Synaptic editing of frontostriatal circuitry prevents excessive grooming in SAPAP3-deficient mice

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Summary

Synaptic dysfunction has been implicated as a key mechanism underlying the pathophysiology of psychiatric disorders. Most pharmacological therapeutics for schizophrenia, autism spectrum disorder, obsessive-compulsive disorder, and major depressive disorder temporarily augment chemical synapse function. Nevertheless, medication non-compliance is a major clinical challenge, and behavioral dysfunction often returns following pharmacotherapeutic discontinuation. Here, we deployed a designer electrical synapse to edit a single class of chemical synapses in a genetic mouse model of obsessive-compulsive disorder (OCD). Editing these synapses in juvenile mice normalized circuit function and prevented the emergence of pathological repetitive behavior in adulthood. Thus, we establish precision circuit editing as a putative strategy for preventative psychotherapeutics.

Alterations to synapse function have been identified across many neuropsychiatric disorders[1-3]. Multiple monogenic mutations that cause synaptic dysfunction have been identified in humans with psychiatric disorders[4]. In other cases, rare monogenic mutations causally implicated in synaptic dysfunction have been identified across psychiatric disorders with shared behavioral phenotypes. For both instances, myriad studies have modeled these monogenic mutations in murine organisms[5,6]. One such example is the *Sapap3-/-* mouse model.

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Sapap3 is a postsynaptic scaffolding protein that is highly expressed in the cortex and striatum[7,8]. The protein is localized to the PSD of excitatory synapses and binds PSD95 and SHANK proteins to coordinate ion channels to the underlying cytoskeleton[9]. Due to this key structural role in excitatory synapses, deficits in Sapap3 yield altered synaptic structure and function[7]. Although the gene does not reach genome wide significance in human disorders, *Sapap3* dysfunction has been associated with neuropsychiatric disorders characterized by repetitive behavior including OCD, trichotillomania, Tourette syndrome, and autism spectrum disorder[9].

A plethora of studies have investigated brain connectivity differences in patients with OCD, indicating several regions of interest. Neuroimaging studies point to anatomical and functional differences in orbitofrontal cortex (OFC) and/or striatum (Str) in patients with OCD [10-12]. Indeed, functional connectivity studies in patients with OCD have revealed altered connectivity of OFC in subjects with OCD and their first-degree relatives, pointing to an underlying genetically mediated process[13] . Further studies have revealed altered functional connectivity between orbitofrontal-striatal regions [14-16], indicating the contributions of the OFC and Str towards behavioral disruption.

OCD is characterized by repetitive behaviors such as excessive hand washing. In some cases, this hand washing can be so severe that it yields skin lesions. Similarly, mice lacking *Sapap3* (*Sapap3* -/- mice) exhibit excessive facial grooming, yielding severe facial lesions compared to healthy littermate controls[7]. OFC and Str dysfunction have been implicated in this behavioral phenotype displayed by *Sapap3*-/- mice. *Sapap3*-/- mice exhibit increased inhibitory neuronal activity in the OFC, and optogenetic stimulation of neuronal activity in OFC or of OFC terminals within Str suppresses their pathological grooming[17]. Within striatum, virally mediated expression of Sapap3 during early development reduces grooming bouts, lesion severity, and anxiety-related behavior in adult *Sapap3*-/- mice[7], and optogenetic stimulation of Str parvalbumin-expressing (PV+) inhibitory neurons acutely suppresses their pathological grooming[18]. This latter stimulus response is enhanced if light stimulation is timed to LFP features in OFC that signal grooming onset[18].

While together, these studies implicate a circuit composed of OFC excitatory projection neurons and Str PV+ interneurons in the grooming phenotype displayed by *Sapap3*-/- mice, definitive evidence for such a pathophysiological mechanism remains lacking. Excitatory projection neurons from OFC form chemical synapses with striatal projection neurons (SPNs, either expressing D1 or D2 receptors) and PV+ interneurons of the dorsal striatum (Figure 1C; Supplemental Fig. S1)[19]. Thus, stimulating OFC terminals in Str drives all three of these subcircuits (OFC→Str D1+ SPN, OFC→Str D2+ SPN, and OFC→Str PV). Timing the stimulation of PV+ interneurons to OFC LFP features that signal grooming onset enhances the potency of this manipulation[18], but it has yet to be established that OFC is the only brain structure that can optimize the behavioral effect of Str PV+ interneuron stimulation. This is a particular concern since OFC LFP activity shows a high degree of spectral synchrony with other brain regions[20,21], and other regions also project to striatum and form synapses with Str PV+ interneurons. Thus, it could be argued that other circuits may also be central to the induction of pathological

89 grooming. Indeed, to directly test this question as to whether deficits in the OFC→Str PV+
90 circuit specifically mediate pathological grooming in the *Sapap3-/-* mice, one must selectively
91 target this circuit while leaving the OFC→Str D1+ SPN and OFC→Str D2+ SPN circuits unaltered.
92 Yet, tools that selectively modify the interaction between a pair of cell types remain scarce.
93
94 We have recently created a tool called Long-term Integration of circuits using Connexins (LinCx).
95 LinCx consists of two proteins, $Cx34.7_{M1}$ and $Cx35_{M1}$, which are designed to exclusively dock
96 with each other and in doing so, form a functional gap junction[22]. Each of the proteins can be
97 targeted to distinct cell membranes that are in close proximity to create an electrical synapse
98 between the pair of cells. Thus, LinCx augments the function of chemical synapses by creating
99 an adjacent electrical synapse. Here, we first targeted LinCx to the circuit involving OFC
100 projection neurons and striatal PV+ interneurons. We crossed PV-Cre mice with *Sapap3-/-*, to
101 generate *Sapap3-/-::PV-Cre* mice. We then generated connexin-T2A-fluorophore expressing
102 viruses such that an epitope-tagged connexin and soluble fluorophore would be produced (Fig.
103 1D). The $Cx34.7_{M1}$ construct was designed under the CaMKIIα promoter (AAV-CaMKII-$Cx34.7_{M1}$-
104 2xHA-T2A-mEmerald), such that it would be expressed in neurons that project to Str and most
105 other neuron types. The Cx35 construct was doubly floxed such that it would only express
106 protein in the presence of Cre recombinase (AAV-hSyn-DIO-$Cx35_{M1}$-FLAG-T2A-mCherry).
107
108 We first verified that adult *Sapap3-/-* mice exhibit excessive grooming and facial lesions (Fig. 1A-
109 B, U=36, p<0.001 for comparison of grooming time and U=18, p<0.001 for comparison of lesions
110 size between 22-week-old *Sapap3+/+* and *Sapap3-/-* mice using a two-tailed Mann-Whitney U
111 test; n=18/17). We then injected AAV-CaMKII-$Cx34.7_{M1}$-2xHA-T2A-mEmerald in OFC and the
112 AAV-DIO-$Cx35_{M1}$-FLAG-T2A-mCherry in Str of *Sapap3-/-::PV-Cre* mice (Fig. 1C-D); we will refer to
113 these mice as "LinCx-edited mice" moving forward. Control mice were injected with similar viral
114 constructs except for the replacement of LinCx proteins with control fluorophores, AAV-CaMKII-
115 EGFP in OFC and AAV-DIO-mCherry in Str. We also tested 4 additional control groups with other
116 unique patterns of $Cx34.7_{M1}$ or $Cx35_{M1}$ expression in OFC and/or Str (described further below in
117 the next paragraph). We were able to identify green processes (from $Cx34.7_{M1}$ expressing cells
118 in OFC) adjacent to red processes ($Cx35_{M1}$ expressing cells in Str; see Fig. 1E), indicating the
119 potential for each component of LinCx to be in close enough proximity to form functional gap
120 junctions. *Sapap3-/-::PV-Cre* mice injected with $Cx34.7_{M1}$ in OFC and DIO-$Cx35_{M1}$ in Str were
121 assessed for grooming behavior at 5, 10, and 15 weeks after viral infection ($F_{1.868, 145.7}$=30.88,
122 p<0.0001 for virus group × time effect for comparison across all 6 viral groups using mixed-
123 effects model ANOVA; Fig. 1F). These mice showed a significant decrease in grooming time at
124 both 5 and 15 weeks, compared to control mice (Dunn's multiple comparison test Z=2.845,
125 adjusted p-value= 0.0222 at 5 weeks; Z=1.773, adjusted p-value=0.3809 at 10 weeks, and
126 Z=2.760, adjusted p-value= 0.0289 at 15 weeks; Fig. 1F; see also Supplemental Fig. S2).
127 Furthermore, this manipulation markedly decreased both the lesion size and the emergence of
128 facial lesions (lesion size: H=28.21, p<0.0001 for comparison of lesion size across all 6 viral
129 groups using Kruskal-Wallis test; post-hoc Dunn's multiple comparison test between
130 fluorophore control and LinCx-edited mice: Z=3.807, adjusted p-value = 0.0007; Fig. 1G-H, see
131 row 1 and 2).
132

