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# Pharmaco-ethological and Hippocampal CA1 region neurohistological study of Sertraline effects on Single-prolonged Stress-induced Rodent model of PTSD



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## ABSTRACT

**Background:** Sertraline is one of the several drugs approved for posttraumatic stress disorder (PTSD). The hippocampal CA1 region is known to be associated with anxiety. Single-prolonged stress (SPS)-treated rats, a rodent model of PTSD, has been found to induce apoptosis of hippocampal pyramidal neurons but the effect of sertraline on anxiety-like behaviors and hippocampal CA1 neurons had not been studied on it. The aim of this study was to investigate the effect of sertraline on classical and ethological variables of anxiety-like behavior, and histomorphological appearance of the hippocampal CA1 region in SPS-induced rats.

**Methods:** Wistar male rats (8-10 weeks; 150-180 g [n = 35]), were randomized into 5 groups. Sertraline was administered for 14 days. Behavioral measures were studied with elevated plus maze test. Hippocampus-containing coronal slices of the brains were HE-stained and analyzed using Optilab.

**Results:** The numbers of closed arm entries and stretched-attend posture (SAP) were not different between groups. Sertraline decreased time spent in closed arms, increased open arm entries, time spent in open arms, and unprotected head dips (UHD). The lowest dose of sertraline exerted the highest level of anxiety as evidenced by the number of protected head dips (PHD), but higher doses ameliorated this effect. The spatial arrangement of CA1 pyramidal neurons was severely impaired in all SPS-exposed groups. Pyknotic, dark-stained, and shrunk neurons were commonly found in sertraline-treated rats, especially at the dose of 18 mg/kg/day.

**Conclusions:** We found that chronic administration of sertraline exerts an anxiolytic effect on single-prolonged stress-induced rats with increased "chaotic" appearance in histomorphology as the dose increased.

**Keywords:** Sertraline, pharmaco-ethological analysis, hippocampal CA1 region, single-prolonged stress, rats

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## INTRODUCTION

Posttraumatic stress disorder (PTSD) is an anxiety disorder in dramatic or life-threatening stress-exposed individuals.<sup>1,2</sup> PTSD is commonly accompanied by comorbidities such as depression and increased risk of suicide.<sup>3</sup> Deficits in cognition and memory dysfunction are often found in PTSD cases.<sup>1,4</sup> These deficits are associated with disturbances in the hippocampus due to its susceptibility to stress.<sup>2</sup>

Han et al. found that single-prolonged stress/SPS (consists of psychological, physical, and biochemical stressors) for 7 days could trigger ER stress-related apoptosis in the rat hippocampus.<sup>2</sup> SPS are often used to model PTSD in rats due to its property in causing interference on hypothalamus-pituitary-adrenal (HPA) axis such as those found in patients with PTSD.<sup>5</sup>

Sertraline is a first-line drug approved by the Food and Drug Administration (FDA) for PTSD.<sup>3</sup> Administration of sertraline to treat PTSD is based on studies that put more emphasis on its role as a pro-serotonergic agents.<sup>6</sup> According to Ravindran and Stein,<sup>7</sup> serotonin dysfunction in PTSD is only partially able to be used as a pathobiological basis to explain PTSD. Thus, it is important to elucidate the pathogenesis of PTSD and the exact molecular mechanisms of sertraline in PTSD patients or models of PTSD.

Up to date, the effects of sertraline on anxiety-like behaviors and specific region of the hippocampus in rodents model of PTSD had never been studied. The purpose of this study was to investigate the effects of sertraline on measures of anxiety-like behavior and histomorphology of hippocampal CA1 region in SPS-induced rodents.

## MATERIALS AND METHODS

### Animal Subjects

The research subjects were Wistar male rats, obtained from LPPT Unit IV UGM with 8-10 weeks of age and weighing 150-180 grams. Rats were maintained in the animal facility of LPPT Unit IV UGM with a temperature of 24 + 2°C and a light-dark cycle of 12 hours. All animals were randomized into five groups and then acclimatized for one week. The normal control group was not exposed with SPS (consisted of 2 hours-immobilization, 20-minutes forced swim (25°C), 15-minutes rest, and exposure to Ether as anesthesia agent [until loss of consciousness]). The positive control group was induced with SPS plus consolidation phase of traumatic memories for seven days. The dose 1 group, dose 2 group, and dose 3 group were induced with SPS for seven days, consolidation phase for seven days, and followed by oral administration of sertraline (4,5 mg, 9 mg, and 18 mg/kg/day, respectively) for 14 days. The rats were given ad libitum access to drinking water and food during treatment period beyond the SPS protocol.

