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Monoamine abnormalities in the SAPAP3 knockout model of obsessive-compulsive disorder-related behaviour

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Obsessive-compulsive disorder (OCD) is a leading cause of illness-related disability, but the neural mechanisms underlying OCD symptoms are unclear. One potential mechanism of OCD pathology is monoamine dysregulation. Because of the difficulty of studying monoamine signalling in patients, animal models offer a viable alternative to understanding this aspect of OCD pathophysiology. We used HPLC to characterize post-mortem monoamine levels in lateral orbitofrontal cortex (OFC), medial OFC, medial prefrontal cortex and dorsal and ventral striatum of SAPAP-3 knockout (KO) mice, a well-validated model of compulsive-like behaviours in OCD. As predicted from previous studies, excessive grooming was significantly increased in SAPAP-3 KO mice. Overall levels of the serotonin metabolite 5-hydroxyindoleacetic acid (HIAA) and the ratio of 5HIAA/serotonin (serotonin turnover) were increased in all cortical and striatal regions examined. In addition, dihydroxyphenylacetic acid/dopamine ratio was increased in lateral OFC, and HVA/dopamine ratio was increased in lateral and medial OFC. No baseline differences in serotonin or dopamine tissue content were observed. These data provide evidence of monoaminergic dysregulation in a translational model of OCD symptoms and are consistent with aberrant cortical and striatal serotonin and dopamine release/metabolism in SAPAP-3 KO mice. These results are guiding ongoing experiments using circuit and cell-type specific manipulations of dopamine and serotonin to determine the contributions of these monoaminergic systems to compulsive behaviours, and serve here as a touchstone for an expanded discussion of these techniques for precise circuit dissection.

This article is part of the discussion meeting issue 'Of mice and mental health: facilitating dialogue between basic and clinical neuroscientists'.

1. Introduction

(a) Obsessive-compulsive disorder is a severe and chronic disorder with limited pharmacologic treatment options

Obsessive compulsive disorder (OCD) is a severe mental illness characterized by intrusive thoughts (obsessions) and repetitive acts (compulsions) [1,2]. These symptoms can be highly disruptive, leading to a significant impact on quality of life. In addition, the annual financial cost associated with OCD in the USA is estimated at $10 billion [3]. Accordingly, the World Health Organization has identified OCD as a major cause of illness-related disability, underscoring both the heavy burden this disorder places on patients and the steep cost to society at large [1,2,4]. Globally, OCD has a lifetime prevalence of 2–3% (i.e. two to three times more common than schizophrenia) [5,6]. Since illness onset often occurs during childhood or adolescence, and symptoms continue throughout life, this chronic disorder leads to significant morbidity [3]. OCD is therefore not only a devastating psychiatric illness, it is also extremely widespread.

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Despite the high prevalence and severity of OCD, the underlying pathophysiologic mechanisms are still unclear. This lack of understanding of the neural basis of pathology limits the development of effective treatments. Current estimates indicate that approximately half of all patients have only partial responses to available pharmacologic treatments, and up to 10% are treatment resistant, meaning even our best treatments leave many patients without relief. Thus, in order to improve treatment of OCD, we need further research to identify the neural substrates of obsessions and compulsions.

In this manuscript, we use original research to highlight basic monoaminergic disruptions in a genetic knockout (KO) mouse model relevant to OCD. We then expand on how nuanced techniques for controlling and monitoring cellular activity could further elucidate the role of these monoaminergic abnormalities in future experiments. Thus, we provide a traditional, static view of dysregulation in the context of an OCD-related model, followed by a look towards cutting-edge approaches that will need to be implemented to expand our knowledge of neuronal circuit abnormalities relevant to OCD-related symptoms.

(b) Evidence from clinical obsessive-compulsive disorder studies suggests dysfunction in monoaminergic systems

While substantial research has focused on the role of cortico-striatal-thalamo-cortical circuits in OCD [1,2,7,8], particularly emphasizing the orbitofrontal cortex (OFC) and striatum, monoamine dysfunction served as a major focus of early human studies of OCD pathophysiology [9–19]. For over 30 years, it has been hypothesized that serotonergic dysfunction is central to OCD pathophysiology [18]. This hypothesis was initially grounded in the finding that serotonin reuptake inhibitors (SRIs), including both clomipramine and selective SRIs, were efficacious in treating a subset of OCD patients [17–19]. Furthermore, symptom exacerbation generally occurs when targeting receptors that negatively regulate presynaptic serotonin release [20,21], suggesting that deficient serotonin signalling contributes to OCD. In addition, associations of serotonin transporter gene polymorphisms with OCD [22–28] have led to speculation that OCD is associated with a hyposerotonergic state.

However, many inconsistencies have made it difficult to isolate specific abnormalities within the serotonergic system. For example, OCD patients and healthy controls show similar levels of plasma serotonin and cerebrospinal serotonin and metabolites [29–31], but recent work indicates that plasma serotonin concentration is positively correlated with OCD symptom reduction [30]. In addition, agonists can reduce or exacerbate OCD symptoms depending on the drug's receptor subtype affinity [28,31]. Because these approaches are unable to detect localized serotonergic disruptions in specific brain regions, they are unable to detect selective dysregulation of specific cortical and striatal serotonin inputs.

Similar challenges have been encountered during attempts to identify OCD-related pathologic changes in the dopaminergic system. Dopaminergic receptor antagonist augmentation of SRIs is efficacious in a subset of subjects with OCD [10,13,32–34]. Coupled with findings of reduced dopamine D1 and D2 receptor binding in the striatum [12,13,35,36] and exacerbation of OCD symptoms by indirect dopamine receptor agonists (cocaine, amphetamine, methylphenidate) [10], these clinical data would be consistent with a potential hyperdopaminergic state in people with OCD. However, these findings have not been consistently replicated [2,10], making the role of dopamine signalling in OCD unclear.

