Supporting Information Appendix:

Supplementary Material and Methods
Figures S1-S8
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Insulin tolerance test

The insulin tolerance test was performed as recommended in literature (1). Animals were subjected to food deprivation for 6h before glucose measurement. Insulin (Sigma-Aldrich, St. Louis, MO, USA) was administered intraperitoneally (i.p.) [0.5 U/kg]. Blood glucose levels were measured at baseline at 30 min intervals post-injection for the following 2h.

Hippocampal measurements of TBARS and NADH/NAD$^+$

At the 10th day of CSD and at 3- and 5 weeks post-CSD, mice were sacrificed and the dorsal hippocampi were dissected, snap frozen in liquid nitrogen and stored at -80°C. Brain tissue was then processed according to the manufacturer's instructions of Lipid Peroxidation (MDA) Assay Kit (Abcam, Cambridge, UK; #Cat.No. ab118970) or NAD/NADH Assay Kit (Abcam, Cambridge, UK; #Cat.No. ab65348). For NADH/NAD$^+$, hippocampal cell suspension was diluted (10 x). MDA and NADH/NAD$^+$ concentrations were normalized to the corresponding protein concentration measured by Pierce BCA protein Assay Kit (Thermo Scientific, #Cat.No. 23225) and expressed as MDA concentration in pmol/µg protein or NADH/NAD$^+$ concentration in pmol/mg protein.

Caloric restriction

All animals were subjected to CSD for 10 days and assigned to the caloric restriction (CR) group, which received 80% of their averaged, regular food consumption during CSD, or to the control group, which received food *ad libitum*. To determine 80% of pre-stress food intake, we measured the amount of individual food intake for 3 days prior to CSD.

CD1-encounter test

CD-1 encounter test was performed in an open field (45 x 45 x 41 cm) with a Plexiglas barred cylinder (∅ 10 cm, H: 20 cm) placed in the center point area of the arena that contained an unknown male CD-1 mouse. Mice were allowed to explore for 5 min and social exploration time was used to validate the impact of our social stress paradigm (2).

Forced Swim test

The forced swim test (FST) was performed as published before (3); between 10 AM and noon, mice were placed in a glass beaker (∅ 13 cm, height, 24 cm) filled with tap water (21± 0.5˚C) to a height of 15 cm. The test lasted 5 min during which floating (immobility) was scored.

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Light Dark box test

The light-dark box consisted of two equisized compartments (20 x 20 x 21 cm); a lit compartment (600 lx) and a covered, dark compartment (15-20 lx), lighting conditions were similar to those published previously (3). Mice were introduced into the lit compartment facing the wall and observed for 5 min. Time [%] spent in the light, latency to enter the dark compartment and the frequency of light-dark transitions was analyzed.

Novel Object Recognition test

Mice were placed in an open field arena (45 x 45 x 41 cm) containing two similar objects at equidistant location and allowed to freely explore for 10 min (familiarization). After a 15 min intertrial interval (i.t.i) in the animals' home cage, mice were replaced in the open field, now containing a single version of the familiar object and a novel object for 5 min (similar in size but different in shape, material, texture and contrast). The arena and objects were cleaned with 5% EtOH in between trials. Object exploration was scored as direct interaction with the object such as sniffing or touching the object with the nose or forepaws. Trials in which total exploration time lasted <5s were considered insufficient and removed from the analysis. The time exploring the novel object divided by the total duration of exploration was taken as novel object recognition index (expressed in %).

Object location task

The object location task took place in the Y-maze as published before (3) with minor modifications. During the habituation phase two identical objects were placed in two arms. The mouse was introduced into the center of the Y-maze and allowed to explore for 15 min after which the animal returned to its home cage for 30 min. Thereafter the mouse was placed back into the center of the Y-maze for an additional 3 min of exploration, but now one object was replaced from its former location into the third, remaining arm. The allocation of the object to a novel position was chosen randomly in order to avoid putative inherent location biases. The setup was cleaned with 5% EtOH in between trials. For reliability, we included only subjects with total exploration time ≥ 5 s. The preference [%] to explore the replaced object was calculated as (replaced object exploration (s))/ (replaced object exploration (s) + exploration for object with kept location (s)) x 100.

