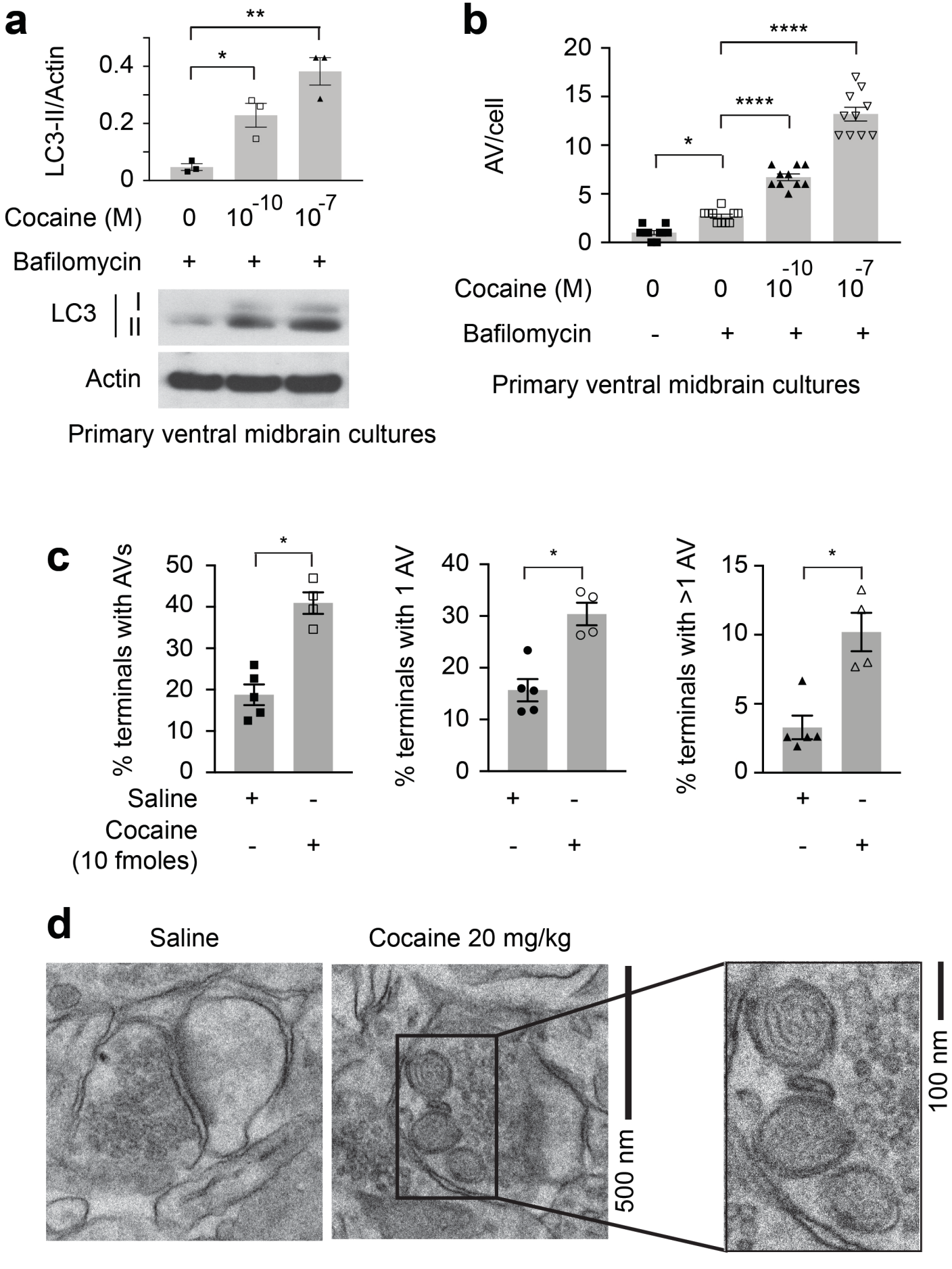
