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Increased tau phosphorylation follows impeded dopamine clearance in a P301L and novel P301L/COMT-deleted (DM) tau mouse model

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Abstract

In Alzheimer's disease, the phosphorylation of tau is a critical event preceding the formation of neurofibrillary tangles. Previous work exploring the impact of a dopamine blocking antipsychotic on tau phosphorylation in a tau transgenic model suggested that extracellular dopamine may play a regulatory role in the phosphorylation state of tau. In order to test this hypothesis, and in order to develop a mouse model of impaired dopamine metabolism and tauopathy, an extant P301L transgenic tau model of Alzheimer's disease and a novel P301L/catechol-*O*-methyltransferase deleted model (DM mouse) were treated with the norepinephrine reuptake inhibitor reboxetine, and prefrontal dopamine concentrations and the phosphorylated state of tau was quantified. In two experiments, male and female P301L+/+//COMT+/+ and P301L+/+//COMT−/− (DM) mice were treated with reboxetine 20 mg/kg IP. In one experiment, acutely following reboxetine injection, the prefrontal cortex of mice were microdialyzed for dopamine, and its metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid, utilizing the MetaQuant technique. In another experiment, acutely following reboxetine injections, tau phosphorylation was quantified in the frontal cortex, striatum, and hippocampus of the mice. Reboxetine injections were followed by significant increases from baseline in extracellular dopamine concentrations in P301L and DM mice, with significantly higher peak levels in the DM mice. Treatment was also followed by increases in tau phosphorylation spread throughout brain regions, with a larger impact on female mice. Extracellular dopamine concentrations exert an influence on the phosphorylation state of tau, with surges in dopamine associating with acute increases in tau phosphorylation.

Keywords

Alzheimer's disease; dopamine; phosphorylation; psychosis; tau

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The phosphorylation of tau is widely believed to be a critical event leading to the formation of paired helical filaments and subsequently neurofibrillary tangles, one of the diagnostic hallmarks of Alzheimer's disease (AD) (Grundke-Iqbal *et al.* 1986). It is estimated that while the normal adult brain contains a ratio of 2–3 moles of phosphate per mole of protein, the AD brain, on average, contains 8–10 moles per mole of protein (Medina and Avila 2015). An understanding of the biologic conditions that promote tau phosphorylation may enable efforts to interrupt this process and in so doing may hold promise for the development of tangle-based treatments in AD.

Dopamine is a catecholamine that acts in the brain as a neurotransmitter along diverse pathways in multiple regions, governing a range of behaviors from movement to learning to motivation (Berke 2018) and is entangled with tau in a complex relationship that has relevance to both Parkinson's disease and AD. The interaction between tau and dopamine appears to be transactional, with tau exerting an influence on dopaminergic neurons, and dopamine influencing phosphorylation patters of tau. With relevance to Parkinson's disease, while tau ablation does not appear to impact dopamine-dependent motor or cognitive deficits in a mouse model (Morris *et al.* 2013), increased tau expression has been associated with a loss of dopaminergic neurons in both *Drosophila* (Wu *et al.* 2013) and mouse models of tauopathy (Chiba *et al.* 2012). With relevance to AD, and from the opposite direction, dopamine signaling may augment phosphorylation states of tau inside of neurons. Elevations of extracellular dopamine have been associated with increased tau phosphorylation. In one study, cocaine treated rats evidenced increased PHF-1 in the hippocampus, cortex, and striatum at 8 and 16 days when compared with vehicle-treated animals (Liu *et al.* 2003). In a dopamine transporter (DAT) knockout mouse model, increased dopaminergic tone has been associated with a mortality rate as high as 36% over 1 year, with motor symptoms including clasping and gait abnormalities preceding death (Cyr *et al.* 2003). Interestingly, symptomatic DAT mice were shown in this study to have elevations of phosphotau as measured with soluble striatal PHF-1, implicating tau hyperphosphorylation in motor dysfunction, supporting the notion that excess dopamine increases tau phosphorylation.

Previously, we reported on a robust decrease in tau phosphorylation in the cortex and striatum of Tg4510 tau mice following 2 and 6 weeks of treatment with haloperidol, a potent dopamine D_2 receptor blocking antipsychotic (Koppel *et al.* 2016). This result suggests that regional extracellular dopamine concentrations may influence tau phosphorylation patterns and that just as agents that block dopamine signaling reduce tau phosphorylation, therapies that increase dopamine signaling may promote it. No previous *in vivo* studies that we are aware of have correlated acute increases in tau phosphorylation with elevations in extracellular dopamine concentrations. In order to investigate the influence of acute increases in extracellular dopamine on tau phosphorylation states, we developed a novel mouse model that combines deficient dopamine metabolism and over-expressed human mutant tau and challenged the model with an agent known to promote frontal dopamine. We then measured tau phosphorylation states with high-sensitivity tau ELISAs that capture acute changes in phosphorylation states in soluble tau at a range of loci associated with neurofibrillary tangles (Acker *et al.* 2013).

The DM mouse comprises a JNPL3 tau model (expressing 0N4R human tau with the P301L tau mutation) (Lewis *et al.* 2000) crossed with a catechol-*O*-methyltransferase (COMT) deleted model (Gogos *et al.* 1998). COMT is an enzyme that is widely distributed in brain and is involved in the enzymatic degradation of dopamine (Napolitano *et al.* 1995). The enzyme has relevance to human psychosis, as the gene is located in the region of the 22q11.2 deletion syndrome, a condition in which one third of individuals develop psychosis (Murphy 2002). Dopamine, expected to be more abundant in conditions of impaired metabolism such as impaired COMT dysfunction, enjoys transdiagnostic relevance to psychotic syndromes including schizophrenia and bipolar disorder in conditions of excess production (Jauhar *et al.* 2017). The COMT knockout model was originally developed in order to investigate the behavioral consequence of COMT enzymatic disruption (Gogos *et al.* 1998). Given that data suggests that extracellular dopamine increases tau phosphorylation, disruption of dopamine clearance associated with COMT deletion in a tau mouse models would be expected to promote an excess of tau phosphorylation in situations of dopamine surge.