133 We then performed control experiments to verify that both halves of the gap junction were
134 needed for this suppression of excessive grooming. Here, mice were injected with either
135 CaMKII-Cx34.7$_{M1}$-2xHA-T2A-mEmerald into OFC or DIO-Cx35$_{M1}$-FLAG-T2A-mCherry into Str. In
136 comparison to the dual fluorophore control, there was no significant difference in grooming
137 time at 5, 10, or 15 weeks for either connexin independently (Dunn's multiple comparison test:
138 Z=0.6029, adjusted p-value=>0.9999 at 5 weeks; Z=0.3016, adjusted p-value=>0.9999 at 10
139 weeks; Z= 0.8154, adjusted p-value=>0.9999 at 15 weeks all for OFC-Cx34.7$_{M1}$-2xHA-T2A-
140 mEmerald. Dunn's multiple comparison test Z=0.5668, adjusted p-value=>0.9999 at 5 weeks;
141 Z=0.8961, adjusted p-value=>0.9999 at 10 weeks; Z=0.1408, adjusted p-value=>0.9999 at 15
142 weeks all for Str-DIO-Cx35$_{M1}$; Supplemental Fig. S2A). Similarly, compared to the control mice,
143 we observed no difference in lesion size when only one connexin was expressed (Dunn's
144 multiple comparison test Z=0.3660, adjusted p-value>0.9999 for OFC-CaMKII-Cx34.7$_{M1}$;
145 Z=0.6533, adjusted p-value >0.9999 for Str-DIO-Cx35$_{M1}$; Fig. 1G-H, see row 4 and 5). We also
146 tested whether selective expression of LinCx across multiple OFC-Str synaptic targets was
147 required to suppress excessive grooming. We created a Cx35$_{M1}$ virus that targeted multiple
148 types of neurons (AAV-CaMKII-Cx35$_{M1}$-FLAG-T2A-mCherry), and injected the Str of *Sapap3-/-*
149 *::PV-Cre* mice (see Supplemental Fig. S3). Mice were also injected with the CaMKII-Cx34.7$_{M1}$
150 virus in OFC. With this strategy, we observed no statistical differences in grooming time at 5-15
151 weeks, compared to the control mice which expressed only fluorophores (Dunn's multiple
152 comparison test Z=2.335, adjusted p-value=0.0978 at 5 weeks; Dunn's multiple comparison test
153 Z=0.9569, adjusted p-value=>0.9999 at 15 Week all for AAV-CaMKII-Cx35$_{M1}$; Supplemental Fig.
154 S2B). Similarly, we found no significant decreases in their lesion size after 15 weeks of viral
155 expression (Dunn's multiple comparison test Z=1.925, adjusted p-value=0.2713; Fig. 1G-H, see
156 row 1 and 3). Next, we developed a virus for which Cx35$_{M1}$ expression is suppressed by Cre-
157 recombinase negative (AAV-DO-Cx35$_{M1}$-FLAG-T2A-mCherry, Fig. 1D, see also Supplemental Fig.
158 S4 and methods). Again, we injected *Sapap3-/-::PV-Cre* mice with CaMKII-Cx34.7$_{M1}$ in OFC and
159 AAV-DO-Cx35$_{M1}$ in Str. We observed significantly lower grooming at 5 weeks, compared to mice
160 that expressed only the fluorophores; however, no statistical differences were observed at 10 or
161 15 weeks (Dunn's multiple comparisons test p=0.0228 at 5 weeks; Z=1.941, adjusted p=0.2610
162 at 10 weeks; Z=0.3811, adjusted p=>0.9999 at 15 weeks all for AAV-DO-Cx35$_{M1}$ in Str;
163 Supplemental Fig. S2B). Similarly, this LinCx targeting approach had no impact on the size of
164 their facial lesions, compared to control mice that expressed both fluorophores (Dunn's multiple
165 comparisons test Z=1.429, p=0.7646; Fig. 1G-H, see row 1 and 6). Thus, our results indicated
166 that LinCx expression across the OFC→Str synapses had to be targeted to PV+ interneurons to
167 prevent pathological grooming behavior.
168
169 To test whether other Str neurons could also prevent this pathological behavior, we examined
170 the impact of LinCx expression across OFC-striatal SPN synapses. We generated two mouse
171 lines, enabling us to independently target D1- or D2-expressing SPNs in the knockouts: *Sapap3-*
172 */-::D1-Cre* and *Sapap3-/-::A2A-Cre* mice. We then injected CaMKII-Cx34.7$_{M1}$ into OFC and DIO-
173 Cx35$_{M1}$ into Str of each line (Fig. 1I and 1M). Control mice were injected with CaMKII-EGFP into
174 OFC and DIO-mCherry into Str. No significant difference in grooming time was found between
175 fluorophore-only and LinCx-edited mice for either line ($F_{2,35}$ = 0.09477; p=0.9098 for virus group
176 × time effect; $F_{1,19}$=0.03, p=0.8646 for *Sapap3-/-::D1-Cre* mice using mixed-effects model

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177 ANOVA. $F_{2,36}$ = 0.1323; p=0.8765 for virus group × time effect; $F_{1,18}$=0.0267, p=0.8731 for
178 *Sapap3-/-::A2A-Cre* mice using mixed-effects model ANOVA, see Fig. 1J and 1N). Similarly, we
179 observed no difference in facial lesion size 15 weeks after viral injection (U=54, p=0.95 for
180 *Sapap3-/-::D1-Cre* mice; U=48, p=0.95 for *Sapap3-/-::A2A-Cre* mice using two-tailed Mann-
181 Whitney U test; Fig. 1K-L and Fig 1O-P). Notably, independent of LinCx expression, we observed
182 a strain effect on *Sapap3 -/-* lesion size. Lesion size for the *Sapap3-/-::D1-Cre* and *Sapap3-/-*
183 *::A2A-Cre* lines were smaller than observed in *Sapap3-/-::PV-Cre* mice (post-hoc Kruskal-Wallis
184 test H=9.802, p=0.0074 for comparison of lesion size across the *Sapap3-/-* fluorophore control
185 groups for the 3 Cre lines), potentially due to a higher degree of backcrossing in the *Sapap3-/-*
186 *::PV-Cre* line. Nevertheless, together, these results showed that only selective expression of
187 LinCx across the OFC→Str PV+ synapses was sufficient to prevent pathological grooming
188 behavior.
189
190 We next tested whether LinCx editing of the OFC→Str PV+ circuit impacted neuronal activity in
191 Str (Fig. 2A-B). We first characterized the impact of Sapap3 deletion using *Sapap3+/+* and
192 *Sapap3-/-* mice. Mice were chronically implanted with microwires targeting OFC and a 1024-
193 channel silicon probe targeting Str (Fig. 2B). After 2 weeks of recovery, OFC local field potential
194 (LFP) activity and Str single-unit activity were obtained concurrently in awake freely behaving
195 mice (Fig. 2C). We classified Str single unit activity based on their waveforms. Single units with a
196 spike half-width between 130 and 250μs and mean firing rates less than 5 Hz were classified as
197 SPNs (Fig. 2D, left). Single units with a spike half-width between 70 and 120 were classified as
198 narrow spiking units (NSUs, Fig. 2D, right). When we compared *Sapap3+/+* and *Sapap3-/-* mice,
199 we found that the *Sapap3-/-* mice exhibited a lower proportion of NSUs ($\chi 2$ = 20.64, df=2,
200 p<0.01 using Chi-square test, Fig. 2E). In contrast to prior work[17], we also found that the firing
201 rate of SPNs was lower in the *Sapap3-/-* mice compared to controls (U=16482, approx. p=0.0057
202 using two-tailed Mann-Whitney U test, Fig. 2G, left). We observed no differences in the firing
203 rate of the NSUs between the groups (U=2886, approx. p=0.2598 using two-tailed Mann-
204 Whitney U test, Fig. 2G, right). Since PV+ interneurons exhibit spike waveforms consistent with
205 NSUs[17], we also tested whether the lower proportion of NSUs observed in *Sapap3-/-* mice was
206 due to a loss of PV+ interneurons. When we quantified PV-expressing cell density by
207 immunofluorescence (Fig. 2I), we found no significant difference in PV-expressing cell density
208 between *Sapap3+/+* and *Sapap3-/-* mice (Unpaired t-test, t=0.4289, df=9, p=0.68, n=5/6, Fig. 2J,
209 left).
210
211 After characterizing the impact of *Sapap3* deletion on Str single-unit firing rates, we tested how
212 LinCx editing of the OFC→Str PV+ circuit impacted cellular firing. *Sapap3-/-::PV-Cre* were virally
213 infected with Cx34.7$_{M1}$ and Cx35$_{M1}$ at 7-8 weeks. Control mice were infected with fluorophores.
214 Mice were then implanted with microwires targeting OFC and a silicon probe targeting Str 15-20
215 weeks later (Fig. 2A-B). Again, we recorded neural activity following surgical recovery, and Str
216 single-unit activity was characterized based on the spike waveforms (Fig. 2C-D). Strikingly, LinCx
217 OFC→Str PV+ circuit expression increased the proportion of NSUs observed in the *Sapap3-/-*
218 *::PV-Cre* mice relative to controls ($\chi 2$ = 24.71, df=2, p<0.01 using Chi-square test, Fig. 2F). LinCx
219 OFC→Str PV+ circuit expression also decreased the firing rate of SPNs but had no impact on the
220 firing rate of NSU between the groups (SPN: U=8925, approx. p=0.0142; NSU: U=233.5,

