Supplementary Information

PEGylation corannulene enhances response of stress through promoting neurogenesis

Xinyu Li,[a, †] Dandan Sun,[b, †] Xu Li,[c, †] Dashuai Zhu,[a] Zhenzhen Jia,[a] Jiajia Jiao,[a] Kai Wang,[a] Deling Kong,[a] Xin Zhao,[b] Lin Xu,[b] Qiang Zhao,[a]* Dongyan Chen,[c]* Xizeng Feng,[a]**

a. State Key Laboratory of Medicinal Chemical Biology, Key Laboratory of Bioactive Materials, Ministry of Education, College of Life Sciences, Nankai University, Tianjin 300071, China.

b. The Institute of Robotics and Automatic Information Systems, Tianjin Key Laboratory of Intelligent Robotics, Nankai University, Tianjin 300071, China.

c. Tianjin Key Laboratory of Tumor Microenvironment and Neurovascular Regulation, Department of Histology and Embryology, School of Medicine, Nankai University, Tianjin 300071, China.

† These authors contributed equally to this work.

*e-mail: xzfeng@nankai.edu.cn.

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1. Experimental design and schedule

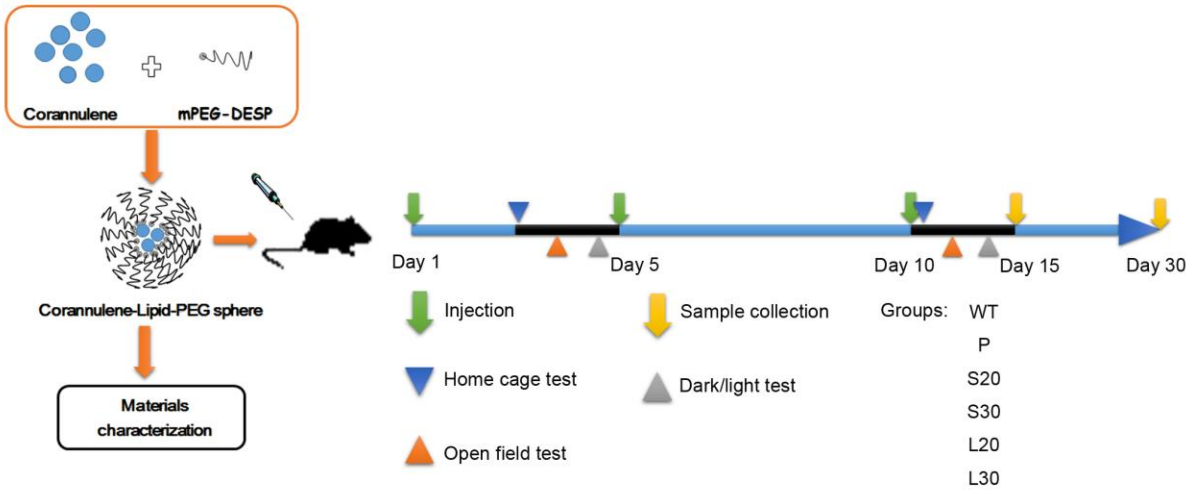

Figure S1. Experimental design and schedule. Corannulene and mPEG-DSPE were dissolved in 500 μL tetrahydrofuran (THF). Then, Corannulene and mPEG-DSPE spontaneously formed to PEGylation corannulene nanoparticles (PEGylation CoNps) under 78^{o}C. Experimental schedule was designed to examine the neural functions of PEGylation CoNps. Two experiments are designed to examine effects of short- and long- term of PEGylation CoNps on living mouse. As the experimental schedule showed home cage test (HC-T), open field test (OF-T) and light-dark transition test (D/L-T) was studied to examined mice behaviors. Mice were sacrificed after behavioral test in Day 15 and Day 30.

2. Behavioral experimental test methods

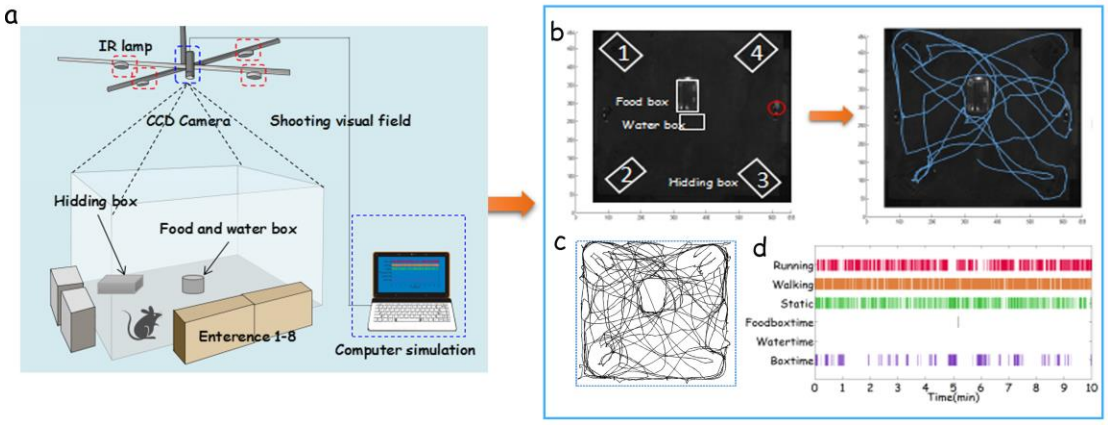

Figure S2. Semi-nature condition was established to examine mice behaviors changes[1]. Mice behaviors was recorded by CCD camera (H0514-MP2, Computar CCD), and analyzed though MATLAB software[2]. The background was subtracted from each video frame, then established coordinate to determine mice position and calculate mice traveled distance and speed. Behaviors pattern was defined according to velocity and distance dynamic as previously published[1, 3, 4]. (a) Semi-nature condition and automated video track system. Mice position was recorded by CCD camera; (b) Coordinate was established by MATLAB software to simulate mice travel trajectories, then create (c); (b) Mice behaviors was defined by frame and established ethogram[1].

