Supplementary Materials for

Adolescent stress leads to glutamatergic disturbance through dopaminergic abnormalities in the prefrontal cortex of genetically vulnerable mice

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Supplementary Figure Legends

Fig. S1 Effects of glucocorticoid receptor antagonist RU486 on behavioral deficits in the DM. **a**, Effects of RU486 on aberrant locomotor activity. **c**, Effects of RU486 on the impaired performances in the social interaction test. **e**, Effects of RU486 on the impaired performance in the novelty preference test. Treatment with RU486 on CTL mice had no effects on the locomotor activity **b**, the performance in the social interaction test **d**, and the performance in the novelty preference test **e**. *Veh* treated with vehicle. N = 13–14 for **a** and **b**, N = 13–15 for **c** and **d**, N = 13–18 for **e** and **f**. Values are means ± SE. Statistical differences were determined using a two-way ANOVA with repeated measures (group, $F_{(2, 42)} = 14.72$, $P < 0.05$ for **a**), and a one-way ANOVA (group, $F_{(2, 42)} = 8.74$, $P < 0.05$ for **c**; $F_{(2, 42)} = 21.73$, $P < 0.05$ for **e**), followed by Bonferroni post hoc tests (*$P < 0.05$).

Fig. S2 Effects of a competitive glutamate transport inhibitor TBOA on behavioral deficits and neurochemical abnormalities in the DM. Treatment with TBOA on CTL mice had no effects on the performance in the forced swim test **a**, the basal extracellular levels of glutamate in the PFc **b**, and the rate of CaMK II phosphorylation in the PFc after the forced swim test **c**. *p*- phospho-, *t*- total-, *Veh* treated with vehicle, *TBOA* treated with *DL*-threo-β-benzyloxyaspartate. N = 10–14 for **a**, N = 7–9 for **b**, N = 5 for **c**. Values are means ± SE.

Fig. S3 Effects of a partial NMDA receptor glycine-site agonist DCS and an inhibitor of *d*-amino acid oxidase, MPC on behavioral deficits and neurochemical abnormalities in the DM. Treatment with DCS on CTL mice had no effects on the performance in the forced swim test **a**, and the rate of CaMK II phosphorylation in the PFc after the forced swim test **b**. Treatment with MPC on CTL

mice had no effects on the performance of the forced swim test **c**, the rate of CaMK II phosphorylation in the PFc after the forced swim test **d**, and the concentration of *d*-serine in the PFc **e**. *p*- phospho-, *t*- total-, *Veh* treated with vehicle, *DCS* treated with *D*-cycloserine, *MPC* treated with 5-Methylpyrazole-3-carboxylic acid. $N = 12$ for **a**, $N = 10$ for **b**, $N = 17$–19 for **c**, $N = 6$ for **d**, $N = 8$–13 for **e**. Values are means ± SE.

Fig. S4 Effects of an atypical antipsychotic drug CLZ on aberrant locomotor activity and METH-induced hyperactivity. **a**, Effects of CLZ on aberrant locomotor activity and METH-induced hyperactivity. **b**, Treatment with CLZ on CTL mice had no effects on the locomotor activity. *Veh* treated with vehicle, *CLZ* treated with clozapine. $N = 9$–13 for **a** and **b**. Values are means ± SE. Statistical differences were determined using a two-way ANOVA with repeated measures (group, $F_{(2, 29)} = 12.64$, $P < 0.05$ for **a**), followed by Bonferroni post hoc tests ($^*P < 0.05$).

Fig. S5 Effects of an atypical antipsychotic drug CLZ on behavioral deficits in the DM. Treatment with CLZ on CTL mice had no effects on the locomotor activity **a**, the performance of the forced swim test **b**, the performance of social interaction test **c**, performance of novelty preference test **d**, performance of prepulse inhibition test **e**, and the rate of CaMK II phosphorylation in the PFc after the forced swim test **f**. *p*- phospho-, *t*- total-, *Veh* treated with vehicle, *CLZ* treated with clozapine. $N = 12$ for **a**, $N = 9$ for **b**, $N = 12$ for **c** to **e**, $N = 7$ for **f**. Values are means ± SE.

Fig. S6 Effects of an atypical antipsychotic drugCLZ on the dopaminergic disturbances in the DM. Treatment with CLZ on CTL mice had no effects on the extracellular levels of dopamine at baseline in the NAc **a**, the extracellular levels of dopamine upon METH challenge in the NAc **b**, the extracellular levels of dopamine at baseline in the PFc **c**, the extracellular levels of dopamine upon

METH challenge in the PFc **d**, the levels of TH in the PFc **e**, and the levels of D2R in the PFc **f**. *Veh* treated with vehicle, *CLZ* treated with clozapine. *N* = 7 for **a** and **b**, *N* = 8 for **c** and **d**, *N* = 6 for **e** and **f**. Values are means ± SE.