221 p=0.3068, using two-tailed Mann-Whitney U test; Fig. 2H). No group differences were observed
222 in the Str PV+ cell density (Unpaired t-test, t=1.778, df=7, p=0.12, n=5/4, Fig. 2J, right). Thus,
223 *Sapap3* deletion suppressed Str SPN activity and LinCx OFC→Str PV+ circuit expression
224 suppressed it further. On the other hand, LinCx expression in the OFC→Str PV+ circuit restored
225 the activity of NSUs that were otherwise silenced by *Sapap3* deletion.
226
227 As LinCx functions by increasing the electrical coupling of neurons, we next wanted to
228 investigate how LinCx editing of the OFC→Str PV+ circuit impacted the entrainment of Str
229 neurons to OFC oscillations. We observed a peak in OFC LFP oscillatory power between 1-5 Hz
230 (Fig. 3A, top); thus, we filtered the LFPs to isolate activity in this frequency range (Fig. 3A,
231 bottom). We then extracted the phase of the filtered OFC LFPs and quantified the relationship
232 between single unit activity at this phasic activity (Fig. 3B-C). Finally, we introduced temporal
233 offsets ranging between -200ms and 200ms between the single-unit activity and the LFP phase,
234 enabling us to also quantify the relationship between Str single unit activity and OFC activity in
235 the immediate past and immediate future[23,24]. Indeed, we found that when we pooled SPN and
236 NSU single units in *Sapap3+/+* mice, Str neurons optimally coupled to OFC activity in the
237 immediate past or immediate future (Fig. 3D). In contrast, *Sapap3* deletion increased coupling
238 to OFC 1-5Hz activity across all temporal offsets (Fig. 3E). Interestingly, when we limited our
239 analysis solely to SPNs, we still observed increased coupling to OFC oscillations ranging from -
240 200ms in the past to 40ms in the future in the *Sapap3-/-* mice (Supplemental Fig. S5A). Thus,
241 the increased coupling observed in the *Sapap3-/-* mice did not simply reflect the decreased
242 portion of NSUs represented by their population activity. Finally, we repeated this analysis in the
243 *Sapap3-/-::PV-Cre* that had been treated with LinCx editing of the OFC→Str PV+ circuit, as well
244 as control mice which solely expressed fluorophores. Strikingly, LinCx editing decreased the
245 coupling of Str neuronal activity to OFC activity in the immediate past and immediate future
246 (Fig. 3F). Similar results were observed when we limited our analysis to SPNs (Supplemental Fig.
247 S5B). Thus, *Sapap3* deletion increased the coupling of SPNs to OFC activity, while LinCx editing
248 of the OFC→Str PV+ circuit decreased this coupling in a temporally selective manner. Together,
249 these findings established the OFC→Str PV+ circuit in mediating pathological grooming in the
250 *Sapap3-/-* mice.
251
252
253 **Discussion**
254
255 Here we set out to test whether circuit-selective assembly of an electrical synapse could be
256 utilized to edit a genetically mediated chemical synaptic disruption and restore normal behavior.
257 Using the *Sapap3-/-* mutant mouse model, we found that LinCx editing of the OFC→Str PV+
258 circuit corrected spike timing between OFC and Str, and it ameliorated the mutant animals'
259 pathological repetitive behavior. Such a behavioral outcome was observed when the striatal
260 portion of LinCx synapse was restricted to PV+ interneurons, highlighting the specificity of the
261 editing approach (Fig. 4). Previous *in vivo* work has implicated hyperactivity of striatal SPNs in
262 mediating the repetitive grooming displayed by *Sapap3-/-* mice[17]. On the contrary, here we
263 observed a decreased SPN firing in *Sapap3-/-* mice compared to controls. Our findings were
264 consistent with the reduced field excitatory postsynaptic potentials described in *Sapap3-/-*

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265 mice[7]. Prior *in vivo* work also implicated hypoactivity of striatal PV+ interneuron activity[17].
266 Though we observed a lower portion of NSUs in *Sapap3-/-* mice using our high-density striatal
267 recordings, *Sapap3-/-* mice did not exhibit a lower NSU firing rate. Interestingly, LinCx editing
268 further lowered the firing rate of striatal SPNs and had no impact on the firing rate of striatal
269 NSUs. Rather, LinCx editing increased the proportion of NSUs we observed in *the Sapap3-/-::PV-*
270 *Cre* mice. Since we observed a similar density of PV+ interneurons compared to controls in our
271 histological analysis, our findings suggest that an overall physiological silencing of Str NSUs may
272 be central to the repetitive grooming phenotype that results from *Sapap3* deletion. Striatal
273 SPNs also showed increased coupling to LFP activity in OFC in the *Sapap3-/-* mice. Such coupling
274 likely reflects a loss of feedforward inhibition in the OFC→Str circuit due to the silencing of
275 NSUs within central Str. LinCx editing of the OFC→Str PV+ circuit both ameliorated the
276 pathological grooming behavior and reduced the increased coupling in the *Sapap3* mutant
277 mice. These findings raise the possibility that altered SPN spike timing across the OFC→Str
278 circuit, rather than simply changes in Str SPN firing rates alone may serve as a core pathological
279 mechanism underlying the repetitive behavioral phenotype in *Sapap3-/-* mice.
280
281 OCD impacts 1-3% of the global population[25]. Yet, standard treatments including cognitive
282 behavioral therapy and SSRI-based pharmacotherapy are only effective in a subset of individuals
283 with OCD[26-28]. Furthermore, these pharmacotherapeutics require chronic administration, with
284 symptoms recurring in the setting of medication non-compliance. Highlighting the predictive
285 validity of the *Sapap3-/-* mouse model for studying OCD, chronic SSRI-based treatment also
286 ameliorates this model's repetitive behavior[7]. Interestingly, anxiety is highly co-morbid with
287 OCD, and it has been reported that *Sapap3-/-* mice have exhibited anxiety-like behavior[7].
288 Further supporting the predictive validity of the *Sapap3-/-* model, their anxiety state is also
289 reduced by chronic SSRI treatment[7]. While we also confirmed an anxiety-like phenotype in
290 *Sapap3-/-* mice (Supplemental Fig. S6), we found that editing of OFC→Str PV+ circuitry had no
291 impact on this behavioral phenotype. Thus, though LinCx precision editing of the OFC→Str PV+
292 circuit yielded amelioration of repetitive behavior, it had no impact on another major psychiatric
293 phenotype of the model. Overall, this finding suggests that the therapeutic impact of chronic
294 SSRI-based treatment on anxiety in OCD may be mediated at other distinct circuits in the brain,
295 either via its direct effects on transporters or via secondary effects whereby released serotonin
296 may interact directly with DNA in a manner that subsequently changes gene expression[29].
297 Future studies in which LinCx is used to edit other chemical synapses in the *Sapap3-/-* brain may
298 help to clarify whether other circuits can be targeted to potentially reduce or prevent the
299 development of anxiety.
300
301 Notably, virally mediated expression of the Sapap3 protein in juvenile *Sapap3-/-* mice prevents
302 the emergence of repetitive grooming and anxiety-like behavior in adulthood[7]. This directly
303 argues that deficient striatal Sapap3 mediates the behavioral dysfunction displayed by *Sapap3-*
304 */-* mice. Moreover, this prior work broadly implicates striatal-dependent circuits in mediating
305 repetitive behavior and anxiety-like phenotypes in OCD. Yet this approach of expressing Sapap3
306 in human Str during early development is highly unlikely to translate to a clinically viable
307 therapeutic, since most individuals with OCD do not have a genetically mediated disruption of
308 Sapap3. Rather, an approach that targets a convergent circuit-based phenotype shared across