### Drug Preparation & Administration

The drug used in this study was sertraline hydrochloride (in tablet form with a brand name of Serlof). The tablets were dissolved in distilled water, and the resulting solution was given orally to the rats with a volume of 2 mL. Sertraline was administered for 14 days at 3 different doses (4.5 mg/kg/day, 9 mg/kg/day, and 18 mg/kg/day) for treatment 1 group, treatment 2 group, and treatment 3 group, respectively.

### Elevated Plus Maze Test

The anxiety-like behavioral measures were investigated using the elevated plus maze (EPM) test in Animal Behavior Laboratory, Department of Physiology, Faculty of Medicine, UGM. Each rat received a 5-minutes single trial. The recorded behaviors comprised the classical as well as ethological measures of anxiety (closed arms entries, time spent in closed arms [measures of total locomotion],

open arms entries, time spent in open arms [inverse measures of anxiety], stretched-attend postures, protected head dips, and unprotected head dips [risk-assessment behaviors/ethological measures of anxiety]). These measures were recorded with a computer-connected video camera for later analysis by two trained observers. EPM apparatus was cleaned with 70% ethanol after each trial to remove potential odor cues.

### Brain Isolation & HE Staining

The animals were anesthetized using ether and perfused intracardially via left ventricle with 0.9% NaCl for 5 minutes, followed by 4% paraformaldehyde (PFA) in PBS for 20 minutes. The whole brain tissues were taken for examination. Brains were subsequently processed and cut into coronal slices with a thickness of 4 µm for hematoxylin and eosin (HE) staining in Pathological Anatomy Laboratory, Faculty of Medicine, Gadjah Mada University. These slices were viewed under a light microscope (magnification x400) using Optilab.

### Statistical Analysis

Data were tested first for normality and variance homogeneity. One-way ANOVA (followed by LSD as the post hoc analysis) or Kruskal-Wallis test (with Mann-Whitney U test as a posthoc analysis) was then applied to the data, where appropriate. Differences with  $p < 0.05$  were considered significant for all tests.

## RESULTS

### Classical and Ethological Measures of Anxiety-like Behaviors

Closed arm entries and time in closed arms were the behavioral measures of total locomotion in this study. There was no significant effect on the number of closed arm entries ( $p > 0.05$ ) between the groups. Time spent in closed arms is higher in group treated with 4.5 mg sertraline/kg/day than normal control ( $p < 0.05$ ). Rats of the SPS group spent slightly higher time in closed arms than normal control, but this effect of SPS is not statistically significant ( $p > 0.05$ ). Sertraline decreases time spent in closed arms in a dose-dependent manner, however, this effect does not differ significantly between the dose of 9 mg and 18 mg/kg/day ( $p > 0.05$ ) (Table 1).

The inverse measures of anxiety in this study are open arm entries and time spent in open arms (%) (Table 2 and 3). The results of statistical analyses on these measures exhibit the same pattern. Both of these measures are higher ( $p < 0.05$ ) in the group treated with sertraline at the dose of 18 mg/kg/day compared to normal control and group treated with lowest sertraline dose (4.5 mg/kg/day).

**Table 1** Time Spent in Closed Arms (%)

Groups	n	Median (Minimum – Maximum)	p
Normal Control	7	92.93 (61.05 – 100)	
SPS	7	93.08 (77.31 – 100)	
SPS + Sertraline Dose 1	7	98.21 (89.53 – 100)	0.003
SPS + Sertraline Dose 2	7	80.67 (0.00 – 100)	
SPS + Sertraline Dose 3	7	72.01 (17.36 – 95.95)	

Post hoc analyses with significant results: Normal Control vs. SPS + Sertraline Dose 1 ( $p = 0.028$ ); SPS vs. SPS + Sertraline Dose 3 ( $p = 0.001$ ); SPS + Sertraline Dose 1 vs. SPS + Sertraline Dose 2 ( $p = 0.011$ ); SPS + Sertraline Dose 1 vs. SPS + Sertraline Dose 3 ( $p = 0.001$ ).