Fig. S7 Effects of a dopamine-D1 receptor agonist SKF on the behavioral deficits and neurochemical abnormalities in the DM. Treatment with SKF on CTL mice had no effects on the performance in the forced swim test **a**, the rate of NR1 phosphorylation in the PFc after the forced swim test **b**, and the rate of CaMK II phosphorylation in the PFc after the forced swim test **c**. *p*- phospho-, *t*- total-, *Veh* treated with vehicle, *SKF* treated with SKF81297. *N* = 8–10 for **a**, *N* = 7 for **b**, *N* = 8 for **c**. Values are means ± SE.

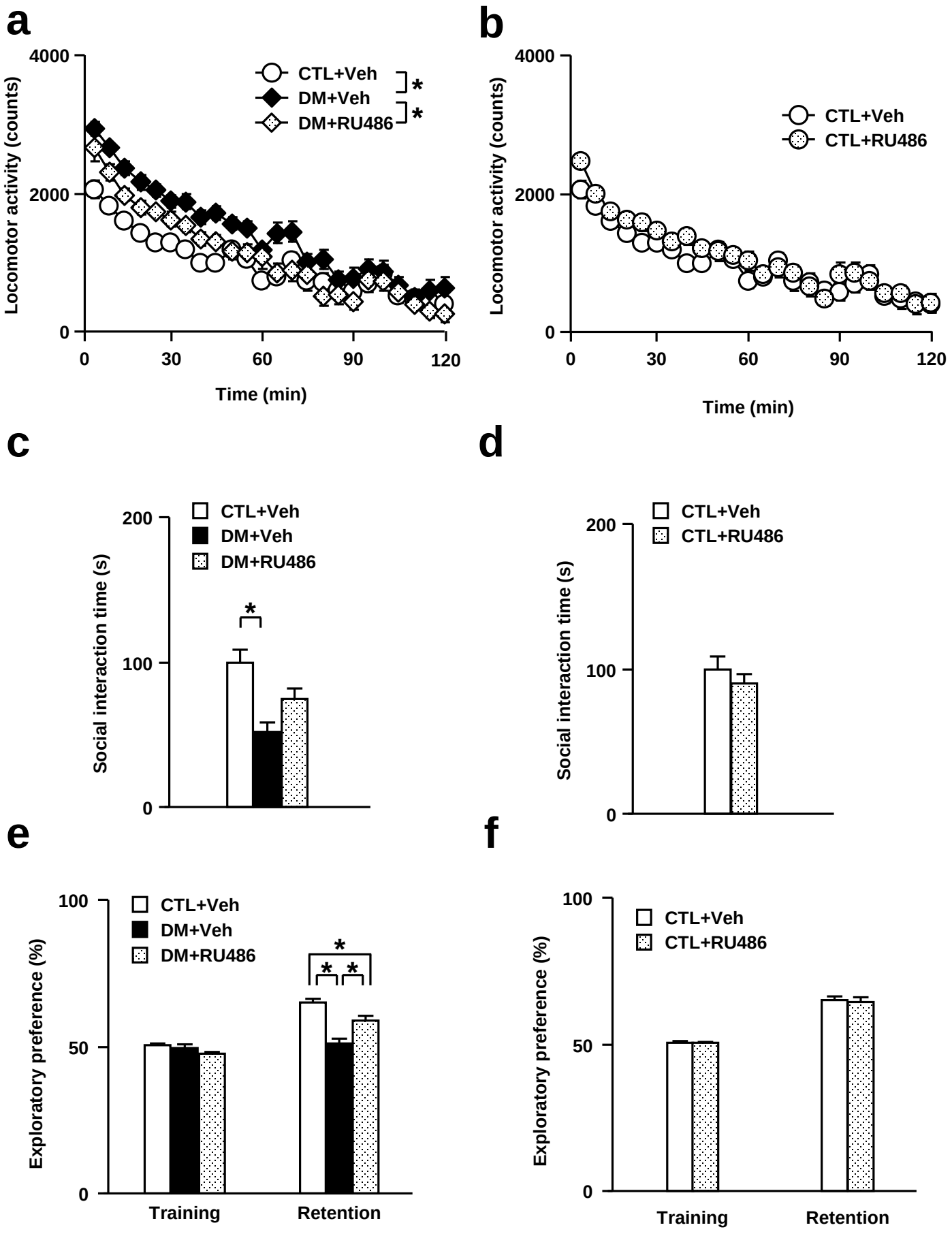

a
4000
2000
0
Locomotor activity (counts)
CTL+Veh
DM+Veh
DM+RU486
*
*
0
30
60
90
120
Time (min)
b
4000
2000
0
Locomotor activity (counts)
CTL+Veh
CTL+RU486
0
30
60
90
120
Time (min)
c
200
100
0
Social interaction time (s)
CTL+Veh
DM+Veh
DM+RU486
*
d
200
100
0
Social interaction time (s)
CTL+Veh
CTL+RU486
e
100
50
0
Exploratory preference (%)
CTL+Veh
DM+Veh
DM+RU486
*
*
*
Training
Retention
f
100
50
0
Exploratory preference (%)
CTL+Veh
CTL+RU486
Training
Retention