In order to evaluate the take advantage of the COMT deficit in promoting frontal dopamine in the DM model, we treated mice with reboxetine. Reboxetine is an antidepressant drug not approved for use in the United States that selectively blocks the norepinephrine transporter (NET) (Montgomery and Schatzberg 1998). In comparison with other brain regions, in the frontal cortex metabolism by COMT and reuptake by NET contribute disproportionately to dopamine concentrations, a byproduct of low DAT density (Carboni *et al.* 1990; Di Chiara *et al.* 1992). A previous study investigating the impact of COMT deficiency reported an escalation in dopamine concentrations in the frontal cortex of mice after being administered reboxetine, with COMT-deleted mice manifesting higher spikes in dopamine than COMT-sufficient mice (Kaenmaki *et al.* 2010). We tested the hypothesis that reboxetine would surge dopamine in cortex of the COMT-deficient DM mouse relative to the COMT-competent P301L tau mouse, and that increased dopamine concentrations would promote an increase in tau phosphorylation.

Materials and methods

All experiments were performed with the approval of the Institutional Animal Care and Use Committee at the Feinstein Institute and the Institutional Care and Use Committee at Charles River Laboratories. The experiment was done in two parts on separate cohorts of mice. In one cohort, DM and JNPL3 (P301L) mice underwent *in vivo* microdialysis in order to measure concentrations of dopamine and dopamine metabolites in the frontal cortex before and immediately following reboxetine treatment. In a separate cohort, DM and P301L mice were treated with reboxetine or vehicle and then killed in order to measure the impact of treatment on tau phosphorylation patterns in the cortex, hippocampus, and striatum. As the severity of tau pathology is sex dependent in the P301L mouse, with female mice exhibiting pathology earlier than males (Buccarello *et al.* 2017), separate cohorts of male and female mice of both genotypes were treated and results were analyzed within sex cohort in the tau experiments.

Animals

COMT-deleted mice on a C57BL/6 background were obtained as a generous donation from Dr. Joseph Gogos at Columbia University (research resource identifier, RRID:MGI:88470). Hemizygous Tg (Prnp-MAPT*P301L) JNPL3Hlmc mice (RRID: MGI: MGI 3604148) on a C57BL/6*DBA/2 background were obtained from Taconic (Taconic Biosciences; Rensselear, NY) (stock #1638). COMT-competent P301L mice were bred with the COMT-deleted mice over several generations to produce a colony of P301L+/+COMT−/− (DM); and P301L+/+COMT+/+ (P301L) mice. Genotyping of mice for COMT deletion and the presence of P301L mutation was performed at Transnetyx (Cordova, TN, USA) from tail samples, using a qPCR-based system to detect the presence or absence of a target sequence within each sample. Mice were bred and housed in the Center for Comparative physiology at the Feinstein Institute for Medical Research where the tau experiments were carried out and at Charles River Laboratories Inc, South San Francisco, CA, USA, where the microdialysis experiments were carried out. Block randomization of mice was used in the reboxetine treatment arm of the study. The study team at Charles River Laboratories Inc. were blinded to the genotypes of the mice undergoing microdialysis as were the study team at the Feinstein Institute during the performance of ELISAs. Codes for mice were only broken at the time of data analysis. Mice were housed three to four per cage, with access to food and water *ad libitum* and maintained on a 12-h light/dark cycle.

Microdialysis

Probe placement—Twenty-two mice (11 DM and 11 P301L) underwent probe placement in the prefrontal cortex for measurement of dopamine and metabolites using the MetaQuant (MQ) technique (Cremers *et al.* 2009). In the first experiment, we interrogated the impact of genotype on dopamine production in response to reboxetine by comparing extracellular dopamine concentrations of the cohort of the DM and the P301L mice, all aged 9 months. As tau phosphorylation patterns could be affected by probe placement, we did not quantify tau phosphorylation in the mice that underwent microdialysis, instead conducting a separate experiment evaluating the impact of reboxetine on tau (see below). We used DM and P301L mice of the same age to directly compare the effect of COMT deletion on dopamine production between the two cohorts of mice, although age has not been shown to impact dopamine response to reboxetine in the COMT-deleted model (Kaenmaki *et al.* 2010). Previous studies focused on the impact of sex on frontal dopamine release in response to reboxetine treatment in COMT-deleted and COMT-sufficient mice have concluded that there is no sex effect; nonetheless, the cohorts were balanced for sex (5 male and 6 female DM and 6 male and 5 female P301L) (Kaenmaki *et al.* 2010).

To minimize distress during microdialysis, mice were anesthetized using isoflurane (2%, 800 mL/min O_2). Lidocaine was used for local anesthesia and carprofen was used for peri-/post-operative analgesia. The animals were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) and MQ microdialysis probe (3 mm exposed polyacrylonitril membrane; BrainLink, Groningen, the Netherlands) were inserted into the Prefrontal Cortex (PFC). Coordinates for the tips of the probes for the PFC were anterior-posterior (AP) = +2.0 mm from the bregma, lateral (*L*) = −0.7 mm from midline and ventral

(*V*) = −4.0 mm from the dura, the toothbar set at 0.0 mm. After surgery animals were housed individually in cages and provided food and water *ad libitum*.

Microdialysis was performed on all animals 1 day after surgery. MQ probes were perfused with solution containing 147 mM NaCl, 3.0 mM KCL, 1.2 mM CaCl2, and 1.2 mM MgCl2 at a flow rate of 0.15 μL/min and a carrier flow rate of 0.9 μL/min. Microdialysis samples were collected over 30 min intervals. Reboxetine mesylate hydrate (Sigma, St Louis, MO, USA, catalogue #R6527) was dissolved in saline and was administered IP at a dose of 20 mg/kg (Kaenmaki *et al.* 2010). Two baseline samples were collected prior to administering reboxetine, and samples were collected for a total of 180 min following injection. Dialysate samples were stored at −80.0°C. Mice were then killed and brain tissue was collected for probe placement verification.