**Table 2 Open Arm Entries**

Groups	n	Median (Minimum – Maximum)	p
Normal Control	7	1 (0 – 3)	
SPS	7	0 (0 – 2)	
SPS + Sertraline Dose 1	7	0 (0 – 1)	0.011
SPS + Sertraline Dose 2	7	1 (0 – 4)	
SPS + Sertraline Dose 3	7	3 (0 – 5)	

Post hoc analyses with significant results: Normal vs SPS + Sertraline Dose 3 ( $p = 0.011$ ) and SPS + Sertraline Dose 1 vs SPS+Sertraline Dose 3 ( $p = 0.007$ ).

**Table 3 Time Spent in Open Arms (%)**

Groups	n	Median (Minimum – Maximum)	p
Normal control	7	5.45 (0.00 – 29.78)	
SPS	7	0.00 (0.00 – 10.59)	
SPS + Sertraline Dose 1	7	0.00 (0.00 – 6.53)	0.017
SPS + Sertraline Dose 2	7	6.36 (0.00 – 30.44)	
SPS + Sertraline Dose 3	7	19.78 (0.00 – 72.07)	

Post hoc analyses with significant results: Normal vs SPS + Sertraline Dose 3 ( $p = 0.011$ ) and SPS + Sertraline Dose 1 vs SPS+Sertraline Dose 3 ( $p = 0.007$ ).

**Table 4 Unprotected Head Dips (UHD)**

Groups	n	Mean (SEM)	p
Normal Control	7	6.14 (1.42)	
SPS	7	3.14 (0.80)	
SPS + Sertraline Dose 1	7	2.29 (0.97)	0.002
SPS + Sertraline Dose 2	7	7.71 (2.45)	
SPS + Sertraline Dose 3	7	14.14 (3.36)	

Post hoc analyses with significant results: Normal Control vs SPS + Sertraline Dose 3 ( $p = 0.010$ ); SPS vs SPS + Sertraline Dose 3 ( $p = 0.001$ ); SPS + Sertraline Dose 1 vs Dose 3 ( $p = 0.000$ ); SPS Sertraline Dose 2 vs. Dose 3 ( $p = 0.034$ ).

**Table 5 Protected Head Dips (PHD)**

Groups	n	Median (Minimum – Maximum)	p
Normal Control	7	1 (0 – 7)	
SPS	7	1 (0 – 2)	
SPS + Sertraline Dose 1	7	3 (1 – 6)	0.024
SPS + Sertraline Dose 2	7	1 (0 – 1)	
SPS + Sertraline Dose 3	7	1 (1 – 3)	

Post hoc analyses with significant results: SPS vs SPS + Sertraline Dose 1 ( $p = 0.004$ ) and SPS + Sertraline Dose 1 vs Dose 2 ( $p = 0.004$ ).

Ethological variables in this study are stretched-attend posture (SAP), unprotected head dips (UHD), and protected head dips (PHD). These variables can be considered as risk-assessment behaviors or direct behavioral measures of “anxiety” in rodents. SAP between groups fails to show any differences ( $p > 0.05$ ). UHD tends to be increasing with sertraline dose increment ( $p < 0.05$ ) (Table 4).

There is no any statistical difference between PHD between normal control and untreated SPS group ( $p > 0.05$ ). Our study finds that the administration of sertraline at the dose of 4.5 mg/kg/day (i.e., dose 1) for 14 days exerts the highest effect on PHD (Table 5).

