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The Sapap3^{-/-} mouse reconsidered as a comorbid model expressing a spectrum of pathological repetitive behaviours

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► To cite this version:

Hugues Lamothe, Christiane Schreiweis, Lizbeth Sirenia Mondragón-González, Sana Rebbah, Oriana Lavielle, et al.. The Sapap3^{-/-} mouse reconsidered as a comorbid model expressing a spectrum of pathological repetitive behaviours. *Translational Psychiatry*, 2023, 13 (1), pp.26. 10.1038/s41398-023-02323-7. hal-03968560

HAL Id: hal-03968560

<https://hal.sorbonne-universite.fr/hal-03968560>

Submitted on 1 Feb 2023

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Running Title

What is the Sapap3^{-/-} mouse a model of?

1 **Title**

2 The Sapap3^{-/-} mouse reconsidered as a comorbid model expressing a spectrum of pathological
3 repetitive behaviours.

4

5

6 **Abstract**

7 Symptom comorbidity is present amongst neuropsychiatric disorders with repetitive
8 behaviours, complicating clinical diagnosis and impeding appropriate treatments. This is of
9 particular importance for obsessive-compulsive disorder (OCD) and Tourette syndrome. Here,
10 we meticulously analysed the behaviour of Sapap3 knockout mice, the recent rodent model
11 predominantly used to study compulsive-like behaviours, and found that its behaviour is more
12 complex than originally and persistently described. Indeed, we detected previously
13 unreported elements of distinct pathologically repetitive behaviours, which do not form part
14 of rodent syntactic cephalo-caudal self-grooming. These repetitive behaviours include
15 sudden, rapid body and head/body twitches, resembling tic-like movements. We also
16 observed that another type of repetitive behaviours, aberrant hindpaw scratching, might be
17 responsible for the flagship-like skin lesions of this mouse model. In order to characterise the
18 symptomatological nature of observed repetitive behaviours, we pharmacologically
19 challenged these phenotypes by systemic aripiprazole administration, a first-line treatment

20 for tic-like symptoms in Tourette syndrome and trichotillomania. A single treatment of
21 aripiprazole significantly reduced the number of head/body twitches, scratching, and single-
22 phase grooming, but not syntactic grooming events. These observations are in line with the
23 high comorbidity of tic- and compulsive-like symptoms in Tourette, OCD and trichotillomania
24 patients.

25

26 **Introduction**

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

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

50 cortico-striatal transmission is dysregulated ¹⁰⁻¹⁵, striatal structure is altered and its activity
51 increased ¹⁶⁻¹⁹, OCD-like relevant behaviour such as excessive self-grooming is aberrantly
52 overexpressed despite deleterious consequences, cognitive parameters such as behavioural
53 flexibility are altered ²⁰⁻²² and anxiety measures are increased ¹³. Pharmacotherapy via
54 selective serotonin reuptake inhibitors, which are applied as first-line therapy in OCD, or
55 targeted deep brain stimulation, which is applied in severe, treatment-resistant OCD cases,
56 decreases compulsive-like behaviours in both OCD patients as well as the Sapap3^{-/-} mouse
57 model ^{13,23-25}. A neurobiological core candidate in human OCD symptomatology is aberrant
58 orbitofrontal cortex (OFC) neuroanatomy and/or activity ^{15,17,26}. Several studies in Sapap3^{-/-}
59 mice have corroborated the potential implication of the OFC ^{16,27}; more specifically the lateral
60 OFC input onto striatal medium spiny neurons (MSN) in Sapap3^{-/-} mice was reduced ²⁸ and
61 their optogenetic excitation restored adapted grooming behaviour and aberrantly elevated
62 striatal firing rates ¹⁶.

63 Dysfunctions of cortico-striatal circuits are consistently reported with the appearance of
64 pathological RBs. These circuits are topographically organised in parallel limbic, associative
65 and sensorimotor loops coursing through the ventral (VS), dorsomedial (DMS) and
66 dorsolateral striatum (DLS), respectively ²⁹⁻³². They are known to dynamically interact across
67 these topographically organised loops and are recruited to different extents during learning
68 and automatised behaviours ³³⁻³⁷. While earlier evidences suggest a specific
69 neuropathological connection between compulsive-like behaviours and the so-called
70 'associative' cortico-striatal loop comprising associative cortical regions such as OFC and the
71 dorsomedial and central striatum, other findings suggest the implication of other,
72 complementary dorsal striatal circuits in the generation of pathological RBs in OCD and other
73 comorbid disorders. In the framework of these dynamically interacting cortico-striatal circuits,

74 OCD has recently been discussed as resulting from an imbalance across associative and
75 sensorimotor CSCs³⁸. This hypothesis is corroborated by studies demonstrating the
76 implication of the 'sensorimotor' cortico-striatal circuits, comprising motor cortices and the
77 dorsolateral striatum, in the generation of pathological RBs^{28,39,40}. Notably, in the same
78 Sapap3^{-/-} model, which has become the main reference mouse model for studying compulsive-
79 like behaviours in rodents, a recent study revealed that synaptic input from the premotor
80 cortex (M2), as observed *in vitro* through slice neurophysiological recording, was strengthened
81 in Sapap3^{-/-} mutants, suggesting thus a potential implication of sensorimotor circuits²⁸. Yet
82 other studies, including some in patients, point to an implication of the entire dorsal striatal
83 circuits in the generation of several types of pathological RBs⁴¹⁻⁴⁸. The hypothesis of generally
84 compromised cortico-dorsostriatal circuitry mediating RBs is also on line with the observed
85 strong comorbidity of tic- and compulsive-like symptoms in patients with Tourette syndrome
86 or OCD^{3,49-52} and this comorbidity is decisive for successful treatment^{49,50,53}.

87 Thus, reconsidering cortico-striatal circuitry as a substrate for pathological RBs and taking into
88 account specific indications of an implication of the sensorimotor cortico-striatal loops also in
89 the Sapap3^{-/-} mice^{28,40}, we here raised the question whether compulsive-like self-grooming in
90 Sapap3^{-/-} mice might be complemented by RBs of different nature. We first performed a
91 detailed multi-angle video screening to seek for distinct types of pathological RB other than
92 compulsive-like self-grooming. We next confirmed predictive validity by pharmacological
93 treatment of the spectrum of observed pathological RBs. These findings are of crucial interest
94 to redefine the Sapap3^{-/-} mouse model as a model of distinct types of RBs in the light of cortico-
95 dorsostriatal circuitry implication. Indeed, these new results are in line with
96 neurophysiological modifications due to whole-striatal Sapap3 expression patterns, the

97 clinical comorbidity observed in patients, and recent work reconsidering the circuitry affected
98 in this mouse model.

99

100 **Materials & Methods**

101 **Animals**

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

111 A total of 92 animals (hereof n=16 females and n=76 males) were used in this study and
112 systematically genotyped for the presence or absence of the Sapap3 protein during weaning
113 period following previously described procedures¹³. In detail, n = 55 Sapap3^{-/-} mice were used
114 for lesion evaluation (hereof, n=17 were used to evaluate the effect of hindpaw nail clipping
115 after two days and n = 16 to evaluate this effect after two weeks); n = 9 of each Sapap3^{-/-}
116 and wildtype mice for screening of repetitive behaviours; and n = 15 Sapap3^{-/-} mice for
117 aripiprazole treatment. Sample sizes were chosen according to previous publications using
118 comparable number of animals for evaluating repetitive behaviours (e.g. see⁵⁴ n = 10 Sapap3^{-/-}
119 mice ; n = 12 Sapap3^{-/-} mice⁵⁵) as well as according pharmacological treatment in the same
120 mouse model (n = 9-11 Sapap3^{-/-} mice¹³). Non-parametric permutation tests were conducted

121 as a robust statistical approach based on resampling in order to increase confidence in the
122 obtained results.

