimenters were blind to the genotype during behavioral testing. Mice were handled by the experimenter on alternate days during the week preceding the test. At least 1 h before any test manipulation, mice were habituated in a room adjacent to the testing room.

**RNA isolation and real-time PCR.** Total RNA was extracted from medial prefrontal cortex (mPFC) and striatum with the RNA miniprep kit (Sigma Aldrich, Milan,Italy) according to the manufacturer's instructions. Total RNA was converted to first-strand cDNA by high-capacity cDNA reverse Transcription kits (Applied Biosystem). The PCR were performed in a final volume of 20 µL with the TaqMan Universal PCR Master Mix with UNG. The target genes have been detected with DRD2 (Mm00438545_m1), DRD3 (Mm00432887_m1), and the reference gene with GAPDH (Mm99999915_g1) TaqMan assay. Amplifications were carried out in 7900ht fast real-time pcr system (Applied Biosystem). Fold change was quantified by the comparative ΔΔCt method.

**In Vivo Microdialysis.** Briefly, as previously performed [22, 27], mice were anaesthetized with a mix of Isoflurane/Oxygene (2%/1%). Using the sterotaxing frame, (Kopf Instrument) the probe was

implanted in the mPFC or dorsal striatum (mPFC: AP+1.9; ML±0.3 DV-3.0; dorsal striatum: AP+1.1; ML±1.7 DV-3.8; (Franklin and Paxinos, 1997). Analytical procedure started 24-26 hours after surgery and probes were perfused with Ringer's solution, 1 µl/min, and 20-min fractions were collected for 1 hour and after acute amphetamine (1.5 mg/kg i.p) for other 3 hours. Extracellular dopamine (DA) levels were quantified using an HPLC apparatus equipped with a reverse-phase column (C83.5 µm, Waters, Milford, USA) and a coulometric electrochemical detector (ESA Coulochem II, Bedford, USA). At the end, mice were sacrificed and their brains were removed and stored in formalin (4%) before histological analysis. Brains were cut in serial coronal slices (100 µm) to locate the placement of the microdialysis probe.

**Behavioral paradigms**.

*Two-chamber operon ID/ED task*. Attentional set-shifting was tested in the two- chamber ID/ED Operon task as previously tested [22, 28]. After random selection of the mice for the ID/ED task, all the behavioral manipulations were obtained blind to the genotype and pharmacological treatments of the animals. "Stuck-in-Set" ID/ED Paradigm. For habituation to the apparatus, in the first 2 days, mice were habituated for 45 min to the apparatus with only neutral stimuli (Habituation 1) and trained to move from one chamber to the other (Habituation 2). Any nose poke into the nose-poke holes resulted in a pellet delivery into the food receptacle. The next day, mice were trained to perform two randomly presented simple discriminations (e.g., between smooth vs. sand cardboard; light on vs. light off; peach vs. sage) so that they were familiar with the stimulus dimensions (Habituation 3). These exemplars were not used again. The mice had to reach a criterion of eight correct choices out of ten consecutive trials to complete this and each following testing stage. Performance was measured in all phases of all experiments using number of trials to reach the criterion; time (in minutes) to reach the criterion; time (in seconds) from breaking the photobeams adjacent to the automated door to a nose-poke response (latency to respond). To conduct the test, we used a stuck-in-set perseveration

paradigm. This procedure is only possible when three dimensions can be manipulated. A session started when a mouse was placed in one of the two chambers where all the stimuli were neutral. Then the transparent door was dropped to give the mouse access to the other chamber where the stimuli cues were on. The series of stages comprised a simple discrimination (SD), compound discrimination (CD), compound discrimination reversal (CDRe), IDS, IDS reversal (IDSRe), a second IDS 2 (IDS2), IDS reversal 2 (IDS2Re), EDS, and EDS reversal (EDSRe). The mice were exposed to the tasks in this order so that they could develop a set, or bias, toward discriminating between the correct and incorrect nose- poke hole.

*Temporal order object recognition test (TOR)*. This task has been performed as previously deacribed [27]. Mice performed two sample phases and one test trial. In the sample phases, mice explored two copies of an identical object for a total of 4 min. Different objects were used for sample phases 1 and 2, with a delay between the sample phases of 1 h. The test trial (3 min) was given 3 h after sample phase 2. During the test trial, a third copy of the objects from sample phase 1 and a third copy of the objects from sample phase 2 were used. The positions of the objects in the test and the objects used in sample phase 1 and sample phase 2 were counterbalanced between the animals. If temporal order memory is intact, the animals will spend more time exploring the object from sample 1 (i.e., the object presented less recently) compared with the object from sample 2 (i.e., the "new" object).

*Acoustic startle response and prepulse inhibition (PPI)*. We used four Startle Response Systems (TSE Systems). Test sessions began by placing the mouse in the chamber for a 5 min acclimation period. Over the next 10.5 min, mice were presented with each of seven trial types across six discrete blocks of trials for a total of 42 trials. The order in which trial types were presented was randomized within each block. The interval between trials was 10–20 s. One trial type measured the response to no stimulus (baseline movement), and another presented the startle stimulus alone (acoustic amplitude), which was a 40 ms, 120 dB sound burst. The other five were acoustic prepulse plus acoustic startle stimulus trials. Prepulse tones were 20 ms at 74, 78, 82, 86, and 90 dB, presented

100 ms before the startle stimulus. The maximum startle amplitude was the dependent variable. A background level of 70 dB white noise was maintained over the duration of the test session.

*Sociability and social novelty tests*. We employed a widely employed test for assaying sociability in mice [27]. We used a classical three-chamber arena. After habituation for 10 minutes, the testing subject was presented, for other 10 minutes, with a white or black plastic object (novel object) contained in one of the two wire cups, placed in one side of the chamber. Simultaneously, an adult conspecific mouse (novel mouse 1), which has had no previous contact with the testing subject, was placed in the wire cup in the other side of the chamber. To measure sociability, the tendency of the subject mouse to spend time with a conspecific, as compared with time spent with an object, a discrimination index was calculated (time spent with novel mouse 1 – time spent with novel object / total time spent with novel mouse1 and novel object). Following sociability test, the object was replaced with a novel mouse (novel mouse 2) and the observer was tested for other 10 minutes to assess the preference for social novelty. This is defined as more time spent in the chamber with novel mouse 2 than time in the chamber with novel mouse 1. Most mice prefer to spend more time near the unfamiliar novel mouse 2. To assess social novelty, we calculated a discrimination index for each mouse (time spent with novel mouse 2 – time spent with novel mouse 1 / total time spent with novel mouse1 and novel mouse 2).

**Postmortem human samples.** For expression of Dys-1 we used RNAseq data from a total of 594 DLPFC from human postmortem brain tissue (for brain quality and demographic characteristics see [22]. The mRNA expression values are referred to Dys-1 NM_183040 gene expression in the human postmortem dorsolateral prefrontal cortex (DLPFC) of normal subjects. The data are available in the open access on-line application "The Brain Cloud", which allows the query of genome-wide gene expression data and their genetic control, http://www.libd.org/braincloud.

Protein expression of Dys1 was assessed by western blot analyses from post mortem human caudate samples from 40 subjects obtained from the NSW Tissue Resource Center. The tissue was processed at Neuroscience Research Australia as approved by the University of New South Wales Human Research Ethics Committee (HREC 12435; Sydney, Australia). The rostral caudate was dissected from anatomically matched fresh frozen coronal sections cut at 60 µm through the head of the caudate. Caudate extracted samples (run in duplicates) were denatured in loading buffer2X, and boiled for 5 min at 95°C, then the denatured samples were centrifuged at 10,000 g for 5 min. Each lane was loaded with 20 mg of total protein.

**Patients with 22q11.2 deletion syndrome.** A neuropsychological and psychopathological assessment was performed by trained neuropsychologists and psychotherapist at the IRCCS Children Hospital Bambino Gesù of Roma, Italy. In particular, the presence of psychotic symptoms was assessed with the Structured Interview for Psychosis-Risk Syndrome (SIPS). The SIPS was composed of 4 scales including a total of 19 symptom constructs (five positive, six negative, four disorganized, and four general symptoms). Evaluation was based on the presence, duration and severity of specific experiences and behaviors. The cognitive performance was assessed with the Wisconsin Card Sorting Test, a widely-used measure of prefrontal cognitive function that is sensitive to a subject's ability to generate hypotheses, establish response sets, and fluently shift sets. The total number of perseverative errors (raw scores were converted into age-specific standard scores) were considered for the analyses. After the neuropsychological task and psychological interview, DNA was extracted and purified from whole peripheral blood using QIAamp DNA Blood Mini Kit (Quiagen). We specifically selected a DYS-1 haplotype composed by the three-marker single nucleotide polymorphisms (SNPs) rs2619538, rs3213207, and rs1047631 or here, the 'Dys1 haplotype' [11, 22]. DNA samples were submitted to genetic analysis for the SNPs on a ABI7900 real-time PCR instrument (Applied Biosystems) using Custom Taqman SNP Genotyping Assays for rs2619538 (C___3114517_10,

Applied Biosystems), for rs3213207 (C__32386418_10, Applied Biosystems), rs1047631 (C___7460562_10, Applied Biosystems). For genotyping the manufacturer's suggested protocol was used. For PCR amplification and allelic discrimination, the ABI Prism 7900 HT Sequence Detection System and SDS software version 2.1 (Applied Biosystems) were used. HWE test and haplotype phase were performed using the software PLINK for Windows http://pngu.mgh.harvard.edu/purcell/plink/. Heterozygous subjects for all the three markers were assigned to the Dys Hap+/− as the probability to be in this group, calculated on the haplotype frequency of the population, was higher than 65–70%. All polymorphisms were in HWH ($p > 0.05$; assessed by online resource http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20%20HW%20calculator.xls; for genotype frequencies, see Supplementary Table 1).

