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1 **Title: Differential enhancement of ERK, PKA and Ca²⁺ signaling in direct**
2 **and indirect striatal neurons of Parkinsonian mice**

3

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14

15 **Abstract:** Parkinson's disease (PD) is characterized by severe locomotor deficits due to the
16 disappearance of dopamine (DA) from the dorsal striatum. The development of PD symptoms
17 and treatment-related complications such as dyskinesia have been proposed to result from
18 complex alterations in intracellular signaling in both direct and indirect pathway striatal
19 projection neurons (dSPNs and iSPNs, respectively) following loss of DA afferents. To identify
20 cell-specific and dynamical modifications of signaling pathways associated with PD, we used a
21 hemiparkinsonian mouse model with 6-hydroxydopamine (6-OHDA lesion) combined with two-
22 photon fluorescence biosensors imaging in adult corticostriatal slices. After DA lesion,
23 extracellular signal-regulated kinase (ERK) activation was found increased in response to DA D1
24 receptor (D1R) or α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)

25 stimulation. The cAMP-dependent protein kinase (PKA) pathway contributing to ERK activation
26 displayed supersensitive responses to D1R stimulation after 6-OHDA lesion. This cAMP/PKA
27 supersensitivity was specific of D1R-responding SPNs and resulted from G α olf upregulation and
28 deficient phosphodiesterase activity. In lesioned striatum, the number of D1R-SPNs with spontaneous
29 Ca²⁺ transients augmented while Ca²⁺ response to AMPA receptor stimulation specifically increased in
30 iSPNs. Our work reveals distinct cell type-specific signaling alterations in the striatum after DA
31 denervation. It suggests that over-activation of ERK pathway, observed in PD striatum, known to
32 contribute to dyskinesia, may be linked to the combined dysregulation of DA and glutamate signaling
33 pathways in the two populations of SPNs. These findings bring new insights into the implication of
34 these respective neuronal populations in PD motor symptoms and the occurrence of PD treatment
35 complications.

36

37 **Competing interests:** no competing interests

38

39 **Keywords:** Parkinson's disease, striatum, striatal projection neuron, 6-hydroxydopamine, mouse, Ca²⁺
40 signaling, cAMP signaling, ERK, biosensors, Förster resonance energy transfer, two-photon
41 microscopy

42

43 **Abbreviations:** 6-OHDA, 6-hydroxydopamine; AAV, adeno-associated virus; ACSF, artificial
44 cerebrospinal fluid; AKAR3; cAMP-dependent protein kinase activity reporter; D1R, dopamine D1
45 receptor; D2R, dopamine D2 receptor; DA, dopamine; EKAR-EV; ERK activity reporter; ERK,
46 extracellular signal-regulated kinase; FRET, Förster resonance energy transfer; L-DOPA, L-3,4-
47 dihydroxyphenylalanine; PD, Parkinson's disease; PKA, cAMP-dependent protein kinase/protein

- 48 kinase A; **SPN**, striatal projection neuron; **dSPN**, direct pathway SPN; **iSPN**, indirect pathway SPN;
- 49 **TH**, tyrosine hydroxylase.

50 1- Introduction

51 The striatum is the main input structure of the basal ganglia, which play a major role in motor control,
52 and habitual and goal-directed actions (Redgrave et al., 2010). The functions of the striatum are based
53 on the balance of two distinct populations of GABAergic striatal projection neurons (SPNs, a.k.a.
54 medium-size spiny neurons). SPN activity is driven by abundant glutamatergic inputs from **the** cerebral
55 cortex and some thalamic nuclei. The direct pathway SPNs (dSPNs) directly project to the basal
56 ganglia output structures (*substantia nigra pars reticulata* and *globus pallidus pars interna*) and
57 promote selected actions. The indirect pathway SPNs (iSPNs), in contrast, project to the same output
58 structures through relays in the *globus pallidus pars externa* and subthalamic nucleus, and suppress
59 unselected actions (Albin et al., 1989). Dopamine (DA) release by afferent neurons from the *substantia*
60 *nigra pars compacta* positively activates dSPNs and inhibits iSPNs, which preferentially express DA
61 D1 (D1R) and D2 (D2R) receptors, respectively (Gerfen et al., 1990).

62 In Parkinson's disease (PD), the progressive loss of DA afferents to the dorsal striatum (caudate
63 nucleus and putamen) is responsible for the motor and possibly non-motor symptoms. Bradykinesia,
64 akinesia, and rigidity are attributed to the loss of dSPN activation and iSPN inhibition (Albin et al.,
65 1989; Alcaccer et al., 2017; Kravitz et al., 2010). Various alterations of signaling pathways have been
66 reported in PD patients and animal models of DA neuron lesion. Extracellular signal-regulated kinase
67 (ERK) can be activated by the stimulation of corticostriatal afferents in the intact striatum (Gerfen et
68 al., 2002; Sgambato et al., 1998), but following lesion of DA neurons, ERK becomes very strongly
69 activated by DA replacement therapy, L-3,4-dihydroxyphenylalanine (L-DOPA) (Darmopil et al., 2009;
70 Gerfen et al., 2002; Pavón et al., 2006; Santini et al., 2007; Westin et al., 2007) in a cAMP/protein
71 kinase A(PKA)- and Ca²⁺-dependent manner (Alcaccer et al., 2012; Fieblinger et al., 2014a; Santini et
72 al., 2007). The cAMP/PKA pathway is strongly activated in response to D1R stimulation in the DA-

73 denervated striatum (Santini et al., 2007). In the dorsal striatum, cAMP concentration reflects the
74 balance between its production, depending on the levels of $G\alpha_{\text{olf}}$ (Hervé, 2011), the G protein subunit
75 which couples D1R to adenylyl cyclase (AC) (Corvol et al., 2001), and its degradation, through
76 phosphodiesterase (PDE) activity (Nishi et al., 2008; Polito et al., 2015). However the dynamics and
77 cell type specificity of signaling alterations in SPNs resulting from the chronic absence of DA are still
78 poorly characterized, hampering our understanding of their pathophysiological consequences.

79 To identify the alterations of ERK, cAMP, and Ca^{2+} -dependent pathways in dSPNs and iSPNs in
80 a chronic rodent model of PD, we used fluorescent biosensors for two-photon imaging of identified
81 living neurons in mouse corticostriatal slices. We used Förster resonance energy transfer (FRET)-based
82 biosensors, ERK activity reporter (EKAR-EV) and cAMP-dependent protein kinase (PKA) activity
83 reporter (AKAR3) (Allen and Zhang, 2006; Castro et al., 2013; Komatsu et al., 2011) and we
84 monitored Ca^{2+} dynamics with the Ca^{2+} indicator (GCaMP6s) (Chen et al., 2013). Striatal DA terminals
85 were lesioned by local microinjection of 6-hydroxydopamine (6-OHDA). The responses in intact and
86 DA-denervated striatum were compared following application of D1R agonist and/or AMPA,
87 mimicking the effects of dopaminergic and glutamatergic afferents. We found specific increased
88 activity of ERK- and cAMP/PKA-dependent pathways in response to D1R stimulation without
89 modification of Ca^{2+} signaling in response to AMPA. In contrast, in iSPNs, AMPA-induced Ca^{2+}
90 transients were increased in the DA-denervated striatum, while the cAMP-dependent pathways was not
91 significantly affected. Hence our results indicate that DA and glutamate-induced responses were
92 differentially disrupted in dSPNs and iSPNs. They also suggest that complications in the PD treatments
93 at late stages may be linked to the inability to appropriately regularize both DA and glutamate
94 responses in both populations.

95 2- Materials and Methods

96 2.1 - Animals

97 C57BL/6JRj mice (Janvier Labs; Le Genest Saint Isle, France) were used for experiments in wild type
98 animals, aged P8-P20 for experiments in young animals and aged from 6 to 8 weeks for experiments in
99 adult males. *Gnal*^{+/-} mice (*Gnal*^{tm1Rax}, (Belluscio et al., 1998)) were mated with C57BL/6J mice (Charles
100 River Lab France; L'Arbresle, France) to produce male and female *Gnal*^{+/-} and *Gnal*^{+/+} littermates.
101 Adult *Drd1::Cre* [Tg(Drd1a-cre)EY262Gsat, (Gong et al., 2007)] and *Adora2a::Cre* [Tg(Adora2a-
102 cre)2MDkde, (Durieux et al., 2009)] mice in which the Cre recombinase is targeted to specific neuronal
103 subtypes, were backcrossed for at least 10 generations on a C57Bl/6J background. The mice were
104 genotyped by PCR analysis of genomic DNA using standard PCR protocols. The mice were kept in
105 groups (maximum five per cage) on a 12 h light/dark cycle at a constant temperature of 22°C with
106 access to food and water *ad libitum*. All experiments were in accordance with the guidelines of the
107 French Agriculture and Forestry Ministry for handling animals (decree 87-848). The animal facility
108 was approved licensed by the *Sous-Direction de la Protection Sanitaire et de l'Environnement de la*
109 *Préfecture de Police (arrêté préfectoral DTPP 2018-20, D 75-05-22)*. The experimental protocols were
110 approved by the *Ministère de l'éducation nationale, de l'enseignement supérieur et de la recherche*
111 (authorization # 02635.02). The principal investigators had a personal agreement (D.H., license C-75-
112 828; J.-A.G., license 75-877).

113 2.2 - 6-OHDA lesions, AAV injections and postoperative care

114 Mice were anesthetized with a mixture of xylazine (10 mg/kg) and ketamine (75 mg/kg) (Centravet)
115 and mounted in a digitalized stereotactic frame (Stoelting Europe) equipped with a mouse adaptor. 6-
116 OHDA-HCl (6.0 mg/ml, Sigma-Aldrich) was dissolved in a solution containing 0.2 g/L ascorbic acid in
117 saline. The AAV stock suspension was diluted 5 times in the 6-OHDA ascorbic acid solution. Mice
118 received a unilateral injection (1.25 µL) of a mix of 6-OHDA and AAV into the right striatum at the

119 following coordinates according to a mouse brain atlas (Paxinos and Franklin; 2001): anteroposterior
120 (AP), +0.3 mm and lateral (L), +2.3 mm from bregma; dorsoventral (DV), -3.4 mm (from the skull
121 surface). Sham mice were injected with vehicle only (ascorbic acid in saline) in which the AAV virus
122 was also diluted 5 times. Before and after surgery, the mice received a subcutaneous injection of a non-
123 steroidal anti-inflammatory drug (flunixin meglumine, 4 mg/kg; Sigma-Aldrich) and were placed on a
124 warm plate during about \approx 10 h after surgery to avoid hypothermia. Mice were allowed to recover for 3
125 weeks before sacrifice and brain slicing. Lesions were assessed at the end of experiments by
126 determining the striatal levels of tyrosine hydroxylase (TH) using immunoblotting (see below) on the
127 striata from the slice or its adjacent slice. Only animals with a TH level reduction by >70% in the
128 lesioned striatal area compared with the control side were included in the analyses.

129 *2.3 - Biosensors and viral vectors*

130 The GECI GCaMP6s (Chen et al 2013), FRET-based A-kinase activity reporter AKAR3 (Allen and
131 Zhang 2006) and ERK activity reporter EKAR-EV (Komatsu et al., 2011) were used in the present
132 study. pAAV.Syn.GCaMP6s.WPRE.SV40 and pAAV.Syn.Flex.GCaMP6s.WPRE.SV40 were a gift
133 from the Genetically Encoded Neuronal Indicator and Effector Project (GENIE) & Douglas Kim
134 (Addgene viral prep # 100843-AAV9 and Addgene viral prep # 100845-AAV1). Plasmid encoding
135 AKAR3 and EKAR-EV were a gift from Jin Zhang and Michiyuki Matsuda respectively.
136 pAAV.hSyn.AKAR3.WPRE was constructed by Ted Abel and pAAV.hSyn.EKAREV.WPRE was
137 synthesized from GenScript HK USA and viral preparations were performed by Upenn Vector Core.
138 AAVs were injected into the striatum as described in the corresponding section and brains were
139 typically sliced 2 to 5 weeks after surgery.

140 *2.4 - Preparation of brain slices*

141 Before brain removal, the animals were anesthetized with a mixture of xylazine (10 mg/kg) and
142 ketamine (75 mg/kg), following the guidelines of our institution. Then ice-cold “cutting” choline-

143 artificial cerebrospinal fluid (choline-ACSF) solution, containing (mM) 110 choline Cl, 0.5 CaCl₂, 7
144 MgCl₂, 1.25 NaH₂PO₄, 25 NaHCO₃, 2.5 KCl, 11.6 ascorbic acid, 3.1 sodium pyruvate and 25 glucose,
145 saturated with 5% CO₂ and 95% O₂, was perfused to the brain by intracardiac perfusion. Brains were
146 quickly isolated and placed in ice-cold “cutting” choline-ACSF solution. Sections (250 μm) were made
147 using a vibrating microtome (Thermo Scientific) in a parahorizontal plane as described previously
148 (Kawaguchi et al., 1989). After cutting, brain slices were transferred 15 min to recover in standard
149 ACSF solution at 35°C, containing (mM): 125 NaCl, 1 CaCl₂, 1 MgCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃,
150 2.5 KCl, and 25 glucose, saturated with 5% CO₂ and 95% O₂. Brain slices were then kept in a custom-
151 made interface chamber on an optic paper lying on a non-woven compress net, placed at the interface
152 between the ACSF solution gassed with 95% O₂/5% CO₂ and incubated for 1 h at room temperature in
153 a 95% O₂/5% CO₂ atmosphere, a time needed to recover a pH/metabolic equilibrium.

154 *2.5 - Two-photon slice imaging*

155 Experiments were performed at the *Institut du Fer à Moulin* Cell and Tissue Imaging Facility. On the
156 microscope stage, a nylon/platinum harp stabilized the slice while suspended on a nylon mesh to
157 facilitate continuous perfusion over the whole slice at 5 mL/min with ACSF at 32°C. Two-photon
158 imaging was performed using an upright Leica TCS MP5 microscope with resonant scanning (8 kHz),
159 a Leica 25X/0.95 HCX IRAPO immersion objective and a tunable Ti:sapphire laser (Coherent
160 Chameleon Vision II) with dispersion correction set to 860 nm for CFP excitation (FRET experiments)
161 and 920 nm for GCaMP6s excitation. The emission path consisted of an initial 700 nm low-pass filter
162 to remove excess excitation light (E700 SP, Chroma Technologies), 505 nm dichroic mirror for
163 orthogonal separation of emitted signal, 483/32 CFP emission filter, 535/30 YFP emission filter for
164 AKAR3 and EKAR-EV imaging, and a 560 nm dichroic mirror for orthogonal separation of emitted
165 signal, 525/50 GFP emission filter for GcAMP6s experiments, and a two-channel Leica HyD detector

166 for simultaneous acquisition. Due to the high quantum efficiency and low dark noise of the HyD
167 photodetectors, detector gain was typically set at 10–20% with laser power at 1–5% (which
168 corresponds to a laser power under the objective of 3 - 5 mW). For AKAR3 and EKAR-EV image
169 acquisition, Z-stack images (12-bit; 512 x 512) were typically acquired every 15 s. The z-step size was
170 1–2 μm and total stack size was typically 40–60 sections depending on the slice (≈ 60 – $120 \mu\text{m}$). For
171 GCaMP imaging, z-stack images (12 bits, 512 x 512) were typically acquired every 1s. The z-step size
172 was 5 μm and total stack size was typically 3 to 5 sections depending on the slice (≈ 10 to $20 \mu\text{m}$).

173 *2.6 - Drug treatments*

174 (RS)-AMPA hydrobromide (0.5 μM ; Tocris), SKF81297 hydrobromide (10 μM , Tocris), and
175 CGS21680 hydrochloride (10 μM , Biotechne) were freshly prepared in ultrapure Milli-Q water.
176 Forskolin (10 μM ; Sigma), U0126 (5 μM , Tocris), and IBMX (10-300 μM ; 3,7-dihydro-1-methyl-3-(2-
177 methylpropyl)-1H-purine-2,6-dione; Tocris) were prepared in 100% DMSO. Concentrated stock
178 solutions were diluted in standard ACSF saturated with 95% O_2 /5% CO_2 and continuously bubbled
179 during perfusion, final concentration of DMSO 0.1% (vol/vol). The imaging chamber of the
180 microscope was continuously perfused with the recording ACSF solution saturated with 95% O_2 /5%
181 CO_2 at a rate of 5 mL/min. For bath application, smoothly switching between different reservoirs
182 allowed for changing the bathing solution to a solution containing drugs, without mechanically
183 disturbing the preparation. For precise time application of the drug, a 18G needle was linked to a
184 Valvebank®4 circuitry (AutoMate Scientific, Inc.) designed for solution-switching use with valve
185 opening at the desired time of compound application with 10 ms accuracy. The pipette holder was
186 mounted onto a micromanipulator, like those used for patch-clamp experiment. The pipette was filled
187 with ACSF or the drug of interest at its final concentration and positioned using the micromanipulator
188 system, in close proximity to the slice.