Quantification of dopamine/DOPAC/HVA—Concentrations of dopamine in dialysate samples were determined by HPLC with tandem mass spectrometry (MS/MS) detection using DA-13C6 as an internal standard. For this purpose, an aliquot of a solution containing the internal standard was mixed with an aliquot of each experimental sample. The resulting mixture was derivatized with SymDAQ™ (Brains On-Line, San Francisco, California) in the autosampler. After a predefined reaction period, an aliquot of the mixture was injected into a Prominence HPLC system (Shimadzu, Maryland, USA) by an automated sample injector (SIL-20ADHT; Shimadzu, Kyoto, Japan). Analytes were separated by liquid chromatography using a linear gradient of mobile phase B at a flow rate of 0.300 mL/min on a reversed-phase CORTECS C18 column (3.0*100 mm, 2.7 μm particle size; Waters, Milford, Massachusetts, USA) held at a temperature of 30°C. Mobile phase A consisted of 98% H_2O, 2% Acetonitrile (ACN) and 0.1% formic acid (FA). Mobile phase B was 90% ACN, 10% MeOH and 0.1% FA. Acquisitions were achieved in positive ionization mode using an API 4000 triple quadrupole (Applied Biosystems, Beverly, Massachusetts, USA) equipped with a Turbo Ion Spray interface. The ion spray voltage was set at 3.0 kV and the probe temperature was 200°C. The collision gas (nitrogen) pressure was held at 2 psi. Data were calibrated and quantified using the Analyst^{tm} data system (Applied Biosystems, version 1.4.2). Concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in dialysate samples were determined by HPLC with tandem mass spectrometry (MS/MS) detection using DOPAC-d5 and HVA-d5 as internal standards. For this purpose, an aliquot of a solution containing the internal standard was mixed with an aliquot of each experimental sample. Dialysate samples (20 μL) were injected into an Infinity 1290 LC system (Agilent, Santa Clara, California, USA) by an automated sample injector (SIL-20AD; Shimadzu, Japan). Analytes were separated by liquid chromatography using a linear gradient of mobile phase Bat a flowrate of 0.300 mL/min on a reversed-phase Luna C18(2)-HST column (3.0*100 mm, 2.5 μm particle size; Phenomenex, Torrance, California, USA) held at a temperature of 30°C. Mobile phase A consisted of H_2O with 5 mM ammonium formateand 0.1% FA. Mobile phase Bwas 70% ACN, 30% H_2O and 0.1% FA. Acquisitions were achieved in negative ionization modeusing a QTrap 5500 (Applied Biosystems) equipped with a Turbo Ion Spray interface. The ion spray voltage was set at −4.5 kV and the probe temperature was 500°C. The collision gas (nitrogen) pressure was held at the Medium setting level. The following MRM transitions were used for

quantification: m/z 167.1/123.0 for DOPAC and m/z 181.1/122.0 for HVA. Data were calibrated and quantified using the Analyst[tm] data system (Applied Biosystems, version 1.4.2).

Quantification of reboxetine—Reboxetine concentrations in the dialysate were quantified with HPLC with tandem mass spectrometry detection in MRM mode. Dialysate samples (5 μL) were injected into a Prominence HPLC system (Shimadzu, USA) by an automated sample injector (SIL-20ADHT, Shimadzu, Japan). Analytes were separated by liquid chromatography using a linear gradient of mobile phase B at a flow rate of 0.300 mL/min on a reversed-phase XBridge BEH Shield C18 column (50*2.1 mm, 2.5 μm particle size; Waters) held at a temperature of 40°C. Mobile phase A consisted of H_2O with 2 mM ammonium acetate and 0.1% FA. Mobile phase B was methanol with 2 mM ammonium acetate and 0.1% FA. Acquisitions were achieved in positive ionization mode using an API 4000 (Applied Biosystems) equipped with a Turbo Ion Spray interface. The ion spray voltage was set at 5.0 kV and the probe temperature was 500°C. The collision gas (nitrogen) pressure was held at 2 psi. The following MRM transition was used for quantification: m/z 314.3/176.2 for Reboxetine. Data were calibrated and quantified using the Analyst[tm] data system (Applied Biosystems, version 1.4.2).

Tau quantification

Reboxetine or saline was administered 1 : 1 to cohorts of 20 female DM and 20 female P301L mice, and 20 male DM and 20 male P301L mice, aged 6–8 months. Tau phosphorylation was quantified in response to injections and comparisons were made within cohorts (reboxetine vs. saline for each cohort of mice of a specific sex),and within each cohort the reboxetine and saline treated groups were age-matched. Reboxetine mesylatehydrate(Sigma,catalogue#R6527)was dissolved in saline and was administered IP at a dose of 20 mg/kg consistent with previous work in the COMT knockout mouse(Kaenmaki *et al.* 2010), at a total volume of 50 μL. Vehicle-treated mice received saline at the same volume, IP. After 2 h, all mice were killed via isoflurane overdose and the brain was removed, and hemisected sagitally. The forebrain region was dissected, and the striatum, frontal cortex and hippocampus were separated. In order to interrogate levels of phosphotau relevant across a spectrum of AD severity from mild to severe stages, a series of sandwich ELISAs employing a battery of monoclonal antibodies that stain neurofibrillary tangles at differing stages of maturity were employed, and ratios of phosphotau/total tau were calculated. A level of total tau was measured with DA31 (RRID:AB 2716724) (Acker *et al.* 2013); Ser396/404 phosphotau, represented in early and late Braak stages and present in a spectrum from early to mature tau aggregates (Mondragon-Rodriguez *et al.* 2013), was measured with PHF-1 (RRID: AB 2315150); Ser202 phosphotau, represented in early neuritic pathology, was measured with CP13(RRID:AB2314223)(Janocko*et al.* 2012);andThr231phospho-tau, a very early conformational epitope phosphorylated in pre-tangles (Augustinack *et al.* 2002) was measured with RZ3 (RRID: AB 2716721), according to previously established protocols established in our lab (Acker *et al.* 2013). Following dissection, the brain was homogenized using appropriate volume of homogenizing buffer: a solution of Tris-buffered saline, pH 7.4 containing 10 mM NaF, 1 mM NaVO3and 2 mMEGTA, including a complete Mini protease inhibitor cocktail (Roche Molecular