(c) Animal models are a powerful tool for investigating obsessive-compulsive disorder pathophysiology

The need for an improved understanding of the role of monoamines in the development, expression and treatment of OCD symptoms is clear. Although human studies are crucial for these efforts, they have inherent limitations due to their non-invasive nature. Animal studies of monoaminergic signalling are therefore essential to complement the human OCD literature by directly addressing questions of mechanism. One of the best currently available OCD-relevant genetic animal models is the SAPAP-3 KO mouse. SAPAP-3 is a post-synaptic density protein found at excitatory synapses, and is highly enriched in striatum relative to other SAPAP gene family members [37–40]. SAPAP-3 has been linked to OCD and related disorders including trichotillomania and skin picking via genetic studies in humans [41,42], strengthening its translational relevance. In addition to face validity (compulsive-like grooming behaviour accompanied by anxiety), SAPAP3-KOs have predictive validity, since chronic fluoxetine treatment ameliorates these behaviours [43]. Furthermore, SAPAP-3 KOs also show increased in vivo baseline striatal activity [44] and decreased fEPSPs in dorsolateral striatum slices [43], providing a potential link to human OCD functional imaging studies. This well-validated OCD-relevant animal model is now being used by numerous groups to investigate the neural mechanisms underlying compulsive-like behaviours [43–45].

Despite the growing use of SAPAP-3 KOs for translational OCD research, monoamine levels and turnover have not yet been examined in this model. To determine whether SAPAP3-KOs have monoaminergic abnormalities in prefrontal cortical and striatal subregions that have been consistently linked to OCD symptoms [1,8] and OCD-related phenotypes in animal models [2,43–47], we used HPLC to characterize dopamine, serotonin and their metabolites in post-mortem OFC (medial and lateral), mPFC, dorsal striatum (DS) and ventral striatum (VS) of SAPAP-3 KO mice and wild-type (WT) controls. This strategy allowed us to determine if global abnormalities in monoamine function are present in SAPAP-3 KOs, an important first step in investigating cortical and striatal neurochemical abnormalities in this OCD model.

2. Methods

(a) SAPAP3-knockout mice

All animal care and testing was approved by the University of Pittsburgh Institutional Animal Care and Use Committee and was in accordance with the *National Institutes of Health's Guide for the Care and Use of Laboratory Animals*. Data were collected from experimentally naive male and female homozygous SAPAP-3 KO (SAPAP-3$^{-/-}$; $n = 9$ females and 8 males) and WT littermate control (SAPAP-3$^{+/+}$; $n = 13$ females and 8 males) mice on a C57BL/6 J background. Original SAPAP-3 KO breeders backcrossed for at least 10 generations were obtained from Dr Guoping Feng (Massachusetts Institute of Technology, Cambridge, MA). Breeding was then performed via heterozygote crosses (SAPAP3$^{+/-}$ × SAPAP3$^{+/-}$), resulting in experimental groups comprised SAPAP3$^{-/-}$ mice and their WT littermate controls. All mice were

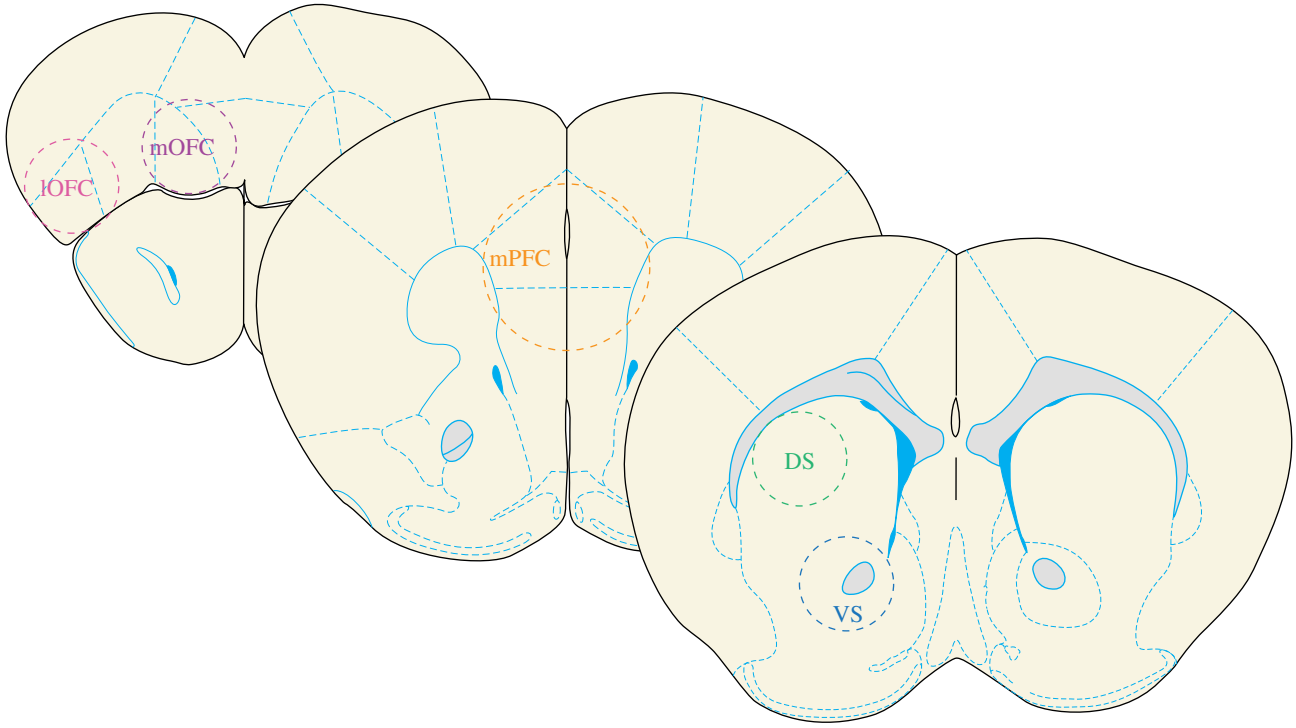

Figure1. Schematic diagram showing location of tissue punches for HPLC. Schematized depictions of tissue collection. In each coronal section, dashed circles depict the approximate location of tissue punches in post-mortem tissue. Sections are depicted anterior to posterior from left to right. (AP: +2.77 mm; +1.97 mm; +1.21 mm) For simplicity, lOFC, mOFC, VS and DS samples are depicted unilaterally, but all tissue was collected bilaterally. Note that there is minimal collection of non-targeted regions using this approach. (Online version in colour.)

group housed on 12 h light-dark schedule (lights on 7.00). Food (LabDiet 5001, LabDiet, St Louis, MO) and water were available ad libitum. Average age at tissue collection was approximately 7 months (207 ± 46 days).