189 *2.7 - Immunoblotting*

190 At the end of the imaging experiments, striata from both sides were separately dissected from each
191 250- μ m-thick corticostriatal slice and were immediately frozen at -80°C. Striata were sonicated in 10
192 g/L SDS, and placed at 100°C for 5 min. Aliquots (5 μ L) of the homogenate were used for protein
193 determination using a bicinchoninic acid assay kit (Pierce Europe). Equal amounts of total protein (20
194 μ g) were separated by SDS-PAGE on 4–15% precast gels (Bio-Rad) and transferred electrophoretically
195 to nitrocellulose membranes (GE Healthcare). The membranes were then incubated in TH chicken
196 polyclonal antibodies (AVES, dilution 1:1000), GFP rabbit polyclonal antibodies (Invitrogen, A-6455)
197 and actin monoclonal mouse and rabbit antibodies (Sigma-Aldrich, dilution 1:5000). Secondary
198 antibodies (1:5000) were IRDye 800CW-conjugated anti-chicken IgG; IRDye 800 CW-conjugated anti-
199 mouse IgG, IRDye 700 CW-conjugated anti-mouse IgG and IRDye 700 CW-conjugated anti-rabbit IgG
200 (Rockland Immunochemical). Bound antibodies were visualized using an Odyssey infrared
201 fluorescence detection system (LI-COR), followed by quantification by Odyssey version 1.2 software.
202 Fluorescence intensity values were normalized to actin values for variations in loading and transfer.

203 *2.8 - Image analysis and post-acquisition processing*

204 Images were processed with ImageJ and Icy software by using maximum z projections (ICY-A9L7V2)
205 followed by translation and rotation registration correction to correct for x/y movements and temporal
206 drift (ICY-E4L7S9). Regions of interest (ROIs) were selected for measurement if they could only be
207 measured for the whole experimental time course for AKAR3 and EKAR-EV experiments. For GCaMP
208 experiments, ROI were selected if they appeared during the time course of the experiment, as GCaMP
209 basal fluorescence is usually low and only increases when a response is observed. ROIs were placed
210 around the periphery of the soma. After ROI placement, raw CFP and YFP or GFP intensity
211 measurements for the entire time course were imported into Microsoft Excel (ICY-Y5X4A1). A
212 fluorescence ratio was calculated for each time point in each ROI series and was normalized to the
213 average baseline ratio for each respective ROI (average of 20 to 30 frames during the period before

214 first stimulus) as ΔR [YFP/CFP]/R0 for AKAR3 or EKAR-EV experiments and $\Delta F/F0$ for GCaMP
215 experiments. In all the FRET experiments, responsive cells are defined by two linked parameters: a
216 clear change in the slope of the FRET emission ratio and a change in the amplitude above the baseline
217 noise. In the GCaMP6s experiments, if the amplitude of the fluorescent signal ($\Delta F/F0$) increased over
218 $>3SD$ (i) during baseline, cell was considered as “spontaneously active” or (ii) after pharmacological
219 stimulation, cell was considered as “responsive to treatment” (see Fig. 5A). Statistical analysis was
220 performed in Matlab or GraphPad Prism. Categorical variables are expressed as the percent of the
221 number of responsive cells to a stimulus over the total number of cells assessed; and continuous
222 variables as mean \pm SEM. Quantitative variables were compared using a one-way ANOVA followed by
223 Tukey’s multiple comparison test in case of Gaussian distribution or Kruskal-Wallis test followed by
224 Dunn’s multiple comparison test in case of non-Gaussian distribution when there were three or more
225 groups. Two-tailed, unpaired t-test was used to compare quantitative variables when two groups were
226 compared. Categorical variables were compared using the chi-square test or with Fisher exact test when
227 numbers were too small.

228

229 **3 - Results**

230 *3.1 - Imaging ERK activity dynamics in striatal neurons*

231 In order to monitor ERK activity in striatal neurons we first compared several FRET-based optical
232 biosensors that allow quantitative real-time analysis of ERK activity dynamics with single cell
233 resolution in tissue to determine which one was best suited for our study. We tested EKAR_{cyto},
234 EKAR2G1, and EKAR-EV (Fritz et al., 2013; Harvey et al., 2008; Komatsu et al., 2011). EKAR_{cyto} is
235 comprised of a fluorescent protein-based FRET pair (mCerulean-mVenus), a phosphorylation substrate
236 peptide containing ERK target sequence (PDVPRTPVGK) and docking site (FQFP), and the proline-
237 directed WW phospho-binding domain (Harvey et al., 2008). EKAR2G1 uses the backbone of

238 EKAR_{cyto} with a substitution of mCerulean at the N terminus and mVenus at the C terminus by variants
239 of mTFP1 (mTFP1/cp227) and Venus (Venus/cp173), respectively (Fritz et al., 2013). EKAR-EV is
240 optimized with the YFP/CFP fluorescent protein variant pair (Ypet/ECFP) and a long, flexible linker
241 between the WW domain and the phosphorylation substrate sequence of EKAR_{cyto}, which was shown to
242 markedly increase the gain of FRET signals (Komatsu et al., 2011). To compare their sensitivity, we
243 transfected striatal neurons in culture (DIV 7) with the three EKAR variants using lipofectamine 2000
244 and we applied on the neurons brain-derived neurotrophic factor (BDNF, 10 ng/mL, for 5 min), which
245 activates ERK signaling through BDNF receptors (Fig. 1A). EKAR-EV exhibited a larger BDNF-
246 induced YFP/CFP emission ratio (FRET ratio) than EKAR_{cyto} and EKAR2G1 (Fig. 1A, middle panel).
247 To quantify FRET responses, we normalized the FRET ratio increases by their corresponding FRET
248 ratio baseline (i.e., $\Delta R/R_0$), as in previous studies (Gervasi et al., 2007, 2010). EKAR-EV showed a
249 higher FRET increase ($12.5 \pm 1.6\%$, mean \pm S.E.M) than EKAR_{cyto} ($6.6 \pm 0.9\%$) and EKAR2G1 ($2.9 \pm$
250 0.3%) (Fig. 1A, right panel). Based on these results, we chose EKAR-EV to monitor ERK activity
251 dynamics in striatal brain slices.

252 Since Sindbis viruses were previously used to transduce biosensors to monitor other signaling
253 pathways in young brain slices (Castro et al., 2013; Gervasi et al., 2007; Luczak et al., 2017), we used
254 them to express EKAR-EV in striatal slice preparations from immature mice (P8-P12) and monitor
255 changes in ERK activity in real time by ratiometric two-photon microscopy. We first checked whether
256 EKAR-EV was able to report ERK activation in striatal neurons after a global depolarization induced
257 by KCl (25 mM) application for 1 min. This treatment produced, in about 90% of the EKAR-EV-
258 expressing neurons, an increase in the FRET ratio, which peaked around 6 min and then decreased
259 slowly (Fig. 1B middle panel). Application of AMPA (5 μ M) for 30 s or KCl for 1 min yielded an

260 increase in the FRET emission ratio of $4.7 \pm 0.5\%$ and $7.8 \pm 0.6\%$ respectively (mean \pm S.E.M., Fig.
261 1C).

262 To study ERK activity dynamics in 6-OHDA-lesioned mice, we needed to perform ERK
263 imaging in mature striatal network (above 8-week old). Since Sindbis virus does not allow effective
264 neuronal infection in adult striatal slices, we produced a recombinant adeno-associated virus (AAV,
265 serotype 2/1) encoding EKAR-EV. The biosensor was expressed in the adult mouse striatum via AAV
266 injection and was subsequently imaged in acute brain slices (2–4 weeks post injection, Fig. 1D).
267 Application of a D1 agonist (SKF81297 10 μ M) for 30 s rapidly increased FRET emission ratio in
268 some of the neuronal somas indicating an increase in ERK activity (Fig. 1E). Subsequent addition of
269 KCl (25 mM) further increased FRET emission ratio ($1.2 \pm 0.2\%$ for SKF81297 versus $3.4 \pm 0.25\%$ for
270 KCl, Fig. 1F). The response to KCl application was used as a positive control for cell health and
271 responsiveness in all experiments. The SKF81297- and KCl-induced increases in FRET emission ratio
272 were dependent on the activity of mitogen-activated protein kinase/ERK kinases, MEK1/2, the kinases
273 activating ERK, since all the responses were abolished in the presence of U0126 (5 μ M), a selective
274 inhibitor of MEK1/2 (Fig. 1D-F). We noticed that each local application, whatever the drug applied,
275 was followed by a transient decrease in the FRET emission ratio. This decrease also occurred after
276 local application of ACSF whereas ACSF did not produce any significant increase in the FRET
277 emission ratio ($0.15 \pm 0.09\%$). Similar decreases were also recorded in the presence of U0126 for all
278 the stimulations (Fig. 1E). We concluded that these transient decreases in FRET ratio were artefactual
279 and since they were short-lived, they did not preclude measurement of ERK activity after their
280 disappearance. In summary, we showed that EKAR-EV biosensor was appropriate to monitor ERK
281 activity in neurons in culture as well as in young and adult striatal slices.

282

283 3.2 - *Biosensor expression and dopamine depletion after 6-OHDA injection into the striatum of adult*
284 *mice*

285 We co-injected 6-OHDA and AAV expressing biosensors into the dorsal striatum of 4-6-week old mice
286 that were allowed to recover for 4 weeks before acute brain slicing and two-photon imaging (Fig. 2A).
287 Striatal depletion of DA terminals following 6-OHDA microinjection was checked by the decrease of
288 tyrosine hydroxylase (TH) immunoreactivity only in the 6-OHDA-injected side, as indicated by
289 immunoblotting (Fig. 2B). Microinjection of 6-OHDA and DA denervation did not alter the expression
290 of AKAR3 biosensors (Fig. 2C). Similar results were observed when we co-injected 6-OHDA and
291 AAVs expressing EKAR-EV or GCaMP6s biosensors (**Fig 2 C**).

292
293 3.3 - *ERK responses are increased after dopamine depletion induced by 6-OHDA lesion*

294 ERK signaling has been reported to be activated by combination of DA D1 and glutamate signals in
295 SPNs in response to drugs of abuse (see (Girault et al., 2007) for a review). Since the activation of ERK
296 is particularly intense after the first L-DOPA treatment in the DA-denervated striatum (Darmopil et al.,
297 2009; Pavón et al., 2006; Santini et al., 2007), we sought to determine whether responsiveness to
298 glutamate or DA or both was increased following the lesion. We compared ERK activity dynamics in
299 response to the application of a DA D1 agonist and/or AMPA stimulation for 30 s, in control and 6-
300 OHDA-lesioned striata. In 6-OHDA-lesioned striata, this maximal increase after D1R stimulation by
301 SKF81297 (10 μ M) was significantly enhanced as compared to non-lesioned control animals (mean \pm
302 SEM: non-lesioned, $0.9 \pm 0.19\%$, lesioned, $2.4 \pm 0.18\%$, $p < 0.0001$, Fig. 3A and B). AMPA (0.5 μ M)
303 application also produced a higher increase in FRET emission ratio in the 6-OHDA-lesioned striata
304 than in the non-lesioned striata (mean \pm SEM: non-lesioned, $1.0 \pm 0.17\%$, lesioned, $1.6 \pm 0.17\%$,
305 $p < 0.05$, Fig. 3A and B). The co-application of AMPA and SKF81297 increased the FRET emission
306 ratio more in 6-OHDA-lesioned striata than in control striata (non-lesioned, $1.0 \pm 0.12\%$, lesioned, 1.8

307 $\pm 0.19\%$, $p < 0.05$, Fig. 3A and B), although the amplitude of the effects was not increased as compared
308 to those of SKF or AMPA alone. In contrast, the 6-OHDA lesion did not modify the FRET emission
309 ratio of striatal neurons in response to ACSF or KCl (Fig. 3B). We also compared the percentage of
310 neurons responsive to these various stimuli among all the EKAR-EV-expressing neurons in the field of
311 view. In all the FRET experiments, responsive cells were defined by a clear change in the slope of the
312 FRET emission ratio and an increase in the amplitude above the baseline noise. In 6-OHDA-lesioned
313 slices, the percentage of responsive neurons was significantly increased as compared to non-lesioned
314 controls, only after AMPA and SKF81297 co-application (non-lesioned, $43 \pm 3\%$, lesioned, $67 \pm 4\%$,
315 $p < 0.05$, Fig. 3C). The proportion of responsive cells after ACSF, SKF81297, AMPA or KCl application
316 was not modified by the lesion (Fig. 3C).

317 These results showed upregulation of ERK signaling in response to D1R agonist and/or to
318 AMPA after DA denervation by 6-OHDA. The upregulation of ERK resulted in an increased response
319 amplitude but did not lead to the recruitment of additional cells following stimulation of AMPA
320 receptor or D1R alone. The percentage of responsive neurons to the combined stimulation of D1R and
321 AMPA receptor was increased and reached about 70% of striatal neurons, indicating that ERK was
322 activated in these conditions in both populations of SPNs. The effects of SKF81297 and AMPA on
323 ERK activation in different SPN populations could explain why the co-application of these drugs had
324 no synergistic effect on the amplitude of ERK activation in SPNs. However it appeared that the co-
325 application of SKF81297 and AMPA was able to recruit additional cells. To address the possible
326 mechanisms of the effects of the DA lesion on ERK signaling, we further investigated, the dynamic
327 changes in two signaling pathways leading to ERK activation in SPN, namely the cAMP/PKA pathway,
328 which is activated after D1R stimulation, and the glutamate-induced Ca^{2+} increase.

329

330 *3.4 - PKA responses to D1R stimulation are increased in dSPN after 6-OHDA lesions*

331 Since an increase in PKA signaling could explain the enhancement of ERK responsiveness to D1R
332 agonist, we investigated PKA responses in control and 6-OHDA-lesioned animals. We injected an AAV
333 vector encoding AKAR3, a biosensor for PKA activity (Allen and Zhang, 2006), into the striatum of
334 adult mice and used corticostriatal slices 3-4 weeks later for real-time imaging of PKA activity (Fig. 4).
335 Since previous studies from other laboratories used very young animals, we first validated our
336 approach in adult mice (8-10-week old). As in those previous studies, we stimulated the slices with
337 D1R and A_{2A}R agonists to differentiate putative dSPNs and iSPNs, expected to respond to D1R and
338 A_{2A}R agonists, respectively (Castro et al., 2013; Polito et al., 2015; Yapo et al., 2017). In the dorsal
339 striatum, application of the A_{2A}R agonist CGS21680 (10 μ M) for 1 min yielded an increase in the
340 FRET emission ratio to $5.9 \pm 0.2\%$ (Fig. 4A-C) in less than half of the neurons present in the field of
341 view ($40 \pm 3\%$, Fig. 4D), revealing the A_{2A}R-expressing neurons. After 10 min of CGS21680 washout,
342 application of the D1R agonist, SKF81297 (10 μ M), for 1 min increased FRET emission ratio to $6.1 \pm$
343 0.3% in about the other half of the neurons present in the field of view ($46 \pm 4\%$) (Fig. 4A-D),
344 revealing the D1R-expressing SPN. This was consistent with studies showing that D1R and A_{2A}R are
345 segregated in the two major subsets of SPNs present in equal proportions and globally corresponding to
346 the direct and indirect pathways, respectively (Bertran-Gonzalez et al., 2008; Schiffmann and
347 Vanderhaeghen, 1993). These results were also in agreement with FRET biosensor studies performed in
348 striatal slices from immature mice (Polito et al., 2015; Yapo et al., 2017). We randomly alternated the
349 order of SKF81297 and CGS21680 applications with no effect on either the amplitude of FRET
350 emission ratio or the proportion of responsive SPNs. In all the experiments, a subsequent application of
351 forskolin (FSK, 10 μ M) that directly activates AC, produced a maximal increase in FRET emission
352 ratio to $11.7 \pm 0.3\%$ (Fig. 4A-D), indicating the cell health and the correct AKAR3 responsiveness to

353 AC activation in all experiments. We also measured the rise time of AKAR3 responses that was shorter
354 for FSK than for SKF81297 and longer for CGS21680 (Fig. 4E).