Cognitive Y-maze

The cognitive Y-maze test has been performed as described before (4,5). During the habituation phase, entry into one of the three identical arms was blocked. The mouse was placed at the distal end of the starting arm (the arm closest to the experimenter) and allowed exploration for 10 min. The animal was then placed in its home cage for 30 min during which the wall blocking the entry to the novel arm was removed. The mouse then returned to the Y-maze and was again placed in the starting arm for 5 min of exploration to assess novel arm preference. The allocation of the novel and familiar arm (left or right arm on the opposite side of the starting arm) was chosen randomly in order to avoid putative inherent location biases. The setup was cleaned with 5% EtOH in between trials. Extensive periods of non-exploratory behavior (>30s of continuous immobility and/or autogrooming) were excluded from the analysis. Novel arm preference [%] was calculated as (novel arm exploration (s))/ (novel arm exploration (s) + familiar arm exploration (s)) x 100.

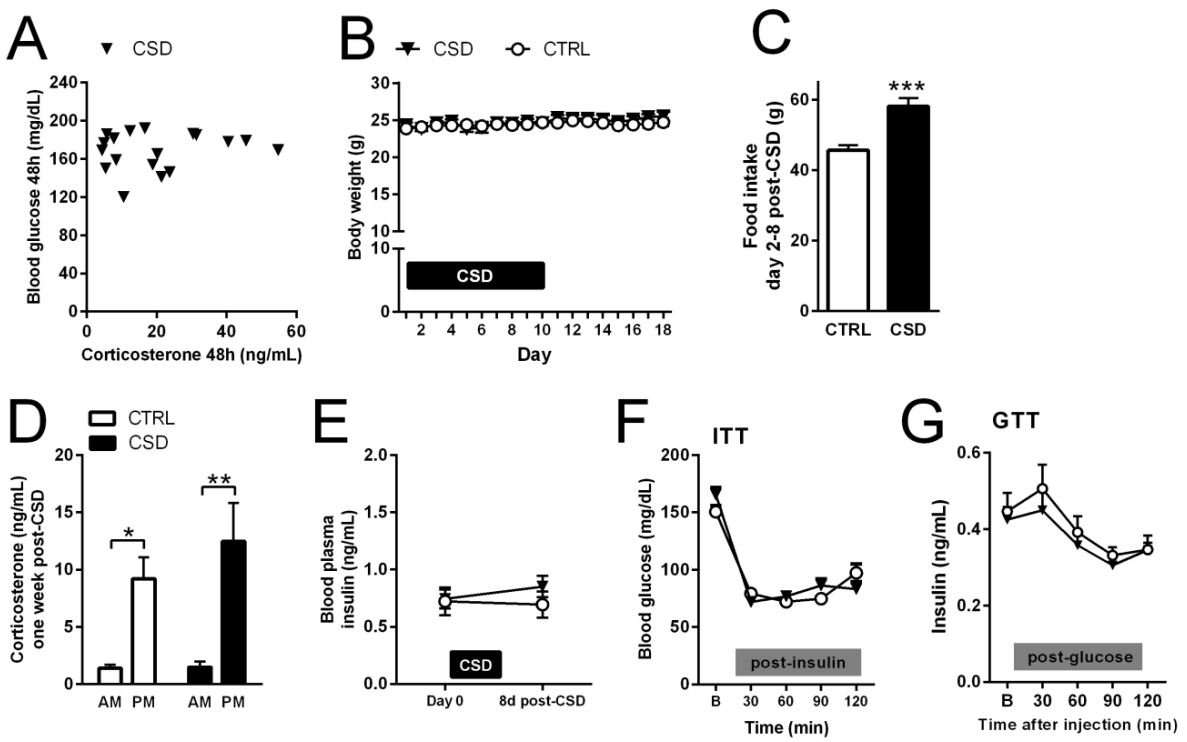

Fig. S1 Effects of corticosterone on peripheral glucose levels and effects of chronic social defeat (CSD) on body weight, food consumption, corticosterone levels and on insulin function. (**A**) Corticosterone was not correlated to blood glucose levels 48h post-CSD (r= 0.16, P= 0.52)). (**B**) Bodyweight did not differ before-, during- or after CSD between treatment groups and was not affected by the stressful paradigm either (treatment:[$F_{(1,10)}$= 0.32, *P*= 