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Running Title

What is the Sapap3-KO a model of?

Title

Not only compulsivity: The SAPAP3-KO mouse reconsidered as a comorbid model expressing a spectrum of pathological repetitive behaviors.

Abstract

Symptom comorbidity is present amongst neuropsychiatric disorders with repetitive behaviours, complicating clinical diagnosis and impeding appropriate treatments. This is of particular importance for obsessive-compulsive disorder (OCD) and Tourette syndrome. Here, we meticulously analysed the behaviour of Sapap3 knockout mice, the recent rodent model predominantly used to study compulsive-like behaviours, and found that its behaviour is more complex than originally and persistently described. Indeed, we detected previously unreported elements of distinct pathologically repetitive behaviours, which do not form part of rodent syntactic cephalo-caudal self-grooming. These repetitive behaviours include sudden, rapid body and head twitches, resembling tic-like movements. We also observed that another type of repetitive behaviours, aberrant hindpaw scratching, is responsible for the flagship-like skin lesions of this mouse model, rather than originally suspected self-grooming. In order to characterize the symptomatological nature of observed repetitive behaviours, we pharmacologically challenged these phenotypes by systemic aripiprazole administration, a first-line treatment for tic-like symptoms in Tourette Syndrome and trichotillomania. A single treatment of aripiprazole significantly reduced the number of tic-like movements but not syntactic grooming events. These observations are in line with the high comorbidity of tic- and compulsive-like symptoms in Tourette, OCD and trichotillomania patients as well as with the hypothesis of shared neurobiological mechanisms.

Introduction

Many neuropsychiatric disorders are characterized by pathological repetitive behaviors (RB) such as compulsions, tics, stereotypies, or mannerisms. The exact nature of pathological RB is not always trivial to distinguish and comorbidities impede correct diagnosis and appropriate subsequent treatment (1–3). This applies especially to two neuropsychiatric disorders with high comorbidity (1,3,4): Tourette Syndrome (TS), a childhood-onset neurodevelopmental disorder characterized by tics, and Obsessive Compulsive Disorder (OCD), a heterogeneous disorder, of which the most typical form is characterized by obsessions and obsession-dependent compulsions (5). Tics are defined as sudden, rapid, recurrent, non-rhythmic, stereotyped motor events or vocalizations (5). Compulsions are clinically described as RBs that individuals feel driven to perform in response to an obsession or according to rules that must be rigidly applied. Although compulsions occur less suddenly than tics, it is not always trivial to correctly distinguish between these two RBs and hence, they could be easily confounded in clinical practice (3,6). Furthermore, a third class of disorders with RBs, trichotillomania (TTM), raises yet another important clinical concern. Although TTM is usually easily diagnosed through abnormal RBs such as hair-pulling or skin-picking, it remains debated amongst experts whether these symptoms are of a tic-like or a compulsive-like nature (7).

Rodent self-grooming is recognized as a relevant behavioural output for mapping and probing neural circuits underlying the generation of repetitive behaviours in translational psychiatric approaches (8,9). Over the last decade, mice lacking the postsynaptic protein SAP90/PSD95-associated protein 3 (*Sapap3*^{-/-}), which is strongly expressed in the striatum, have been used as the main reference mouse model for compulsive-like behaviours since their phenotype matches with human OCD symptomatology in many ways. In both OCD patients as well as *Sapap3*^{-/-} mice, neurophysiological and behavioural components are similarly affected:

cortico-striatal transmission is dysregulated (10–15), striatal activity is increased (16,17), OCD-like relevant behaviour such as excessive self-grooming is aberrantly overexpressed despite deleterious consequences, and anxiety measures are increased (13). Pharmacotherapy via selective serotonin reuptake inhibitors, which are applied as first-line therapy in OCD, or targeted deep brain stimulation, which is applied in severe, treatment-resistant OCD cases, decreases compulsive-like behaviours in both OCD patients as well as the *Sapap3^{-/-}* mouse model (13,18–20). A neurobiological core candidate in human OCD symptomatology is aberrant orbitofrontal cortex (OFC) neuroanatomy and/or activity (15,17,21). Several studies in *Sapap3^{-/-}* mice have corroborated the potential implication of the OFC; more specifically the lateral OFC input onto striatal medium spiny neurons (MSN) in *Sapap3^{-/-}* mice was reduced (22) and their optogenetic excitation restored adapted grooming behaviour and aberrantly elevated striatal firing rates (16).

Dysfunctions of cortico-striatal circuits are consistently reported with the appearance of pathological RBs. These circuits are topographically organized in parallel limbic, associative and sensorimotor loops coursing through the ventral (VS), dorsomedial (DMS) and dorsolateral striatum (DLS), respectively (23–26). They are known to dynamically interact across these topographically organized loops and are recruited to different extents during learning and automatization of behaviours (27–31). While earlier evidences suggest a specific neuropathological connection between compulsive-like behaviours and the so-called ‘associative’ cortico-striatal loop comprising associative cortical regions such as OFC and the dorsomedial and central striatum, other findings suggest the implication of other, complementary dorsal striatal circuits in the generation of pathological RBs in OCD and other comorbid disorders. In the framework of these dynamically interacting cortico-striatal circuits, OCD has recently been discussed as resulting from an imbalance across associative and

sensorimotor CSCs (32). This hypothesis is corroborated by studies demonstrating the implication of the ‘sensorimotor’ cortico-striatal circuits, comprising motor cortices and the dorsolateral striatum, in the generation of pathological RBs (22,33). Notably, in the same *Sapap3*^{-/-} model, which has become the main reference mouse model for studying compulsive-like behaviours in rodents, a recent study revealed that synaptic input from the premotor cortex (M2), as observed *in vitro* through slice neurophysiological recording, was strengthened in *Sapap3*^{-/-} mutants, suggesting thus a potential implication of sensorimotor circuits (22). Yet other studies, including some in patients, point to an implication of the entire dorsal striatal circuits in the generation of several types of pathological RBs (34–41). The hypothesis of generally compromised cortico-dorsostriatal circuitry mediating RBs is also on line with the observed strong comorbidity of tic- and compulsive-like symptoms in patients with Tourette syndrome or OCD (3,42–45) and this comorbidity is decisive for successful treatment (42,43,46).

Thus, reconsidering cortico-striatal circuitry as a substrate for pathological RBs and taking into account specific indications of an implication of the sensorimotor cortico-striatal loops also in the *Sapap3*^{-/-} mice (22), we here raised the question whether compulsive-like self-grooming in *Sapap3*^{-/-} mice might be complemented by RBs of different nature. We first performed a detailed multi-angle video screening to seek for distinct types of pathological RB other than compulsive-like self-grooming. We next confirmed predictive validity by pharmacological treatment of the spectrum of pathological RBs, which we observed and the interaction of which we describe. These findings are of crucial interest to redefine the *Sapap3*^{-/-} mouse model as a model of distinct types of RBs in the light of cortico-dorsostriatal circuitry implication. Indeed, these new results are in line with neurophysiological modifications due

to whole-striatal Sapap3 expression patterns, the clinical comorbidity observed in patients, and recent work reconsidering the circuitry affected in this mouse model.

Materials & Methods

Animals

All experimental procedures followed national and European guidelines and have been approved by the institutional review boards (French Ministry of Higher Education, Research and Innovation; APAFiS Protocol no. 1418-2015120217347265). Animals were group-housed in ventilated standard cages in groups of up to six animals per cage; they were maintained in a 12-hour light/dark cycle (lights on/off at 8:00am/8:00 pm, respectively), and had *ab libitum* food and water access. Sapap3^{-/-} mutant mice and Sapap3^{+/-} littermates (wt) were generated in heterozygous breeding trios of C57BL/6J background in the animal facility of the Paris Brain Institute. Founders for the Sapap3^{-/-} colony were kindly provided by Dr. G. Feng, MIT, Cambridge, USA.

A total of 85 animals (hereof n=16 females and n=69 males) were used in this study and systematically genotyped for the presence or absence of the Sapap3 protein during weaning period following previously described procedures (13). In detail, n = 55 Sapap3^{-/-} mice were used for lesion evaluation; out of this pool for lesion evaluations, n = 16 mice were recycled and complemented by n = 4 additional mice for the nails clipping study; n = 18 of each Sapap3^{-/-} (n = 9) and wildtype (n = 9) mice for screening of repetitive behaviours; n = 8 Sapap3^{-/-} mice for aripiprazole treatment.