123 Animals for naïve behaviour were chosen randomly from the available colony pool of Sapap3⁻
124 ^{/-} adult mice (> 4 months of age) and age-matched wildtype littermates. For the aripiprazole
125 experiment, adult animals were briefly (1-2 minutes) observed in their homecages for signs of
126 increased grooming activity, for signs of anxiety (e.g. eye squinting, anxious crouching,
127 freezing, hiding away from the experimenter) and general quality of fur and skin. The animals
128 were selected in a range between mild to moderate phenotypes. For the nail clipping
129 experiments, we selected adult Sapap3⁻^{/-} animals showing a range of mild to severe skin
130 lesions of different shapes and locations.

131

132 **Video acquisition**

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

145

146 **Pharmacological treatments with aripiprazole.**

147 Sapap3^{-/-} mice (n=15) were weighted and placed inside the video acquisition system at 10am.

148 Animals were habituated to the environment for 30 hours prior to injections as well as to

149 handling and restraining procedures. At 4pm the following day, half the animals were injected

150 first with vehicle solution (0.9% sterile solution with 1% Tween 80 and 1% sterile DMSO;

151 0.1ml/10g) and 24 hours later with aripiprazole (1.5mg/kg in vehicle solution, 0.1ml/10g)^{56,57}.

152 The other half of the animals received first an aripiprazole injection, followed by a vehicle

153 solution injection a week later to allow for a sufficient washout period of aripiprazole. In that

154 condition, animals were taken out of the video recording apparatus 24hours after aripiprazole

155 treatment and re-habituated one week later to handling, restraining and to the apparatus for

156 30hrs prior to vehicle injections.

157

158 **Video analysis**

159 For behavioural assessment, videos were manually analysed offline using a freely available

160 scoring software (Kinovea, 0.8.15, www.kinovea.org), which allows to tag each individual

161 scored event and to export timestamps of tagged behaviours¹⁶. The experimenter scoring the

162 behaviour was blind to genotype and treatment and the order of the scored videos was

163 randomized.

164 For detailed behaviour characterisation in naïve mice, four time-segments of 30 minutes were

165 defined across 24 hours: 10-10h30 am, 6-6:30pm, 9-9h30pm, and 4-4h30am (Fig 1C). This

166 selection of time segments comprised dark/light cycle episodes as previous studies including

167 those using automated assessment of grooming⁵⁴ and was primarily based on the first study

168 reporting the excessive grooming phenotype in the Sapap3^{-/-} mice¹³.

169 For the behavioural assessment under aripiprazole, one time segment per mouse was selected
170 for video analyses according to the pharmacokinetics of the compound and following
171 procedures of previously published assays^{56,57}. The segment started at 9PM and lasted until
172 reaching 30 minutes of active behaviour. Concretely, an independent person randomized the
173 order of videos for mice and treatment (vehicle or aripiprazole) and relabelled the videos in a
174 pseudorandom manner. The expert scorer was blind to genotype and treatment during the
175 entire scoring process.

176 The proportion of sleep episodes, interspersed during 30 minutes of active behaviour, was
177 additionally quantified both in the behavioural assessment of naïve wildtype and Sapap3^{-/-}
178 mice as well as in aripiprazole-treated Sapap3^{-/-} mice.

179

180 **Motion estimation using DeepLabCut**

181 To quantify animal motion during vehicle and aripiprazole treatment, we used an open-source
182 Python package for body part tracking: DeepLabCut (version 2.2.1)^{58,59}, with CUDA Toolkit
183 (11.2) and Tensorflow (2.8.0). We used the DeepLabCut toolbox according to the protocol
184 published in⁵⁹. Briefly, the DeepLabCut toolbox was used to extract frames from selected
185 videos, manually annotate body parts of interest from those frames, form a training dataset
186 to train a convolutional neural network, train the neural network and evaluate the
187 performance of the network. Specifically, we labelled 200 frames per mouse (n=15, Sapap3^{-/-}
188) taken from one video per animal, with all videos corresponding to the hour directly following
189 the video recording used to assess the vehicle or aripiprazole effect. To capture gait and head-
190 turning while standing still, we targeted the hump on the centre back as estimate for body
191 centre, and the middle site between the ears as a marker for head location. 90% of the frames
192 were used to form a database of training. We used a ResNet-50-based neural network for

193 30,000 iterations⁶⁰. We validated with 50,000 number of shuffles and found a test error of
194 9.07 pixels and a training error of 6.19 pixels (image size was 704 by 576). We then used a p-
195 cut-off of 0.6. This network was then applied to analyse 15 one-hour videos that we used to
196 assess repetitive behaviours in both the vehicle and aripiprazole conditions.

197 To estimate the activity and locomotion of the mice during the awake states, the X and Y
198 coordinates of the tracked head and centre back marker, determined with DeepLabCut, were
199 imported into Python (v.3.8.10) and processed with custom scripts. The instantaneous speed
200 of the head and centre back marker was determined between two frames (25 fps) by deriving
201 the markers' positions over time. The activity and distance travelled was estimated with the X
202 and Y coordinates of the head and centre back marker by calculating the Euclidian distance
203 between two frames and its cumulative total distance. The pixel-to-cm conversion for each
204 video was determined by taking as a scale reference the distance between the head and
205 centre back markers.

206

207 **Ethogram**

208 ***Self-Grooming.*** Self-grooming behaviour is defined as a rostro-caudal sequence of four typical,
209 distinct, often intermittently executed phases as previously described in the literature for
210 rodent syntactic grooming^{61,62}. In our study we distinguished two different types of grooming
211 bouts. Short grooming bouts (<3sec) predominantly composed of only one of the four
212 grooming phases (Supplementary Figure 1, Supplementary Video 1), and long grooming bouts
213 (>3sec) composed of multiple self-grooming phases separated by less than 1 second from each
214 other.

215

216 **Head/body twitches.** Head/body twitches were defined as rapid, sudden repetitive
217 behaviours, consisting of a single movement and correspond to axial jerks as described in
218 mouse models of tic-like behaviours^{63,64} (Supplementary Videos 1, 2).

219
220 **Scratching behaviour.** We defined scratching behaviour as a rhythmic movement of the hind
221 limbs interacting with more rostral parts of the body⁶⁵. The targeted body parts varied
222 between individuals in snout, area around the eyes, upper forehead, neck, between shoulders
223 and on the back (Supplementary Videos 1, 3).

224
225 **Nail clipping assay**
226 We selected 20 mice (13 male and 7 female) *Sapap3*^{-/-} mice with lesions of different severity
227 grades to perform hindpaw nail clipping under isoflurane anesthesia (Isovet, Centravet,
228 1000mg/g). Using small surgery scissors, we removed the pointy part of the hindpaw claws
229 without hurting the nail bed. Clipped nails were disinfected with 10% betadine solution
230 (Vétédine, Vétquinol) and mice were placed back into their homecages with their littermates.
231 Lesion were scored at three different time points: before nail clipping procedure, two days
232 and two weeks after nail clipping. Hereby, a common pool of n = 13 mice was assessed on all
233 three time points; n = 4 additional mice were assessed only prior to and two days after nail
234 clipping; n = 1 mouse was additionally assessed only prior to and two weeks after nail clipping
235 treatment. Lesion scores were determined according to the following definitions: absence of
236 lesions (score 1); mild fur and skin lesions without blood crusts (score 2); moderate fur and
237 skin lesions with blood crusts (score 3); tissue missing with blood crusts or open, wet skin
238 (severe lesion) (score 4).

