Supplementary Methods:

1. Diet induced obese (DIO) mice:
Male C57BL/6 mice 4-6 weeks of age (Harlan, UK) were group housed in polypropylene cages with ad libitum access to a high fat diet (D12451 45% of Kcal derived from fat; Research Diets, New Jersey, USA) and water. Animals were maintained at 21-23^{o}C with a 12h light-dark cycle. After 14 weeks diet exposure, animals were singly housed in polypropylene cages. Animals were exposed to high fat diet for 27 weeks in total prior to start of the procedures and then continued throughout the study period. The average weight of the DIO mice at the start of the study was 48.7±0.82g.

2. Measurement of locomotor activity:
The ambulatory activity of each animal was measured simultaneously using the optical beam technique (Opto M3, Columbus Instruments). The optical beam technique was carried out with animals individually housed in Plexiglas cages (width 20.5cm, length 32.0cm and depth 19.5cm) in a 24-chamber open-circuit Oxymax comprehensive lab animal monitoring system (CLAMS; Columbus Instruments, Columbus, OH) as previously described (23). All rats were acclimatized to their cages for 2 days prior to the study day.

Supplementary Table 1: Coordinates used for placement of intranuclear hypothalamic cannulae in male Wistar rats
Rats were implanted with permanent 26-gauge stainless steel guide cannulae projecting into the SON, ARC, PVN, AHA, VMN, DMN, SCN and LHA of the hypothalamus, according to coordinates of Paxinos and Watson.

Area	**Posterior** from bregma (mm)	**Lateral** from midline (mm)	**Depth** (mm)
SON	1.1	1.8	9.3
ARC	3.4	0.5	10.0
PVN	1.8	0.3	8.0
AHA	1.3	0.8	8.5
VMN	2.8	0.7	9.6
DMN	3.3	0.6	9.0
SCN	0.7	0.1	9.2
LHA	2.2	2.0	8.5

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Supplementary Table 2: Effect of ICV administration of PK2 on food intake in rats

Supplementary Table 2A: Effect of ICV administration of saline or PK2 at doses of 0.005, 0.015, 0.05 and 0.15nmol/rat to rats (n=10-12/group) injected at the beginning of the dark phase. Data presented as mean ± SEM food weight in grams (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. saline).

Treatment (nmol)	Food intake following injection (hours)				
	0-1 hr	0-2 hr	0-4 hr	0-8 hr	0-24 hr
Saline	2.93 ± 0.48	3.44 ± 0.35	4.91 ± 0.60	9.00 ± 1.67	18.91 ± 2.29
PK2 0.005	2.75 ± 0.39	4.14 ± 0.58	4.71 ± 0.81	9.06 ± 1.51	20.13 ± 0.98
PK2 0.015	2.41 ± 0.43	3.95 ± 0.59	5.67 ± 1.10	10.89 ± 2.65	18.39 ± 3.94
PK2 0.05	1.53 ± 0.45*	2.55 ± 0.58	5.50 ± 1.33	12.23 ± 1.91	22.40 ± 1.98
PK2 0.15	0.79 ± 0.12***	1.27 ± 0.32**	1.54 ± 0.49*	5.03 ± 1.07	15.65 ± 2.91

Supplementary Table 2B: Effect of ICV administration of saline or PK2 at doses of 0.15, 0.5 and 1.5nmol/rat to rats (n=10-12/group) injected at the beginning of the dark phase. Data presented as mean ± SEM food weight in grams (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. saline).