Video acquisition

For the detailed behavioural characterization in naïve mice, animals were temporarily separated from their littermates for a continuous video recording session of 24 hours in video recording apparatuses. An innovative recording setup has been custom-made for the purpose of our experiment to allow for detailed behavioural analysis. The setup was equipped with four behavioural boxes (black acrylic side walls, opaque front wall, transparent back wall; 20 cm(l) x 20 cm (w) x 25 cm(h)). Each box was equipped with a side and a top camera (25 fps) and connected to a digital video recording system (KKMoon, Shenzhen Tomtop Technology) (Figure 1A, B). The boxes were filled with standard wood bedding; *ad libitum* water and food was provided. As in the animals' regular housing conditions, light was on between 8am-8pm and infrared illumination was on between 8pm-8am. Additionally, a commercially available system with similar specificities (StereoScan, CleverSys®, Reston, VA, USA) has been used to complement our video-recording boxes.

Pharmacological treatments with aripiprazole.

Sapap3^{-/-} mice (n=8) were weighted and placed inside the video acquisition system at 10am. Animals were habituated to the environment for 30 hours prior to injections as well as to handling and restraining procedures. At 4pm the following day, half the animals were injected first with vehicle solution (0.9% sterile solution with 1% Tween 80 and 1% sterile DMSO; 0.1ml/10g) and 24 hours later with aripiprazole (1.5mg/kg in vehicle solution, 0.1ml/10g) (47,48). The other half of the animals received first an aripiprazole injection, followed by a vehicle solution injection a week later to allow for a sufficient washout period of aripiprazole. In that condition, animals were taken out of the video recording apparatus 24hours after

aripiprazole treatment and re-habituated one week later to handling, restraining and to the apparatus for 30hrs prior to vehicle injections.

Video analysis

For spontaneous behavioural assessment, videos were manually analysed offline using a freely available scoring software (Kinovea, 0.8.15, www.kinovea.org), which allows to tag each individual scored event and to export timestamps of tagged behaviours (16). The experimenter scoring the behaviour was blind to genotype and treatment.

For detailed behaviour characterisation in naïve mice, four time segments of 30 minutes were defined across 24 hours: 10-10h30 am, 6-6:30pm, 9-9h30pm, and 4-4h30am (Fig 1C). This selection of time segments comprised dark/light cycle episodes as previous studies including those using automated assessment of grooming (49) and was primarily based on the first study reporting the excessive grooming phenotype in the Sapap3^{-/-} mice (13).

For the behavioural assessment under aripiprazole, one time segment per mouse was selected for video analyses according to the pharmacokinetics of the compound and following procedures of previously published assays (47,48). The segment started at 9PM and lasted until reaching 30 minutes of active behaviour. The proportion of sleep episodes, interspersed during 30 minutes of active behaviour, was additionally quantified.

Ethogram

Self-Grooming. Self-grooming behaviour is defined as a rostro-caudal sequence of four typical, distinct, often intermittently executed phases as previously described in the literature for rodent syntactic grooming (50,51). In our study we distinguished two different types of grooming bouts. Short grooming bouts (<3sec) predominantly composed of only one of the

four grooming phases (Supplementary Figure 1, Supplementary Video 1), and long grooming bouts (>3sec) predominantly composed of multiple self-grooming phases separated by less than 1 second from each other.

Tic-like behaviour. Tic-like behaviours were defined as rapid, sudden repetitive behaviours, consisting of a single movement. These behaviours typically included head-body twitches, i.e. axial jerks as described in other mouse models of tic-like behaviours (52,53) (Supplementary Videos 1, 2).

Scratching behaviour. We defined scratching behaviour as a rhythmic movement of the hind limbs interacting with more rostral parts of the body (54). The targeted body parts varied between individuals in snout, area around the eyes, upper forehead, neck, between shoulders and on the back (Supplementary Videos 1, 3).

Nail clipping assay

We selected 20 mice (13 male and 7 female) Sapap3^{-/-} mice with lesions of different severity grades to perform hindpaw nail clipping under isoflurane anesthesia (Isovet, Centravet, 1000mg/g). Using small surgery scissors, we removed the pointy part of the hindpaw claws without hurting the nail bed. Clipped nails were disinfected with 10% betadine solution (Vétédine, Vétquinol) and mice were placed back into their homecages with their littermates. Lesion were scored at three different time points: before nail clipping procedure, two days and two weeks after nail clipping. Hereby, a common pool of n = 15 mice was assessed on all three time points; n = 4 additional mice were assessed only prior to and two days after nail clipping; n = 1 mouse was additionally assessed only prior to and two weeks after nail clipping treatment. Lesion scores were determined according to the following definitions: absence of lesions (score 1); mild fur and skin lesions without blood crusts (score 2); moderate fur and

skin lesions with blood crusts (score 3); tissue missing with blood crusts or open, wet skin (severe lesion) (score 4).

Statistical analysis

For statistical analysis, we used the following non-parametric tests under R version 3.4.0 (<https://www.r-project.org/>): Spearman tests for assessing correlations, Mann Whitney U testing for between-group comparisons, Wilcoxon signed rank test for evaluating treatment effects (nail clipping, aripiprazole) and Aligned Rank Transformation Analysis of Variance for testing factor interactions (package ARTool v0.10.6). For a comprehensive listing of statistical analyses of all results, see Supplementary Table 1. For estimating the most reliable separation of single versus syntactic grooming events consisting of distinct grooming phases, we used a receiver operating characteristic (ROC) curve, indicating the optimal true-positive rate (sensitivity) of a finding given the least possible probability of a false positive (1 - specificity). The R packages used for the ROC analysis were pROC (v3.6.3) and epiR (v3.6.1). For graphical illustration, we used the packages ggplot2 (v3.2.0.) and reshape2 (v1.4.3.).

Results

Sapap3^{-/-} mice express aberrant tic-like behaviours

Given the clinical reality of tic-like and compulsive-like comorbidity and recent publications reconsidering the purely compulsive-like nature of aberrant self-grooming in the Sapap3^{-/-} mouse (22), we performed a precise screening for other RBs than self-grooming, especially those, which might resemble tic-like movements. Indeed, we detected a very short and sudden type of repetitive behaviour, which is nearly absent in wildtype but significantly

present in $Sapap3^{-/-}$ mice (median_{wt} = 6.3 vs. median _{$Sapap3^{-/-}$} = 49.7; $W = 76$, $p = 0.002$) (Figure 2A). These repetitive behaviours consist of rapid head twitches and/or body jerks. This observed sudden, rapid recurrent, nonrhythmic execution of a single movement in the $Sapap3^{-/-}$ model strongly resembles the clinical definition of tics in human patients (5) as well as what has been described for rodent models of tic-like behaviours (52), suggesting face validity of the observed phenotype.

Typical skin lesions of $Sapap3^{-/-}$ mice are provoked by excessive scratching, a repetitive behaviour distinct from syntactic self-grooming

In addition to phenomenologically tic-like head and body twitches, we furthermore detected a prominent number of scratching events, which consist of the rapid, repeated beating of the hindpaw against various body parts (such as snout, areas surrounding the eyes and the ears, the neck, between the shoulders etc.), and should be distinguished from syntactic grooming, a stereotypically enchainned sequence of segregate phases, which is well-conserved in its choreography in all rodents (9,50,51). The amount of scratching events was significantly increased in $Sapap3^{-/-}$ compared to wildtype mice (median_{wt} = 5.7 vs. median _{$Sapap3^{-/-}$} = 106.3; $W = 73$, $p = 0.003$) (Figure 2B; Supplementary Videos 1, 3). The duration of scratching, significantly larger in $Sapap3^{-/-}$ mice, further corroborates the importance of this phenotype (median_{wt} = 0.3 min/hour_{activity} vs. median _{$Sapap3^{-/-}$} = 7.1 min/hour_{activity}; Mann Whitney U: $W = 76$, $p = 0.0008$) (Figure 2C). The number of tic-like head/body twitches correlated significantly with the number of scratching events, likely suggesting a common pathophysiological foundation of the two newly observed phenotypes (Spearman correlation – wt: $S = 34.64$, $\rho = 0.71$, $p = 0.03$; $Sapap3^{-/-}$: $S = 4$, $\rho = 0.97$, $p = 0.0002$) (Figure 2D).