355 Since these experiments showed that our experimental approach reliably allowed studying PKA
356 responses in adult striatal slices, we then compared PKA activation in control and 6-OHDA-lesioned
357 striata. We co-injected 6-OHDA with an AAV expressing AKAR3 in the dorsal striatum of 4-6-week
358 old mice. In 6-OHDA-lesioned striata, the increase in FRET emission ratio after the application of
359 SKF81927 was significantly higher than in non-lesioned striata (non-lesioned, 4.4 ± 0.3 %, lesioned,
360 7.7 ± 0.3 %, $p < 0.001$, Fig. 4F). In contrast, no significant change was detected after application of
361 CGS21680 (non-lesioned, 4.7 ± 0.2 %, lesioned, 4.9 ± 0.3 %) nor after application of FSK (non-
362 lesioned, 11 ± 0.5 %, lesioned, 10.6 ± 0.4 %, Fig. 4F). There was no significant change in the percentage
363 of cells responsive to the D1R agonist after 6-OHDA lesion (non-lesioned, 44 ± 5 %, lesioned, 50 ± 5 %,
364 Fig. 4G). The lesion did not alter either the percentage of cells responsive to the A_{2A}R agonist (non-
365 lesioned, 52 ± 4 %, lesioned, 54 ± 5 %) or FSK (non-lesioned 94 ± 3 %, lesioned, 98 ± 2 %, Fig. 4G).
366 Together, these results show that PKA responses are specifically amplified in D1R-expressing SPNs
367 after DA denervation by 6-OHDA lesion, with no change in the number of responsive cells. We then
368 investigated the possible mechanism of this amplification.

369

370 *3.5 - G α_{olf} protein contributes to cell type-specific 6OHDA-induced increase in PKA activation*

371 In the dorsal striatum, D1R activates AC through its coupling to G α_{olf} (Corvol et al., 2001; Hervé et al.,
372 1993). Increase of G α_{olf} protein levels in the dorsal striatum has been reported after 6-OHDA-lesion
373 (Alcacer et al., 2012; Hervé et al., 1993; Ruiz-DeDiego et al., 2015) and in the putamen of PD patients
374 (Corvol et al., 2004). Therefore, we used G α_{olf} gene knockout mice to investigate the mechanism of
375 increased PKA activity after 6-OHDA lesion. Homozygous G α_{olf} gene knockout mice (*Gnal^{-/-}*) have a

376 severe phenotype combining olfactory and striatal deficits (Belluscio et al., 1998; Corvol et al., 2001;
377 Zhuang et al., 2000). These mice usually die in the early postnatal period and could not be used in our
378 study. In contrast, *Gnal*^{+/-} mice, which develop and breed normally, provide an interesting model
379 because they display a decrease of about 50% in G α_{olf} protein levels (Alcacer et al., 2012; Corvol et al.,
380 2007).

381 We co-injected saline or 6-OHDA with AAV expressing AKAR3 into the striatum of 4-6 week-
382 old *Gnal*^{+/-} and *Gnal*^{+/+} littermates. When DA innervation was intact, the FRET emission ratio in
383 response to CGS21680 (*Gnal*^{+/+}, 5.2 \pm 0.2%, *Gnal*^{+/-}, 3.6 \pm 0.2%, p<0.001, Fig. 5A) and SKF81297
384 application (*Gnal*^{+/+}, 5.9 \pm 0.2%, *Gnal*^{+/-}, 3.6 \pm 0.3%, p<0.001, Fig. 5B) was lower in *Gnal*^{+/-} mice than
385 in wild type littermates. No change was detected after application of FSK (*Gnal*^{+/+}, 11.4 \pm 0.3%, *Gnal*^{+/-},
386 11.5 \pm 0.2%) meaning that AC was not altered and could still be directly activated by FSK in the *Gnal*^{+/-}
387 mice (Fig. 5C). No significant change was observed in the percentage of responsive cells in *Gnal*^{+/-}
388 mice after CGS21680 (*Gnal*^{+/+}, 36 \pm 3%, *Gnal*^{+/-}, 39 \pm 2%), SKF81297 (*Gnal*^{+/+}, 50 \pm 3%, *Gnal*^{+/-}, 48 \pm
389 2%), or FSK (*Gnal*^{+/+}, 91 \pm 1%, *Gnal*^{+/-}, 92 \pm 2%). Our results show that the activation of AC by D1R
390 or A2_AR is markedly impaired when G α_{olf} protein is reduced, leading to a decreased PKA activation in
391 both populations of SPNs. Our results are in contrast to a study in young mice (P8-12) that do not show
392 any change in PKA responses in *Gnal*^{+/-} mice (Castro et al., 2013). This is likely due to the fact that the
393 G α_s /G α_{olf} switch has not yet fully taken place at P8-12 (Iwamoto et al., 2004) and that AC responses to
394 D1R or A2_AR agonists in young mice are less dependent on G α_{olf} levels.

395 In 6-OHDA-lesioned *Gnal*^{+/-} mice no modification of the FRET emission ratio was observed
396 when CGS21680 was applied when compared to non-lesioned *Gnal*^{+/-} mice (non-lesioned, 3.7 \pm 0.2%,
397 lesioned, 3.6 \pm 0.3%, Fig. 5A). In contrast, SKF81297 increased the FRET emission ratio to a higher
398 level in 6-OHDA-lesioned *Gnal*^{+/-} mice than in non-lesioned mutant mice (non-lesioned, 3.6 \pm 0.2%,

399 lesioned, $6.1 \pm 0.5\%$, $p < 0.001$, Fig. 5B). However, this increase in FRET response did not reach the
400 level attained in 6-OHDA-lesioned striata from wild type mice ($7.8 \pm 0.3\%$ in 6-OHDA-lesioned wild
401 type striata, indicated by a green dashed line in Fig. 5B). No significant change was observed in the
402 FRET emission ratio after FSK application (non-lesioned, $11.1 \pm 0.3\%$, lesioned, $11.9 \pm 0.5\%$, Fig. 5C).
403 In addition, the percentage of responsive cells was unaffected by the lesion in *Gnal*^{+/-} mice after
404 CGS21680 (non-lesioned, $39 \pm 2\%$, lesioned, $42 \pm 2\%$), SKF81297 (non-lesioned, $48 \pm 2\%$, lesioned,
405 $45 \pm 2\%$), or FSK (non-lesioned, $92 \pm 1\%$, lesioned, $89 \pm 2\%$).

406 These results suggest that in 6-OHDA-lesioned animals, increased PKA activity after D1R
407 stimulation is compatible with an increase in $G\alpha_{olf}$ levels in D1R-expressing SPNs. This increase is cell
408 type-specific because no modification was observed in A2_AR-expressing SPNs.

409

410 *3.6 - Cell type-specific decrease in PDE activity contributes to DA lesion-induced increase in PKA*

411 PDEs are important negative regulators of PKA activity. Regulation of the striatal expression of PDEs
412 has been reported in PD patients and animal models of PD, particularly a down-regulation of PDE4 and
413 PDE10 (Heckman et al., 2018; Niccolini et al., 2015, 2017). Hence, we tested if PDE activity could be
414 implicated in the cell type-specific upregulation of PKA activity observed after DA denervation. We
415 examined the effects of application of a broad-spectrum PDE inhibitor, 3-isobutyl-1-methylxanthine
416 (IBMX). We first observed that, as expected, AKAR3 was activated by IBMX in SPNs of adult non-
417 lesioned mice in a dose dependent-manner (Fig. 5D). These results were similar to those previously
418 reported in immature mice (Polito et al., 2015). They showed that in our conditions, cAMP was
419 tonically produced in striatal slices and that PDEs constantly degraded it.

420 We then investigated PKA responses in adult striatal slices in the presence of a low
421 concentration of IBMX ($30 \mu\text{M}$ for 10 min), which had no effects on basal FRET emission ratio (Fig.

422 5D). At this concentration, IBMX enhanced the PKA responses to CGS21680 (no IBMX, $5.4 \pm 0.3\%$,
423 IBMX $9.3 \pm 0.5\%$, $p < 0.001$, Fig. 5E), SKF81297 (no IBMX, $5.0 \pm 0.3\%$, IBMX $9.0 \pm 0.3\%$, $p < 0.001$,
424 Fig. 5F) and FSK (Fig. 5G and H). This confirmed that PDE activity exerted a strong negative tuning
425 on PKA responses in adult SPNs. In 6-OHDA-lesioned animals, IBMX ($30 \mu\text{M}$) also increased the
426 amplitude of FRET emission ratio in response to CGS21680 (no IBMX, $4.1 \pm 0.3\%$, IBMX, $8.2 \pm$
427 0.3% , $p < 0.001$, Fig. 5E). In contrast, it did not further increase the response to SKF81297 (no IBMX,
428 $7.5 \pm 0.3\%$, IBMX $8.2 \pm 0.5\%$, Fig. 5F). Pretreatment with IBMX did not alter the proportion of
429 responsive cells to the D1R agonist in the 6-OHDA-lesioned striata as compared to non-lesioned ones
430 (non-lesioned, no IBMX, $39 \pm 7\%$, IBMX, $53 \pm 4\%$; lesioned, no IBMX, $48 \pm 6\%$, IBMX, $54 \pm 5\%$) or
431 $A_{2A}R$ agonist-responsive SPNs (non-lesioned, no IBMX, $44 \pm 7\%$, IBMX, $49 \pm 9\%$, lesioned, no
432 IBMX, $49 \pm 3\%$, IBMX, $48 \pm 4\%$). Our data show a loss of responsiveness of PKA activity to a PDE
433 inhibitor in D1R agonist-responsive SPNs after 6-OHDA lesion, but not in $A_{2A}R$ -responsive SPN.
434 Even if the global response to FSK was apparently not changed in lesioned SPNs (see Fig. 4F), we
435 analyzed separately FSK-induced PKA activity in the two types of SPNs to test whether the loss of
436 responsiveness to IBMX was restricted to the D1R pathway or was more generalized in D1R-
437 expressing- SPNs. Pretreatment with IBMX, increased the responses to FSK in the $A_{2A}R$ agonist-
438 responsive SPNs in non-lesioned (no IBMX, $8.9 \pm 0.4\%$, IBMX, $12.4 \pm 0.8\%$, $p < 0.001$) and 6-OHDA-
439 lesioned slices (no IBMX, $8.2 \pm 0.4\%$, IBMX, $13.7 \pm 0.5\%$, $p < 0.001$, Fig. 5G). The pretreatment with
440 IBMX also increased the responses to FSK in the D1R-expressing SPNs of non-lesioned slices (no
441 IBMX, $9.6 \pm 0.5\%$, IBMX, $11.3 \pm 0.4\%$, $p < 0.05$, Fig. 5H). However, the effect of IBMX was not
442 observed in the 6-OHDA-lesioned slices (no IBMX, $11.3 \pm 0.5\%$, IBMX, $10.7 \pm 0.4\%$, Fig. 5H and
443 Table S1). This suggested an occlusion of the effect of IBMX by the lesion of DA neurons. The lack of
444 effect of the PDE inhibitor specifically in D1R-responsive neurons, following 6-OHDA lesion can be

445 explained by a decrease in PDE activity specifically in these neurons. This change in PDE activity is
446 likely to contribute to the enhanced PKA activation in response to D1R or AC stimulation.

447

448 *3.7 - Spontaneous Ca²⁺ transient activity is increased in 6-OHDA-lesioned D1R-expressing striatal*
449 *neurons*

450 Intracellular Ca²⁺ increase has been implicated in ERK activation in many models ranging from *C.*
451 *elegans* (Tomida et al., 2012) to CA1 pyramidal neurons in rodents (Zhai et al., 2013) and DA-
452 denervated SPNs (Fieblinger et al., 2014a). Hence, we investigated intracellular free Ca²⁺ in striatal
453 neurons of non-lesioned and 6-OHDA-lesioned mice using the biosensor GCaMP6s. We co-injected 6-
454 OHDA or vehicle with an AAV expressing GCaMP6s (Chen et al., 2013) in the dorsal striatum of 4-6-
455 week old mice, and 3-4 weeks later, acute striatal slices were imaged under a 2-photon microscope. In
456 our experimental conditions, some SPNs were spontaneously active and showed transient increases in
457 intracellular Ca²⁺ detected by the normalized fluorescence ratio ($\Delta F/F_0$). We therefore sorted the striatal
458 neurons on the basis of their spontaneous activity during the baseline recording period into two
459 categories, as described in the Methods section, spontaneously active and non-spontaneously active
460 SPNs (Fig. 6A). The number of spontaneously active SPNs was higher in 6-OHDA-lesioned than in
461 non-lesioned striata (non-lesioned, 13.1%, n= 274/2085, lesioned, 18.2%, n= 319/1748, p<0.001, Fig.
462 6B). To determine whether this higher spontaneous activity affected dSPNs and/or iSPNs, we
463 microinjected an AAV Cre-dependently expressing GCaMP6s (AAV-flex-GCaMP6s), into the striatum
464 of *Drd1::Cre* (*D1Cre*) and *Adora2_A::Cre* (*A2_ACre*) mice. In the 6-OHDA-lesioned *D1Cre* mice, cells
465 expressing GCaMP6s were spontaneously more active than in the non-lesioned animals (non-lesioned,
466 11.3%, n= 58/512, lesioned, 19.7%, n= 179/910, p<0.001, Fig. 6B). In contrast, in the *A2_ACre* mice, no
467 significant difference was observed between lesioned and non-lesioned striata (non-lesioned 15.4%, n=

468 80/518, lesioned, 18.2%, n= 69/379, Fig. 6B). These results suggested that a higher proportion of
469 dSPNs had spontaneous Ca²⁺ activity in DA-depleted striatum.

470

471 *3.8 - Specific enhancement of AMPA-induced intracellular Ca²⁺ dynamics in A_{2A}R-expressing striatal*
472 *neurons after 6-OHDA lesion*

473 As mentioned above, co-application of AMPA and SKF81297 activated ERK in a larger number of
474 neurons in the 6-OHDA-lesioned striatum than in the intact striatum (see Fig. 3B). These effects could
475 be indicative of alterations in Ca²⁺ responses to AMPAR stimulation in the DA-denervated striatum. To
476 address this question, we monitored intracellular Ca²⁺ dynamics following a 30 s AMPA (0.5 μM)
477 application in 6-OHDA-lesioned and control mice. We focused our analysis on the non-spontaneously
478 active SPNs because spontaneous activity rendered the drug-induced Ca²⁺ responses difficult to
479 evaluate. In the non-lesioned animals, AMPA-induced Ca²⁺ responses, evaluated by the normalized
480 fluorescence ratio, were very variable from one neuron to another, but the average response was small
481 (Fig. 7A middle panel). In contrast, in the 6-OHDA-lesioned animals, we observed a prolonged
482 increase of the fluorescence ratio in response to AMPA application, showing an overall increase in Ca²⁺
483 responses (area under the curve [AUC]: non-lesioned $1.97 \pm 0.26 \times 10^3$ %·s, lesioned, $12.55 \pm 0.63 \times 10^3$
484 %·s, Fig. 7A right lower panel). When KCl (25 mM, 30 s) was applied at the end of all experiments, it
485 produced a general and transient activation of virtually all neurons expressing GCaMP6s in the slices
486 (Fig. 7A-C left panels). This stimulation allowed us to test the viability of striatal neurons in brain
487 slices and also to determine the total number of responsive cells and, thus, the percentage of cells
488 responsive to the application of AMPA. This calculation revealed a significant increase in the
489 percentage of responsive cells following 6-OHDA lesion (non-lesioned 44.2%, n=206/466, lesioned,
490 and 61.2%, n= 120/196, Fig. 7A right upper panel).

491 To determine in which SPN population(s) the increase in AMPA-induced Ca^{2+} transients
492 occurred in the DA-denervated striatum, we first microinjected AAV-flex-GCaMP6s into the striatum
493 of *D1Cre* mice. In these mice the lesion did not modify the AMPA-induced increase in normalized
494 fluorescence ratio (AUC: non-lesioned, $5.06 \pm 0.93 \times 10^3$ %s, lesioned, $3.50 \pm 0.52 \times 10^3$ %s, Fig. 7B
495 right lower panel). In addition, the percentage of responsive cells was not changed (non-lesioned,
496 23.8%, n=62/261, lesioned, 25.6%, n= 103/403, Fig. 7B right upper panel).

497 We then microinjected AAV-flex-GCaMP6s into the striatum of *A2_ACre* mice to selectively
498 study the iSPNs. In these mice, AMPA markedly increased the normalized fluorescence ratio in 6-
499 OHDA-lesioned compared to non-lesioned mice (AUC: non-lesioned, $7.62 \pm 1.04 \times 10^3$ %s, lesioned,
500 $18.38 \pm 3.14 \times 10^3$ %s, Fig. 7C right lower panel). In addition, the percentage of AMPA-responsive
501 iSPNs was strongly enhanced (non-lesioned, 57.8%, n=52/90, lesioned, and 83.9%, n= 47/56, Fig. 7C
502 right upper panel). These results indicated a pronounced iSPN-specific increase in AMPA-induced
503 intracellular Ca^{2+} transients after 6-OHDA lesion.

504 In conclusion, this series of experiments shows that 6-OHDA lesion increases the amplitude of
505 AMPA-induced Ca^{2+} responses and the number of AMPA-responsive cells, and that this effect is
506 selectively taking place in *A2_AR*-expressing cells, presumably iSPNs.