Fig. S1

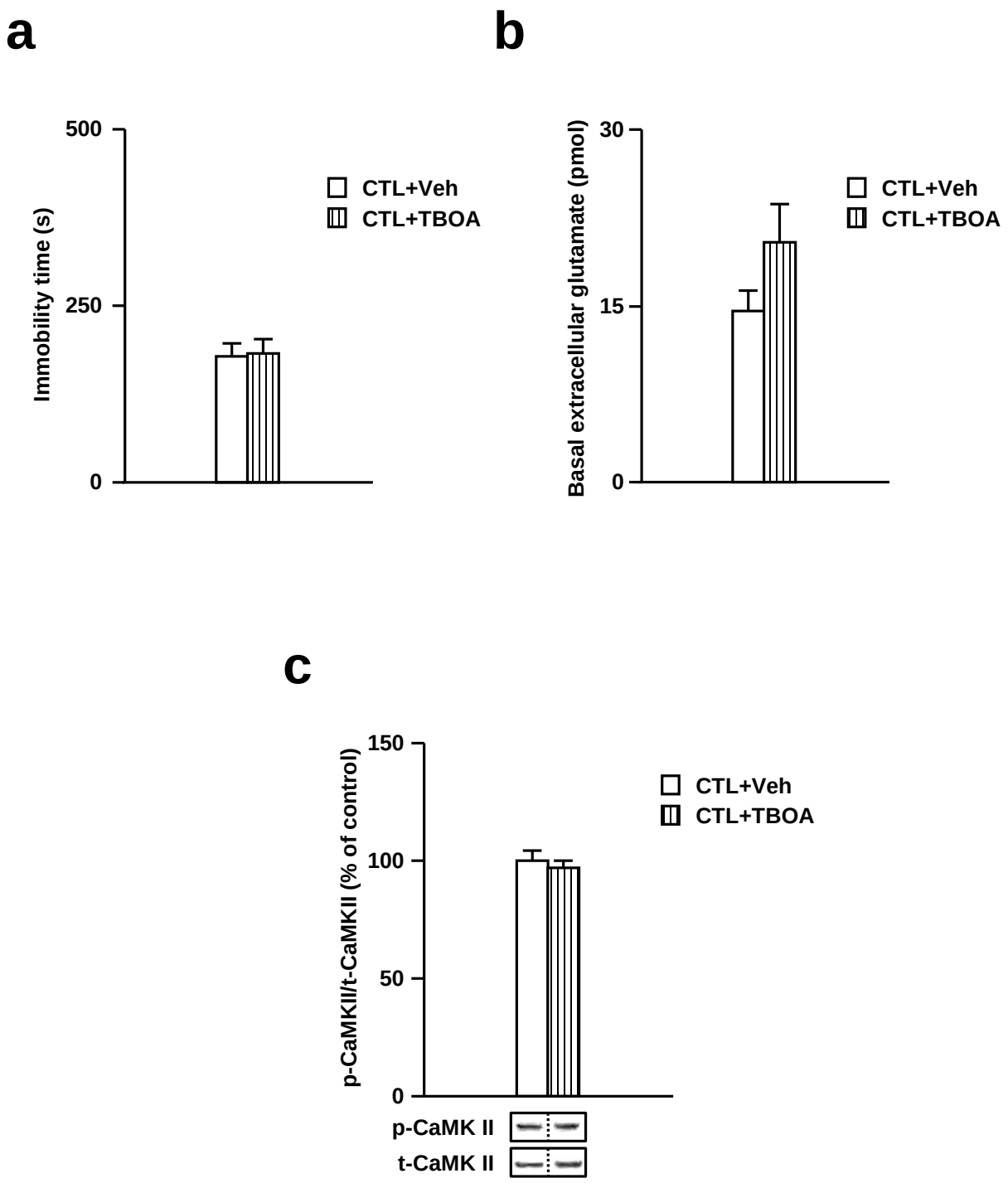

a
500
250
0
Immobility time (s)
CTL+Veh
CTL+TBOA
b
30
15
0
Basal extracellular glutamate (pmol)
CTL+Veh
CTL+TBOA
c
150
100
50
0
p-CaMKII/t-CaMKII (% of control)
CTL+Veh
CTL+TBOA
p-CaMK II
t-CaMK II

