**Supplementary Information:**

Subfield-specific Effects of Chronic Mild Unpredictable Stress on Hippocampal Astrocytes

Garima Virmani1, Priyal D’almeida1, Arnab Nandi1, Swananda Marathe1,2

1Centre for Neuroscience, Indian Institute of Science, Bangalore, India 560012

2Correspondence may be addressed to: Dr. Swananda Marathe, Centre for Neuroscience, Indian Institute of Science, Old TIFR building, Gulmohar Marg, Bangalore, India 560012

email: swanand@iisc.ac.in or swanandlab@gmail.com

**Supplementary Table 1**

|  |  |  |
| --- | --- | --- |
|  | First Stressor | Second Stressor |
| Day 0 | Forced Swim Stress | |
| Day 1 | Overcrowding | Cage Tilt |
| Day 2 | Tail Suspension Stress | Cold Exposure |
| Day 3 | Overcrowding | Cage Tilt |
| Day 4 | Wet Bedding | Restraint |
| Day 5 | Forced Swim Stress | Food Water Deprivation |
| Day 6 | Cold Exposure | |
| Day 7 | Tail Suspension Stress | |
| Day 8 | Wet Bedding | Cage Tilt |
| Day 9 | Shaker Stress | Tone |
| Day 10 | Forced Swim Stress | White Noise |
| Day 11 | Overcrowding | Tone |
| Day 12 | Tail Suspension Stress | Cold Exposure |
| Day 13 | Restraint | |
| Day 14 | Wet Bedding | |
| Day 15 | Forced Swim Stress | Cage Tilt |
| Day 16 | Overcrowding | Tone |
| Day 17 | Tail Suspension Stress | Wet Bedding |
| Day 18 | Shaker Stress | Food Water Deprivation |
| Day 19 | Forced Swim Stress | Cage Tilt |
| Day 20 | Overcrowding 🡨---------------------------SPT-----------------------------🡪 | |
| Day 21 | Food Water Deprivation OFT | |
| Day 22 | Sacrifice | |

**Supplementary Figure and Table Captions:**

*Supplementary Figure 1: Analysis of locomotor behavior in the open field test*

Mice were subjected to 21 days of Chronic Mild Unpredictable Stress (CMUS), while the controls were handled similarly without exposure to stress. The mice were subjected to the Open Field Test (OFT) on day 21 to assess locomotor behavior. Movements were recorded from top-mounted video camera and the trajectories were tracked **(A)**. CMUS caused a statistically significant increase in locomotor activity as compared to the control mice **(B)**. On the other hand, we did not observe any difference in the maximum speed achieved within 1 second time bins between the two groups **(C)**. n = 6-8 mice per group. Data represented as mean ± SEM. \* represents p < 0.05. All comparisons using unpaired student’s t-test.

*Supplementary Figure 2: double immunolabeling with GFAP and S100β*

The sections were double immunolabeled with GFAP and S100β and confocal images were obtained. Representative maximum intensity projection images of molecular layer (ML) of the dentate gyrus, Hilus, *stratum lucidum* layer of CA3 (CA3 s.l.) and *stratum radiatum* region of CA1 (CA1 s.r.) showing immunostaining for GFAP (red), S100β (green) and Merged channels showing a near complete overlap between cells expressing GFAP and S100β. All scale bars are 100µm.

*Supplementary figure 3: Image analysis pipeline*

Images of GFAP-stained hippocampal sections were captured on a confocal microscope and maximum intensity projection was generated. The images were then binarized by using global dynamic thresholding. The background was eliminated using size exclusion. Single cells were cropped out and surface area was measured by pixel count. Next, the cells were skeletonized to generate cells with single pixel-wide processes. The pixel count of the skeletonized cell was calculated to estimate the total process length. Surface area of every cell was divided by its total process length to generate the approximate average process width for that cell. Skeletonized images of astrocytes were used to generate Sholl analysis vectors. A 5-period moving average was calculated for every cell to generate smooth curves for Sholl analysis.

*Supplementary Table 1*

Table shows the schedule of different stressors employed during the 21-day CMUS paradigm. On day 22, 24 hours after the last stressor, mice were sacrificed.