Treatment (nmol)	Food intake following injection (hours)				
	0-1 hr	0-2 hr	0-4 hr	0-8 hr	0-24 hr
Saline	2.16 ± 0.16	4.27 ± 0.44	6.20 ± 0.91	10.56 ± 1.75	20.60 ± 2.96
PK2 0.15	0.36 ± 0.13***	1.67 ± 0.42***	2.63 ± 0.54***	6.98 ± 1.31	12.09 ± 2.75*
PK2 0.5	0.32 ± 0.04***	1.23 ± 0.28***	3.35 ± 0.70**	8.08 ± 1.38	19.3 ± 2.56
PK2 1.5	0.30 ± 0.05***	1.13 ± 0.14***	4.01 ± 0.82*	8.45 ± 1.75	17.3 ± 2.97

Supplementary Table 2C: Effect of ICV administration of saline or PK2 at doses of 0.15, 1.5 or 4.5nmol/rat to rats (n=10-12/group) fasted for 24 hours injected in the early light phase. Data presented as mean ± SEM food weight in grams (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. saline).

Treatment (nmol)	Food intake following injection (hours)				
	0-1 hr	0-2 hr	0-4 hr	0-8 hr	0-24 hr
Saline	7.28 ± 1.30	9.84 ± 1.04	10.00 ± 1.03	14.30 ± 1.41	36.75 ± 3.20
PK2 0.15	4.37 ± 0.52***	7.96 ± 0.69	10.49 ± 1.27	15.38 ± 1.25	37.84 ± 1.51
PK2 0.5	4.67 ± 0.31**	7.41 ± 0.32**	7.54 ± 0.31	9.90 ± 1.17*	24.75 ± 3.53**
PK2 1.5	1.67 ± 0.36***	2.64 ± 0.61***	6.16 ± 1.19*	9.48 ± 1.85*	27.50 ± 4.30*

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Supplementary Table 3: PK2 does not alter behavior in rats.
Adult male Wistar rats weighing 200-250g (n=10-12 per group) were injected ICV with saline or PK2 1.5 nmol/rat. After injection, behavioral patterns were monitored continuously for 120 minutes after injection by observers blinded to the experimental treatment Data are presented as median (interquartile range). ** P < 0.01 vs. saline.

	0-1hour		**1-2 hour**		**0-2 hour**	
	saline	**PK2**	**saline**	**PK2**	**saline**	**PK2**
Feeding	5 (3-6)	2 (0-4)	6 (6-7)	3 (3-4)	10 (10-12)	6 (4-7)**
Drinking	0 (0-2)	1 (0-3)	0 (0-3)	0 (0-0)	2 (0-4)	1 (0-3)
Grooming	6 (6-8)	5 (4-8)	7 (3-10)	6 (5-8)	14 (10-18)	13 (9-16)
Burrowing	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Rearing	16 (16-20)	16 (16-23)	7 (4-11)	15 (9-16)	22 (20-34)	32 (29-37)
Locomotion	3 (2-4)	4 (3-7)	2 (1-3)	2 (1-4)	5 (4-6)	5 (4-6)
Sleep	0 (0-0)	0 (0-0)	2 (0-3)	0 (0-0)	2 (0-3)	0 (0-0)
Head down	1 (0-3)	1 (0-2)	11 (4-16)	4 (1-10)	12 (5-19)	5 (1-13)

Supplementary Table 4: Effect of ICV administration of anti-PK2 antibody on food intake
Rats (n = 10-12/group) were injected ICV with either control IgG or anti-PK2 antibody (10 or 30pmol) in the early light phase. Data presented as mean ± SEM food weight in grams (* p < 0.05 vs. control Ab). Ab = antibody

Treatment (pmol)	Food intake following injection (hours)					
	0-1 hr	0-2 hr	2-4 hr	0-4 hr	0-8 hr	0-24 hr
Control Ab 10	0.22 ± 0.10	0.55 ± 0.22	0.04 ± 0.02	0.60 ± 0.22	1.74 ± 0.69	23.95 ± 2.95
Control Ab 30	0.18 ± 0.10	0.54 ± 0.24	0.02 ± 0.01	0.55 ± 0.24	2.57 ± 0.49	29.3 ± 1.32
Anti-PK2 Ab 10	0.52 ± 0.15	0.62 ± 0.13	0.12 ± 0.06	0.74 ± 0.19	2.35 ± 0.31	23.7 ± 1.47
Anti-PK2 Ab 30	0.17 ± 0.04	0.21 ± 0.06	0.56 ± 0.21*	0.77 ± 0.19	3.71 ± 0.45	27.03 ± 0.97