0.582], interaction: [$F_{(17,170)}$= 1.16, *P*= 0.30], n= 6/group). (**C**) CSD-treatment increased food consumption after the stressful period compared to controls (t= 4.37, df= 19, *P*<0.001, n= 10-11/group). (**D**) One week post-CSD, morning (AM) and afternoon (PM) corticosterone levels did not differ between treatment groups (time: [$F_{(1,15)}$= 26.69, *P*<0.001], treatment: [$F_{(1, 15)}$= 0.75, *P*= 0.40], n= 8-9/group). PM corticosterone levels increased in both treatment groups compared to AM corticosterone levels (CTRL [t= 3.13, df= 15, *P*<0.05], CSD [t= 4.15, df= 15, *P*<0.01]). (**E**) Blood plasma insulin levels were not different before- and after CSD nor between treatment groups (time: [$F_{(1,25)}$= 0.25, *P*= 0.62], treatment: [$F_{(1,25)}$= 0.53, *P*= 0.47], n= 13-14/group). (**F**) In the insulin tolerance test (ITT), a bolus of insulin (0.5 U/kg i.p.) reduced blood glucose levels similarly in control- and CSD-treated mice (time: [$F_{(4,56)}$= 110.7, *P*<0.001], n= 8/ group), but no treatment effect ([$F_{(1,14)}$= 0.17, *P*= 0.68]) and (**G**) In the glucose tolerance test (GTT), a bolus of glucose (2 g/kg i.p.) reduced insulin levels (time: $F_{(4,44)}$= 9.17, *P*<0.001) but no differences were found between control- and CSD-treated mice (treatment: $F_{(1,11)}$= 0.42, *P*= 0.53 and interaction: $F_{(4,44)}$= 0.22, *P*= 0.93). Data are presented as mean ± SEM (**P*<0.05, ***P*<0.01, ****P*<0.001, Pearson correlation coefficient [**A**], two-way repeated measures ANOVA with Bonferroni´s post-test [**B, D-G**], Student´s t-test [**C**]).