**Synthesis, characterization, and functionalization of 40 nm AuNPs.** 40 nm citrate capped gold nanoparticles (AuNPs) were synthesized as previously reported [29]. The AuNPs were characterized by UV-vis spectroscopy, Dynamic Light Scattering (DLS), TEM and SEM. The AuNPs were functionalized with DNA probes according to published protocols [30], by conjugating the AuNPs with thiolated probe oligonucleotides consisting of sequence of thymines (5' T(30)-(O-$CH_2$-$CH_2$)$_3$-SH3') (purchased from IDT DNA). Briefly, the DNA probes were digested with 10 mM tris(2-carboxyethyl) phosphine (TCEP) for 3 hours at room temperature, and then they were incubated with 40 nm AuNPs in a molar ratio 2000:1, overnight at room temperature under mild shaking. The DNA-AuNP mixture was then brought to 0.3 M NaCl in 10 mM phosphate buffer, pH 7.4, 0.01% SDS, with a stepwise increment of salt over the course of 8h. After another overnight incubation, the functionalized AuNPs were centrifuged and washed thrice with 0.3 M NaCl in 10 mM phosphate buffer plus 0.01% SDS, to remove the excess unbound DNA. These conjugates were stored at 4 °C until their use. Their concentration was determined by UV-vis spectroscopy.

**PCR amplification and gel electrophoresis.** Traditional PCR was used to amplify genomic DNA to obtain approximately 100 bp fragments containing the SNPs rs1047631, RS 2619538 and RS 3213207 in the Dystrobrevin binding protein (DYS-1) gene and rs4680 in the Catechol-O-methyl-transferase (COMT) gene. The PCR reaction mixture contained 100 pg/µl of genomic DNA, 100 nM FWD primer, 100 nM biotinylated REV primer, 2.5 mM $MgCl_2$, 1x reaction buffer, 200 µM dNTPs and 0.05 units/µl of Taq DNA polymerase. Sequences of all primers used are listed in Table1. The thermal cycling conditions used for the DNA amplification was as follows: 95 °C for 5 min, followed by 38 cycles of 95 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min, with a final extension at 72 °C for 5 min. The PCR products were electrophoresed on an 18% denaturing polyacrylamide gel, run in 1X Tris- Borate- EDTA (TBE) buffer and stained with Gel red nucleic acid stain (Biotium), to assess the specificity of the primer sets and exclude unspecific amplification.

**Colorimetric discrimination of single nucleotide polymorphisms (SNPs).** The naked-eye discrimination of the SNPs was achieved by a colorimetric assay, with some optimizations. Briefly, each amplification product related to each SNP was analyzed in two separate samples, to achieve visual discrimination of the results after hybridization with the two discriminating probes (IDT DNA) matching the two possible allelic variants (Table 1). For each sample, 15 µl of the biotinylated PCR amplicons were incubated for 5 min at room temperature with 5 µl Dynabeads M-280 Streptavidin (Invitrogen) paramagnetic microbeads, which were washed twice with hybridization buffer (HB)(1x PBS, pH 7.4, 5% w/v PEG-600) and resuspended in equal volume of HB. The microbeads coated with biotinylated PCR products were incubated with 50 µl of 0.15 N NaOH for 5 min to denature the double stranded PCR amplicon. The interfering non-biotinylated strand was removed by magnetic separation after denaturation. The suspension was washed with 100 µl 0.15 N NaOH and subsequently with 100 µl HB and finally resuspended in 20 µl HB. 10 µl of 10 µM solution of one of

the two discriminating probes were added to the two samples and incubated at room temperature for 10 min. This suspension was incubated for 5 min at room temperature, with 300 fmol of AuNPs conjugated with detection probes. This was followed by a magnetic wash with 100 µl HB at room temperature. For different SNPs, the mixture was incubated at different temperatures (as mentioned below) for 5 min and then resuspended in 25 µl of HB for the readout of the colorimetric result. The incubation temperature for the SNPs were as follows: 47 °C for rs1047631, 46 °C for RS2619538 and RS3213207 and 44 °C for rs4680.

**Stastistics.** No statistical methods were used to pre- determine sample sizes, although sample sizes were consistent with those from previous studies [22, 27]. No explicit randomization method was used to allocate animals to experimental groups and mice were tested and data processed by an investigator blind to animal treatment identity. Statistical analyses were performed using GraphPad Prism 8 and STATISTICA-StaSoft 12. Normality was assessed using Shapiro-Wilk tests. Results are expressed as mean ± standard error of the mean (S.E.M.) throughout. For analysis of variance, we used one- or two-way ANOVA, as appropriate. Post hoc analyses were conducted using Tukey test with multiple comparisons corrections when statistical significance emerged in the main effects or interactions. Single variable comparisons were made with two-tailed unpaired t-test. Statistical tests used for each graph are indicated in the figure legends. The accepted value for significance was $p<0.05$.

## Results

### COMT-Dys1 interaction differentially alters dopamine/D2 levels in the PFC versus the striatum

Previous findings pointed to Dys1-selective modulation of D2-like receptors trafficking [11, 14, 22]. Thus, we first analyzed in single Dys1 (dys$^{+/-}$ COMT$^{+/+}$), single COMT (dys$^{+/+}$ COMT$^{+/-}$) and double dys*COMT mutant (dys$^{+/-}$ COMT$^{+/-}$) mice littermates the expression levels of D2 in both PFC and striatum, principal areas thought to be involved in psychiatric-relevant dopamine dysfunctions.

In agreement with previous findings [17, 22], partial disruption of either COMT or Dys1 did not alter D2 expression in the PFC (Fig. 1a); in contrast, dys$^{+/-}$ COMT$^{+/-}$ mice show increased D2 expression levels (Fig. 1a) compared to wild-type littermates (dys$^{+/+}$ COMT$^{+/+}$). In the striatum, partial disruption of COMT did not change D2 expression while Dys1 reduction led to increased D2 expression, (Fig. 1b), while dys$^{+/-}$ COMT$^{+/-}$ mice showed decreased levels of D2 receptors in the striatum (Fig. 1b). These data show that, unlike what is expected by single gene variations, concomitant hypofunctioning of COMT and Dys1 can produce an opposite and counterintuitive pattern of D2 expression between the PFC and striatal regions.

### Dopamine release is reduced in mPFC, but increased in the striatum of COMT*Dys1 double hypofunctioning mice

To directly explore the consequence of Dys1-by-COMT interaction on dopamine transmission, we performed *in vivo* microdialysis in the mPFC or striatum of freely moving dys$^{+/+}$ COMT$^{+/+}$ and dys$^{+/-}$ COMT$^{+/-}$ mice. The basal extracellular dopamine levels in both the mPFC and striatum were not changed in dys$^{+/-}$ COMT$^{+/-}$ compared to their dys$^{+/+}$ COMT$^{+/+}$ littermates (Supplementary Fig. 1). However, following an acute low systemic dose of amphetamine, we found that compared to dys$^{+/+}$ COMT$^{+/+}$ mice, dys$^{+/-}$ COMT$^{+/-}$ mice manifest relatively reduced increases in dopamine release in mPFC (Fig. 1c), while this increase was higher in the striatum (Fig. 1d). These data support the

evidence COMT and Dys1 genes interaction generates distinct dopaminergic effects between cortical and subcortical regions. Notably, this same pattern of opposite amphetamine-induced dopamine release between cortical and striatal regions is evident in patients with schizophrenia [31], and in schizophrenia-relevant mouse models [27].