Supplementary Table 5: Effects of PK2 on the release of hypothalamic factors known to regulate appetite. Hypothalamic explants were taken from adult male Wistar rats weighing 200-250g and incubated in artificial CSF (basal) for 45 minutes, followed by incubation in PK2 (10, 100 or 1000nM) for 45 minutes. n = 9-12 per treatment. Peptide release is expressed as mean ± SEM and as a percentage of basal release.

Peptide release	**Concentration of PK2 incubated with hypothalamic explants**		
	10nM	**100nM**	**1000nM**
NPY	109.8 ± 8.6	107.8 ± 10.1	103.3 ± 11.9
AgRP	105.2 ± 8.9	101.2 ± 9.8	105.5 ± 10.3
CART	108.3 ± 10.8	106.0 ± 10.1	113.9 ± 9.5
TRH	113.5 ± 14.0	89.1 ± 11.9	116.2 ± 11.2
CRH	110.0 ± 10.1	107.1 ± 7.1	114.0 ± 14.2

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Supplementary Table 6: Effect of acute peripheral administration of PK2 on food intake in lean rats and mice

Supplementary Table 6A: Effect of intraperitoneal (ip) injection of saline or PK2 at doses of 2.3, 7 or 20nmol/kg to rats (n=10-12/group) at the beginning of the dark phase. Data presented as mean ± SEM food weight in grams (* $p < 0.05$ vs. saline).

Treatment (nmol/kg)	Food intake following injection (hours)				
	0-1 hr	0-2 hr	0-4 hr	0-8 hr	0-24 hr
Saline	3.57 ± 0.26	6.83 ± 0.50	12.34 ± 0.66	19.23 ± 0.59	30.68 ± 0.49
PK2 2.3	3.28 ± 0.34	6.54 ± 0.54	11.50 ± 0.74	17.95 ± 0.98	28.80 ± 1.14
PK2 7	3.10 ± 0.50	5.39 ± 0.73	10.59 ± 0.71	17.94 ± 0.81	29.20 ± 0.97
PK2 20	2.04 ± 0.43*	5.23 ± 0.60	10.61 ± 0.66	18.04 ± 0.76	28.63 ± 1.07

Supplementary Table 6B: Effect of ip injection of saline or PK2 at doses of 7, 20, 60, 180 or 540nmol/kg to C57BL/6 mice (n=10-12/group rats (n=10-12/group) at the beginning of the dark phase. Data presented as mean ± SEM food weight in grams (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. saline).

Treatment (nmol/kg)	Food intake following injection (hours)				
	0-1 hr	0-2 hr	0-4 hr	0-8 hr	0-24 hr
Saline	0.15 ± 0.01	0.53 ± 0.07	1.27 ± 0.05	2.45 ± 0.08	4.11 ± 0.11
PK2 7	0.12 ± 0.05**	0.38 ± 0.06	0.99 ± 0.11*	2.12 ± 0.21*	3.73 ± 0.41
PK2 20	0.06 ± 0.01***	0.34 ± 0.05*	1.03 ± 0.07**	2.12 ± 0.09***	3.87 ± 0.09**
PK2 60	0.04 ± 0.01***	0.21 ± 0.04***	0.79 ± 0.10***	1.86 ± 0.13***	3.49 ± 0.20***
PK2 180	0.04 ± 0.01***	0.21 ± 0.03**	0.65 ± 0.10***	1.63 ± 0.21***	3.37 ± 0.24***
PK2 540	0±0***	0.03 ± 0.01***	0.28 ± 0.06***	1.27 ± 0.13***	2.95 ± 0.14***