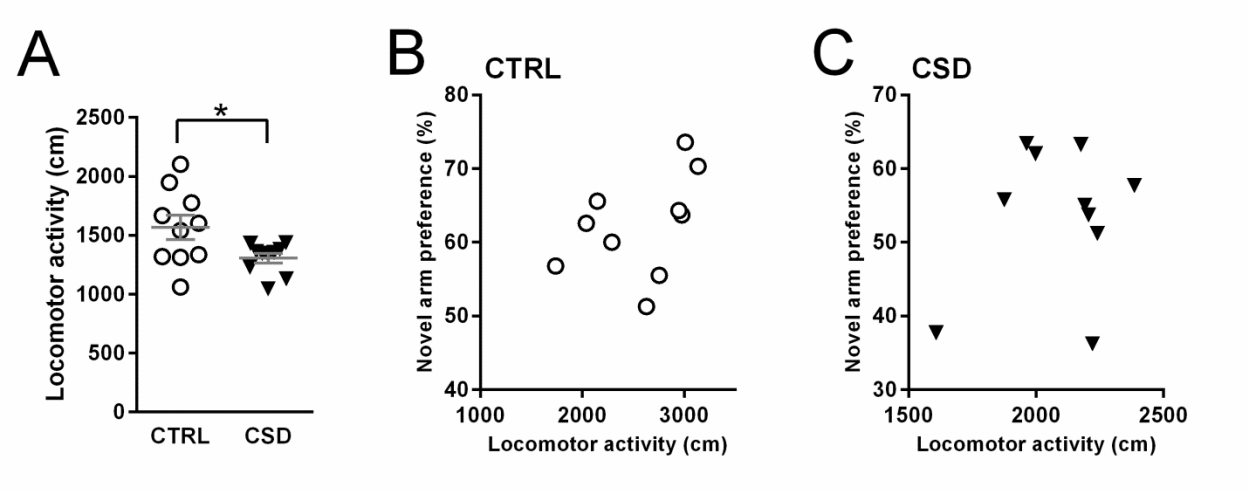

Fig. S2 Effects of CSD on locomotor activity**.** (**A**) In the Y-maze, CSD-treatment decreased locomotor activity compared to controls (t= 2.39, df= 18, *P*= 0.03, n= 10/group). (**B**) However, locomotor activity did not correlate with Y-maze performance for CTRL-animals (r= 0.43, r^2= 0.18, *P*= 0.22) or for (**C**) CSD-treated animals (r= 0.24, r^2= 0.06, *P*= 0.50). Data are presented as mean ± SEM (**P*<0.05, Student´s t-test [**A**], Pearson correlation coefficient [**B, C**]).

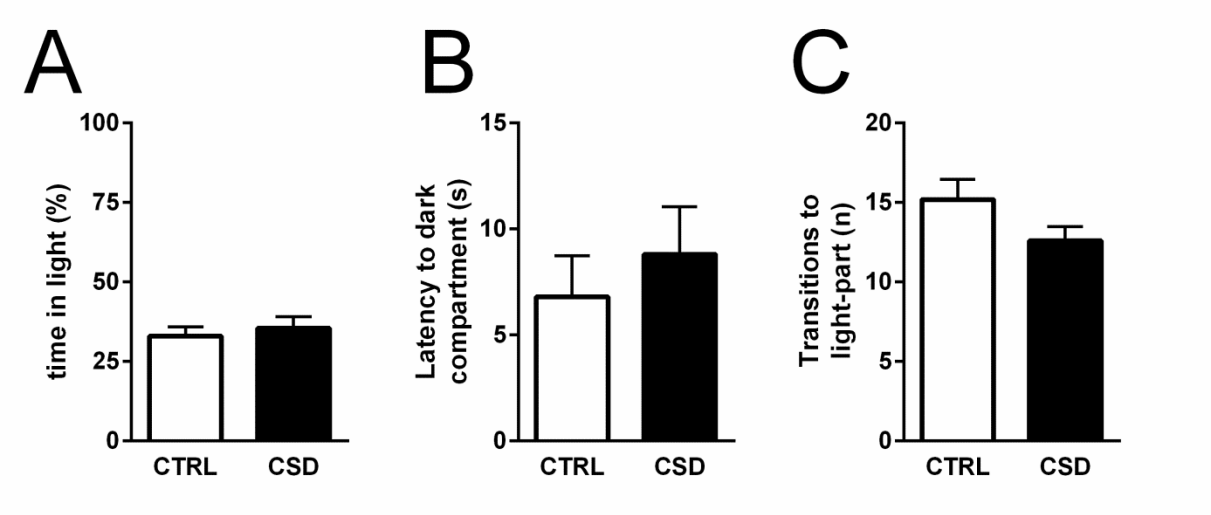

Fig. S3 Effects of CSD on anxiety-like behavior in the light-dark box. (**A**) Time spent in the lit compartment of the light dark box did not differ between CTRL and CSD-treated mice (t= 0.55, df= 18, *P*= 0.59) and (**B**) nor did the latency to enter the dark compartment (t= 0.67, df= 18, *P*= 0.51). (**C**) The frequencies of light-dark transitions were similar when comparing CTRL to CSD-treated animals (t= 1-7, df= 18, *P*= 0.11). Data are presented as mean + SEM, Student´s t-test [**A-C**]).