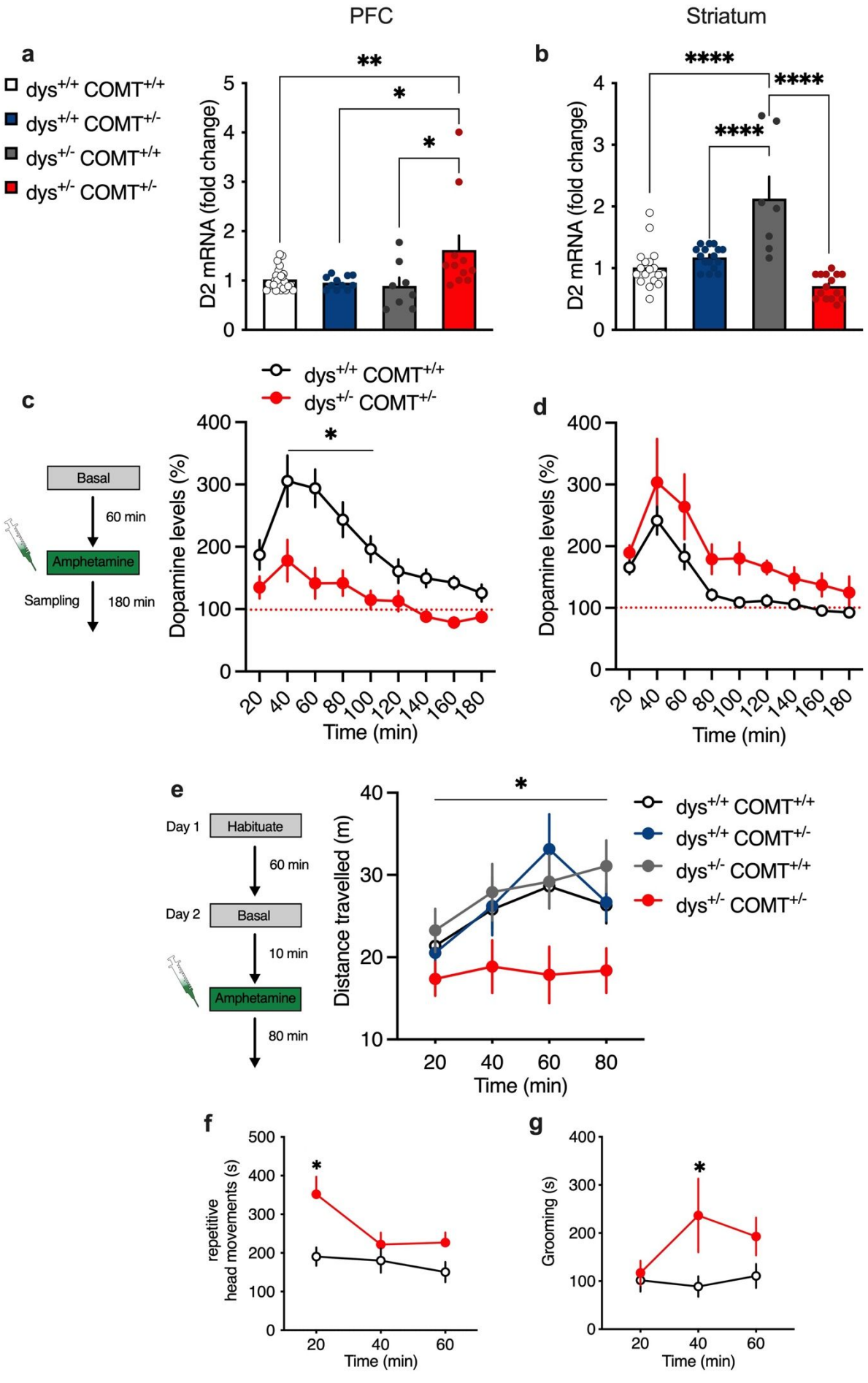

PFC
Striatum
a
dys+/+ COMT+/+
dys+/+ COMT+/-
dys+/- COMT+/+
dys+/- COMT+/-
D2 mRNA (fold change)
b
c
Basal
60 min
Amphetamine
Sampling
180 min
Dopamine levels (%)
Time (min)
d
e
Day 1
Habituate
60 min
Day 2
Basal
10 min
Amphetamine
80 min
Distance travelled (m)
f
repetitive head movements (s)
g
Grooming (s)

**Figure 1. Cortical/striatal dopamine dichotomy caused by COMT*Dys1 epistasis.** Abundance of D2 receptor mRNA in the mPFC (**a**) and striatum (**b**) of Dys+/+ COMT+/+ (n=19-25), Dys+/+ COMT+/− (n=8-11), Dys+/− COMT+/+ (n=7-8), Dys+/− COMT+/− (n=8-11) littermates measured by quantitative RT-PCR. Mean fold changes are expressed relative to transcript levels in control mice (Dys+/+ COMT+/+). One-way ANOVAs revealed a genotype effect for D2 expression in the mPFC ($F_{3,65}$=5.23; P<0.003) and striatum ($F_{3,52}$=20.76; P<0.0001). **c,d**. *In vivo* microdialysis was performed on freely moving mice surgically implanted in the mPFC (two-way RM ANOVA, time x genotype, $F_{(12,156)}$=4.63, p<0.0001; *n*=6/9 each group) or in the striatum (two-way RM ANOVA, time, $F_{12,108}$=15.17, p<0.0001; genotype, $F_{1,108}$=7.90, p=0.020; *n*=6/5 each group) with a concentric dialysis probe. 24 hours after surgery probes were perfused with Ringer's solution, 1 µl/min, and 20-min fractions were collected after acute amphetamine administration. Extracellular dopamine levels were quantified by HPLC with ESA electrochemical detection. Results are indicated as change in dopamine extracellular levels expressed as the percentage of basal value (red dotted line). **e**, Ambulatory distance in 20minute intervals, **f**, repetitive head movements in a stationary and **g**, repetitive grooming displayed by wild-type (Dys+/+ COMT+/+) and COMT*dys1 double heterozygous mice during the 60 minutes after the amphetamine injection, during the second exposure to the open field arena. Post hocs: *P<0.05, **P<0.005, ***P<0.0005, ****P<0.00005. Each histogram shows the mean ± s.e.m.

**Amphetamine locomotor supersensitivity in COMT*Dys1 double hypofunctioning mice**

Behaviorally, as compared to dys$^{+/+}$ COMT$^{+/+}$, genetic hypofunction of only the COMT gene, only the Dys1 gene or both did not produce any effect in the distance travelled during the first exposure to an open field arena (Supplementary Fig. 1c). In contrast, testing these same mice on the next day in the same open field following treatment with amphetamine, revealed a genotype effect. In agreement with previous reports [14, 32], neither COMT nor Dys1 single genetic reduction affected the motor responses to amphetamine (Fig. 1e). In contrast, dys$^{+/-}$ COMT$^{+/-}$ mice showed reduced locomotor activity when compared to wild-type littermates (Fig. 1e). This behavioral response to amphetamine in the COMT*dys double hypofunctioning mice did not appear to be due to a reduced locomotor response to this psychostimulant drug but, conversely, to a heightened sensitivity to amphetamine. Indeed, higher level of stereotyped repetitive head movements (Fig. 1f) and grooming behaviors (Fig. 1g) were reported in these mice compared with wild-type littermates following amphetamine treatment. Our results are consistent with earlier data in C57BL/6J mice showing a bimodal effect of amphetamine on locomotor activity, whereby lower doses increase the distance traveled while higher doses decreased the distance traveled because of the increase in stereotypic behaviors [33]. Thus, COMT*Dys1 double hypofunctioning mutant mice present a right-shift in the inverted U-shaped motor behavioral responses to amphetamine. Notably, the exaggerated stereotypic behavior in response to amphetamine in COMT*Dys1 hypofunctioning mice are consistent with the increased striatal dopaminergic outflow we observed and again it is a phenotype not predicted by the effects of individual reductions in either gene.

**COMT-Dys1 interaction impairs PFC-dependent cognitive functions and pre-pulse inhibition, but not social behavior**

To investigate the functional effects of COMT*Dys1 genetic interaction on cognitive functions, we tested dys$^{+/+}$ COMT$^{+/+}$, dys$^{+/-}$ COMT$^{+/+}$, dys$^{+/+}$ COMT$^{+/-}$ and dys$^{+/-}$ COMT$^{+/-}$ littermates on a temporal order object recognition (TOR) task, which assess recency memory [27]. The performance of dys$^{+/-}$

COMT$^{+/-}$ was significantly worse than control mice (Fig. 2a). While dys$^{+/+}$ COMT$^{+/+}$ littermates spent more time exploring the object presented least recently, dys$^{+/-}$ COMT$^{+/-}$ mice failed to show any preference for the less recent object. No differences were evident in the total amount of time spent exploring the objects in all sessions of the TOR task (Supplementary Fig. 2). This cognitive ability measured by the TOR paradigm has been shown to depend on mPFC functions, and to be disrupted in patients with schizophrenia [11, 27, 34, 35].

We then tested the ability of dys$^{+/-}$ COMT$^{+/-}$ on the attentional Set Shifting Task (ID/ED Task), a mice analogue of the human 'Wisconsin Card Sorting Task', which have been associated with the functioning of the mPFC [22, 28]. We found that dys$^{+/-}$ COMT$^{+/-}$ needed more trials and time to complete the extradimensional shift stage (EDS), compared to all their control and single mutant littermates (Fig. 2c,d,e). The EDS has been linked with cortical dopamine/D2 signaling [22, 36]. Moreover, executive function deficits in patients with schizophrenia are particularly evident in the EDS [37]. Dys$^{+/-}$ COMT$^{+/-}$ also required more trials to complete the EDS compared to control mice (Fig. 2d). We did not observe other differences in other stages of the task (Supplementary Fig. 3). Instead, similar to previous reports from both rodents and humans studies [28], genetic reduction of COMT produces specific cognitive advantages in the EDS, and not in the IDS (Fig. 2d,e and Supplementary Fig. 3), consistent with a functional specialization of the PFC, where COMT exert a major role compared to other areas.