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Supplementary Table 7: Chronic administration of PK2 results in sustained reduction in food intake in lean mice.
Ad libitum fed adult male C57BL/6 mice weighing 20-25g were randomized into two treatment groups: saline or PK2 180nmol/kg. Each group of animals was given twice daily i.p. injections (at the beginning of the light phase and just prior to the beginning of the dark phase) for 5 days. Light = food intake 4 hours following the injection administered at the beginning of the light phase. Dark = food intake 1 hour after the injection administered just prior to the dark phase. Data are presented as mean ± SEM. * P < 0.05 vs. saline, ** P < 0.01 vs. saline, n=10 per group.

Food consumed (g)	Day 1		Day 2		Day 3		Day 4		Day 5	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Saline	0.19 ± 0.04	0.23 ± 0.02	0.21 ± 0.05	0.26 ± 0.04	0.14 ± 0.04	0.19 ± 0.02	0.31 ± 0.08	0.14 ± 0.02	0.19 ± 0.06	0.20 ± 0.03
PK2 180 nmol/kg	0.04 ± 0.01**	0.10 ± 0.03**	0.03 ± 0.02**	0.12 ± 0.02*	0.07 ± 0.04	0.08 ± 0.02**	0.24 ± 0.12	0.12 ± 0.02	0.05 ± 0.02*	0.10 ± 0.03*

Supplementary Table 8: Chronic administration of PK2 results in sustained reduction in food intake in obese mice.
Adult male C57BL/6 DIO mice 31-33 weeks of age were randomized into three groups: (i) saline treated with *ad libitum* access to food, (ii) PK2 treated (540nmol/kg per injection) with *ad libitum* access to food, (iii) pair fed group (saline treated but food restricted to the median food intake consumed by the PK2 treated mice (group ii) over the previous 24 hour period). Each group of animals was given twice daily i.p. injections (at the beginning of the light phase and just prior to the beginning of the dark phase) for 5 days. Food intake is shown only for the saline control group and PK2 treated group as a fixed amount of food was given to animals in the pair fed group. Light = food intake 4 hours following the injection administered at the beginning of the light phase. Dark = food intake 1 hour after the injection administered just prior to the dark phase. Data are presented as mean ± SEM. * P < 0.05 vs. saline, ** P < 0.01 vs. saline, *** P < 0.001 vs. saline, n=10 per group.

Food consumed (g)	Day 1		Day 2		Day 3		Day 4		Day 5	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Saline	0.26 ± 0.05	0.63 ± 0.03	0.22 ± 0.03	0.57 ± 0.07	0.22 ± 0.03	0.68 ± 0.07	0.16 ± 0.03	0.62 ± 0.07	0.16 ± 0.04	0.59 ± 0.08
PK2 540 nmol/kg	0.09 ± 0.02***	0.22 ± 0.03***	0.09 ± 0.01**	0.31 ± 0.03*	0.10 ± 0.03*	0.41 ± 0.05*	0.07 ± 0.03	0.31 ± 0.04**	0.09 ± 0.02	0.36 ± 0.06*

