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

Nevena Todorović
Mentor: dr Dragana Filipović

Laboratory of Molecular Biology and Endocrinology, Institute of Nuclear Sciences Vinča, University of Belgrade, Serbia
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 21 days, 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

<table>
<thead>
<tr>
<th>drug</th>
<th>Dose (mg/kg/day)</th>
<th>rat serum level (ng/ml)</th>
<th>therapeutically effective level (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>fluoxetine</td>
<td>15</td>
<td>280 ± 50</td>
<td>100–700</td>
</tr>
<tr>
<td>clozapine</td>
<td>20</td>
<td>123 ± 18</td>
<td>100–700</td>
</tr>
</tbody>
</table>

Materials and methods

Medial prefrontal cortex

- PrCm, medial precentral area;
- Cg1, cingulate cortex, area1;
- 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)
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.
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