Disruption of prepulse inhibition is associated with severity of compulsive behavior and nucleus accumbens dopamine system changes in Sapap3 knockout mice

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Highlights

- Disruption of PPI is associated with severity of compulsive grooming in Sapap3-KOs
- D1 and D2/3 receptors in NAcC are negatively correlated with PPI and grooming
- Chronic fluoxetine normalizes PPI and grooming
- Therapeutic efficacy of fluoxetine on PPI correlates with attenuated DAT in NAc
- NAc dopamine changes may underly severity of PPI in Sapap3-KOs

Abbreviations

CMS= centromedial striatum; DAT = Dopamine transporter; FLX= Fluoxetine; KOs = Knockout mice; NAc= nucleus accumbens; NAcC= NAc core; OCD= Obsessive compulsive disorder; PPI=prepulse inhibition; PP=prepulse; SSRI= selective serotonin reuptake inhibitor; VEH= vehicle; WT= wild-type.

Abstract

Obsessive compulsive disorder (OCD) is associated with disruption of sensorimotor gating, which may contribute to difficulties inhibiting intrusive thoughts and compulsive rituals. Neural mechanisms underlying these disturbances are unclear; however, striatal dopamine signalling is implicated in both OCD pathophysiology and the neural regulation of sensorimotor gating. The goal of this study was to examine the relationships between sensorimotor gating, compulsive behavior, and dopamine system functioning in Sapap3 knockout mice (KOs), a widely used preclinical model system for OCD research. Using prepulse inhibition (PPI), a translational measure of sensorimotor gating, we found no differences between Sapap3-KOs and controls when comparing group means. However, there was significant heterogeneity in PPI levels across Sapap3-KOs, which was negatively correlated with severity of compulsive grooming. Disruption of PPI and compulsive grooming were associated with reduced dopamine D1 and D2/3 receptor density in the nucleus accumbens core (NAcC) measured via autoradiography. Finally, chronic treatment with a first-line pharmacotherapy for OCD, the selective serotonin reuptake inhibitor (SSRI) fluoxetine, normalized PPI; these therapeutic effects were associated with reduced density of dopamine transporter in NAcC. This is the first detailed characterization of heterogeneity across different OCD-relevant behavioral and neurochemical measures in a transgenic model system, highlighting associations between severity of compulsive behavior and sensorimotor gating disturbances. Our findings also suggest that changes in NAcC dopamine functioning may be critical for the pathology and treatment of OCD-relevant sensorimotor gating impairments.

1. Introduction

Obsessive compulsive disorder (OCD) is a severe neuropsychiatric disorder affecting 1-3% of the population (Kessler et al., 2005, Koran, 2000, Koran et al., 2007). Intrusive thoughts (obsessions) and uncontrollable actions (compulsions) are the central clinical features of OCD. Sensorimotor gating is an information processing mechanism that is disrupted in OCD (Ahmari et al., 2012, Ahmari et al., 2016, Steinman et al., 2016, Hoenig et al., 2005, Swerdlow et al., 1993), and is thought to profoundly influence both the content and organization of what is consciously perceived by an individual, through filtering of less relevant information. It has been proposed that sensorimotor gating impairments in OCD may facilitate intrusive thoughts and disrupt inhibitory control over compulsive actions, although these relationships have not been clearly demonstrated in patients.

Elevated dopamine signalling in both dorsal and ventral striatum causes disruption of PPI in preclinical rodent models (Rodrigues et al., 2017, Wan and Swerdlow, 1996, Swerdlow et al., 1990). In addition, dopamine system disturbances have been described in the striatum in patients with OCD (Perani et al., 2008, Denys et al., 2004, Denys et al., 2013, van der Wee et al., 2004, Kim et al., 2007). *In vivo* molecular imaging with single photon emission computerized tomography (SPECT) and positron emission tomography (PET) has demonstrated that binding of striatal dopamine D1 and D2/3 receptors is reduced in OCD patients (Denys et al., 2013, Denys et al., 2004, Olver et al., 2009, Perani et al., 2008), and dopamine transporter binding is increased (van der Wee et al., 2004, Kim et al., 2003). It has been proposed that these alterations represent compensatory changes in response to elevated dopamine tone, consistent with convergent evidence that trans-diagnostic compulsive behaviors are associated with elevated striatal dopamine. Specifically, increased cue-evoked striatal dopamine release has been observed via PET imaging in cocaine users, people with binge eating disorder, and patients with Parkinson’s disease who develop compulsive behavior following dopamine agonist treatment (Wang et al., 2011, Wong et al.,

2006, Volkow et al., 2006, O'Sullivan et al., 2011). There is also evidence that drugs that increase dopamine signalling can exacerbate compulsive behavior in OCD patients (Jhanda et al., 2016). Further supporting the role of elevated dopamine in the manifestation of OCD symptoms, dopamine antagonists can be useful as adjunct treatments in some OCD patients (Dold et al., 2015, Keuneman et al., 2005). In addition, treatment with the leading pharmacotherapy for OCD, selective serotonin reuptake inhibitors (SSRIs), has been shown to attenuate striatal dopamine tone in rodents (Sawyer et al., 2012, Ichikawa and Meltzer, 1995, Morelli et al., 2011, Horne et al., 2008) and normalize dopamine receptor and transporter changes in OCD patients measured using *in vivo* molecular imaging approaches (Kim et al., 2007, Moresco et al., 2007). Together, these results suggest a relationship between alterations in striatal dopamine signalling, sensorimotor gating disturbances, and OCD symptoms.

Preclinical model systems can be used to gain mechanistic insight into the relationship between dopamine signalling, impaired sensorimotor gating, and other OCD-relevant behaviors using a straightforward translational paradigm: prepulse inhibition (PPI) of the acoustic startle response (Geyer and Dulawa, 2003). For example, studies in mouse models suggest that elevated striatal dopamine and disruption of dorsal striatum function may have relevance to disruption of PPI in Tourette's Syndrome (Baldan Ramsey et al., 2011, Baldan et al., 2014). Although prior studies have demonstrated PPI deficits in pharmacologic models of relevance to OCD (Shanahan et al., 2009, Shanahan et al., 2011), sensorimotor gating deficits and their relationship to compulsive behaviors have not been characterized in transgenic mouse models. Here, we examined PPI in Sapap3 knockout mice (KOs), a widely used model system for OCD research (Welch et al., 2007, Burguiere et al., 2013, Ade et al., 2016) that displays compulsive grooming, anxiety (Welch et al., 2007), and impaired reversal learning (van den Boom et al., 2019, Manning et al., 2019). Both compulsive grooming and anxiety are normalized following treatment with effective

interventions for OCD, including chronic administration of the SSRI fluoxetine (Welch et al., 2007) and deep-brain stimulation (Pinhal et al., 2018). Sapap3-KOs also show hyperactivity in the dorsal and centromedial striatum (CMS) (Welch et al., 2007, Ade et al., 2016, Burguiere et al., 2013), which could be predicted to influence PPI; the ventral striatum has not yet been thoroughly characterized in this model. The goal of this study was therefore to begin to probe the mechanistic relationship between PPI, striatal dopamine signalling, and compulsive behavior in Sapap3-KOs, using a combination of behavioral testing, ex *vivo* autoradiography, and pharmacological treatment studies.