(b) Grooming assessments

All testing took place in the same room during the light cycle. In each of two behavioural sessions (7 and 3 days prior to tissue collection), animals were allowed to acclimate to the testing room for 1 h in home cages. Each animal was then placed in one of eight clear acrylic chambers (20.32 × 20.32 × 30.48 cm, Collecting Warehouse, Fairfield, OH), which were cleaned with disinfectant spray (Virox, Oakville, ON, Canada) just prior to testing. Laminated white paper barriers were placed around the back and sides of the chambers to prevent mice from seeing each other. A video camera was placed in front of each chamber to capture behaviour for offline analysis. Collection of behavioural data was performed by an experimenter blinded to genotype. This procedure was repeated a second time for comparison with naive behaviour.

Video data were analysed offline by a pair of highly trained raters blinded to experimental conditions. Thirty minute videos were scored in 5 min bins for time spent grooming (defined as clearly visible cleaning or smoothing of the skin and fur with the mouth, front paws or hind paws). Each rater scored videos independently and without knowledge of the other rater's scores to ensure behavioural assessment was robust to errors in subjective ratings. Reported data are averaged between assessments and raters, except where otherwise noted.

(i) Tissue acquisition

Animals were sacrificed 3 days after grooming assessments during the light cycle using cervical dislocation, and brains were rapidly removed for slicing in ice-cold phosphate buffered saline. 0.7 mm thick coronal sections were cut at +2.77 mm, +1.97 mm and +1.21 mm anterior to Bregma [48]. Each section was then rapidly frozen on dry ice. 1.0 mm diameter tissue punches were then obtained from both hemispheres of lateral orbitofrontal cortex (lOFC) and medial orbitofrontal cortex (mOFC) (figure 1). A 1.75 mm diameter punch was taken from the midline of a medial prefrontal cortex (mPFC) section containing the prelimbic and infralimbic mPFC (figure 1). 1.0 mm diameter punches were obtained from both hemispheres of VS and DS. The DS section contained both dorsolateral and dorsomedial striatal subregions (figure 1). For a single subject, sections from both hemispheres were pooled together in a tube, and stored at −80°C prior to HPLC analysis.

(ii) Neurochemical detection via HPLC

All neurochemical assays were conducted at the University of Vanderbilt Neurochemistry Core (Biogenic Amines Assay); samples were shipped on dry ice. Brain sections were homogenized using a tissue dismembrator in 100–750 µl 0.1 M TCA (10–2 M sodium acetate, 10–4 M EDTA, 5 ng ml^{-1} isoproterenol (as internal standard); 10.5% methanol (pH 3.8)). Ten microlitres of homogenate were used for each protein assay. Samples were spun in a microcentrifuge at 10 000 g for 20 min and supernatant was removed for biogenic monoamine analysis with HPLC. Remaining samples were stored at −80°C for future use.

Biogenic amines were determined by a specific HPLC assay using an Antec Decade II (oxidation: 0.65) electrochemical detector operated at 33°C. 20 µl samples of the supernatant were injected using a Water 2707 autosampler onto a Phenomenex Kintex (2.6u, 100A) C18 HPLC column (100 × 4.60 mm). Biogenic amines were eluted with a mobile phase consisting of 89.5% 0.1 M TCA, 10–2 M sodium acetate, 10–4 M EDTA and 10.5% methanol (pH 3.8). Solvent was delivered at 0.6 ml min^{-1} using a Waters 515 HPLC pump. Using this HPLC solvent, biogenic amines elute in the following order: noradrenaline, adrenaline, 3,4-dihydroxyphenylacetic acid (DOPAC), dopamine,

5-hydroxyindoleacetic acid (5HIAA), homovanillic acid (HVA), serotonin 5-HT and 3-methoxytyramine (3-MT). HPLC control and data acquisition were managed by Empower software.

Protein concentration was determined by BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA). 10 µl of tissue homogenate from each sample was placed into a 96-well plate; 200 µl of mixed BCA reagent (25 ml of Protein Reagent A mixed with 500 µl of Protein Reagent B) was then added. The plate was incubated at room temperature for 2 h for colour development while a BSA standard curve was run. Absorbance was measured on a plate reader (POLARstar Omega; BMG LABTECH, Ortenberg, Germany).

(iii) Data analysis

All data analysis was conducted with custom Matlab scripts (ver. 2016a, MathWorks, Natick, MA) and SPSS statistical analysis software (IBM, Armonk, NY). Each animal's grooming data were averaged across both raters' assessments of both test sessions. Thus, each animal's grooming was an average of four ratings to minimize bias between raters and variability between test sessions. Time spent grooming was not normally distributed and non-parametric statistics were used as appropriate. Effects of sex and genotype on total grooming were assessed via a pair of Mann–Whitney tests with Bonferroni corrections for multiple comparisons. Data are reported as corrected *p*-values. Genotype effects on time spent grooming were also analysed for males and females separately using a Mann–Whitney procedure. Inter-rater reliability was calculated via the intraclass correlation (single measures; absolute agreement, one-way random raters model), and differences between rater's scores were assessed via Wilcox signed-rank test. Consistency across days within a rater was assessed with Spearman's correlation of each animal's total time grooming in assessment 1 and 2.