3. Supplementary materials characterization

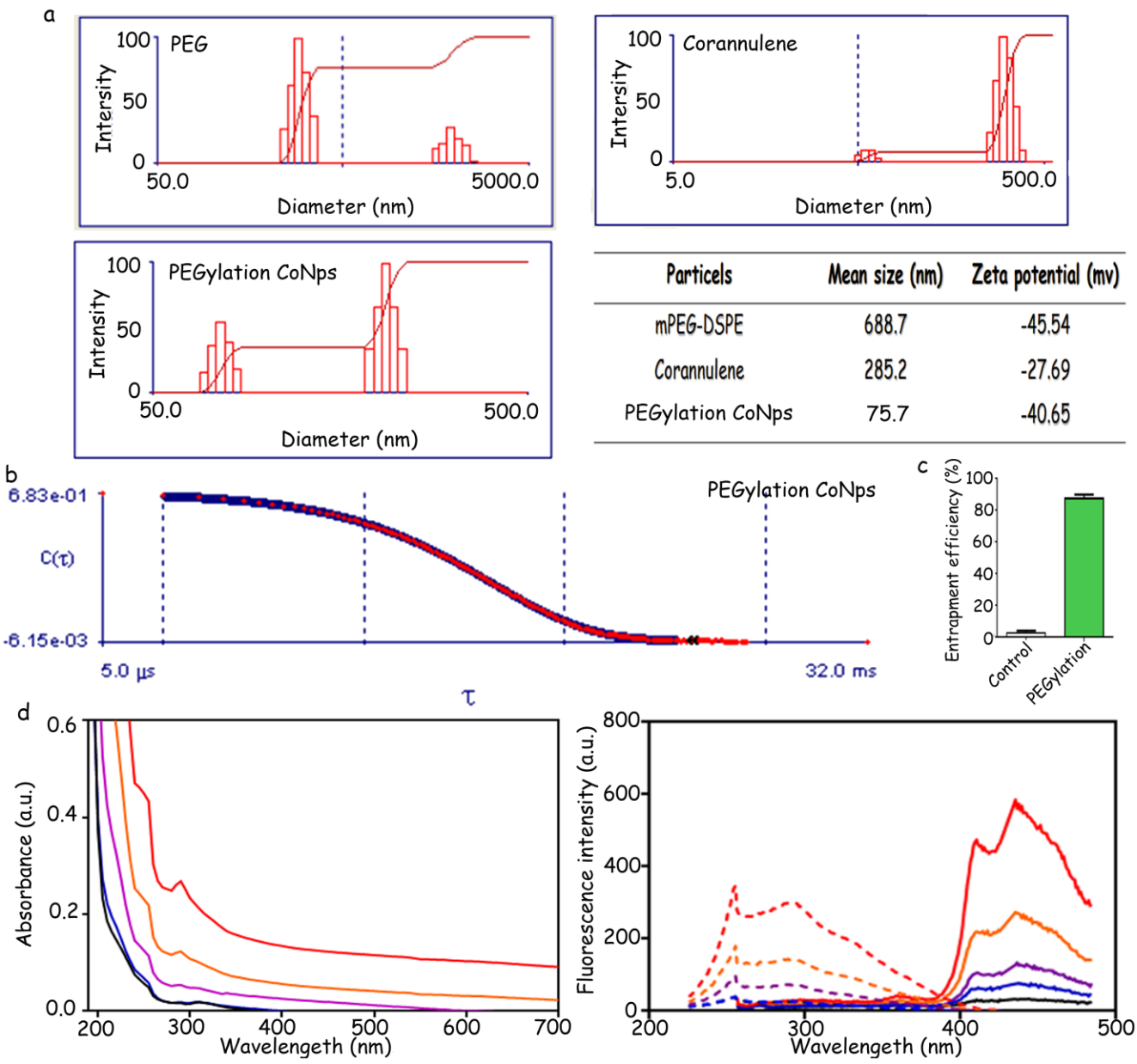

Figure S3. The characterization of materials. (a) Dynamic light scattering (DLS) data. DLS results showed the average size of PEGylation CoNps are 75.7 nm, and zeta potential is -40.65 mv in saline solution. Corannulene is 285.2 and zeta potential is -27.96 (mv) in THF due to corannulene insoluble in non-organic solvents, and the DLS was examined in corannulene/THF solution. (b) The intensity of PEGylation CoNps. DLS results showed that there are large particles interference, but the smaller PEGylation CoNps exhibited most contribution in particles' number and displayed good curve fitting. (c) Entrapment efficiency of

PEGytion. The results showed that the entrapment efficiency of PEG is about 87%. (d) Absorption spectrum (190 nm-700 nm), emission spectrum and excitation spectrum of PEGylation CoNps. As the results showed the excitation spectrum is 250 nm and emission are 438 nm.