2. Materials and Methods

2.1 Animals

Male and female Sapap3-KO and wild-type (WT) littermates were bred from heterozygous x heterozygous breeding pairs on a C57/BL6 background, and were derived from a colony initially established at MIT by Dr Guoping Feng (Welch et al., 2007). Mice were housed in groups of 2-5 same-sex mice in individually ventilated cages (except for fluoxetine-treated mice, which were transferred to group housed filter top cages), with *ad libitum* access to standard chow and drinking water, in 12:12 light/dark cycle. All behavioral testing was conducted in the light cycle. All experiments were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh in compliance with National Institutes of Health (NIH) guidelines for the care and use of laboratory animals. Cohort details can be found in the Supplementary Materials.

2.2 Drugs

Fluoxetine (NIMH Chemical Synthesis and Drug Supply Program, Bethesda, MD) was administered in the drinking water at 100-150mg/L to attain approximately 18mg/kg dose (based on recorded fluid consumption of ~160mL/kg/day); this achieves plasma concentrations within the clinically effective range for the treatment of OCD (Dulawa et al., 2004). D1 antagonist SCH-23390 [R(+)-SCH-23390-HCl, Sigma, St Louis, MO)] was dissolved in saline (1mg/kg) and administered by intraperitoneal injection (5ml/kg)(Doherty et al., 2008).

2.3 Prepulse Inhibition testing

PPI was tested using SR-LAB startle response system (San Diego Instruments, San Diego, CA) with methods similar to those previously described (Geyer and Dulawa, 2003, Ahmari et

al., 2013). All mice were tested in startle only session (10 x 120dB startle only trials) prior to first PPI session for habituation to testing procedures. Mice were excluded from analysis if PPI data suggested poor hearing [(average of startle only trial amplitudes in blocks 2&3) < (3 x average of no stimulus trial amplitudes)].

2.4 Grooming testing

Prior to first grooming test, mice were habituated to acrylic testing chambers (8 x 8 x 12 inches) for 2 consecutive days (20 mins/session). Mice were then videotaped for a 20 minute session, and grooming was manually scored offline by trained experimenters blind to genotype, PPI values, and fluoxetine treatment (where applicable). Detailed timeline in Supplementary Materials.

2.5 Lesion severity scoring

A trained experimenter blind to genotype and mouse behavioral scores rated each animal for evidence of skin lesions on the day of PPI testing. Mice that had regions on the face or body where skin was raw, inflamed, or bloody were classified in the “lesion” group. In line with institutional IACUC protocols, mice classified with lesions were treated using topical antibiotic (Neomycin and Polymyxin B Sulfates, and Bacitracin Zinc Opthalmic Ointment; Akorn, Lake Forest, IL), with the exclusion of the day of and prior to behavioral testing.

2.6 Autoradiography

Fresh frozen brains were obtained from mice assessed in PPI and grooming tests, and processed according to standard protocols to detect dopamine D1 and D2/3 receptors and dopamine transporter (DAT) binding (Jupp et al., 2013, Mennicken et al., 1992). Technical details are provided in supplementary materials (Table S1 and Figure S1).

2.7 Statistical analysis

Nonparametric t-tests were used for analyses of data that were not normally distributed (e.g. when KOs were analysed as one heterogeneous population). Repeated measures ANOVAs were used to compare the effects of group or genotype across different prepulse (PP) intensities; however, analysis of group differences examined average PPI across the 3 PP intensities. Pearson's R was used to assess correlations between different measurements. 3-way repeated measures ANOVAs were calculated using SPSS (IBM, Armonk, NY; version 25); and t-tests, correlations, and 1 or 2-way ANOVAs were performed using GraphPad Prism (La Jolla, CA; version 7.03). Mice were excluded if PPI values were more than 2 standard deviations outside their group mean or PPI data indicated poor hearing (as defined above). Sexes were combined for all behavioral analyses after it was determined that no significant sex differences were observed (Figure S2). Graphs show mean ± 95% confidence interval unless otherwise stated.

3. Results

3.1 Disruption of PPI is associated with compulsive grooming phenotype in Sapap3-KOs

PPI was tested in male Sapap3-KO and WT mice at ~8 months of age, when approximately half of KOs (10/18) showed lesions resulting from compulsive grooming. Analysis of average %PPI revealed no significant differences between genotypes (Figure 1A; Mann-Whitney test: $p=0.29$), with both groups showing increased PPI with higher PP intensities (Figure S3A; main effect PP, $F_{(2,56)}= 87.43$, $p<0.0001$). However, visual inspection demonstrated more variability in KOs compared to WTs, prompting us to determine whether this heterogeneity within KOs mapped onto other behavioral measures. As expected, Sapap3-KOs groomed

significantly more than WTs (Figure 1B; Mann-Whitney test: WT: M=38.5, KO: M=628, $p<0.0001$). In addition, we determined that a subgroup of Sapap3-KOs with skin lesions (KO-L, n=10, unfilled symbols Figure 1B) showed elevated grooming relative to KOs without lesions (KO-NL, n=8, filled symbols), supporting the idea that lesions result from elevated grooming. However, KO-NL also groomed significantly more than WTs (Bonferroni post-hoc test, $p=0.012$; also see Figure S5 for data from an independent cohort).

Given the significant heterogeneity in the KO compulsive grooming phenotype, the relationship between PPI and grooming levels was assessed to determine whether PPI deficits were observed in mice with more severe compulsive grooming. In Sapap3-KOs, average %PPI was significantly negatively correlated with time spent grooming (Figure 1C; $R=-0.69$, $p=0.0015$), and was selectively impaired in KO-L relative to WTs and KO-NL (Figure 1D; main effect of group, $F_{(2,27)}=8.5$, $p=0.0013$). Importantly, startle amplitude during blocks 2 and 3, which is used to calculate %PPI, did not differ between groups (main effect of group, $p=0.43$; Figure S3B).

3.2 Comparison of the developmental progression of compulsive grooming, lesions, and PPI impairment

To examine the relationship between the emergence of compulsive grooming and disruption of PPI in Sapap3-KOs, PPI and grooming were both assessed monthly from 2 months (before the development of lesions) until 8 months of age (when approximately half of KOs have lesions). Time spent grooming progressively increased with age (Figure 2A, genotype x age interaction: $F_{(6,270)}=15.8$, $p<0.0001$), with significantly elevated grooming detected at 5 months. The number of Sapap3-KOs with lesions resulting from severe compulsive grooming increased each month (6 months: n=5; 7 months: n=9, 8 months; n=12). Note, in other cohorts tested weekly at an earlier developmental stage, a modest elevation in

grooming was detected as early as 7 weeks of age [Figure S4, (Corbit et al., 2019)]; however, this small effect-size difference was not detectable during the less frequent testing performed in the 2-8 months of age study.