Prepulse inhibition (PPI) may relate to overactive dopamine/D2 signaling in basal ganglia [38-40], to PFC dopamine [41], and is impaired in schizophrenia [27, 42]. In line with this hypothesis, we found that Dys$^{+/-}$ COMT$^{+/-}$ have decreased PPI compared to their control littermates (Fig. 2g), while single mutants did not show any alterations. Acoustic startle reactivity was not affected by any of the genetic variants (Fig. 2f).

Social deficits are common features of many psychiatric conditions, thus we analyzed social behavior of Dys$^{+/-}$ COMT$^{+/-}$ and single mutants and control littermates, using a well-validated three-chamber test [27, 42]. Both sociability, that is the preference to engage with a novel mouse than with a

novel object, and social novelty, which is a form of social recognition, were intact in $Dys^{+/-}$ $COMT^{+/-}$ (Fig. 2h,i).

These results suggest that dys*COMT genetic interaction have detrimental effects on PFC-dependent cognitive abilities and sensorimotor gating processes, both related to dopamine pathways.

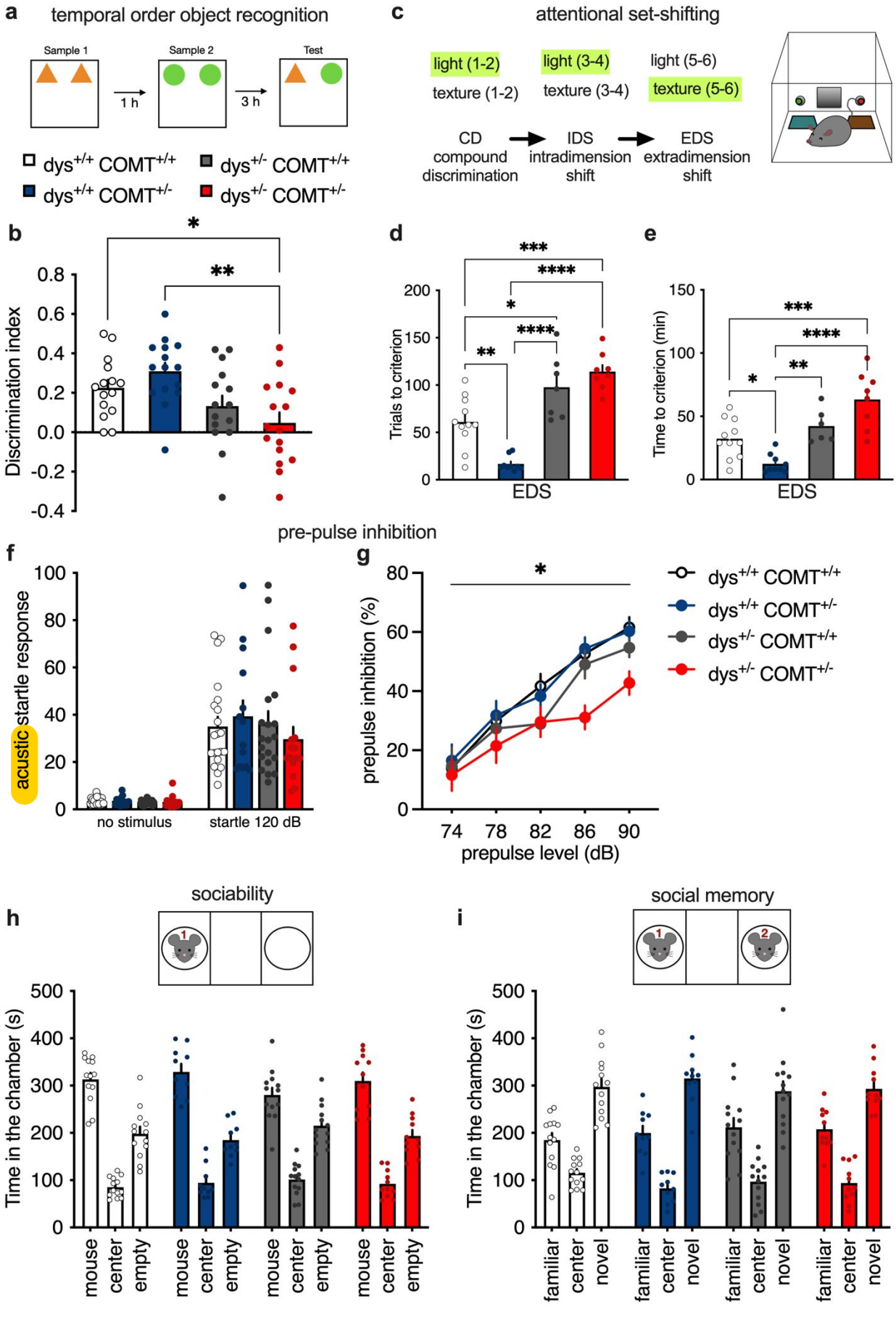

a
temporal order object recognition
Sample 1
Sample 2
Test
1 h
3 h
dys+/+ COMT+/+
dys+/- COMT+/+
dys+/+ COMT+/-
dys+/- COMT+/-
c
attentional set-shifting
light (1-2)
texture (1-2)
light (3-4)
texture (3-4)
light (5-6)
texture (5-6)
CD
compound
discrimination
IDS
intradimension
shift
EDS
extradimension
shift
b
Discrimination index
d
Trials to criterion
EDS
e
Time to criterion (min)
EDS
pre-pulse inhibition
f
acustic startle response
no stimulus
startle 120 dB
g
prepulse inhibition (%)
prepulse level (dB)
dys+/+ COMT+/+
dys+/+ COMT+/-
dys+/- COMT+/+
dys+/- COMT+/-
sociability
h
Time in the chamber (s)
mouse
center
empty
social memory
i
Time in the chamber (s)
familiar
center
novel

**Figure 2. Dys1 and COMT concomitant hemideletion impairs PFC-dependent cognitive abilities. a,** procedure of the temporal order object recognition. **b,** decreased discrimination index in dys$^{+/-}$ COMT$^{+/-}$ mice (one-way ANOVA, $F_{(3,57)}$=5.78, p=0.0016; *n*=15/16 each group. **c**, attentional set-shifting task. Mice were trained to perform multiple discrimination using pairs of stimuli under reinforcement of the same dimension (i.e. light 1-2). A second set of stimuli was used as confounding dimension (i.e. texture 1-2). During the EDS mice were required to shift their attention to previously irrelevant dimension (i.e. texture). The green highlighted dimension is the relevant one. **d**, increased number of trials to reach the criterion during the EDS in dys$^{+/-}$ COMT$^{+/-}$ mice compared to control mice control (one-way ANOVA, $F_{(1,30)}$=27.02, p<0.0001; *n*=6/11 each group). **e**, increased time to reach the criterion during the EDS in dys$^{+/-}$ COMT$^{+/-}$ mice compared to control mice control (one-way ANOVA, $F_{(1,30)}$=15.56, p<0.0001; *n*=6/11 each group). **f**, acoustic startle response did not differ between groups (two-way ANOVA, genotype, $F_{(3,67)}$=0.54, p=0.6533; *n*=14/21 each group). **g**, reduced PPI in dys$^{+/-}$ COMT$^{+/-}$ mice (two-way ANOVA, genotype, $F_{(3,67)}$=2.98, p=0.037164; prepulse level, $F_{(4,268)}$=66.20, p<0.0001; *n*=14/21 each group). **h**, dys$^{+/-}$ COMT$^{+/-}$ have normal sociability behavior (two-way ANOVA, chamber (mouse, center, empty), $F_{(2,86)}$=183.3, p<0.00001; *n*=10/13 each group). **i**, dys$^{+/-}$ COMT$^{+/-}$ have intact social memory (two-way ANOVA, chamber (mouse, center, empty), $F_{(2,86)}$=112.9, p<0.00001; *n*=10/13 each group). Post hocs: *P<0.05, **P<0.005, ***P<0.0005, ****P<0.00005. Each histogram shows the mean ± s.e.m.



**Concomitant reduced COMT and Dys1 in patients with 22q11.2DS is associated with worst executive function performance and increased motor disturbances**

To assess whether the COMT-Dys1 interacting effects found in mice could have any clinical relevance, we evaluated this genetic epistasis in individuals with 22q11.2DS. The 22q11.2DS is one of the major risk factors for schizophrenia, and all subjects have a hemi-deletion of the COMT gene and abnormal dopamine function [23, 26, 43, 44].