During scratching, the hindpaw exerts a strong power onto targeted body areas, including body areas such as the neck or back, which are not touched by the forepaws during the self-grooming sequence. The quality of this event is rather violent and best described as a “beating” of the hindpaw against the body (55). Given the large frequency and duration of scratching behavior in *Sapap3^{-/-}* mice, the occasional detection of blood underneath the hindpaw claws of mice with lesions, the inherent violence of the movement and the observation that a proportion of principal lesions were detected in the neck and/or back of the animals, i.e. body locations, which are not prominently involved in self-grooming behavior, we established the alternative hypothesis that the flagship-like phenotype of facial and body lesions in *Sapap3^{-/-}* might be provoked by scratching instead of self-grooming. We therefore screened a large number of *Sapap3^{-/-}* mutants in the colony (n = 55 *Sapap3^{-/-}* mice) to revisit the most prominent lesion locations on their bodies and found that more than 30% of the lesions were indeed in body locations, which are not touched during the syntactic self-grooming sequence, namely the neck or back (Supplementary Figure 1A). We analyzed a subpopulation of these animals (n = 32) more in detail and found that in about 81% of these animals, the principal lesion was accompanied by further lesions at multiple sites including the snout (12.3%), eyes (16.4%), ears (34.2%), top of the head (2.7%), neck (24.7%) or back of the animals (9.6%) (Supplementary Figure 1B).

Out of the colony pool used to evaluate the lesion locations, we next selected *Sapap3^{-/-}* mice with representative lesions of various degrees of severity (n = 20; see methods section for details). In these representative individuals, we clipped the sharp tip of exclusively the hind-not forepaw nails without hurting the nailbed. We assessed the severity of the lesions longitudinally, prior to nail clipping, and two days or two weeks after hindpaw nail clipping. We applied a lesion score determined by the absence of fur, skin or tissue (see materials &

methods section for details). Stark improvement of lesion scores was already clearly detectable in all mice after only two days following nail clipping treatment ($n = 19$ mice; Wilcoxon signed rank test, paired; $V = 0$, $p = 0.0005$) (Fig. 2E, F), and further improved when screened after two weeks ($n = 16$; Wilcoxon signed rank test, paired; $V = 0$, $p = 0.0002$) (Fig. 2E, G).

Single-phase grooming events are more exaggerated than syntactic grooming in Sapap3^{-/-} mice

Having detected two novel RB phenotypes in the Sapap3^{-/-} mice and having observed that the prominent, typical lesions are inflicted by hindpaw scratching and not syntactic self-grooming, we revisited in detail the self-grooming behaviour in these mice, a highly stereotypical enchainment of four distinct phases (9,51,56). Increased self-grooming in Sapap3^{-/-} mice is usually quantified in the literature either via increased number of grooming events (13,49) or via increased grooming duration (13,19,49). In our detailed analysis, we decided to pay particular attention to the qualitative grooming heterogeneity observed in mice. We distinguished between both classical syntactic grooming composed of distinct rostro-caudal phases chained in sequence, and a deviating type consisting of a more sudden isolated short single-phase grooming event. When these two types of grooming were merged together, we observed a significantly increased number of grooming events in Sapap3^{-/-} mice (median_{wt} = 24.9 vs. median_{Sapap3^{-/-}} = 96.7; Mann Whitney U: $W = 80$, $p = 0.00008$ (Fig. 3A). However, surprisingly, we did not observe a significant difference in grooming duration between wildtype and mutant mice (median_{wt} = 11.6 min/hour_{activity} vs. median_{Sapap3^{-/-}} = 16.4 min/hour_{activity}, Mann Whitney U, $W = 51$, $p = 0.39$) (Fig. 3B). We first excluded that differences in sleep duration between Sapap3^{-/-} and wildtype mice might cause a confound in our

grooming dataset (sleep: median_{wt} = 33.2 min vs. median_{Sapap3^{-/-}} = 34.7 min; Mann Whitney U: $W = 41$, $p = 1$) (Supplementary Figure 2A). Thus, we next systematically investigated the distribution and quality of individual grooming events. We indeed detected a difference in the distribution of grooming bout lengths between Sapap3^{-/-} and wildtype controls with a substantial number of grooming events falling into the short event spectrum of the distribution (Fig. 3C). To analyse whether these short grooming events corresponded to short events consisting of a single grooming phases only, we performed a fine-scale scoring analysis, distinguishing individual grooming phases ($n = 608$ number of grooming events in $n = 4$ Sapap3^{-/-} mice; Supplementary Figure 2B). Applying receiver operating characteristic (ROC) curve estimations to our full-second binned data, we calculated that short events in our dataset consisting of a single grooming phase and those being composed of distinct grooming phases were best separated by a duration of 3 seconds (true positive rate / sensitivity_{3s} = 87.2%; false positive rejection rate / Specificity_{3s} = 61.5%; Supplementary Figure 2C). When classifying all scored grooming events ($n = 1737$ in $n = 9$ mice per genotype) into these two categories, Sapap3^{-/-} mice showed an aberrantly higher number of both short and long grooming bouts (short single-phase grooming bouts: median_{Sapap3^{-/-}} = 61.9; median_{wt} = 7.2, Mann Whitney U: $W = 81$, $p = 0.0004$; long syntactic grooming bouts: median_{Sapap3^{-/-}} = 56.0; median_{wt} = 18.9, Mann Whitney U: $W = 71$, $p = 0.006$; Figure 3D). Although this effect was present in both types of grooming events, the genotype effect depended on the type of grooming (Aligned Ranks Transformation ANOVA (ART ANOVA): $p_{GT*Grooming\ category} = 0.01$; Figure 3D). The proportion of short single phase to long syntactic grooming was genotype-dependent: while single phase grooming events formed about half the number of all grooming events in the Sapap3^{-/-} mice (grooming < 3 s : median_{Sapap3^{-/-}} = 56.3%; median_{wt} = 21.8%, $W = 79$, p -value = 0.0002), wildtype mice had a significantly higher proportion of long, syntactic

grooming events (grooming > 3 s : median_{Sapap3^{-/-}} = 44.7%; median_{wt} = 77.4%, $W = 2$, p -value = 0.0002 (Aligned Ranks Transformation ANOVA (ART ANOVA): $p_{GT*Grooming\ category} = 8.7*10^{-10}$; Supplementary Figure 2D). Lastly, we explored potential confounds between self-grooming and other types of RB such as scratching, which we report here as a novel type of RB. Indeed, when summing up total grooming duration as well as scratching duration, we confirmed that the total duration of RBs in Sapap3^{-/-} was also significantly increased in our dataset (Mann Whitney U: $W = 65$, $p = 0.003$), consistent with previous studies (13). Taken together, besides the increased number of syntactic self-grooming events previously described, we demonstrated here that exaggerated self-grooming reported in Sapap3^{-/-} mice was prominently due to elevated onsets of the sub-category of short grooming events.

Excessive tic-like, scratching and short grooming events are associated in Sapap3^{-/-} mice

Next, we analysed the distribution between the four different types of observed RBs, namely tic-like movements, scratching, short and long self-grooming events, as well as the correlations amongst them. While all four RBs formed part of a normal phenotype in wildtype mice, they were significantly more present in Sapap3^{-/-} mice and their distribution was also significantly different (Pearson's Chi-squared test: Chi-squared = 44.1, $df = 3$, $p = 1.5*10^{-09}$; Figure 4A). Tic-like events positively correlated with short grooming events in Sapap3^{-/-} mice only (Spearman correlation: Sapap3^{-/-}: $S = 32$, $\rho = 0.73$, $p = 0.03$; wt: $S = 53.7$, $\rho = 0.55$, $p = 0.12$), but not with long grooming sequences (Spearman correlation: Sapap3^{-/-}: $S = 60$, $\rho = 0.5$, $p = 0.18$; wt: $S = 173$, $\rho = -0.44$, $p = 0.23$) (Figure 4B), hinting towards a shared pathological neurobiological nature of tic-like and short grooming onsets.