Biochemicals, Indianapolis, IN, USA, catalogue #11836153001). Samples were stored at −80°C. Heat stable preparations were used to obtain soluble tau levels, by adding 5% β-Mercaptoethanol and 200 mM NaCl to brain homogenate. Samples were then heated at 100°C for 10 min, cooled at 4°C for 15 min onice, and then centrifuged at 13000 *g* at 4°C for 15 min and supernatants were collected. 96-well plates (Fisher Scientific, Pittsburgh, PA, USA, catalogue #12-565-135) were, respectively, coated with DA31 (RRID: AB2716724),PHF1(RRID:AB2315150),CP13(RRID:AB2314223), and RZ3 (RRID: AB 2716721) at a concentration of 6 μg/mL in coating buffer,foratleast48 hat4°C.Plates were washed3×in wash buffer,and blocked for 1 h at 20°C using StartingBlock (Thermo Scientific, Waltham, Massachusetts catalogue # PI37542) to avoid non-specific binding. After the 1 h block, each plate was washed 5×and 50 μL of the appropriate sample was added to the wells, with 50 μL of DA9 (RRID: AB 2716723)-horseradish peroxidase detection antibody. Plates were incubated O/N shaking at 4°C and washed 99 in wash buffer. 1-Step ULTRA TMB-ELISA (Thermo Scientific, catalogue #34028) was addedfor30′ at20°Cbefore stopping the reaction with 2 MH_2SO_4.Plates were read with an Infinite m200 plate reader (Tecan, Mannedorf, Switzerland) at 450 nm.

Statistical analysis

Power calculations were carried out assuming a β risk of 0.8, and an α risk of 0.05, with primary outcomes in the microdialysis experiment being the difference in dopamine concentrations over time post-reboxetine injection between COMT-competent and COMT-deficient mice, and in the tau experiments being the difference in ratio of p-tau/total tau post-injection between reboxetine treated and untreated mice. Data representation was performed using GraphPad Prism 7.03 and statistical evaluation using SigmaStat 4.0 (SigmaStat, version 4, San Jose California) and GraphPad Prism (GraphPad Prism, San Diego, California). The effects of genotype on dopamine, DOPAC and HVA was evaluated using analysis of variance with repeated measures (RM_{ANOVA}). Further individual comparisons were evaluated using Bonferroni's *post hoc* test. The Grubbs' test was employed to identify outliers, and the Shapiro–Wilk test was run as a test of normality. No mice were excluded from any of the analyses. Student's *t*-test was employed to evaluate differences in tau phosphorylation patterns between reboxetine- and saline-treated cohorts, followed by effect size calculations using Cohen's d for any significant differences. The level of statistical significance was set at $p < 0.05$ for all tests.

Results

Microdialysis

Treatment with reboxetine was well tolerated. An increase in extracellular dopamine concentrations from baseline in prefrontal cortex was significant at 60 min following reboxetine injection in both DM and P301L mice (Fig. 1). Starting from 90 min, dopamine concentrations were significantly higher in DM mice relative to P301L mice (Fig. 1.) Extracellular DOPAC levels in the prefrontal cortex were significantly elevated in the DM mice from baseline and across time points (Fig. 2.) As expected, given COMT deficiency, HVA was not detectable in the DM mice. No significant sex differences in dopamine,

DOPAC, or HVA concentrations were observed. Reboxetine levels reached Cmax at 150 min after injection in both DM and P301L mice and did not differ between genotypes (Fig. 3).

Tau

In response to reboxetine treatment when compared with saline injections, elevations in tau phosphorylation in female DM mice were observed in the cortex with a range of phosphotau antibodies that stain early (RZ3), mid (CP13), and late (PHF-1) tangle formations (Table 1). Reboxetine did not increase phosphorylation in the striatum in these mice, and in the hippocampus only CP13 phosphotau ratios were increased. In female P301L mice, there were robust elevations of tau phosphorylation in the cortex, striatum, and hippocampus, as measured with PHF-1, RZ3, and CP13 (Table 2). Reboxetine had no discernable impact on tau phosphorylation in male DM mice, with no significant changes in phosphotau in any region (Table 3). In male P301L mice, reboxetine treatment increased tau phosphorylation in the striatum and hippocampus, but not the cortex where there were no changes in phosphotau (Table 4).

Discussion

In the current report, we found that in mice homozygous for the P301L transgene, treatment with reboxetine was followed acutely by significant increases in extracellular dopamine concentrations, and contemporaneous increases in the phosphorylation of tau. In our previous work with the Tg4510 mouse, a forebrain expressed P301L model of tauopathy (Santacruz *et al.* 2005), a reduction in phosphorylation of tau was observed following the blockade of the dopamine D_2 receptor with haloperidol (Koppel *et al.* 2016). Similar to the current report, but in a different direction, reductions were observed in PHF1, RZ3, and CP13 phosphotau ratios. However, in the previous study, in two different experiments, there was an effect of treatment in the cortex and the striatum, but not the hippocampus, suggesting a regional role for dopamine neurotransmission. In the current report, reboxetine, which has previously been shown to promote dopamine efflux in the hippocampus (Borgkvist *et al.* 2012), promoted tau phosphorylation in the hippocampus. It may be that under basal conditions dopamine plays a larger regulatory role in tau phosphorylation states in the cortex and striatum, but in situations of dopamine surge this extends to other regions as well. Together, these studies suggest that dopamine does play some regulatory role in the phosphorylation state of tau.