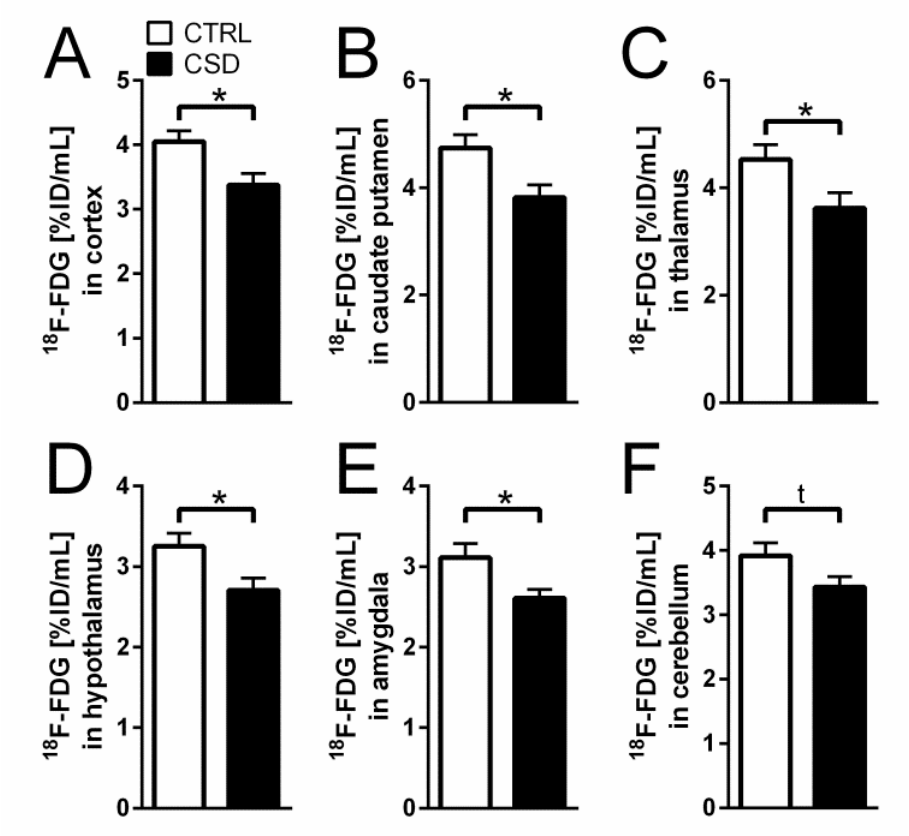

Fig. S4 Effects of CSD on glucose uptake in individual brain areas. ^{18}F-FDG uptake was reduced in selected brain regions of mice that underwent chronic social defeat (CSD, n= 8-9/group for all brain areas) including (**A**) the cortex (t= 2.77, df= 15, *P*= 0.014), (**B**) caudate putamen (t=2.69, df= 15, *P*= 0.017), (**C**) thalamus (t= 2.28, df= 15, *P*= 0.038), (**D**) hypothalamus (t= 2.44, df=15, *P*= 0.028), (**E**) amygdala (t= 2.43, df= 15, *P*= 0.028) and tended to be decreased in (**F**) the cerebellum (t= 1.88, df= 15, *P*= 0.079). Data are presented as mean + SEM (**P*<0.05, ^{t}*P*<0.1, Student´s t-test [**A-F**]).