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309   the many different genetic mediators of OCD bears greater potential for preventative therapy.
310   Here, we demonstrate that precision circuit editing using an electrical synapse can override a
311   genetically mediated synaptic deficit and prevent the emergence of behavioral disruption
312   relevant for OCD and other neuropsychiatric disorders in adulthood. Future work will be
313   necessary to test whether such a circuit-editing framework extends to models of other genetic
314   lesions linked to OCD, and to models of other psychiatric disorders.
315
316
317   **Figure Legends**
318
319   **Figure 1. LinCx expression in the OFC→Str-PV interneuron circuit selectively reduces**
320   **pathological grooming in *Sapap3-/-* mice. A)** Quantification of grooming in *Sapap3-/-* mice and
321   their littermate controls (*$p<0.001$ using Mann-Whitney test). **B)** Facial lesion size in *Sapap3-/-*
322   and littermate controls at age 22 weeks (left, *$p<0.001$ using Mann-Whitney test). The
323   percentage of mice with facial lesions is shown in the center, and representative images of
324   *Sapap3+/+* and *Sapap3-/-* mice are shown to the right. **C)** Schematic of targeted OFC→Str
325   circuitry involving excitatory pyramidal neuron synapses onto each of 3 main cell types in Str:
326   D1+ SPNs, D2+ SPNs, and PV+ interneurons. Within Str, PV+ interneurons synapse onto D1 and
327   D2 SPNs and each other (left). Schematic of intended heteromeric gap junction. Green
328   represents mEmerald/EGFP, while red represents mCherry. The Cx34.7$_{M1}$ (light grey)
329   hemichannel and mEmerald are expressed in OFC projection neurons. Striatal neurons express
330   the reciprocal hemichannel, Cx35$_{M1}$ (dark grey) and mCherry. Together, the Cx34.7$_{M1}$ and
331   Cx35$_{M1}$ constitute a heterotypic gap junction, creating a connection between the intracellular
332   space of OFC projection neurons and striatal neurons (right). **D)** Diagram of AAV9 viral
333   constructs used to target OFC and Str. **E)** Diagram of viral manipulation strategy into OFC and Str
334   in PV-Cre mice. AAV-Cx34.7$_{M1}$ or AAV9-GFP was injected into OFC bilaterally. Additionally, AAV-
335   DIO-Cx35$_{M1}$, AAV-DO-Cx35$_{M1}$, or AAV-DIO-mCherry was injected into Str bilaterally (left).
336   Representative image of LinCx viral expression. Green fluorescence indicates AAV-CamK2-
337   Cx34.7$_{M1}$-T2A-mEmerald expression in OFC soma, and in OFC terminals in Str. Red fluorescence
338   indicates Str soma that express AAV-hSyn-DIO-Cx35$_{M1}$-T2A-mCherry (center). A confocal
339   rendering of OFC (green) projections contacting PV+ interneurons (red) in Str in a *Sapap3-/-::PV-*
340   *Cre* mouse is also shown (right- grey box enlarged on bottom right). **F)** Quantification of
341   grooming in *Sapap3-/-::PV-Cre* mice following OFC-STR PV+ circuit editing (*$p<0.05$ using mixed
342   effects model ANOVA followed by Dunn's correction; note that other control groups are shown
343   in Supplemental Fig. S2). Data shown as mean ± s.e.m. **G)** Quantification of facial lesions in
344   *Sapap3-/-::PV-Cre* mice 15 weeks post-viral manipulation (*$p<0.0001$ using Kruskal-Wallis test).
345   The middle "LinCx" column indicates which rows are tests of LinCx manipulations (+) vs.
346   hemichannel or fluorophore-only control groups (-). **H)** The percentage of *Sapap3-/-::PV-Cre*
347   mice with facial lesions is shown for each group (left), and representative images of a LinCx-
348   edited *Sapap3-/-::PV-Cre* mouse and a non-edited control mouse are shown (right). **I)** Viral
349   injection strategy into OFC and striatum of *Sapap3-/-::D1-Cre* mice. Representative image of
350   LinCx viral expression (center) and confocal rendering of OFC (green) projections contacting D1-
351   expressing striatal projection neurons (red) in Str in a *Sapap3-/-::D1-Cre* mouse (right- grey box
352   enlarged on bottom right). **J)** Quantification of grooming p=0.9098 for viral group x time effect,

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353 p=0.8635 for viral group effect, and p=0.0012 for time effect using mixed effects model; data
354 shown as mean ± s.e.m.) and **K)** facial lesions in viral-manipulated mice (p=0.95 using Mann-
355 Whitney test). **L)** The percentage of *Sapap3-/-::D1-Cre* mice with facial lesions is shown for each
356 group (left), and representative images of a LinCx-edited mouse and a non-edited control
357 mouse are shown (right). **M)** Viral injection strategy into OFC and Str of *Sapap3-/-::A2A-Cre*
358 mice. Representative image of LinCx viral expression (center) and confocal rendering of OFC
359 (green) projections contacting A2A-expressing SPNs (red) in Str in a *Sapap3-/-::A2A-Cre* mouse
360 (right- grey box enlarged on bottom right). **N)** Quantification of grooming (P=0.8765 for viral
361 group x time effect using mixed effects model; data shown as mean ± s.e.m.) and **O)** facial
362 lesions in viral-manipulated mice (p=0.95 using Mann Whitney test). **P)** The percentage of
363 *Sapap3-/-::A2A-Cre* mice with facial lesions is shown for each group (left), and representative
364 images of a LinCx-edited mouse and a non-edited control mouse are shown (right). For box and
365 whisker plots, the central mark is the median, the edges of the box are the 25th and 75th
366 percentiles, the whiskers extend to the most extreme datapoints the algorithm considers to be
367 not outliers, and the outliers are plotted individually as a "+".
368
369
370 **Figure 2: OFC→Str-PV circuit editing recovers striatal narrow spiking unit activity in *Sapap3-/-***
371 **mice. A)** Viral target strategy for OFC→Str PV+ circuit. **B)** Schematic and representative image of
372 OFC electrode targeting (left). Green fluorescence indicates cells expressing Cx34.7$_{M1}$-T2A-
373 mEmerald. Schematic and representative image of silicon probe targeting Str (right). Green
374 fluorescence indicates terminals from OFC expressing Cx34.7$_{M1}$-T2A-mEmerald. Red
375 fluorescence indicates PV+ interneuron soma that expresses AAV-hSyn-DIO-Cx35$_{M1}$-T2A-
376 mCherry. **C)** Representative OFC LFPs and raster plot depicting activity of 302 single units
377 recorded concurrently from central Str. **D)** Waveforms distribution for putative striatal
378 projection neurons (SPNs, spike half-with between 130 and 250μs, and mean firing rate < 5Hz,
379 left) and narrow spiking units (NSU, spike half-with between 70 and 120μs, right). **E)** Portion of
380 cells characterized as SPNs or NSUs in *Sapap3-/-* vs. *Sapap3+/+* mice (p<0.05 using Chi-square
381 test). **F)** Portion of cells characterized as SPNs or NSUs in LinCx edited *Sapap3-/-::PV-Cre* mice
382 vs. non-edited EGFP/mCherry fluorophore-only controls (p<0.05 using Chi-square test). **G-H)**
383 Mean firing rates of SPNs and NSUs in G) *Sapap3-/-* vs. *Sapap3+/+* mice, and H) LinCx edited
384 *Sapap3-/-::PV-Cre* mice vs. non-edited controls (*p<0.05 using two wailed Mann-Whitney U
385 test). The purple dashed line indicates the firing threshold classically utilized to characterize
386 putative PV+ interneurons. **I)** Representative images of immunohistochemistry against PV in
387 *Sapap3+/+* and *Sapap3-/-*. Red boxes indicate region-enlarged insets to the right. **J)**
388 Quantification of PV cell density in Str based on immunohistochemistry (left: t=0.4289, df=9,
389 p=0.68 using a two-tailed unpaired t-test; right: t=1.1778, df=7, p=0.12 using a two-tailed
390 unpaired t-test). For box and whisker plots, the central mark is the median, the edges of the box
391 are the 25th and 75th percentiles, the whiskers extend to the most extreme datapoints the
392 algorithm considers to be not outliers, and the outliers are plotted individually as a "+".
393
394
395 **Figure 3: LinCx circuit editing moderates OFC→Str spike timing deficits in *Sapap3-/-* mice. A)**
396 Representative power spectral density for OFC LFPs recorded from a *Sapap3+/+* mouse. Note