507

508 **4 - Discussion**

509 Our work reveals the spatiotemporal dynamics of Ca^{2+} , PKA and ERK signaling using multiphoton
510 biosensor imaging in the DA-denervated striatum of adult mice. Our results show that 6-OHDA lesion
511 increases ERK and PKA activation in response to D1R stimulation. The increased activation of PKA
512 results at least in part from an increase in $G\alpha_{\text{olf}}$ combined with a deficit in phosphodiesterase activity
513 selectively in dSPNs. Monitoring Ca^{2+} signals revealed that the spontaneous Ca^{2+} transients are
514 increased in D1R-expressing dSPNs of the DA-denervated striatum. In contrast, although their

515 spontaneous activity is unchanged, the Ca^{2+} transients induced by stimulation of AMPA glutamate
516 receptors in iSPNs is highly increased. Our work reveals distinct cell type-specific signaling alterations
517 in the two populations of SPNs and suggests possible mechanisms for these alterations.

518

519 4.1 - The activity of D1R- $G\alpha_{\text{olf}}$ -PKA pathway is increased in dSPNs after 6-OHDA lesion

520 D1R signal transduction in SPNs is mediated by $G\alpha_{\text{olf}}$, the G protein that activates AC in these neurons
521 (Corvol et al., 2001; Hervé et al., 1993; Zhuang et al., 2000). We found that after DA denervation the
522 PKA response was specifically amplified in SPNs responsive to a D1R agonist, presumably dSPNs.
523 Studies in transgenic mice expressing GFP under the control of the D1R gene promoter have shown
524 that the D1-SPNs project to the *substantia nigra pars reticulata* and *globus pallidus pars interna*
525 (reviews in Gerfen and Surmeier, 2011; Valjent et al., 2009). In contrast less than 1% of striatonigral
526 neurons express D2R (Matamales et al., 2009). However, in mice expressing GFP under the control of
527 D1R promoter, a low GFP-positive innervation was also observed in the *globus pallidus pars externa*
528 (Cazorla et al., 2014; Matamales et al., 2009) corresponding to axon collaterals of dSPNs (Cazorla et
529 al., 2015; Parent et al., 2000). Finally, in the dorsal striatum the of SPNs expressing both D1Rs and
530 D2Rs, is very low, less than 2% (Gagnon et al., 2017). Therefore it is possible to conclude that, in the
531 mouse dorsal striatum, D2-SPN are virtually exclusively iSPN while D1-SPNs are dSPNs but they can
532 contribute a minor component of the indirect pathway, mostly as collateral projection.

533 The PKA pathway upregulation did not occur in the iSPNs since no modification of PKA
534 signaling was observed after $A2_A$ R stimulation. This can account for the previously reported enhanced
535 phosphorylation of PKA substrates, DARPP-32 Thr-34 and GluA1 Ser-845, after acute administration
536 of L-DOPA (Santini et al., 2007). Striatal levels of D1R (Hurley et al., 2001) and other mediators of
537 D1R signaling (Girault et al., 1989) show no major modifications after DA denervation. In contrast, the

538 levels of $G\alpha_{olf}$ are increased in the striatum of DA-denervated rodents and in postmortem samples from
539 PD patients (Alcacer et al., 2012; Corvol et al., 2004; Ruiz-DeDiego et al., 2015). DA lesion selectively
540 increases $G\alpha_{olf}$ amounts associated with D1Rs, leaving unaffected those associated with $A2ARs$
541 (Morigaki et al., 2017). Striatal $G\alpha_{olf}$ levels are regulated by DA and D1R utilization, presumably
542 through post-translational mechanisms (Hervé et al., 2001; Ruiz-DeDiego et al., 2015). In *Gnal^{+/-}* mice,
543 which display a decrease of $\approx 50\%$ in $G\alpha_{olf}$ protein levels (Alcacer et al., 2012; Corvol et al., 2007), we
544 found a decrease in PKA activation in response to D1R agonist confirming that $G\alpha_{olf}$ is a rate-limiting
545 factor for the D1R-dependent cAMP/PKA pathway activation (Corvol et al., 2007). The PKA response
546 to the D1R agonist was increased in 6-OHDA-lesioned *Gnal^{+/-}* mice as compared to non-lesioned
547 mutant mice, but remained lower than in 6-OHDA-lesioned wild type mice, in agreement with previous
548 biochemical results (Alcacer et al., 2012). Our present observations combined with previous results
549 show that increased striatal $G\alpha_{olf}$ levels are an important factor leading to sensitized PKA responses to
550 D1R stimulation in the DA-denervated striatum. Importantly, such $G\alpha_{olf}$ upregulation was detected in
551 the putamen of PD patients (Corvol et al., 2004) showing that similar pathological processes occur in
552 human. Our study identifies another factor contributing to enhanced cAMP signaling in dSPNs
553 following DA lesion, namely a selective decrease in PDE activity. PDEs are a family of enzymes that
554 degrade cAMP and/or cGMP, participate in the regulation of their intracellular levels, and directly
555 contribute to the spatial and temporal dynamics of cAMP/PKA pathway in neurons (Castro et al., 2010;
556 Gervasi et al., 2007, 2010). In adult striatal neurons, cAMP is constantly produced by ACs and
557 degraded by PDEs since phosphodiesterase inhibition with IBMX enhances cAMP levels and activates
558 PKA in both dSPNs and iSPNs, as revealed by an increase of AKAR3 FRET fluorescence in our study.
559 In non-lesioned striatum a low concentration of IBMX (30 μM), devoid of effect by itself, enhanced
560 AKAR3 signal in response to stimulation of D1R, $A2AR$ or AC. However, this effect was specifically

561 lost in D1R-responsive neurons after DA denervation. A possible explanation of this loss is that the
562 effects of 30 μ M IBMX were occluded by a preexisting decrease in endogenous PDE activity. This
563 putative decrease in PDE activity only occurred in D1R-expressing SPNs since the low concentration
564 of PDE inhibitor remained effective in the A_{2A}R-expressing neurons of 6-OHDA-lesioned striatum,
565 increasing the PKA responses to A_{2A}R agonist or forskolin. Several PDE families are expressed in the
566 striatum, including PDE1, PDE4, and PDE10, and play a critical role in modulating cAMP-mediated
567 DA signaling. Knockout of PDE1B in mice increases locomotor activity and responses to DA agonists
568 (Ehrman et al., 2006), but in 6-OHDA-lesioned mice an upregulation of PDE1B was reported
569 (Sancesario et al., 2004). In contrast, studies in PD patients point to a reduced expression of PDE10A
570 which correlates with PD duration and severity of motor symptoms (Niccolini et al., 2015) and a
571 decrease in PDE4 (Niccolini et al., 2017). However, in the striatum, PDE4 is predominantly active in
572 DA terminals, regulating TH phosphorylation (Nishi et al., 2008). The improvement observed in PD
573 following treatment with a PDE4 inhibitor (Rolipram) was attributed to a protective effect on DA
574 neuron degeneration (Yang et al., 2008). In rodents, PDE10A mRNA and protein levels are decreased
575 in 6-OHDA-lesioned striatum (Giorgi et al., 2008). This reduction in PDE10A levels is associated with
576 higher cAMP-dependent phosphorylation in response to D1R stimulation (Mango et al., 2014). Thus,
577 decreased PDE10A activity is a strong candidate to explain our observations and further work is needed
578 to test this hypothesis.

579 Although not investigated in the present study, changes in ACs may also contribute to the
580 increased cAMP responses in DA-lesioned mice. Among the ten different ACs, AC5 is the most
581 abundant subtype in the striatum (Iwamoto et al., 2004). Work in rat showed that DA denervation
582 increases activity and expression of AC5 in the striatum (Rangel-Barajas et al., 2011). In conclusion,
583 the combination of several alterations, including increases in G α_{olf} and AC5 and decrease in PDE

584 activity may account for the increased responsiveness of the PKA pathway in D1R-expressing SPNs
585 following dopaminergic lesion.

586 4.2 - Differential changes in intracellular Ca^{2+} transients in SPNs after 6-OHDA lesion

587 In this study, we observed that in DA-denervated striatal slices SPNs displayed more spontaneous
588 intracellular Ca^{2+} transients than in non-lesioned slices, a modification that occurred predominantly in
589 dSPNs. It has been previously shown that spontaneous Ca^{2+} transients and firing rate of SPNs are
590 enhanced after DA denervation (Jáidar et al., 2010), without identification of the SPN population. This
591 increased activity could be linked to the elevated intrinsic excitability of dSPN after 6-OHDA lesion,
592 attributed to a possible homeostatic response to the loss of excitatory D1R signaling (Fieblinger et al.,
593 2014b; Suárez et al., 2014; Suarez et al., 2016, 2018). It is noteworthy that the intrinsic excitability is
594 higher in iSPNs than in dSPNs in basal conditions, which correlates with a more frequent occurrence of
595 Ca^{2+} transients in iSPNs than in dSPNs observed in the non-lesioned striata (Gertler et al., 2008). It was
596 proposed that DA denervation reduces this difference between excitability of dSPNs and iSPNs, mostly
597 by increasing dSPN excitability (Maurice et al., 2015). Accordingly, in our experiments the proportions
598 of spontaneously active neurons in the dSPN and iSPN populations appeared to equalize following DA
599 denervation.

600 The increased spontaneous activity of dSPNs in *ex vivo* corticostriatal slices contrasts with *in*
601 *vivo* observations. A recent study reported that following 6-OHDA lesion, dSPN activity in awake mice
602 was decreased while iSPN activity was increased when animals were immobile (Ryan et al., 2018).
603 These observations confirmed and expanded previous reports in anaesthetized rodents (Mallet et al.,
604 2006). The increased basal activity of iSPNs *in vivo* is attributed to their enhanced sensitivity to cortical
605 inputs (Escande et al., 2016; Mallet et al., 2006; Ryan et al., 2018). In our slice study we observed an
606 increase in AMPA-induced intracellular Ca^{2+} responses specifically in iSPNs of lesioned mice, without

607 change in dSPN. These findings are in agreement with several works that identified corticostriatal
608 synaptic reorganization following lesion of DA neurons. Indeed, DA denervation induces a pruning of
609 cortical synapses associated with an increase of dendritic excitability specifically in iSPN, resulting in
610 an enhancement of the average amplitude of corticostriatal synaptic responses (Fieblinger et al.,
611 2014b). iSPNs express functional Ca^{2+} -permeable AMPA receptors at corticostriatal synapses and
612 AMPA receptor subunit phosphorylation, trafficking, and alternative splicing are enhanced in animal
613 models of PD, possibly contributing to an enhanced function of AMPA receptors (Ba et al., 2011;
614 Kobylecki et al., 2013). These various modifications could provide potential mechanisms for the
615 enhancement of AMPA-induced intracellular Ca^{2+} transients we observed in iSPNs. Increased Ca^{2+}
616 transients can have numerous impacts on neuronal function including regulation of synaptic strength,
617 cellular excitability, and gene expression, as well as modulation of calcium-activated potassium
618 channels that control the duration and intervals of action potentials (Trusel et al., 2015). Synaptic
619 plasticity in iSPN has been shown to be calcium-dependent (Trusel et al., 2015). Inflammation
620 increases AMPA responses through Ca^{2+} -permeable AMPA receptors and voltage-gated calcium
621 channels specifically in iSPNs of the dorsal striatum (Winland et al., 2017). Since DA lesion triggers an
622 inflammatory response (Cicchetti et al., 2002), it will be important to examine its contribution to the
623 altered responsiveness of iSPNs.

624 It is remarkable that although our understanding of the basal ganglia circuits is much more
625 complete and complex than it was 30 years ago, the in vivo results in 6-OHDA-lesioned mice as well
626 as our observations in slices are consistent with the model proposed for primates by DeLong in which
627 the loss of striatal DA resulted in an increase in transmission through the indirect pathway (DeLong,
628 1990).

629 *4.3 - ERK activity is increased after 6-OHDA lesion*

630 Phosphorylation of ERK is triggered in neurons by various external stimuli, including
631 neurotransmitters and growth factors, leading to a wide range of plastic responses through activation of
632 cytosolic and nuclear targets [reviews in (Girault, 2012)]. In the striatum ERK activation is essential for
633 instrumental learning (Shiflett et al., 2010) and long-lasting effects of addictive drugs (Valjent et al.,
634 2000). Conditional deletion of ERK1/2 in dSPNs or iSPNs induces pathway-specific alterations in
635 motor function, synaptic properties, and plasticity-related gene expression, emphasizing the importance
636 of ERK for SPNs function (Hutton et al., 2017). In non-lesioned animals D1R activation leads to a
637 modest activation of ERK in SPNs (Fieblinger et al., 2014a; Gerfen et al., 2002). In contrast, in DA-
638 lesioned mice, treatment with D1R agonists or L-DOPA results in pronounced and sustained activation
639 of ERK that depends on the canonical PKA signaling pathway and MEK1/2 (Gerfen et al., 2002)
640 (Darmopil et al., 2009; Fieblinger et al., 2014a; Pavón et al., 2006; Santini et al., 2007). Our imaging
641 experiments with ERK biosensor confirm the limited responses to D1R agonist in non-lesioned striata
642 and the upregulation of ERK responses after 6-OHDA lesion. ERK activation was detected in close to
643 half of the cells in both conditions indicating that in the DA-denervated striatum, the increase in D1R-
644 induced ERK responses is mostly attributable to an increased response in a specific set of SPNs, and
645 not to the recruitment of additional SPNs. In hippocampal neurons, ERK can be activated after
646 glutamate receptor stimulation through increases in intracellular calcium (Zhai et al., 2013). In the
647 striatum, we showed that pharmacological AMPA receptor stimulation, mimicking cortical or thalamic
648 glutamatergic inputs, can also activate ERK in a small population of SPNs. This is in agreement with
649 previous reports showing that excitatory glutamatergic synaptic transmission and corticostriatal
650 stimulation activate ERK in the striatum, mainly in iSPNs (Gerfen et al., 2002; Sgambato et al., 1998).
651 AMPA-induced ERK activation was increased after DA-denervation. Since our imaging study
652 indicated that AMPA-induced intracellular Ca^{2+} increase was limited to iSPN after 6-OHDA lesion, we
653 can hypothesize that ERK activation by AMPA is due to Ca^{2+} increase in these neurons. This hypothesis

654 is in line with previous findings by Gerfen and colleagues who found that after DA-denervation,
655 corticostriatal stimulation elicited ERK activation in iSPN, identified by histochemical localization of
656 enkephalin mRNA (Gerfen et al., 2002). In D1R-expressing dSPNs, Ca²⁺ and cAMP signaling
657 pathways synergize to activate ERK in response to addictive drugs and, possibly in physiological
658 circumstances, leading to long-term changes (Girault et al., 2007), including modification of neuronal
659 excitability, changes in activity-induced gene expression and modulation of dendritic spine density
660 (Cerovic et al., 2013). In our experiments, the combination of D1R and AMPA receptor agonists,
661 produced an ERK activation of comparable amplitude as the D1R agonist alone, in non-lesioned or
662 DA-denervated mice. This is in agreement with data indicating that D1R agonist-induced activation of
663 ERK signaling in DA-denervated striatum is not completely depending on ionotropic glutamate
664 receptors (Fieblinger et al., 2014a; Gerfen et al., 2002). However, the use of ERK imaging at the single
665 cell level allowed us to detect an increase in the number of responsive cells. When AMPA and D1R
666 agonist were co-applied, ERK was activated in 43% of the SPNs in the non-lesioned striatum and 67%
667 after 6-OHDA lesion. This result implies that ERK activation took place in the two SPN populations
668 after 6-OHDA lesion.

669 *4.4 - Conclusions*

670 Our work using 2-photon biosensor imaging in the DA-denervated striatum of adult mice underlines
671 the complex signaling dysregulations in SPNs in the absence of DA inputs. It reveals distinct cell type-
672 specific alterations of cAMP, Ca²⁺ and ERK responses in the two populations of SPNs. These results
673 emphasize the need to take into consideration these differences for the development of treatments in PD
674 and the importance of acting both dSPNs and iSPNs for the normalization of signaling pathway
675 dynamics after DA denervation.