When comparing PPI between WTs and KOs across development (not taking grooming severity into account), there was a trend towards reduced PPI in KOs (Figure S5A, p=0.095), but no interaction with age (p=0.93) [note: PPI increased with age consistent with previous reports (Shoji et al., 2016, Young et al., 2010); main effect of age: $F_{(6,270)}$=29.2, p<0.0001]. However, analysis of startle amplitude revealed age-dependent genotype differences (Figure S5B, genotype x age interaction $F_{(6,270)}$=3.8, p=0.001). Whereas startle amplitude decreased in WT mice as they aged [consistent with other studies in mice (Young et al., 2010, Shoji et al., 2016)], KOs had relatively stable startle amplitude over time. Since group differences in startle amplitude confound interpretation of PPI, we therefore focused analysis on 6-8 months of age when startle amplitude did not differ between the groups (Figure S5C-E). PPI was not significantly different between groups at 6 months (Figure 2B, $F_{(2,41)}$=0.7, p=0.49); however, KO-L showed reduced PPI relative to the other 2 groups at 7 and 8 months (Figure 2C-D, 7 months: $F_{(2,40)}$=9.2, p=0.0005; 8 months: $F_{(2,41)}$=7.8, p=0.0013).

3.3 PPI and grooming abnormalities in Sapap3-KOs are associated with changes in striatal dopamine receptors

To determine whether disruption of PPI and grooming severity in Sapap3-KOs were associated with changes in expression of dopamine receptors, autoradiography was used to measure *ex vivo* binding of D1 and D2/3 in CMS and ventral striatum (nucleus accumbens core; NAcC) of animals from experiment 1 (see Figures 3A,E for regions of interest). Binding levels were then correlated with behavioral measures in Sapap3-KOs, to determine whether heterogenous severity of OCD-relevant phenotypes was associated with striatal dopamine

receptor changes. D1 receptor density was unaffected by genotype (Figure 3B, p=0.74); however, in Sapap3-KOs D1 density in NAcC was positively correlated with PPI (Figure 3C, NAcC: R=0.54, p=0.021). D1 density in CMS also showed a trend for correlation with PPI (Figure S6A, CMS: R=0.40, p=0.096). There was also a trend for negative correlation of grooming with D1 density in NAcC (Figure 3D, NAcC: R=-0.43, p=0.074), whereas D1 density in CMS was not associated with grooming (Figure S6B, CMS: p=0.16).

D2/3 receptor density was altered between the genotypes (Figure 3F; main effect genotype, $F_{(1,25)}$=4.6, p=0.042), and there was a trend for interaction with striatal region of interest suggesting that this effect was region specific (genotype x region interaction, p=0.097). Planned post-hoc tests revealed selective downregulation of D2/3 in NAcC of Sapap3-KOs (NAcC: $t_{(50)}$=2.7; p=0.017; CMS: p=0.74). In Sapap3-KOs, D2/3 density in NAcC was positively correlated with PPI (Figure 3G, R=0.59, p=0.017) and negatively associated with grooming (Figure 3H, R=-0.68, p=0.004). In contrast, D2/3 levels in CMS showed no correlation with behavior (Figure S6C-D; PPI: P=0.46; grooming: p=0.72).

3.4 Acute D1R blockade reverses PPI impairments in SAPAP3-KOs

We next hypothesized that lower levels of D1 and D2 receptors reflected compensatory downregulation in response to hyperdopaminergic tone in Sapap3-KOs. To begin to investigate this possibility, we tested whether increased signalling through dopamine D1 receptors is involved in disruption of PPI in Sapap3-KOs. Mice received acute treatment with the D1 antagonist SCH-23390 or vehicle in a cross-over design, and PPI was tested 10 minutes later. There were main effects of group and drug treatment (Figure 4A, $F_{(1,28)}$=5.3, p=0.011 and $F_{(2,28)}$=29.0, p<0.0001 respectively), and a trend for interaction ($F_{(2,28)}$=2.2, p=0.134). Planned post-hoc comparisons demonstrated decreased PPI in KO-L following vehicle administration ($t_{(56)}$=3.6, p=0.002), consistent with Fig.1D, but no difference between

KO-L and WT following SCH administration ($t_{(56)}$=1.9, p=0.17). Importantly, startle amplitude was not affected by 1mg/kg SCH-23390 (Figure 4B, main effect of drug: p=0.21; main effect of group: p=0.11; interaction p=0.33).

3.5 Normalization of PPI disruption and compulsive grooming following chronic fluoxetine treatment

Because compulsive grooming in Sapap3-KO mice is normalized following repeated treatment with the SSRI fluoxetine, we next investigated the impact of fluoxetine on disruption of PPI. Fluoxetine was administered in drinking water at a high dose (~18mg/kg), consistent with previous studies in preclinical models that have modelled doses required to achieve therapeutic response in patients (Shanahan et al., 2009, Shanahan et al., 2011, Ahmari et al., 2013, Bloch et al., 2010). PPI and grooming were assessed weekly to determine the onset of therapeutic effects, and repeated measures ANOVA was used to examine the effects of fluoxetine and lesion group across the treatment period. This analysis revealed a group-dependent effect of fluoxetine treatment on PPI that differed across the treatment period (Figure 5A; time x group x treatment interaction, $F_{(10, 255)}$= 2.1, p=0.027). To determine when the treatment groups began to differ, separate ANOVAs were applied to each timepoint (test details in Table S2). At 3 and 4 weeks, significant group x treatment interactions were detected; Bonferroni post-hoc tests demonstrated that these interactions reflected increased PPI in KO-L treated with fluoxetine compared to vehicle (3 weeks: t_{51}=3.6, p=0.002; 4 weeks: t_{51}=3.4, p=0.004). There was also a significant group-dependent effect of fluoxetine treatment on grooming behavior that differed across the treatment period (Figure 5B, time x group x treatment interaction, $F_{(10, 255)}$= 5.9, p<0.001); however, separate ANOVAs at each timepoint demonstrated more rapid emergence of significant therapeutic effects (Table S3). Significant treatment x group interactions were detected from 24 hours onwards, with post-hoc tests demonstrating that this was due to decreased grooming in KO-L treated with fluoxetine compared to vehicle (24 hours: t_{51}=2.8, p=0.024; 1 week: t_{51}=3.6,

p=0.002; 2 weeks: t_{51}=3.8, p=0.001; 3 weeks: t_{51}=4.7, p<0.0001; 4 weeks: t_{51}=4.9, p<0.0001).

3.6 DAT expression in NAcC is correlated with therapeutic effect of fluoxetine on PPI in Sapap3-KOs

Chronic fluoxetine treatment causes changes in striatal dopamine signalling, including reduction of dopamine concentrations (Morelli et al., 2011) and reduced DAT density (Horne et al., 2008). In OCD patients, DAT is increased at baseline (Kim et al., 2003), and decreases following treatment with SSRIs (Kim et al., 2007), suggesting that DAT may be a useful marker of dopamine system sensitivity to the therapeutic effects of fluoxetine. *Ex vivo* binding of DAT was therefore performed in animals from experiments 1 and 4 (Figure 1 and Figure 5) to examine the relationship between DAT binding and OCD-relevant behaviors at baseline and following fluoxetine treatment. In untreated mice (experiment 1), DAT was increased in Sapap3-KOs (Figure 6B, main effect of genotype, $F_{(1,29)}$=4.8; p=0.037). There was a trend for genotype x striatal subregion interaction, suggesting that this effect was larger in NAcC (p=0.09). NAcC and CMS DAT density were not correlated with grooming or PPI (Figure 6C-D, NAcC: grooming p=0.52; PPI p=0.358; Figure S6E-F, CMS: grooming p=0.61; PPI p=0.71).