The number of scratching events positively correlated with short but not long grooming events in Sapap3^{-/-} mice (Spearman correlation: short grooming events: $S = 20$, $\rho = 0.83$, $p = 0.008$;

long grooming events: $S = 54$, $\rho = 0.55$, $p = 0.13$) (Figure 4C); a similar tendency was also observed in wildtype mice (Spearman correlation: short grooming events: $S = 40$, $\rho = 0.67$, $p = 0.06$; long grooming events: $S = 132$, $\rho = -0.1$, $p = 0.81$) (Figure 4B, C), again suggesting a potentially shared neurobiology between short grooming onset events and hindpaw scratching. Finally, the number of scratching and tic-like events significantly correlated positively in both genotypes (Spearman correlation: $Sapap3^{-/-}$: $S = 4$, $\rho = 0.97$, $p = 0.0002$; wt: $S = 34.6$, $\rho = 0.71$, $p = 0.03$) (Figure 2E).

Taken together, these results suggest a symptomatological association between tic-like behaviours, scratching and short grooming events but not syntactic grooming sequences, with a stronger effect in $Sapap3^{-/-}$ than wildtype mice.

Tic-like and short grooming bouts were selectively reduced by aripiprazole, a first-line pharmacological treatment for Tourette syndrome

Although face validity, i.e. the close phenomenological similarity of tics in human patients and rapid recurrent repetitive behaviours observed in the $Sapap3^{-/-}$ mice, seems to point to a recapitulation of a common etiology, it is insufficient to draw conclusions about the nature of the observed rodent behavior. On top, face validity remains the most intuitive but at the same time subjective and prone to anthropomorphic interpretations (57). Thus, in order to investigate the nature of tic-like head-body twitches, scratching, short and long grooming events, and to question if they belong to the same symptomatologic categories, we pharmacologically challenged the predictive validity on these different types of RB observed in $Sapap3^{-/-}$ mice for a potential tic-like nature. Therefore, we applied the first-line pharmacological treatment for tic-like symptoms, aripiprazole (58–62). Aripiprazole is an atypical antipsychotic medication with a high in vitro affinity for dopamine 2 receptors (D2R)

and has a mixed effect as partial agonist and antagonist on type 1A and 2A serotonin receptors, respectively (63,64). Aripiprazole has an elimination half-life of approximately 75 hours and stable brain-to-serum concentration is achieved after 6 hours following acute injection (65). We applied a dose of 1.5mg/kg aripiprazole, which previously had been used to successfully reduce tic-like movements in according rodent models (47,48). We evaluated the effect of acutely administered aripiprazole on the different types of repetitive behaviours observed in the Sapap3^{-/-} mice, comparing treatment effect to the behavioural baseline of systemic injection of its vehicle solution. Acute aripiprazole treatment significantly lowered the number (Wilcoxon signed rank test, paired; $V = 1$, $p = 0.035$) and total duration of short grooming events (Wilcoxon signed rank test, paired; $V = 3$, $p = 0.04$) as well as the number of tic-like movements such as sudden head twitches and body jerks (Wilcoxon signed-rank test, paired; $V = 3$, $p = 0.04$) in Sapap3^{-/-} mice ($n = 8$ males) (Figure 5A, B). We observed a tendency of a decrease in number and duration of scratching, although these findings remained non-significant (Wilcoxon signed rank test, paired; $V = 8$, $p_{\text{number of events}} = 0.2$; Wilcoxon signed-rank test, paired; $V = 8$, $p_{\text{duration of events}} = 0.2$) (Figure 5A). However, such a tendency for a decrease was absent for the number and total duration of long grooming events (Wilcoxon signed-rank test, paired; $V = 12$, $p_{\text{number of events}} = 0.46$; Wilcoxon signed-rank test, paired; $V = 13$, $p_{\text{duration of events}} = 0.55$) (Figure 5A). This suggests that at least two out of four repetitive behaviours, which we observed as significantly present in the Sapap3^{-/-} mouse model, responded to a pharmacological treatment, which has proven success in treating tic-like movements both in Tourette syndrome in humans as well as in corresponding rodent models (47,48,58–61,66). Thus, we provide evidence that two RBs, namely tic-like body twitches and short single-phase grooming events, which symptomatologically are characterized as short, sudden movements

composed out of one single movement, - corresponding to the clinical definition of a tic - additionally possess predictive validity for tic-like symptoms.

Discussion

Here, we reconsidered the current main reference mouse model of compulsive-like behaviours, the Sapap3^{-/-} mouse, in light of the cortico-striatal circuitry as a substrate for pathological RBs. Recent studies indicate that not only the associative but also the sensorimotor CSCs might be implicated in the often comorbid occurrence of compulsive-like and tic-like RBs (3,22,67,68). Concretely, we performed a detailed, behavioural re-analysis of this mouse model, discovered previously undescribed types of pathologically RBs and pharmacologically challenged their clinical/neurobiological nature using aripiprazole, the first-line treatment for tic-like movements.

The here detected previously unreported RBs in the Sapap3^{-/-} mice consisted of single movements, which were repeatedly executed. This included sudden, rapid head/body twitches as well as hindpaw scratching, both occurring at an aberrantly high rate in Sapap3^{-/-} mice. The suddenness and rapidity of head-body twitches and their successful pharmacological treatment using aripiprazole hint straight to a classification of these RBs as tic-like RBs. The presence of both tic- and compulsive-like behaviours in the same model is in line with the clinical observation of tic-like comorbidities in both patients with Tourette Syndrome as well as with OCD (3,42,67,69). Indeed, some forms of OCD can be etiologically related to chronic tic disorders and 10-40% of OCD cases diagnosed in childhood or during adolescence are defined as belonging to a tic-related OCD subtype (44,45,68,70–75). Patients with tic-related OCD more likely report sensory phenomena such as “just right” perceptions

associated with sensory stimuli or the feeling of an “urge” (67,72,76) and may respond better to neuroleptic augmentation treatment (46,77). Within this clinical context, it is important to detect necessary subtlety in the phenotype of applied research models. Hence, the presence of both tic- and compulsive-like phenotypes in the Sapap3^{-/-} model increases its importance for studying the neurobiological basis of tic- and compulsive like comorbidities in various disorders or these pathologically RBs.

Hindpaw scratching, nearly absent in wildtype mice, occurred at an even higher frequency than tic-like head-body twitches. The significance of this RB is furthermore elevated by the pronounced improvement of the well-reported, flagship-like phenotype of skin lesions in this mouse model upon hindpaw claw dulling; our experiments suggest that it is actually the previously unconsidered RB of scratching and not syntactic self-grooming, which is crucially implicated in the most visible pathological phenotype of this mouse model. Having observed these previously unreported RBs in the Sapap3^{-/-} mouse model, we last revisited the syntactic self-grooming phenotype, the sole defined RB which had led to the definition of these mice as a compulsive-like model. Indeed, we confirmed the well-reported compulsive-like phenotype of an increased number of grooming bouts in these mice, however, couldn’t replicate the increased duration of self-grooming RB, which represents the most often reported pathological parameter in Sapap3^{-/-} mice (13,19,49). Most likely, the incongruence of our findings with previous reports is caused by a distinction of scratching and self-grooming behaviour, which was first performed in this study. Indeed, pooling of these two RBs has been previously mentioned (13). Yet, self-grooming is a highly stereotyped linear action sequence *stricto sensu*, which follows a predictable order (51), while scratching as a single isolated action does not share these properties of linearity and predictability. Thus, pooling of two qualitatively very distinct forms of behaviour causes confounds in the behavioural

phenotyping and in drawing conclusions for translational approaches. The pathophysiological definition of scratching is less clear. Can we define scratching as a tic-like behaviour due to its phenomenological appearance? Indeed, this pathologically repetitive behaviour consists of a sudden, rapidly repeated single movement and its frequency correlates with tic-like events in both wildtype and mutant mice. However, despite a tendency, aripiprazole did not significantly improve scratching frequency nor duration. Hence, scratching demonstrates tic-like elements but may not be entirely defined as such. Scratching may be considered similar to pathological hair-pulling and skin-picking, which has propagated a wave of clinical discussion concerning these phenotypes in human trichotillomania patients as well as frequently comorbid OCD and/or TS patients with hair-pulling and/or skin-picking pathologies (7). Indeed, it has been reported that patients with tic-related OCD also have higher rates of TTM (43,69). Interestingly, although no direct link was found between genetic *SAPAP3* variants and OCD, identified single nucleotide polymorphisms were associated with grooming disorders such as pathologic nail biting, pathologic skin picking, and/or trichotillomania, an obsessive-compulsive related disorder (78,79). These genetic studies underline the potential involvement of *SAPAP3/Sapap3* in the generation of hair-pulling or other grooming disorders, which occur in TTM or as a comorbidity in PCD and TS patients (78). TTM possesses clinical characteristics, which overlap with TS and OCD, e.g. the premonitory urge and temporary relief after completion of individual repetitive behaviours (80).