4. PEGylation enhanced biocompatibility of corannulene

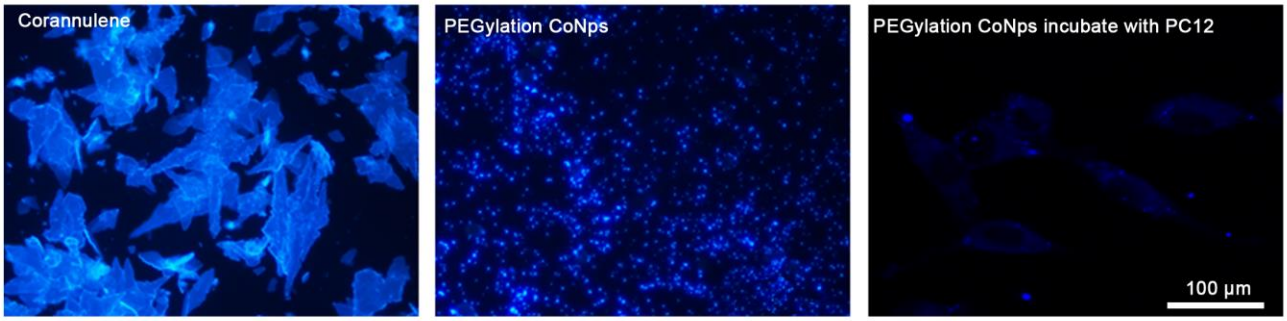

Figure S4. PEGylation enhanced biocompatibility of corannulene. PEGylation reduced the aggregation of corannulene. In addition, PC-12 cells were incubated with PEGylation CoNps for 4h. Confocal image showed that PEGylation CoNps can be up-taken by PC-12 cells. These results indicate that PEGylation enhanced biocompatibility of corannulene.