In fluoxetine-treated mice from experiment 4, which included males and females, there was a trend for a sex difference in the effect of genotype on DAT binding ($F_{(1,46)=}$3.5, p=0.068), suggesting that DAT was higher in female KOs than in male KOs (Figure S7). Fluoxetine treatment did not lead to significant changes in DAT density (Figure 6E, main effect of treatment, p=0.129; treatment x region interaction p=0.894). To determine whether DAT was associated with the therapeutic effects of fluoxetine, DAT density was correlated with change in behavior measurements between baseline testing and the end of the

treatment period in KO-L treated with fluoxetine (who showed the most robust therapeutic effect). Reduced DAT in NAcC was associated with larger improvement in PPI (Figure 6G, R=-0.74, p=0.036). However, DAT in NAcC showed no relationship with changes in grooming (Figure 6H, p=0.81). There was no association between DAT in CMS and PPI or grooming (Figure S6G-H; p=0.52 and 0.76 respectively).

4. Discussion

Here, we demonstrated that Sapap3-KOs show impaired sensorimotor gating as measured by disruption of PPI, but found that these deficits are only manifested when mice have progressed to a severe compulsive grooming phenotype. Autoradiography revealed that disruption of PPI was associated with reduced NAcC D1 and D2/3 receptor binding. Similarly, severity of compulsive grooming was associated with reduced D2/3 binding in NAcC. These findings are consistent with a compensatory response to striatal hyperdopaminergic tone. This idea is further supported by our finding of normalization of PPI by acute D1-blockade in Sapap3-KOs with lesions. Furthermore, chronic treatment with the SSRI fluoxetine normalized PPI, and the magnitude of this therapeutic effect was associated with reduced density of NAcC DAT in fluoxetine treated KO-L mice. This is consistent with studies demonstrating that fluoxetine reduces striatal dopaminergic innervation (Horne et al., 2008) and dopamine concentrations (Morelli et al., 2011), and supports the idea that striatal dopamine changes are involved in the therapeutic efficacy of fluoxetine in OCD. These findings suggest that the neural mechanisms underlying disruption of PPI and compulsive behaviors may overlap, and that alterations in dopamine signalling within the NAcC may contribute to both the development and treatment of disrupted PPI in Sapap3-KOs.

4.1 Relationship between compulsive behaviour and sensorimotor gating

The observed association between severity of compulsive grooming and disruption of PPI in the Sapap3-KO model could reflect that one of these behaviors plays a causal role in the presentation of the other, or that a common neural mechanism(s) contributes to the development or manifestation of both behaviors. A clear relationship between PPI and other OCD symptoms has not been established in previous clinical studies. Early studies in medicated patients demonstrated that severity of PPI impairment was significantly associated with severity of OCD symptoms as measured by the Yale-Brown Obsessive Compulsive Scale (Y-BOCS) (Hoenig et al., 2005, Swerdlow et al., 1993). Although these associations have not been replicated in more recent studies examining unmedicated OCD patients (Steinman et al., 2016, Ahmari et al., 2012), reduced PPI in patients with tics was noted in one of these samples, supporting the hypothesis that disruption of PPI may be associated with aspects of OCD related to lack of inhibitory control over compulsions/tics (Ahmari et al., 2012, Ahmari et al., 2016). Interestingly, the two earlier studies examined patients with moderate symptom levels and greater heterogeneity (Swerdlow et al. 1993: Y-BOCS = 22.91 ± 4.25; Hoenig et al. 2005: 16.9 ± 9.86) compared to the more recent studies that had Y-BOCS scores in the severe range (Steinman et al. 2016 = 24.98 ± 3.46; Ahmari et al. 2012, 2016: 27 ± 5.5). This observation suggests that examination of correlations between OCD symptoms and other behavioral measures may require sampling across a broad range of patient severity, similar to our current examination of PPI in Sapap3-KOs displaying a range of severity of compulsive behavior.

Interestingly, we recently demonstrated that disruption of cognitive flexibility in a reversal learning task is not associated with severity of compulsive grooming in Sapap3-KOs (Manning et al., 2019); these findings have been replicated by an independent laboratory (van den Boom et al., 2019). This suggests that while overlapping neural disturbances may

contribute to compulsive grooming and disruption of PPI, distinct mechanisms may underlie impaired reversal learning. The Sapap3-KO model therefore serves as a useful system for further dissection of the contributions of overlapping versus distinct circuits associated with different OCD-relevant behavioral domains.

4.2 Dopamine system changes associated with OCD-relevant behaviors

Striatal dopamine signalling is implicated in both the pathophysiology of OCD and regulation of PPI. *Ex vivo* autoradiography was therefore used to examine the relationship between D1 and D2/3 receptor levels, PPI disruption, and compulsive grooming in Sapap3-KOs (Figure 2). These studies demonstrated reduced D2/3 receptor density in NAcC of Sapap3-KOs, and correlation of NAcC D2/3 receptor levels with both disruption of PPI and severity of compulsive grooming. Reduced NAcC D1 receptor binding was also associated with disruption of PPI. Together these results are consistent with PET imaging findings demonstrating reduced D2/3 and D1 binding in striatum of OCD patients (Denys et al., 2004, Denys et al., 2013, Olver et al., 2009). Compulsive behavior and disruption of PPI are both typically associated with a hyperdopaminergic state in striatum (Doherty et al., 2008, Jhanda et al., 2016, Wang et al., 2011, Wong et al., 2006, Volkow et al., 2006, O'Sullivan et al., 2011, Swerdlow et al., 1990), and PPI deficits were not observed in OCD patients undergoing adjunct treatment with dopamine receptor antagonists (Hoenig et al., 2005). These findings suggest that compulsive behavior and impairment of PPI are associated with increased dopamine tone, and that correlations between these OCD-relevant behaviors and reduced dopamine receptor density in Sapap3-KOs may reflect compensatory downregulation. In support of this theory, acute administration of the D1 antagonist SCH-23390 improved PPI in KO-L. Note that acutely administered antagonists are not ideal for investigating the involvement of specific dopamine receptor subtypes in more complex motor behaviors like grooming, because pharmacological manipulations of striatal dopamine have

significant effects on motor control. Genetic dissection of the involvement of distinct dopamine receptors in different OCD-relevant behavioral domains is therefore warranted.

Interestingly, our prior studies assessing the post-mortem concentration of dopamine and its metabolites in striatum using high performance liquid chromatography (HPLC) did not provide evidence of hyperdopaminergic tone in Sapap3-KO mice (Wood et al., 2018). This suggests that measurement of behavior-associated dopamine release *in vivo* may be necessary to detect the changes in striatal dopamine suggested by our data. This possibility is in line with clinical studies demonstrating that cues associated with compulsive behavior are necessary to evoke increased dopamine in disorders associated with compulsive behaviour (Wang et al., 2011, Wong et al., 2006, Volkow et al., 2006, O'Sullivan et al., 2011).