As a last major finding of our study, we observed that self-grooming events in *Sapap3*^{-/-} mice were not always conform with syntactic rodent self-grooming *stricto sensu*, i.e. composed of a syntactic chain of different, well-defined grooming phases (9,51,56). Instead, the majority of *Sapap3*^{-/-} self-grooming events were of short duration and seemed to consist of a single grooming phase only, i.e. a repeatedly executed, short and single movement. Both short and

long grooming events distinguish Sapap3^{-/-} from wildtype mice given their aberrant frequency, but their neurobiological nature seems to differ. Indeed, aripiprazole significantly reduced short but not long grooming events. Both the symptomatologic description of short grooming events and a decrease in their frequency upon aripiprazole treatment, i.e. face and predictive validity both suggest that short single-phase grooming events could be considered as tic-like events. On the other hand, longer grooming events, which mostly consisted of a syntactic sequence of different grooming phases, were not affected by acute aripiprazole administration. This distinction suggests a different neurobiological nature of these two types of grooming events. We thus report important evidences that self-grooming behaviour should not be considered a homogeneous behaviour and stress that a detailed characterization is essential to capture its neurobiological nature. Ushering a paradigm shift in the definition of rodent self-grooming might provide deeper insights into the pathological nature of RBs. This is important for the Sapap3^{-/-} mouse as we exemplarily analysed, but might need to be considered also for other mouse models, for which aberrantly elevated self-grooming behaviour had been reported (38,81,82). Differentiating distinct forms of self-grooming could help researchers to more adequately define the neurobiology of self-grooming and make them less prone to interpret their results in an anthropomorphic way.

Taken together, we observed distinct types of repetitive behaviours in the Sapap3^{-/-} mouse model, half of which can be defined as tic-like behaviours according to face and predictive validity criteria. We confirm previously reported excessive self-grooming sequences in Sapap3^{-/-} mice, but highlight the necessity to distinguish these from more sudden and simple repetitive behaviours. Indeed, we conclude that excessive number of grooming onsets rather than their duration characterizes the pathological phenotype of Sapap3^{-/-} mice. This observation of exaggerated grooming onsets is in line with previous studies suggesting that

Sapap3^{-/-} mice lack inhibition of executing an acquired motor sequence (16,22). This phenotype seems to be anchored in a diminished number of striatal parvalbumine-positive interneurons (16), which form a strong feed-forward inhibitory striatal regulatory network (83), as well as an increased striatal input of premotor cortico-striatal projections (22), a pathway which has been shown to be important for initiating behavioural sequences (84).

Altogether, the here newly reported comorbidity of different RBs in Sapap3^{-/-} mice is in line with the numerous clinical reports of comorbidity of tics and compulsions in OCD as well as TS patients (3) as well as with the current literature on disorders of repetitive behaviours, including fundamental neuroscience studies highlighting the potential implication of sensorimotor cortico-striatal circuits. Comorbidity findings of tic- and compulsive-like behaviours in Sapap3^{-/-} further corroborate the current hypothesis of a common neurobiological basis in disorders with repetitive behaviours. Re-defining the Sapap3^{-/-} mouse as a mouse model of RBs instead of compulsive-like behaviours raises its translational value in defining the proposed common neurobiological mechanism of tic- and compulsive-like symptoms.

References

1. Freeman RD, Soltanifar A, Baer S. Stereotypic movement disorder: easily missed. *Dev Med Child Neurol.* 2010 Aug;52(8):733–8.
2. Jiujiang M, Kelley E, Hall L. Restricted, Repetitive Behaviors in Autism Spectrum Disorder and Obsessive-Compulsive Disorder: A Comparative Review. *Child Psychiatry Hum Dev.* 2017;48(6):944–59.
3. Worbe Y, Mallet L, Golmard J-L, Béhar C, Durif F, Jalenques I, et al. Repetitive behaviours in patients with Gilles de la Tourette syndrome: tics, compulsions, or both? *PloS One.* 2010;5(9):e12959.

4. Leckman JF, Bloch MH, King RA. Symptom dimensions and subtypes of obsessive-compulsive disorder: a developmental perspective. *Dialogues Clin Neurosci.* 2009;11(1):21–33.
5. American Psychiatric Association. Diagnostic and statistical manual of mental disorders (DSM-V). 5th ed. American Psychiatric Publishing. Arlington, VA.; 2013.
6. Kircanski K, Woods DW, Chang SW, Ricketts EJ, Piacentini JC. Cluster analysis of the Yale Global Tic Severity Scale (YGTSS): symptom dimensions and clinical correlates in an outpatient youth sample. *J Abnorm Child Psychol.* 2010 Aug;38(6):777–88.
7. Lamothe H, Baleyte J-M, Mallet L, Pelissolo A. Trichotillomania is more related to Tourette disorder than to obsessive-compulsive disorder. *Rev Bras Psiquiatr Sao Paulo Braz* 1999. 2019 Sep 26;
8. Kalueff AV, Stewart AM, Song C, Berridge KC, Graybiel AM, Fentress JC. Neurobiology of rodent self-grooming and its value for translational neuroscience. *Nat Rev Neurosci.* 2016 Jan;17(1):45–59.
9. Berridge KC, Whishaw IQ. Cortex, striatum and cerebellum: control of serial order in a grooming sequence. *Exp Brain Res.* 1992;90(2):275–90.
10. Ting JT, Feng G. Neurobiology of obsessive-compulsive disorder: insights into neural circuitry dysfunction through mouse genetics. *Curr Opin Neurobiol.* 2011 Dec;21(6):842–8.
11. Milad MR, Rauch SL. Obsessive-compulsive disorder: beyond segregated cortico-striatal pathways. *Trends Cogn Sci.* 2012 Jan;16(1):43–51.
12. Pittenger C, Bloch MH, Williams K. Glutamate abnormalities in obsessive compulsive disorder: neurobiology, pathophysiology, and treatment. *Pharmacol Ther.* 2011 Dec;132(3):314–32.
13. Welch JM, Lu J, Rodriguiz RM, Trotta NC, Peca J, Ding J-D, et al. Cortico-striatal synaptic defects and OCD-like behaviours in Sapap3-mutant mice. *Nature.* 2007;448(7156):894–900.
14. Wan Y, Ade KK, Caffall Z, Ilcim Ozlu M, Eroglu C, Feng G, et al. Circuit-selective striatal synaptic dysfunction in the Sapap3 knockout mouse model of obsessive-compulsive disorder. *Biol Psychiatry.* 2014 Apr 15;75(8):623–30.
15. Pauls DL, Abramovitch A, Rauch SL, Geller DA. Obsessive-compulsive disorder: an integrative genetic and neurobiological perspective. *Nat Rev Neurosci.* 2014 Jun;15(6):410–24.
16. Burguière E, Monteiro P, Feng G, Graybiel AM. Optogenetic stimulation of lateral orbitofronto-striatal pathway suppresses compulsive behaviors. *Science.* 2013;340(6137):1243–6.
17. Rauch SL, Jenike MA, Alpert NM, Baer L, Breiter HC, Savage CR, et al. Regional cerebral blood flow measured during symptom provocation in obsessive-compulsive disorder using oxygen 15-labeled carbon dioxide and positron emission tomography. *Arch Gen Psychiatry.* 1994 Jan;51(1):62–70.
18. Pinhal CM, van den Boom BJG, Santana-Kragelund F, Fellingner L, Bech P, Hamelink R, et al. Differential Effects of Deep Brain Stimulation of the Internal Capsule and the Striatum on Excessive Grooming in Sapap3 Mutant Mice. *Biol Psychiatry.* 2018 Dec 15;84(12):917–25.

