

**Chronic social isolation reduces the parvalbumin-positive interneurons in the medial prefrontal cortex of adult rats: protection by fluoxetine and clozapine**

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# Depression – the silent epidemic

- Serious mood disorder
- One of the leading causes of disability worldwide
- Affect 350 million people (WHO, 2012)
- Theories of depression
- **Disregulation of GABAergic system**

# GABAergic system dysfunction in mood disorders

- '80s – Emrich et al.
- Decreased GABA levels in serum and CSF of depressive patients (Petty et al., 1992)
- MDD patients - lower density and size of GABAergic interneurons (Rajkowska et al., 2007)

# Parvalbumin (PV) – positive interneurons

- Subtype of GABAergic interneurons
- 40% of the GABAergic cortical interneuron population
- PV – 11 kDa
  - calcium – binding protein

# Goals



- whether chronic social isolation of adult male Wistar rats for a period of 21day, which represents an animal model of depression, affects the number of PV-positive interneurons in medial prefrontal cortex
- whether the treatment with antidepressant fluoxetine or antipsychotic clozapine may offer the protection from eventual isolation-induced alternation in number of PV-positive GABAergic interneurons

# Chronic social isolation stress

- Adult male Wistar rats
  - 21 day
- Absence of any visual or tactile contact



## depressive- and anxiety-like behavior

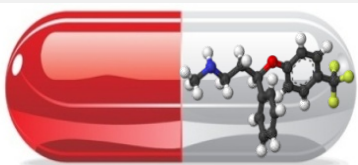
(Sucrose preference test,  
Marble burying test,  
Forced swim test)



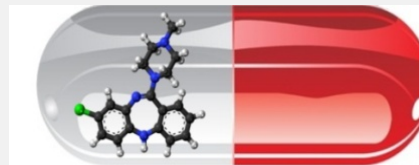
# Materials and methods

21 day

Vehicle control  
Control + Fluoxetine-hydrochloride (15mg/kg/day)  
Control + Clozapine (20mg/kg/day)  
Vehicle social isolation  
Social isolation + Fluoxetine-hydrochloride (15mg/kg/day )  
Social isolation + 20mg/kg/day Clozapine



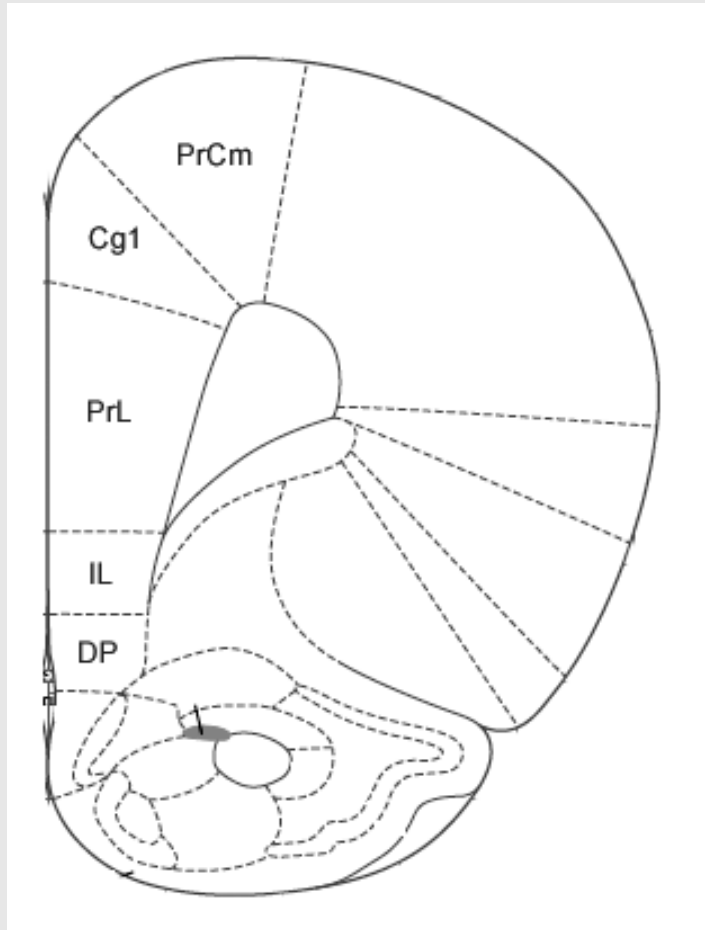
Fluoxetine - a  
selective serotonin  
reuptake inhibitor