239
12

240 **Statistical analysis**

241 For statistical analysis, we used the following non-parametric tests under R version 3.4.0
242 (<https://www.r-project.org/>): Spearman tests for assessing correlations, Mann Whitney U
243 testing for between-group comparisons, Wilcoxon signed rank test for evaluating treatment
244 effects (nail clipping, aripiprazole), and Aligned Rank Transformation Analysis of Variance for
245 testing factor interactions (package ARTool v0.10.6). We additionally calculated Wilcoxon
246 effects sizes for all repetitive behaviours under aripiprazole treatment, and conducted non-
247 parametric, paired or unpaired permutation tests to analyse each response variable of the
248 aripiprazole or naïve behavioural dataset, respectively, which did not meet the assumptions
249 of normality and homogeneity of variance. Hereby, the number of iterations was set to 10000.
250 The level of statistical significance was set at p-values < 0.05. Permutation tests were
251 conducted using R version 4.1.0 (R Development Core Team, 2021). Briefly, permutation tests
252 are robust statistical approaches based on resampling and thus rely on the empirical and not
253 a theoretical distribution. Thus, they can provide more accurate p-values and can help control
254 the overall type I error rate Finally, after having verified that the assumptions of normality of
255 distribution and homoscedasticity were fulfilled, we used a linear mixed model (LMM)
256 approach to explain the repetitive behavioural variables by treatment and either sedation or
257 injection order as well as their interactions. To account for individual variability, we
258 implemented subject as weight in the model, and performed Type II Wald chi-square tests to
259 test the significance of the main effects and interactions. For a comprehensive listing of all
260 conducted statistical analyses and their results, see Supplementary Table 1. For estimating the
261 most reliable separation of single versus syntactic grooming events consisting of distinct
262 grooming phases, we used a receiver operating characteristic (ROC) curve, indicating the
263 optimal true-positive rate (sensitivity) of a finding given the least possible probability of a false

264 positive (1 - specificity). The R packages used for the ROC analysis were pROC (v3.6.3) and epiR
265 (v3.6.1). For graphical illustration, we used the packages ggplot2 (v3.2.0.) and reshape2
266 (v1.4.3.).

267

268 **Results**

269 **Sapap3^{-/-} mice express aberrant head/body twitches**

270 Given the clinical reality of tic-like and compulsive-like comorbidity and recent publications
271 reconsidering the purely compulsive-like nature of aberrant self-grooming in the Sapap3^{-/-}
272 mouse^{28,40}, we performed a precise screening for other RBs than self-grooming, especially
273 those, which might resemble tic-like movements. Indeed, we detected a very short and
274 sudden type of repetitive behaviour, which is nearly absent in wildtype but significantly
275 present in Sapap3^{-/-} mice (median_{wt} = 6.3 vs. median_{Sapap3^{-/-}} = 49.7; Mann Whitney U: W = 76,
276 $p = 0.002$; non-parametric permutation test: $p = 0.01$) (Figure 2A; Supplementary Videos 1, 2).

277 These repetitive behaviours consist of rapid head/body twitches. This observed sudden, rapid
278 recurrent, non-rhythmic execution of a single movement in the Sapap3^{-/-} model strongly
279 resembles the clinical definition of tics in human patients⁵ as well as what has been described
280 for rodent models of tic-like behaviours^{57,63}, suggesting face validity of the observed
281 phenotype.

282

283 **Typical skin lesions of Sapap3^{-/-} mice are likely provoked by excessive scratching, a repetitive** 284 **behaviour distinct from syntactic self-grooming**

285 In addition to head/body twitches, we furthermore detected a prominent number of
286 scratching events, which consist of the rapid, repeated beating of the hindpaw against various
287 body parts (such as snout, areas surrounding the eyes and the ears, the neck, between the

288 shoulders etc.), and which have to be distinguished from syntactic grooming, a stereotypically
289 enchain sequence of segregate phases, which is well-conserved in its choreography in all
290 rodents^{9,61,62}. The amount of scratching events was significantly increased in Sapap3^{-/-}
291 compared to wildtype mice (median_{wt} = 5.7 vs. median_{Sapap3^{-/-}} = 106.3; Mann Whitney U: W =
292 73, p = 0.003; non-parametric permutation test: p = 0.02) (Figure 2B; Supplementary Videos
293 1, 3). The duration of scratching, significantly larger in Sapap3^{-/-} mice, further corroborates the
294 importance of this phenotype (median_{wt} = 0.3 min/hour_{activity} vs. median_{Sapap3^{-/-}} = 7.1
295 min/hour_{activity}; Mann Whitney U: W = 76, p = 0.0008; non-parametric permutation test: p =
296 0.01) (Figure 2C). The number of head/body twitches correlated significantly with the number
297 of scratching events (Spearman correlation – wt: S = 34.64, rho = 0.71, p = 0.03; Sapap3^{-/-}: S =
298 4, rho = 0.97, p = 0.0002) (Figure 2D).

299 During scratching, the hindpaw exerts a strong power onto targeted body areas, including
300 body areas such as the neck or back, which are not touched by the forepaws during the self-
301 grooming sequence. The quality of this event is rather violent and best described as a
302 "beating" of the hindpaw against the body⁶⁶. Given the large frequency and duration of
303 scratching behaviour in Sapap3^{-/-} mice, the occasional detection of blood underneath the
304 hindpaw claws of mice with lesions, the inherent violence of the movement and the
305 observation that a proportion of principal lesions were detected in the neck and/or back of
306 the animals, i.e. body locations, which are not prominently involved in self-grooming
307 behaviour, we established the alternative hypothesis that the flagship-like phenotype of facial
308 and body lesions in Sapap3^{-/-} might be provoked by scratching instead of self-grooming. We
309 therefore screened a large number of Sapap3^{-/-} mutants in the colony (n = 55 Sapap3^{-/-} mice)
310 to revisit the most prominent lesion locations on their bodies and found that more than 30%
311 of the lesions were indeed in body locations, which are not touched during the syntactic self-

312 grooming sequence, namely the neck or back (Supplementary Figure 1A). We analysed a
313 subpopulation of these animals (n = 32) more in detail and found that in about 81% of these
314 animals, the principal lesion was accompanied by further lesions at multiple sites including the
315 snout (12.3%), eyes (16.4%), ears (34.2%), top of the head (2.7%), neck (24.7%) or back of the
316 animals (9.6%) (Supplementary Figure 1B).

317 Out of the colony pool used to evaluate the lesion locations, we next selected *Sapap3*^{-/-} mice
318 with representative lesions of various degrees of severity. In these representative individuals,
319 we clipped the sharp tip of exclusively the hind- not forepaw nails without hurting the nailbed.
320 We assessed the severity of the lesions longitudinally, prior to nail clipping, and two days or
321 two weeks after hindpaw nail clipping. We applied a lesion score determined by the absence
322 of fur, skin or tissue (see materials & methods section for details). Stark improvement of lesion
323 scores was already clearly detectable in all mice after only two days following nail clipping
324 treatment (n = 17 mice; Wilcoxon signed rank test, paired; $V = 0$, $p = 0.0005$) (Fig. 2E, F), and
325 further improved when screened after two weeks (n = 16; Wilcoxon signed rank test, paired;
326 $V = 0$, $p = 0.0002$) (Fig. 2E, G).