Given the larger magnitude of dopamine surge in DM mice, and in light of the hypothesized relationship between dopamine and tau phosphorylation, we expected that following reboxetine treatment the DM mice would have more robust patterns of tau phosphorylation than similarly treated P301L mice, especially in the frontal cortex. This was not the case, and may reflect a threshold effect of dopamine on phosphorylation. Curiously, in male mice the treatment effect of reboxetine on tau phosphorylation patterns was only substantial in P301L mice, and not significant in any region of the male DM mice. Furthermore, there were no discernable effects of reboxetine treatment on phosphorylation in the cortex of male mice of either the P301L or DM genotype. This may be related in part to the lower levels of tau pathology expressed in the male mice carriers of the P301L genotype, a finding that has

been reported previously (Buccarello *et al.* 2017), and the more hippocampal distribution of pathology in the P301L model. The resistance in male mice to the development of tau pathology given commensurate transgene expression may also explain the more consistent cortical response in the female mice to dopamine. In female mice, phosphorylation of tau was increased in the cortex and hippocampus of both DM and P301L mice in response to reboxetine, while in the striatum only the P301L female mice evidenced a treatment effect. A blunting of phosphorylation response to reboxetine in the DM mice relative to the P301L mice in the striatum where an impact on phosphotau was entirely absent across the spectrum of epitopes in the DM mice of both sexes-was observed. It may be that compensatory mechanisms activated in COMT-deficient DM mice in the region of highest basal dopamine concentration buffer against dynamic dopamine-driven phosphorylation events that are observed in COMT-competent P301L mice. However, the striatum, densely populated with DAT, is not known to rely on NET; nonetheless a significant reboxetine effect on tau phosphorylation in the striatum of P301L mice was observed. It is possible that NET participation in striatal dopamine metabolism was captured, or the impact on tau of norepinephrine itself, which may have contributed to the impact on phosphorylation in other brain regions as well. Concentrations of norepinephrine have been reported to be evenly distributed between the prefrontal cortex, hippocampus and striatum (Ihalainen *et al.* 1999).

The DM model was conceived in part as a candidate preclinical behavioral model of psychosis in AD. The psychotic syndrome, comprising delusions and hallucinations, occurs in approximately 50% of those affected with AD (Koppel and Greenwald 2014) and has been associated with tau (Koppel *et al.* 2013a,b; Murray *et al.* 2013), especially in the frontal cortex, a region that has been shown to be uniquely impacted when interrogated with functional imaging (Koppel *et al.* 2013a,b) and neurocognitive testing (Koppel *et al.* 2012, 2013a,b). This has led to the development of a frontal, tau-driven model of disease (Koppel and Greenwald 2014). Dopamine has been independently linked with psychotic states in general via a predictable response to psychostimulant intoxication (Angrist *et al.* 1991), and an increased synthesis capacity in those with psychosis in the context of schizophrenia (Kesby *et al.* 2018), bipolar disorder (Jauhar *et al.* 2017), and temporal lobe epilepsy (Reith *et al.* 1994). Merging the phosphorylated tau pathology of the P301L mouse and the enhanced extracellular dopamine of the COMT-deleted mouse integrates neuropathologic and transdiagnostic neurochemical (Jauhar *et al.* 2017) risk factors for psychosis in AD, providing construct validity for the DM model as a novel model of behavioral symptoms in AD. In a previous human study of post-mortem AD brain, we reported on a sex-specific elevation in frontal phosphotau in females but not males who suffered with psychotic symptoms (Koppel *et al.* 2014a,b). In the current experiment, the female DM response to reboxetine-focal cortical hyperphosphorylation of tau in the absence of hippocampal or striatal alterations in response to treatment reifies the topography of tau-driven deficit abstractly rendered in the frontal model of psychotic AD (Koppel and Greenwald 2014). Following this biochemical characterization, a behavioral analysis of reboxetine-treated DM mice will be an important next step in the evaluation of the face validity of the model as a model of psychotic AD, to determine whether there are changes in the ethogram relevant to psychosis, for instance, in frontal-mediated sensorimotor gating (Koppel *et al.* 2014a,b) and

social cognitive impairment (Koppel *et al.* 2018) that would support the preclinical validity of the model.

This study has limitations. In the microdialysis experiment, the study was underpowered to find significant differences related to the effects of sex on dopamine metabolism within genotype. Larger numbers may have been helpful in exploring sex-based differences in dopamine concentrations that may elucidate some of the tau outcomes that differed between sexes within genotype. Quantitation of dopamine concentrations in the hippocampus and striatum in response to reboxetine injections would have been helpful in correlating changes with phosphotau alterations, but was not feasible in the current experiment. Long-term studies focused on the impact of reboxetine on neurofibrillary tangle production would be helpful to discern whether changes in phosphorylation states of tau seen acutely have neuropathological consequence in long-term treatment.

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All experiments were conducted in compliance with the ARRIVE guidelines.

Abbreviations used:

AD	Alzheimer's disease
COMT	catechol-*O*-methyltransferase
DM mouse	COMT−/− P301L+/+
DOPAC	3,4-dihydroxyphenylacetic acid
HVA	homovanillic acid
PD	Parkinson's disease
p-tau	phosphotau
RRID	Research Resource Identifier

