

Corticosterone and Exogenous Glucose Alter Blood Glucose levels, Neurotoxicity,  
and Vascular Toxicity Produced by Methamphetamine

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Supplemental File 1. Raw counts of FJc- labeled neurons for each evaluator that  
that were used in determining treatment effects on degeneration.

**Supplemental File 1**

Treatment	Evaluators	Brain Region Parietal Cortex				Brain Region PV Thalamus				Brain Region IL/ VM/ VL Thalamus			
		KT	JFB	KK JPO	Slice#	KT	JFB	KK JPO	Slice#	KT	JFB	KK JPO	Slice#
Saline	Rat ID FG 255	0	0	0	#1	0	2	0	#4	0	0	0.5	#4
		0	0	0.5	#2	0	1	0	#5	0	0	0	#5
		0	0	1.5	#3	0	0	3	#6	0	0	0.5	#6
Saline	Rat ID FG 264	0	0	0.5	#1	0	0	0	#6	0	0	0	#6
		0	0	3	#2	0	0	0	#7	0	0	0	#7
		0	0	0	#3	0	0	0	#8	0	0	0	#8
		0	0	0	#4	0	0	0	#9	0	0	0	#9
Saline	Rat ID FG 275	0.5	0	0	#2	0	0	0	#6	1	0	0	#6
		1	0	0	#3	0	0	0	#8	3	0	0.5	#8
		0	0	0	#4	0	0	0	#9	1.5	0.5	0	#9
		0	0	0	#5	0	0	0	#10	0	1	0	#10
Saline	Rat ID FG 295	0	0	0	#1	0	0	0	#5	0	0	0	#5
		0	0	0	#2	0	0	0	#6	0	0	0	#6
		0	0	0	#3	0	0	0	#7	0	0	0	#7
		0	0	0	#4	0	1	0	#8	0	0	0	#8
Saline	Rat ID FG 307	3.5	0	1	#1	0	0	0	#5	0.5	0	0	#5
		1.5	0	1	#2	0	0	2	#6	0	0	2.5	#6
		0	0	2.5	#3	0	0	1	#7	0.5	0	2.5	#7
		2	0	4	#4	0	0	2	#8	1	0	1	#8
Glucose	Rat ID FG 256	0	0	0.5	#1	0	0	0	#3	0	0	0	#3
		0	0	0.5	#2	0	0	0	#4	0	0	0	#4
		0	0	0.5	#3	0	0	0	#5	0	0	0	#5
Glucose	Rat ID FG 257	1.5	0	0.5	#1	2	0	0	#5	0	0	0	#5
		0	0	2	#2	0	0	0	#6	0	0	0	#6
		2.5	0	0	#3	0	0	0	#7	0	0	0	#7
		0	0	0	#8	3	0	0	#9	0	0	0	#9
Glucose	Rat ID FG 265	0	0	0	#1	0	0	0	#5	0	0	0	#5
		0	0	0	#2	0	0	0	#6	0	0	0	#6
		0	0	0	#3	0	0	0	#7	0	0	0.5	#7
		0	0	0	#4	0	0	0	#8	0	0	0	#8

Glucose	<b>Rat ID FG 297</b>	0	0	0	#1		0	0	0	#5		0	0	0	#5
		0	0	0	#2		0	0	0	#6		0	0	0	#6
		0	0	0	#5		0	0	0	#7		0	0	0	#7
		0	0	0	#8		0	0	0	#9		0	0	0	#9

Glucose	<b>Rat ID FG 309</b>	0	0	0.5	#1		0	0	0	#5		0	0	0	#5
		1	0	0	#2		0	0	0	#6		0	0	0	#6
		1	0	0	#3		0	0	0	#7		0	0	0	#7
		0	0	0	#4		0	0	0	#8		0	0	0	#8

CORT	<b>Rat ID FG 276</b>	0	0	0	#1		0	0	0	#5		0	0	0	#5
		0	0.5	0	#2		0	0	0	#6		0	0	0	#6
		0	0	0	#5		0	0	0	#7		0	0.5	0	#7
		0.5	0	0	#6		0	0	0	#9		0	0	0	#9

CORT	<b>Rat ID FG 277</b>	0	0	0	#2		0	0	0	#6		0	0	0	#6
		0	0	0	#3		0	0	0	#7		0	0.5	0	#7
		0	0.5	0	#4		0	0	0	#8		0	0	0	#8
		0	1	1.5	#5		0	0	0	#9		0	0	0	#9

CORT	<b>Rat ID FG 287</b>	0	0	0	#9		0	0	0	#8		0	0	0	#8
		0	0	0	#10		0	0	0	#13		0	0	0	#13
		0	0	0	#11		0	0	0	#14		0	0	0	#14
		0	0	0	#12		0	0	0	#15		0	0	0	#15