5. Mice home-cage resting bouts in light and dark condition

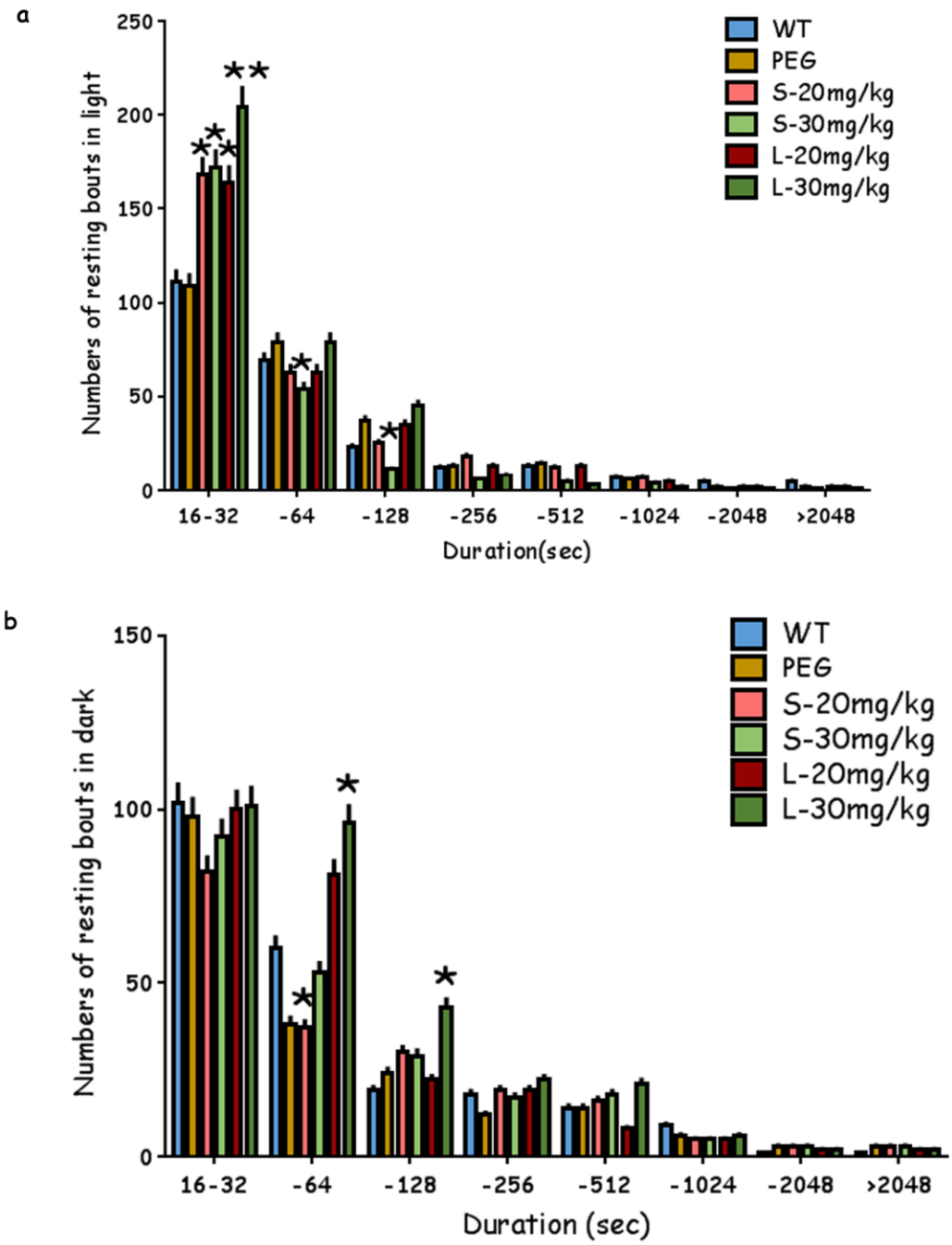

Figure S5. (a) Numbers of resting bouts in light. Injection groups displayed more resting bouts than WT groups in 16-32 s resting sections[5]. (b) Numbers of resting bouts in dark. Injection groups showed more resting bouts in -64 s and -128 s resting section than in light. As the results showed that PEGylation CoNps mainly increased short resting time in light and lead to more total resting bouts injected groups than WT groups[5] (Tukey Test, *p<0.05,**p<0.01, ***p<0.001).

6. General linear model of mice behaviors

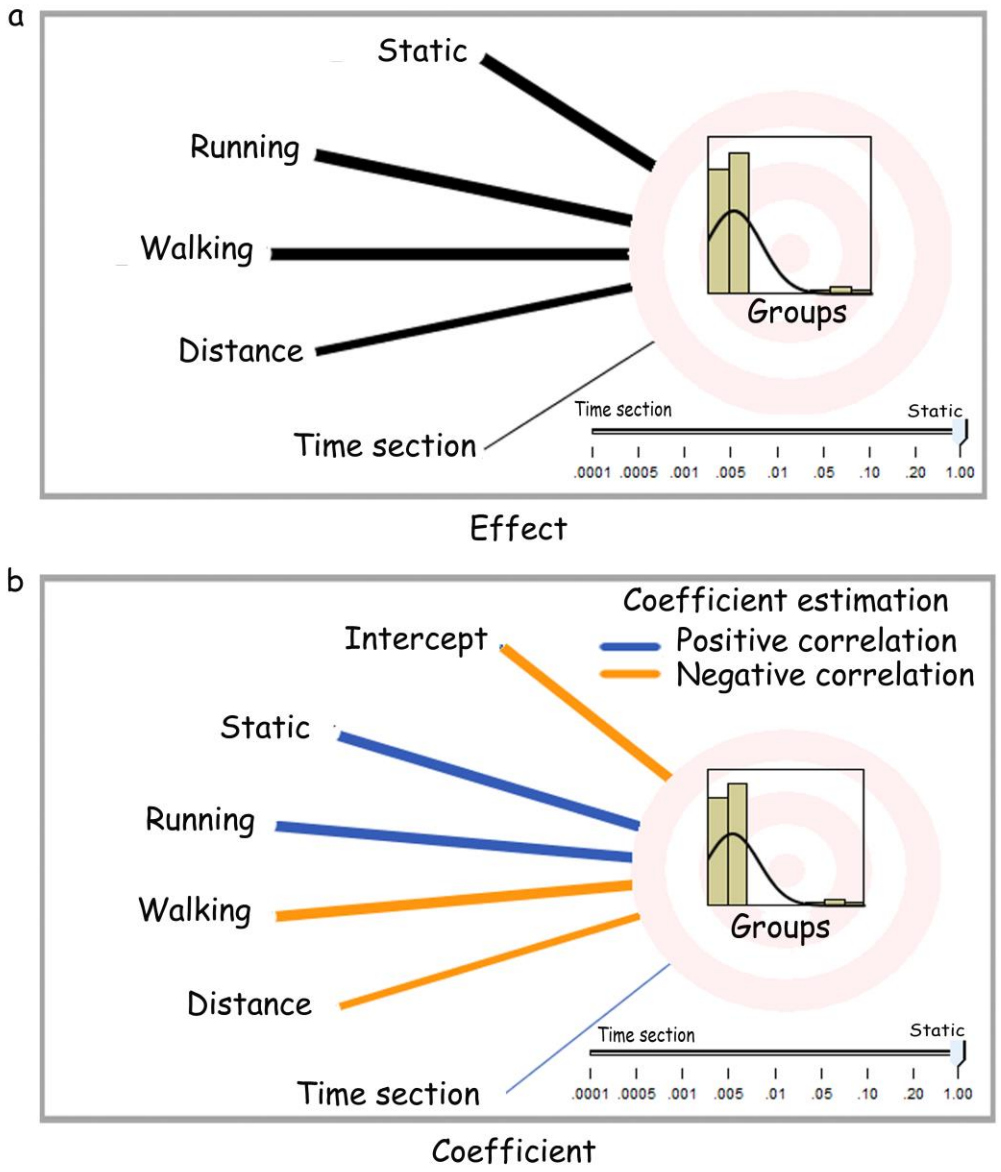

Figure S6. General linear model analysis the effect and coefficient of mice behaviors on experimental groups which indicate the contribution degrees of mice behavioral states to experimental groups. (a) The effects of mice behaviors on experimental groups, line model was established under 1 significant value; (b) The influence coefficient of mice behaviors to experimental groups, the mice static time, running time and time section were positive correlation to experimental groups and the other were negative correlation.