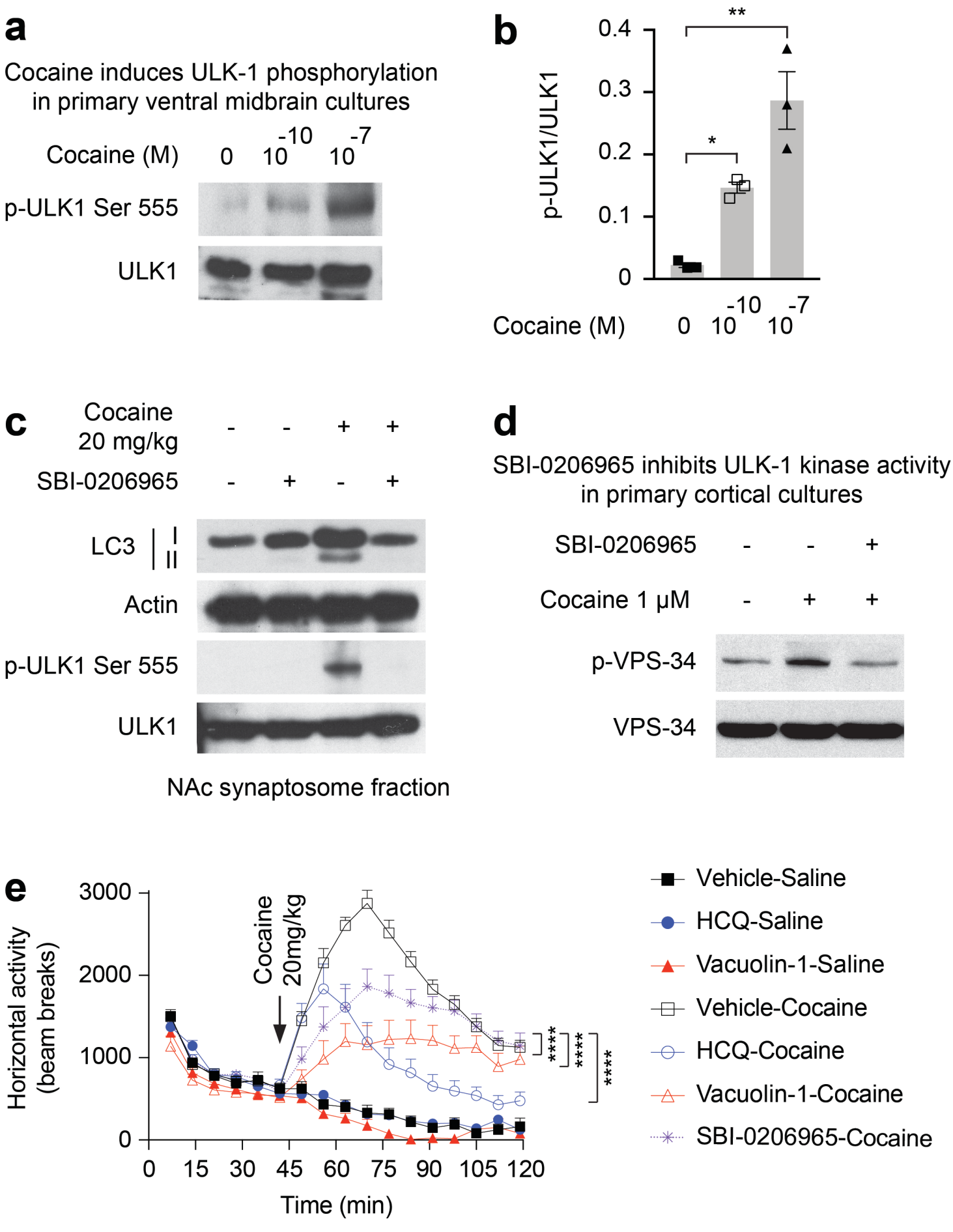