19. Manning EE, Wang AY, Saikali LM, Winner A, Ahmari SE. Disruption of prepulse inhibition is associated with severity of compulsive behavior and nucleus accumbens dopamine system changes in Sapap3 knockout mice. *bioRxiv*. 2019 Jul 7;694935.
20. Mallet L, Polosan M, Jaafari N, Baup N, Welter M-L, Fontaine D, et al. Subthalamic nucleus stimulation in severe obsessive-compulsive disorder. *N Engl J Med*. 2008 Nov 13;359(20):2121–34.
21. Chamberlain SR, Menzies L, Hampshire A, Suckling J, Fineberg NA, del Campo N, et al. Orbitofrontal dysfunction in patients with obsessive-compulsive disorder and their unaffected relatives. *Science*. 2008 Jul 18;321(5887):421–2.
22. Corbit VL, Manning EE, Gittis AH, Ahmari SE. Strengthened inputs from secondary motor cortex to striatum in a mouse model of compulsive behavior. *J Neurosci Off J Soc Neurosci*. 2019 Feb 8;
23. Hintiryan H, Foster NN, Bowman I, Bay M, Song MY, Gou L, et al. The mouse cortico-striatal projectome. *Nat Neurosci*. 2016 Aug;19(8):1100–14.
24. McGeorge AJ, Faull RL. The organization of the projection from the cerebral cortex to the striatum in the rat. *Neuroscience*. 1989;29(3):503–37.
25. Pan WX, Mao T, Dudman JT. Inputs to the Dorsal Striatum of the Mouse Reflect the Parallel Circuit Architecture of the Forebrain. *Front Neuroanat [Internet]*. 2010 Dec 27 [cited 2016 Jun 3];4. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3014656/>
26. Alexander GE, DeLong MR, Strick PL. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu Rev Neurosci*. 1986;9:357–81.
27. Jin X, Costa RM. Start/stop signals emerge in nigrostriatal circuits during sequence learning. *Nature*. 2010 Jul 22;466(7305):457–62.
28. Miyachi S, Hikosaka O, Miyashita K, Kárádi Z, Rand MK. Differential roles of monkey striatum in learning of sequential hand movement. *Exp Brain Res*. 1997 Jun;115(1):1–5.
29. Miyachi S, Hikosaka O, Lu X. Differential activation of monkey striatal neurons in the early and late stages of procedural learning. *Exp Brain Res*. 2002 Sep;146(1):122–6.
30. Thorn CA, Atallah H, Howe M, Graybiel AM. Differential dynamics of activity changes in dorsolateral and dorsomedial striatal loops during learning. *Neuron*. 2010 Jun 10;66(5):781–95.
31. Yin HH, Mulcare SP, Hilário MRF, Clouse E, Holloway T, Davis MI, et al. Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. *Nat Neurosci*. 2009 Mar;12(3):333–41.
32. Gillan CM, Pappmeyer M, Morein-Zamir S, Sahakian BJ, Fineberg NA, Robbins TW, et al. Disruption in the balance between goal-directed behavior and habit learning in obsessive-compulsive disorder. *Am J Psychiatry*. 2011 Jul;168(7):718–26.
33. Xu M, Kobets A, Du J-C, Lenington J, Li L, Banasr M, et al. Targeted ablation of cholinergic interneurons in the dorsolateral striatum produces behavioral manifestations of Tourette syndrome. *Proc Natl Acad Sci U S A*. 2015 Jan 20;112(3):893–8.

34. Kalanithi PSA, Zheng W, Kataoka Y, DiFiglia M, Grantz H, Saper CB, et al. Altered parvalbumin-positive neuron distribution in basal ganglia of individuals with Tourette syndrome. *Proc Natl Acad Sci U S A*. 2005 Sep 13;102(37):13307–12.
35. Kataoka Y, Kalanithi PSA, Grantz H, Schwartz ML, Saper C, Leckman JF, et al. Decreased number of parvalbumin and cholinergic interneurons in the striatum of individuals with Tourette syndrome. *J Comp Neurol*. 2010 Feb 1;518(3):277–91.
36. Peñagarikano O, Abrahams BS, Herman EI, Winden KD, Gdalyahu A, Dong H, et al. Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. *Cell*. 2011 Sep 30;147(1):235–46.
37. Rapanelli M, Frick LR, Pogorelov V, Ota KT, Abbasi E, Ohtsu H, et al. Dysregulated intracellular signaling in the striatum in a pathophysiologically grounded model of Tourette syndrome. *Eur Neuropsychopharmacol J Eur Coll Neuropsychopharmacol*. 2014 Dec;24(12):1896–906.
38. Shmelkov SV, Hormigo A, Jing D, Proenca CC, Bath KG, Milde T, et al. Slitrk5 deficiency impairs corticostriatal circuitry and leads to obsessive-compulsive-like behaviors in mice. *Nat Med*. 2010 May;16(5):598–602, 1p following 602.
39. Nordstrom EJ, Burton FH. A transgenic model of comorbid Tourette's syndrome and obsessive-compulsive disorder circuitry. *Mol Psychiatry*. 2002;7(6):617–25, 524.
40. Baldan LC, Williams KA, Gallezot J-D, Pogorelov V, Rapanelli M, Crowley M, et al. Histidine decarboxylase deficiency causes tourette syndrome: parallel findings in humans and mice. *Neuron*. 2014 Jan 8;81(1):77–90.
41. Xu M, Li L, Ohtsu H, Pittenger C. Histidine decarboxylase knockout mice, a genetic model of Tourette syndrome, show repetitive grooming after induced fear. *Neurosci Lett*. 2015 May 19;595:50–3.
42. Leckman JF, Denys D, Simpson HB, Mataix-Cols D, Hollander E, Saxena S, et al. Obsessive-compulsive disorder: a review of the diagnostic criteria and possible subtypes and dimensional specifiers for DSM-V. *Depress Anxiety*. 2010 Jun;27(6):507–27.
43. Petter T, Richter MA, Sandor P. Clinical features distinguishing patients with Tourette's syndrome and obsessive-compulsive disorder from patients with obsessive-compulsive disorder without tics. *J Clin Psychiatry*. 1998 Sep;59(9):456–9.
44. Cath DC, Spinhoven P, Hoogduin CAL, Landman AD, van Woerkom TCAM, van de Wetering BJM, et al. Repetitive behaviors in Tourette's syndrome and OCD with and without tics: what are the differences? *Psychiatry Res*. 2001 Mar 25;101(2):171–85.
45. Jaisoorya TS, Reddy YCJ, Srinath S, Thennarasu K. Obsessive-compulsive disorder with and without tic disorder: a comparative study from India. *CNS Spectr*. 2008 Aug;13(8):705–11.
46. Bloch MH, Landeros-Weisenberger A, Kelmendi B, Coric V, Bracken MB, Leckman JF. A systematic review: antipsychotic augmentation with treatment refractory obsessive-compulsive disorder. *Mol Psychiatry*. 2006 Jul;11(7):622–32.
47. Rizzo F, Abaei A, Nespoli E, Fegert JM, Hengerer B, Rasche V, et al. Aripiprazole and Riluzole treatment alters behavior and neurometabolites in young ADHD rats: a longitudinal 1H-NMR spectroscopy study at 11.7T. *Transl Psychiatry*. 2017 01;7(8):e1189.