7. Velocity frequency distribution of OF-T

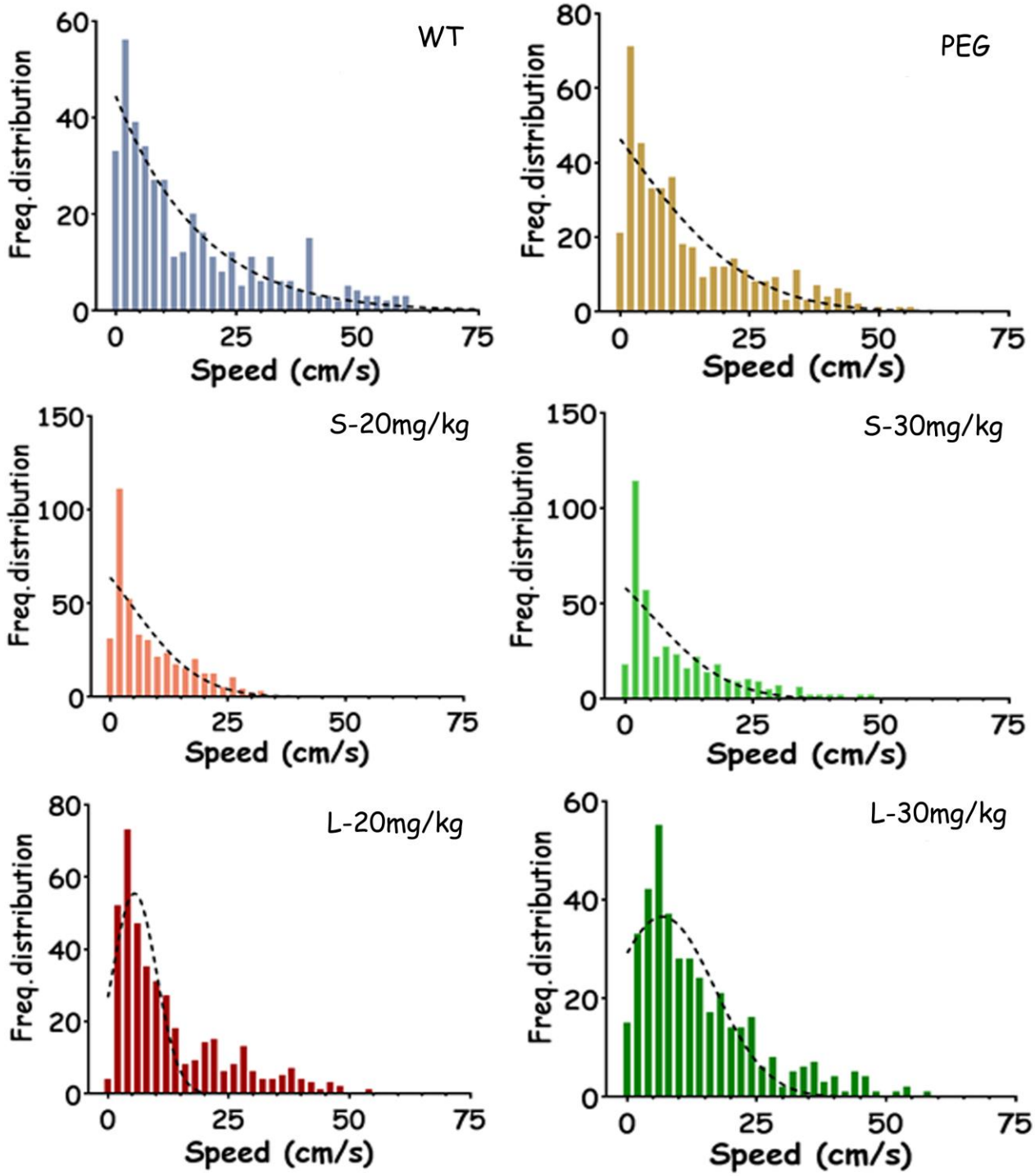

Figure S7. Velocity Frequency distribution over 400s in OF-T. As the results showed that WT and PEG groups displayed more 5 cm/s than S20, S30 and L20 in 400s. Although the speed of L30 is more than 5 cm/s, but there were little >5 cm/s distribution within it. It is means that the PEGylation CoNps reduced mice velocity and lead to decreasing locomotor activities.