CORT	<b>Rat ID FG 296</b>	0	0		#1		0	0		#4		0	0		#4
		0	0		#2		0	0		#5		0	0		#5
		0	0		#3		0	0		#6		0	0		#6
		0	0.5		#4		0	0		#7		0	0		#7

CORT	<b>Rat ID FG 308</b>	4	0	0.5	#1		0	0	0	#5		0	0	0	#5
		1	0.5	0	#2		0	0	0	#6		0	0	0	#6
		1.5	0	0	#3		0	0	3	#7		0	0	0	#7
		2	0.5	0	#4		0	0	3	#8		0	0	0	#8

METH	<b>Rat ID FG 227</b>	10	8.5	5.5	#2		0	3	2	#4		19	21	19.5	#8
		10.5	10	10.5	#4		3	3	9	#8		8	7	2.5	#11
		7.5	7	4.5	#7		2	2	1	#12		7	13	14.5	#12
		7	7.5	2	#10		1	2	3	#13		6	10	9	#13

METH	<b>Rat ID FG 228</b>	16	6	15	#2		6	5	5	#4		154	169.5	180.5	#4
		12.5	9	13.5	#3		5	6	3	#8		195	146	173	#5
		11.5	7	7.5	#6		8	6	28	#9		178	139.5	162.5	#8
		9	6.5	8.5	#7		4	8	18	#11		145.5	123.5	179.5	#10

METH	<b>Rat ID FG 267</b>	1	2	0	#1		8	9	1	#5		26.5	39.5	39.5	#10
		4	4	3.5	#2		7	9	4	#10		36	60	54.5	#11

4.5	6	3.5	#7		10	7	11	#11		26.5	24.5	36.5	#12
5.5	4	7.5	#14		6	6	8	#13		29.5	32.5	54	#13

METH  
Rat ID  
FG 268

8	7.5	8	#1		2	0	1	#8		1	4	0	#8
10.5	10	5	#2		0	0	0	#12		4	5.5	2.5	#12
5.5	13.5	3	#4		0	0	0	#15		0	1	0	#15
11.5	13	8	#6		0	0	0	#17		0	1.5	0	#17

METH  
Rat ID  
FG 310

3.5	3	3	#2		3	4	3	#5B		76.5	86	76.5	#5A
2.5	3.5	2.5	#3		5	5	5	#6		24.5	44.5	24.5	#5B
2.5	4.5	2	#4		5	5	5	#10		72.5	72.5	72.5	#10
2	8	1	#8		6	7	6	#11		35.5	31	35.5	#11

METH  
Rat ID  
FG 311

8.5	7	6.5	#1		6	6	6	#6		60.5	46.5	56	#7
5	5	3.5	#2		6	6	7	#7		42.5	49.5	25.5	#8
3	5	2	#3		4	5	5	#13		62.5	78	57.5	#13
4.5	4.5	6.5	#10		10	8	12	#14		47.5	57	41.5	#14

METH + Vehicle  
Rat ID  
FG 280

2	6	2.5	#1		2	3	2	#7		11	13	16	#6
4	7	3.5	#3		6	3	6	#12		10	15.5	16	#7
5	5.5	5	#8		4	4	6	#13		14	17	20	#10
5	5	4	#9		5	4	4	#14		17	15.5	19	#14

METH + Vehicle  
Rat ID  
FG 288

3.5	2	6	#3		2	4	2	#5		19.5	17	15	#7
2	2	1.5	#6		2	5	2	#8		17	19.5	13.5	#9
4	4.5	2	#13		6	4	7	#10		16	19.5	17	#16
1.5	2	0.5	#15		0	2	1	#16		17	22.5	18	#17

METH + Vehicle  
Rat ID  
FG 289

17	14.5	13	#4		4	5	2	#6		47	68.5	47	#7
10	7.5	6	#5		10	7	7	#7		9.5	11.5	12.5	#13
15.5	12.5	14	#10		2	5	2	#8		54.5	48.5	49	#14
7	12.5	7.5	#11		0	8	0	#13		39.5	38	43	#15

METH + Vehicle  
Rat ID  
FG 298

10	11	8.5	#3		0	3	0	#6		2	3.5	2	#9
9	10.5	8	#4		2	4	3	#7		6	4	4.5	#10
12	9	9.5	#6		3	2	3	#8		4	5	4	#11
12	11	10.5	#7		2	1	1	#9		8	9	7	#12

METH + Vehicle  
Rat ID  
FG 299

2	3	1.5	#3		0	0	0	#8		9.5	7.5	9.5	#8
6	5	4.5	#5		2	0	0	#9		7	6.5	6.5	#9
5.5	4.5	4	#7		3	2	3	#10		10.5	8	9.5	#11
3	2.5	3	#13		1	0	1	#15		6	5	5	#15