Fig. S2

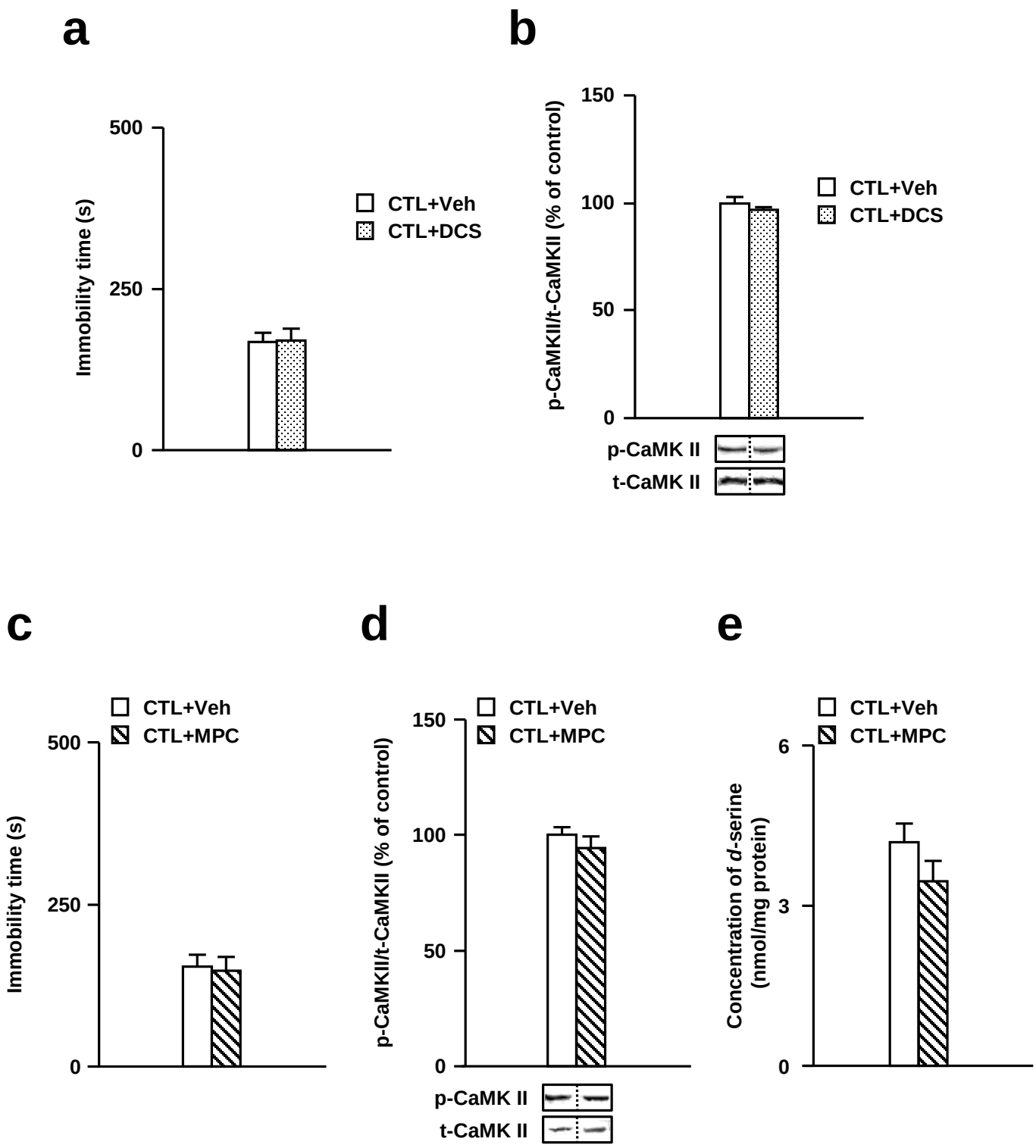

a
Immobility time (s)
500
250
0
CTL+Veh
CTL+DCS
b
p-CaMKII/t-CaMKII (% of control)
150
100
50
0
CTL+Veh
CTL+DCS
p-CaMK II
t-CaMK II
c
Immobility time (s)
500
250
0
CTL+Veh
CTL+MPC
d
p-CaMKII/t-CaMKII (% of control)
150
100
50
0
CTL+Veh
CTL+MPC
p-CaMK II
t-CaMK II
e
Concentration of d-serine (nmol/mg protein)
6
3
0
CTL+Veh
CTL+MPC