327
328 **Single-phase grooming events are more exaggerated than syntactic grooming in *Sapap3*^{-/-}**
329 **mice**

330 Having detected two novel RB phenotypes in the *Sapap3*^{-/-} mice and having observed that the
331 prominent, typical lesions are inflicted probably by hindpaw scratching, we revisited in detail
332 the self-grooming behaviour in these mice, a highly stereotypical enchainment of four distinct
333 phases^{9,62,67}. Increased self-grooming in *Sapap3*^{-/-} mice is usually quantified in the literature
334 either via increased number of grooming events^{13,54} or via increased grooming duration
335^{13,24,54}. In our detailed analysis, we decided to pay particular attention to the qualitative

336 grooming heterogeneity observed in mice. We distinguished between both syntactic
337 grooming composed of distinct rostro-caudal phases chained in sequence, and a deviating
338 type consisting of a more sudden isolated short single-phase grooming event. When these two
339 types of grooming were merged together, we observed a significantly increased number of
340 grooming events in *Sapap3*^{-/-} mice (median_{wt} = 24.9 vs. median_{*Sapap3*^{-/-}} = 96.7; Mann Whitney U:
341 $W = 80$, $p = 0.00008$; non-parametric permutation test: $p = 0.004$) (Fig. 3A). However,
342 surprisingly, we did not observe a significant difference in grooming duration between
343 wildtype and mutant mice (median_{wt} = 11.6 min/hour_{activity} vs. median_{*Sapap3*^{-/-}} = 16.4
344 min/hour_{activity}, Mann Whitney U, $W = 51$, $p = 0.39$; non-parametric permutation test: $p = 0.4$)
345 (Fig. 3B). We first excluded that differences in sleep duration between *Sapap3*^{-/-} and wildtype
346 mice might be a confounding factor in our grooming dataset (sleep: median_{wt} = 33.2 min vs.
347 median_{*Sapap3*^{-/-}} = 34.7 min; Mann Whitney U: $W = 41$, $p = 1$) (Supplementary Figure 2A). Thus,
348 we next systematically investigated the distribution and quality of individual grooming events.
349 We indeed detected a difference in the distribution of grooming bout lengths between
350 *Sapap3*^{-/-} and wildtype controls with a substantial number of grooming events falling into the
351 short event spectrum of the distribution (Fig. 3C). To analyse whether these short grooming
352 events corresponded to short events consisting of a single grooming phases only, we
353 performed a fine-scale scoring analysis, distinguishing individual grooming phases ($n = 608$
354 number of grooming events in $n = 4$ *Sapap3*^{-/-} mice; Supplementary Figure 2B). Applying
355 receiver operating characteristic (ROC) curve estimations to our full-second binned data, we
356 calculated that short events in our dataset consisting of a single grooming phase and those
357 being composed of distinct grooming phases were best separated by a duration of 3 seconds
358 (true positive rate / sensitivity_{3s} = 87.2%; false positive rejection rate / Specificity_{3s} = 61.5%;
359 Supplementary Figure 2C). When classifying all scored grooming events ($n = 1737$ in $n = 9$ mice

360 per genotype) into these two categories, Sapap3^{-/-} mice showed an aberrantly higher number
361 of both short and long grooming bouts (short single-phase grooming bouts: median_{Sapap3^{-/-}} =
362 61.9; median_{wt} = 7.2, Mann Whitney U: $W = 81$, $p = 0.0004$, non-parametric permutation test:
363 $p = 0.004$; long syntactic grooming bouts: median_{Sapap3^{-/-}} = 56.0; median_{wt} = 18.9, Mann
364 Whitney U: $W = 71$, $p = 0.006$, non-parametric permutation test: $p = 0.008$; Figure 3D).
365 Although this effect was present in both types of grooming events, the genotype effect
366 depended on the type of grooming (Aligned Ranks Transformation ANOVA (ART ANOVA):
367 $p_{GT*Grooming\ category} = 0.01$; Figure 3D). The proportion of short single phase to long syntactic
368 grooming was genotype-dependent: while single phase grooming events formed about half
369 the number of all grooming events in the Sapap3^{-/-} mice (grooming < 3 s : median_{Sapap3^{-/-}} =
370 56.3%; median_{wt} = 21.8%, Mann Whitney U: $W = 79$, p-value = 0.0002), wildtype mice had a
371 significantly higher proportion of long, syntactic grooming events (grooming > 3 s :
372 median_{Sapap3^{-/-}} = 44.7%; median_{wt} = 77.4%; Mann Whitney U: $W = 2$, p-value = 0.0002; Aligned
373 Ranks Transformation ANOVA (ART ANOVA): $p_{GT*Grooming\ category} = 8.7*10^{-10}$; Supplementary
374 Figure 2D). Lastly, we explored potential confounds between self-grooming and other types
375 of RB such as scratching, which we report here as a novel type of RB. Indeed, when summing
376 up total grooming duration as well as scratching duration, we confirmed that the total
377 duration of RBs in Sapap3^{-/-} was also significantly increased in our dataset (Mann Whitney U:
378 $W = 65$, $p = 0.003$), consistent with previous studies¹³. Taken together, besides the increased
379 number of syntactic self-grooming events previously described, we demonstrated here that
380 exaggerated self-grooming reported in Sapap3^{-/-} mice was prominently due to elevated onsets
381 of the sub-category of short grooming events.

382

383 **Excessive head/body twitches, scratching and short grooming events are associated in**
384 **Sapap3^{-/-} mice**

385 Next, we analysed the distribution between the four different types of observed RBs, namely
386 head/body twitches, scratching, short and long self-grooming events, as well as the
387 correlations amongst them. While all four RBs formed part of a normal phenotype in wildtype
388 mice, they were significantly more present in Sapap3^{-/-} mice and their distribution was also
389 significantly different (Pearson's Chi-squared test: Chi-squared = 44.1, df = 3, $p = 1.5 \times 10^{-09}$;
390 Figure 4A). Head/body twitches positively correlated with short grooming events in Sapap3^{-/-}
391 mice only (Spearman correlation: Sapap3^{-/-}: $S = 32$, $\rho = 0.73$, $p = 0.03$; wt: $S = 53.7$, $\rho =$
392 0.55 , $p = 0.12$), but not with long grooming sequences (Spearman correlation: Sapap3^{-/-}: $S =$
393 60 , $\rho = 0.5$, $p = 0.18$; wt: $S = 173$, $\rho = -0.44$, $p = 0.23$) (Figure 4B).

394 The number of scratching events positively correlated with short but not long grooming events
395 in Sapap3^{-/-} mice (Spearman correlation: short grooming events: $S = 20$, $\rho = 0.83$, $p = 0.008$;
396 long grooming events: $S = 54$, $\rho = 0.55$, $p = 0.13$) (Figure 4C); no such significant correlation
397 was found in wildtype mice (Spearman correlation: short grooming events: $S = 40$, $\rho = 0.67$,
398 $p = 0.06$; long grooming events: $S = 132$, $\rho = -0.1$, $p = 0.81$) (Figure 4C). Finally, the number
399 of scratching events and head/body twitches significantly correlated positively in both
400 genotypes (Spearman correlation: Sapap3^{-/-}: $S = 4$, $\rho = 0.97$, $p = 0.0002$; wt: $S = 34.6$, $\rho =$
401 0.71 , $p = 0.03$) (Figure 2D).