We recruited 102 patients with 22q11.2DS which were genotyped for the three-marker haplotype (rs2619538-rs3213207-rs1047631) that has been associated with reduced Dys-1 gene expression in human brain (Dys1 Hap)[22], and for the COMT rs4680 functional genetic variation, i.e. the Val-Met single-nucleotide polymorphism (SNP) that affects enzyme activity in brain [45]. In agreement with Dys1 Hap modulation of Dys1 mRNA levels [11, 22], here we confirmed this association also at the protein level from postmortem human brain samples (Fig. 3a). Moreover, we show that there is one SNP of the Dys1 Hap (the rs1047631) that could be used to predict Dys-1 mRNA expression (Fig. 3b), but we didn't find this correlation at the protein level (Fig. 3c).

For the genotype-phenotype analyses in 22q11.2DS individuals, we considered the groups with the more consistent change in Dys1 expression excluding patients heterozygous for the Dys1 Hap. Moreover, we considered in a parallel analysis the subdivision by the rs1047631 SNP, as this allowed us to lose fewer individuals. For COMT genetic variation, having only one copy of this gene, we were able to split all our population in only two different groups, the Met carriers with relatively lower COMT levels than Val carriers [46]. Demographic characteristics for the patients with 22q11.2DS revealed no significant differences in sex, age, psychiatric diagnosis, medications, and general IQ based on either COMT of Dys1 genotypes (Supplementary Table 1).

All participants were interviewed with the Structured Interview for Psychosis-Risk Syndromes (SIPS) to assess the severity of positive, negative, disorganization, and general symptoms of psychosis. No genotype-dependent differences were evident in items related to the positive, negative and disorganization symptoms. Based on the mouse data, we then tested executive functions in 22q11.2DS by the Wisconsin Card Sorting Task (WCST; analogous to the mice attentional set shifting task), which revealed a genotype effect. Specifically, patients carrying genetic variants decreasing Dys1 as well as COMT expression made more errors in the WCST than subjects with relative higher levels of both these genes (Fig. 3d). The same result was evident even considering the single genetic variants (rs1047631 SNP) associated with a reduced Dys1 (T-carriers) in combination with low level of COMT expression (met-carriers) (Fig. 3g).

Patients carrying genetic variants decreasing Dys1 as well as COMT expression had also greater sleep disturbance scores than patients with relative higher level of both these genes (Fig. 3f). In these patients we also observes higher motor disturbance scores, although this effect was only close to statistical significant (Fig. 3e). However, considering only one functional Dys1 SNP did not show any genotype-dependent difference in these two SIPS items (Fig. 3h,i). Overall, in agreement with mouse data, these findings corroborate a detrimental effect of the concomitant hypofunctioning of COMT-Dys1 in measures of motor and executive functions in 22q11.2DS.

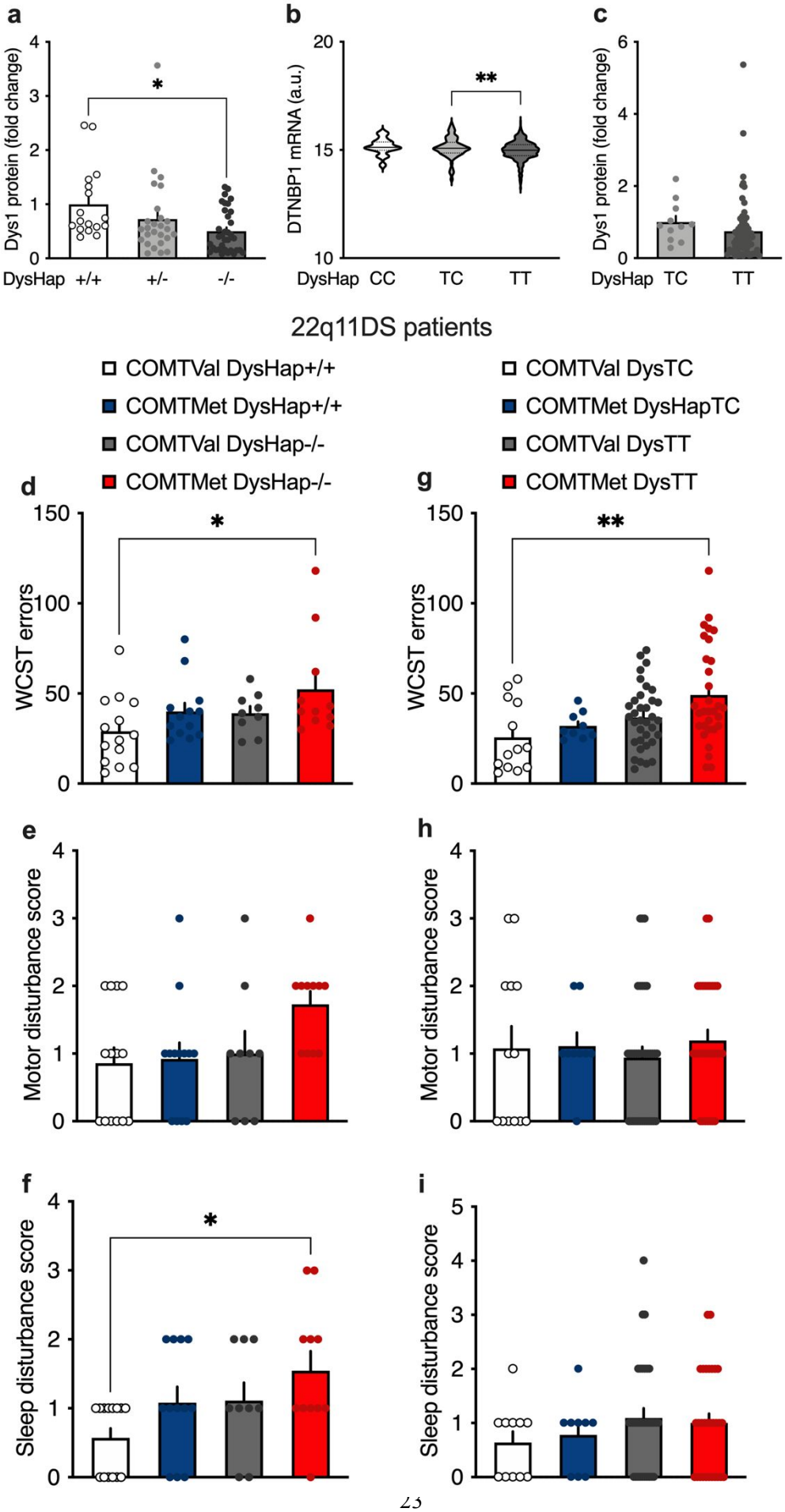

a
Dys1 protein (fold change)
DysHap +/+ +/- -/-
b
DTNBP1 mRNA (a.u.)
DysHap CC TC TT
c
Dys1 protein (fold change)
DysHap TC TT
22q11DS patients
COMTVal DysHap+/+
COMTMet DysHap+/+
COMTVal DysHap-/-
COMTMet DysHap-/-
COMTVal DysTC
COMTMet DysHapTC
COMTVal DysTT
COMTMet DysTT
d
WCST errors
e
Motor disturbance score
f
Sleep disturbance score
g
WCST errors
h
Motor disturbance score
i
Sleep disturbance score