METH + Vehicle  
Rat ID  
FG 300

13	17.5	23.5	#3		0	1	0	#8		5	4.5	3.5	#9
20	14.5	18	#7		2	2	2	#9		5.5	4.5	4	#10
18.5	17	16.5	#6		3	3	2	#13		4	5.5	5.5	#11
27	20	24	#7		1	1	1	#14		5	4	5	#16

METH+Glucose

<b>Rat ID</b>	1	2.5	0	#1		2	2	2	#5		18.5	30	14.5	#10
<b>FG 269</b>	4.5	2	0	#3		1	1	2	#6		18.5	28.5	11	#11
	1.5	2	0.5	#8		3	3	3	#12		17	39.5	34.5	#13
	2	2	2.5	#14		6	0	2	#13		21.5	19	13	#15

METH+Glucose

<b>Rat ID</b>				#3		0	1	0	#8		0	8	1.5	#8
<b>FG 271</b>	5.5	6	4.5	#4					#9		0	4	0	#9
	10	10.5	9	#5		1	1	1	#11					#11
	9.5	8.5	6.5	#7					#12		2.5	3	0	#12
	9.5	8	8.5	#10		1	1	0	#15		1.5	3	1.5	#15
						1	1	1	#16					#16

METH+Glucose

<b>Rat ID</b>	2	3		#3		2	2		#4		4.5	14.5		#4
<b>FG 282</b>	3	3		#12		5	5		#14		3	8.5		#5
	4	5.5		#13		7	7		#15		8	8.5		#20
	5	4		#18		3	3		#20		8.5	8.5		#23

METH+Glucose

<b>Rat ID</b>	14	14.5	14	#4		16	18	18	#5		116.5	108.5	119.5	#7
<b>FG 291</b>	19	12	13	#12		39	25	41	#6		123.5	87.5	117.5	#8
	9.5	7.5	9.5	#13		38	22	38	#7		104.5	75.5	106.5	#9
	10.5	9.5	9.5	#14		13	20	16	#8		158	95.5	148	#10

METH+Glucose

<b>Rat ID</b>	10	7.5	10.5	#1		3	3	3	#7		47.5	35	44	#7
<b>FG 314</b>	8	8	4.5	#3		4	3	3	#8		44	46.5	42	#8
	14	8	12	#4		4	3	4	#9		36.5	26.5	34.5	#9
	12	11	12	#12		2	2	1	#13		34	33.5	33.5	#13

METH+Glucose

<b>Rat ID</b>	20.5	20	16	#1		74	42	63	#7		348.5	121	305.5	#7
<b>FG 315</b>	14.5	13	2.5	#2		92	65	57	#8		378.5	280.5	332.5	#8
	18	12	7	#4		87	33	74	#9		381.5	382	395.5	#9
	21	10	16.5	#10		56	37	54	#13		367.5	249	359	#13

METH+CORT

<b>Rat ID</b>	29	10	22.5	#3		0	0	0	#7		1.5	2.5	0.5	#7
<b>FG 272</b>	23	12	20	#4		1	1	0	#8		2	3.5	1.5	#8
	19.5	11.5	12.5	#5		0	0	1	#9		0.5	4.5	0	#9
	29	13	17.5	#10		0	0	0	#14		0	1.5	0	#14

METH+CORT

<b>Rat ID</b>	9	9	3	#5		3	8	8	#11		0	8	0	#8
<b>FG 285</b>	11	12	11.5	#6		0	6	5	#12		0.5	12.5	1	#9
	9	10	8	#7		4	6	5	#14		0	11	1	#13
	9.5	9.5	9.5	#15		0	7	5	#17		2.5	9.5	1.5	#19

METH+CORT

<b>Rat ID</b>	12	13		#3		20	17		#6		55	75.5		#7
<b>FG 286</b>	13.5	14.5		#11		18	18		#7		118	126.5		#8
	8	13.5		#12		25	25		#9		105.5	132		#9
	12.5	16		#13		16	14		#10		124	119		#14

METH+CORT

<b>Rat ID</b>	16	17	16.5	#3		8	15	8	#8		227.5	201	128.5	#12
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FG 304	17	19	17.5	#6		5	8	5	#10		199.5	239	185	#13
	38	27.5	26	#14		10	9	10	#17		71.5	247.5	53	#17
	24	16	26.5	#15		11	12	9	#18		82.5	195	72.5	#18

METH+CORT

Rat ID FG 306	21.5	18	17.5	#2		7	7	7	#4		15	13	14.5	#4
	20.5	16.5	18	#3		5	5		#5		7.5	8.5	7.5	#9
	13	12	9.5	#10		5	4	4	#11		2.5	8.5	0.5	#11
	14	12	13	#17		4	5	4	#13		11	11.5	10	#18