Clozapine - an  
atypical  
antipsychotic

drug	Dose (mg/kg/day)	rat serum level (ng/ml)	therapeutically effective level (ng/ml)
fluoxetine	15	280 ± 50	100–700
clozapine	20	123 ± 18	100–700

# Materials and methods



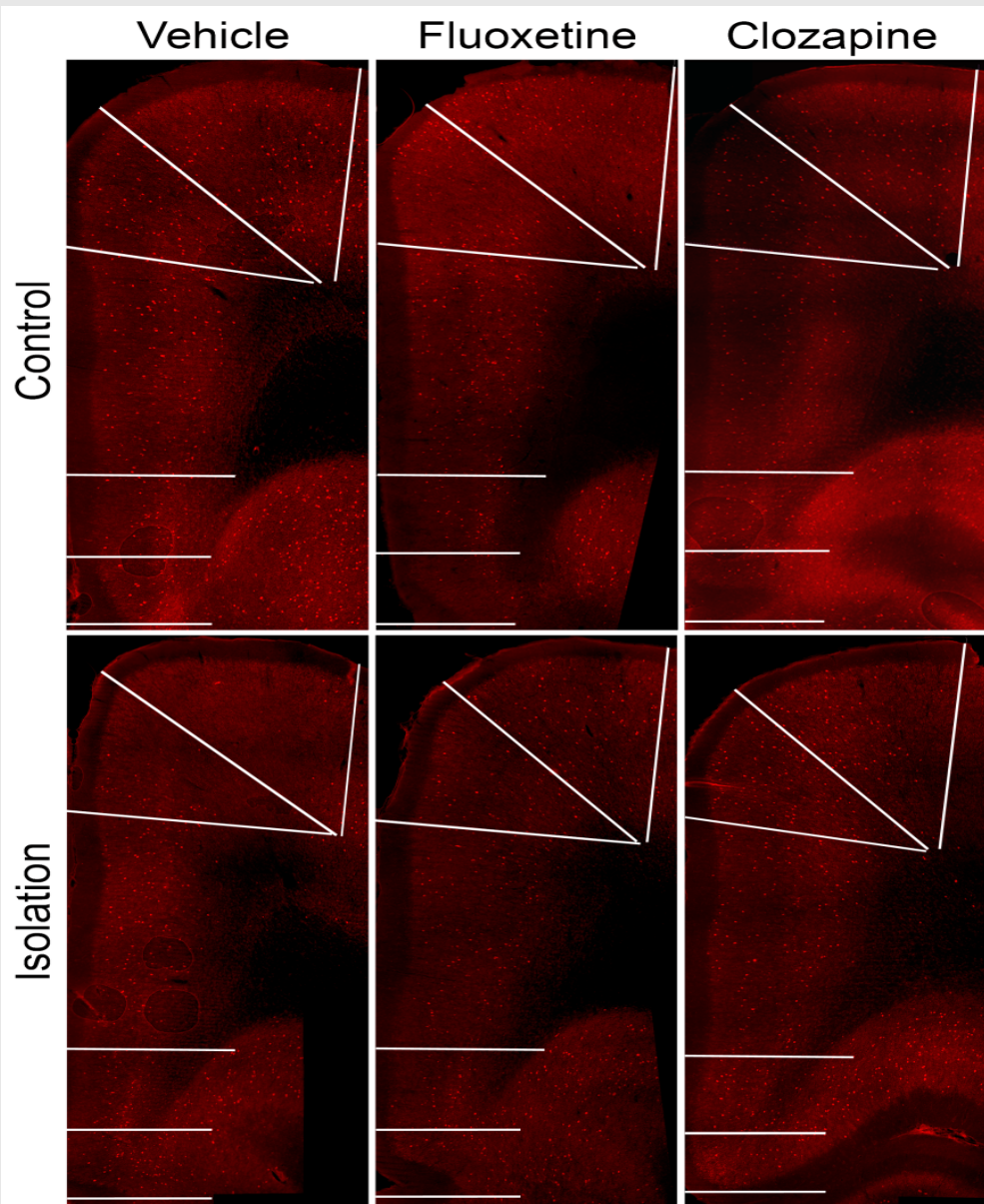
## Medial prefrontal cortex

- PrCm, medial precentral area;
- Cg1, cingulate cortex, area 1;
- PrL, prelimbic area;
- IL, infralimbic area;
- DP, dorsal peduncular cortex

(+3.72 mm from bregma,  
Paxinos and Watson, 1997).