9

397 the peak in activity between 1 and 5 Hz (top). Representative OFC LFP with the 1-5 Hz filtered LFP
398 signal shown in red (bottom). **B)** Plot showing a 1-5 Hz filtered OFC LFP (top), the extracted
399 oscillatory phase (middle), and a concurrently recorded Str single unit (bottom). **C)** The firing of
400 the same Str unit relative to the phase of the OFC 1-5 Hz oscillation. **D)** Phase locking of Str units
401 recorded in *Sapap3+/+* mice. Heatmap depicts the mean resultant length (MRL) of the 83/433
402 units that phase-locked to OFC oscillations with temporal shifts ranging from -200 to 200ms.
403 The black line highlights the optimal temporal offset for each phase-locked cell. The histogram
404 above depicts the distribution of peak temporal offsets for the 83 neurons. Significance was
405 determined using the Rayleigh test at $\alpha=0.05/21$ temporal shifts. **E-F)** Coupling of pooled Str
406 SPN and NSU units to OFC 1-5 Hz oscillations at temporal offset ranging from -200 to 200ms. E)
407 Str phase-coupling in *Sapap3-/-* vs. *Sapap3+/+* mice and F) LinCx-edited *Sapap3-/-::PV-Cre* mice
408 vs. non-edited controls. The dashed blue line highlights offsets at which significant differences
409 were observed between groups using a Mann-Whitney U test with a false discovery rate
410 correction for comparisons across the 21 offsets. Data shown as mean ± s.e.m.
411
412 **Figure 4: Summary of LinCx precision circuit editing manipulations and corresponding effects**
413 **on repetitive behavior in *Sapap3* deficient mice.** Expression of Cx34.7$_{M1}$ (green cylinder) and
414 Cx35$_{M1}$ (red cylinder) in cell type-specific OFC$\rightarrow$Str circuits, with arrows indicating alterations (if
415 any) to grooming and lesion formation.
416
417
434
435 **Author contributions**

436 Conceptualization and Methodology – K.W.C., N.C., and K.D.; Formal Analysis – K.W.C., H.A.S.,
437 and K.D.; Investigation – K.W.C., and H.A.S., K.D.; Resources – N.C., and K.D.; Writing – Original
438 Draft – K.W.C., and K.D.; Writing – Review & Editing – K.W.C, N.C., and K.D.; Visualization – K.W.C,

439 and K.D.; Supervision – K.W.C, and K.D.; Project Administration and Funding Acquisition – K.W.C,
440 N.C., and K.D.;
441
442 See Supplemental materials for detailed author contributions.
443
444

445 **Declaration of Interests**

446 The authors declare no competing interests.

447
448
449 **Methods:**

450 **Animal Care and Use**

451 All experimental procedures were performed in compliance with approved protocols from the
452 Duke University Institution of Animal Care and Use Committee at Duke University. *Sapap3-/-*
453 mice and littermate controls were bred from heterozygous breeding pairs. *Sapap3-/-*::Cre
454 recombinase mice were generated by crossing *Sapap3-/-* and *+/-* mice to respective Cre+ mice:
455 PV+ interneurons targeted using B6 Pvalb-IRES-Cre (Jackson Laboratory #017320); D1+ striatal
456 projection neurons targeted using D1-Cre (Jackson Laboratory #037156); and D2+ striatal
457 projection neurons targeted using Adora2a-Cre (gift from Dr. Henry Yin; MMRC 34744). *Sapap3-*
458 */-*::Cre+ mice were crossed together to generate *Sapap3-/-*::Cre+ offspring used for
459 experiments. Mice were genotyped prior to weaning using standard protocols for Cre and
460 Sapap3-(Transnetyx). 2-5 mice were housed together by sex and age. Mice were given
461 unrestricted access to food and water and were maintained on a 12-hour light cycle. All
462 experiments were conducted during the light-on phase of their light cycle. Humane endpoints
463 were defined as follows: animals that exhibited a 20% weight loss over a week, active bleeding
464 for more than 5 minutes, or bilateral lesions over 5mm in size. 3 mice from the *Sapap3-/-*::*PV-*
465 *Cre* mice virally transduced with $Cx34.7_{M1}$-only group met criteria for euthanasia prior to the
466 completion of the 15-week behavioral experimental protocol.

467 **Molecular Cloning and Viral Production**

468 pAAV-CaMKIIa-$Cx34.7_{M1}$-2xHA-T2A-mEmerald was generated by producing a gBlock with
469 $Cx34.7_{M1}$-2xHA-T2A-mEmerald (Integrated DNA Technologies) and ligating together with BamHI-
470 HF and EcoRI-HF digested pAAV-CaMKIIa-EGFP (Addgene # 50469, a gift from Bryan Roth).
471 Similarly, pAAV-CaMKIIa-$Cx35_{M1}$-FLAG-T2A-mCherry was generated likewise but using a gblock
472 containing $Cx35_{M1}$-FLAG-T2A-mCherry. pAAV-hSyn-DO-$Cx35_{M1}$-FLAG-T2A-mCherry(off)-
473 iRFP670(on)-WPRE was generated by amplifying $Cx35_{M1}$-FLAG-T2A-mCherry using AscI and NheI
474 and iRFP670 with NheI and ligating step-wise into pAAV-hSyn-DIO-mCAR(off)-CheTA(on)-WPRE
475 (Addgene # 11387, a gift from Adam Kepecs[30]).

476   Phusion using GC Phusion buffer under standard conditions was used to amplify DNA
477   fragments. All restriction enzymes were from NEB. Ligations were completed with standard In-
478   Fusion protocol (Takara Bio). Plasmids were transformed into Stbl3 competent *E. coli*
479   (ThermoFisher). Final vectors were full length sequenced prior to viral production (Eton Bio).

480   All Cx viruses were AAV2/9 serotype and produced at the Duke Viral Vector Core (graded for *in*
481   *vivo* use).

482

483   **Surgery**

484   Viral Manipulation Surgery

485   Seven-week-old mice were anesthetized with isoflurane (1%) and then secured in a stereotaxic
486   device. A blunt end 33-gauge Hamilton syringe was used to deliver 100nL of AAV9-CaMKII-
487   Cx34.7$_{M1}$-T2A-mEmerald (5 x 10^{13} vg/ml) or AAV9-CaMKII-EGFP (Addgene viral prep # 50469-
488   AAV9, titer = 2.8 x 10^{13} vg/ml, a gift from Bryan Roth) bilaterally, into orbital frontal cortex (OFC;
489   +2.6mm AP and ±2.0mm ML from bregma, and -1.49DV from brain surface at a 10° angle) and
490   250nL AAV9-hSyn-DIO-Cx35$_{M1}$-T2A-mCherry (1.26 x 10^{13} vg/ml)[22], AAV9-hSyn-DIO-mCherry
491   (Addgene viral prep # 50459-AAV9, titer = 2.3 x 10^{13} vg/ml, a gift from Bryan Roth), AAV9-
492   CaMKII-Cx35$_{M1}$-T2A-mCherry (6.24 X 10^{13} vg/ml), or AAV9-hSyn-DO-Cx35$_{M1}$-T2A-mCherry (3.5 x
493   10^{12} vg/ml) into striatum (Str; +0.3mm AP and ±2.35mm ML from bregma, and -2.35mm DV
494   from brain surface at a 10° angle). Virus was delivered at a rate of 100nL/min using a
495   microinjection syringe pump (WPI Micro2T SMARTouch with UMP3 pumps). Carprofen (5mg/kg,
496   s.c.) injections were administered once prior to surgery and again every 24 hours for three days
497   following viral injection surgery.