a
LC3-II/Actin
0.4
0.2
0
**
*
Cocaine (M)
0
10-10
10-7
Bafilomycin
+
+
+
LC3
I
II
Actin
Primary ventral midbrain cultures
b
AV/cell
20
15
10
5
0
****
****
*
Cocaine (M)
0
0
10-10
10-7
Bafilomycin
-
+
+
+
Primary ventral midbrain cultures
c
% terminals with AVs
50
40
30
20
10
0
*
Saline
+
-
Cocaine
(10 fmoles)
-
+
% terminals with 1 AV
40
30
20
10
0
*
+
-
-
+
% terminals with >1 AV
15
10
5
0
*
+
-
-
+
d
Saline
Cocaine 20 mg/kg
500 nm
100 nm

Supplementary Materials:

Figures S1-S6

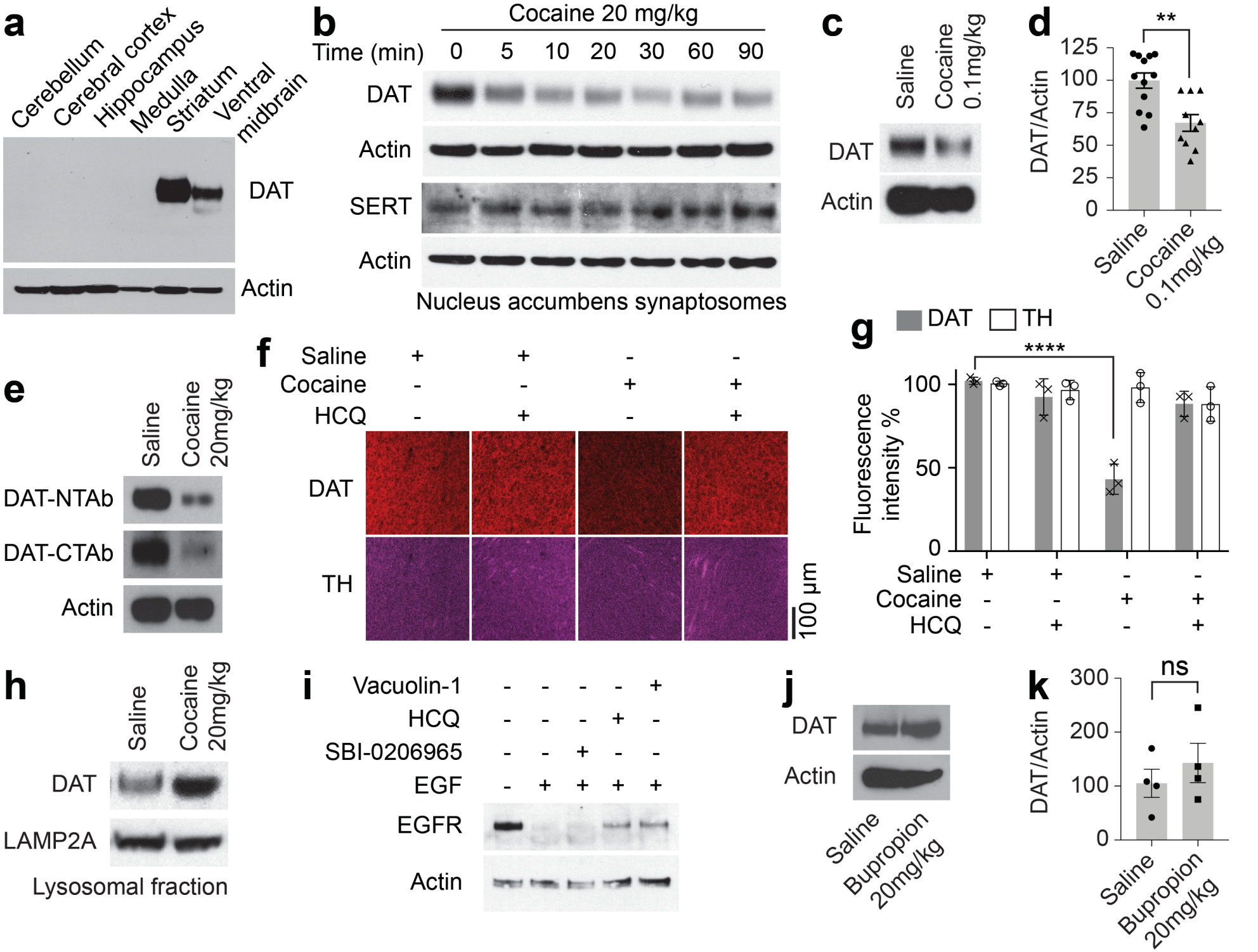
Supplementary Figure 1.