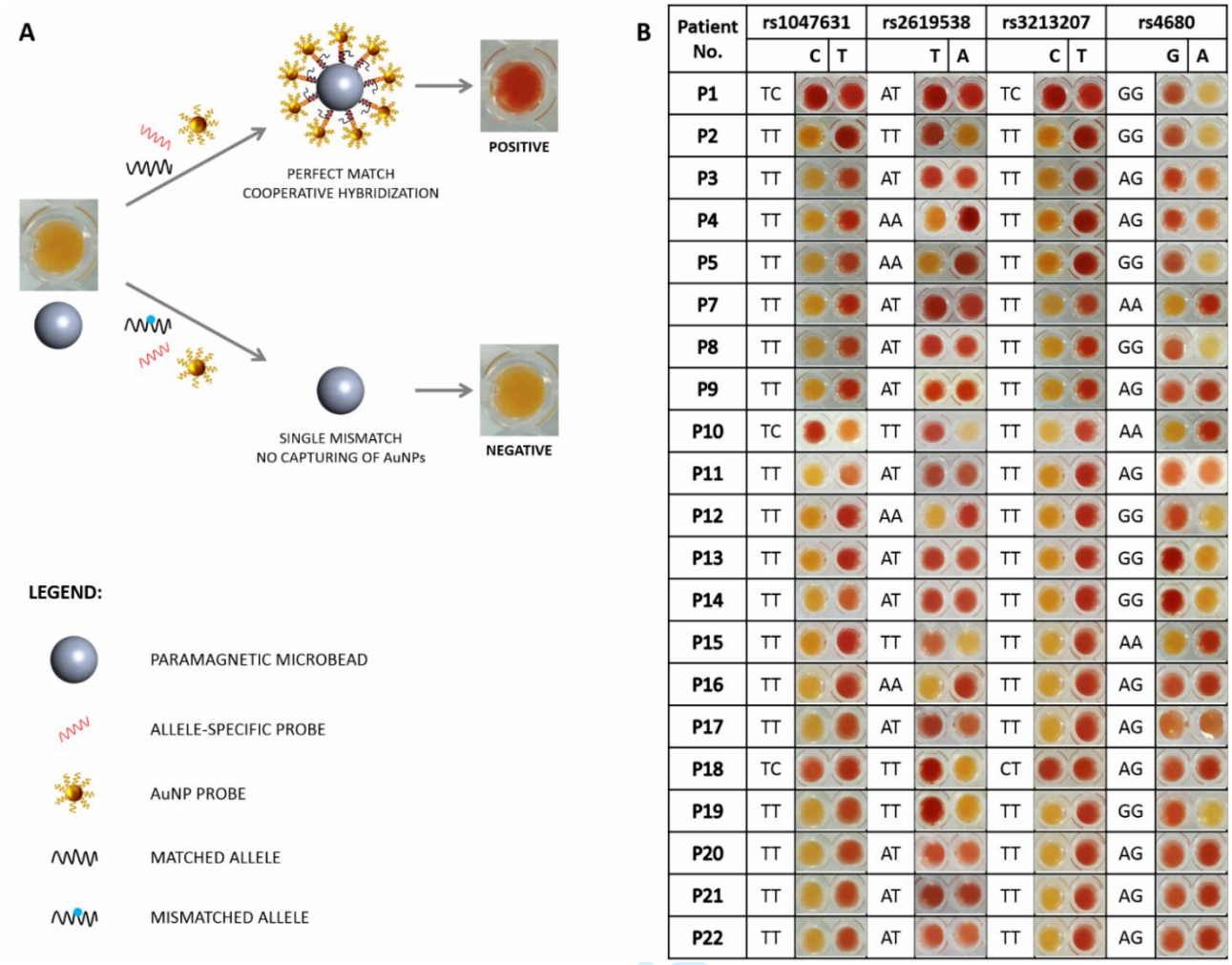
**Figure 3. Concomitant reduced levels of COMT and Dys1 in patients with 22q11.2DS is associated with worst executive function performance and increased motor disturbances. a,** Dys-1 protein levels (expressed as fold change in DysHap +/+ (n=18), +/- (n=28) and -/- (n=34) subjects (one-way ANOVA, $F_{(2,74)}$=4.25, p=0.017). **b**, Dys1 mRNA expression levels in DysHap CC (n=16), CT (n=214) and TT (n=508) subjects (one-way ANOVA, $F_{(2,735)}$=6.9, p=0.010). **c**, Dys-1 protein levels (expressed as fold change) in DysHap CT (n=12) and TT (n=66) subjects (two-tailed unpaired t-test, t=0.93, df= 74, p=0.35). **d**, WCST errors (one-way ANOVA, $F_{(2,43)}$=2.81, p=0.05) **e**, motor disturbances (one-way ANOVA, $F_{(3,43)}$=2.62, p=0.062), and **f**, sleep disturbance score (one-way ANOVA, $F_{(2,43)}$=3.47, p=0.024) in COMTVal DysHap +/+ (n=14), COMTMet DysHap +/+ (n=12), COMTVal DysHap -/- (n=9), COMTMet DysHap -/- (n=11) subjects. **g**, WCST errors (one-way ANOVA, $F_{(3,84)}$=4.64, p=0.004) **h**, motor disturbances (one-way ANOVA, $F_{(3,84)}$=0.40, p=0.748), and **i**, sleep disturbance score (one-way ANOVA, $F_{(3,79)}$=0.81, p=0.490) in COMTVal DysTC (n=11/13), COMTMet DysTC (n=9), COMTVal DysTT (n=33/35), COMTMet DysTT (n=31) subjects. Post hocs: *P<0.05, **P<0.005. Each histogram shows the mean ± s.e.m.

**Colorimetric detection of COMT and Dys1 functional genetic variants in humans**

To allow large-scale screening of Dys1 rs2619538-rs3213207-rs1047631 and COMT rs4680 SNP signature we then set up and validated a rapid and low-cost diagnostic test. This test exploits DNA-functionalized gold nanoparticles (AuNPs) and streptavidinated paramagnetic microbeads to realize a colorimetric strategy, which allows naked-eye discrimination of each SNPs using minimal instrumentation [47].

Genomic DNA from patients was amplified by standard PCR to obtain different short amplicons, each containing one of the SNP of interest and biotinylated at their 5' ends. The amplicons were then captured on the surface of paramagnetic microparticles and analyzed through a one-tube assay, based on two sequential hybridization reactions. The first reaction (discrimination step) exploits a SNP discriminating probe, specific for one of the two versions of each SNP tested, while the second reaction (detection step) employs universal AuNP probes. The binding of the discriminating probe to a complementary amplicon allows the capturing of the AuNP probe, which turns the yellow microparticle suspension to brilliant red, due to the plasmonic properties of AuNPs. Thus, each amplicon is interrogated with a pair of discriminating probes for the SNP of interest, and the corresponding sample turns red only when a probe matches the target. A yellow/red result indicates a homozygous sample, while a red/red result indicates a heterozygous sample (Fig. 4a; Supplementary Table 2).

The test was performed on 22 blood human samples (Fig. 4b). To validate the test results, rt-PCR was performed on the same samples with standardized protocols. The agreement between the results of the two tests was 100%. This strongly supports the potential of the colorimetric test for future applications for large-scale screening in the general population.




| Patient No. | rs1047631 | | rs2619538 | | rs3213207 | | rs4680 | |
|---|---|---|---|---|---|---|---|---|
| | C | T | T | A | C | T | G | A |
| P1 | TC | | AT | | TC | | GG | |
| P2 | TT | | TT | | TT | | GG | |
| P3 | TT | | AT | | TT | | AG | |
| P4 | TT | | AA | | TT | | AG | |
| P5 | TT | | AA | | TT | | GG | |
| P7 | TT | | AT | | TT | | AA | |
| P8 | TT | | AT | | TT | | GG | |
| P9 | TT | | AT | | TT | | AG | |
| P10 | TC | | TT | | TT | | AA | |
| P11 | TT | | AT | | TT | | AG | |
| P12 | TT | | AA | | TT | | GG | |
| P13 | TT | | AT | | TT | | GG | |
| P14 | TT | | AT | | TT | | GG | |
| P15 | TT | | TT | | TT | | AA | |
| P16 | TT | | AA | | TT | | AG | |
| P17 | TT | | AT | | TT | | AG | |
| P18 | TC | | TT | | CT | | AG | |
| P19 | TT | | TT | | TT | | GG | |
| P20 | TT | | AT | | TT | | AG | |
| P21 | TT | | AT | | TT | | AG | |
| P22 | TT | | AT | | TT | | AG | |

**Figure 4.** Colorimetric detection of SNPs signatures. **a.** Assay strategy: SNPs detection occurs on paramagnetic microbeads, which are yellow at the beginning of the assay. When the allele-specific probe hybridizes with a matched target, cooperative hybridization with the AuNP probe stabilizes the binding and allows their efficient capturing on the bead surface. The AuNPs confer to the beads a red colour (positive result). Conversely, when the target does not match the allele specificity of the probe, the AuNP probes are not captured on the beads surface, which remains yellow (negative result). **b.** Colorimetric detection of SNPs signatures in 22 patients.

## Discussion

The main finding of this study is evidence of a non-linear gene-gene interaction between COMT and Dys1 with a concomitant alteration and inversion of functions at the level of cortical and striatal dopaminergic system and related behaviors. This epistatic interaction has potential clinical relevance for dopamine-related genetic pathologies, as illustrated in the context of cognitive and motor disturbances present in 22q11.2DS.

Unexpectedly, and in contrast to the effects of single gene mutations, the hypofunction of both COMT and Dys1 genes produced a hypodopaminergic vulnerable state in the PFC, contraposed to a hyperdopaminergic vulnerable state in striatal regions. The opposite effects of the COMT-Dys1 genetic interaction in amphetamine-induced dopamine release in PFC versus striatal regions might originate from a circuit mechanism, involving inversely coupled dopamine transmission between the two regions [27, 48-50], and direct anatomical connections with opposite feedbacks to each other [51]. Remarkably, the COMT-Dys1-dependent mesocortical and mesostriatal dopaminergic alterations resemble abnormalities considered key features in patients with schizophrenia [50, 52, 53]. Thus, our findings suggest that COMT-Dys1 functional genetic interaction is important for establishing the proper activity balance between mesocortical and mesostriatal dopaminergic circuits. Moreover, our studies further suggest that dopaminergic cortical/striatal dichotomy could arise from the same genetic background. This has been difficult to determine in humans where methodological limitations prevent to simultaneously image dopamine dynamics in striatal and cortical regions [54]. Notably, we found that these dopaminergic alterations are relevant in the context of 22q11.2DS. These results complement previous evidence suggesting dopaminergic alterations in subjects with 22q11.2DS, because of their COMT hemideletion and altered dopamine functioning [24-26, 44]. Future studies will be needed to differentiate the circuit- and cell-specific effects of the COMT-Dys1 genetic interactions.