676

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693

694 **Legends**

695

696 **Fig 1. Single-cell spatiotemporal dynamics of ERK activity in SPNs in culture and brain slices.**

697 In all cases, ERK responses were imaged by two-photon microscopy of the indicated FRET biosensor.

698 (A) Comparison of three ERK biosensors in cultured striatal neurons. EKAR_{cyto}, EKAR2G1 or EKAR-

699 EV biosensors were transfected into striatal neurons in culture (DIV 7) using Lipofectamine 2000.

700 Twenty-four hours after lipofection, brain-derived neurotrophic factor (BDNF, 10 ng/mL) was bath-

701 applied for 5 min. **Left panel**, representative FRET images of ERK biosensors in neurons before

702 (Baseline) and after stimulation with BDNF. All images are pseudo-colored according to the same

703 FRET scale to show the differences in response amplitudes across biosensors. **Middle panel**,

704 representative time course traces and **right panel**, maximal amplitude responses ($\Delta R/R_0$ in %) of the

705 indicated ERK biosensors. **p < 0.01 for EKAR2G1 versus EKAR-EV (Kruskal–Wallis test followed

706 by Dunn's test, see Table S1). **(B)** ERK imaging in striatal slice preparations from neonatal mice (P8-
707 P12) using a recombinant Sindbis virus expressing EKAR-EV biosensor. ERK responses were recorded
708 16-20 h after viral infection. **Left panel**, two-photon image of YFP channel obtain with a maximal
709 projection of a z stack in the dorsal striatum (top left) and representative FRET images of EKAR-EV
710 before (Baseline, a), 300 s (b) after the beginning of the stimulation with KCl (25 mM, 1 min) and after
711 the recovery (c). **Middle panel**, representative time course of normalized FRET ratio following 25 mM
712 KCl application (indicated by a horizontal bar) experiment (a, b and c indicate the time at which left
713 panel pictures were taken). **(C)** Maximal amplitude responses to AMPA (5 μ M, 30 s) and KCl (25 mM,
714 1 min) responses. **(D-E)** ERK activation in adult mice striatal slices. Recombinant AAV virus
715 expressing EKAR-EV was stereotaxically injected into the striatum 3-5 weeks before imaging. **(D)**
716 Two-photon image of YFP channel with maximal projection of a z-stack in the dorsal striatum (left
717 pictures) and representative FRET images of EKAR-EV biosensor before (Baseline, a) and after
718 stimulation with SKF81297 (SKF, 10 μ M, b) and KCl (25 mM, c) in ACSF (Control, upper row), or in
719 the presence of a MEK inhibitor (U0126, 5 μ M, lower row). **(E)** Representative time course of a typical
720 FRET experiment in control condition (red) and with U0126 (black). a, b and c indicate the time at
721 which pictures in **D** were taken. **(F)** Maximal amplitudes of FRET ratio responses after application of
722 ACSF (30 s), SKF81297 (10 μ M, 30 s) and KCl (25 mM, 30 s). * $p < 0.05$ for SKF81297 versus
723 SKF81297+U0126 and *** $p < 0.001$ for KCl versus KCl+U0126 (Kruskal–Wallis test followed by
724 Dunn's test, see Table S1). In **A**, **B**, and **E** lines represent the mean value and shaded envelopes indicate
725 SEM. Scale bars, 20 μ m.

726

727

728 **Fig 2. Biosensor and tyrosine hydroxylase expression after DA-denervation in the dorsal**
729 **striatum.**

730 (A) General experimental design. Mice (4-6-week-old) were injected with a solution containing the
731 biosensor-expressing AAV with or without 6-OHDA in the right striatum. After a 4-week recovery, 2-
732 photon imaging was performed on acute parahorizontal corticostriatal slices. At the end of each
733 experiment, striata were homogenized for TH quantification by immunoblot. (B) Example of
734 immunoblot and quantification of TH levels by immunoblotting. Data are expressed as percentage of
735 the mean in non-lesioned (NL) side and are means \pm SEM. *** $p < 0.001$ for NL versus L side (paired t-
736 test, see Table S1). (C) Stereotaxic co-injection with 6-OHDA of AAV viruses encoding biosensors did
737 not modify biosensor expression. Wide field image of the dorsal striatum in Dodt gradient contrast
738 mode (upper left panel) and in the YFP channel (lower left panel). Scale bar 100 μm . Two-photon
739 images of the dorsal striatum in the YFP excitation/emission channel in mice injected with AAV-
740 AKAR3, EKAR-EV and GCaMP6s with and without 6-OHDA. Scale bar 20 μm .

741

742 **Fig 3. ERK activity induced by a D1 agonist and AMPA is increased after 6-OHDA lesion in the**
743 **striatum**

744 (A) Time course of responses to SKF81297 (SKF, 10 μM , 30 s), AMPA (2.5 μM , 30 s) and
745 SKF+AMPA (30 s) in non-lesioned and 6-OHDA lesioned striatum. Gray bars represent the drug
746 application time. The non-specific FRET ratio responses to drug application were removed for a better
747 visualization of normalized ratio changes. (B) ERK maximal amplitude response after application of
748 ACSF (30 s), SKF (10 μM , 30 s), AMPA (2.5 μM , 30 s) and KCl (25 mM, 30 s) in 6-OHDA-lesioned
749 (+) and non-lesioned (-) corticostriatal slices (drugs were applied on different slices except for KCl that

750 was added at the end of all the experiments). Mann-Whitney test, see Table S1. (C) Percentage of
751 responsive cells among the total number of EKAR-EV-expressing cells, after the indicated treatments
752 as in **B**, in 6-OHDA-lesioned (+) and control (-) striatal slices. Mann-Whitney test, see Table S1. *p <
753 0.05, ***p<0.001.

754

755 **Fig 4. PKA responses to D1R stimulation are increased in dSPNs after 6-OHDA lesion in the**
756 **striatum**

757 **(A-B)** Identification of putative dSPNs and iSPNs in the dorsal striatum using AKAR3 PKA biosensor
758 2-photon imaging. **(A)** Representative FRET pseudocolor images of AKAR3 before (Baseline, a) and
759 after stimulation with an A_{2A} agonist, CGS21680 (CGS, 10 μM, b), then a D1 agonist, SKF81297
760 (SKF, 10 μM, c) and forskolin (FSK, 10 μM, d). Scale bar, 20 μm. **(B)** Time course of a typical
761 AKAR3 experiment. After recording a FRET baseline, sequential application of CGS and SKF was
762 used to activate PKA signaling. Based on their agonist selective responses, neurons were classified as
763 A_{2A}R-expressing SPNs (red) and D1R-expressing SPNs (blue). At the end of the experiment FSK was
764 applied to directly activate AC in both types of neurons. a, b, c and d indicate the time at which pictures
765 in **A** were taken. Traces for cells responsive to CGS or SKF were separated with different y axes, but
766 they correspond to neurons in the same field and the time x axis is the same. **(C)** AKAR3 maximal
767 responses after application of ACSF, CGS21680 (10 μM), SKF81297 (10 μM), and FSK (10 μM). The
768 order of SKF and CGS application was alternated between slices. Only neurons in which the AKAR3
769 response to the specific drug or FSK was significant were considered. Kruskal–Wallis followed by
770 Dunn’s test, see Table S1. **(D)** Percentage of responsive cells after treatments as in **B**. Kruskal–Wallis
771 followed by Dunn’s test, see Table S1. **(E)** Rise time (10-90%) of the same treatments as in **B**. Kruskal–

772 Wallis followed by Dunn's test, see Table S1. **(F-G)** Comparison of AKAR3 responses between non-
773 lesioned and 6-OHDA-lesioned striatal slices. **(F)** Maximal FRET emission ratio responses after
774 application of ACSF, CGS21680 (10 μ M), SKF81297 (10 μ M), and FSK (10 μ M) in non-lesioned mice
775 (-, light color) and 6-OHDA-lesioned slices (+, dark color). Mann-Whitney test, see Table S1. **(G)**
776 Percentage of responsive cells among the total number of AKAR3-expressing cells. Same conditions as
777 in **F**. **(C-G)** Error bars indicate SEM, statistical significance of pairwise comparisons, * $p < 0.05$,
778 *** $p < 0.001$.

779

780 **Fig 5. Role of $G\alpha_{\text{olf}}$ and PDEs in the upregulation of PKA response to D1R agonist in the 6-**
781 **OHDA-lesioned striatum**

782 **(A-C)** AKAR3 responses in A2AR-agonist- and D1R-agonist responsive SPNs in *Gnal* heterozygous
783 mice. **(A)** Maximal AKAR3 FRET emission ratio in response to CGS21680 (CGS, 10 μ M) in *Gnal*^{+/-}
784 heterozygous mice (expressing 50% of the normal $G\alpha_{\text{olf}}$ levels) without (-) or with (+) 6-OHDA lesion
785 and non-lesioned wild type littermates (*Gnal*^{+/+}) (as in figure 4F). For comparison, FRET emission ratio
786 response observed in the 6-OHDA-lesioned striatum of wild type animals (data from figure 3F) are
787 indicated by a green dashed line. One-way ANOVA followed by Tukey's test (see Table S1). **(B)** Same
788 as in **A**, but in response to SKF81297 (SKF, 10 μ M). **(C)** Same as in **A** but in response to forskolin
789 (FSK, 10 μ M). **(D-H)** Effects of a phosphodiesterase (PDE) inhibitor on AKAR3 responses. **(D)**
790 Maximal AKAR3 FRET emission ratio in response to the indicated concentrations of the broad-
791 spectrum PDE inhibitor, 3-isobutyl-1-methylxanthine (IBMX), in non-lesioned striatum of wild type
792 mice. Kruskal–Wallis test followed by Dunn's test, see Table S1. **(E)** Effects of a low concentration of
793 IBMX (30 μ M) on maximal FRET emission ratio responses to CGS21680 (CGS, 10 μ M) in non-

794 lesioned and 6-OHDA-lesioned striatal slices. Kruskal–Wallis test followed by Dunn’s test, see Table
795 S1. (F) Same as in E but in response to SKF81297 (SKF, 10 μ M). Kruskal–Wallis test followed by
796 Dunn’s test, see Table S1. (G) Effects of 30 μ M IBMX on FSK-induced responses in CGS-responsive
797 cells. One-way ANOVA followed by Tukey’s test, see Table S1. (H) Effects of 30 μ M IBMX on FSK-
798 induced responses in SKF-responsive cells. One-way ANOVA followed by Tukey’s test, see Table S1.
799 (A–H) Post-hoc pairwise comparisons * p <0.05, *** p <0.001.

800

801 **Fig 6. Spontaneous Ca^{2+} transients are increased in D1R-expressing neurons of 6-OHDA-lesioned**
802 **striatum**

803 (A) Sorting neurons based on their spontaneous activity and response to AMPA stimulation. Maximal
804 change of normalized fluorescence ratio ($\Delta F/F_0$) of GCaMP6s biosensor was calculated for all the cells
805 in the fields of view and all the time courses for the neurons (**All recorded SPNs**) from different slices
806 were plotted in 3D (one black line per neuron). Cells were separated in two groups based on the
807 baseline activity. Cells were classified as spontaneously active if during baseline recording they
808 presented a $\Delta F/F_0$ increase >3 standard deviation (SD) calculated on the basal activity of all the cells
809 (red curves). The other cells (non-spontaneously active) were further sorted according to their increase
810 in $\Delta F/F_0$ after the application of AMPA. They were classified as responsive if they presented a $\Delta F/F_0$
811 increase >3 SD after AMPA application (0.5 μ M, 30 s, green curves) and as non-responsive if not (blue
812 curves). (B) Comparison of the percentage of spontaneously active cells in non-lesioned (NL) and 6-
813 OHDA-lesioned striatal slices. **Left panel:** wild type C57BL/6 mice injected with an AAV expressing
814 GCaMP6s in all neurons. **Middle panel:** *Adora2a::Cre* ($A2_A\text{Cre}$) mice injected with a Cre-dependent

815 AAV (AAV-flex-GCaMP6s). **Right panel:** *Drd1::Cre* (*D1Cre*) mice injected with AAV-flex-
816 GCaMP6s. Two-tailed Chi-square test (see Table S1), *** $p < 0.001$.

817

818 **Fig 7. Specific upregulation of AMPA-induced intracellular Ca^{2+} dynamics in $A2AR$ -expressing**
819 **neurons in 6-OHDA-lesioned striatum**

820 Intracellular Ca^{2+} increase after AMPA application in non-spontaneously active cells (see figure 6A)
821 measured with GCaMP6s biosensor in striatal slices of non-lesioned (NL) and 6-OHDA-lesioned (6-
822 OHDA) mice. (A) Wild type C57Bl/6 mice expressing GCaMP6s in all neurons. Left panel:
823 representative pseudocolor-coded images representing $\Delta F/F_0$ during the baseline (a), after AMPA (0.5
824 μM , 30 s, b), then KCl (25 mM) application. Middle panel: time course of AMPA-induced intracellular
825 Ca^{2+} dynamics in non-lesioned and 6-OHDA-lesioned striatal slices. a and b indicate the time at which
826 left panel pictures were taken. Right upper panel: Percentage of responsive cells, test two-tailed Chi-
827 square see (Table S1). Right lower panel: area under the curve (AUC) of Ca^{2+} responses in striatal
828 neurons after application of AMPA (0.5 μM , 30 s), Mann-Whitney test, see Table S1. (B) Same as in A,
829 in *Drd1::Cre* mice (*D1Cre*) injected with a Cre-dependent AAV (AAV-flex-GCaMP6s) (see Table S1
830 for statistical analysis). (C) Same as in A, in *Adora2A::Cre* mice ($A2AR$ Cre) injected with AAV-flex-
831 GCaMP6s. (see Table S1 for statistical analysis). (A-C) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

832

833 **Supplementary material**

834 **Table S1. Statistical analysis of results shown in the Figures 1-7**

Figure 1.

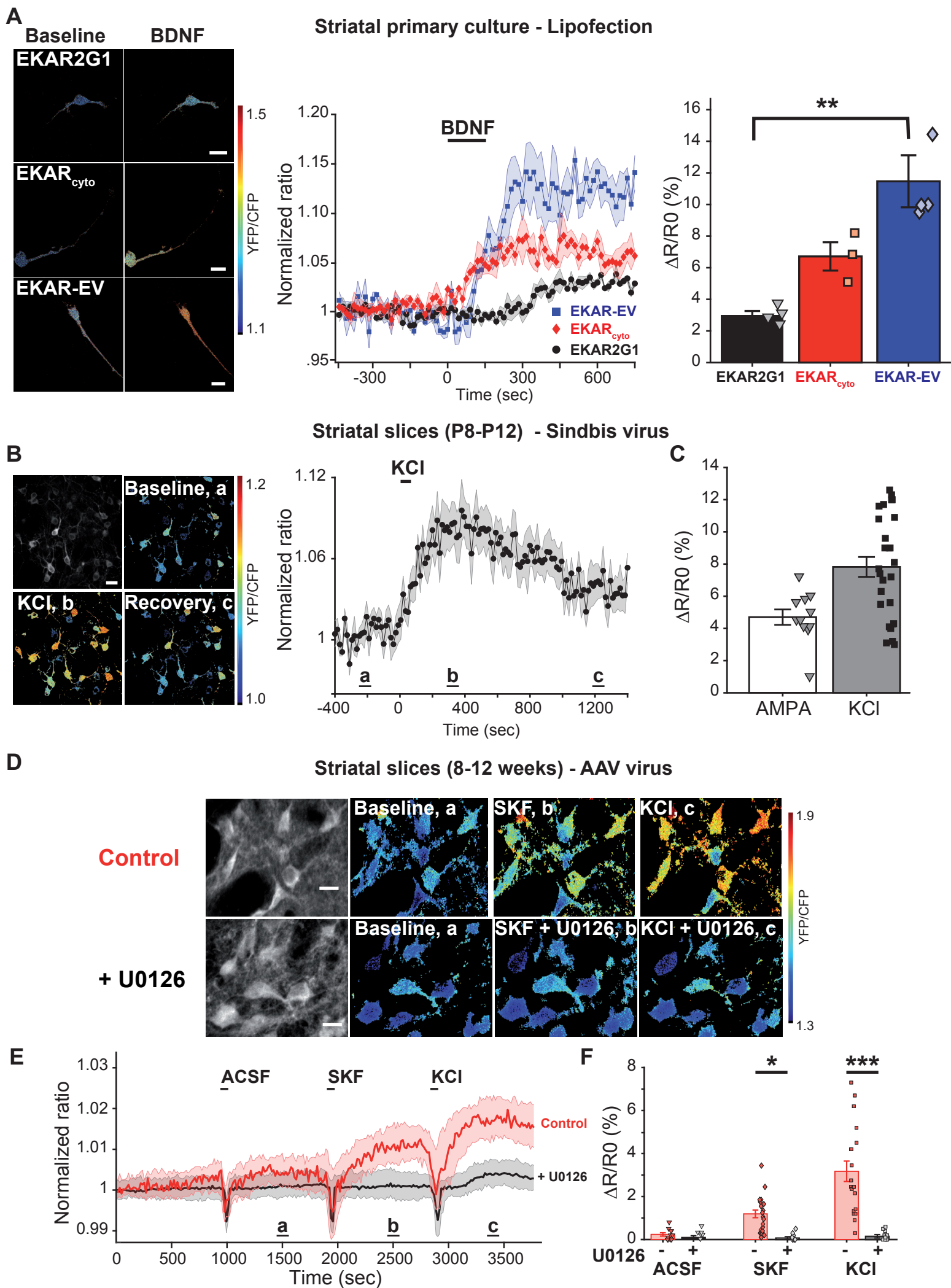
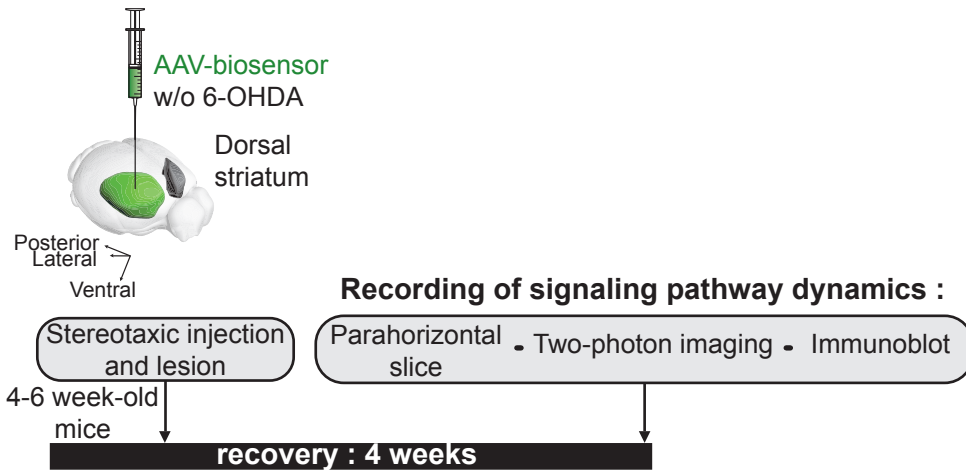
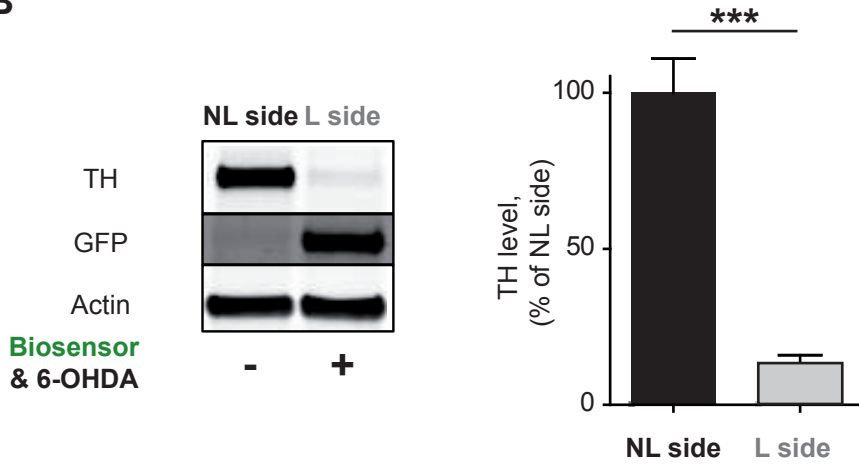