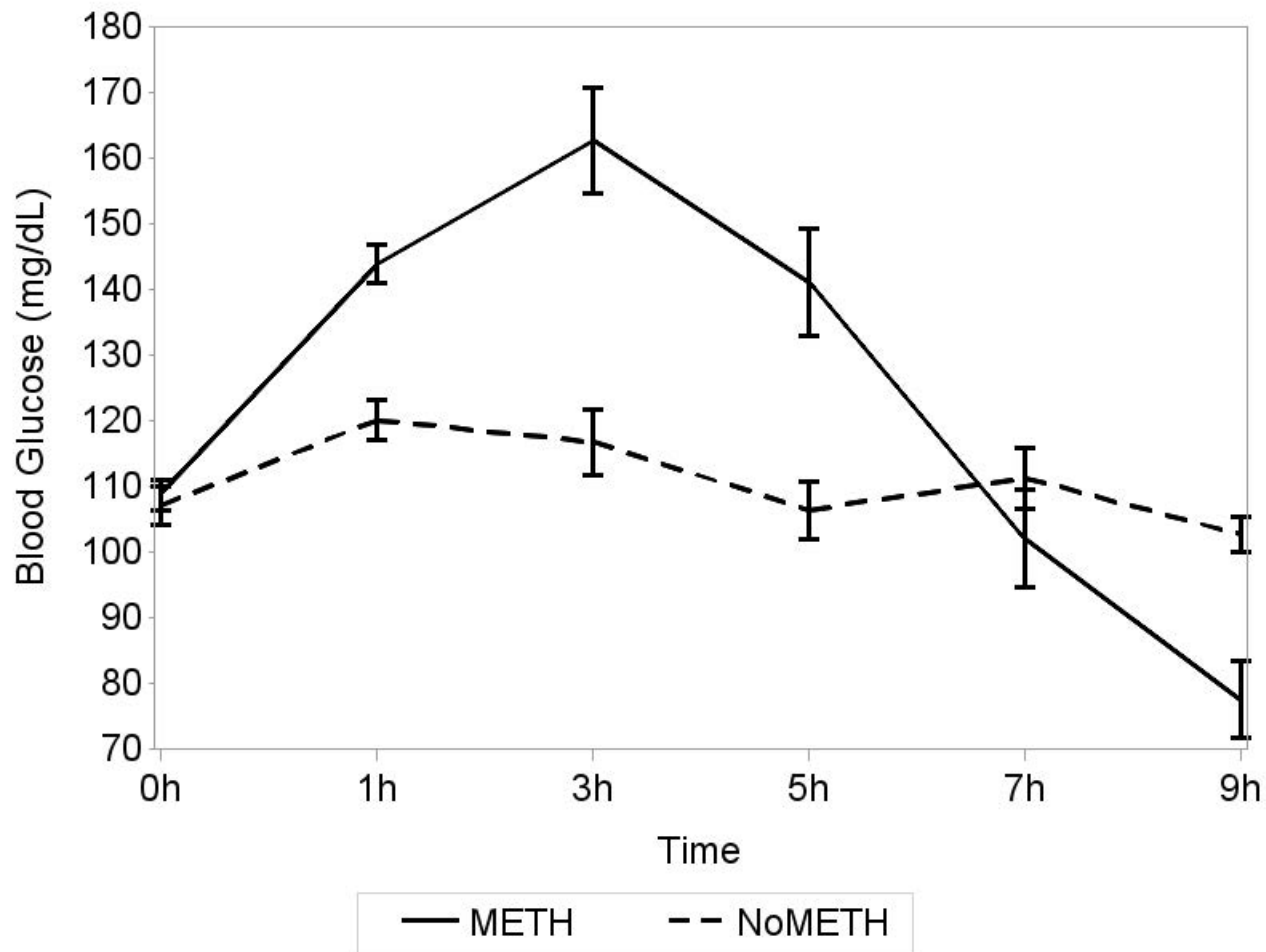
METH+CORT

Rat ID FG 316	28.5	26.5	29.5	#3		0	1	0	#7		122	155.5	117.5	#7
	19	26	17.5	#4		0	1	0	#8		142	212	141	#8
	44.5	30	40.5	#10		4	1	5	#9		171	196	168.5	#9
	28	26.5	27	#12		0	2	0	#13		146	205	145	#13