498

499   Viral Tracing Surgery

500   At 22 weeks, mice were anesthetized with isoflurane (1%) and secured in a stereotaxic device. A
501   blunt end 33-gauge Hamilton syringe was used to deliver 250nL of AAV8-DIO-hSyn-TC66T-2A-
502   mRuby3-2A-OG (2.16 x 10^{10} vg/ml, UCI Center for Neural Circuit Mapping Viral Core) into Str
503   (+0.3mm AP and ±2.35mm ML from bregma, and -2.35mm DV from brain surface at a 10° angle)
504   using a microinjection syringe pump (WPI Micro2T SMARTouch with UMP3 pumps) at a rate of
505   100nL/min. After 21 days, mice were then injected with 250nL EnvA-SAD-B19-RVdG-
506   mNeonGreen (5 x 10^7 vg/mL, UCI Center for Neural Circuit Mapping Viral Core) as with the same
507   conditions and location as the AAV8 delivery method previously reported [31]. Carprofen (5mg/kg,
508   s.c.) injections were administered once prior to each surgery and again every 24 hours for three
509   days following each viral injection surgery.

510

511   Electrode Implantation and Opsin Viral Surgery

512   After 15 weeks post-viral injection, a subset of mice was anesthetized with isoflurane (1%),
513   placed in a stereotaxic device, and metal ground screws were secured to the anterior cranium
514   and posterior cortex. A single ground wire was connected to the front screw and to one of the

12

515 posterior screws. As before, viral injections were performed using a blunt end 33-gauge
516 Hamilton syringe to deliver 150nL of AAV1-CAG-fDIO-ChR2-mCherry (Addgene viral prep #
517 75470-AAV1, titer = 2.3 x 10^{13} vg/ml, a gift from Connie Cepko[32]) unilaterally into OFC (+2.6mm
518 AP and -2.0mm ML from bregma, and -1.49mm DV from brain surface at a 10° angle) and 250nL
519 rAAV-EF1a-FLPo (Addgene viral prep # 55637-AAVrg, titer = 1.7 x 10^{13} vg/ml, a gift from Karl
520 Deisseroth[33]) unilaterally into Str (+0.3mm AP and -2.35mm ML from bregma, and -2.35DV from
521 brain surface at a 10° angle). Virus was delivered at a rate of 100nL/min using a microinjection
522 syringe pump (WPI Micro2T SMARTouch with UMP3 pumps). Following viral infusion, a custom
523 8-microwire bundle (built in a 0.25mm spaced square grid using 2x2 arrangement with 2 wires
524 in each hole with 0.2mm DV stagger for the wires in the same hole) with an 100μm optic fiber in
525 the center (MFC_100/1.5-0.22_8mm_MF2.5_FLT, Doric Lenses) connected to a 32-pin omnetics
526 connector was inserted into OFC (+2.5mm AP, -1.5mm ML, -1.7mm DV) and secured with dental
527 cement to the anterior screw. After drying, a 1024-channel silicon probe (NeuroNexus,
528 SINAPS_4S_1024) was inserted into Str (+0.5mm AP, -3mm through -1mm ML based on
529 placement of medial probe at -1.0mm ML from bregma, and -4mm DV from the brain surface).
530 Dental cement was liberally applied to secure the probe to the skull. Dental cement and
531 rubberized glue were used to secure the implants in place. Again, carprofen (5mg/kg, s.c.)
532 injections were administered once prior to surgery along with 0.1ml 10% dextrose and 0.6ml
533 saline and again every 24 hours for three days following viral injection surgery.

534

535 **Behavior**

536 Grooming behavior was quantified in *Sapap3-/-* and control mice at age 23 weeks and anxiety
537 behaviors were quantified at 17 weeks. Grooming behavior was quantified in virally injected
538 mice at 5, 10, and 15 weeks post-surgery (ages 13, 18, 23 weeks). Anxiety-related behavior was
539 assessed in the same animals 10 weeks post-surgery (~age 17 weeks) as described below.

540

541 Open Field Test for Grooming

542 Mice were habituated to handling for 3 days prior to experimentation and placed into the
543 experimental room at least 1 hour prior to behavioral testing. Lighting was set for 50lux inside
544 of either a black or white open field box (45cm x 45cm x 30cm) based on coat color to provide
545 maximal contrast for tracking purposes. Mice were placed into the box and allowed to explore
546 freely for 5 min (baseline). Immediately after, mice were misted with a single spray of tap water
547 (~300μL) to induce grooming (i.e., water mist-induced grooming). This data was not utilized in
548 this study. Videos were recorded for an additional 10 min. Experimental videos were analyzed
549 using Ethovision XT17 with mouse behavior recognition module (Noldus). Grooming was
550 identified by the Ethovision software with threshold settings based on >90% agreement with a
551 blinded observer for 2 videos for each set of experimental settings (arena and detection
552 settings). Behavioral videos that were not able to be tracked with Ethovision were manually
553 scored by a blinded observer (n=6).

554 Bright Open Field Test

13

555 The bright open field test was conducted as before[34]. In brief, mice were habituated to the
556 behavioral room at least 1 hour prior to behavioral testing. Lighting was set for 500lux at the
557 center of the open field box (45cm x 45cm x 30cm). Mice were placed into the periphery of the
558 box and allowed to freely explore for 5min. Mouse position was tracked using Ethovision XT17
559 (Noldus) and center time was calculated based on presence of the body center in the innermost
560 third of the arena.

561 Light/Dark Emergence Test

562 The light dark emergence test was conducted in a rectangular arena (60cm x 20cm x 20cm),
563 where a small portion (20cm x 20cm) was covered with a dark plastic lid that allowed for IR
564 imaging and was closed by a small door covering an opening (5cm x 5cm) (Noldus). Room light
565 was set to 500lux. Mice were placed in the room for at least 1 hour prior to testing. During
566 testing, mice were placed into the dark side of the chamber. The door was removed and mice
567 were allowed to freely explore for 5 min. Videos were tracked using Ethovision XT17 (Noldus)
568 and time in light side of the chamber was measured based on center of the mouse. Videos that
569 were not able to be tracked in Ethovision were manually scored for time in light chamber by a
570 blinded observer (n=16).

571 Elevated Plus Maze

572 The elevated plus maze test was conducted as previously described[34]. In brief, we used a cross-
573 shaped arena comprised of four arms wherein 2 opposing arms are enclosed by walls 16.5cm
574 high (closed arm), and the other two arms are opened with a 1mm rim (open arm). All arms
575 were 30cm long and 5cm wide, connected by a center region measuring 5cm x 5cm. The arena
576 was elevated 91.4cm above the floor, and lighting at the ends of the open arms was set to
577 175lux. Mice were placed into the center of the arena with their nose in a closed arm, and they
578 were allowed to freely explore for 5min. Video recordings were tracked on Ethovision XT17
579 (Noldus). Duration in the open arm was calculated based on presence of the nose in either
580 open arm.

581

582 **Facial Lesion Scoring**

583 Facial lesions were assessed at 24 weeks of age or earlier for mice that reached a humane
584 endpoint (n=3). Mice were anesthetized and photographed on profile from both sides. Lesions,
585 identified by red exposed skin, were measured and summed for each side of the face using a
586 standard ruler and rounded to the nearest millimeter. Lesion scoring was based on the average
587 size of lesions on the left and right side of the face (i.e., total mm of lesions/2).

588

589 **Acquisition and Analysis of Neural Activity**

590 Mice were habituated to the recording room in a new homecage for at least 60 minutes over
591 multiple days prior to testing. On the recording day, mice were connected to a headstage
592 (Blackrock Microsystems, UT, USA) without anesthesia and placed in the home cage used for
593 habituation. Mice were also connected to a mezzanine board and SiNAPS interface cable
594 (Neuronexus, MI USA). Mice were habituated to the recording room for at least 60 minutes on

14

595   the testing day. For each mouse, the last 10 minutes of neural data, which corresponded to low
596   activity periods, were used for subsequent electrophysiological analysis. We selected these
597   periods since our prior work demonstrated that phase coupling is diminished during novelty
598   exposure and exploration[35,36].