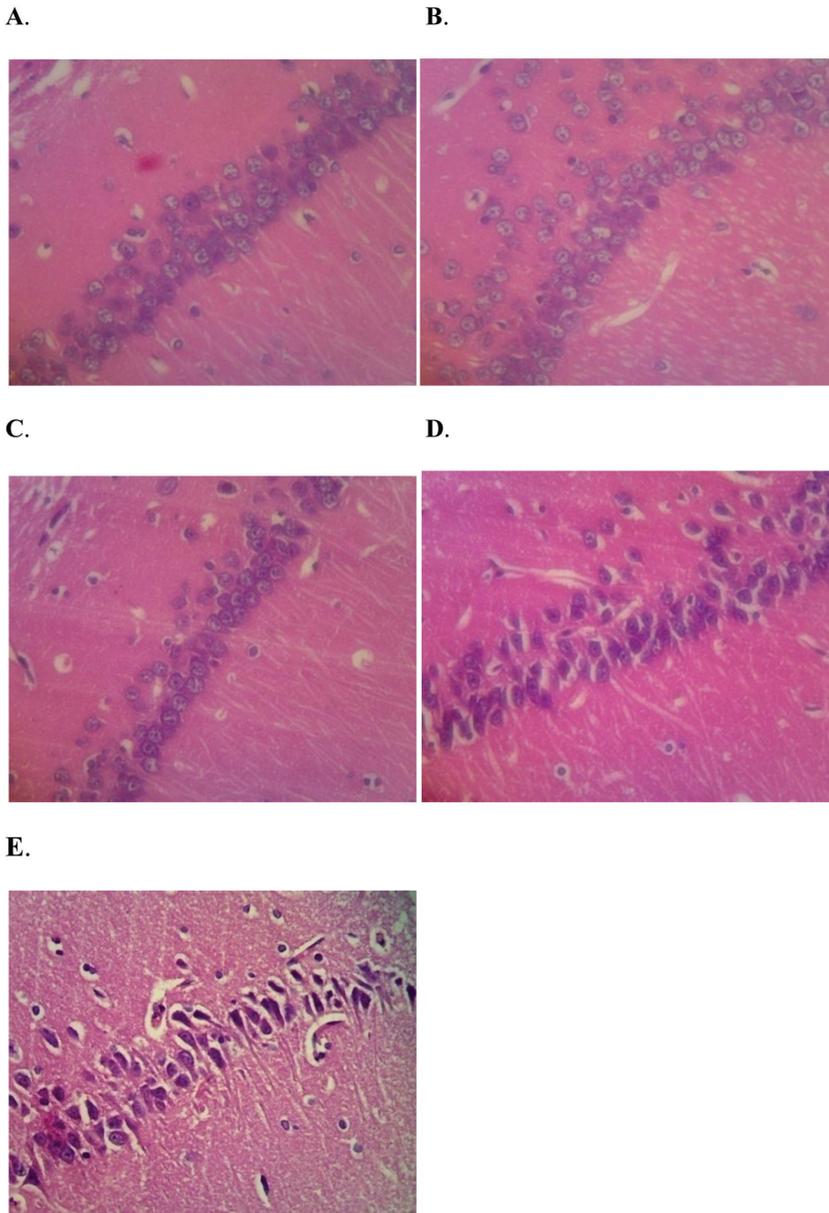
### Neurohistological Analysis of Hippocampal CA1 Region

Using HE staining method, the morphology and pathological changes in all groups were observed as follows (Figure 1): (A) There was a tight and orderly arrangement of pyramidal cells in normal control group. This group exhibited normal morphology (round appearance) of neurons, light staining, and clear nucleoli. Deformations (nuclear pyknosis, unclear nucleoli, and dark staining) were rarely seen in this group. (B) SPS-induced groups showed disordered and very sparse arrangements of pyramidal neurons. There were also many deformed neurons. (C and D) SPS+Sertraline-treated groups also showed similar pathological changes with SPS-only group but with a tighter arrangement of pyramidal neurons. Group treated with dose 1 (4.5 mg/kg/day of sertraline) showed a more rounded morphology of neurons than the group treated with dose 2 (9 mg/kg/day of sertraline). Dose 2-treated group tends to shows elongated, spindle-shaped, or polygonal morphology (this effect is even more prominent in dose 3-treated group). (E) Shranked and deformed pyramidal neurons were commonly found especially in the group treated with dose 3 or the highest dose of sertraline (18 mg/kg/day). Subjectively, we observe a more “chaotic” picture of neuron morphology and spatial arrangement in this group, compared to dose 2-treated group.