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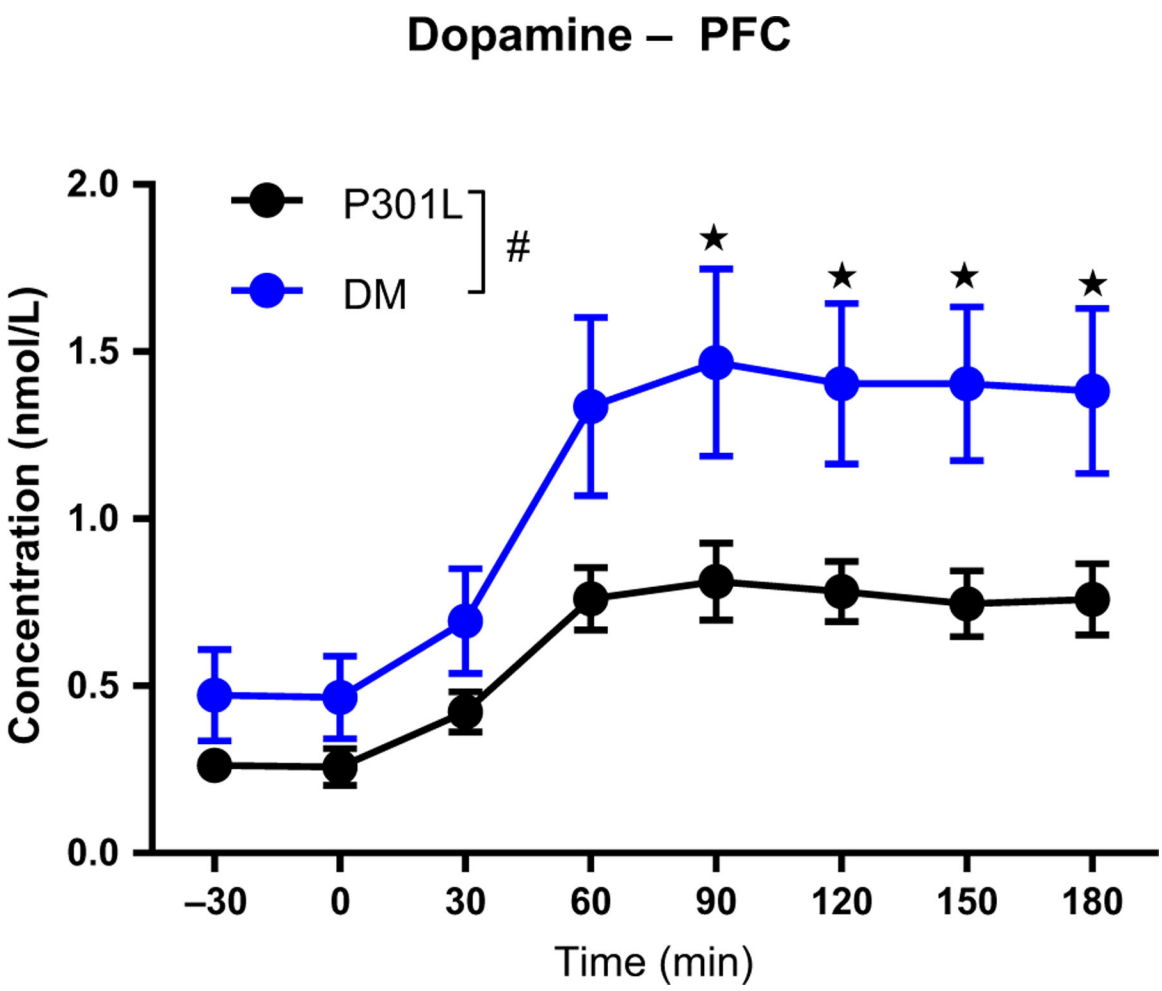

Fig. 1.

Effect of catechol-*O*-methyltransferase-deficiency on DA levels in the PFC after reboxetine treatment. Interstitial fluid from the PFC was collected from P301L and DM mice using Metaquant microdialysis. Mice received reboxetine treatment (20 mg/kg, i.p.) at $T = 0$. Data are expressed as DA concentration (nM) ± SEM. $N = 11$/genotype. *$p < 0.05$ DM vs. P301L mice, interaction genotype 9 time effect. #$p < 0.05$ DM vs. P301L mice, main effect genotype.

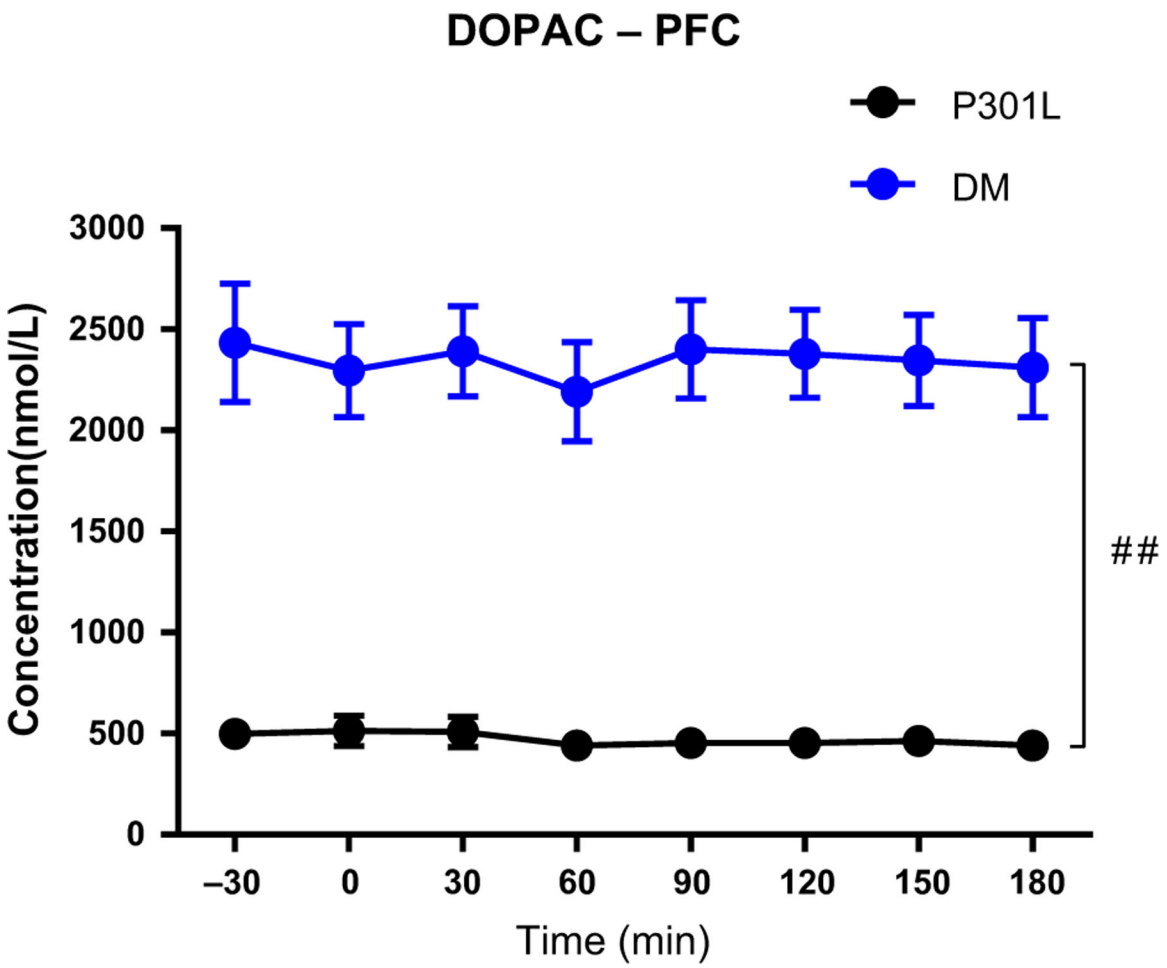

Fig. 2.