599   Neuronal activity was sampled from OFC at 30kHz using the Cerebus acquisition system
600   (Blackrock Microsystems Inc., UT). Local field potentials (LFPs) were bandpass filtered at 0.5–
601   250Hz and stored at 1000Hz. An online noise cancellation algorithm was applied to reduce 60Hz
602   artifact. Neural data was also sampled at 20kHz using a SmartBox Pro acquisition system
603   (NeuroNexus, MI USA). All neurophysiological recordings were referenced to a ground wire
604   connected to both ground screws. Finally, a TTL pulse was generated using the digital output
605   from the Cerebus acquisition system. This signal was stored at 10Hz using the analog input on
606   the Cerebus acquisition system and at 20Hz using analog input on the acquisition on the
607   SmartBox Pro acquisition system.

608   To extract single-unit activity, neural activity was converted to *Neurodata Without Borders*
609   *(NWB)* format, high-pass filtered at 300Hz, and automatically sorted with Kilisort4[37]. Single units
610   were identified based on ISI violations < 0.5, presence ratio > 0.9, an amplitude cutoff < 0.1[38,39],
611   a peak-valley ratio < 1.1. Single striatal projection neurons (SPN) neurons were classified by
612   mean firing rate less than 5Hz and spike-width (at half-peak amplitude) within a range of 130–
613   250µs[17]. Narrow spiking units (NSUs) were classified by a spike width within a range of 70–
614   120µs[17]. Only single units that mapped to the central striatum targeted channels were used for
615   analysis.

616   We solely utilized neuronal activity that occurred within the last 10 minutes of the recording for
617   analysis. This approach controlled for differences in recording length across animals. LFP activity
618   was averaged across all the OFC microwires and then filtered using a 3rd-order Butterworth
619   bandpass filter to isolate 1-5Hz oscillations. The instantaneous phase of the filtered-averaged
620   LFP was then determined using the Hilbert transform, and phase locking was calculated using
621   the MATLAB (MathWorks) circular statistics tool box (i.e., the 'circ_r' function for the mean
622   resultant length and the 'circ_rtest' function for the Rayleigh test of uniformity analyses). We
623   also calculated phase locking after introducing temporal shits ranging from -100ms to 20ms
624   between the OFC oscillation and the striatal spikes. Notably, only the last 200 spiking events
625   were used to determine the phase locking of each neuron. Neurons that fired less than 200
626   times within the 10-minute recording experiment were excluded from further analysis. This
627   approach ensured that any putative difference in firing rates would not skew our quantification
628   and analysis of phase locking. For these analyses, the TTL signal was used to synchronize the
629   Cerebus and SmartBox Pro acquisition systems.

630   Mice were also subjected to optogenetic stimulation of OFC neurons that project to Str
631   following the initial neural recordings. These data were not utilized as part of this study.

632   **Histology**

633   At the end of experiments, mice were fully anesthetized using 150mg/kg ketamine, 12mg/kg
634   xylazine, and 3mg/kg acepromazine in saline and then perfused transcardially with PBS followed
635   by 4% paraformaldehyde (PFA, EM Sciences) in 1xPBS. Brains were postfixed overnight at room

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636 temperature in 4% PFA in PBS and then transferred to 1x PBS + 0.01% Sodium azide and stored
637 at 4°C until sectioning. Brains were sectioned coronally using a vibratome (Leica) at 40um
638 thickness and mounted onto charged glass slides with Mowiol mounting media [24% (w/v)
639 glycerol, 9.6% (w/v) Mowiol 4-88, 0.1M Tris-HCl pH 8.5] with 1μg/ml Hoechet 33342 and
640 coverslipped. Images were obtained on a slide scanner microscope (Olympus VS200) using 4x or
641 10x objective. Confocal images were obtained on a confocal microscope (Zeiss 780 inverted)
642 using 63x objective (accessed through Duke LMCF). Confocal z-stack images were rendered
643 using Imaris 9.3.1.

644 Immunohistochemistry

645 After tissue was sectioned, slices were placed 1xPBS + 0.01% sodium azide (PBS+azide). All
646 tissue incubation steps were conducted at room temperature with rotational agitation unless
647 otherwise noted. Tissue was briefly permeabilized by placing in 0.5% TritonX-100 in PBS+azide
648 for 15 minutes. Tissue was then washed in PBS+azide and placed into blocking buffer (5% goat
649 serum in PBS+azide+0.1%Tween-20) for three hours. Tissue was then placed into the diluted
650 primary antibody [1:1000 Rabbit α-PV (Abcam ab11427)] in blocking buffer overnight at 4°C.
651 The following day, tissue was washed in PBS+azide and incubated in secondary antibody [1:1000
652 Goat α-Rabbit IgG-AlexaFluor Plus 647 (ThermoScientific A32733TR and 1μg/ml Hoechet 33342
653 or 1:1000 Goat α-Rabbit IgG-AlexaFluor 405 (ThermoScientific A-31556)] diluted with blocking
654 buffer for 2 hours. Following secondary incubation, tissue was washed with PBS+azide and
655 mounted onto charged glass slides with Mowiol mounting media and coverslipped.

656 Micrograph images were obtained on a slide scanner microscope (Olympus VS200) using 4x or
657 10x objective. Confocal images were obtained on a confocal microscope (Zeiss 780 inverted)
658 using 63x objective (accessed through Duke LMCF). Confocal z-stack images were rendered
659 using Imaris 9.3.1.
660
661 For viral tracing studies, mice were perfused nine days after the injection of pseudorabies, and
662 brain tissue was collected as described above. Brains were then sliced, mounted, and imaged on
663 the slide scanning microscope using a 4x objective.
664
665 **Statistics**
666
667 All Statistical tests were conducted using Graph Pad Prism (v10.1.1) or Matlab (R2021b). For
668 unpaired data between two groups with non-Gaussian distributions, a two-tailed Mann-
669 Whitney rank sum test was used. For paired data with non-Gaussian distributions and more
670 than two groups, a mixed effects model (REML) was fit, and posthoc comparisons with Dunn's
671 multiple comparison tests were conducted accounting for multiple hypothesis testing using
672 Dunnett's method. For unpaired data with non-Gaussian distributions among more than two
673 groups, a Kruskal-Wallis test was performed with Dunn's multiple comparison tests as post-hoc
674 tests with Dunnett's method for multiple hypothesis testing correction. Relative proportions
675 with small sample size (facial lesions) were tested with Fisher's exact test. Relative proportions
676 with large sample size (single units) were analyzed via Chi-square tests. Normally distributed,
677 unpaired data between two groups with similar variance was analyzed using an unpaired, two-
678 tailed t-test. Normally distributed, unpaired data between two groups with unequal variance
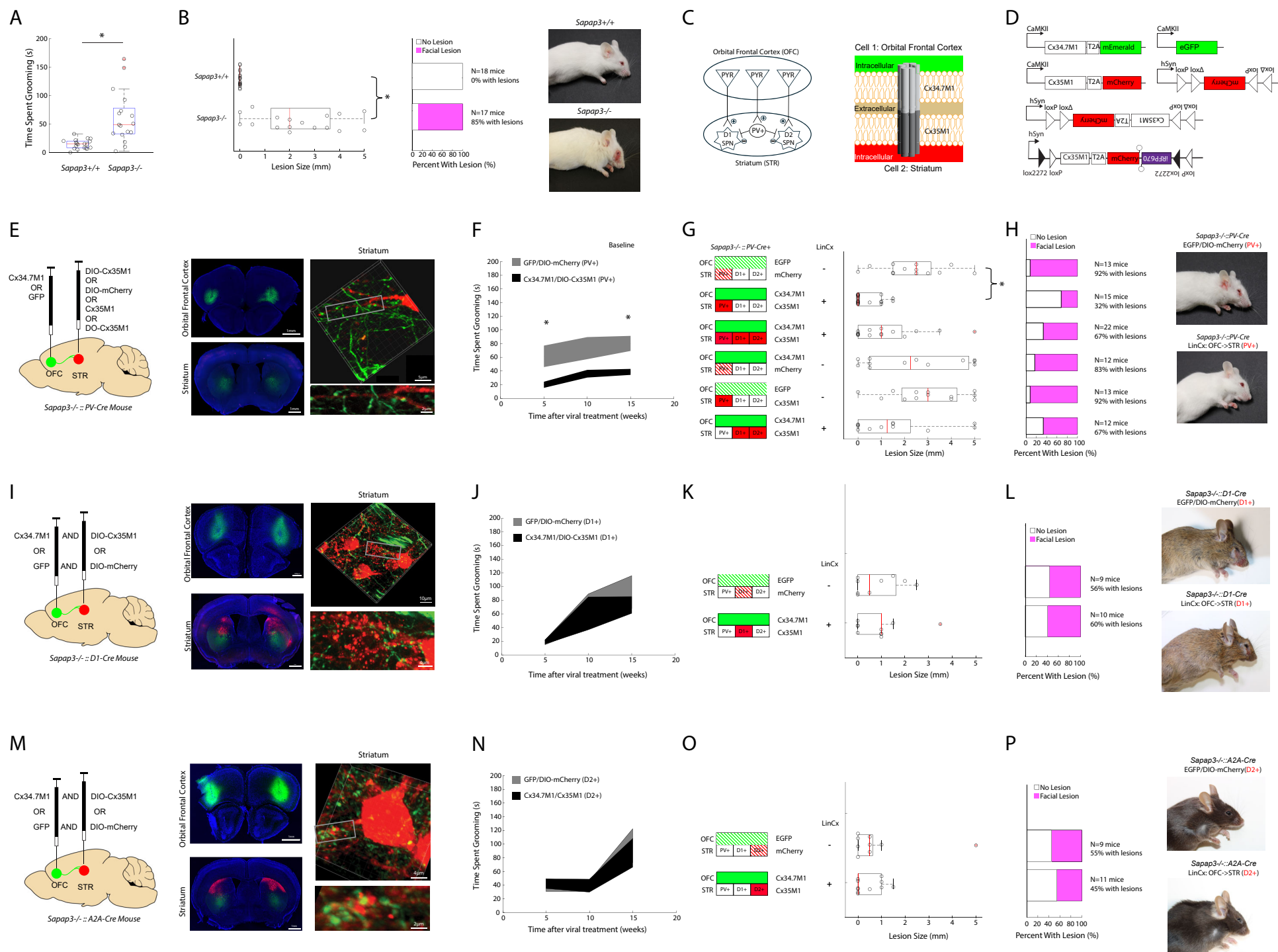
16