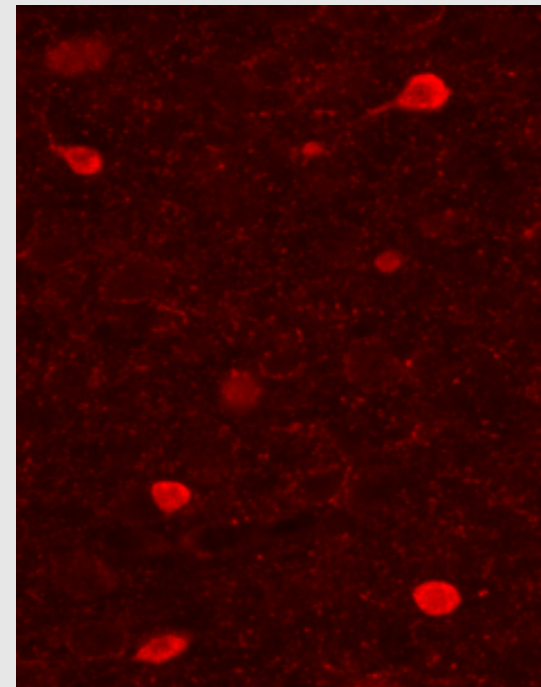


# Representative images of PV-positive interneurons in medial prefrontal cortex

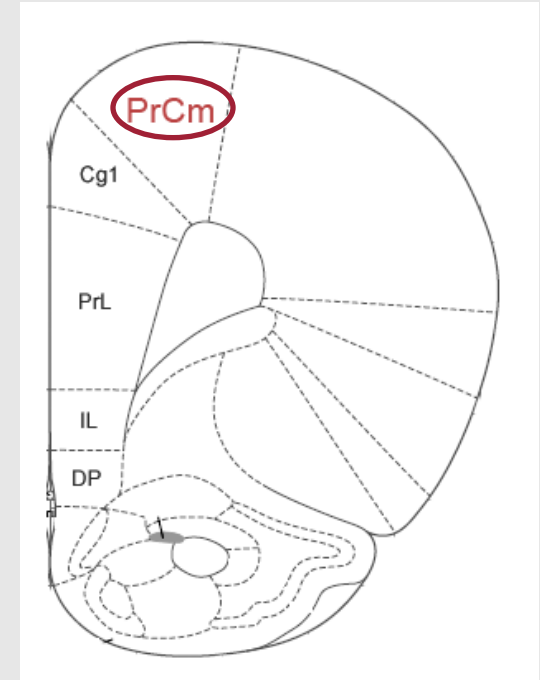
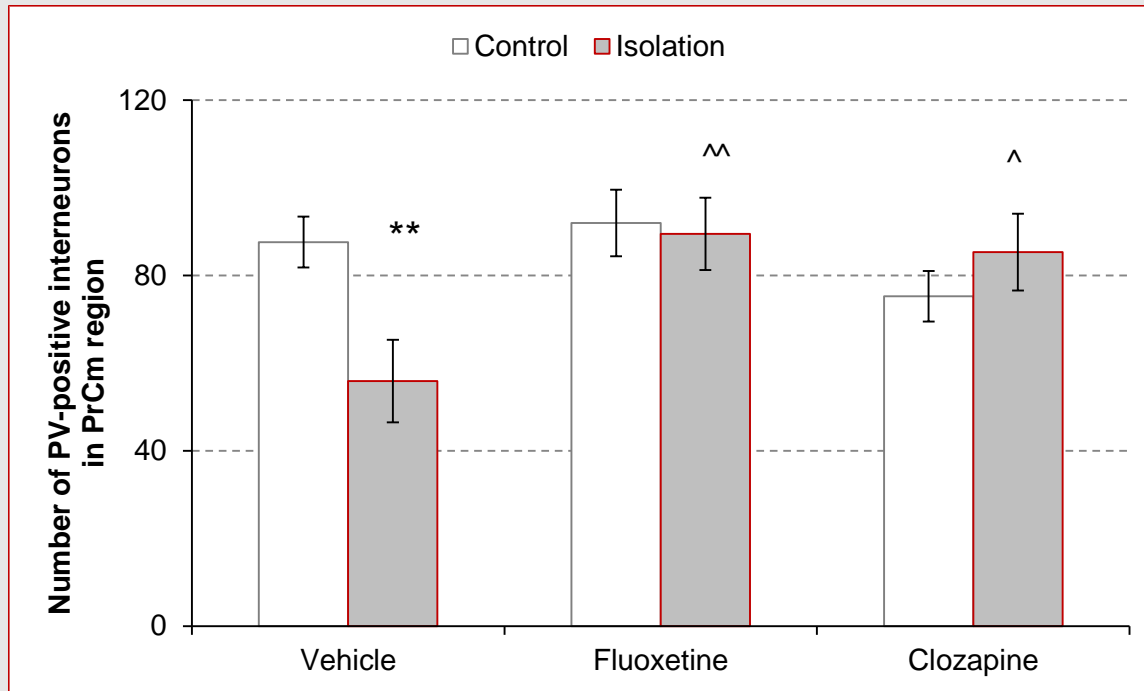


**Figure 1.** Representative images of PV-positive interneurons in the

- medial precentral area (PrCm)
- cingulate cortex, area 1 (Cg1)
- prelimbic (PrL),
- infralimbic area (IL)
- dorsal peduncular cortex (DP)