48. Rizzo F, Nespoli E, Abaei A, Bar-Gad I, Deelchand DK, Fegert J, et al. Aripiprazole Selectively Reduces Motor Tics in a Young Animal Model for Tourette's Syndrome and Comorbid Attention Deficit and Hyperactivity Disorder. *Front Neurol*. 2018;9:59.
49. van den Boom BJG, Pavlidi P, Wolf CJH, Mooij AH, Willuhn I. Automated classification of self-grooming in mice using open-source software. *J Neurosci Methods*. 2017 Sep 1;289:48–56.
50. Berridge KC. Comparative fine structure of action: rules of form and sequence in the grooming patterns of six rodent species. *Behaviour*. 1–2(113):21–56.
51. Berridge KC, Fentress JC, Parr H. Natural syntax rules control action sequence of rats. *Behav Brain Res*. 1987 Jan;23(1):59–68.
52. Santangelo A, Bortolato M, Mosher LJ, Crescimanno G, Di Giovanni G, Cassioli E, et al. Behavioral fragmentation in the D1CT-7 mouse model of Tourette's syndrome. *CNS Neurosci Ther*. 2018 Aug;24(8):703–11.
53. Pogorelov V, Xu M, Smith HR, Buchanan GF, Pittenger C. Corticostriatal interactions in the generation of tic-like behaviors after local striatal disinhibition. *Exp Neurol*. 2015 Mar;265:122–8.
54. Orito K, Chida Y, Fujisawa C, Arkwright PD, Matsuda H. A new analytical system for quantification scratching behaviour in mice. *Br J Dermatol*. 2004 Jan;150(1):33–8.
55. Inagaki N, Igeta K, Shiraishi N, Kim JF, Nagao M, Nakamura N, et al. Evaluation and Characterization of Mouse Scratching Behavior by a New Apparatus, MicroAct. *Skin Pharmacol Physiol*. 2003;16(3):165–75.
56. Berridge KC, Aldridge JW, Houchard KR, Zhuang X. Sequential super-stereotypy of an instinctive fixed action pattern in hyper-dopaminergic mutant mice: a model of obsessive compulsive disorder and Tourette's. *BMC Biol*. 2005;3:4.
57. Bortolato M, Pittenger C. Modeling tics in rodents: Conceptual challenges and paths forward. *J Neurosci Methods*. 2017 Dec 1;292:12–9.
58. Cox JH, Seri S, Cavanna AE. Safety and efficacy of aripiprazole for the treatment of pediatric Tourette syndrome and other chronic tic disorders. *Pediatr Health Med Ther*. 2016;7:57–64.
59. Yang C-S, Huang H, Zhang L-L, Zhu C-R, Guo Q. Aripiprazole for the treatment of tic disorders in children: a systematic review and meta-analysis. *BMC Psychiatry*. 2015 Jul 29;15:179.
60. Sallee F, Kohegyi E, Zhao J, McQuade R, Cox K, Sanchez R, et al. Randomized, Double-Blind, Placebo-Controlled Trial Demonstrates the Efficacy and Safety of Oral Aripiprazole for the Treatment of Tourette's Disorder in Children and Adolescents. *J Child Adolesc Psychopharmacol*. 2017 Nov;27(9):771–81.
61. Hartmann A, Worbe Y. Pharmacological treatment of Gilles de la Tourette syndrome. *Neurosci Biobehav Rev*. 2013 Jul;37(6):1157–61.
62. Pringsheim T, Holler-Managan Y, Okun MS, Jankovic J, Piacentini J, Cavanna AE, et al. Comprehensive systematic review summary: Treatment of tics in people with Tourette syndrome and chronic tic disorders. *Neurology*. 2019 07;92(19):907–15.

63. Shapiro DA, Renock S, Arrington E, Chiodo LA, Liu L-X, Sibley DR, et al. Aripiprazole, a novel atypical antipsychotic drug with a unique and robust pharmacology. *Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol*. 2003 Aug;28(8):1400–11.
64. Wood M, Reavill C. Aripiprazole acts as a selective dopamine D2 receptor partial agonist. *Expert Opin Investig Drugs*. 2007 Jun;16(6):771–5.
65. Kirschbaum KM, Uhr M, Holthoewer D, Namendorf C, Pietrzik C, Hiemke C, et al. Pharmacokinetics of acute and sub-chronic aripiprazole in P-glycoprotein deficient mice. *Neuropharmacology*. 2010 Nov;59(6):474–9.
66. Nespoli E, Rizzo F, Boeckers T, Schulze U, Hengerer B. Altered dopaminergic regulation of the dorsal striatum is able to induce tic-like movements in juvenile rats. *PLOS ONE*. 2018 Apr 26;13(4):e0196515.
67. Miguel EC, do Rosário-Campos MC, Prado HS, do Valle R, Rauch SL, Coffey BJ, et al. Sensory phenomena in obsessive-compulsive disorder and Tourette's disorder. *J Clin Psychiatry*. 2000 Feb;61(2):150–6; quiz 157.
68. Diniz JB, Rosario-Campos MC, Hounie AG, Curi M, Shavitt RG, Lopes AC, et al. Chronic tics and Tourette syndrome in patients with obsessive-compulsive disorder. *J Psychiatr Res*. 2006 Sep;40(6):487–93.
69. Coffey BJ, Miguel EC, Biederman J, Baer L, Rauch SL, O'Sullivan RL, et al. Tourette's disorder with and without obsessive-compulsive disorder in adults: are they different? *J Nerv Ment Dis*. 1998 Apr;186(4):201–6.
70. Leonard HL, Lenane MC, Swedo SE, Rettew DC, Gershon ES, Rapoport JL. Tics and Tourette's disorder: a 2- to 7-year follow-up of 54 obsessive-compulsive children. *Am J Psychiatry*. 1992 Sep;149(9):1244–51.
71. George MS, Trimble MR, Ring HA, Sallee FR, Robertson MM. Obsessions in obsessive-compulsive disorder with and without Gilles de la Tourette's syndrome. *Am J Psychiatry*. 1993 Jan;150(1):93–7.
72. Leckman JF, Grice DE, Barr LC, de Vries AL, Martin C, Cohen DJ, et al. Tic-related vs. non-tic-related obsessive compulsive disorder. *Anxiety*. 1994 1995;1(5):208–15.
73. Holzer JC, Goodman WK, McDougle CJ, Baer L, Boyarsky BK, Leckman JF, et al. Obsessive-compulsive disorder with and without a chronic tic disorder. A comparison of symptoms in 70 patients. *Br J Psychiatry J Ment Sci*. 1994 Apr;164(4):469–73.
74. Zohar AH, Pauls DL, Ratzoni G, Apter A, Dycian A, Binder M, et al. Obsessive-compulsive disorder with and without tics in an epidemiological sample of adolescents. *Am J Psychiatry*. 1997 Feb;154(2):274–6.
75. Eichstedt JA, Arnold SL. Childhood-onset obsessive-compulsive disorder: a tic-related subtype of OCD? *Clin Psychol Rev*. 2001 Feb;21(1):137–57.
76. Prado HS, Rosário MC, Lee J, Hounie AG, Shavitt RG, Miguel EC. Sensory phenomena in obsessive-compulsive disorder and tic disorders: a review of the literature. *CNS Spectr*. 2008 May;13(5):425–32.

77. Ipser JC, Carey P, Dhansay Y, Fakier N, Seedat S, Stein DJ. Pharmacotherapy augmentation strategies in treatment-resistant anxiety disorders. *Cochrane Database Syst Rev*. 2006 Oct 18;(4):CD005473.
78. Bienvenu OJ, Wang Y, Shugart YY, Welch JM, Grados MA, Fyer AJ, et al. Sapap3 and pathological grooming in humans: Results from the OCD collaborative genetics study. *Am J Med Genet Part B Neuropsychiatr Genet Off Publ Int Soc Psychiatr Genet*. 2009 Jul 5;150B(5):710–20.
79. Boardman L, van der Merwe L, Lochner C, Kinnear CJ, Seedat S, Stein DJ, et al. Investigating SAPAP3 variants in the etiology of obsessive-compulsive disorder and trichotillomania in the South African white population. *Compr Psychiatry*. 2011 Apr;52(2):181–7.
80. Novak CE, Keuthen NJ, Stewart SE, Pauls DL. A twin concordance study of trichotillomania. *Am J Med Genet Part B Neuropsychiatr Genet Off Publ Int Soc Psychiatr Genet*. 2009 Oct 5;150B(7):944–9.
81. Nagarajan N, Jones BW, West PJ, Marc RE, Capecchi MR. Corticostriatal circuit defects in Hoxb8 mutant mice. *Mol Psychiatry*. 2018 Sep;23(9):1868–77.
82. Mei Y, Monteiro P, Zhou Y, Kim J-A, Gao X, Fu Z, et al. Adult restoration of Shank3 expression rescues selective autistic-like phenotypes. *Nature*. 2016 Feb 25;530(7591):481–4.
83. Silberberg G, Bolam JP. Local and afferent synaptic pathways in the striatal microcircuitry. *Curr Opin Neurobiol*. 2015 Aug;33:182–7.
84. Rothwell PE, Hayton SJ, Sun GL, Fuccillo MV, Lim BK, Malenka RC. Input- and Output-Specific Regulation of Serial Order Performance by Corticostriatal Circuits. *Neuron*. 2015 Oct 21;88(2):345–56.