METH+CORT

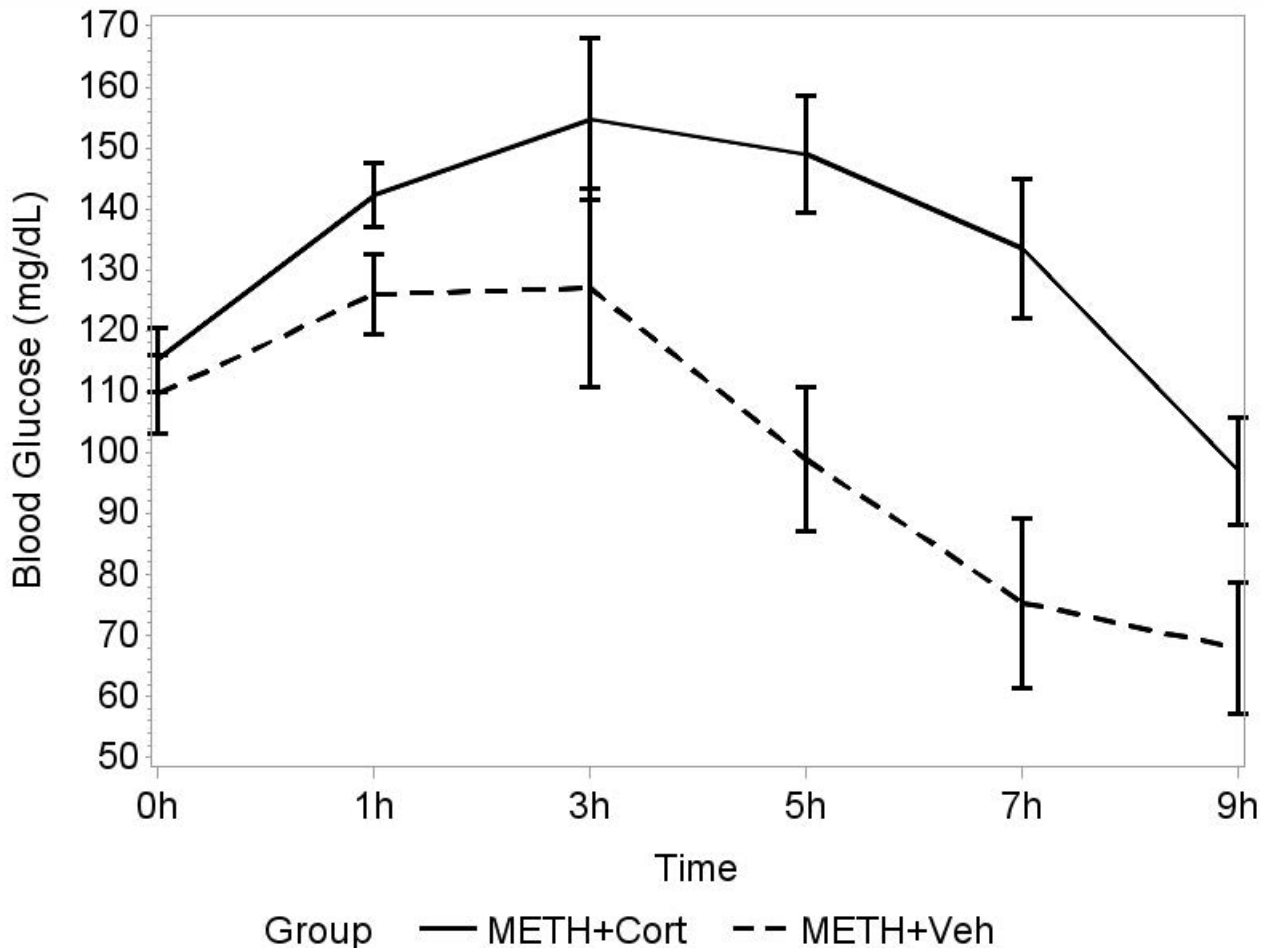
Rat ID FG 317	20	14	12.5	#1B		7	5	7	#5					#4
	16	16	12	#9		5	4	4	#6		64.5	45.5	64.5	#5
	25.0	19.5	29.0	#10B		5	5	5	#7		45.5	37	45.5	#6
	23	20.5	21	#11		9	10	8	#10		22	26.5	22	#7
											64	67.5	57	#10A

Supplemental Figure 1. METH vs noMETH blood glucose levels



Mean ( $\pm$  SEM) blood glucose levels for METH (Saline, Glucose, and CORT) and noMETH (METH, METH+Glucose, and METH+CORT) treatment groups. Blood glucose was determined 1h after each of injection of METH and also at 3 h after the last set injection from blood obtained from the hindfoot toe pad.