Fig. S3

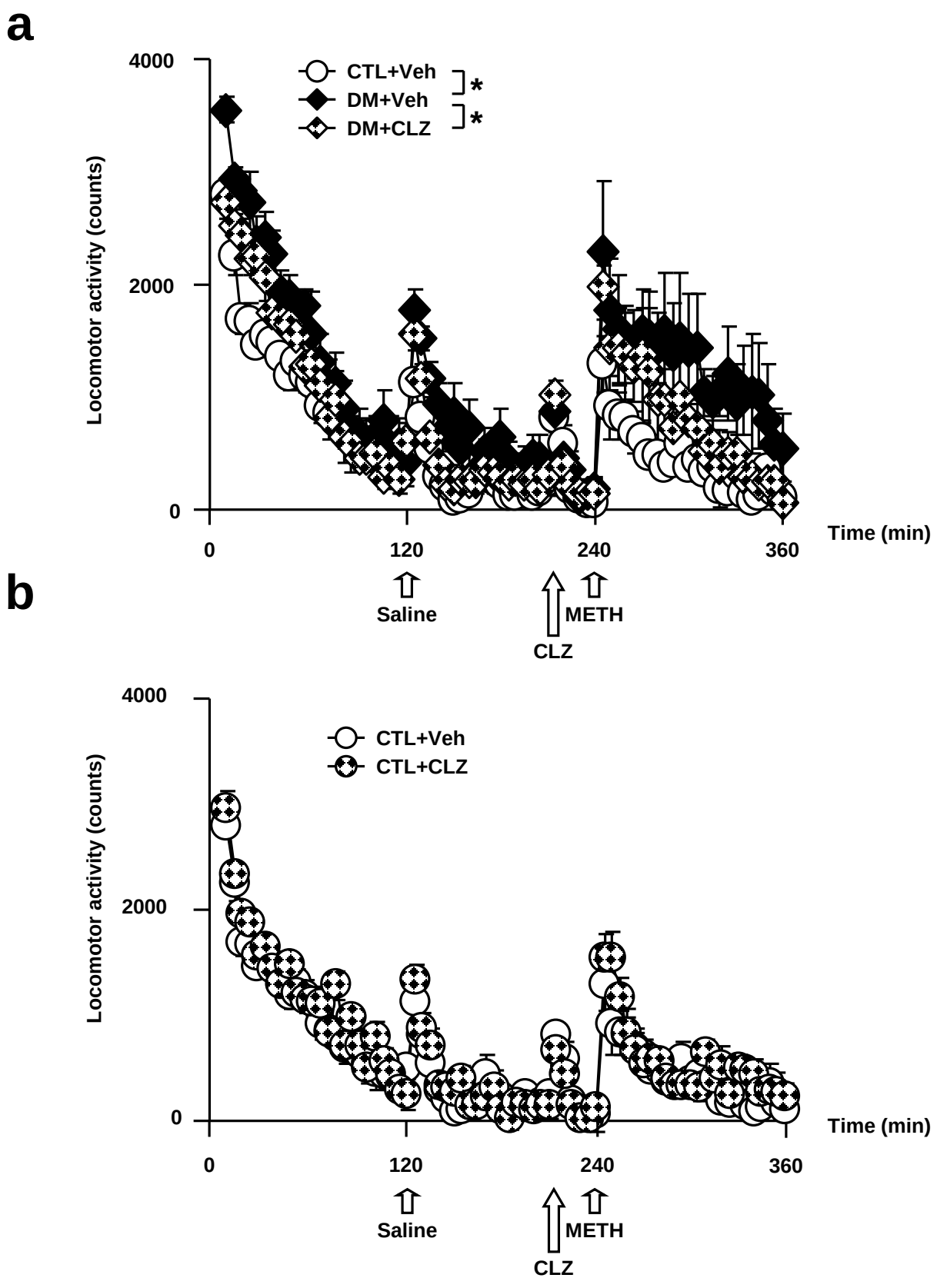

a
4000
2000
0
Locomotor activity (counts)
CTL+Veh
DM+Veh
DM+CLZ
*
*
0
120
240
360
Time (min)
Saline
METH
CLZ
b
4000
2000
0
Locomotor activity (counts)
CTL+Veh
CTL+CLZ
0
120
240
360
Time (min)
Saline
METH
CLZ