8. Trajectories of OF-T over 400 s

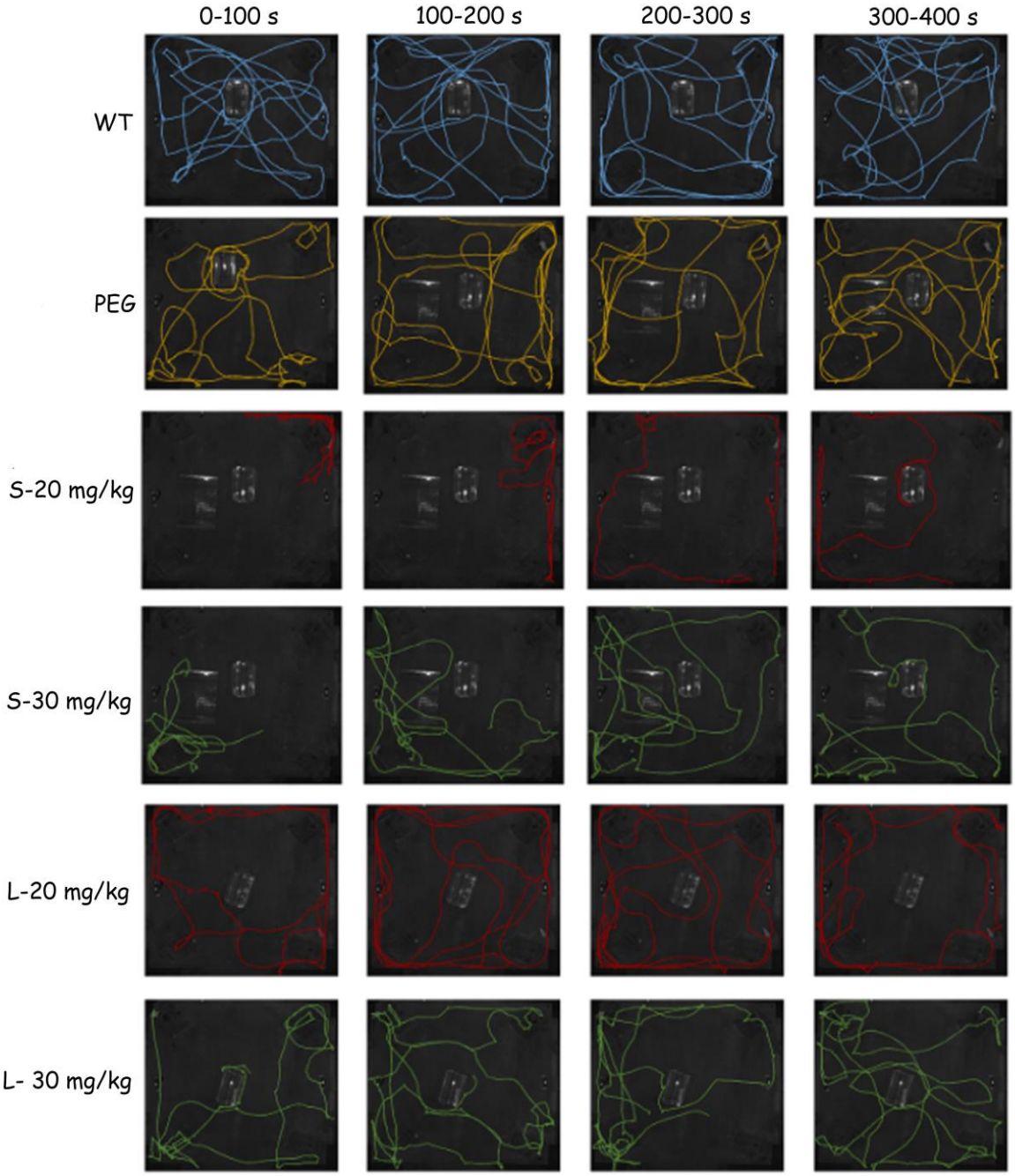

Figure S8. Trajectories of OF-T from 0-100 s section to 300-400 s section[6]. WT and PEG groups showed more travel distance than injection groups in each section what demonstrated PEGylation CoNps take down mice locomotor activity. Furthermore, WT and PEG groups mice have entered the central region, but vividly occurred in injected groups. This results illustrated that mice exhibited anxiety-like behaviors[6].