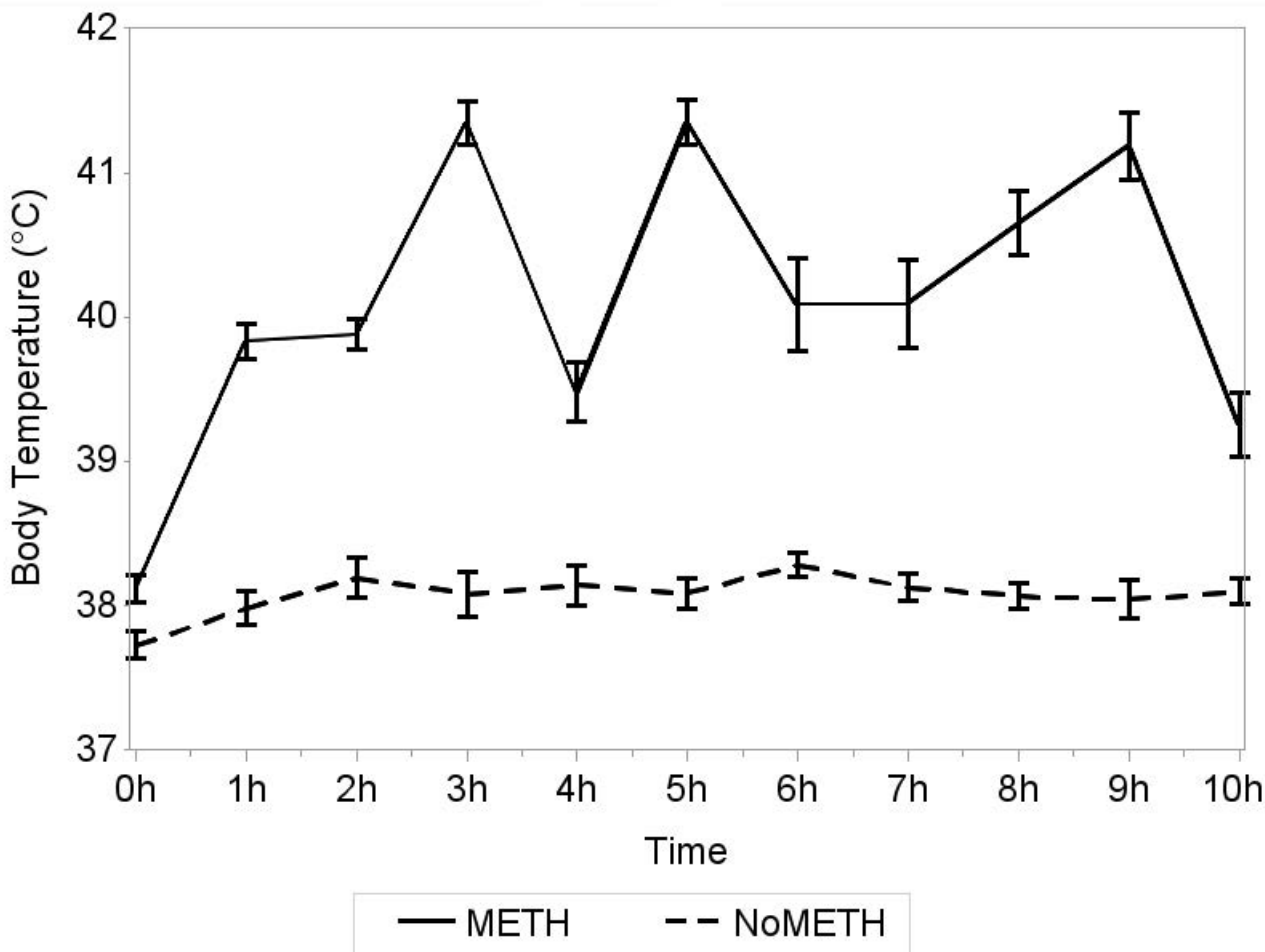
Supplemental Figure 2. METH+CORT vs METH+Veh blood glucose levels



Mean ( $\pm$  SEM) blood glucose levels are shown for the METH+CORT and METH+Veh treatment groups. Blood glucose was determined 1h after each of injection of METH and also at 3 h after the last set injection from blood obtained from the hindfoot toe pad.