Fig. S4

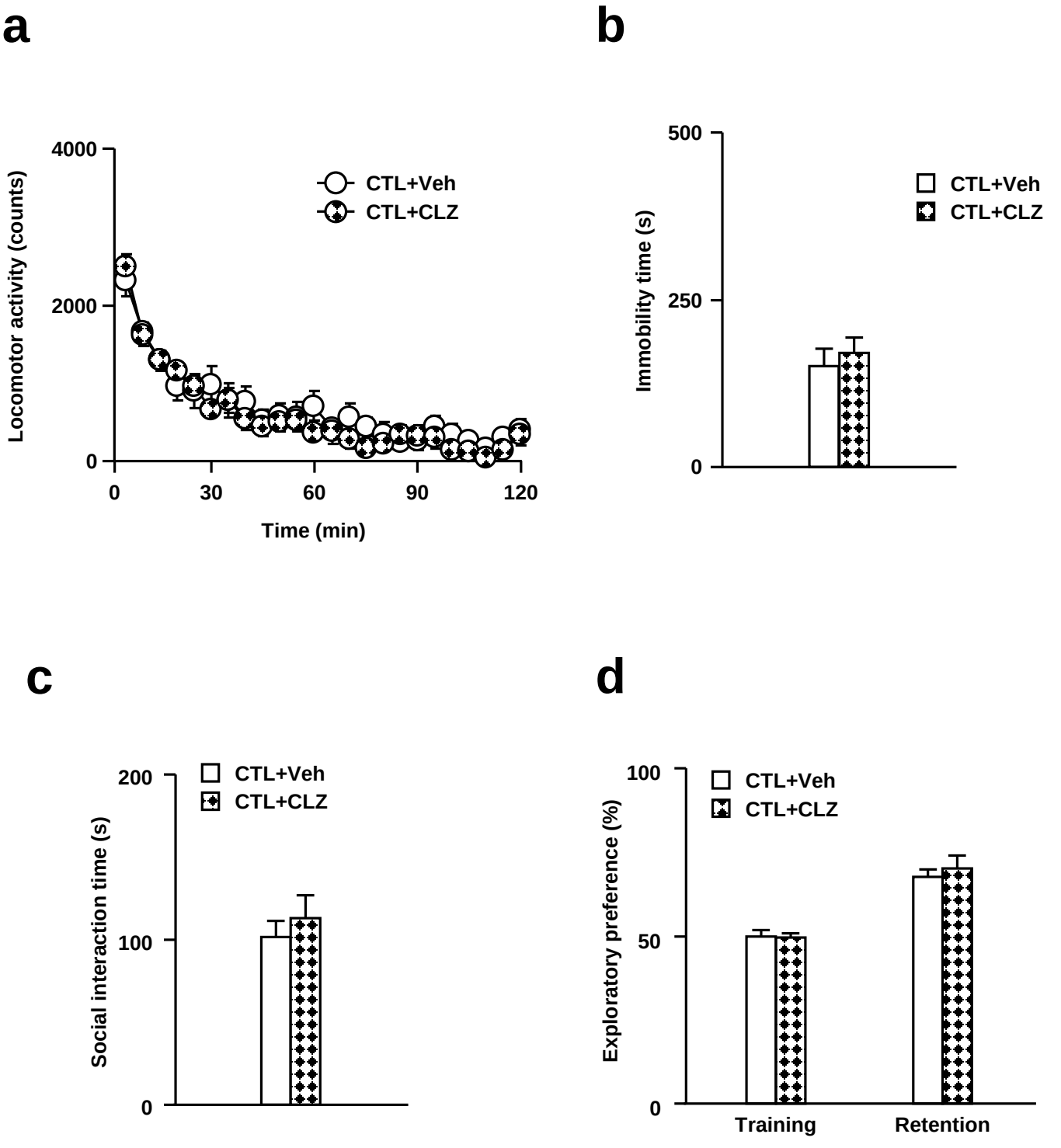
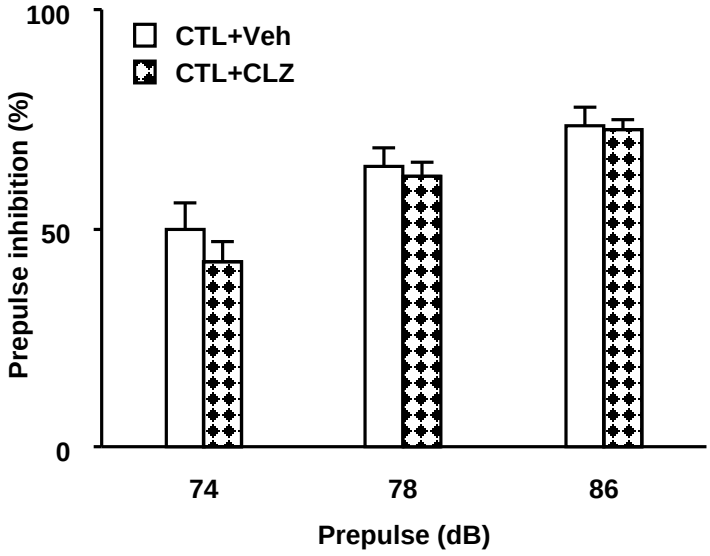

a
Locomotor activity (counts)
4000
2000
0
0
30
60
90
120
Time (min)
CTL+Veh
CTL+CLZ
b
Immobility time (s)
500
250
0
CTL+Veh
CTL+CLZ
c
Social interaction time (s)
200
100
0
CTL+Veh
CTL+CLZ
d
Exploratory preference (%)
100
50
0
CTL+Veh
CTL+CLZ
Training
Retention