In contrast to what is expected by hypo-functioning of the single COMT or Dys1 genes (current data and [11, 14, 17, 22]), concomitant hypofunction of both genes in the same subject recapitulated many behavioral abnormalities that are considered rodent correlates of schizophrenia-relevant behavioral abnormalities. In particular, deficits of COMT*Dys1 double heterozygous in PPI are analogous to those found in patients with schizophrenia [27, 42]. Furthermore, COMT*Dys1 double heterozygous-dependent impairments in the temporal order object recognition tasks and in the EDS stage of the attentional set shifting task are key cognitive processes altered in patients with schizophrenia [22, 27, 35, 37, 55, 56]. Finally, COMT*Dys1 double heterozygous mice's supersensitivity to amphetamine might parallel the psychotic agitation of patients, amphetamine's psychogenic property in healthy subjects, and exacerbation of psychotic experiences mediated by amphetamine in patients with schizophrenia [27, 57]. Notably, apart from an EDS deficit in Dys1+/- mice, these alterations were different in single mutant mice for COMT or Dys1. COMT*Dys1 double heterozygous, however, did not show any deficit in sociability and social novelty which has also been used as a potential correlate of social withdrawal in schizophrenia [27, 58]. However, we employed a three-chamber task, which has been designed to catch more autism-relevant social deficits that might be less relevant to the distinct socio-cognitive deficits present in patients with schizophrenia [59]. Further studies might address this in the future using more refined socio-cognitive tasks [60, 61].

The investigated COMT-by-Dys1 epistasis is expected to be only one of several different genetic interactions relevant to the development of schizophrenia or other neuropsychiatric disorders. Indeed, while in 22q11.2DS patients we found a consistent prediction of the COMT-Dys1 genetic interaction in executive functions and motor defects, no clear effects were evident in relationship to psychiatric diagnosis (Supplementary Table 1). Perspective clinical studies in larger and older populations might better address this latter topic. Indeed, our sample was composed mostly by teenagers, while psychotic events are expected to appear later in life [44, 62]. Nevertheless, our evidence of non-linear gene-gene interaction underlies the importance of considering this kind of epistatic events that goes undetected in common GWAS studies. This could be one of the factors contributing

to the missing heritability evident in psychiatric disorders [9], which would deserve further investigations. In particular, the functional genetic interaction by COMT and Dys1 common variants here reported might have general implications to a number of pathologies linked with altered dopaminergic signaling (e.g. schizophrenia, ADHD, Parkinson's etc.) as well as for a number of therapies targeting the dopaminergic system (e.g. antipsychotics and psychostimulants).

In summary, the COMT-Dys1 functional genetic interaction recapitulate important features relevant to schizophrenia dopaminergic neuropathology. We describe previously unexplored non-linear effects of this specific genetic interaction in the modulation of dopaminergic transmission, consistent with schizophrenia-relevant endophenotypes. Moreover, we confirmed in human subjects with 22q11.2DS the behavioral effects of this epistasis predicted by mouse studies, finally providing new tools to simply and cheaply check the targeted common genetic variants. Thus, our experimental model provides the conceptual need to address different gene-gene interactions, and the clinical relevance for these epistatic interactions at the base of complex behavioral traits.

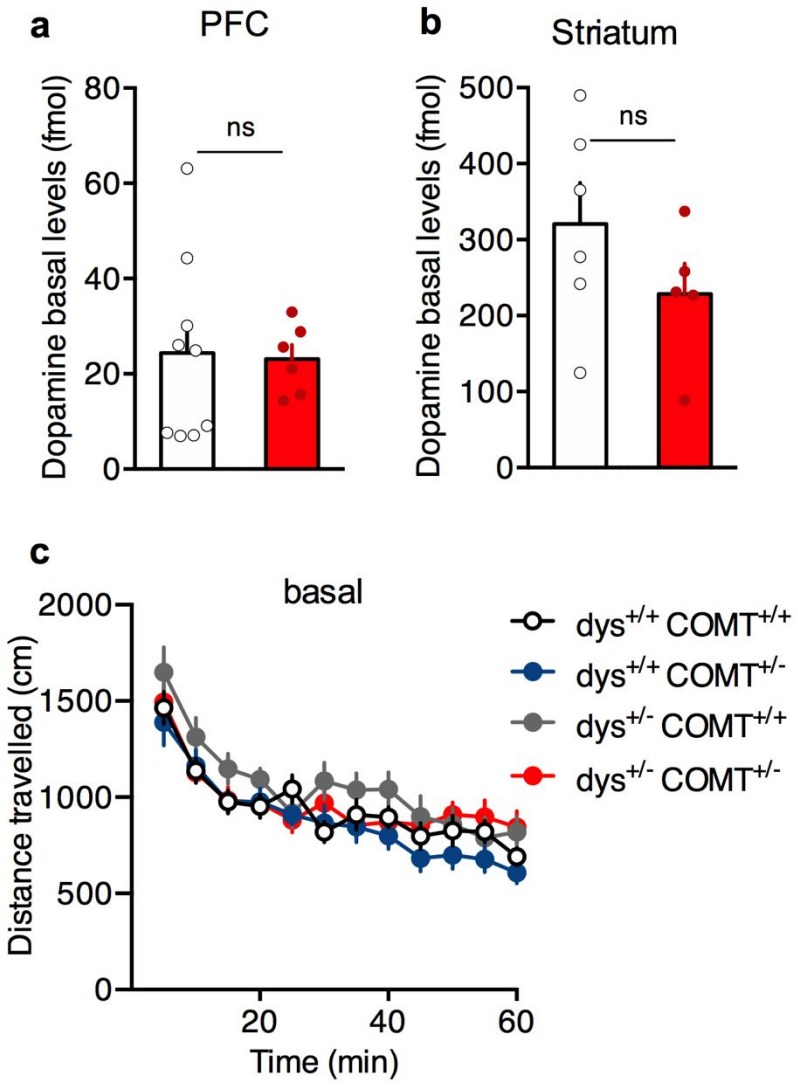
**Funding.** This work was supported by funding from the Istituto Italiano di Tecnologia, the Ministero della Salute italiano (project GR-2016-02362413), and Fondazione Telethon Italia (project GGP19103) to FP.

**Competing Interests' Statement.** None. The authors declare that they have no conflict of interest.

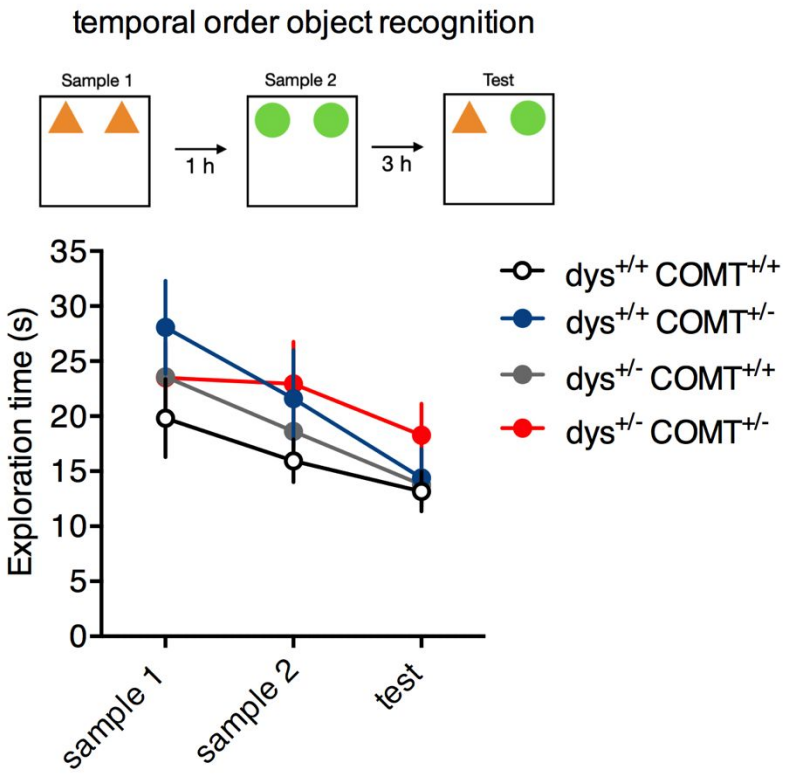
**Acknowledgments.** We thank M. Morini, D. Cantatore, G. Pruzzo, B. Chiarenza, A. Monteforte and C. Chiabrera, for technical support.

**Author Contributions.** Conceptualization, FM, DS and FP; Methodology and Investigation, FM, DS, MP, MM, RM, GU, PV, MCT, PPP, MADL and FP; Resources, FM, DRW, CSW, PPP, MADL, SV and FP; Writing, all authors; Visualization, FM, DS, PV and FP; Supervision, FP; Funding Acquisition, DRW, CSW, PPP, SV and FP.