4.3 SSRI mechanisms of action

We demonstrated that SSRI treatment normalizes PPI in Sapap3-KOs. Receptor binding studies demonstrated that larger fluoxetine treatment response in KO-L (change in %PPI from baseline) was associated with lower density of NAcC DAT. This is consistent with PET studies demonstrating that DAT is elevated in OCD patients (van der Wee et al., 2004, Kim et al., 2003), and that reduced DAT in the basal ganglia following fluoxetine treatment is correlated with improved Y-BOCS scores (Kim et al., 2007). It is unclear whether changes in DAT binding density in our experiments and clinical imaging studies reflect reduction of the number of dopamine terminals innervating the striatum, which would correspond to reduced NAcC dopaminergic tone. Alternatively, these changes could reflect downregulation of DAT at existing terminals, which would correspond to increased NAcC dopamine tone due to decreased reuptake. Preclinical studies have demonstrated reduced striatal dopamine following chronic SSRI administration using *ex vivo* and *in vivo* measurements (Sawyer et

al., 2012, Morelli et al., 2011), supporting the former option. Further investigation of striatal dopamine in Sapap3-KOs *in vivo* while they undergo fluoxetine treatment may provide new insight about the changes in striatal DA system function supporting therapeutic effects in OCD.

4.4 Regionally specific dopamine system changes in striatum of Sapap3-KOs

Prior striatal investigations in Sapap3-KO mice have largely focused on dorsal and centromedial striatum (Ade et al., 2016, Burguiere et al., 2013, Corbit et al., 2019), demonstrating hyperactivity in striatal spiny projection neurons (SPNs). Given these previous findings in Sapap3-KOs in CMS, it is interesting that our current findings have demonstrated that dopamine system measures in NAcC are correlated with severity of compulsive grooming and PPI deficits and therapeutic efficacy of fluoxetine, whereas the same measurements in CMS are not. Although SPN activity in ventral striatum has not yet been characterized in Sapap3-KO mice, deep brain stimulation in ventral striatum reduces grooming in Sapap3-KOs, suggesting potential baseline abnormalities in neural activity in this region that may be related to behavioral dysfunction (Pinhal et al., 2018). Direct investigation of striatal SPN neural activity and dopaminergic signaling in CMS vs. NAcC in awake-behaving Sapap3-KOs will aid in dissecting the relationship between neural signalling in striatal subregions and OCD-relevant behaviors.

4.5 Progressive development of compulsive grooming

These studies are the first to thoroughly investigate the progressive development of the compulsive grooming phenotype in Sapap3-KOs, and raise the important question of whether there are distinct neural changes that lead to low grooming vs high grooming KOs. We show that grooming progressively escalates to a point where it continues despite the

presence of skin lesions, and that mice with lesions show significantly elevated grooming relative to non-lesioned KO mice (Figure S6F-H). Given the significant heterogeneity in compulsive grooming severity in Sapap3-KOs, observed both within animals (across time) and between animals at a given age, it will be important to report grooming severity in future research detailing the molecular, cellular and circuit disturbances that are present in this model.

5. Conclusions

These studies are the first to demonstrate an association between the development of OCD-relevant compulsive grooming and disruption of PPI in a transgenic mouse model. Through *ex vivo* neurochemical analysis of brain tissue from mice assessed in these distinct OCD-relevant behaviors, we have identified a potential role of reduced NAcC dopamine receptors in the manifestation of these maladaptive behaviors. In contrast, reduced density of NAcC DAT may be involved in the therapeutic effects of fluoxetine on PPI. By leveraging heterogeneity in the penetrance and progression of OCD-relevant behaviors in this preclinical model system, we have highlighted associations that may be relevant to neural changes occurring in distinct subsets of patients across the heterogeneous diagnosis of OCD and related disorders.

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Competing interests

All authors have no competing interests to report.

Figure legends

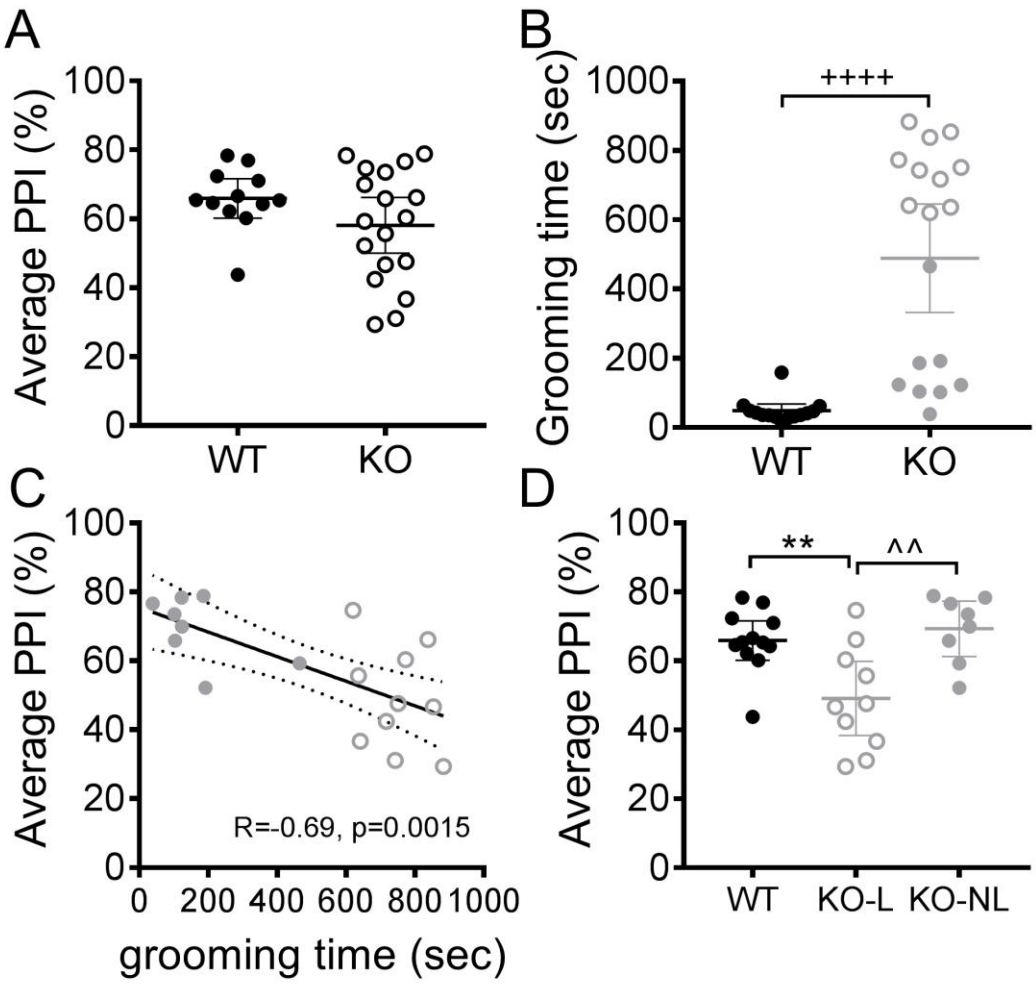

Figure 1: Disruption of PPI is associated with compulsive grooming phenotype in Sapap3-KOs

A) PPI (average % across 3 prepulse intensities tested) did not differ between Sapap3-KO and WT mice. B) Time spent grooming was significantly elevated in Sapap3-KO relative to WT, as previously described. Unfilled symbols denote KO-L, filled symbols denote KO-NL. C) Average PPI was significantly negatively correlated with time spent grooming, suggesting disruption of PPI in Sapap3-KOs with the most severe compulsive grooming phenotype. D) When Sapap3-KOs were separated based on the presence (KO-L) or absence (KO-NL) of lesions resulting from compulsive grooming, significant disruption of PPI in KO-L was revealed relative to WT and KO-NL (Bonferroni post-hoc tests: WT vs KO-L, p=0.006; WT vs

KO-NL, p>0.99, KO-L vs KO-NL p=0.003). + indicates significant difference between WT and Sapap3-KOs, * indicates significant difference between WT and KO-L, ^ indicates a significant difference between KO-L and KO-NL. ++++ p<0.0001; **/^^ p<0.01. n= 12 WT (14 for panel B), 18 KO (10 KO-L, 8 KO-NL). PPI = prepulse inhibition, KO= knockout, KO-L= KOs with lesions, KO-NL= KOs without lesions, WT= wild-type.