Figure 2.

A



B



C

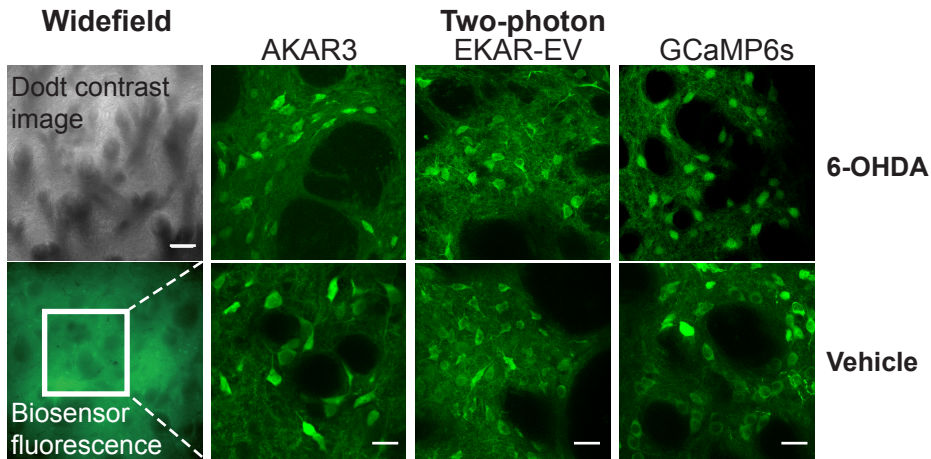
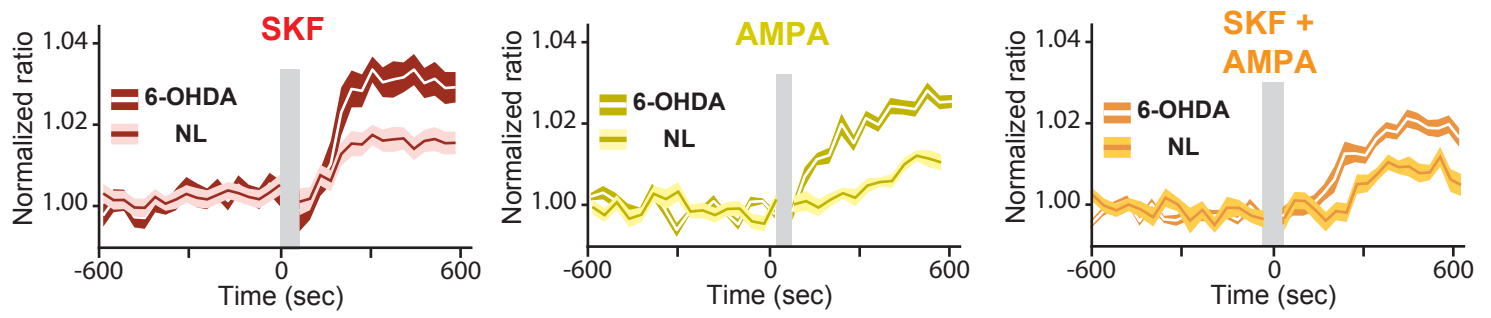
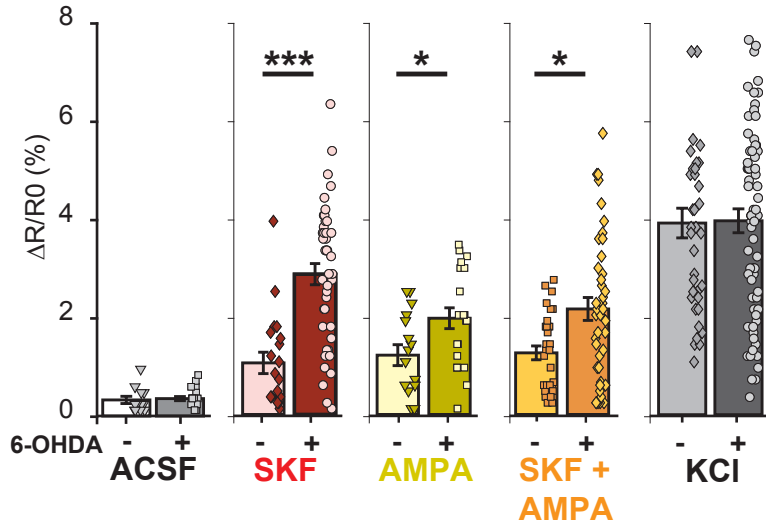


Figure 3.

A



B



C

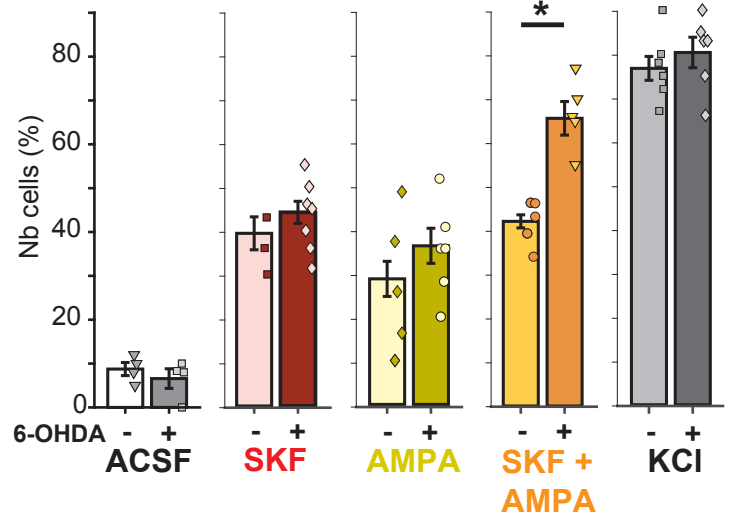
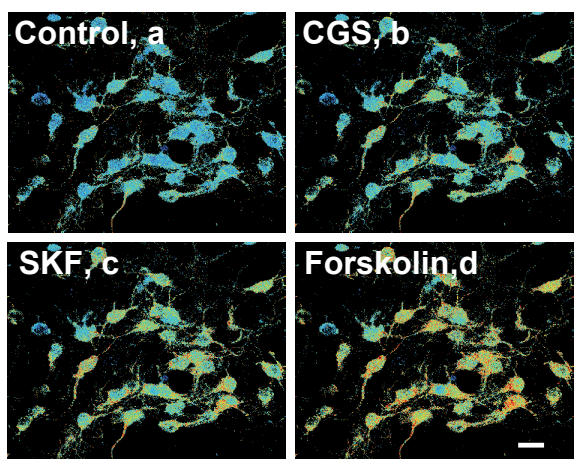
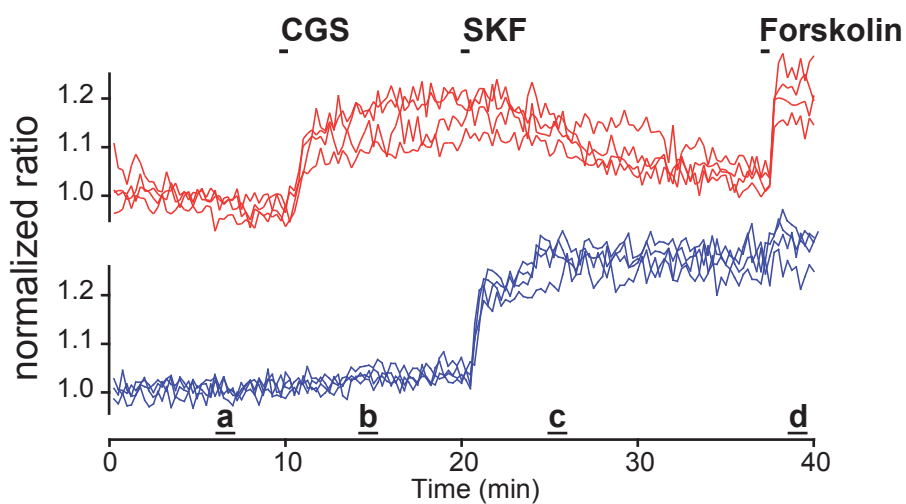


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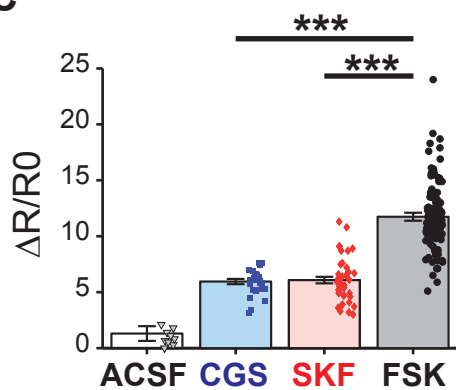
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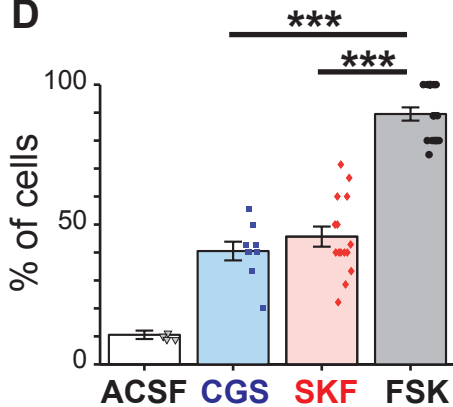
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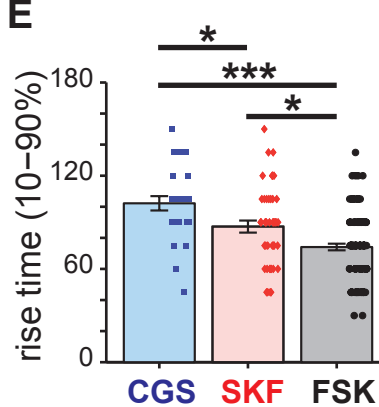
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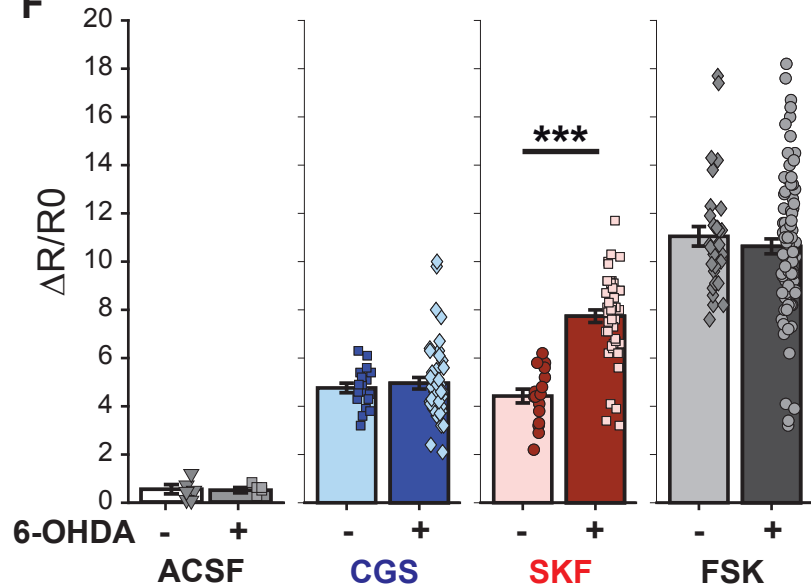
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E



F



G

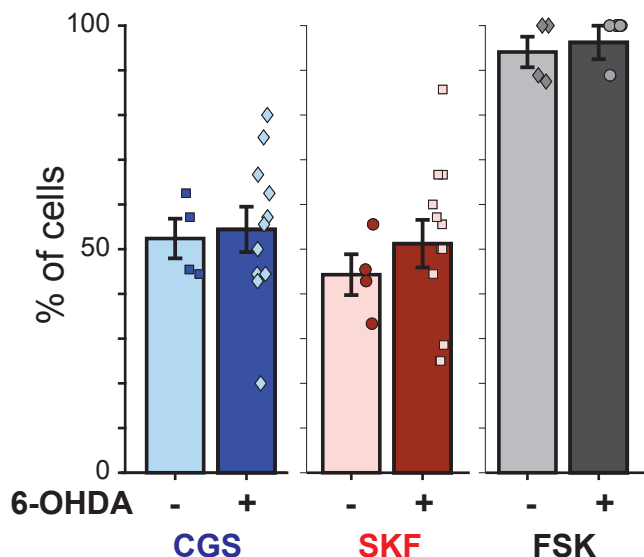


Figure 5.