Contributions

HL, CS, LM, EB conceptualized the research; HL, CS, OL conducted experiments; HL, CS, EB analyzed data; CS, HL, EB wrote the article; HL, CS, LM, EB edited the manuscript.

Acknowledgements

All animal work was conducted at the PHENO-ICMice facility. This work furthermore benefitted from the equipment and services from the iGenSeq core facility at ICM for the genotyping of the animals. We thank Drs. G. Feng and Ann M. Graybiel for kindly providing founders of the Sapap3^{-/-} colony, and Dr. Ester Nespola for her experimental advice on aripiprazole

preparation. This work was realized with the following funding: Agence Nationale de la Recherche (ANR-16-INSERM-SINREP, ANR-19-ICM-DOPALOOPS) (EB); Fondation de France (EB), and the L'Oréal-UNESCO Fellowship 2016 (CS). The core facilities were supported by "Investissements d'avenir" (ANR-10-IAIHU-06 and ANR-11-INBS-0011-NeurATRIS) and "Fondation pour la Recherche Médicale".

Conflict of interest.

The authors declare no conflict of interest.

Figure Legends

Figure 1. Behavioural assessment of Sapap3^{-/-} mice

(A) Photographs of custom-made apparatus for behavioural assessment, consisting of four acrylic chambers, each equipped with top and side cameras, connected to a digital video recording system. (B) Detailed graphic illustration of a single video chamber with *ad libitum* water and food access. (C) Time scale of behavioural assessment. Mice were video-recorded in the behavioural apparatus for 24hrs. Four intermittent time bins of 30 minutes each (i.e. a total of two hours) were manually analysed offline for repetitive behaviours including self-grooming, head-body twitches and hindpaw scratching. The scored time bins were distributed regularly across the light/dark circadian following previous protocols (Welch et al., 2007).

Figure 2. Sapap3^{-/-} mice express aberrant tic-like and scratching behaviours

(A) Sapap3^{-/-} mice execute a significant amount of tic-like head-body twitches, which are nearly absent in wildtype mice (Mann-Whitney U test, $p < 0.001$). (B) Sapap3^{-/-} mice show a significant amount of hindpaw scratching compared to wildtype control mice (Mann-Whitney U test, $p < 0.001$). (C) The duration of hindpaw scratching is significantly elevated in Sapap3^{-/-} in comparison to wildtype mice (Mann-Whitney U test, $p < 0.001$). (D) The number of tic-like movements and scratching bouts correlate positively in both wildtype (Spearman correlation, $p < 0.05$) and Sapap3^{-/-} mice (Spearman correlation, $p < 0.001$). (E) Photographs of three individual mice with representative lesions before, and two days or two weeks after hindpaw nail clipping treatment. (F) Lesions, assessed through a lesion score ranging from no lesions (score = 1) to severe lesions (score = 4), significantly improved already two days after clipping the hindpaw claws (Wilcoxon signed-rank test, paired, $p < 0.001$). (G) Lesions are further

improved two weeks after clipping the hindpaw claws as assessed through a significantly lowered lesion score (Wilcoxon signed-rank test, paired, $p < 0.001$). ** = $p < 0.01$, *** = $p < 0.001$.

Figure 3. Short, single-phase grooming events are more exaggerated than syntactic grooming in Sapap3^{-/-} mice

(A) Sapap3^{-/-} mice show significantly more grooming events compared to wildtype controls (Mann-Whitney U test, $p < 0.001$). **(B)** Total grooming duration is comparable between Sapap3^{-/-} and wildtype mice (Mann-Whitney U test, $p = 0.55$). **(C)** Self-grooming behavior of Sapap3^{-/-} mice compared to wildtype mice is characterized by a large proportion of grooming events of short duration. The x-axis is depicted on a log₁₀ scale. **(D)** Both short grooming events (< 3 second duration) as well as long grooming events (>3 second duration) were significantly enhanced in Sapap3^{-/-} mice compared to wildtype controls (Mann-Whitney U test, $p < 0.001$ and $p < 0.01$, respectively). Self-grooming behavior depended both on genotype and bout length (ART ANOVA, $p_{\text{genotype} \times \text{grooming type}} < 0.01$). Box plots illustrate the first and third quartiles; whiskers indicate the minimum and the maximal value of each data set at no further than 1.5 interquartile range. The indicated average is the median. Quartiles of Sapap3^{-/-} and wildtype mice are plotted in grey or white, and individual data points in filled black and empty black dots, respectively. ** = $p < 0.01$; *** = $p < 0.001$; ns = non-significant.

Figure 4. Excessive tic-like, scratching and short grooming events are associated in Sapap3^{-/-} mice

(A) The proportion of novel detected repetitive behaviours in Sapap3^{-/-} mice outweighs previously reported syntactic self-grooming behavior (Pearson's Chi-squared test, $p < 0.001$).

(B) Tic-like bouts positively correlate with short, single-phase grooming but not long, syntactic grooming bouts in Sapap3^{-/-} mice (Spearman correlation, $p < 0.05$, $p = ns$, respectively). **(C)** Scratching bouts also correlate positively with short, single-phase grooming but not long, syntactic grooming bouts in Sapap3^{-/-} mice (Spearman correlation, $p < 0.01$, $p = ns$, respectively). Box whisker plots are designed as in Figure 2. Correlation estimates are plotted in a grey solid line or a dotted black line for wildtype or Sapap3^{-/-} mice, respectively.

Figure 5. Tic-like and short grooming bouts were selectively reduced by aripiprazole

(A) Acute treatment with aripiprazole (1.5mg/kg) significantly reduced the number of single-phase grooming and the number of tic-like events (Wilcoxon signed-rank test, both $p < 0.05$), but not the number of syntactic grooming events nor the number of hindpaw scratching events (Wilcoxon signed-rank test, both $p = \text{non significant}$). The number of events under aripiprazole treatment are normalized to vehicle treatment (100%) of individual mice. **(B)** Aripiprazole significantly improves exaggerated single-phase but not syntactic grooming behaviour in Sapap3^{-/-} mice. The x-axis is depicted on a $\log_{10}$ scale. * = $p < 0.05$, ns = non-significant.

Supplementary Figure 1. Skin lesions in Sapap3^{-/-} include body areas that are not touched during syntactic grooming

(A) Distribution of most prominent skin lesions (in %) detected in a large colony pool of Sapap3^{-/-} mice ($n = 55$), including snout, eye, ear, upper head, neck and back body areas. **(B)**

In more than 80% of animals analyzed in detail (n = 32), the principal lesion was accompanied by further lesions at multiple sites.

Supplementary Figure 2. Distinction between short and long grooming episodes

(A) Sleep duration was comparable in wildtype and Sapap3^{-/-} mice (Mann-Whitney U, p = ns).

(B) Grooming events of short duration (<3 seconds) tend to consist of single-phase grooming events, while longer grooming events (>3 second) tend to consist of syntactic grooming events.

(C) Receiver operating characteristic (ROC) curve estimation of the best cut-off separating single- and syntactic grooming bouts according to their duration at 3 seconds (true positive rate / sensitivity_{3s} = 87.2%; false positive rejection rate / Specificity_{3s} = 61.5%). **(D)** Sapap3^{-/-} mice and wildtype mice significantly differ in the proportion of short, single-phase and long, syntactic grooming events (ART ANOVA, p_{genotype*grooming type} < 0.001). *** = p < 0.001, ns = non-significant.

Figure 2

Figure 3

Figure 4

A

B

C

Supplementary Figure 1

Supplementary Figure 2