Fig. S5

e


100
50
0
Prepulse inhibition (%)
CTL+Veh
CTL+CLZ
74
78
86
Prepulse (dB)

f

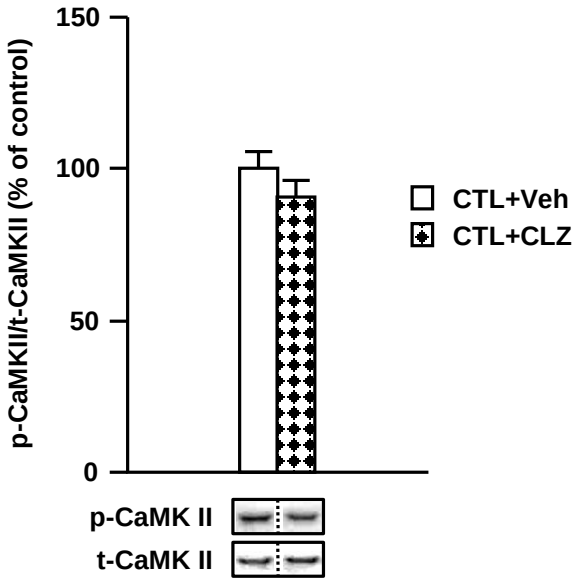

150
100
50
0
p-CaMKII/t-CaMKII (% of control)
CTL+Veh
CTL+CLZ
p-CaMK II
t-CaMK II

Fig. S5

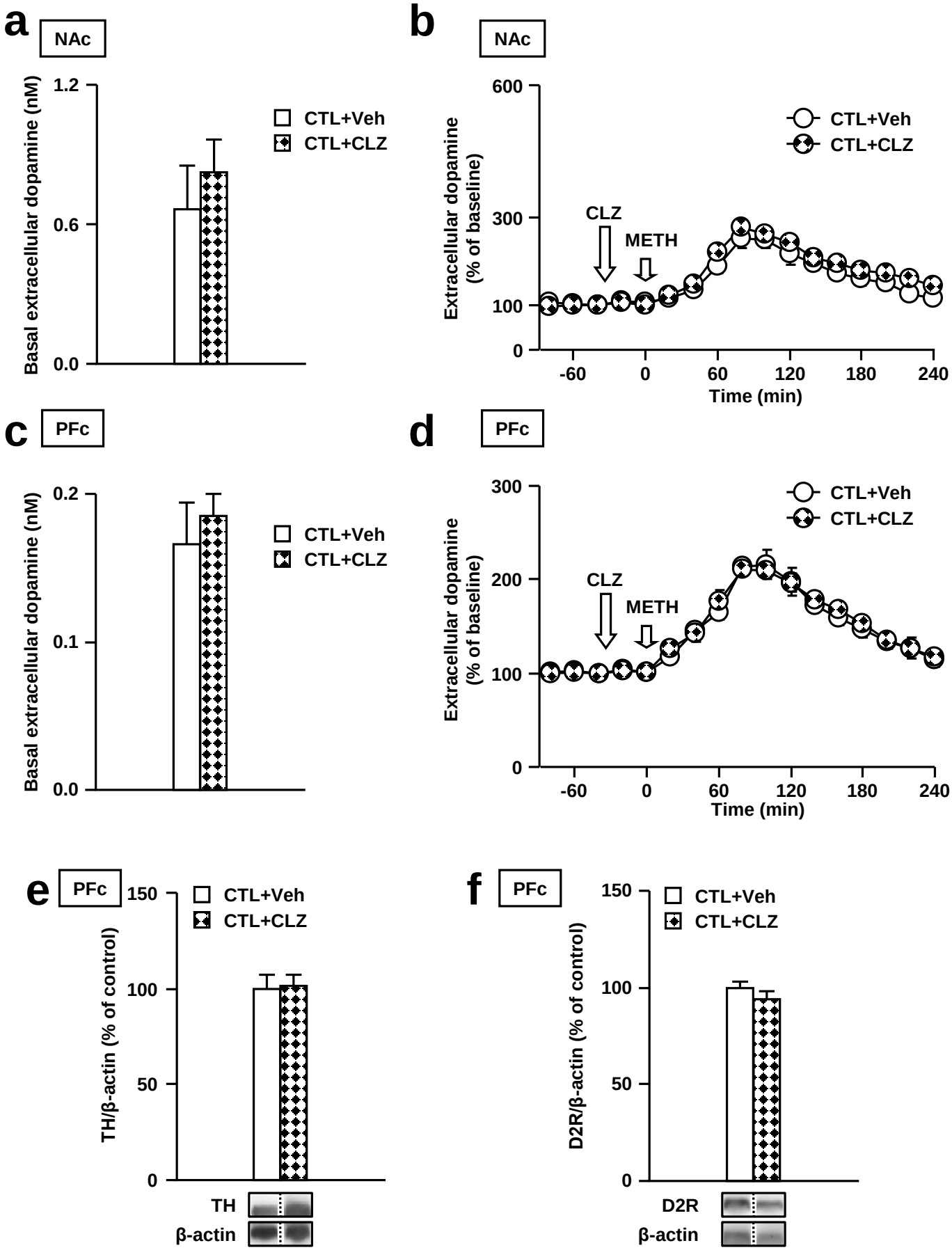

a
NAc
Basal extracellular dopamine (nM)
1.2
0.6
0.0
CTL+Veh
CTL+CLZ
b
NAc
Extracellular dopamine (% of baseline)
600
300
100
0
CLZ
METH
CTL+Veh
CTL+CLZ
-60
0
60
120
180
240
Time (min)
c
PFc
Basal extracellular dopamine (nM)
0.2
0.1
0.0
CTL+Veh
CTL+CLZ
d
PFc
Extracellular dopamine (% of baseline)
300
200
100
0
CLZ
METH
CTL+Veh
CTL+CLZ
-60
0
60
120
180
240
Time (min)
e
PFc
TH/β-actin (% of control)
150
100
50
0
CTL+Veh
CTL+CLZ
TH
β-actin
f
PFc
D2R/β-actin (% of control)
150
100
50
0
CTL+Veh
CTL+CLZ
D2R
β-actin