Effect of catechol-*O*-methyltransferase-deficiency on free 3,4-dihydroxyphenylacetic acid (DOPAC) level in the PFC after reboxetine treatment. Data are expressed as DOPAC concentration (nM) ± SEM. $N = 11$/genotype. $^{\#\#}p < 0.005$ DM vs. P301L mice, main effect genotype.

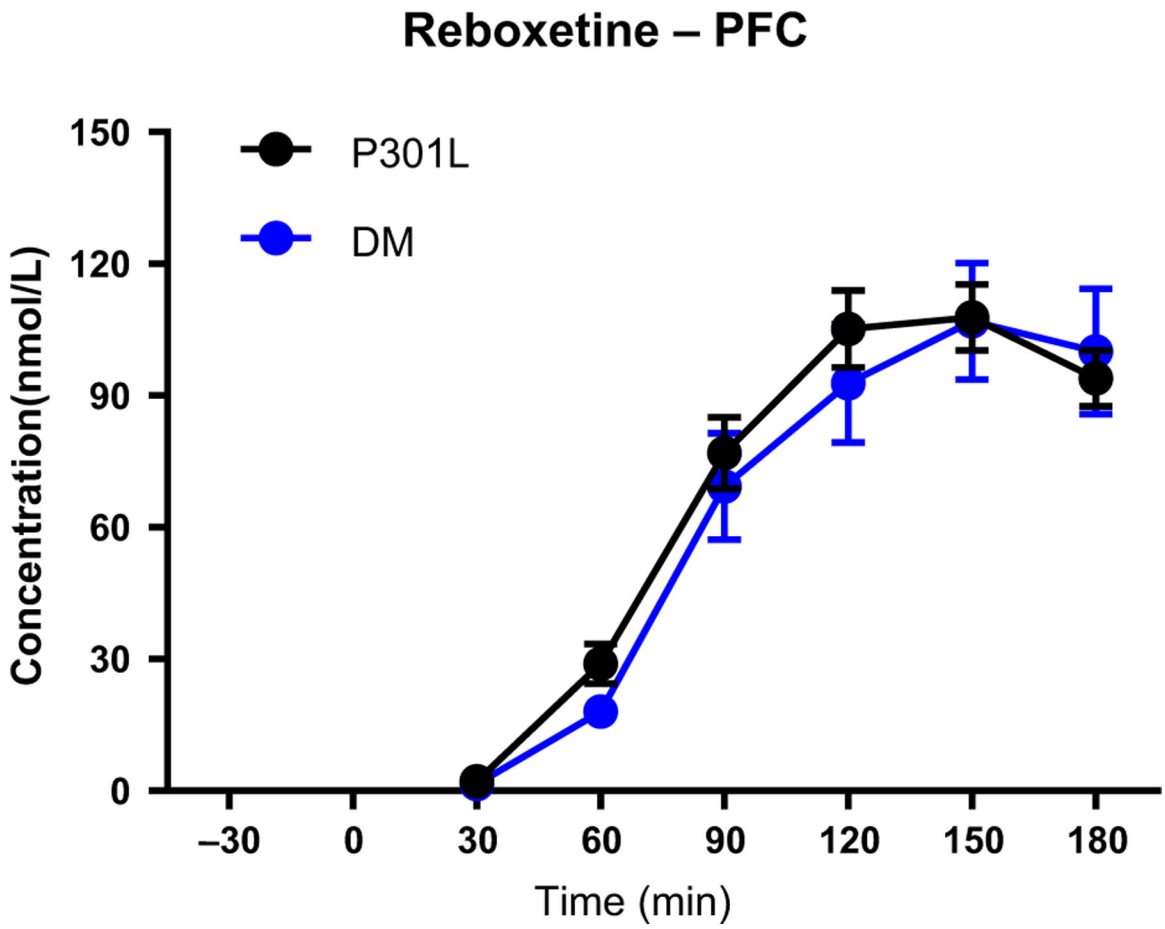

Fig. 3.

Effect of catechol-*O*-methyltransferase-deficiency on free reboxetine level in the PFC. Data are expressed as reboxetine concentration (nM) ± SEM. *N* = 11/genotype.

Table 1

DM females

Region	Saline *N* = 10	Reboxetine *N* = 10	Δ p-tau/total	*p*	Effect size (95% CI)
Cortex					
PHF-1/DA31	46.06 ± 11.59	63.77 ± 23.99	17.71 ± 8.42	0.05	0.94 (0.016,1.86)
RZ3/DA31	16.09 ± 3.35	21.13 ± 5.77	5.04 ± 2.11	0.028	1.07 (0.13,2.01)
CP13/DA31	26.90 ± 7.22	36.45 ± 7.01	9.55 ± 3.18	0.008	1.34 (0.37,2.31)
Striatum					
PHF-1/DA31	52.41 ± 21.83	47.43 ± 21.05	−4.98 ± 9.59	0.61	NS
RZ3/DA31	20.89 ± 12.68	17.33 ± 3.56	−3.56 ± 4.17	0.40	NS
CP13DA31	31.28 ± 15.85	40.26 ± 14.5	8.98 ± 6.79	0.20	NS
Hippocampus					
PHF-1/DA31	23.79 ± 6.074	28.30 ± 8.14	4.51 ± 3.21	0.18	NS
RZ3/DA31	5.73 ± 5.76	5.92 ± 2.92	0.19 ± 2.04	0.93	NS
CP13/DA31	14.85 ± 8.85	21.83 ± 5.17	6.98 ± 3.24	0.045	0.96 (0.037,1.89)

NS, non-significant.

Total tau (DA31) and phosphotau were quantified with ELISA. Phosphotau (p-tau) was quantified using an ensemble of antibodies: PHF-1(pSer396/ 404), CP13 (pSer202), and RZ3(pThr231); and a ratio of p-tau/DA31 was calculated for each antibody in each region. Changes (Δ p-tau/total) in the ratio of p-tau/DA31 (arbitrary units) represent the difference between mean p-tau/DA31 of vehicle-treated mice and mean p-tau/DA31 of reboxetine treated mice. A value of $p < 0.05$ was considered significant, and for each significant difference effect sizes were calculated with 95% confidence intervals.