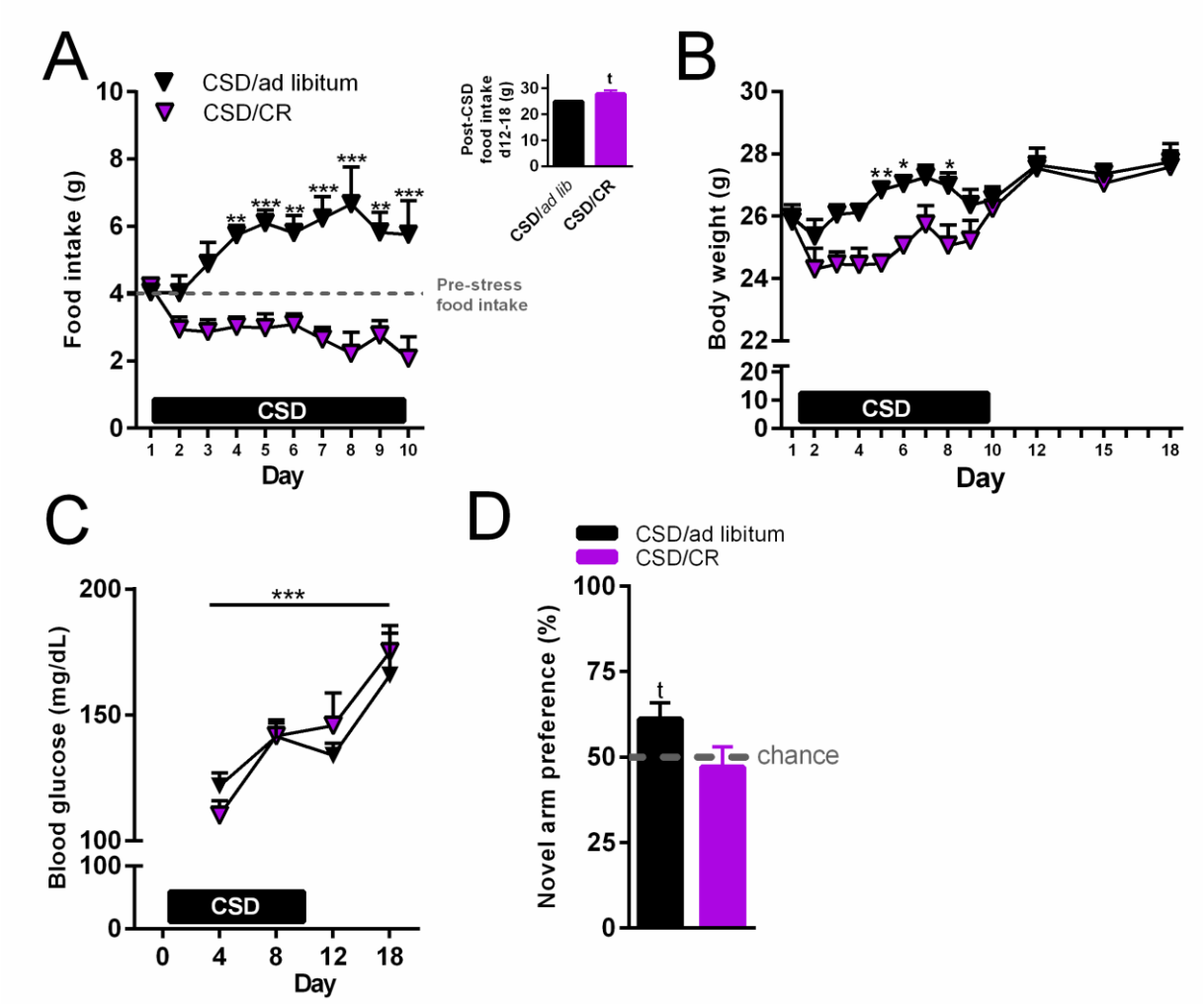

Fig. S5 Effects of caloric restriction on food intake, body weight, blood glucose and cognition in socially stressed mice. (**A**) Food intake was reduced for CSD-mice submitted to caloric restriction (CR) as compared to CSD-mice fed *ad libitum* during 10 days CSD (treatment: [$F_{(1,77)}$= 118.1, *P*<0.001], n= 5/group and significant post-test on day 4 [t= 3.63, df= 77, *P*<0.01], 5 [t= 4.16, df= 77, *P*<0.001], 6 [t= 3.62, df= 77, *P*<0.01], 7 [t= 4.52, df= 77, *P*<0.001], 8 [t= 5.92, df= 77, *P*<0.001], 9 [t= 3.85, df= 77, *P*<0.01] and 10 [t= 4.62, df= 77, *P*<0.001]). After the stressful period, food consumption tended to be increased for CSD-CR treated mice (see insert; U= 4, *P*= 0.095). (**B**) CSD-CR mice exhibited a decreased body weight in comparison to CSD-*ad libitum* throughout CSD (treatment: [$F_{(1,8)}$= 8.74, *P*=0.018], n= 5/ group with significant post-tests on day 5 [t= 3.57, df= 72, *P*<0.01], 6 [t= 2.99, df= 72, *P*<0.05] and 8 [t= 2.90, df= 72, *P*<0.05]) but quickly regained bodyweight thereafter (when food was freely available again for CSD-CR) up to control values (treatment: [$F_{(1,8)}$= 0.06, P= 0.81]). (**C**) Blood