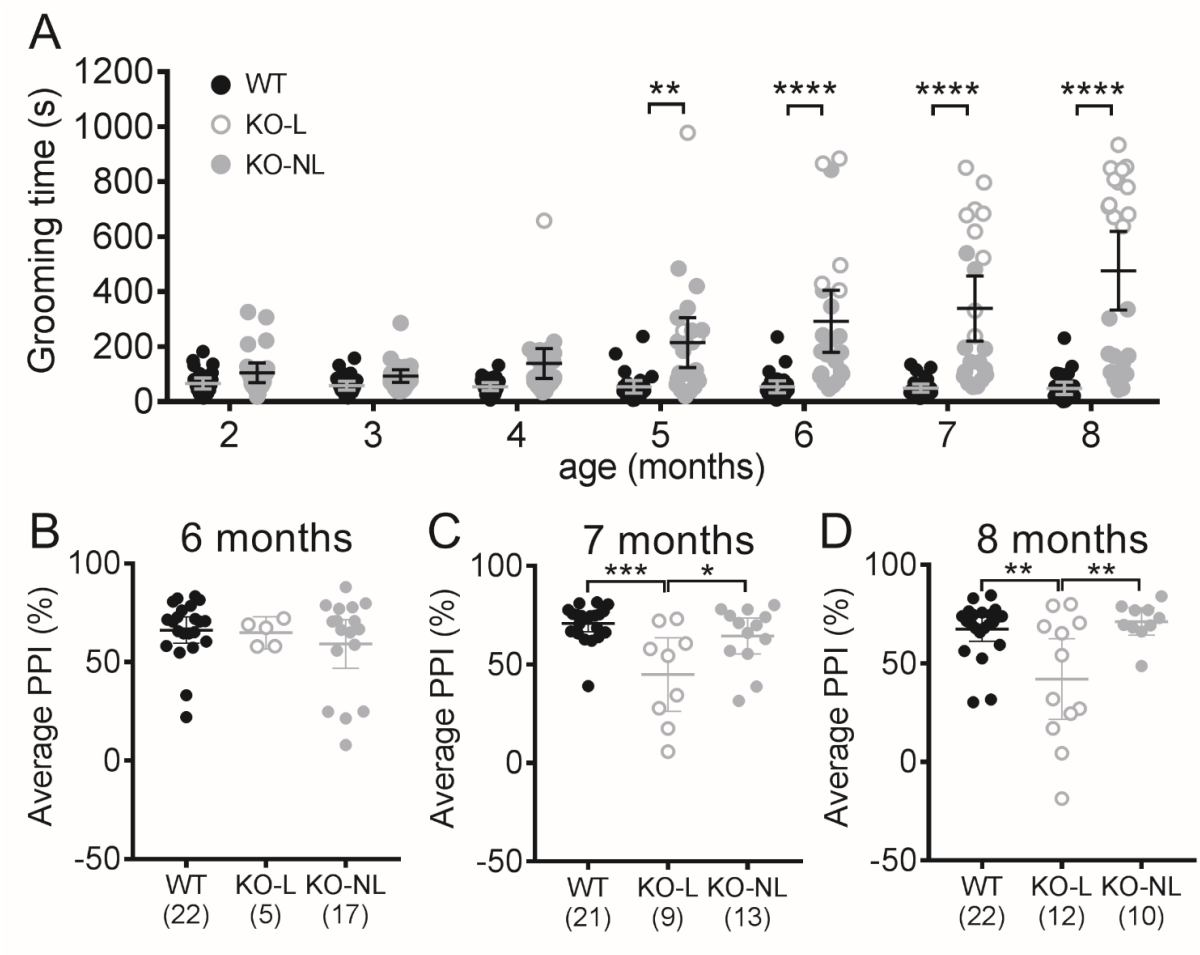

Figure 2: Comparison of the developmental progression of excessive grooming, lesions, and PPI impairment

A) Longitudinal progression of compulsive grooming phenotype in Sapap3-KOs from 2-8 months of age. Unfilled symbols are KO-L; filled symbols are KO-NL. B-D). PPI was compared between groups at 6-8 months of age when startle did not significantly differ between genotypes (see Figure S5B). Although there are no group differences at 6 months of age, PPI disruption emerges in KO-L at 7 months of age. * indicates significant Bonferroni post-hoc test comparing WT to a KO comparison group. **** $p<0.0001$, ** $p<0.01$, * $p<0.05$. n= 24 WT, 23 KO (panel A). KO= Sapap3 knockout, KO-L= KOs with lesions, KO-NL= KOs without lesions, WT= wild-type, PPI= prepulse inhibition.

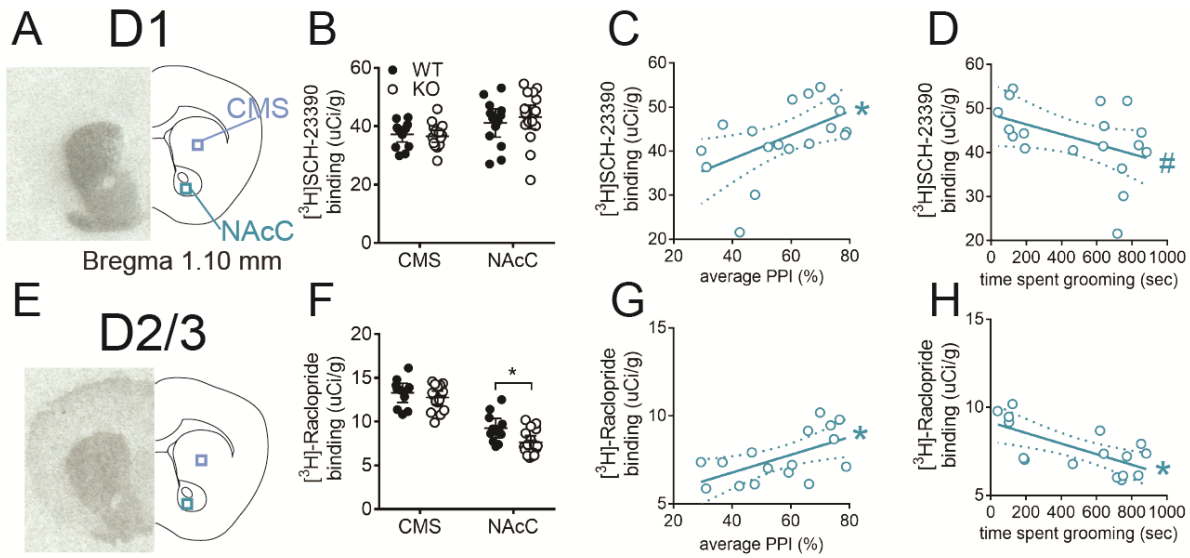

Figure 3: Dopamine system changes associated with disruption of PPI in Sapap3-KOs