Cocaine induces autophagy *in vivo*. (a) Activation of autophagy analyzed by western blot of LC3II in primary ventral midbrain cultures. (b) Quantitative analysis of autophagic vacuoles in primary ventral midbrain cultures using TEM. * ($P < 0.05$), ** ($P < 0.01$), one-way ANOVA, Tukey's multiple comparisons test. TEM of induction of autophagic vacuoles in axonal terminals in NAc after cocaine injection. The number of presynaptic terminals with autophagic vacuoles (AVs) in NAc is plotted. The number of AVs was quantified in a blinded fashion. n = at least 4 mice per group. At least 75 synapses were quantified per mouse. * ($P < 0.05$), two-tailed t test. Error bars = +/-SEM. **(d)** TEM of Induction of autophagic vacuoles in axonal terminals of NAc after intraperitoneal cocaine injection (20 mg/kg).


a
Cocaine induces ULK-1 phosphorylation
in primary ventral midbrain cultures
Cocaine (M)
0
-10
10
-7
10
p-ULK1 Ser 555
ULK1
b
p-ULK1/ULK1
0.4
0.3
0.2
0.1
0
**
*
Cocaine (M)
0
-10
10
-7
10
c
Cocaine
20 mg/kg
SBI-0206965
LC3
I
II
Actin
p-ULK1 Ser 555
ULK1
NAc synaptosome fraction
d
SBI-0206965 inhibits ULK-1 kinase activity
in primary cortical cultures
SBI-0206965
Cocaine 1 µM
p-VPS-34
VPS-34
e
Horizontal activity
(beam breaks)
3000
2000
1000
0
Cocaine
20mg/kg
****
****
****
0
15
30
45
60
75
90
105
120
Time (min)
Vehicle-Saline
HCQ-Saline
Vacuolin-1-Saline
Vehicle-Cocaine
HCQ-Cocaine
Vacuolin-1-Cocaine
SBI-0206965-Cocaine

Supplementary Figure 2.

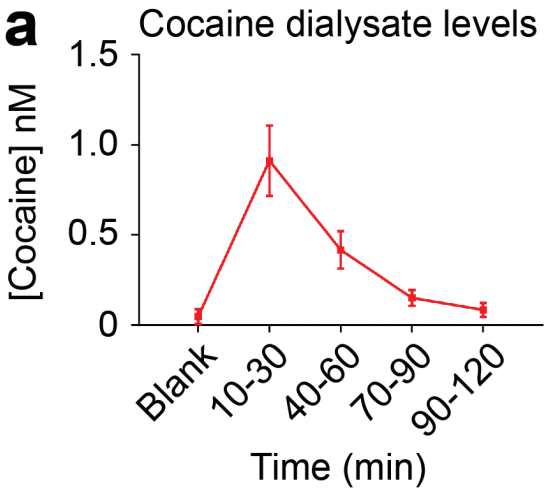
Validation of the ULK1 inhibitor SBI-0206965. (a) Western blot of ULK ser 555 in primary ventral midbrain cultures after treatment of cocaine and **(b)** densitometric analysis. * ($P < 0.05$), ** ($P < 0.01$), one-way ANOVA, Tukey's multiple comparisons test. **(c)** Western blot of p-VPS34 to validate the efficacy of ULK1 inhibitor SBI-0206965 in primary cortical cultures. **(d)** Western blot of LC3II and ULK Ser 555 in NAc synaptosomes after treatment with cocaine and SBI-0206965. **(e)** The complete time course for the open field behavioral experiments before and after cocaine injection (20 mg/kg) with and without hydroxychloroquine (HCQ) (2 mg/kg), vacuolin-1 (2 mg/kg), and SBI-0206965 (2 mg/kg). Vacuolin-1 inhibits the fusion between lysosome and autophagosomes. SBI-0206965 is an inhibitor of ULK. HCQ is a lysosomal inhibitor. Vehicle-saline $n = 8$, Vehicle-cocaine $n = 40$, Vacuolin-1-saline $n = 4$, SBI-0206965 or Vacuolin-1-cocaine $n = 16$, HCQ-saline $n = 20$, HCQ-cocaine $n = 14$, one-way ANOVA, $P < 0.0001$, Tukey's multiple comparisons test, **** ($P < 0.0001$). Error bars = +/-SEM.