glucose levels increased for CSD-treated animals, leading to hyperglycemic conditions on day 18 without differences between treatment groups (time: [$F_{(3,24)}$= 15.27, *P*<0.001], treatment: [$F_{(1,8)}$= 0.070, *P*= 0.80]). (**D**) CSD-*ad libitum* show a tendency for novel arm preference whereas the performance of CSD-CR was at chance level and no differences were observed between groups, indicating that CR was not beneficial for hippocampal-related spatial memory (CSD-*ad libitum* against chance [t= 1.93, df= 9, *P*= 0.09], CSD-CR against chance [t= 0.48, df= 4, *P*= 0.65] and CSD-*ad libitum* versus CSD-CR [t= 1.48, df= 13, *P*= 0.16], n= 5-10/ group). Data are presented as mean + SEM (^{t}*P*<0.1, **P*<0.05, ***P*<0.01, ****P*<0.001, two-way repeated measures ANOVA with Bonferroni´s post-test [**A-C**], Mann-Whitney test [**A**, insert], Student´s t-test (**D**) or one-sample t-test against chance [**D**]).

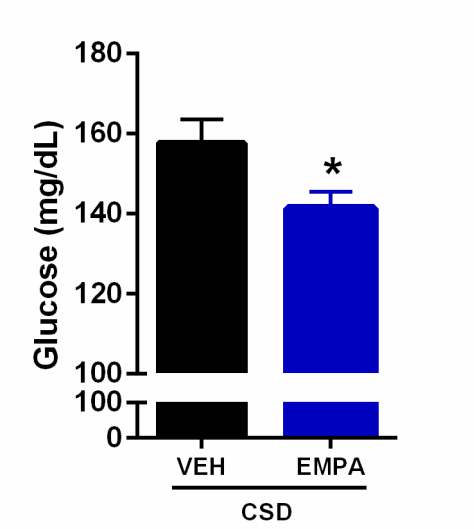

Fig. S6 EMPA treatment significantly reduced peripheral blood glucose in stressed animals seven days post-CSD (t= 2.32, df= 22, *P*= 0.03, n= 11-13/group). Data are presented as mean + SEM (**P*<0.05, Student´s t-test).