679   was analyzed using an unpaired, two-tailed t-test with Welch's correction. Normally distributed,
680   unpaired data between more than two groups with unequal variance was analyzed using a
681   Welch's ANOVA test with post-hoc Dunnett's T3 multiple comparisons test and p-value
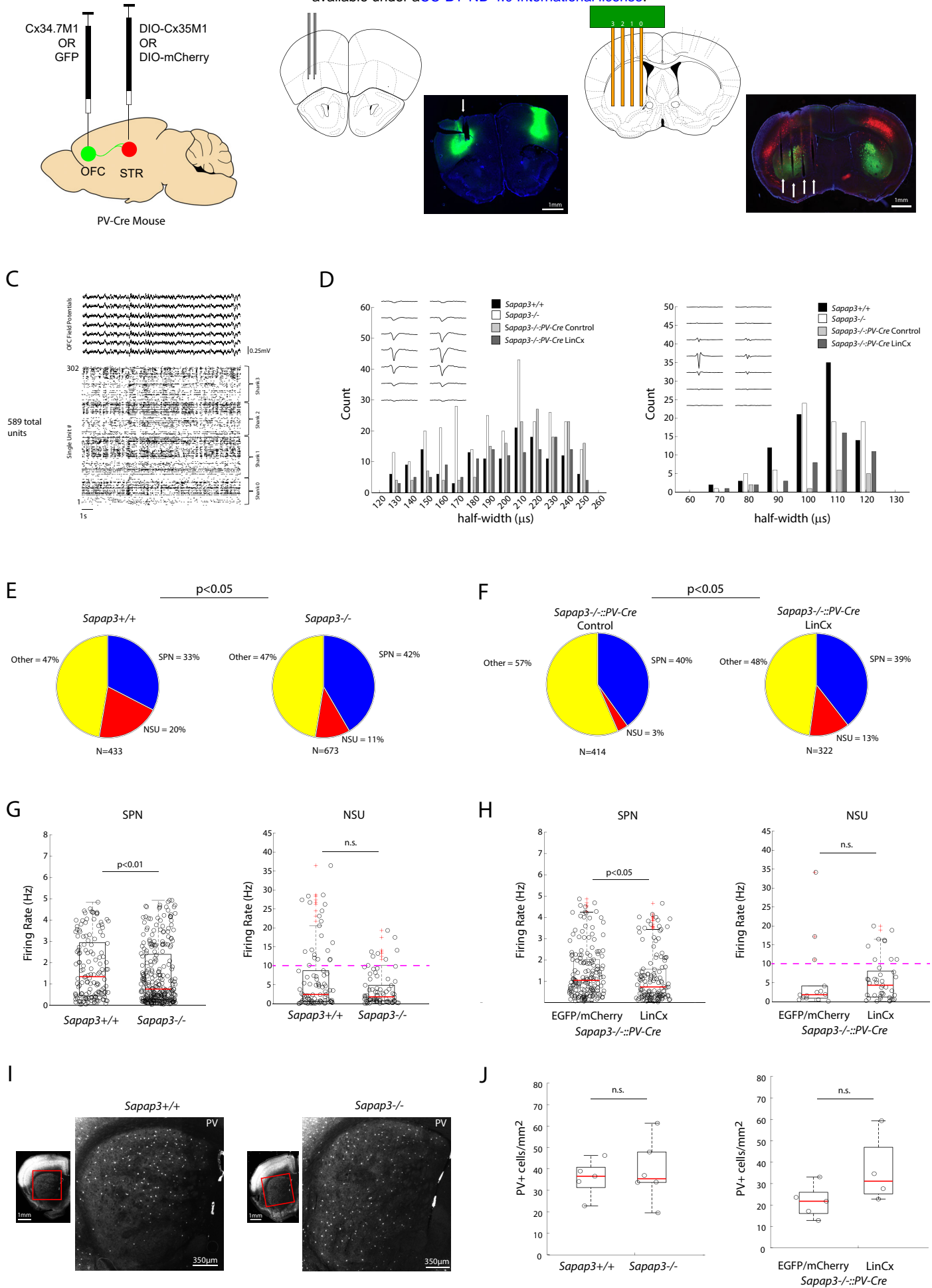682   thresholds were adjusted using a Bonferroni correction for multiple hypotheses testing.
683
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685
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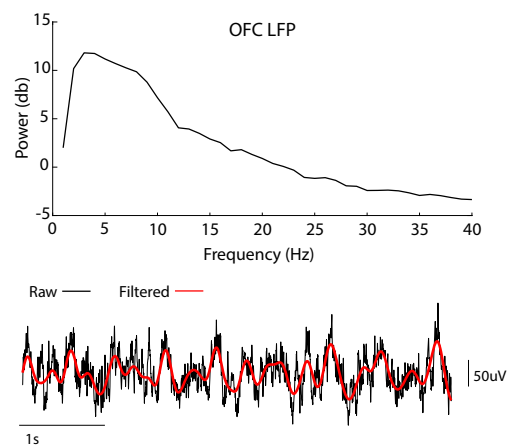
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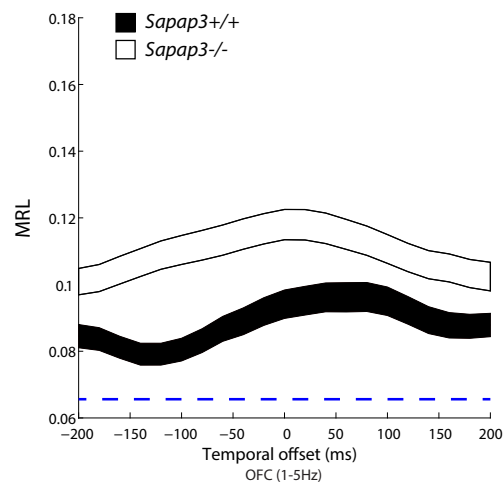789   s.html> (
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CamKII+ OFC

Grooming/
Lesion
Unchanged

CamKII+ Striatum

Cx34.7$_{M1}$

Cx35$_{M1}$

CamKII+ OFC

Grooming/
Lesion
Reduced

PV+ Striatum

CamKII+ OFC

Grooming/
Lesion
Unchanged

D1+ Striatum

CamKII+ OFC

Grooming/
Lesion
Unchanged

A2A+ Striatum

CamKII+ OFC

or

Grooming/
Lesion
Unchanged

PV+ Striatum

CamKII+ OFC

Not PV

Grooming/
Lesion
Unchanged

PV+ Striatum- Cre OFF