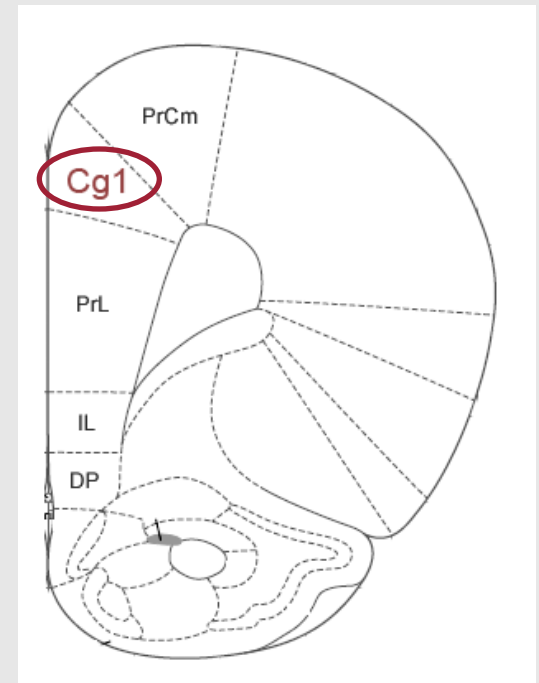
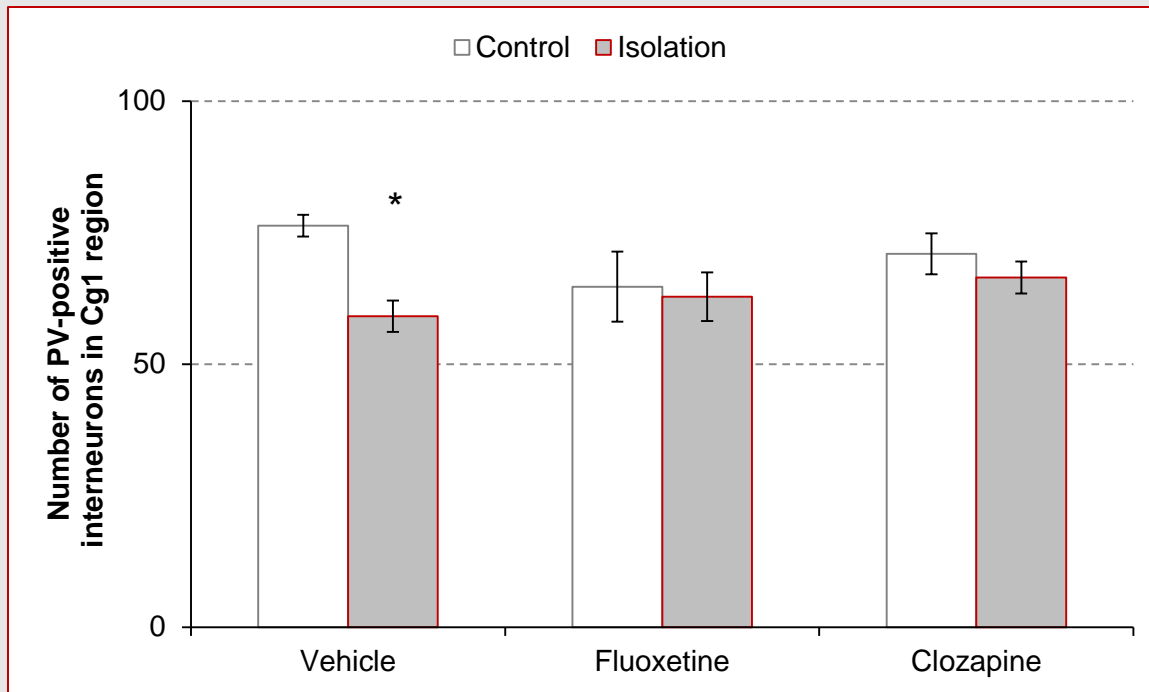