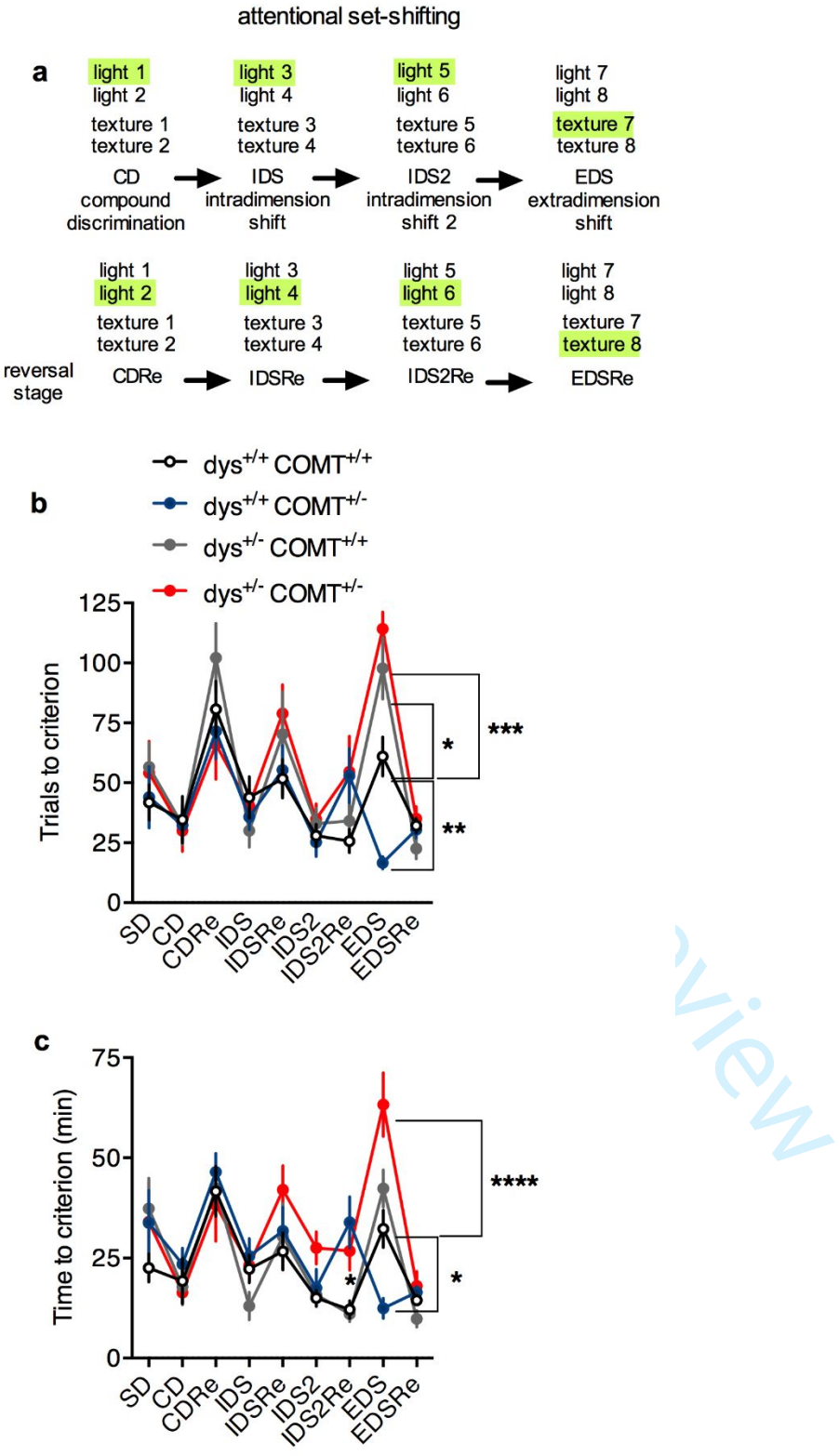
**Supplementary Figures**




**Supplementary Figure 1. a,** no difference in basal level of dopamine in the PFC between dys$^{+/+}$ COMT$^{+/+}$ and dys$^{+/-}$ COMT$^{+/-}$ (two-tailed unpaired t-test, t=0.14, d.f.=13, p=0.8834, *n*=6/9 each group). **b,** basal level of dopamine in the striatum did not differ between dys$^{+/+}$ COMT$^{+/+}$ and dys$^{+/-}$ COMT$^{+/-}$ (two-tailed unpaired t-test, t=1.31, d.f.=9, p=0.2200, *n*=5/6 each group). **c,** Ambulatory distance in 5-minute intervals displayed by COMT*dys double ko mice during the first 1 hour exposure to the open field arena (two-way ANOVA, genotype, $F_{(3,57)}$=1.67, p=0.1836; n=9-19/group).




**Supplementary Figure 2.** Exploration time (in seconds) during each phase (sample 1, sample 2 and test) of the temporal order object recognition task in all groups (two-way ANOVA, genotype, $F_{(3,162)}$=2.10, p=0.1014; n=11-17/group).




**Supplementary Figure 3. a**, attentional set-shifting task procedure. Mice were trained to perform multiple discrimination and reversal stages under reinforcement of the same dimension (i.e. light stimuli). During the EDS mice were required to shift their attention to previously irrelevant dimension (i.e. texture). **b**, increased number of trials to reach the criterion during the EDS in dys$^{+/-}$ COMT$^{+/-}$ mice compared to control mice control (two-way ANOVA, genotype x stage, $F_{(24,248)}$=2.10, p<0.0001; *n*=6/11 each group). **e**, increased time to reach the criterion during the EDS in dys$^{+/-}$ COMT$^{+/-}$ mice compared to control mice control (two-way ANOVA, genotype x stage, $F_{(24,248)}$=3.00, p<0.0001).

| | COMTVal DysHap+/+ | COMTMet DysHap+/+ | COMTVal DysHap-/- | COMTMet DysHap-/- | P values |
|---|---|---|---|---|---|
| Ns (F/M) | 14 (5/9) | 13 (5/8) | 9 (2/7) | 11 (4/7) | |
| Age | 16.8±1 | 16.4±2 | 16.2±2 | 16.7±2 | 0.99 |
| Psychiatric diagnoses | 12/14 | 8/13 | 5/9 | 7/11 | |
| Medications | 2/14 | none | none | 2/11 | |
| IQ | 86.2±3 | 79.0±4 | 85.7±6 | 84.4±4 | 0.60 |

| | COMTVal DysTC | COMTMet DysTC | COMTVal DysTT | COMTMet DysTT | P values |
|---|---|---|---|---|---|
| Ns (F/M) | 13 (9/4) | 9 (3/6) | 35 (7/28) | 31 (13/18) | |
| Age | 17.7±2 | 13.2±1 | 18.1±2 | 18.7±1 | 0.34 |
| Psychiatric diagnoses | 9/13 | 6/9 | 24/35 | 21/31 | |
| Medications | 2/13 | 1/9 | 3/35 | 2/31 | |
| IQ | 85.6±3 | 77.9±5 | 85.0±2 | 83.5±2 | 0.41 |

**Supplementary Table 1.** Demographic and genetics data on the cohort of patients with 22q11.2DS.

| PCR primers | | | | | |
|---|---|---|---|---|---|
| | | **Sequence (5'->3')** | **Length** | **T melting (°C)** | **GC%** |
| **DTNBP1** | **rs1047631** | GGAAAGCCAGGTTGTTTTATAGAGG | 25 | 55.8 | 44.0 |
| | | Biot – TCTCAGTTTACCGTCCTCACACT | 23 | 57.4 | 47.8 |
| | **RS2619538** | GATTGGATGAGGCCAGTGAG | 20 | 55.4 | 55.0 |
| | | Biot – TCATTGCTGGGGATGCAAAG | 20 | 56.0 | 50.0 |
| | **RS3213207** | CATGGTATATTCTAAATGTATTAGGGAAC | 29 | 51.9 | 31.0 |
| | | Biot – CTACCACTAACAACCAAAAAGAAAAC | 26 | 53.0 | 34.6 |
| **COMT** | **rs4680** | CCATCGAGATCAACCCCGAC | 20 | 57.6 | 60.0 |
| | | Biot – TTTCCAGGTCTGACAACGGG | 20 | 57.3 | 55.0 |

| Discriminating probes | | |
|---|---|---|
| **DTNBP1** | **rs1047631** | 5’ TAC **G** TAATTGCCAATAAAAAAAAAAAAAAAAAAAAAAAAAA **3’** |
| | | 5’ TAC **A** TAATTGCCAATAAAAAAAAAAAAAAAAAAAAAAAAAA 3’ |
| | **RS2619538** | 5’ CCC **T** GATGTAAACTGAAAAAAAAAAAAAAAAAAAAAAAAAA 3’ |
| | | 5’ CCC **A** GATGTAAACTGAAAAAAAAAAAAAAAAAAAAAAAAAA 3’ |
| | **RS3213207** | 5’ CCA **G** TAATTACCCGTAAAAAAAAAAAAAAAAAAAAAAAAAA 3’ |
| | | 5’ CCA **A** TAATTACCCGTAAAAAAAAAAAAAAAAAAAAAAAAAA 3’ |
| **COMT** | **rs4680** | 5’ GGC **G** TGAAGGACAAAAAAAAAAAAAAAAAAAAAAAAAAAA 3’ |
| | | 5’ GGC **A** TGAAGGACAAAAAAAAAAAAAAAAAAAAAAAAAAAA 3’ |

**Supplementary Table 2. Sequences of primers and discriminating probes.**

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