A) Autoradiography was performed in striatal tissue to quantify density of D1 receptors in CMS and NAcC. Representative autoradiograph for total binding is shown in the left panel, with schematic of coronal brain section on the right. B) D1 density was unchanged in Sapap3-KOs; however, in Sapap3-KOs, reduced D1 density in NAcC was associated with lower PPI (C). D) Reduced D1 in NAcC also showed a trend for association with severity of compulsive grooming. E) Representative autoradiograph showing D2/3 density. F) D2/3 was reduced in NAcC of KOs relative to WT, whereas CMS binding was unchanged. Reduced D2/3 binding in NAcC was associated with lower %PPI (G) and increased severity of compulsive grooming (H) in Sapap3-KOs. In panel F, * indicates significant difference between WT and KO comparison group, in panels C, D, G, H * indicates significant correlation, # indicates trend for correlation ($0.05<p<0.10$). n= 13 WT D1, 11 WT D2/3, 18 KO D1, 16 KO D2/3. PPI = prepulse inhibition, CMS= centromedial striatum, NAcC= Nucleus accumbens core, KO= Sapap3 knockout, WT= wild-type.

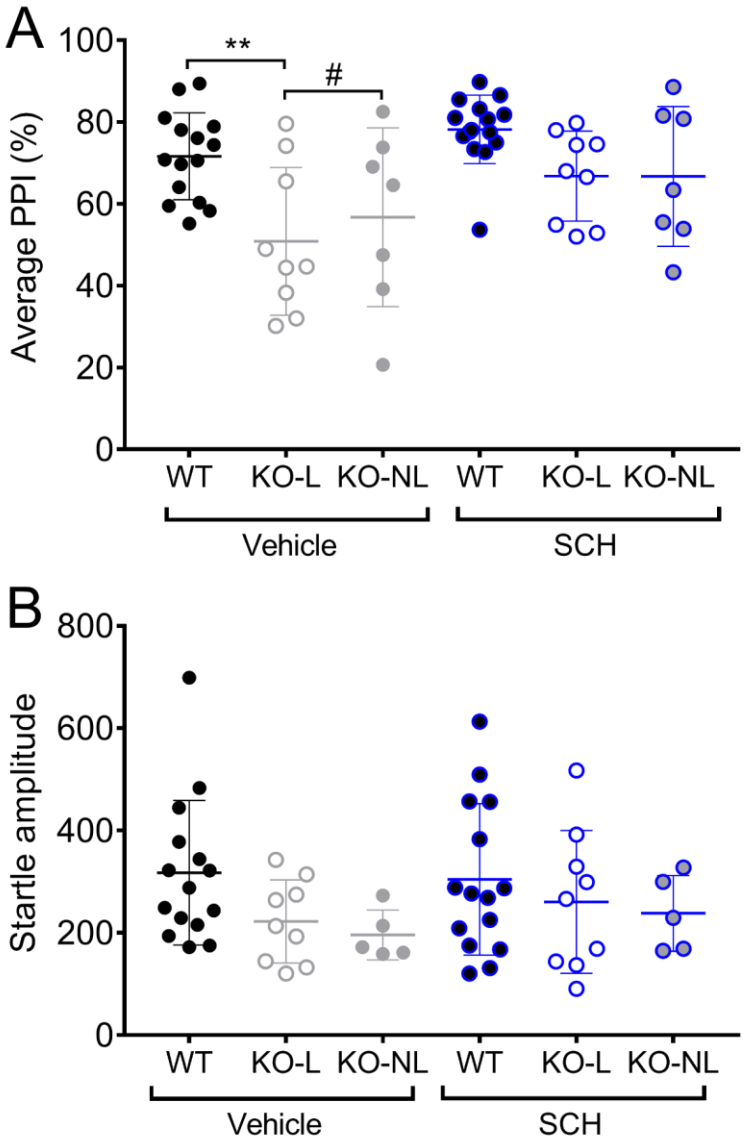

Figure 4: Acute blockade of D1 receptors normalizes PPI in KO-L group

Using a repeated measures design, mice received acute administration of D1 antagonist SCH-23390 or vehicle (1 week between tests, randomized order). A) SCH treated mice showed higher PPI (main effect $p<0.0001$), and a weak trend for differences in this effect between the groups ($p=0.13$). Planned post-hoc comparisons revealed that whereas KO-L showed disruption of PPI relative to WT during vehicle treatment session, KO-L PPI was normalized during SCH treatment session and did not differ from WT. B) Startle amplitude showed no difference between the groups or across treatment sessions. n=15 WT (8 males),