# Number of PV-positive interneurons in PrCm region



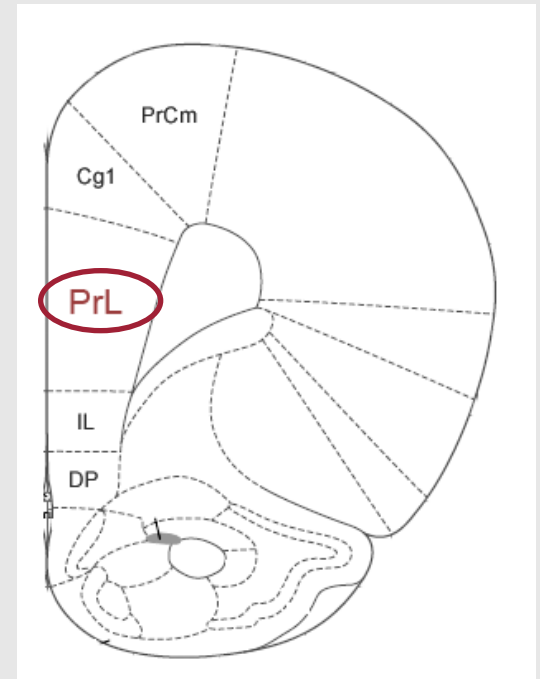
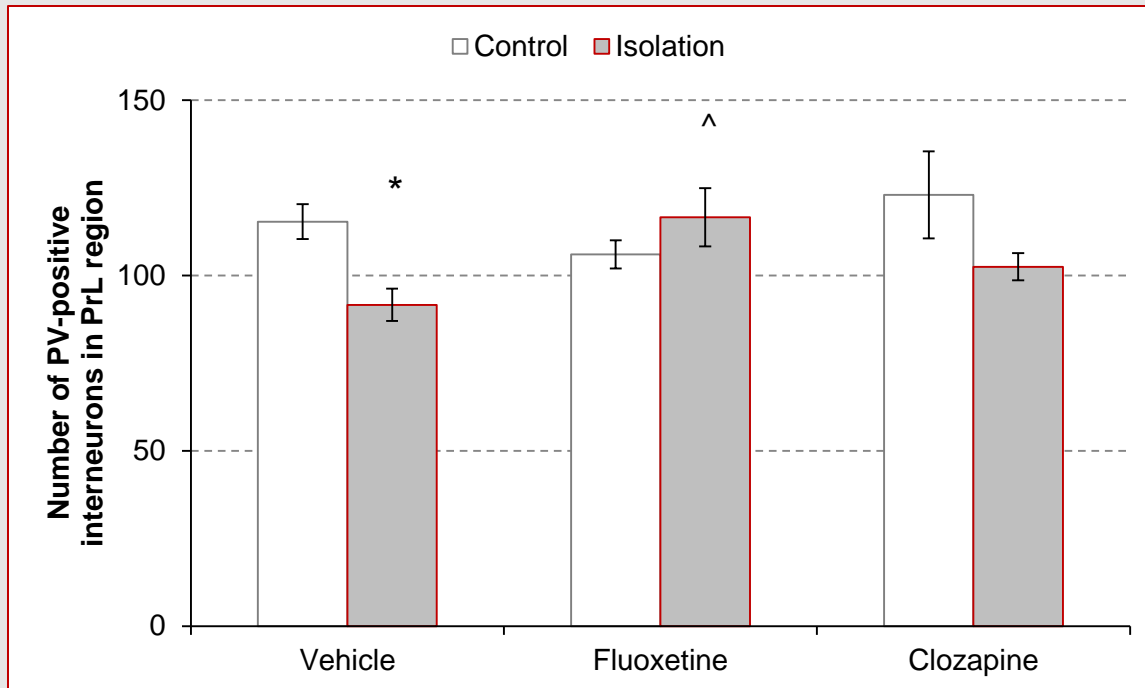
**Figure 2.** Number of PV-positive interneurons in medial precentral area, PrCm of medial prefrontal cortex of controls and treated rats. Symbols indicate significant differences between: treated experimental group and vehicle control \*\* $p < 0.01$ ; drug-treated and vehicle isolation ^ $p < 0.05$ , ^^ $p < 0.01$ .