Table 2

P301L females

Region	Saline *N* = 10	Reboxetine *N* = 10	Δ p-tau/total	*p*	Effect size (95% CI)
Cortex					
PHF-1/DA31	55.05 ± 12.74	83.29 ± 19.77	28.24 ± 7.44	0.0013	1.70 (0.68,2.72)
RZ3/DA31	9.80 ± 1.62	12.15 ± 3.20	2.35 ± 1.13	0.053	NS
CP13/DA31	40.29 ± 8.09	55.30 ± 19.25	15.01 ± 6.61	0.035	1.02 (0.085,1.95)
Striatum					
PHF-1/DA31	41.99 ± 8.75	54.84 ± 15.38	12.85 ± 5.94	0.033	1.03 (0.094,1.96)
RZ3/DA31	13.14 ± 2.67	27.47 ± 9.24	14.34 ± 3.04	0.0002	2.11 (1.014,3.20)
CP13DA31	33.56 ± 3.05	49.62 ± 7.11	16.06 ± 2.45	< 0.0001	2.94 (1.67,4.20)
Hippocampus					
PHF-1/DA31	65.25 ± 22.15	90.56 ± 22.87	25.31 ± 10.07	0.021	1.12 (0.18,2.07)
RZ3/DA31	25.79 ± 6.75	56.38 ± 43.83	30.60 ± 14.02	0.042	0.98 (0.048,1.90)
CP13/DA31	39.69 ± 11.37	56.66 ± 10.38	16.97 ± 4.87	0.003	1.56 (0.56,2.56)

NS, non-significant.

Total tau (DA31) and phosphotau were quantified with ELISA. Phosphotau (p-tau) was quantified using an ensemble of antibodies: PHF-1(pSer396/404), CP13 (pSer202), and RZ3(pThr231); and a ratio of p-tau/DA31 was calculated for each antibody in each region. Changes (Δ p-tau/total) in the ratio of p-tau/DA31 (arbitrary units) represent the difference between mean p-tau/DA31 of vehicle-treated mice and mean p-tau/DA31 of reboxetine treated mice. A value of $p < 0.05$ was considered significant, and for each significant difference effect sizes were calculated with 95% confidence intervals.

Table 3

DM males

Region	Vehicle *N* = 10	Reboxetine *N* = 10	Δ p-tau/total	*p*	Effect size
Cortex					
PHF-1/DA31	48.97 ± 14.97	65.04 ± 24.01	16.07 ± 8.95	0.089	NS
RZ3/DA31	14.50 ± 2.18	19.38 ± 9.07	4.88 ± 2.95	0.11	NS
CP13/DA31	91.62 ± 30.57	83.75 ± 45.30	−7.87 ± 17.28	0.65	NS
Striatum					
PHF-1/DA31	25.49 ± 15.16	34.16 ± 7.561	8.67 ± 5.36	0.12	NS
RZ3/DA31	19.04 ± 10.38	19.13 ± 8.81	0.09 ± 4.31	0.98	NS
CP13DA31	26.75 ± 20.28	36.93 ± 11.18	10.18 ± 7.32	0.18	NS
Hippocampus					
PHF-1/DA31	81.74 ± 24.62	101.0 ± 23.09	19.25 ± 10.67	0.088	NS
RZ3/DA31	12.17 ± 3.301	13.91 ± 6.86	1.73 ± 1.67	0.48	NS
CP13/DA31	43.52 ± 12.65	55.98 ± 22.24	12.46 ± 8.09	0.14	NS

NS, non-significant.

Total tau (DA31) and phosphotau were quantified with ELISA. Phosphotau (p-tau) was quantified using an ensemble of antibodies: PHF-1(pSer396/404), CP13 (pSer202), and RZ3(pThr231); and a ratio of p-tau/DA31 was calculated for each antibody in each region. Changes (Δ p-tau/total) in the ratio of p-tau/DA31 (arbitrary units) represent the difference between mean p-tau/DA31 of vehicle-treated mice and mean p-tau/DA31 of reboxetine treated mice. $p < 0.05$ was considered significant, and for each significant difference effect sizes were calculated with 95% confidence intervals.

Table 4

P301 males

Region	Saline *N* = 10	Reboxetine *N* = 10	Δ p-tau/total	*p*	Effect size (95% CI)
Cortex					
PHF-1/DA31	70.19 ± 17.34	84.54 ± 22.82	14.35 ± 9.06	0.13	NS
RZ3/DA31	35.35 ± 6.84	46.19 ± 18.44	10.84 ± 6.22	0.098	NS
CP13/DA31	38.24 ± 14.28	51.44 ± 14.35	13.20 ± 6.40	0.054	NS
Striatum					
PHF-1/DA31	38.13 ± 5.95	46.57 ± 5.62	8.44 ± 2.59	0.0043	1.46 (0.47,2.44)
RZ3/DA31	20.04 ± 2.97	30.39 ± 7.74	10.35 ± 2.62	0.0009	1.69 (0.67,2.71)
CP13DA31	51.21 ± 19.62	51.91 ± 30.31	0.7 ± 11.42	0.95	NS
Hippocampus					
PHF-1/DA31	38.41 ± 10.27	58.86 ± 11.49	20.45 ± 4.87	0.0005	1.87 (0.82,2.92)
RZ3/DA31	20.28 ± 2.079	32.84 ± 9.57	12.56 ± 3.10	0.007	1.81 (0.77,2.85)
CP13/DA31	54.19 ± 16.39	78.29 ± 17.29	24.10 ± 7.53	0.005	1.43 (0.45,2.41)

NS, non-significant.

Total tau (DA31) and phosphotau were quantified with ELISA. Phosphotau (p-tau) was quantified using an ensemble of antibodies: PHF-1(pSer396/404), CP13 (pSer202), and RZ3(pThr231); and a ratio of p-tau/DA31 was calculated for each antibody in each region. Changes (Δ p-tau/total) in the ratio of p-tau/DA31 (arbitrary units) represent the difference between mean p-tau/DA31 of vehicle-Vehicle-treated treated mice and mean p-tau/DA31 of reboxetine treated mice. A value of $p < 0.05$ was considered significant, and for each significant difference effect sizes were calculated with 95% confidence intervals.