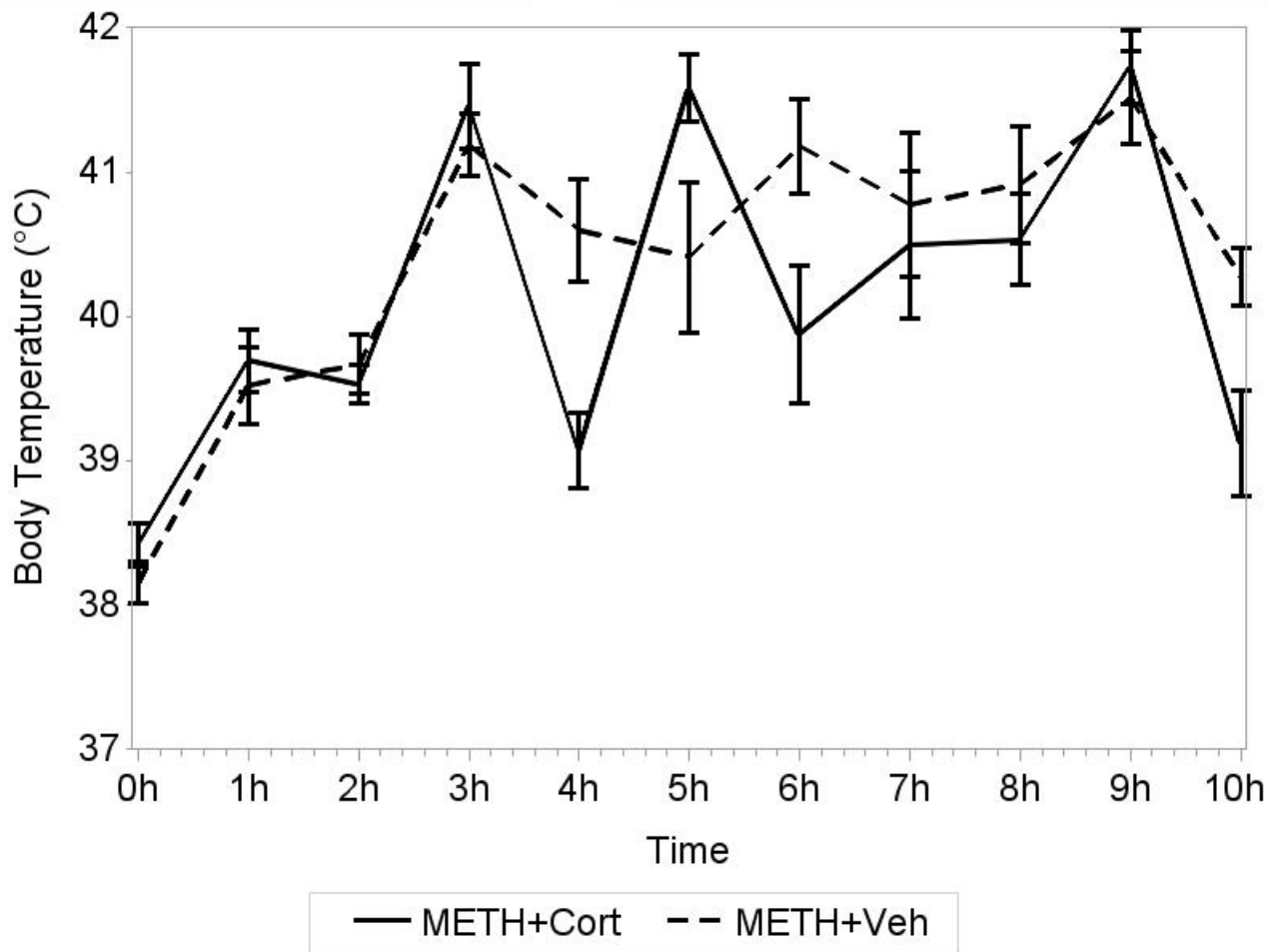


Supplemental Figure 3. METH vs noMETH body temperature levels



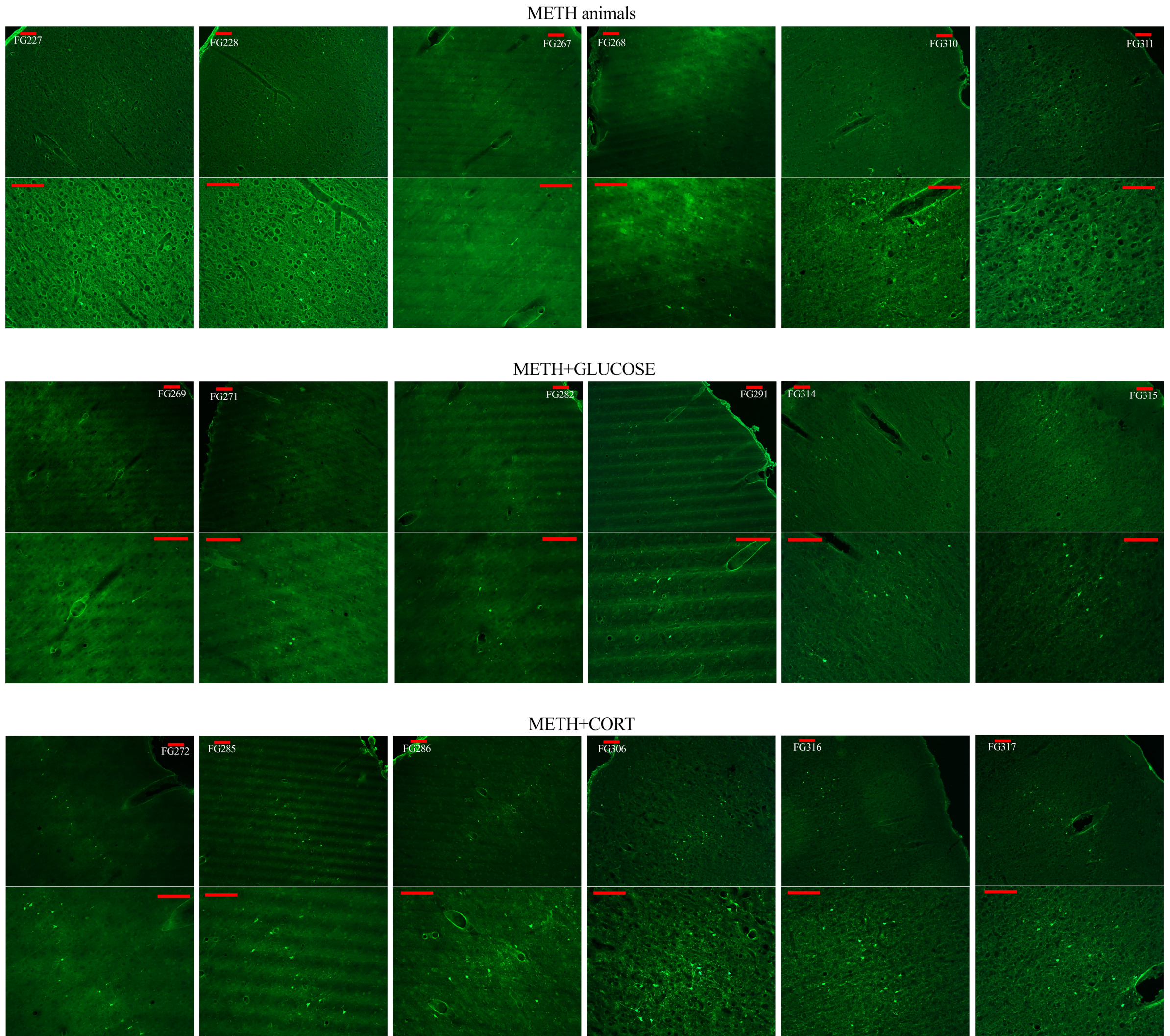
Mean ( $\pm$  SEM) body temperature levels for METH (Saline, Glucose, and CORT) and noMETH (METH, METH+Glucose, and METH+CORT) treatment groups. Upper colonic temperature was determined every hour.