# Number of PV-positive interneurons in Cg1 region



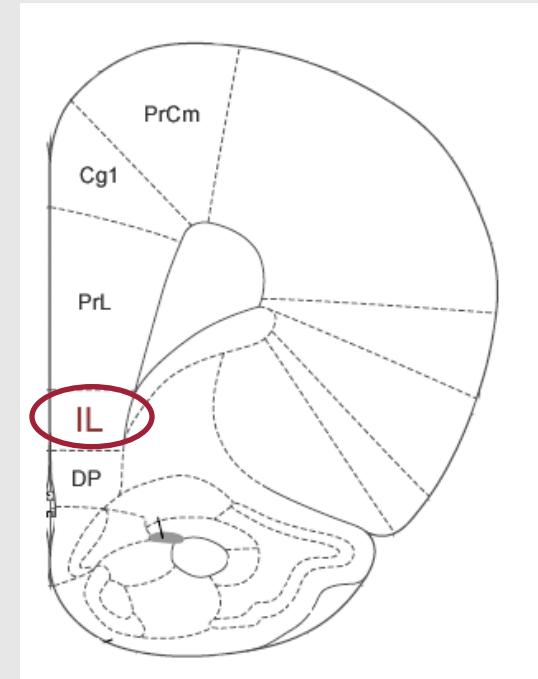
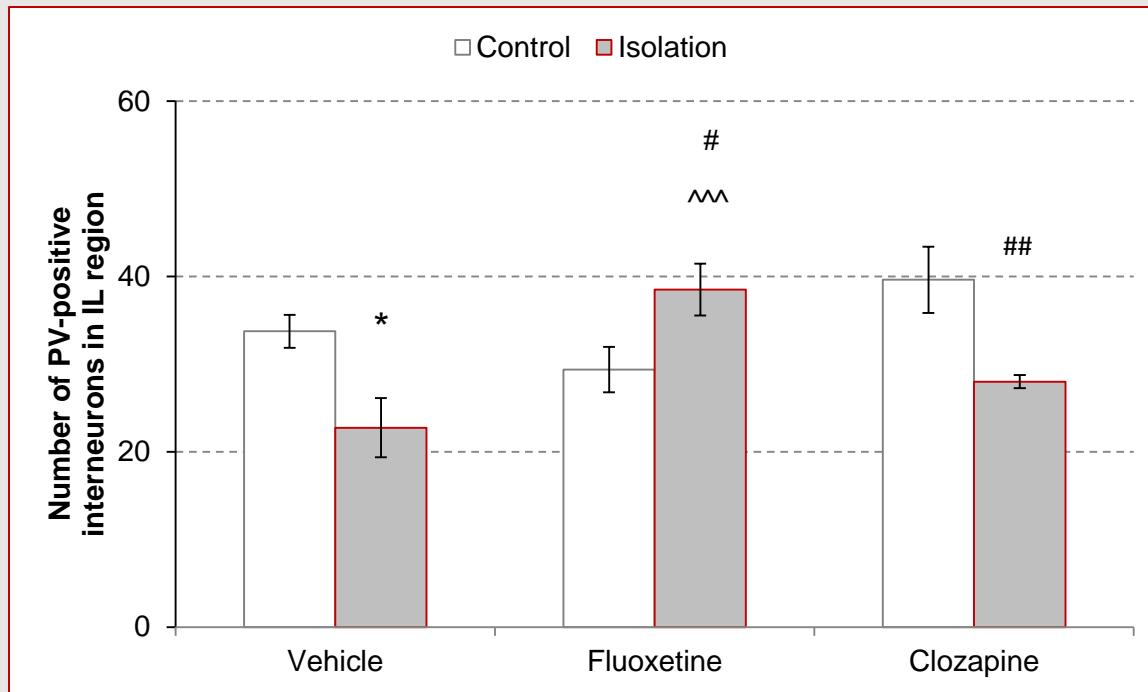
**Figure 3.** Number of PV-positive interneurons in cingulate cortex, area 1, Cg1 of rat medial prefrontal cortex of controls and treated rats. Symbol indicates significant differences between: treated experimental group and vehicle control \* $p < 0.05$ .