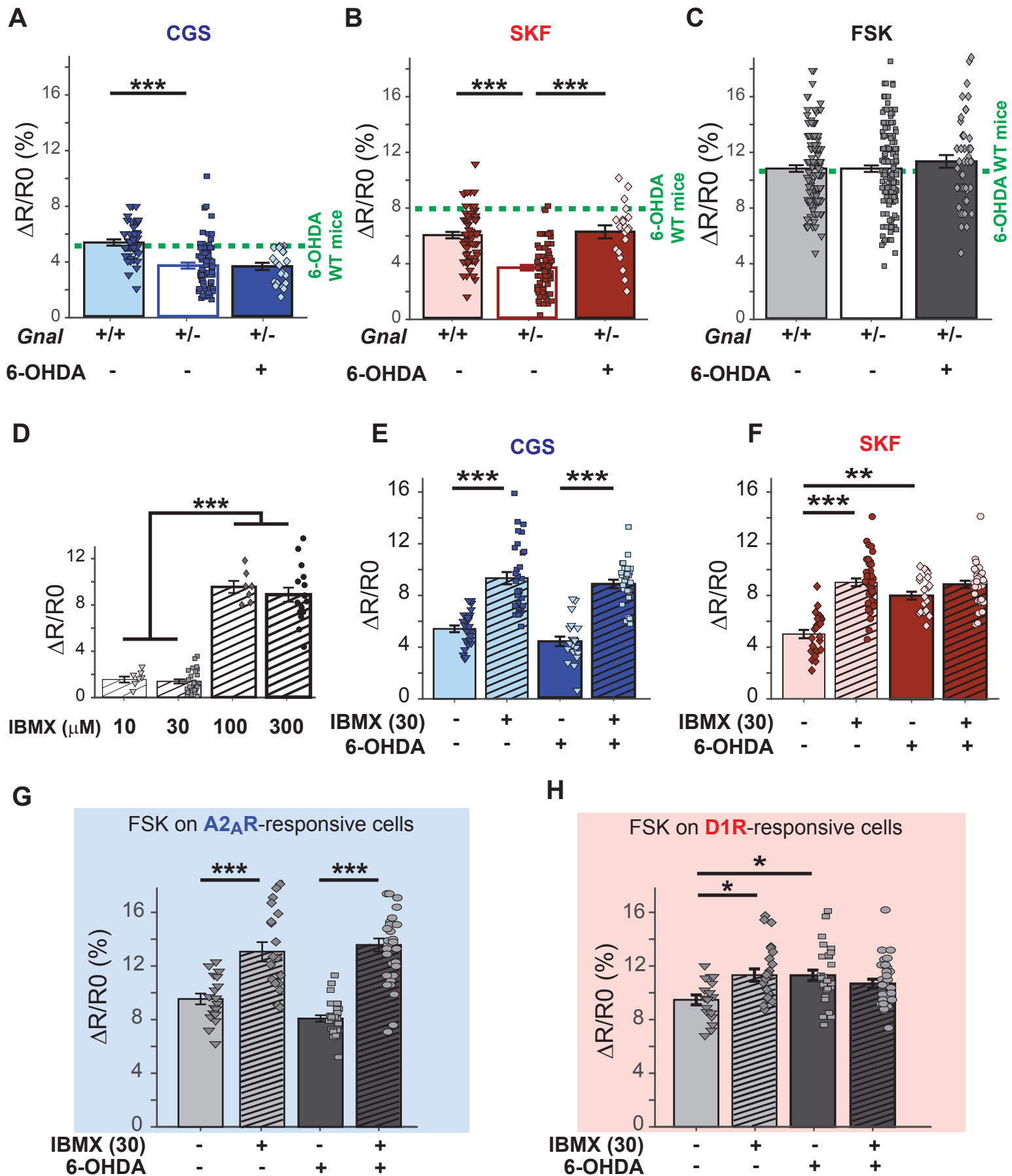
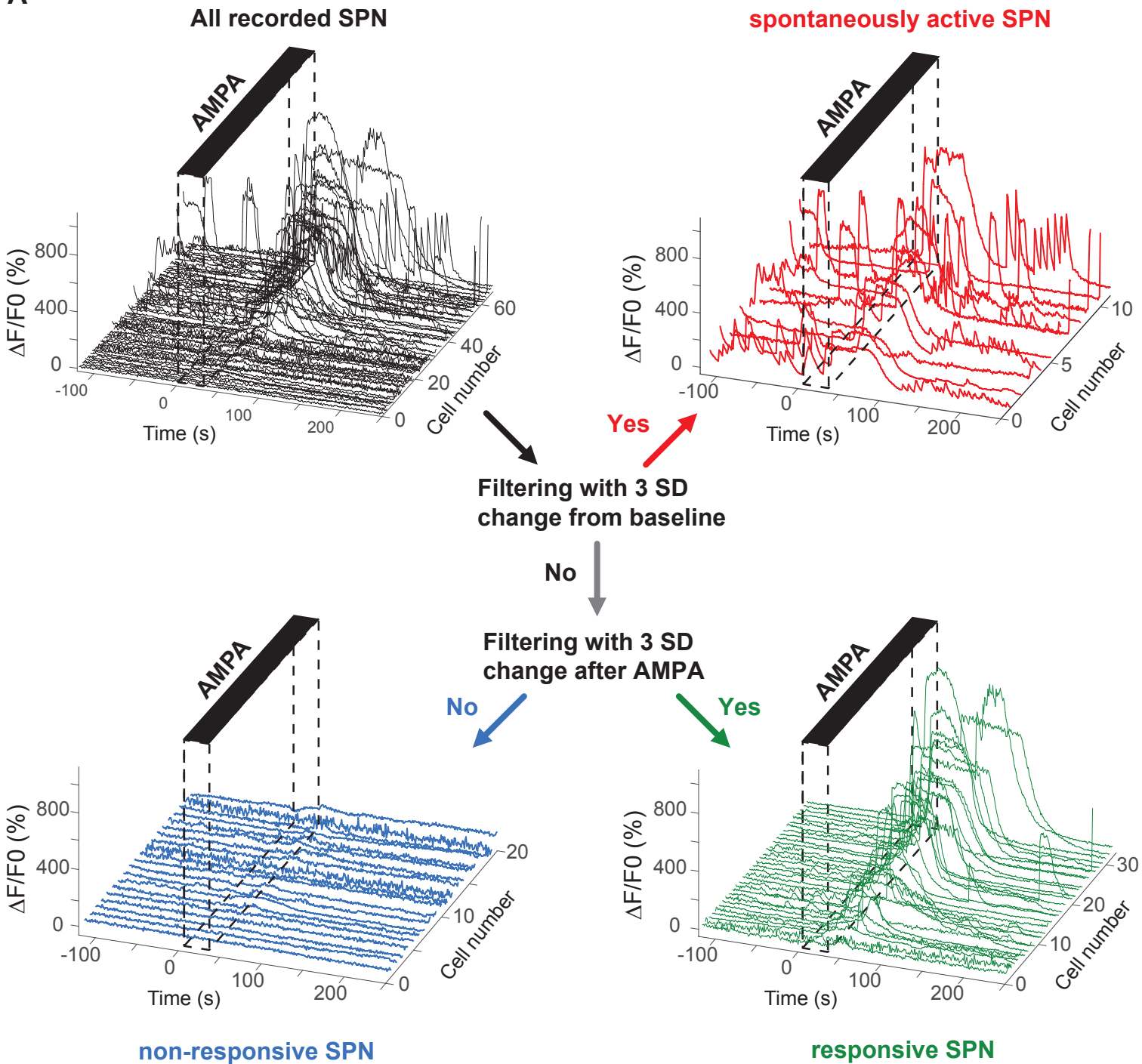


Figure 6.

A



B

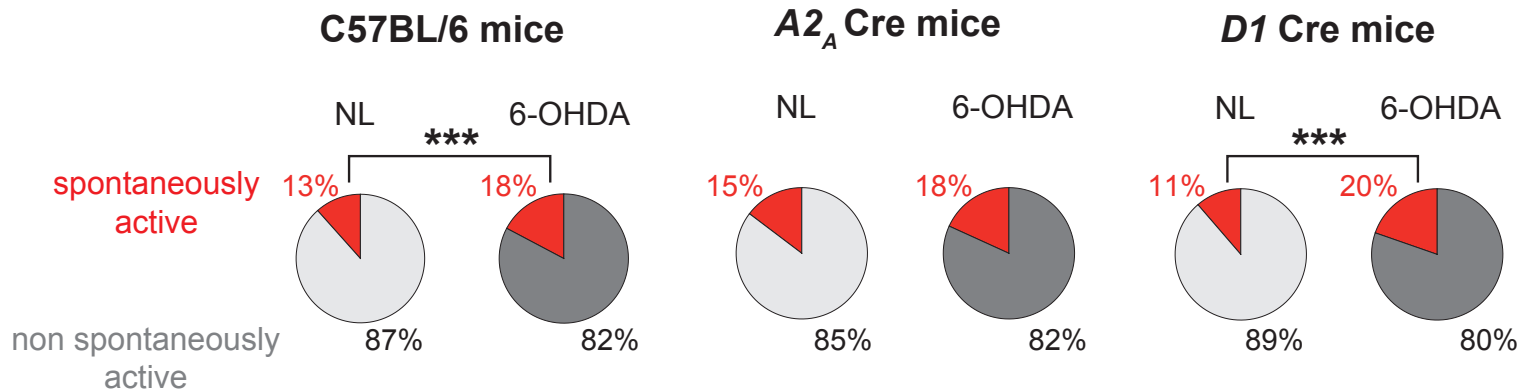
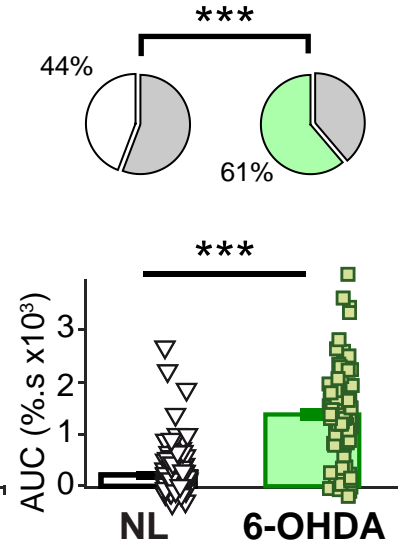
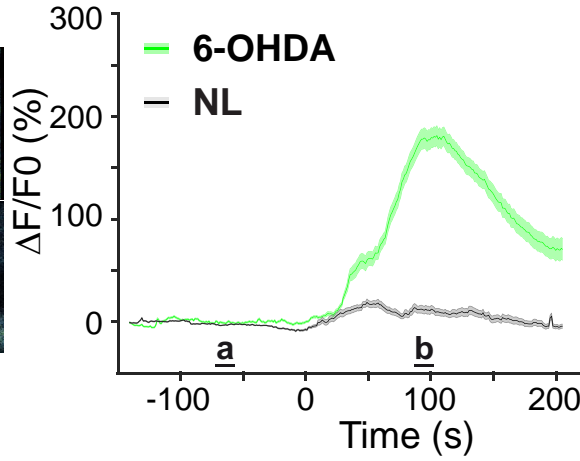
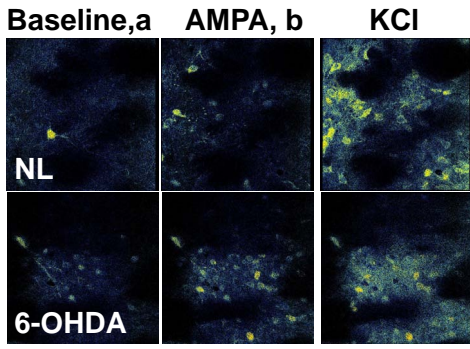
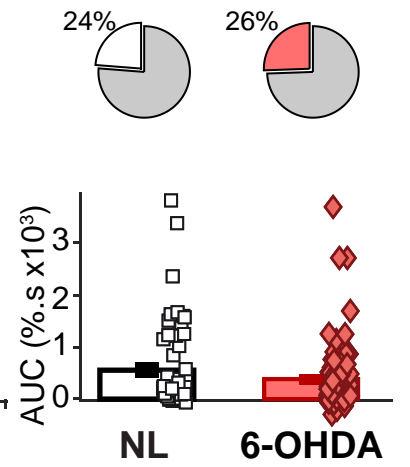
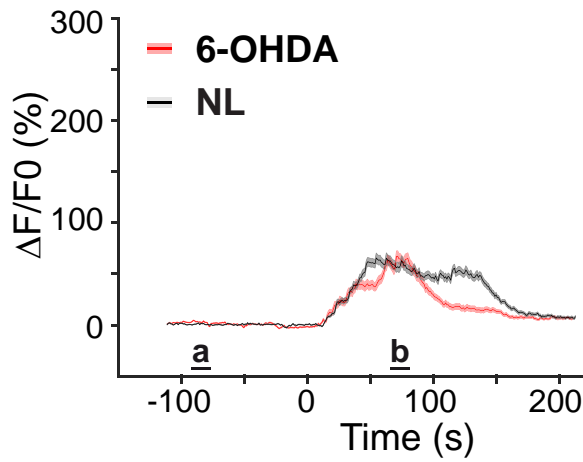
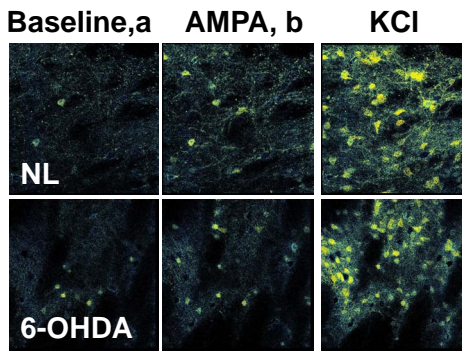


Figure 7.

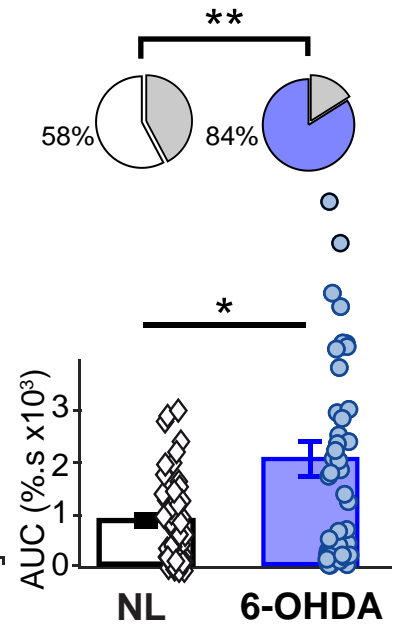
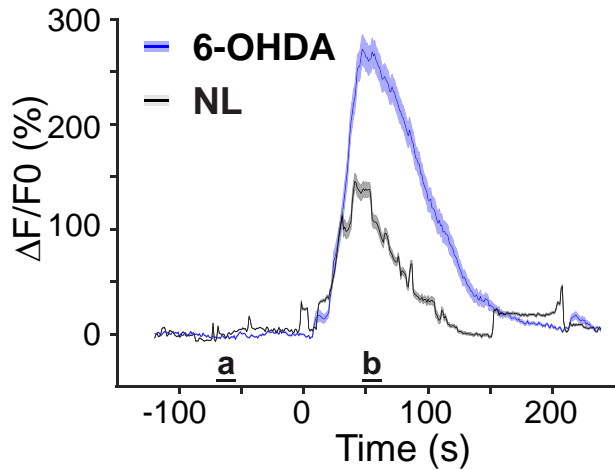
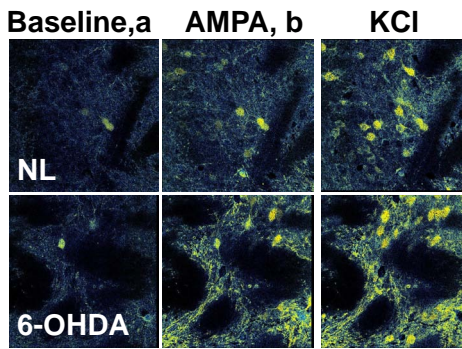
A - C57BL/6