Differences in neurochemical content were assessed via analysis of variance (ANOVA) with sex, genotype and brain region as fixed factors ($2 \times 2 \times 5$ ANOVA). Main effects of brain region are not of central interest, as effects related to genotype were the main focus of the current study. For a given neurochemical, significant interactions between region and genotype were analysed with Bonferroni corrected post hoc independent samples *t*-tests on each region's data from SAPAP-3 KO and WT mice. All reported post hoc *p*-values were Bonferroni corrected.

We calculated the Spearman's rank correlation between neurochemical levels and grooming for samples that were significantly different according to genotype, pooling WT and SAPAP3$^{-/-}$ animals. All resulting *p*-values for this exploratory analysis are reported prior to Bonferroni correction.

3. Results

(a) SAPAP-3 knockouts demonstrate excessive grooming

Consistent with previous reports [43,44], time spent grooming was increased in SAPAP-3 KOs relative to WT controls (figure 2; $U = 56$, $Z = -3.596$, $p < 0.001$). These effects were similar when male ($U = 2$, $Z = -3.151$, $p = 0.004$) and female ($U = 28$, $Z = -2.037$, $p = 0.042$) animals were considered separately, and there was no effect of sex on time spent grooming ($U = 130$, $Z = -1.360$, $p = 0.348$). Total time grooming was highly correlated between both raters (*intraclass correlation* $ICC_{37,38} = 0.994$, $p < 0.001$), and grooming values did not significantly differ between them (Wilcox signed-rank test, $Z_1 = 1.617$, signed rank = 482, $p = 0.106$, median rater 1: 300.40, median rater 2 = 284.43). For both raters, each animal's total time grooming was highly correlated across days (rater 1, $r = 0.784$, $p < 0.001$; rater 2, $r = 0.823$, $p < 0.001$).

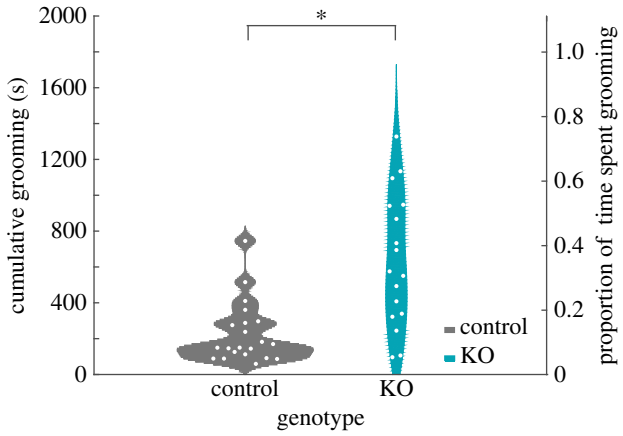

Figure 2. SAPAP-3 KOs display significantly increased grooming compared with WT mice. SAPAP-3 KO animals demonstrate increased grooming compared with WT littermate controls ($Z = -3.596$, $p < 0.001$). Data are depicted as violin plots of the cumulative time spent grooming (left *Y* axis) and proportion of time spent grooming (right *Y* axis) over a 30 min observation period. Each histogram represents the smoothed distribution of data points at each location on the *Y* axis. White dots represent each animal's grooming data plotted individually. Note that data are distributed non-normally. (Online version in colour.)

(b) Tissue samples

In order to assess post-mortem differences in monoamine neurochemistry between SAPAP-3 KO and WT mice, we collected bilateral lOFC, mOFC, mPFC, DS and VS from males and females of both genotypes. Total weight of protein recovered from each brain region did not differ according to sex or genotype (region, $F_{4,169} = 36.068$, $p < 0.001$; genotype, $F_{1,169} = 0.163$, $p = 0.687$; sex, $F_{1,169} = 0.023$, $p = 0.879$; all interactions non-significant), suggesting no gross differences in tissue quality or preparation between SAPAP-3 KO versus WT and male versus female.

(c) SAPAP-3 knockouts display broadly increased serotonin turnover in cortical and striatal regions

We observed that post-mortem basal 5-HT levels did not differ between SAPAP-3 KO and WT mice (figure 3; region, $F_{4,169} = 20.179$, $p < 0.001$; genotype, $F_{1,169} = 2.103$, $p = 0.149$; sex, $F_{1,169} = 0.392$, $p = 0.532$; all interactions non-significant). However, levels of the 5-HT metabolite, 5HIAA, were increased in SAPAP-3 KOs (figure 3; region, $F_{4,169} = 16.173$, $p < 0.001$; genotype, $F_{1,169} = 37.591$, $p < 0.001$; sex, $F_{1,169} = 3.370$, $p = 0.68$; all interactions non-significant), indicating dysregulation of 5-HT that is not packaged in vesicles and therefore is not shielded from catabolism by monoamine oxidase (MAO). The ratio of 5HIAA to 5-HT, commonly referred to as serotonin turnover, partially reflects serotonergic transmission and metabolism [49]. Using this measure, we detected increased serotonin turnover in SAPAP-3 KOs versus WT (figure 3; region, $F_{4,168} = 11.993$, $p < 0.001$; genotype, $F_{1,168} = 14.304$, $p < 0.001$; sex, $F_{1,168} = 3.536$, $p = 0.62$; all interactions non-significant).

(d) SAPAP-3 knockouts display dopaminergic abnormalities in orbitofrontal cortex

There were no differences in post-mortem levels of dopamine (figure 4; region, $F_{4,169} = 503.545$, $p < 0.001$; genotype,