17 KO (9 males). KO-L= KOs with lesions, KO-NL= KOs without lesions, WT= wild-type, PPI= prepulse inhibition.

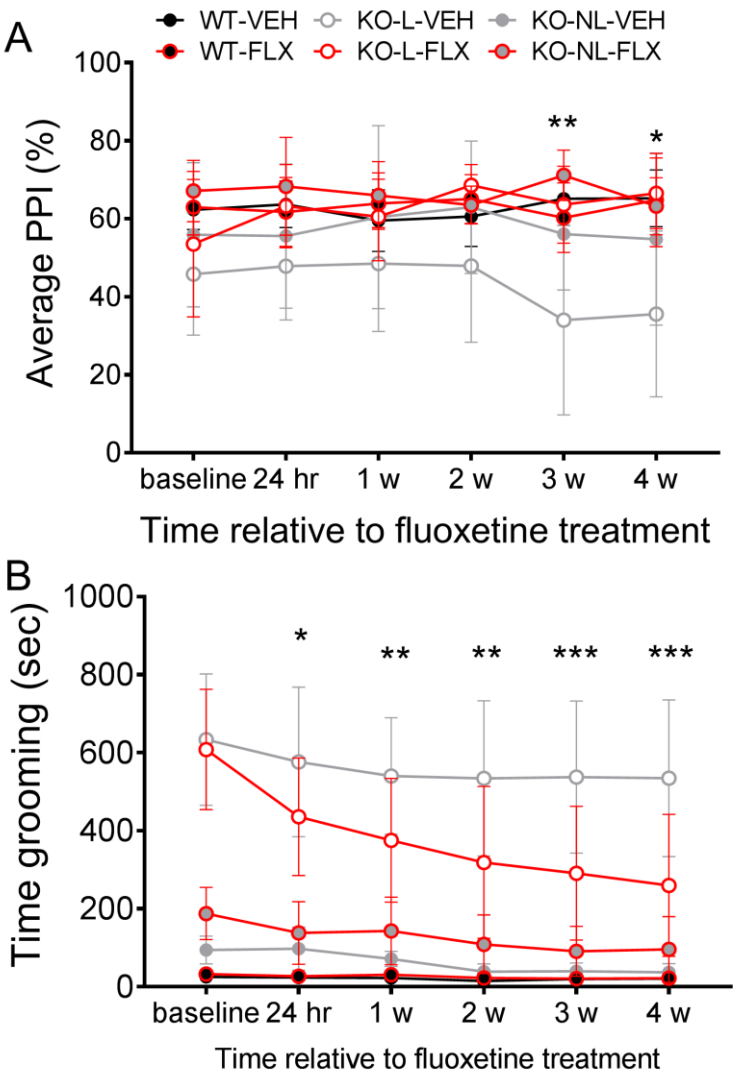

Figure 5: Fluoxetine normalizes PPI deficits and compulsive grooming

A) PPI was measured at baseline and across 4 weeks of fluoxetine administration in drinking water. At 3 and 4 week timepoints, KO-L treated with fluoxetine showed significantly increased PPI relative to vehicle treated KO-L mice. B) Grooming was also examined during fluoxetine treatment. After 24 hours fluoxetine administration, KO-L showed a modest reduction in time spent grooming relative to vehicle treated mice; this effect increased over 4 weeks of treatment (See Table S2 for interaction effect sizes and text for post-hoc comparison of KO-L treated with fluoxetine and vehicle). Post-hoc comparisons at timepoints with significant treatment x group interactions found no differences between vehicle- and fluoxetine-treated WT or KO-NL for either behavior. * significant treatment x group interaction at specified time-point. *p<0.05, ** p<0.01, *** p<0.001. n= 17 WT vehicle, 14 WT

FLX, 7 KO-L vehicle, 7 KO-L FLX, 6 KO-NL vehicle, 6 KO-NL FLX. PPI = prepulse inhibition, FLX= fluoxetine, KO= Sapap3 KO, KO-L= KOs with lesions, KO-NL= KOs without lesions, WT= wild-type, VEH= vehicle.

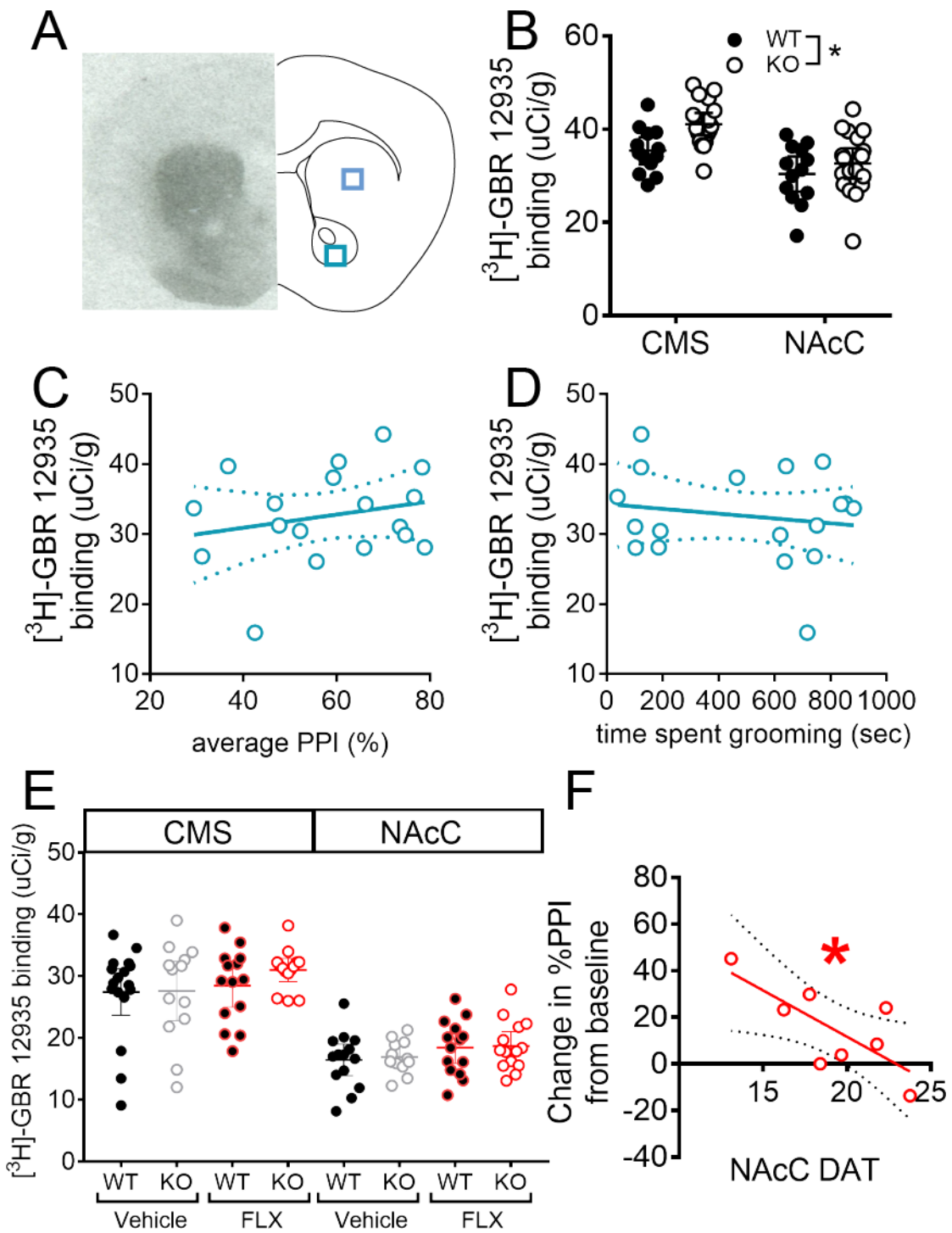

Figure 6: NAcC DAT expression is correlated with fluoxetine response in Sapap3-KOs

A) Representative autoradiograph for total [^{3}H]-GBR 12935 binding in striatal tissue used to quantify DAT density in CMS (blue square) and NAcC (green square). B) DAT density was elevated in Sapap3-KOs (main effect genotype, p=0.037). DAT binding in NACc was not correlated with disruption of PPI (C) or grooming severity (D) in Sapap3-KOs. E) After treatment with chronic fluoxetine, mean DAT density was unchanged in CMS and NAcC

(main effect of treatment, p=0.129). F) In Sapap3-KOs with lesions treated with fluoxetine, NAcC DAT density was negatively correlated with improvement in PPI following treatment (R=-0.74, p=0.036). In panel B, * indicates significant difference between WT and KO comparison group (p<0.05); in panel F, * indicates significant correlation (p<0.05). Panel B: n= 13 WT, 18 KO; panels C-D: n= 18 KO; Panel E (CMS): n= 17 WT-vehicle, 14 WT-FLX, 13 KO-vehicle, 14 KO-FLX; Panel E (NAcC): n= 14 WT-vehicle, 14 WT-FLX, 12 KO-vehicle, 14 KO-FLX; Panel F: n=8 KO-L-FLX. PPI = prepulse inhibition, CMS= centromedial striatum, NAcC= Nucleus accumbens core, DAT= dopamine transporter, KO= Sapap3 knockout, KO-L= KO mouse with lesions, WT= wild-type, FLX= fluoxetine.

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