B - D1 Cre



C - A2_A Cre



| Fig | Condition | Median | Interquartile (25-75%) | Comparison | Statistical Analysis | DF | Test value | P value | n | Number of Animal / slice or culture |
|-----|---------------------|--------|------------------------|--|------------------------------|----|------------|-------------------|----|-------------------------------------|
| 1a | Biosensor EKAR2G1 | 2.85 | 2.575 – 3.515 | EKAR2G1 vs EKARcyto EKAR2G1 vs EKAR-EV EKARcyto vs EKAR-EV | Kruskal–Wallis test | 2 | K = 8.909 | 0.0116 | 4 | 2 / 4 |
| | Biosensor EKARcyto | 6.8 | 5.1 – 8.1 | | | | | | | |
| | Biosensor EKAR-EV | 11 | 10.7 – 15.8 | | | | | | | |
| 1e | ACSF | 0 | 0 - 0.4 | ACSF vs SKF ACSF vs KCl SKF vs KCl ACSF vs ACSF + U0126 SKF vs SKF + U0126 KCl vs KCl + U0126 | Kruskal–Wallis test | 5 | K = 75.13 | <0.0001 | 11 | 3 / 3 |
| | ACSF + U0126 | 0 | -0.2 - 0.3 | | | | | | | |
| | SKF | 1.2 | 0.5 – 1.5 | | | | | | | |
| | SKF + U0126 | 0 | 0 – 0.2 | | | | | | | |
| | KCl | 3.2 | 2.25 – 4.25 | | | | | | | |
| | KCl + U0126 | 0.1 | 0 - 0.4 | | | | | | | |
| 2b | NL | 92.95 | 63.14 – 131.7 | NL vs 6-OHDA | Wilcoxon matched-paired test | | Rs = 0.57 | 0.0005 | ? | 15 / 30 |
| | 6-OHDA | 10.3 | 2.3 – 17.3 | | | | | | | |
| 3b | ACSF | 0.2 | 0.1 – 0.4 | ACSF vs ACSF + 6-OHDA SKF vs SKF + 6-OHDA AMPA vs AMPA + 6-OHDA SKF + AMPA SKF + AMPA + 6-OHDA | Two-tailed Mann Whitney test | | U = 102 | 0.37 | 12 | 3 / 5 |
| | ACSF + 6-OHDA | 0.3 | 0.2 – 0.35 | | | | | | | |
| | SKF | 0.7 | 0.3 – 1.4 | | | | | | | |
| | SKF + 6-OHDA | 2.4 | 1.5 – 2.3 | | | | | | | |
| | AMPA | 1 | 0.425 – 1.675 | | | | | | | |
| | AMPA + 6-OHDA | 1.7 | 0.9 – 2.5 | | | | | | | |
| | SKF + AMPA | 1.1 | 0.5 – 1.6 | AMPA vs AMPA + 6-OHDA | Mann Whitney test two-tailed | | U = 98 | 0.0327 | 16 | 3 / 5 |
| | SKF + AMPA + 6-OHDA | 1.7 | 0.675 – 2.6 | | | | | | | |
| | | | | | | | | | | |
| | KCl | 3.2 | 2 – 4.2 | SKF + AMPA vs SKF + AMPA + 6-OHDA | Mann Whitney test two-tailed | | U = 422 | 0.0153 | 39 | 5 / 8 |

| | KCl + 6OHDA | 3.3 | 2 – 4.5 | KCl vs KCl + 6-OHDA | Mann Whitney test two-tailed | U = 1285 | 0.70 | 69 | 7 / 11 |
|---------------------------------|---------------------------------|------|-------------|---------------------|------------------------------|-----------|---------|----|--------|
| 3c | ACSF | 10 | 4.1 – 16 | KCl vs KCl + 6-OHDA | Mann Whitney test two-tailed | U = 1285 | 0.70 | ? | 3 / 5 |
| | ACSF + 6-OHDA | 8.3 | 0 – 10 | | | | | | |
| | SKF | 41 | 33 – 45 | | | | | | |
| | SKF + 6-OHDA | 46 | 39 – 50 | | | | | | |
| | AMPA | 25 | 13 – 45 | | | | | | |
| | AMPA + 6-OHDA | 37.5 | 31 – 43.5 | | | | | | |
| | SKF + AMPA | 45 | 36.5 – 60 | | | | | | |
| | SKF + AMPA + 6-OHDA | 66.7 | 50 – 73.5 | | | | | | |
| | KCl | 75 | 69 – 80 | | | | | | |
| | KCl + 6OHDA | 77 | 75 – 83 | | | | | | |
| 4c | ACSF | 0.75 | 0.25 – 1.17 | KCl vs KCl + 6-OHDA | Mann Whitney test two-tailed | U = 35 | 0.48 | ? | 7 / 11 |
| | CGS | 6.1 | 5.2 – 6.9 | | | | | | |
| | SKF | 6.1 | 4.75 – 6.8 | | | | | | |
| | FSK | 10.8 | 9.35 – 13.2 | | | | | | |
| | ACSF vs CGS | | | | | | | | |
| | ACSF vs SKF | | | | | | | | |
| | ACSF vs FSK | | | | | | | | |
| | CGS vs SKF | | | | | | | | |
| | CGS vs FSK | | | | | | | | |
| | SKF vs FSK | | | | | | | | |
| 4d | ACSF | 9 | 7.2 – 10.8 | KCl vs KCl + 6-OHDA | Mann Whitney test two-tailed | U = 35 | 0.48 | ? | 7 / 11 |
| | CGS | 40 | 36.6 – 46.4 | | | | | | |
| | SKF | 40 | 40 – 60 | | | | | | |
| | FSK | 89 | 80 – 100 | | | | | | |
| | ACSF vs CGS | | | | | | | | |
| | ACSF vs SKF | | | | | | | | |
| | ACSF vs FSK | | | | | | | | |
| | CGS vs SKF | | | | | | | | |
| | CGS vs FSK | | | | | | | | |
| | SKF vs FSK | | | | | | | | |
| 4d | ACSF | 9 | 7.2 – 10.8 | KCl vs KCl + 6-OHDA | Kruskal–Wallis test | K = 130.1 | <0.0001 | ? | 3 / 4 |
| | CGS | 40 | 36.6 – 46.4 | | | | | | |
| | SKF | 40 | 40 – 60 | | | | | | |
| | FSK | 89 | 80 – 100 | | | | | | |
| | Dunn's multiple comparison Test | | | | | | | | |
| | Dunn's multiple comparison Test | | | | | | | | |
| | Dunn's multiple comparison Test | | | | | | | | |
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| | Dunn's multiple comparison Test | | | | | | | | |
| Dunn's multiple comparison Test | | | | | | | | | |
| 4d | ACSF | 9 | 7.2 – 10.8 | KCl vs KCl + 6-OHDA | Kruskal–Wallis test | K = 35.49 | <0.0001 | ? | 3 / 4 |
| | CGS | 40 | 36.6 – 46.4 | | | | | | |
| | SKF | 40 | 40 – 60 | | | | | | |
| | FSK | 89 | 80 – 100 | | | | | | |
| | Dunn's multiple comparison Test | | | | | | | | |
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|----|--|---|--|--|--|---|---|--|--|--|
| 4e | CGS SKF FSK | 105 90 75 | 90 – 120 63.5 – 105 60 – 90 | CGS vs SKF CGS vs FSK SKF vs FSK | Kruska–Wallis test Dunn's multiple comparison Test Dunn's multiple comparison Test Dunn's multiple comparison Test | 2 K = 28.84 | | | 27 44 101 | 5 / 9 8 / 15 10 / 17 |
| 4f | ACSF ACSF + 6-OHDA CGS CGS + 6-OHDA SKF SKF + 6-OHDA FSK FSK + 6-OHDA | 0.25 0.45 4.6 4.55 4.45 8 10.7 10.35 | 0 – 0.57 0 – 0.77 4.2 – 5.4 3.92 – 5.57 3.42 – 5.5 6.6 – 8.9 9.7 – 11.7 8.85 – 12.2 | ACSF vs ACSF + 6-OHDA CGS vs CGS + 6-OHDA SKF vs SKF + 6-OHDA FSK vs FSK + 6-OHDA | Mann Whitney test two-tailed Mann Whitney test two-tailed Mann Whitney test two-tailed Mann Whitney test two-tailed | U = 40 U = 388 U = 52 U = 1344 | 0.468 0.9 <0.0001 0.53 | | 10 10 18 44 16 47 33 88 | 2 / 4 3 / 11 3 / 4 5 / 11 3 / 4 5 / 12 2 / 4 5 / 11 |
| 4g | CGS CGS + 6-OHDA SKF SKF + 6-OHDA FSK FSK + 6-OHDA | 51 55 43.5 52.5 94.5 100 | 44.2 – 60.7 44 – 66 35.2 – 52.5 32 – 64.5 87.5 – 100 100 – 100 | CGS vs CGS + 6-OHDA SKF vs SKF + 6-OHDA FSK vs FSK + 6-OHDA | Mann Whitney test two-tailed Mann Whitney test two-tailed Mann Whitney test two-tailed | U = 21 U = 16.5 U = 14 | 0.94 0.39 0.207 | | ? ? ? ? ? ? | 3 / 4 5 / 11 3 / 4 5 / 12 2 / 4 5 / 11 |
| 5a | Gnal +/- _ CGS Gnal +/- _ CGS Gnal +/- + 6-OHDA _ CGS | 5.3 3.67 3.75 | 4.4 – 6.0 2.4 – 4.8 2.37 – 4.72 | Gnal +/- vs Gnal +/- Gnal +/- vs Gnal +/- + 6-OHDA Gnal +/- vs Gnal +/- + 6OHDA | 1-way Anova Tukey's multiple comparison Test Tukey's multiple comparison Test Tukey's multiple comparison Test | 2 F = 18.84 | 0.09 <0.0001 <0.0001 0.972 | | 47 68 22 | 4 / 6 5 / 9 3 / 4 |
| 5b | Gnal +/- _ SKF Gnal +/- _ SKF Gnal +/- + 6-OHDA _ SKF | 6.0 3.5 6.45 | 4.25 – 7.2 2.35 – 4.55 4.75 – 7.15 | | | | | | 65 84 20 | 5 / 8 6 / 11 3 / 4 |

| | | | | | <i>Gnal</i> +/- vs <i>Gnal</i> +/- <i>Gnal</i> +/- vs <i>Gnal</i> +/- + 6-OHDA <i>Gnal</i> +/- vs <i>Gnal</i> +/- + 6OHDA | | Tukey's multiple comparison Test Tukey's multiple comparison Test Tukey's multiple comparison Test | | <0.0001 0.821 <0.0001 | | 12 / 18 13 / 20 5 / 8 |
|----|--|----------------------------|---|--|--|---|---|-----------|---|--|--|
| 5c | <i>Gnal</i> +/- _ FSK <i>Gnal</i> +/- _ FSK <i>Gnal</i> +/- + 6-OHDA _ FSK | 11.1 11.3 12.2 | 9.1 – 13.08 9.7 – 13.75 9.7 – 14 | | <i>Gnal</i> +/- vs <i>Gnal</i> +/- <i>Gnal</i> +/- + 6-OHDA <i>Gnal</i> +/- + 6OHDA | | Tukey's multiple comparison Test Tukey's multiple comparison Test Tukey's multiple comparison Test | | 122 157 47 | | |
| | | | | | <i>Gnal</i> +/- vs <i>Gnal</i> +/- <i>Gnal</i> +/- + 6-OHDA <i>Gnal</i> +/- + 6OHDA | 2 | 1-way Anova Tukey's multiple comparison Test Tukey's multiple comparison Test Tukey's multiple comparison Test | F = 0.48 | 0.618 0.852 0.71 0.72 | | |
| 5d | IBMX 10 µM IBMX 30 µM IBMX 100 µM IBMX 300 µM | 1.7 1.2 9.5 8.45 | 1.2 – 2.1 0.575 – 2.225 8.1 – 10.6 7.3 – 10.2 | | IBMX 10 µM vs IBMX 30 µM IBMX 10 µM vs IBMX 100 µM IBMX 10 µM vs IBMX 300 µM IBMX 30 µM vs IBMX 100 µM IBMX 30 µM vs IBMX 300 µM IBMX 100 µM vs IBMX 300 µM | 3 | Kruskal–Wallis test Dunn's multiple comparison Test Dunn's multiple comparison Test Dunn's multiple comparison Test Dunn's multiple comparison Test Dunn's multiple comparison Test Dunn's multiple comparison Test | K = 42.41 | <0.0001 >0.99 0.0155 0.0081 <0.0001 <0.0001 >0.99 | | 7 30 7 16 2 / 2 3 / 6 1 / 2 2 / 3 |
| 5e | CGS CGS + IBMX CGS + 6-OHDA CGS + IBMX + 6-OHDA | 5.45 8.4 3.9 8.55 | 4.45 – 6.35 7.2 – 11.4 3.15 – 4.95 7.3 – 9.175 | | CGS vs CGS + IBMX CGS vs CGS + 6-OHDA CGS vs CGS + IBMX + 6-OHDA CGS + IBMX vs CGS + 6-OHDA CGS + IBMX vs CGS + IBMX + 6-OHDA CGS + 6-OHDA vs CGS + IBMX + 6-OHDA | 3 | Kruskal–Wallis test Dunn's multiple comparison Test Dunn's multiple comparison Test Dunn's multiple comparison Test Dunn's multiple comparison Test Dunn's multiple comparison Test | K = 65.79 | <0.0001 <0.0001 0.539 <0.0001 <0.0001 >0.99 <0.0001 | | 24 42 22 32 3 / 4 4 / 7 3 / 4 3 / 6 |
| 5f | SKF SKF + IBMX SKF + 6-OHDA SKF + IBMX + 6-OHDA | 5.1 9.0 7.25 8.5 | 3.725 – 6.175 7.55 – 10.4 6.3 – 8.8 7.125 – 9.05 | | SKF vs SKF + IBMX SKF vs SKF + 6-OHDA | 3 | Kruskal–Wallis test Dunn's multiple comparison Test Dunn's multiple comparison Test | K = 50.30 | <0.0001 <0.0001 0.0027 | | 24 41 22 32 3 / 4 4 / 7 3 / 4 3 / 6 |

| | | | | | | | | |
|----|--|-----------------------------|--|--|--|---|----------------------|--------------------------|
| 5g | FSK FSK + IBMX FSK + 6-OHDA FSK + IBMX + 6-OHDA | 8.6 11.7 8.15 13.7 | 7.725 – 9.825 9.1 – 15.3 7.2 – 8.8 11.7 – 15.55 | SKF vs SKF + IBMX + 6-OHDA SKF + IBMX vs SKF + 6-OHDA SKF + IBMX vs SKF + IBMX + 6-OHDA SKF + 6-OHDA vs SKF + IBMX + 6-OHDA | Dunn's multiple comparison Test Dunn's multiple comparison Test Dunn's multiple comparison Test Dunn's multiple comparison Test | <0.0001 0.0324 0.904 0.913 | 18 19 28 30 | 3/3 2/3 4/5 3/5 |
| | | | | FSK vs FSK + IBMX FSK vs FSK + 6-OHDA FSK vs FSK + IBMX + 6-OHDA FSK + IBMX vs FSK + 6-OHDA FSK + IBMX vs FSK + IBMX + 6-OHDA FSK + 6-OHDA vs FSK + IBMX + 6-OHDA | Tukey's multiple comparison Test Tukey's multiple comparison Test Tukey's multiple comparison Test Tukey's multiple comparison Test Tukey's multiple comparison Test Tukey's multiple comparison Test | <0.0001 <0.0001 0.757 <0.0001 <0.0001 0.2 <0.0001 | 3 | F = 37.39 |
| 5h | FSK FSK + IBMX FSK + 6-OHDA FSK + IBMX + 6-OHDA | 9.5 11 10.9 10.6 | 8.35 – 10.9 9.45 – 12.85 10.2 – 12.7 9.45 – 11.85 | FSK vs FSK + IBMX FSK vs FSK + 6-OHDA FSK vs FSK + IBMX + 6-OHDA FSK + IBMX vs FSK + 6-OHDA FSK + IBMX vs FSK + IBMX + 6-OHDA FSK + 6-OHDA vs FSK + IBMX + 6-OHDA | Tukey's multiple comparison Test Tukey's multiple comparison Test Tukey's multiple comparison Test Tukey's multiple comparison Test Tukey's multiple comparison Test Tukey's multiple comparison Test | 0.0201 0.036 0.024 0.269 >0.99 0.594 0.547 | 17 21 29 33 | 3/3 2/3 4/5 3/5 |
| 6b | C57BL/6 NL C57BL/6 6-OHDA | 274/1811 319/1429 | | NL vs 6-OHDA | Chi-square test two-tailed | <0.0001 | 2085 1748 | 11/40 11/35 |
| 6b | A2 _A cre NL A2 _A cre 6-OHDA | 80/438 69/310 | | NL vs 6-OHDA | Fisher's exact test | 0.277 | 518 379 | 4/13 4/9 |
| 6b | D1 cre NL D1 cre 6-OHDA | 58/454 179/731 | | NL vs 6-OHDA | Fisher's exact test | <0.0001 | 512 910 | 3/6 4/14 |
| 7a | C57BL/6 NL C57BL/6 6-OHDA | 206/466 120/196 | | NL vs 6-OHDA | Chi-square test two-tailed | <0.0001 | 466 196 | 7/11 3/4 |

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|----|--|-------------------|------------------------------|-----------------------|------------------------------|----------|---------|------------|-----------------|
| 7a | AMPA AMPA + 6-OHDA | 1103 12347 | 325 – 2274 9158 – 15671 | AMPA vs AMPA + 6-OHDA | Mann Whitney test two-tailed | U = 1862 | <0.0001 | 206 120 | 7 / 11 3 / 4 |
| 7b | D1 cre NL D1 cre 6-OHDA | 62/261 103/403 | | NL vs 6-OHDA | Fisher's exact test | | 0.646 | 261 403 | 2 / 3 4 / 7 |
| 7b | AMPA AMPA + 6-OHDA | 1911 1696 | 875 – 8071 713 – 4734 | AMPA vs AMPA + 6-OHDA | Mann Whitney test two-tailed | U = 2954 | 0.422 | 62 103 | 2 / 3 4 / 7 |
| 7c | A2 _A cre NL A2 _A cre 6-OHDA | 52/90 47/56 | | NL vs 6-OHDA | Fisher's exact test | | <0.001 | 90 56 | 2 / 3 2 / 2 |
| 7c | AMPA AMPA + 6-OHDA | 6154 12220 | 1342 – 11130 1585 – 27070 | AMPA vs AMPA + 6-OHDA | Mann Whitney test two-tailed | U = 903 | 0.0256 | 52 47 | 2 / 3 2 / 2 |
| | In blue responsive / non responsive | | | | | | | | |