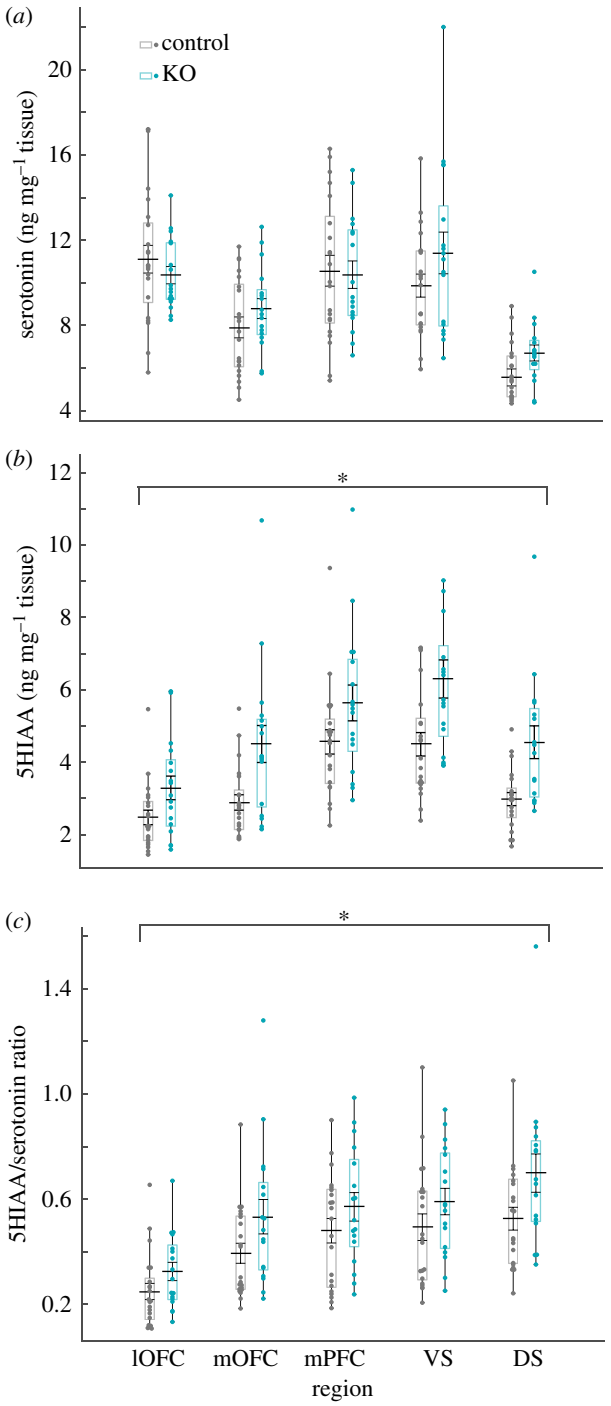

Figure 3. SAPAP-3 KOs demonstrate increased serotonin turnover in cortical and striatal regions. SAPAP-3 KO mice have elevated post-mortem 5HIAA and 5HIAA/serotonin ratios in frontal cortex and striatum. (*a*) Data depicted as box plot of ng of serotonin per mg of tissue in each region. Each box plot represents inner quartiles (25–75%) of distribution (bars indicate 9% and 91% of distribution). Black line and error bars inside box plots represent the mean ± s.e. Each data point is plotted in each distribution. (*b*) 5HIAA (serotonin metabolite) per mg of tissue in each region. Data plotted identically to (*a*). Main effect of genotype indicates that 5HIAA is increased in KO mice ($F_{1,169} = 37.591$, $^*p < 0.001$). (*c*) The ratio of 5HIAA/serotonin, representing metabolized serotonin relative to serotonin content, is increased in SAPAP-3 KO mice ($F_{1,168} = 14.304$, $^*p < 0.001$); this is potentially indicative of increased serotonin release and metabolism. Note different scales on *Y* axes are used throughout figure 3. (Online version in colour.)

$F_{1,169} = 1.046$, $p < 0.308$; sex, $F_{1,169} = 0.261$, $p = 0.610$; all interactions non-significant) or dopamine metabolites DOPAC and 3-MT between SAPAP-3 KO and WT mice in any region

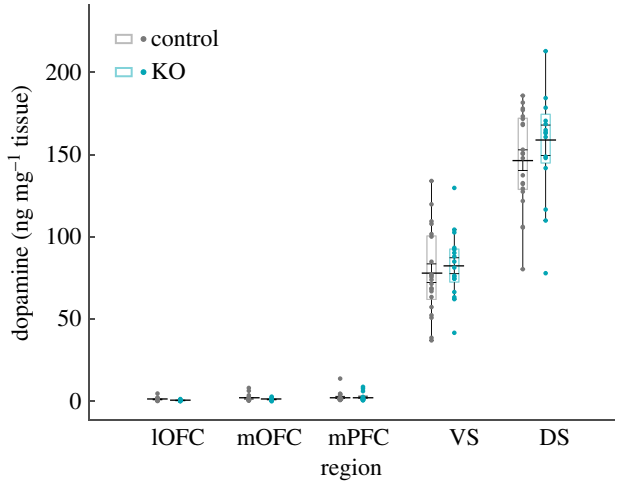

Figure 4. No differences in cortex or striatum dopamine levels in SAPAP-KOs. No significant differences are observed in post-mortem dopamine levels in frontal cortex and striatum between SAPAP-3 KO mice and littermate controls (genotype, $F_{1,169} = 1.046$, $p = 0.308$). Data depict ng of dopamine per mg of tissue in each region, and are plotted identically to figure 3. (Online version in colour.)

(figure 4; *DOPAC*, region, $F_{4,169} = 89.528$, $p < 0.001$; genotype, $F_{1,169} = 0.107$, $p = 0.744$; sex, $F_{1,169} = 0.592$, $p = 0.443$; all interactions non-significant; *3-MT*, region, $F_{4,169} = 341.641$, $p < 0.001$; genotype, $F_{1,169} = 0.189$, $p = 0.664$; sex, $F_{1,169} = 0.848$, $p = 0.359$; all interactions non-significant) (figure 5).

Both DOPAC and 3-MT are precursors to further metabolic conversion into HVA by either catechol-*O*-methyl transferase or MAO, respectively. Therefore, we also examined HVA levels in all brain regions as a marker of dopaminergic release and metabolism [50,51]. HVA was weakly, but significantly, increased in SAPAP-3 KO versus WT mice (region, $F_{4,169} = 198.169$, $p < 0.001$; genotype, $F_{1,169} = 5.012$, $p = 0.026$; sex, $F_{1,169} = 0.107$, $p = 0.744$; all interactions non-significant). These results indicate there are modest differences in overall dopaminergic tone in post-mortem SAPAP-3 KO tissue.