402
403 **Head/body twitches, short grooming bouts and scratching events were selectively reduced**
404 **by aripiprazole, a first-line pharmacological treatment for Tourette syndrome**

405 Although face validity, i.e. the close phenomenological similarity of tics in human patients and
406 rapid recurrent repetitive behaviours observed in the Sapap3^{-/-} mice, seems to point to a

407 recapitulation of a common etiology, it is insufficient to draw conclusions about the nature of
408 the observed rodent behaviour. On top, face validity remains the most intuitive but at the
409 same time subjective and prone to anthropomorphic interpretations ⁶⁸. Thus, in order to
410 investigate the nature of head-body twitches, scratching, short and long grooming events, and
411 to question if they belong to the same symptomatologic categories, we pharmacologically
412 challenged the predictive validity on these different types of RB observed in Sapap3^{-/-} mice for
413 a potential tic-like nature. Therefore, we applied the first-line pharmacological treatment for
414 tics, aripiprazole ⁶⁹⁻⁷³. Aripiprazole is an atypical antipsychotic medication with a high in vitro
415 affinity for dopamine 2 receptors (D2R) and has a mixed effect as partial agonist and
416 antagonist on type 1A and 2A serotonin receptors, respectively ^{74,75}. Aripiprazole has an
417 elimination half-life of approximately 75 hours and stable brain-to-serum concentration is
418 achieved after 6 hours following acute injection ⁷⁶. We applied a dose of 1.5mg/kg
419 aripiprazole, which previously had been used to successfully reduce what has been reported
420 as tic-like movements in according rodent models ^{56,57}. We evaluated the effect of acutely
421 administered aripiprazole on the different types of repetitive behaviours observed in the
422 Sapap3^{-/-} mice, comparing treatment effect to the behavioural baseline of systemic injection
423 of its vehicle solution $\left(\frac{\text{number of RB after aripiprazole}}{\text{(number of RB after aripiprazole + number of RB after vehicle)}}\right)$. Acute
424 aripiprazole treatment significantly lowered the number (Wilcoxon signed rank test, paired: V
425 = 8, $p = 0.006$; non-parametric, paired permutation test: $p_{\text{permutation; short grooming}} = 0.0023$) and
426 total duration of short grooming events (Wilcoxon signed rank test, paired; $V = 12$, $p = 0.004$)
427 (Figure 5A). This decrease was most visible the shorter the grooming events (Figure 5B). We
428 additionally found a reduction in the number of head/body twitches (Wilcoxon signed-rank
429 test, paired: $V = 8$, $p = 0.006$; non-parametric, paired permutation test: $p_{\text{permutation; head/body}}$
430 twitches = 0.0032) as well as a decrease in number and duration of scratching (Wilcoxon signed

431 rank test, paired; $V = 7$, $p_{\text{number of scratching bouts}} = 0.001$; non-parametric, paired permutation test:
432 $p_{\text{permutation; scratching events}} = 0.0011$; Wilcoxon signed-rank test, paired; $V = 21$, $p_{\text{duration of scratching}} =$
433 0.029) in Sapap3^{-/-} mice under aripiprazole treatment (Figure 5A). However, despite a
434 tendency, such effect was absent for the number and total duration of long grooming events
435 (Wilcoxon signed-rank test, paired; $V = 29$, $p_{\text{number of long groomings}} = 0.083$; non-parametric, paired
436 permutation test: $p_{\text{permutation; long groomings}} = 0.087$; Wilcoxon signed-rank test, paired; $V = 38$,
437 $p_{\text{duration of long groomings}} = 0.23$) (Figure 5A). Additionally, we calculated effect sizes of all four RBs,
438 which showed a lower effect on long grooming events when compared to the three other RBs
439 (Wilcoxon effect sizes: $r_{\text{short grooming}} = 0.73$; $r_{\text{head/body twitches}} = 0.78$; $r_{\text{scratching}} = 0.72$; $r_{\text{long grooming}} =$
440 0.45 ; Supplementary Table 1). Given potential sedative effects of aripiprazole, in addition to
441 assessing repetitive behaviours only during awake active phases, as control parameters, we
442 quantified the duration of sleep episodes interspersed between active behavioural episodes,
443 which did not differ between vehicle-treated and aripiprazole-treated animals ($n = 15$ Sapap3^{-/-}
444 mice; Wilcoxon signed rank test, paired; $V = 73$, $p = 0.2$; non-parametric, paired permutation
445 test: $p = 0.45$) (Supplementary Figure 3A). We further excluded potential sedation effects by
446 assessing trunk centre and head centre movements as a proxy for forward locomotion as well
447 as general activity applying the DeepLabCut toolbox, which also did not differ between the
448 vehicle and the aripiprazole condition (Wilcoxon signed rank test, paired; $V_{\text{trunk}} = 36$, $p = 0.2$;
449 $V_{\text{head}} = 53$, $p = 0.5$; non-parametric, paired permutation test: $p_{\text{trunk}} = 0.2$; $p_{\text{head}} = 0.39$)
450 (Supplementary Figure 3B-C). No correlations were observed between repetitive behaviours
451 and activity parameters (Spearman correlation: all $p > 0.1$; all detailed information is available
452 in Supplementary Table 1), nor did we observe any significant interaction between these
453 activity parameters and treatment (LMM: all $p > 0.2$, all detailed information is available in
454 Supplementary Table 1), furthermore excluding potential sedation effects in our assay. Lastly,

455 to estimate the potentially confounding effect of potential handling and injection stress, we
456 also tested for the interaction of treatment with injection order, but did not observe
457 significant interactions (LMM: all $p > 0.1$; all detailed information is available in Supplementary
458 Table 1; Supplementary Figure 3D). Taken together, our findings suggest that specifically three
459 out of four repetitive behaviours, which we observed as significantly present in the *Sapap3*^{-/-}
460 mouse model, responded to a pharmacological treatment, which has proven success in
461 treating tic-like movements both in Tourette syndrome in humans as well as in corresponding
462 rodent models ^{56,57,69–72,77}. Thus, we provide evidence that three types of RBs, namely
463 head/body twitches, short single-phase grooming events and scratching, additionally possess
464 predictive validity for tic-like symptoms.

465

466 **Discussion**

467 Here, we reconsidered the current main reference mouse model of compulsive-like
468 behaviours, the *Sapap3*^{-/-} mouse, in light of the cortico-striatal circuitry as a substrate for
469 pathological RBs. Recent studies indicate that not only the associative but also the
470 sensorimotor CSCs might be implicated in the often comorbid occurrence of compulsive-like
471 and tic-like RBs ^{3,28,40,78,79}. Concretely, we performed a detailed, behavioural re-analysis of this
472 mouse model, discovered previously undescribed types of pathologically RBs and
473 pharmacologically challenged their nature using aripiprazole, the first-line treatment for tic-
474 like movements ⁷³.

475 The here detected previously unreported RBs in the *Sapap3*^{-/-} mice consisted of single
476 movements, which were repeatedly executed. This included sudden, rapid head/body
477 twitches as well as hindpaw scratching, both occurring at an aberrantly high rate in *Sapap3*^{-/-}
478 mice. The suddenness and rapidity of head-body twitches and their successful

479 pharmacological treatment using aripiprazole hint straight to an interpretation of these RBs
480 as tic-like RBs. As a marginal sedative effect, which does not impede a normal life in society,
481 have been reported in some patients ⁷³, we analysed and excluded potential sedation side
482 effects of aripiprazole accounting for changes in head/body twitches and other RBs in our
483 dataset. Replication of our pioneering findings in a larger cohort would be recommended to
484 further substantiate our findings. The presence of both tic- and compulsive-like behaviours in
485 the same model is in line with the clinical observation of tic-like comorbidities in both patients
486 with Tourette Syndrome as well as with OCD ^{3,49,79,80}. Indeed, some forms of OCD can be
487 etiologically related to chronic tic disorders and 10-40% of OCD cases diagnosed in childhood
488 or during adolescence are defined as belonging to a tic-related OCD subtype ^{51,52,78,81-86}.
489 Patients with tic-related OCD more likely report sensory phenomena such as "just right"
490 perceptions associated with sensory stimuli or the feeling of an "urge" ^{79,83,87} and may respond
491 better to neuroleptic augmentation treatment ^{53,88}. Such observation is interesting given the
492 recent reports of increased neuronal activity of striatal projection neurons expressing
493 dopamine D2 receptors in the Sapap3^{-/-} mouse model ¹⁸. Within this clinical context, it is
494 important to detect necessary subtlety in the phenotype of applied research models. Hence,
495 the presence of both tic- and compulsive-like phenotypes in the Sapap3^{-/-} model increases its
496 importance for studying the neurobiological basis of tic- and compulsive-like comorbidities in
497 various disorders or these pathologically RBs.