Supplemental Figure 4. METH+CORT vs METH+Veh body temperature levels



Mean ( $\pm$  SEM) body temperature levels are shown for the METH+CORT and METH+Veh treatment groups. Upper colonic temperature was determined every hour.

Supplemental Figure 5. FJc-labeling of degenerating neurons in three of the METH treatment groups



Representative images of histological sections labeled with FJc for animals in the METH, METH+Glucose, and METH+CORT groups are presented. There was a significant difference in neurodegeneration in the parietal cortex when comparing METH+CORT to METH, METH+Glucose, and METH+Veh (See Table 2 in text) Saline and CORT groups are not shown since there was virtually no FJc-labeling present.

# Supplemental Figure 6. "Screen Shot" of Nikon NIS Display of an image of the parietal cortex and setting an parameters used for image analysis.

The screenshot displays the Nikon NIS Elements software interface. The main window shows a photomicrograph of a parietal cortex section with a red ellipse highlighting a region of interest (ROI). The software interface includes various toolbars and panels:

- DS-R11 Settings:** Resolution: 1280x1024 Fast, Normal; Quality: 1280x1024 Fine - 8bit; Exposure: Auto Exposure, 1 s; Analog Gain: 4.0x; AE Compensation: -1.3 EV; Color: Auto White; Scene Mode: > Commands.
- Object Count:** Threshold RGB; Restrictions: EqDiameter (µm); Result: Total Area: 1.231e+007 µm<sup>2</sup>; Measured Area: 3.18 %; **3251** objects detected.
- Object Count Table:**

ROI ID	Binary ID	#	Area	EqDiameter	Perimeter	Length	Width
ROI 1	Threshold (2)	681	583.93	27.27	231.28	110.55	5.29
ROI 1	Threshold (2)	698	329.12	20.47	124.22	56.26	5.85
ROI 1	Threshold (2)	705	631.70	28.36	170.03	76.79	8.23
ROI 1	Threshold (2)	748	26.54	8.81	18.90	7.46	3.56
ROI 1	Threshold (2)	749	451.22	23.97	157.67	72.62	6.21
ROI 1	Threshold (2)	761	599.85	27.64	166.41	75.23	7.57
ROI 1	Threshold (2)	769	10.62	3.69	11.08	5.56	1.91

A representative "screen shot" of a photomicrographs (4X) which shows an image of cortical layers II through Layer V of the vibrissae somatosensory S1 barrel fields and a portion of the more dorsal S2 barrel fields. NIS Elements Software (Nikon) was used to detect and measure microglia within the analyzed region of interest (ROI) shown enclosed by the red ellipse. The ROI was standardized and kept constant. The intensity range selection points for detecting the microglia were set to eliminate (as much as possible) as many of the minor processes not connected to somas. However, many of the darkly stained isolated distal process were still captured. Setting intensity capture parameters to exclude all of the darkly stained distal processes was not an option since it resulted in exclusion of smaller or more lightly stained microglia soma and adjoining processes.