a
Cerebellum
Cerebral cortex
Hippocampus
Medulla
Striatum
Ventral midbrain
DAT
Actin
b
Cocaine 20 mg/kg
Time (min) 0 5 10 20 30 60 90
DAT
Actin
SERT
Actin
Nucleus accumbens synaptosomes
c
Saline
Cocaine 0.1mg/kg
DAT
Actin
d
**
DAT/Actin
125
100
75
50
25
0
Saline
Cocaine 0.1mg/kg
e
Saline
Cocaine 20mg/kg
DAT-NTAb
DAT-CTAb
Actin
f
Saline + + - -
Cocaine - - + +
HCQ - + - +
DAT
TH
100 μm
g
DAT
TH
****
Fluorescence intensity %
100
50
0
Saline + + - -
Cocaine - - + +
HCQ - + - +
h
Saline
Cocaine 20mg/kg
DAT
LAMP2A
Lysosomal fraction
i
Vacuolin-1 - - - - +
HCQ - - - + -
SBI-0206965 - - + - -
EGF - + + + +
EGFR
Actin
j
DAT
Actin
Saline
Bupropion 20mg/kg
k
ns
DAT/Actin
300
200
100
0
Saline
Bupropion 20mg/kg

Supplementary Figure 3.

Cocaine selectively induces autophagic degradation of DAT. (a) Western blot of DAT to verify the antibody showing positive bands in the striatal and ventral midbrain lysates but not other areas of the brain. **(b)** Western blot of DAT and SERT in NAc synaptosome fraction following cocaine injection. **(c)** Western blot of NAc synaptosomes showing rapid DAT depletion following cocaine 0.1 mg/kg intraperitoneal injection. **(d)** Quantification of DAT/actin in panel **c** is plotted. Saline n = 12, cocaine n = 10, ** ($P < 0.01$, two tailed *t* test. **(e)** Western blot of DAT using anti-N-terminus (NTAb) and anti-C-terminus (CTAb) antibodies to verify the depletion of DAT in NAc synaptosomal fraction following cocaine injection. **(f)** Confocal imaging of DAT and tyrosine hydroxylase (TH) in NAc. Mice were treated with saline or cocaine (20 mg/kg) +/- HCQ, followed by harvesting tissue and immunostaining for DAT and TH. DAT but not TH is rapidly depleted (30 minutes) following cocaine injection. This effect is rescued by HCQ. Scale bar: 100 μm. **(g)** Quantification of immunofluorescence staining of DAT and TH in NAc. $n = 3$. one-way ANOVA, $P < 0.0001$, Holm-Sidak's multiple comparisons test, **** ($P < 0.0001$). Error bars = +/-SEM. **(h)** Western blot of DAT and LAMP2A in ventral midbrain lysosomal fraction following cocaine injection. **(i)** EGFR is endocytosed and degraded by the lysosome following EGF stimulation. EGFR degradation is not rescued by SBI-0206965 and modestly rescued by HCQ and vacuoiln-1. This suggests that SBI-0206965 does not affect the endocytic degradation pathway. **(j)** Western blot of NAc synaptosomes showing no change in DAT levels following intraperitoneal of 20 mg/kg bupropion. **(k)** Quantification of DAT/actin in panel **i** is plotted. $n = 4$/group, *p* value NS, two tailed *t* test.