498 Hindpaw scratching, nearly absent in wildtype mice, occurred at an even higher frequency
499 than head/body twitches. The importance of this RB is furthermore elevated by the systematic
500 and consistent improvement of skin lesions in this mouse model upon hindpaw claw dulling,
501 suggesting at least a major and maybe even a causal role of this RB in the well-reported,
502 flagship-like phenotype of Sapap3^{-/-} mutant mice. Further support for such interpretation

503 comes from the observation that a large proportion of skin lesions is found on body parts,
504 which are not touched at all during syntactic self-grooming. As the sharp nail tips grow back
505 during the second week after nail clipping treatment, the observation of remaining skin lesions
506 two weeks after hindpaw nail clipping are likely a consequence of reappearing deleterious
507 scratching effects. However, we cannot exclude at least a contribution to skin lesion
508 maintenance due to rodent self-grooming. Taken together, our experiments suggest that
509 hindpaw scratching most likely provokes or is at least crucially implicated in the most visible
510 pathological phenotype of this mouse model. Can scratching pathophysiologically be defined
511 as a tic-like behaviour? Indeed, this RB consists of a sudden, rapidly repeated single movement
512 and its frequency correlates with head/body twitches in both wildtype and mutant mice.
513 Aripiprazole treatment significantly decreased both scratching frequency as well as duration.
514 Scratching may be considered similar to pathological hair-pulling and skin-picking, which has
515 propagated a wave of clinical discussion concerning these phenotypes in human
516 trichotillomania patients as well as frequently comorbid OCD and/or TS patients with hair-
517 pulling and/or skin-picking pathologies⁷. Indeed, it has been reported that patients with tic-
518 related OCD also have higher rates of TTM^{50,80}. Interestingly, although no direct link was found
519 between genetic *SAPAP3* variants and OCD, identified single nucleotide polymorphisms were
520 associated with grooming disorders such as pathologic nail biting, pathologic skin picking,
521 and/or trichotillomania, an obsessive-compulsive related disorder^{89,90}. These genetic studies
522 underline the potential involvement of *SAPAP3/Sapap3* in the generation of hair-pulling or
523 other grooming disorders, which occur in TTM or as a comorbidity in OCD and TS patients⁸⁹.
524 TTM possesses clinical characteristics, which overlap with TS and OCD, e.g. the premonitory
525 urge and temporary relief after completion of individual repetitive behaviours⁹¹.

526 Having observed these previously unreported RBs in the Sapap3^{-/-} mouse model, we last
527 revisited the syntactic self-grooming phenotype, the sole defined RB which had led to the
528 definition of these mice as a compulsive-like model. Indeed, we confirmed the well-reported
529 compulsive-like phenotype of an increased number of grooming bouts in these mice, however,
530 couldn't replicate the increased duration of self-grooming RB, which represents the most
531 often reported pathological parameter in Sapap3^{-/-} mice^{13,24,54}. Most likely, the incongruence
532 of our findings with previous reports is caused by a distinction of scratching and self-grooming
533 behaviour, which was first performed in this study. Indeed, pooling of these two RBs has been
534 previously mentioned¹³ and pooling these two distinct repetitive behaviours in our datasets
535 indeed results in a significant genotype-dependent difference (Mann-Whitney U: $W = 65$, $p =$
536 0.003) (Supplementary Table 1). Yet, self-grooming is a highly stereotyped linear action
537 sequence, which follows a predictable order⁶², while scratching as a single isolated action
538 does not share these properties of linearity and predictability. Thus, pooling of two
539 qualitatively very distinct forms of behaviour causes confounds in the behavioural
540 phenotyping and in drawing conclusions for translational approaches.

541 As a last major finding of our study, we observed that self-grooming events in Sapap3^{-/-} mice
542 were not always conform with syntactic rodent self-grooming *stricto sensu*, i.e. composed of
543 a syntactic chain of different, well-defined grooming phases^{9,62,67}. Instead, the majority of
544 Sapap3^{-/-} self-grooming events were of short duration and seemed to consist of a single
545 grooming phase only, i.e. a repeatedly executed, short and single movement. Both short and
546 long grooming events distinguish Sapap3^{-/-} from wildtype mice given their aberrant frequency,
547 but their neurobiological nature seems to differ. Indeed, aripiprazole significantly reduced
548 short but not long grooming events. Both the symptomatologic description of short grooming
549 events and a decrease in their frequency upon aripiprazole treatment, i.e. face and predictive

550 validity both suggest that short single-phase grooming events could be considered as tic-like
551 events. On the other hand, longer grooming events, which mostly consisted of a syntactic
552 sequence of different grooming phases, might form a category of RBs apart from the others:
553 first, despite a tendency, this category was the only that was not significantly reduced by acute
554 aripiprazole administration. Secondly, effect size of the long grooming category was much
555 smaller than the comparable effect sizes of the other three RB categories. While these results
556 might suggest a different neurobiological nature of these two types of grooming events,
557 conclusions of our findings on long grooming remain limited and will benefit from a follow-up,
558 dedicated study with much higher sample number. First, despite our negative findings, a
559 tendency of decrease in long grooming behaviours was still detected, indicating a possible
560 aripiprazole response also in long grooming events, maybe due to individual heterogeneity,
561 which has previously been reported in our own work and that of others^{20,22}. Second, although
562 in our analysis, we statistically excluded the confounding factor of handling and injection
563 stress, we cannot entirely rule out such effects in this mouse model with marked anxiety.
564 Altogether, our results report important evidences that self-grooming behaviour should not
565 be considered a homogeneous behaviour and pronounce that a detailed characterisation is
566 essential to capture its neurobiological nature. Ushering a paradigm shift in the definition of
567 rodent self-grooming might provide deeper insights into the pathological nature of RBs. This
568 is important for the *Sapap3*^{-/-} mouse as we exemplarily analysed, but might need to be
569 considered also for other mouse models, for which aberrantly elevated self-grooming
570 behaviour had been reported^{45,92,93}. Thus, differentiating distinct forms of self-grooming or
571 other behavioural phenotypes could help researchers to more adequately investigate the
572 neurobiology of RBs^{20,22,94}.

573 Taken together, we observed distinct types of repetitive behaviours in the Sapap3^{-/-} mouse
574 model, three of which can be labelled as tic-like behaviours according to face and predictive
575 validity criteria ⁹⁵. We confirm previously reported excessive self-grooming sequences in
576 Sapap3^{-/-} mice, but highlight the necessity to distinguish these from more sudden and simple
577 repetitive behaviours. Indeed, we conclude that excessive number of grooming onsets rather
578 than their duration characterises the pathological phenotype of Sapap3^{-/-} mice. This
579 observation of exaggerated grooming onsets is in line with previous studies suggesting that
580 Sapap3^{-/-} mice lack inhibition of executing an acquired motor sequence ^{16,28}. This phenotype
581 seems to be anchored in a diminished number of striatal parvalbumin-positive interneurons
582 ¹⁶, which form a strong feed-forward inhibitory striatal regulatory network ⁹⁶, as well as an
583 increased striatal input of premotor cortico-striatal projections ²⁸, a pathway which has been
584 shown to be important for initiating behavioural sequences ⁹⁷.