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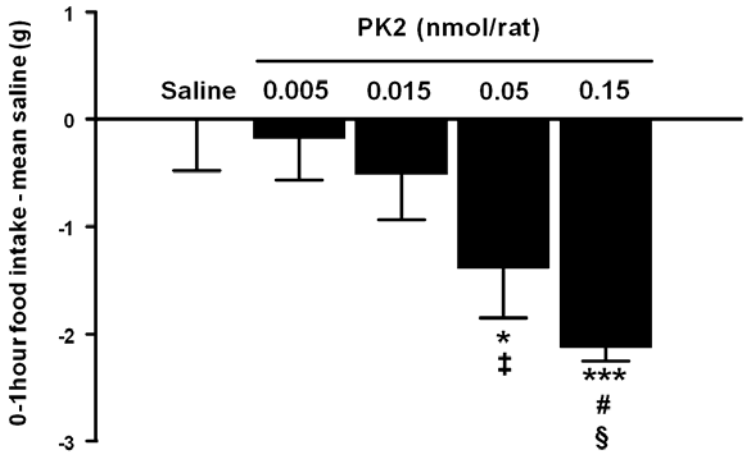
Supplementary Figure 1: Effect of ICV administration of saline or PK2 at doses of 0.005, 0.015, 0.05 and 0.15nmol/rat to rats (n=10-12/group) injected at the beginning of the dark phase. Data presented as mean ± SEM 0-1hr food weight as a change compared to saline injected animals (*= p < 0.05 PK2 0.05nmol vs. saline; ‡ = P<0.05 PK2 0.05nmol vs. PK2 0.005nmol; *** = p < 0.001 PK2 0.15nmol vs. saline; # = p < 0.01 PK2 0.15nmol vs. PK2 0.005nmol; § = p < 0.01 PK2 0.15nmol vs. PK2 0.015nmol).



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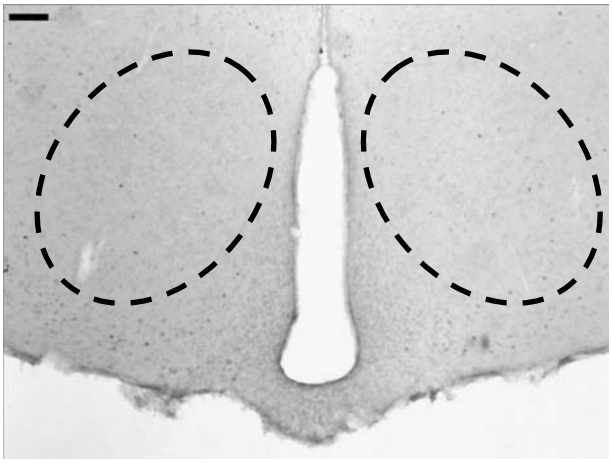
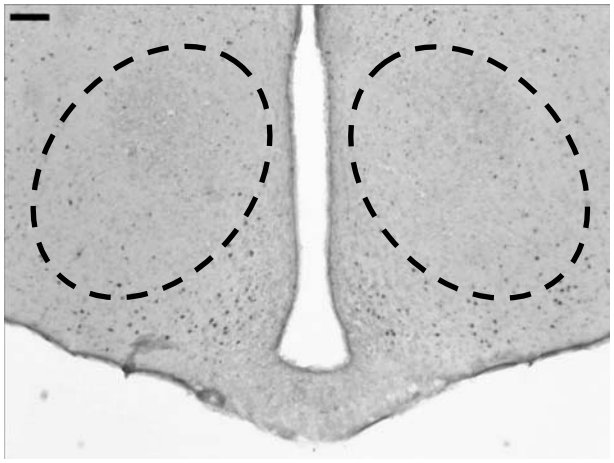
Supplementary Figure 2: ICV administration of PK2 to *ad libitum* fed rats does not cause cfos activation in the VMN, DMN, SCN or LHA of the hypothalamus.
(A-D) Representative brain sections showing cfos expression in the VMH (A), DMN (B), SCN (C) and LHA (D), from rats injected into the lateral ventricle with saline or PK2 (1.5nmol/rat) in the early light phase. Scale bar 100 µm. Numbers of c-fos positive cells were expressed as activated cells per section per nucleus. Brain sections from rats injected with saline are shown in the panels on the left and those from rats injected with PK2 on the right in the figure below. Fx = fornix, Opt = optic tract, 3v = third ventricle. The dashed line demarcates the nucleus.