There was a significant interaction between genotype and brain region on DOPAC relative to dopamine (region, $F_{4,168} = 18.667$, $p < 0.001$; genotype, $F_{1,168} = 18.749$, $p < 0.001$; sex, $F_{1,168} = 1.897$, $p = 0.170$; Genotype*Region, $F_{4,168} = 6.334$, $p < 0.001$). Post hoc analyses revealed that both lOFC and mOFC were associated with increased DOPAC turnover in SAPAP-3 KOs (lOFC, $t_{36} = -3.243$, $p = 0.015$; mOFC, $t_{35} = -3.061$, $p = 0.02$; VS, DS, and MPFC non-significant). Similarly, there was a significant interaction between genotype and brain region on HVA relative to dopamine ($F_{4,168} = 32.217$, $p < 0.001$; genotype, $F_{1,168} = 16.277$, $p < 0.001$; sex, $F_{1,168} = 0.055$, $p = 0.815$; Genotype*Region, $F_{4,168} = 8.207$, $p < 0.001$). Post hoc analyses indicated that lOFC HVA/dopamine was significantly greater in SAPAP-3 KO lOFC compared with WT mice (*lOFC*, $t_{36} = -3.353$, $p = 0.01$; mOFC, VS, DS, and mPFC: non-significant).

(e) Relationship between grooming levels and monoaminergic abnormalities in SAPAP3-knockouts

As a first attempt to elucidate the relationship between neurochemical alterations and behavioural abnormalities in SAPAP3-KOs, we performed an exploratory analysis to correlate grooming with each HPLC sample that differed

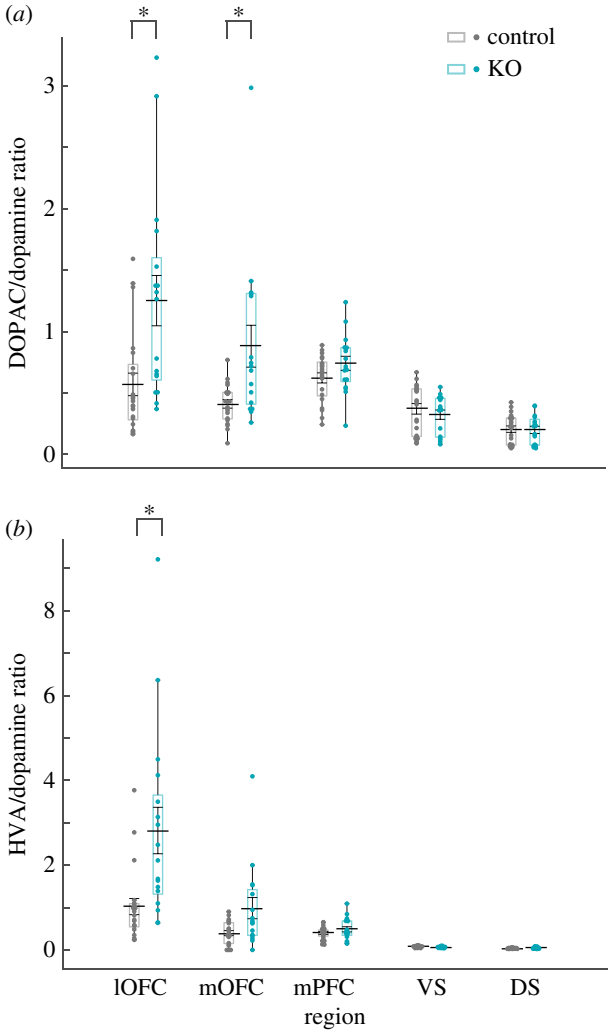

Figure 5. SAPAP-3 KO mice have elevated post-mortem metabolized dopamine ratios in OFC. (*a*) Data depict the ratio of the dopamine metabolite DOPAC to dopamine, and are plotted identically to figure 3. Interaction between genotype and region ($F_{4,168} = 6.334$, $p < 0.001$) that resulted in significantly greater ratios in both OFC regions in SAPAP-3 KOs (*lOFC*, $t_{36} = -3.243$, $p = 0.015$; mOFC, $t_{35} = -3.061$, $p = 0.02$). Asterisks represent within-region significant differences in DOPAC/dopamine ratio. (*b*) Data depict the ratio of HVA to dopamine, plotted identically to figure 2. A significant interaction between genotype and region ($F_{4,168} = 8.207$, $p < 0.001$) was due to increased HVA/dopamine ratios in lOFC of SAPAP-3 KOs (*lOFC*, $t_{36} = -3.353$, $p = 0.01$). Note different scales on *Y* axis are used throughout figure 5. (Online version in colour.)

significantly according to genotype. No neurochemical was significantly correlated with grooming following corrections for multiple comparisons (table 1). However, 5HIAA levels were correlated with grooming in all regions except mPFC prior to correction (table 1). Note that *p*-values displayed in table 1 are *uncorrected* for multiple comparisons.

4. Discussion

As expected based on previous studies, SAPAP-3 KO mice displayed excessive grooming [43]. In the same animals, we found evidence of serotonergic dysregulation (5HIAA and 5HIAA/serotonin ratio) in mOFC, lOFC, mPFC and dorsal and VS. We also observed evidence of dopamine abnormalities in OFC. Specifically, the ratio of HVA/dopamine was increased in lateral and medial OFC, and the ratio of DOPAC/dopamine was increased in lateral OFC. These findings are suggestive of increased cortical and striatal monoamine release in SAPAP-3 KO mice. They also align with a larger literature associating OCD with aberrant monoamine signalling [2,10,15,16,18] and documenting dysregulated neural activity in frontal cortex and striatum of both OCD patients and several animal models relevant to OCD [19,52–54].