9. Ethogram of mice in open field test over 400 s

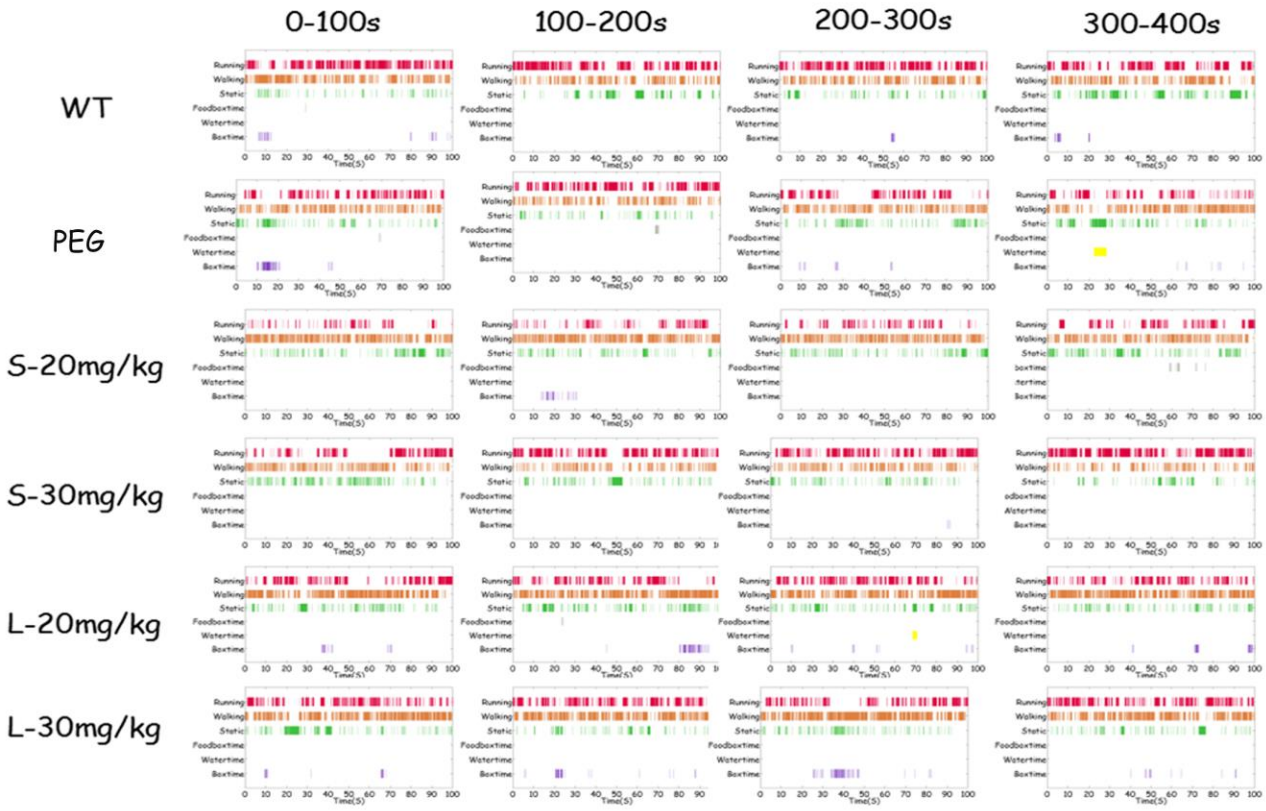

Figure S9. Ethogram of mice states over 400 s[1]. As the results showed that the food time, water time and box time was very little and neglected. We discuss the average running time, walking time and static time in 100 s and showed that treatment of PEGylation CoNps reduced average running time and increased walking time[1, 2]. The result suggests that the locomotion activities of mice were decreased after injected PEGylation CoNps[6].

9. Trajectories and analysis of D/L-T

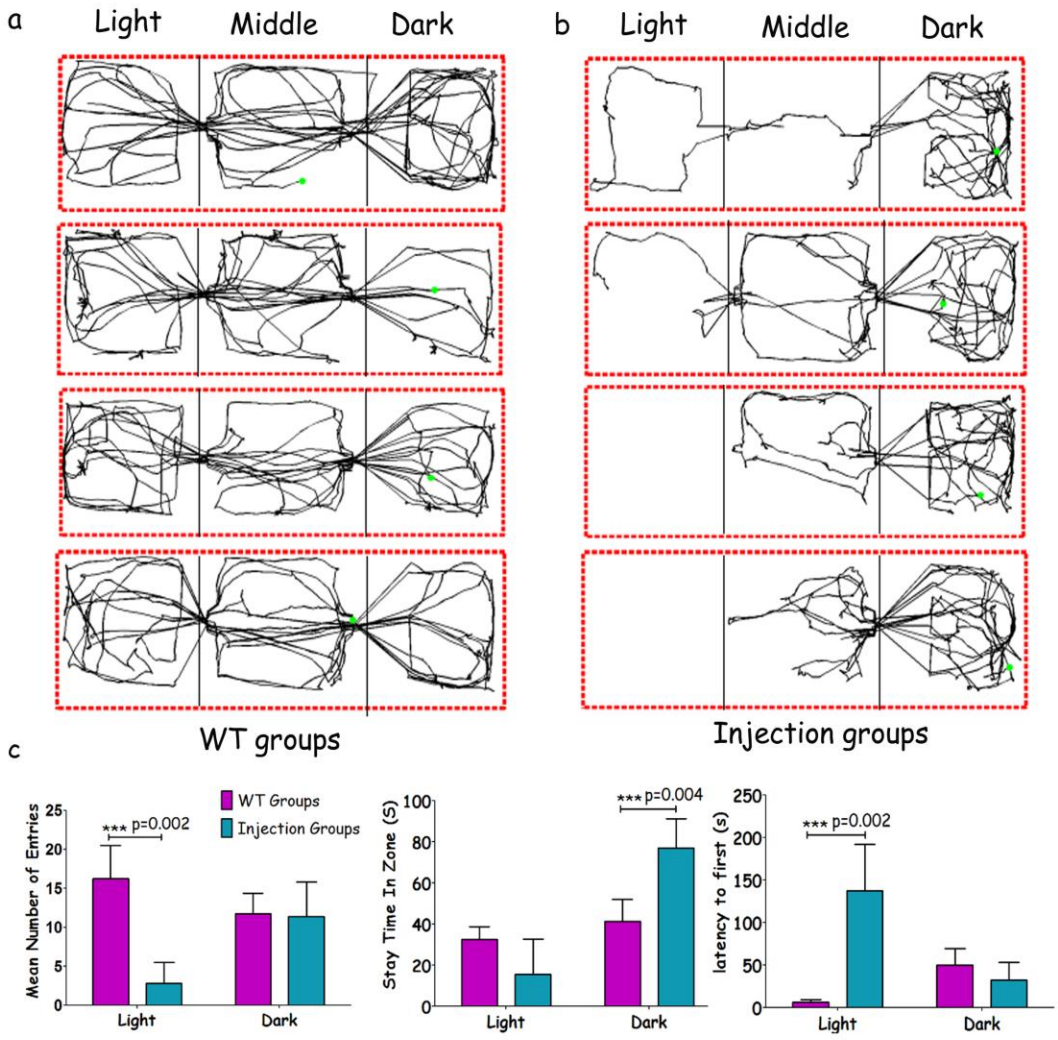

Figure S9. (a) Trajectories of each experimental groups. The results showed that the trajectory was related with dose-dependent of PEGylation CoNps. (b) Analyzing the mean number of entries times, stay time and latency to first entre in light and dark zone. The results showed there was a significant difference between WT and injection groups.