Fig. S6

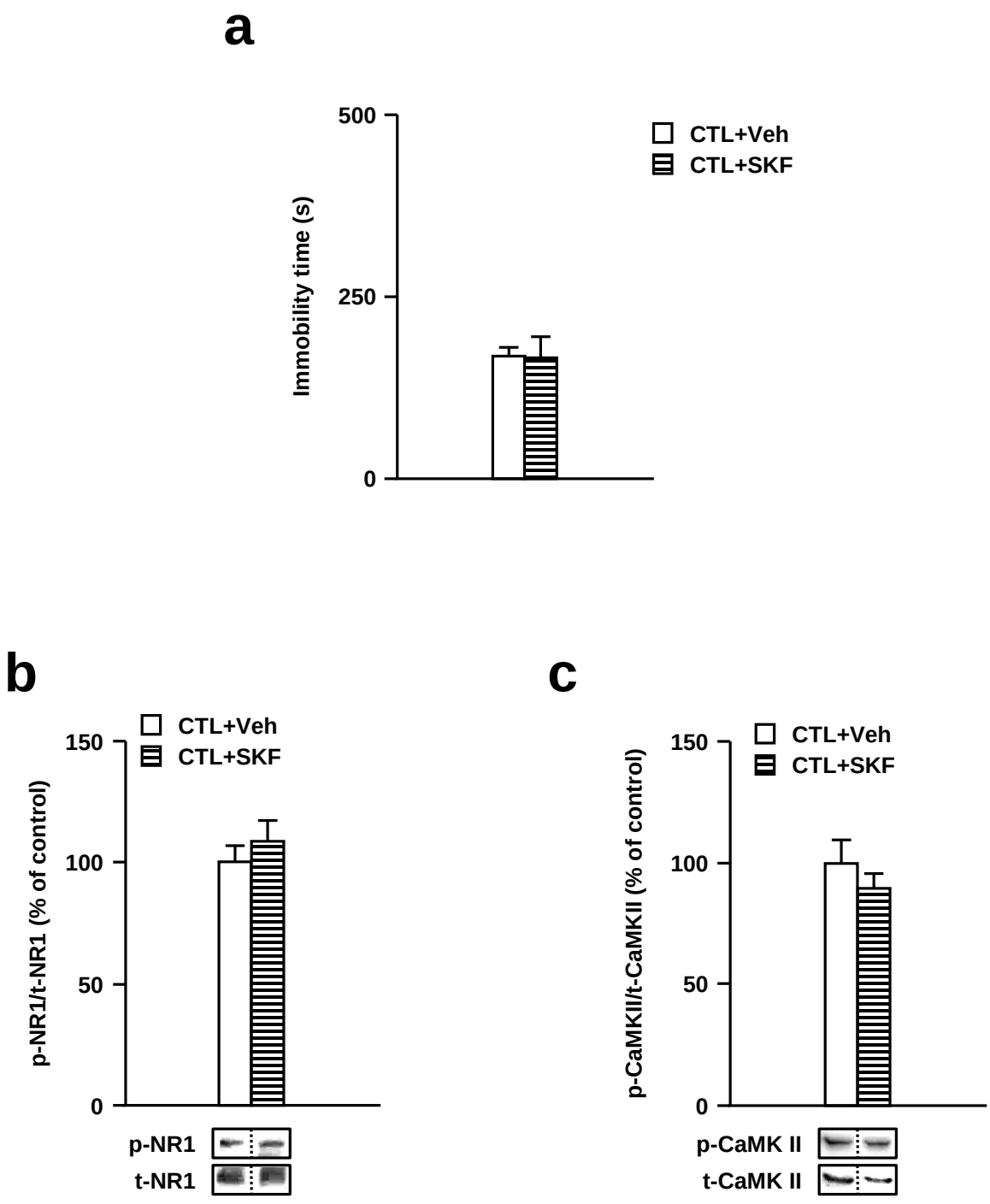

a
500
250
0
Immobility time (s)
CTL+Veh
CTL+SKF
b
150
100
50
0
p-NR1/t-NR1 (% of control)
CTL+Veh
CTL+SKF
p-NR1
t-NR1
c
150
100
50
0
p-CaMKII/t-CaMKII (% of control)
CTL+Veh
CTL+SKF
p-CaMK II
t-CaMK II

Fig. S7