A





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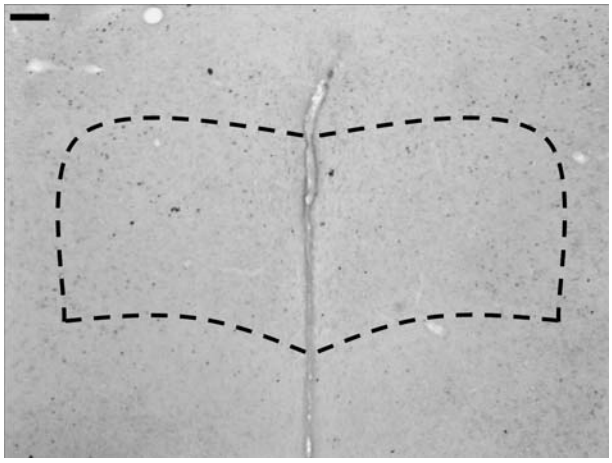

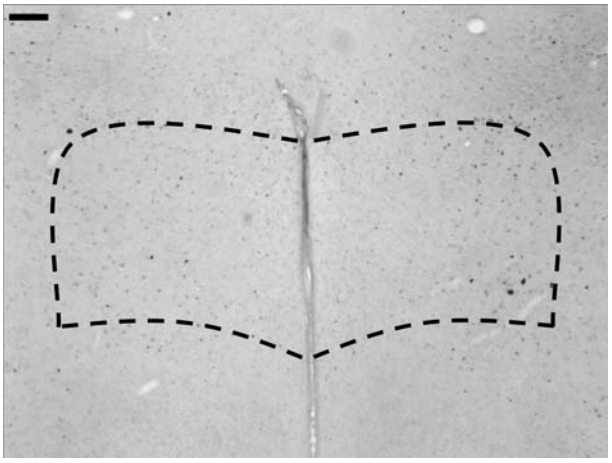

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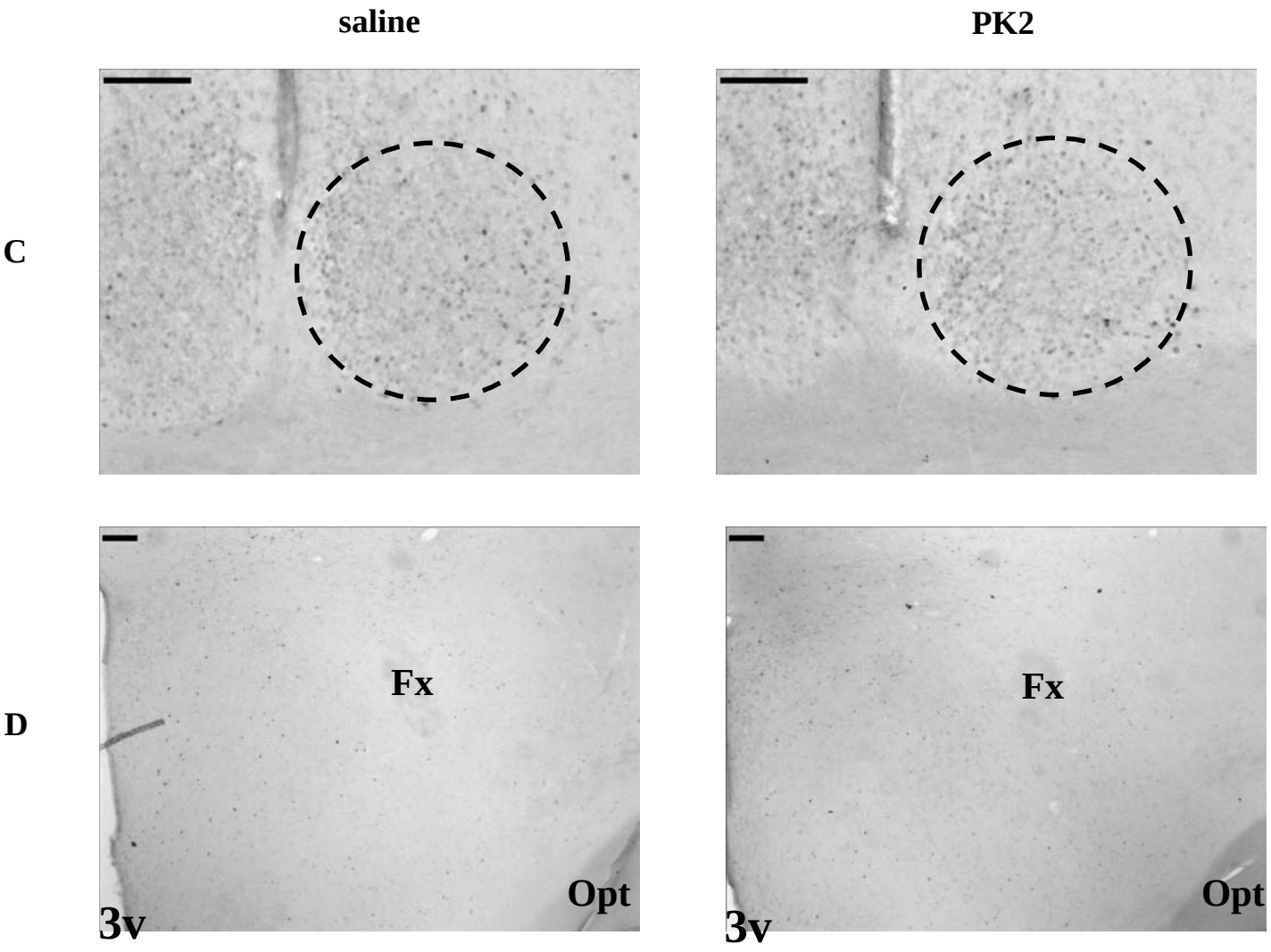
Supplementary Figure 2 continued