# Number of PV-positive interneurons in PrL region



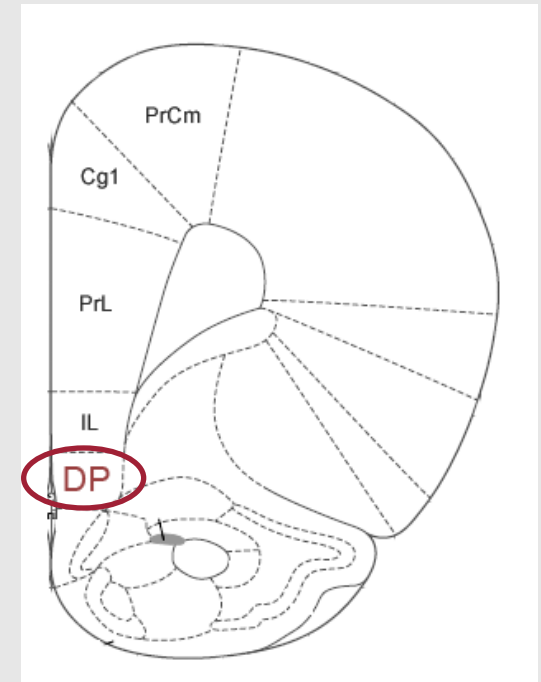
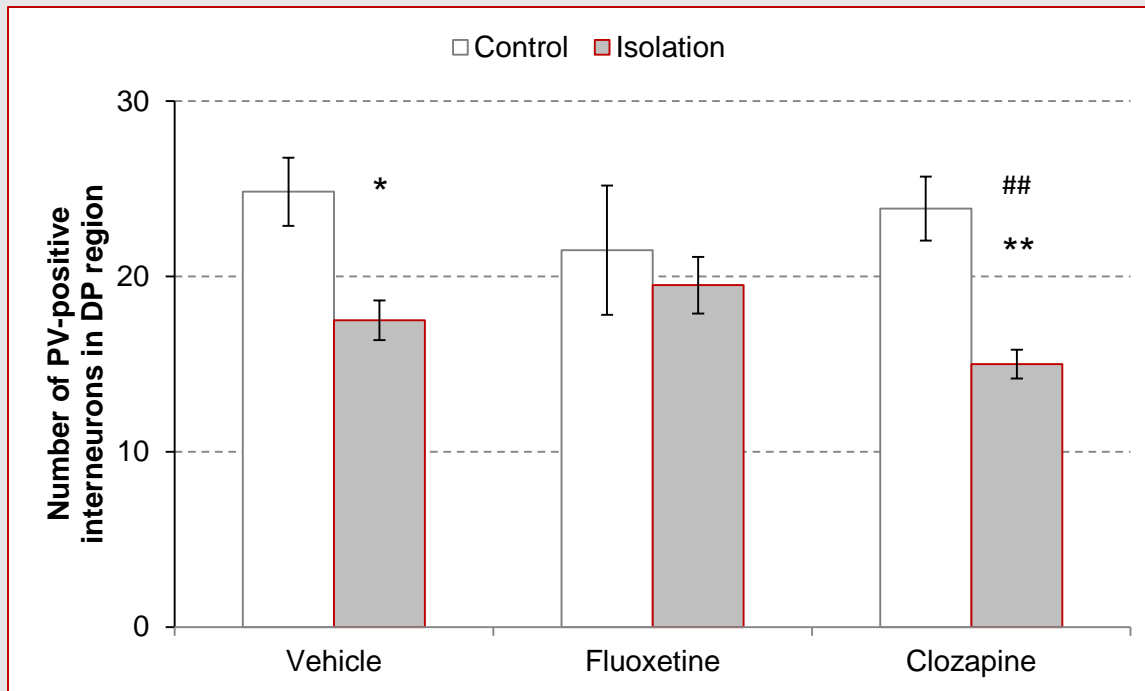
**Figure 4.** Number of PV-positive interneurons in prelimbic area, PrL of rat medial prefrontal cortex of controls and treated rats. Symbol indicates significant differences between: treated experimental group and vehicle control \* $p < 0.05$ ; drug-treated and vehicle isolation ^ $p < 0.05$ .

# Number of PV-positive interneurons in IL region



**Figure 5.** Number of PV-positive interneurons in infralimbic area, IL of rat medial prefrontal cortex of controls and treated rats. Symbol indicates significant differences between: treated experimental group and vehicle control \* $p < 0.05$ ; drug-treated and vehicle isolation ^^ $p < 0.001$ ; drug-treated isolation and respective drug-treated control # $p < 0.05$ , ## $p < 0.01$ .

# Number of PV-positive interneurons in DP region



**Figure 6.** Number of PV-positive interneurons in dorsal peduncular cortex, DP of rat medial prefrontal cortex of controls and treated rats. Symbol indicates significant differences between: treated experimental group and vehicle control \* $p < 0.05$ , \*\* $p < 0.01$ ; drug-treated isolation and respective drug-treated control ## $p < 0.01$ .

# Discussion and conclusions

- Chronic social isolation stress reduced the number of PV-positive interneurons in all regions of rat brain mPFC;
- Fluoxetine demonstrated protective effect in PrCm, PrL and IL region, while clozapine had the same effect only in PrCm.

# Discussion and conclusions

- Reduction of PV-positive interneurons???

## Oxidative stress

- **↓** Total SOD activity, **↑** MDA (Zlatković et al., 2014)
- Compromised GSH-dependent defense (Todorović et al., 2014)
- Mice with GSH deficit – impairment of PV interneurons (Kulak et al., 2013)



Thank you for your attention