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

594

595

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597

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845

846 **Contributions**

847 HL, CS, LM, EB conceptualised the research; HL, CS, OL conducted experiments; HL, CS, EB, SR,
848 SLM analysed data; CS, HL, EB wrote the article; HL, CS, LM, EB edited the manuscript.

849

850 **Acknowledgements**

851 All animal work was conducted at the PHENO-ICMice facility. This work furthermore benefitted
852 from the equipment and services from the iGenSeq core facility at ICM for the genotyping of
853 the animals, as well from the statistical advice and help by Drs. Sana Rabbeh and François-
854 Xavier Lejeune from the statistics core facility. We thank Dr. Ester Nespoli for her experimental

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

860

861 **Conflict of interest.**

862 The authors declare no conflict of interest.

863 **Figures Legends**

864 **Figure 1. Behavioural assessment of Sapap3^{-/-} mice. (A)** Photographs of custom-made
865 apparatus for behavioural assessment, consisting of four acrylic chambers, each equipped
866 with top and side cameras, connected to a digital video recording system. **(B)** Detailed graphic
867 illustration of a single video chamber with *ad libitum* water and food access. **(C)** Time scale of
868 behavioural assessment. Mice were video-recorded in the behavioural apparatus for 24hrs.
869 Four intermittent time bins of 30 minutes each (i.e. a total of two hours) were manually
870 analysed offline for repetitive behaviours including self-grooming, head-body twitches and
871 hindpaw scratching. The scored time bins were distributed regularly across the light/dark
872 circadian following previous protocols (Welch et al., 2007).

873

874 **Figure 2. Sapap3^{-/-} mice express aberrant head/body twitches and scratching behaviours.**
875 **(A)** Sapap3^{-/-} mice execute a significant amount of head/body twitches, which are nearly
876 absent in wildtype mice (n = 9 mice per genotype; Mann-Whitney U, p < 0.01). **(B)** Sapap3^{-/-}
877 mice show a significant amount of hindpaw scratching compared to wildtype control mice (n
878 = 9 mice per genotype; Mann-Whitney U test, p < 0.01). **(C)** The duration of hindpaw scratching
879 is significantly elevated in Sapap3^{-/-} in comparison to wildtype mice (n = 9 mice per genotype;
880 Mann-Whitney U test, p < 0.001). **(D)** The number of head/body twitches and scratching bouts
881 correlate positively in both wildtype (Spearman correlation, p < 0.05) and Sapap3^{-/-} mice (n =
882 9 mice per genotype; Spearman correlation, p < 0.001). **(E)** Photographs of three individual
883 mice with representative lesions before, and two days or two weeks after hindpaw nail
884 clipping treatment. **(F)** Lesions, assessed through a lesion score ranging from no lesions (score
885 = 1) to severe lesions (score = 4), significantly improved already two days after clipping the
886 hindpaw claws (n = 17 Sapap3^{-/-} mice; Wilcoxon signed-rank test, paired, p < 0.001). **(G)** Lesions
887 are further improved two weeks after clipping the hindpaw claws as assessed through a
888 significantly lowered lesion score (n = 16 Sapap3^{-/-} mice; Wilcoxon signed-rank test, paired, p
889 < 0.001). Box plots illustrate the first and third quartiles; whiskers indicate the minimum and
890 the maximal value of each data set at no further than 1.5 interquartile range. The indicated
891 average is the median. Quartiles of Sapap3^{-/-} and wildtype mice are plotted in grey or white,

892 and individual data points in filled black and empty black dots, respectively. ** = $p < 0.01$, ***
893 = $p < 0.001$.

894

895 **Figure 3. Short, single-phase grooming events are more exaggerated than syntactic**
896 **grooming in Sapap3^{-/-} mice. (A)** Sapap3^{-/-} mice show significantly more grooming events
897 compared to wildtype controls (Mann-Whitney U test, $p < 0.001$). **(B)** Total grooming duration
898 is comparable between Sapap3^{-/-} and wildtype mice (Mann-Whitney U test, $p = ns$). **(C)** Self-
899 grooming behaviour of Sapap3^{-/-} mice compared to wildtype mice is characterised by a large
900 proportion of grooming events of short duration. The x-axis is depicted on a \log_{10} scale. **(D)**
901 Both short grooming events (< 3 second duration) as well as long grooming events (>3 second
902 duration) were significantly enhanced in Sapap3^{-/-} mice compared to wildtype controls (Mann-
903 Whitney U, $p < 0.001$ and $p < 0.01$, respectively). Self-grooming behaviour depended both on
904 genotype and bout length (ART ANOVA, $p_{\text{genotype} * \text{grooming type}} < 0.01$). All plots illustrate data
905 from $n = 9$ Sapap3^{-/-} and $n = 9$ wildtype mice; box whisker plots were designed as described in
906 legend of Figure 2. ** = $p < 0.01$; *** = $p < 0.001$; ns = non-significant.

907

908 **Figure 4. Excessive head/body twitches, scratching and short grooming events are**
909 **associated in Sapap3^{-/-} mice. (A)** The proportion of novel detected repetitive behaviours in
910 Sapap3^{-/-} mice outweighs previously reported syntactic self-grooming behaviour (Pearson's
911 Chi-squared test, $p < 0.0001$). **(B)** Head/body twitches positively correlate with short, single-
912 phase grooming but not long, syntactic grooming bouts in Sapap3^{-/-} mice (Spearman
913 correlation, $p < 0.05$, $p = ns$, respectively). **(C)** Scratching bouts also correlate positively with
914 short, single-phase grooming but not long, syntactic grooming bouts in Sapap3^{-/-} mice
915 (Spearman correlation, $p < 0.01$, $p = ns$, respectively). Correlation estimates are plotted in a

916 grey solid line or a dotted black line for wildtype or Sapap3^{-/-} mice (n = 9 animals per genotype),
917 respectively.

918

919 **Figure 5. Short grooming bouts, head/body twitches and scratching were reduced by**

920 **aripiprazole. (A)** Acute treatment with aripiprazole (1.5mg/kg) significantly reduced the

921 number of single-phase grooming, head/body twitches and scratching (Wilcoxon signed-rank

922 test: all $p < 0.01$; non-parametric, paired permutation test: all $p < 0.01$), but not the number

923 of syntactic grooming events (Wilcoxon signed-rank test: $p = 0.08$ and non-parametric, paired

924 permutation test: $p = 0.09$). Plotted are the proportions of number of RB events under

925 aripiprazole treatment and the sum of the number of RB events (vehicle + aripiprazole) of

926 individual mice. **(B)** Aripiprazole in particular shorter grooming events in Sapap3^{-/-} mice. The

927 x-axis is depicted on a \log_{10} scale. Box whisker plots were designed as described in the legend

928 of Figure 2. Vehicle and aripiprazole conditions are colour-coded in blue and red, respectively.

929 * = $p < 0.05$, ** = $p < 0.01$, ns = non-significant.