(a) Serotonergic disruptions in SAPAP-3 knockout mice

These data are the first evidence of serotonergic abnormalities in SAPAP-3 KO mice, a validated animal model of compulsive-like behaviours [43,47,55]. To date most studies using SAPAP-3 KOs have focused on cortical and striatal systems [43–45,56]; our results therefore set the stage for more expansive investigation of the monoaminergic mechanisms underlying compulsive-like behaviours. Though SAPAP-3 is enriched in striatal regions relative to other SAPAP gene family members, it is also present in other brain regions [43,57]. Constitutive knock out of SAPAP-3 could therefore potentially impact monoaminergic signalling via multisynaptic circuits either including or bypassing the striatum. For example, although this hypothesis has not yet been tested, SAPAP-3 deletion could generate dysregulated activity throughout cortico-striatal-thalamo-cortical circuits [58–61], leading to hyperactivity in descending projections from cortex to serotonin neurons in the raphe [62,63]. In turn, this could ultimately cause increased serotonin release in cortical and striatal regions. Further work is needed to directly elucidate how SAPAP-3 genetic deletion affects monoaminergic neural activity.

Our data suggest that serotonin release and metabolism are increased in cortex and striatum of SAPAP-3 KO mice. These preclinical data may seem somewhat at odds with some clinical data. For instance, the utility of SRIs in treating OCD symptoms [19,64], decreased serotonin transporter availability [28,31,65–69], and associations of polymorphisms of the genes encoding serotonin transporter [22–28] and serotonin 2A receptor [23] proteins with OCD have led to speculation that OCD is associated with a hypo-serotonergic state. However, it is important to note that the serotonin hypothesis has mixed support [1]. For instance, though the majority of the available clinical data suggest that OCD is associated with hypo-serotonergia, acute doses of meta-chlorophenylpiperazine (a serotonergic agonist) increase symptoms in OCD patients [70]. These data led to speculation that chronic treatment with SRIs induces adaptive hyposerotonergic states [71]. Further supporting the notion that OCD symptoms can be driven by increased serotonin release, SRI administration in OCD patients is correlated with decreased 5HIAA in cerebrospinal fluid [72] and decreased platelet serotonin concentrations [73].

There are several important considerations for interpreting the current data and comparing them to clinical observations. First, HPLC measurements from post-mortem tissue combine intracellular and extracellular serotonin, leading to challenges in making direct comparisons to human studies monitoring extracellular peripheral levels of neurotransmitter. HPLC also directly measures neurochemical levels, without inferring function through genetic expression or transporter availability. Second, increased serotonin turnover in SAPAP3-KOs could result from increased serotonin metabolism without changes in release. Third, assessment of these neurochemicals is often done in conjunction with a manipulation of neuronal activity

Table 1. Correlation between monoamines and grooming. Table displays the Spearman's rank order correlation between monoamine neurochemical levels and total time grooming in SAPAP-3 KO and WT mice. The *p*-values displayed are *uncorrected*. None of the correlations were significant following Bonferroni correction.

	lOFC	mOFC	mPFC	VS	DS
5HIAA	$r = 0.406$	$r = 0.441$	$r = 0.270$	$r = 0.369$	$r = 0.392$
	$p = 0.011$	$p = 0.006$	$p = 0.101$	$p = 0.023$	$p = 0.017$
5HIAA/5-HT	$r = 0.335$	$r = 0.263$	$r = 0.161$	$r = 0.353$	$r = 0.332$
	$p = 0.040$	$p = 0.111$	$p = 0.334$	$p = 0.030$	$p = 0.048$
HVA	$r = 0.240$	$r = 0.254$	$r = 0.346$	$r = 0.164$	$r = 0.174$
	$p = 0.146$	$p = 0.124$	$p = 0.033$	$p = 0.324$	$p = 0.303$
HVA/DA	$r = 0.352$	$r = 0.012$	$r = -0.135$	$r = -0.160$	$r = 0.009$
	$p = 0.030$	$p = 0.944$	$p = 0.418$	$p = 0.338$	$p = 0.958$
DOPAC/DA	$r = 0.312$	$r = 0.174$	$r = -0.028$	$r = -0.047$	$r = -0.123$
	$p = 0.056$	$p = 0.304$	$p = 0.867$	$p = 0.78$	$p = 0.469$

(e.g. a drug or stimulation of a neuronal system), and it may therefore be more difficult to interpret the basal neurochemical levels obtained in our study. Fourth, it is unclear if these neurochemical abnormalities disrupt brain or behaviour function, and they could instead represent a compensatory process. This could be consistent with the absence of correlations between post-mortem monoamine content and the observed grooming phenotype that survived multiple comparisons. Finally, because mice cannot model all aspects of a human psychiatric disorder, it is possible that the SAPAP3-KO model does not accurately reflect serotonergic pathology in OCD patients. In vivo studies in SAPAP3-KOs examining the relationship between monoaminergic signalling and behaviour will yield more insight into these possibilities.

(b) Evidence of dopaminergic abnormalities in SAPAP-3 knockout orbitofrontal cortex

Our data are consistent with dysregulation of dopamine specifically in OFC of SAPAP3-KO mice, lending further support to an extensive literature associating abnormal dopamine transmission with dysregulated behavioural organization. For example, systemic administration of D1-receptor agonists or constitutive knockdown of the dopamine transporter can lead to compulsive-like grooming behaviour [74–77]. In addition, compulsive-like checking behaviours can be induced by D2-receptor agonists [78,79]. Our observation of increased dopamine turnover in OFC could contribute to compulsive-like behaviours in several ways. For example, OFC is essential for performing reward value comparisons and stimulus-outcome associations [80–85]. Dysregulated dopamine signalling in OFC could therefore lead to deficits in comparison of actual and expected rewards [86,87], ultimately leading to repeated perseverative performance of an action. In addition, OFC is strongly associated with behavioural flexibility in the context of reversal learning, and OCD patients have consistently demonstrated abnormal performance and/or neural activity on this task [52,88]. Thus, impaired dopamine signalling in OFC could impact the ability to learn new rules, contributing to cognitive inflexibility in OCD.