a



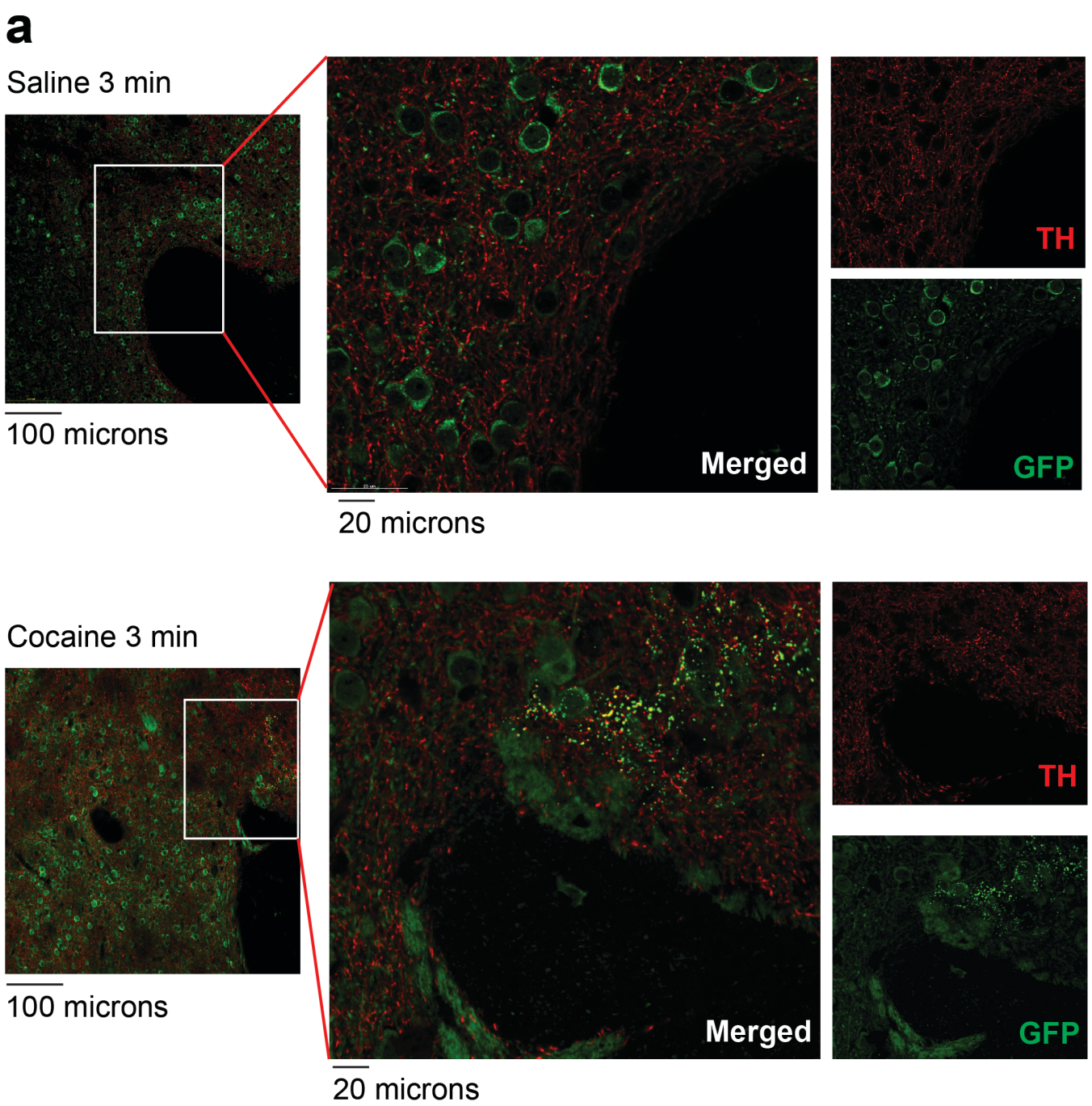
b

Cocaine Pharmacokinetic Parameters			
C_{max} (nM)*	T_{max} (min)	$T_{1/2}$ (min)	Corrected AUC_{0-t} (nM x h)*
911 ± 195	10 - 30	35.2 ± 9.86	1543 ± 181

* Corrected Cmax and AUC0-t are calculated from the % recovery values obtained in in vitro diaysate samples

Supplementary Figure 4.