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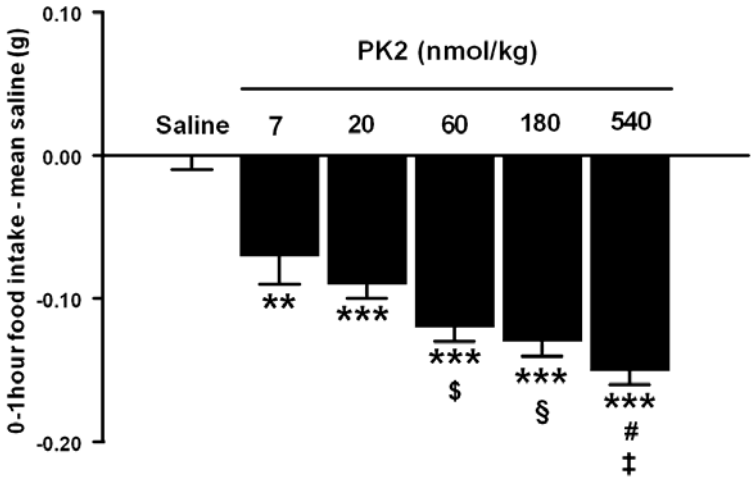
Supplementary Figure 3: Effect of ip injection of saline or PK2 at doses of 7, 20, 60, 180 or 540nmol/kg to C57BL/6 mice (n=10-12/group rats (n=10-12/group) at the beginning of the dark phase. Data presented as mean ± SEM 0-1hr food weight as a change compared to saline injected animals (** = p < 0.01 PK2 7nmol/kg vs. saline; *** = p < 0.001 PK2 20nmol/kg or PK2 60nmol/kg or PK2 180nmol/kg or PK2 540nmol/kg vs. saline; $ = p < 0.05 PK2 60nmol/kg vs. PK2 7nmol/kg; § = PK2 180nmol/kg vs. PK2 7nmol/kg; # = p < 0.01 PK2 540nmol/kg vs. PK2 7nmol/kg; ‡ = P<0.01 PK2 540nmol/kg vs. PK2 20nmol/kg).



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