Although we selectively observed increased dopamine turnover in OFC, aberrant behavioural selection is less commonly associated with dopamine signalling within the OFC, and more commonly associated with striatal and mPFC dopamine signalling [86,89–92]. Our data may therefore point to a unique regional mechanism of monoaminergic dysfunction relevant to OCD. Of particular interest in this context is the fact that dopaminergic innervation of OFC is relatively weak compared with striatum and mPFC [93]; thus, modest abnormalities in OFC dopamine levels could represent a strong functional change in OFC. It will therefore be important to use in vivo approaches to precisely measure or control OFC dopamine signalling and examine the impact on performance of tasks impaired in OCD.

(c) Emerging technologies to further understand monoaminergic dysfunction in obsessive-compulsive disorder

More work is necessary to relate our findings to dynamic interactions between networks and resulting behaviours. For instance, monoaminergic abnormalities were not correlated with the compulsive grooming phenotype after corrections for multiple comparisons. However, this does not rule out a role for these changes in abnormal grooming, since a static and non-time-locked alteration in monoamine levels may not necessarily be directly related to a temporally fluctuating behavioural phenotype like grooming. The use of optogenetics to precisely control dopamine and serotonin signalling is an effective way to solve this problem, and this approach will be essential for elucidating the role of monoaminergic signalling in compulsive-like behaviour. However, it is also critical to develop strategies to optimize use of this technology by determining (1) *when* to optogenetically modulate these systems in relationship to behaviour, and (2) *how* to pattern optical stimulation, in order to elucidate the neural mechanisms that give rise to compulsive-like phenotypes.

Timing of light delivery: After over a decade of advances, neuroscientists are now poised to refine optogenetics to explore increasingly complex questions regarding the relationship between neural activity and behaviour. Behavioural state-dependent optogenetics is a nuanced approach to understanding *when* to optogenetically target monoaminergic activity relative to naturalistic behaviours. The strength of this

technique was recently demonstrated in an important study from Costa and co-workers [94], in which the authors observed a complex relationship between movement initiation and execution in direct pathway dorsal striatum neurons [94]. This projection system is traditionally linked to movement initiation [60,95]. A clear prediction is that stimulating direct pathway neurons should therefore initiate and sustain behavioural sequences [96]. Stimulation of the direct pathway sustained ongoing behavioural sequences as predicted. However, stimulation also delayed action initiation when applied prior to sequence execution. The latter finding is contrary to theory and demonstrates that optogenetic effects of stimulation depended on the animal's ongoing behaviour. Simply stimulating without taking behavioural state into account would not have uncovered this important distinction. This demonstrates a more subtle relationship between action execution/movement and the direct pathway than previously theorized.

A similar approach has been used to reveal how the amygdala contributes to an opposing relationship between grooming and attack behaviours [97]. Specifically, optogenetic modulation of medial amygdala glutamatergic and GABAergic neurons during distinct behavioural states revealed that the circuits controlling self-grooming and attack behaviours are mutually antagonistic. These examples highlight the potential utility of state-dependent optogenetics to reveal complex relationships between compulsive-like grooming and monoaminergic activity that would be difficult to disentangle using standard optogenetic approaches.

State-dependent optogenetics can be further enhanced by 'closed-loop' approaches, which automatically detect ongoing neural activity or behaviours of interest to trigger optogenetic modulation [98–105]. Although this approach was first applied in an in vitro electrophysiological setting [100], more recent applications have focused on real-time in vivo modulation of seizure activity [98,101,102,105], memory consolidation [103,106] and somatosensation [99]. Building on this work, on-line detection of behavioural phenotypes, closed-loop optogenetics, and state-dependent modulations of neural activity will be important tools for elucidating the relationship between monoamine signalling and naturalistic compulsive-like phenotypes in SAPAP-3 KO mice.

Characteristics of light stimulation patterns: *How* optogenetic stimulation is patterned is another crucial parameter for optimization. While non-physiological modulation patterns have led to extremely important advances in neuroscience, it is clear that mimicking naturally occurring patterns of activity and determining how these patterns contribute to complex behaviours is an important next step. Step-function opsins offer one approach for studying how naturalistic patterns of activity impact behavioural phenotypes. These opsins create a stable step in membrane potential when activated by light, and therefore modulate excitability while preserving local circuit dynamics and the relative weight of excitatory and inhibitory inputs [107–111]. Thus, step-function opsins make targeted neurons more responsive to excitatory neurotransmitter release without directly causing neural firing. Frame-projected independent-fibre photometry is an alternative approach that allows naturally occurring levels of activity to be imaged and then optogenetically mimicked in the same population of neurons [112]. We propose that the use of these tools to naturalistically modulate the excitability of dopamine and serotonin neurons will be a powerful approach for elucidating the role of monoaminergic neurons in development and expression of compulsive-like phenotypes.

5. Conclusion

Our findings highlight disruptions in monoamine signalling in the OCD-relevant SAPAP3-KO mouse model, and serve as a starting point for future circuit-based investigation of the role of serotonin and dopamine signalling in compulsive-like behaviours. The current data suggest that dopamine signalling from ventral tegmental area to OFC may be a specific source of pathology in SAPAP-3 KO mice, and that serotonergic signalling may be impacted across cortical and striatal circuits, potentially pointing to global dysregulation in serotonergic tone. This study is therefore an important first step towards targeting distinct cortico-striatal circuits and monoamine systems with advanced optogenetic techniques to facilitate dynamic modulation. With this expanded technological toolbox and sophisticated behavioural paradigms, future studies will implement behaviour state-dependent and naturalistic optogenetic manipulations of dopaminergic and serotonergic neurons to assess how their dysfunction may play a role in compulsive-like behaviours.

Data accessibility. Data available from the Dryad Digital Repository: doi:10.5061/dryad.h6qm7.

Authors' contributions. J.W. and S.E.A. designed experiments; J.W. collected and analysed data, and J.W., Z.F.L. and S.E.A. wrote the manuscript.

Competing interest. Dr Ahmari has no conflicts of interest to disclose.

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