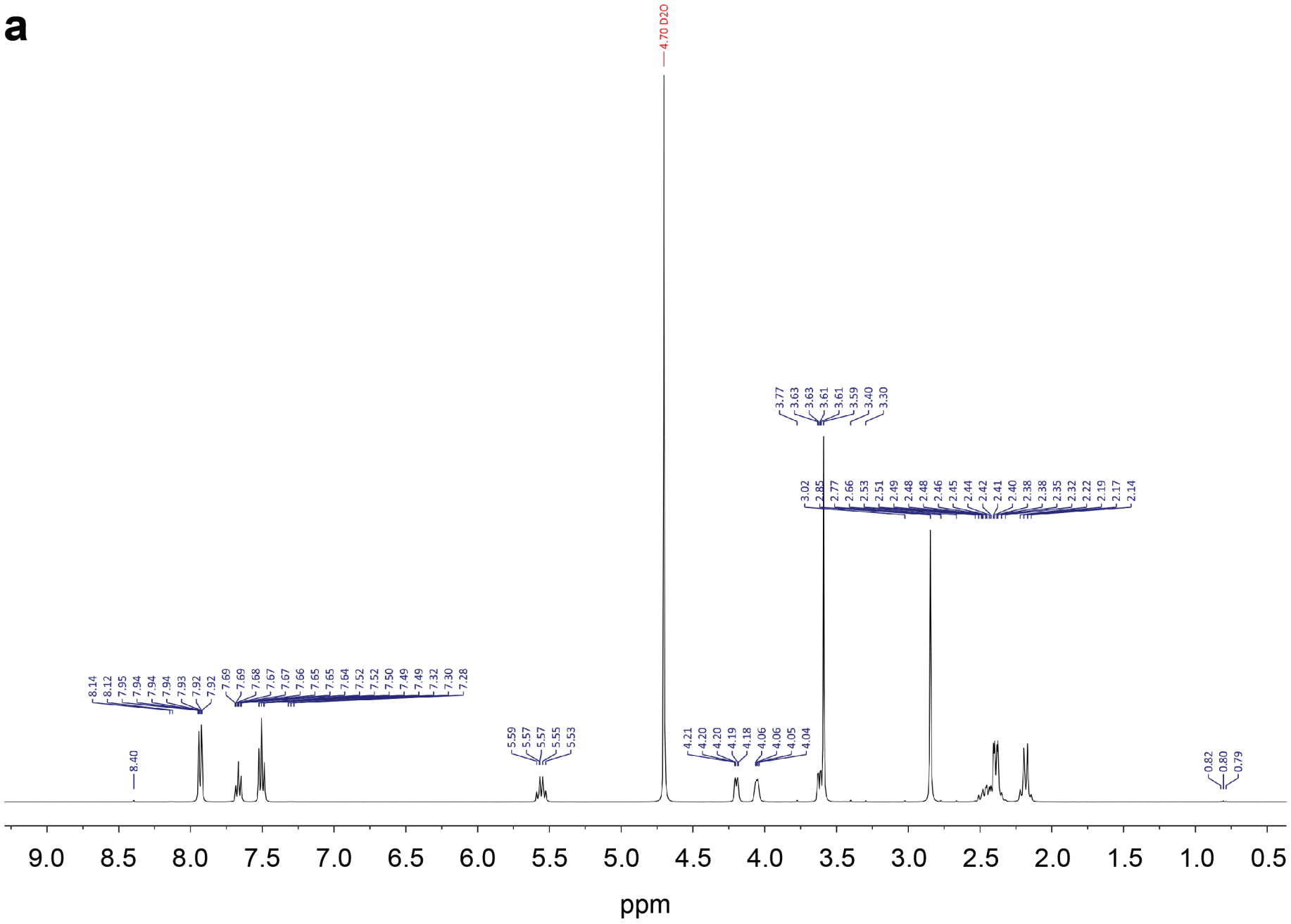
Cocaine levels in the nucleus accumbens following peripheral administration in mice. (a) Mean concentration vs. time profile of cocaine in dialysate from the nucleus accumbens following 20 mg/kg intraperitoneal injection in mice. Cocaine dialysate levels were calculated after normalization using recovery correction. **(b)** Pharmacokinetic parameters of cocaine in the NAc following 20 mg/kg intraperitoneal injection as assessed by microdialysis experiments.


a
Saline 3 min
100 microns
Merged
TH
GFP
20 microns
Cocaine 3 min
100 microns
Merged
TH
GFP
20 microns
b
Pearson correlation coefficient
****
0.3
0.2
0.1
0
- 0.1
- 0.2
Saline 3 min
Cocaine 3 min
20 mg/kg

Supplementary Figure 5.

Cocaine induces autophagy in tyrosine hydroxylase positive nerve terminals in the nucleus accumbens. (a) NAc tissue sections from GFP-LC3 mice immuno-stained with anti-GFP and anti-tyrosine hydroxylase (TH) antibodies. The brains were harvested 3 minutes following saline or cocaine injections and immediately fixed in 4°C then cryopreserved before sectioning and immunostaining. **(b)** Colocalization analysis for GFP-LC3 and TH. Pearson's correlation coefficients of images of tissue sections immuno-stained with GFP-LC3 and TH. n = 3 mice/group, **** ($P < 0.00001$, two-tailed t-test. Error bars = +/-SD.

^{1}H NMR spectrum of cocaine used in our studies



Supplementary Figure 6.

^{1}H NMR spectrum of cocaine. (a) ^{1}H NMR spectrum and for cocaine hydrochloride used in our studies in D2O/TSP at 400 MHz. The results conform to cocaine structure and show no impurities. The cocaine-HCl batch specifications data form MilliporeSigma shows 100% purity by TLC. Using proton nuclear magnetic resonance (^{1}H NMR) and carbon-13 NMR (^{13}C NMR) analysis, MilliporeSigma reported that the spectrum conforms to structure.