Figure 1

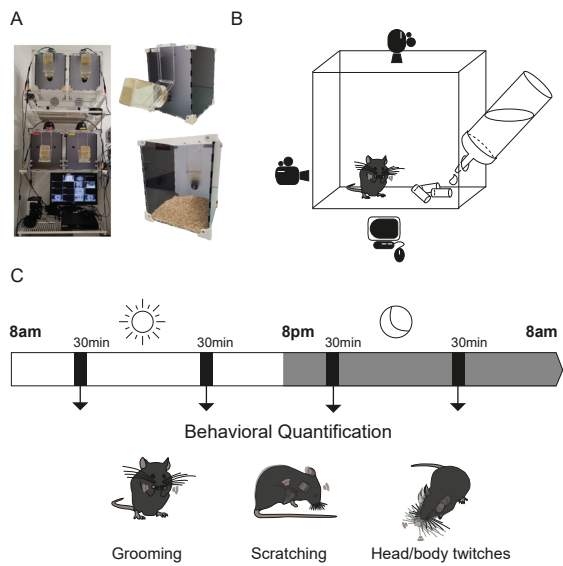


Figure 2

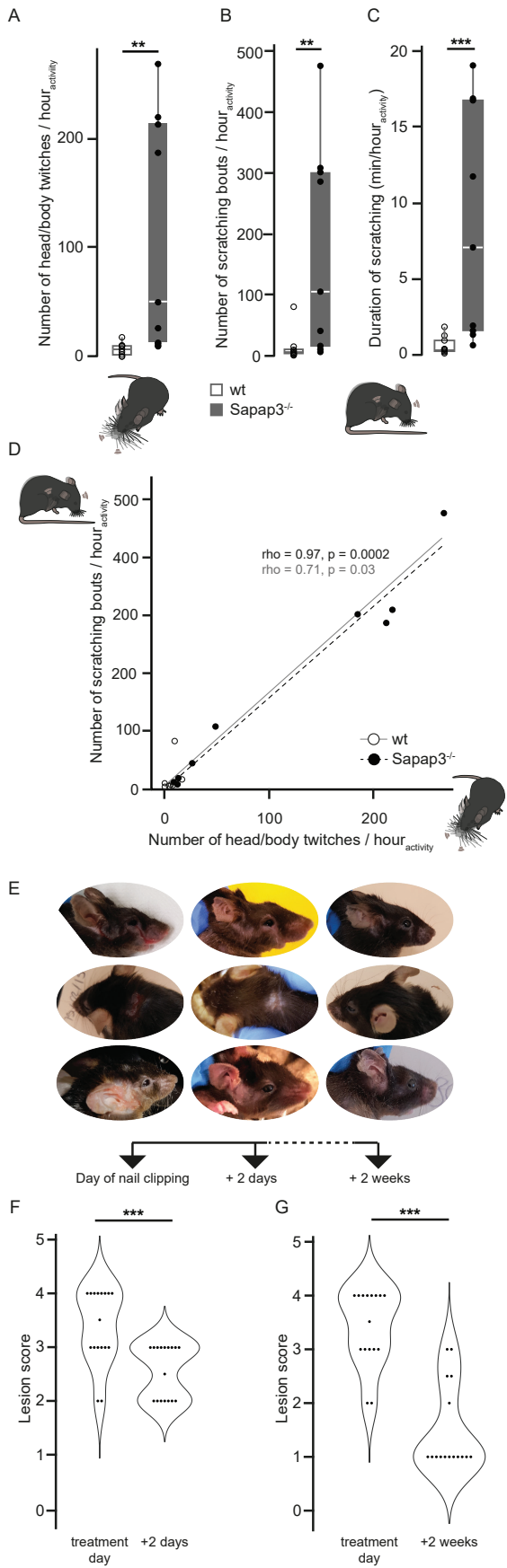


Figure 3

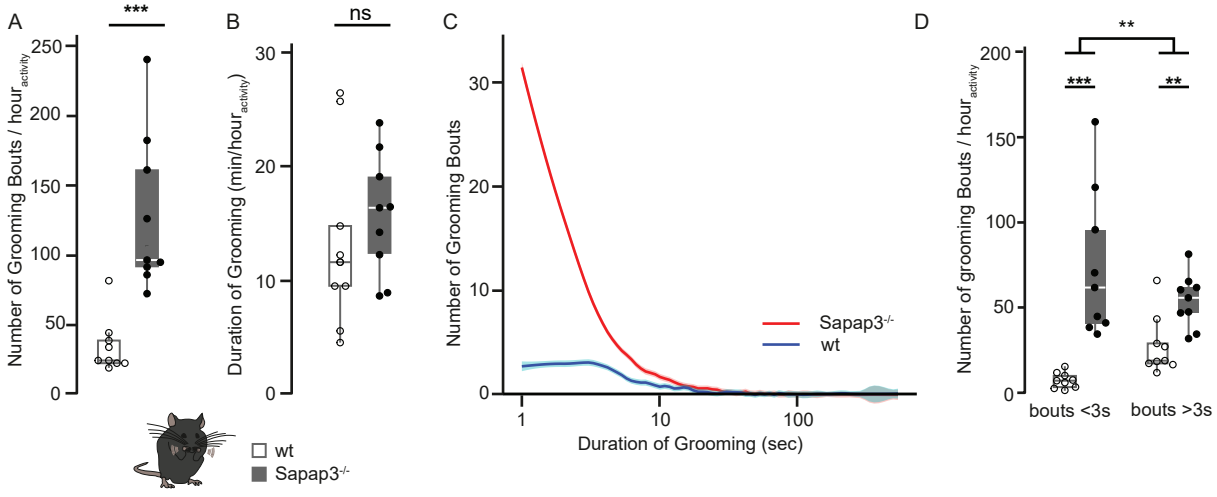
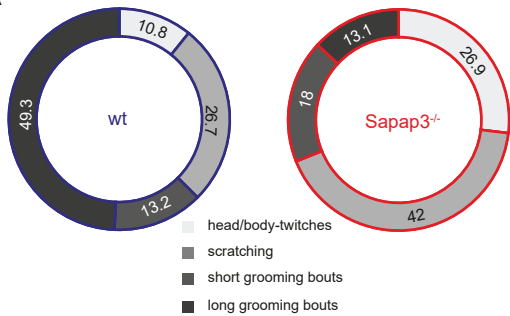
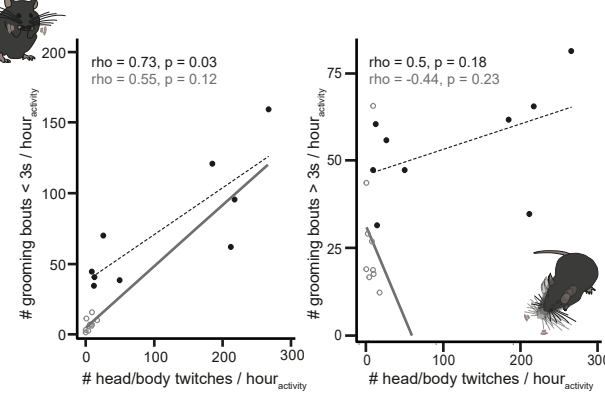


Figure 4

A



B



C

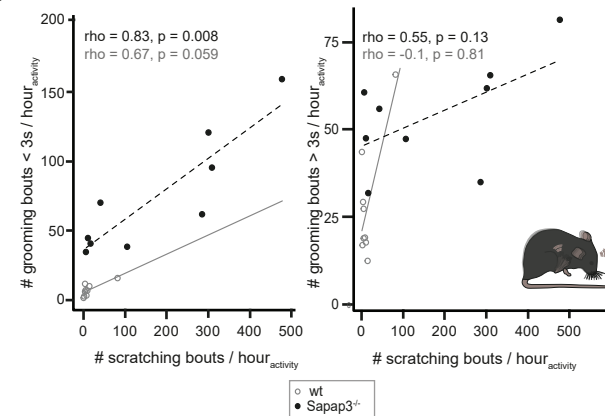


Figure 5

A