10. DCX expression of 30 days PEGylation CoNps treatment

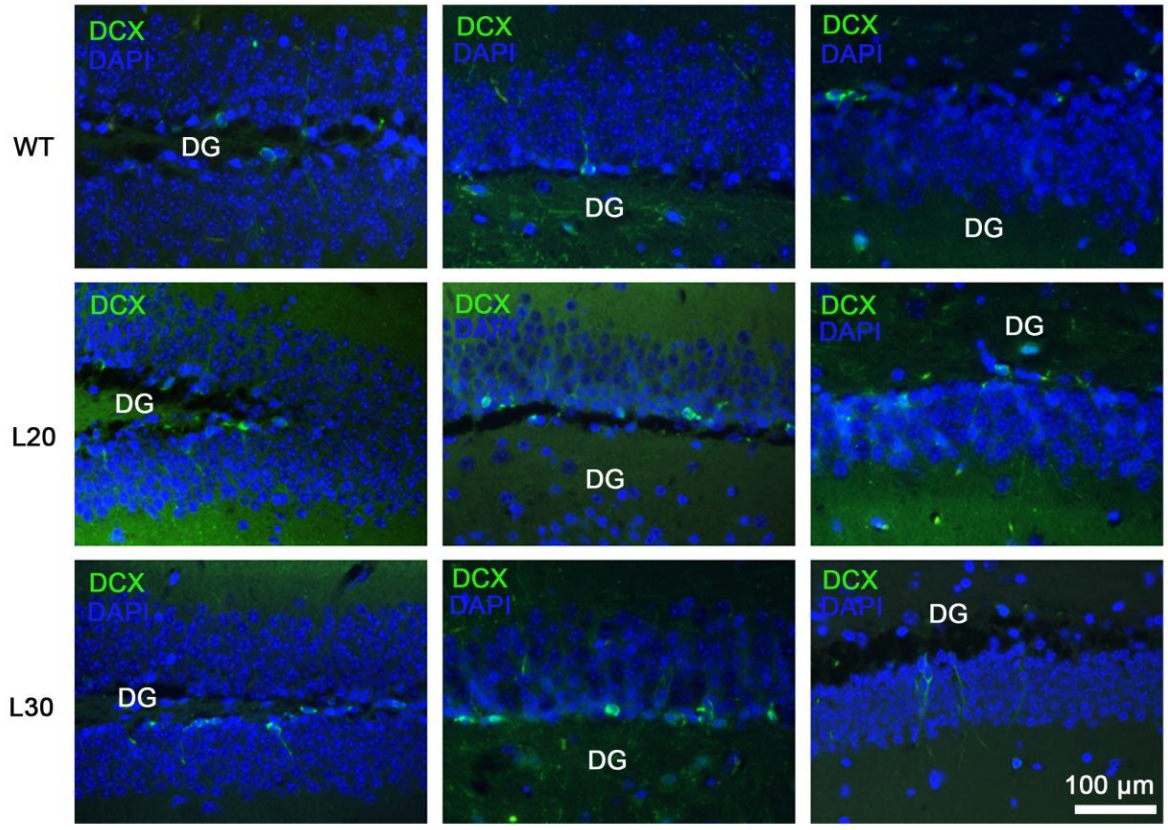

Figure S10. DCX expression of 30 days PEGylation CoNps treatment. DCX is the marker of new-born neuron that is 1 to 28 days of cells age. Therefore, DCX expression was examined at 30 days. The results showed that DCX expression was increased after PEGylation CoNps treatment for 30 days.

Supplementary reference

1. A. Weissbrod, *nature communications*, 2013.
2. W. Hong, A. Kennedy, X. P. Burgos-Artizzu, M. Zelikowsky, S. G. Navonne, P. Perona and D. J. Anderson, *Proceedings of the National Academy of Sciences*, 2015, 112, E5351-E5360.
3. H. Jhuang, E. Garrote, J. Mutch, X. Yu, V. Khilnani, T. Poggio, A. D. Steele and T. Serre, *Nature Communications*, 2011, 1, 626-626.
4. N. Matsuo, K. Tanda, K. Nakanishi, N. Yamasaki, K. Toyama, K. Takao, H. Takeshima and T. Miyakawa, *Frontiers in Behavioral Neuroscience*, 2009, 3, 3-3.
5. H. Zhi-Li, M. Takatoshi, Q. Wei-Min, H. Zong-Yuan, W. Takeshi, U. Yoshihiro and H. Osamu, *Proceedings of the National Academy of Sciences*, 2006, 103, 4687-4692.
6. A. L. Samson, L. Ju, H. A. Kim, S. R. Zhang, J. A. Lee, S. A. Sturgeon, C. G. Sobey, S. P. Jackson and S. M. Schoenwaelder, *Scientific reports*, 2015, 5.
7. M. L. Schipper, N. R. Nozomi, C. R. Davis, N. W. S. Kam, C. Pauline, L. Zhuang, S. Xiaoming, D. Hongjie and S. S. Gambhir, *Nature Nanotechnology*, 2008, 3, 216-221.
8. K. Yang, H. Gong, X. Shi, J. Wan, Y. Zhang and Z. Liu, *Biomaterials*, 2013, 34, 2787–2795.