## DISCUSSION

### Sertraline Effect on Anxiety-like Behaviors in SPS-induced Rats

This study was performed to investigate the effects of sertraline on classical and ethological measures of anxiety using the SPS-induced model of PTSD. Using EPM paradigm to investigate these effects in SPS and SPS plus sertraline treatment for 14 days, we found that the drug produced an anxiolytic-like effect on some behavioral measures. Sertraline decreased time spent in closed arms. However, SPS-only rats in our study showed the different result to that of Miao et al.<sup>8</sup> regarding some closed arm entries. We found that there was no significant effect of SPS-exposure on the number of closed arm entries between groups. The present study shows that sertraline does not affect locomotion in all groups since locomotion-enhancing (-lowering)



**Figure 1** Histological Sections of CA1 Region. (A) Normal control; (B) SPS-induced rats; (C) SPS + sertraline-induced groups (4,5 mg, 9 mg, and 18 mg/kg/day, respectively) (HE staining, x400)

drug should increase (decrease) the number of closed arm entries.<sup>9</sup>

Sertraline increases open arm entries and percentage of time spent in open arms. The results of the present study were equivocal to that of the study done by Varty et al.,<sup>9</sup> except for the open arm entries. Since they used gerbils to model anxiety and administered fluoxetine and paroxetine in their study, we assume that the effect of SSRIs on these inverse measures of anxiety are drug- and species-specific.

Sertraline exerts varied effects on the ethological measures of anxiety-like behaviors in SPS-induced rats. SPS protocol and sertraline treatment in our

study do not affect the number of SAP. This measure is known as a more sensitive measure of an anti-anxiety drug than classical indices of anxiety in EPM.<sup>10</sup> Head dips in open arms (UHD) is another ethological measure of risk-assessment behavior. This measure contributes to the enhanced sensitivity of EPM in studying the anxiolytic or anxiogenic effect of an agent.<sup>11</sup> Sertraline is also found to increase UHD, which is inversely correlated with anxiety. The lowest dose of sertraline in our study exerted the highest level of anxiety as evidenced by the number of protected head dips (PHD). However, higher doses of this drug ameliorate the anxiogenic effect. Thus, sertraline shows the biphasic effect on the number of PHD in SPS-induced rats. This effect warrants further investigation to elucidate the exact mechanism of sertraline in modulating this type of risk-assessment behavior.

#### Sertraline Effect on Neurohistology of Hippocampal CA1 Region in SPS-induced Rats

The findings in this study are different from the findings of studies by Aboukhatwa et al. and Taler et al.<sup>12,13</sup> Both teams of researchers stated that the administration of SSRIs might protect hippocampal neurons from stress. Hence, SSRIs are assumed as neuroprotective agents. Administration of sertraline, as a SSRI, is expected to protect hippocampal neurons from excessive stress by enhancing serotonin level in the hippocampus and maintaining the excitability of the hippocampal neurons.<sup>14</sup> However, in the present study, it is found that there is a trend of dose-effect relationship between the sertraline treatment and impairment of hippocampal CA1 region histomorphology. The hippocampal impairment tends to be progressively enhanced by the increasing dose of sertraline. Therefore, we assume that an alternative pharmacodynamics of sertraline is operative in SPS-induced rats.

We predict that sertraline may modulate ER stress pathway as an alternative mechanism of a hippocampal defect in this SPS-induced model of PTSD. SPS protocol *per se* has been shown to induce apoptosis of hippocampal neurons through excessive ER stress, which is associated with a decrease in the hippocampal GRP78 pool.<sup>2</sup> In dormant condition, GRP78 forms an intraluminal complex or colocalizes with Sig1R, which is associated with PTSD pathobiology.<sup>15</sup> In the presence of ER stress or Sig1R agonist, there will be dissociation of this complex and activation of both GRP78 and Sig1R as chaperones.<sup>16</sup> Sig1R activation will inhibit pro-apoptotic protein so that the adverse effects of ER stress on survival of neurons can be mitigated.<sup>17</sup> Inhibition or antagonism towards Sig1R will lead to stabilization of Sig1R-GRP78 complex and reduce chaperoning activities of each component

under stress conditions.<sup>18</sup> We suggest investigation of sertraline's effect on ER stress markers in SPS-induced rats to confirm the prediction that sertraline is an ER stress modulator.

## CONCLUSIONS

We conclude that chronic administration of sertraline exerts an anxiolytic effect on single-prolonged stress-induced rats. Increasing dose of sertraline is associated with increasing "chaotic" appearance of hippocampal CA1 region histomorphology. Further study is needed to elucidate the exact molecular mechanisms of sertraline on behavioral measures and histomorphological impairment of hippocampus in this rodent model of PTSD.

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