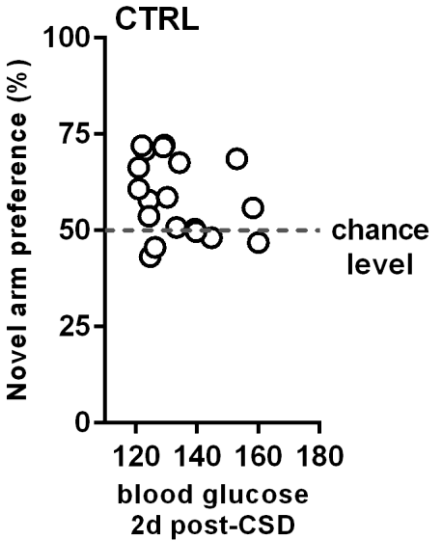

Fig. S7 Y-maze performance in control mice did not correlate with peripheral glucose measured two days post-CSD (Spearman correlation coefficient: r= -0.34, r^2= 0.08, *P*= 0.15).

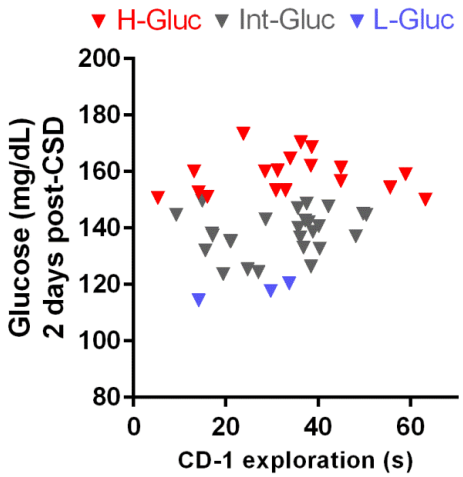

Fig. S8 The duration of exploration in the CD-1 confrontation test did not predict the level of peripheral blood glucose, taken two days after Chronic Social Defeat (Pearson correlation coefficient: r= 0.20, r^2= 0.04, *P*= 0.19). Indicated in color are the mice that were identified with high- (H-Gluc: >150 mg/dL), intermediate- (Int-Gluc: >125 and <150 mg/dL) or low glucose levels (L-Gluc: <125 mg/dL).

Supplementary Table 1. Hippocampal measurements for levels of NAD^+, NADH and MDA (TBARS), taken at day 10 of CSD and at 3- and 5 weeks post-CSD did not differ between control and CSD-treated mice. Group comparisons were made using Student's t-tests.

	CTRL	**CSD**			
NAD^+ (pmol/mg protein)	mean ± sem	mean ± sem	t	df	*P*
CSD-day 10	15.9 ± 1.89	14.8 ± 1.87	0.41	8	0.69
3 wks post-CSD	11.9 ± 1.39	13.1 ± 1.20	0.62	16	0.54
5 wks post-CSD	13.4 ± 1.17	13.9 ± 1.42	0.26	11	0.80
NADH (pmol/mg protein)					
CSD-day 10	2.78 ± 0.29	2.62 ± 0.56	0.25	8	0.81
3 wks post-CSD	2.04 ± 0.32	2.14 ± 0.33	0.22	16	0.83
5 wks post-CSD	2.58 ± 0.17	3.20 ± 0.62	0.97	8	0.36
MDA (pmol/µg protein)					
CSD-day 10	22.1 ± 1.73	25.4 ± 2.11	1.19	6	0.28
3 wks post-CSD	28.9 ± 2.21	23.3 ± 4.36	0.98	8	0.36
5 wks post-CSD	26.7 ± 1.73	27.4 ± 2.43	0.24	10	0.81

Supplementary Table 2. Hippocampal cytosolic protein levels for GluT-1, GluT-3 and GluT-4 did not differ between control (n= 8) and CSD-treated mice (n= 9). Values are normalized to CTRL-values (set at 100) and group comparisons were made using Student's t-tests.

	CTRL	**CSD**			
	mean ± sem	mean ± sem	t	df	*P*
GluT-1	100 ± 9.8	108.3 ± 13.8	0.48	15	0.64
GluT-3	100 ± 14.1	95.6 ± 10.8	0.25	15	0.80
GluT-4	100 ± 19.2	123.4 ± 18.6	0.87	15	0.40

References

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