An Improved Elevated Platform for Simultaneously Assessing Rodent Locomotor Activity and Anxiety

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Elevated plus maze (EPM) has been the golden standard for assessing rodent anxiety, which consists of two enclosed arms and two open arms elevated 100 cm above the floor. The two closed arms are closed and dark space with high edges (>30 cm). The two open arms are open space with low edges (0.5 cm). Animals prefer exploring unknown environment but will feel fearful in the open arms; thus, the psychiatric conflict between intrinsic exploring desire and fearful challenge produced by the 100 cm height reflects the rodent anxiety level [1–3]. However, the existing EPM has some limitations. Firstly, time spent in the center zone was usually neglected for the much smaller area than the four arms,

which may greatly decrease its sensitivity in assessing anxiety. Secondly, the route from the closed arm to open arm is not linear, and high walls of closed arm make the open arm invisible for animals in the closed arm, which may decrease the animal exploring activity and thus affect the sensitivity. This drawback even makes some animals stay in the closed arms during all the test duration in some experiment without entering the open arm. Thirdly, it is difficult to handle mouse/rat when test finish, which may increase animal stress and further affect the subsequent behavior test. Thus, a more sensitive method should be developed for better assessing animal anxiety.

Figure 1 The Elevated platform apparatus. **A** and **B**: The side and top views of the elevated platform apparatus. The round platform was elevated 100 cm above the floor, and the diameter is 45 cm. **C**: The platform was divided into three consecutive zones using Any-Maze tracking system, including outer zone, middle zone and center zone. There are no real boundaries between different zones. Before test, mouse was placed in the center zone and then the entries into each zone and time spent in the three zones were recorded using Any-Maze tracking system.

Figure 2 Comparison of elevated platform (EP) and EPM in assessing anxiety difference between female and male mice. **A-A'**: There is no significant difference between female and male C57/BL6J mice in spontaneous activity using OF task. **A-A''**: There is no significant difference between female and male C57/BL6J mice in spontaneous activity using EP task. **B-B'**: There was no significant difference between female and male C57/BL6J mice in time spent in the center, closed arms, and open arms of EPM. **B-B''**: There was no significant difference between female and male C57/BL6J mice in entries into the center, closed arms, and open arms of EPM, but compared with female, slightly more entries into the open arm were found in the male group mice ($P = 0.0542$, very close to statistical significance). **C-C'**: There was no significant difference of time spent in the center zone between female and male mice, but significant difference of time spent in the middle zone and the outer zone were observed ($*P < 0.05$). **C-C''**: Significantly, more entries into center zone of EP were observed ($P < 0.05$), and slightly increased entries into the middle zone of EP were found in female group mice ($P = 0.0544$, very close to statistical significance). Data are mean $\pm$ SEM.

Here, for the purpose of improving the EPM, we designed a novel equipment to assess animal anxiety based on the EPM task, which was named elevated platform (EP). Like EPM, for assessing rodent anxiety level, the EP task also utilizes the psychiatric conflict between exploring desire and fear produced by the 100 cm height. As shown in Figure 1A, similar with EPM, a round platform (diameter = 45 cm) is elevated 100 cm above the floor for producing fearful challenge, and the 0.3 cm height of edge is used to prevent animals slipping down. As shown in Figure 1B, to make the rodent explore freely on the platform, the total area of the platform (area = $\pi \times 22.5^2$) is designed to be almost same as the area of our open field task (OF, 40 $\times$ 40 cm). As Figure 1C, the platform was divided into three consecutive zones using Any-Maze tracking system, including center, middle and outer zone. Actually, there are no real boundaries among these zones, which will facilitate the rodent to explore freely on the platform. The center zone is the safest zone (which is equivalent to the closed arm of EPM), and the middle zone (which is equivalent to the center of EPM) is middle safe zone, while the outer zone (which is

equivalent to the open arm of EPM) is the most unsafe zone. According to our designed principle, the operated animals will be placed at the center zone of EP, and then, the exploring activity will be recorded using tracking video system (Any-Maze was used in this experiment). The animals with higher anxiety level will spend more time exploring in the center zone, while the animals with less anxiety level will spend more time in the outer zone. Thus, the animal anxiety level will be assessed by analyzing the time spent in each zone and the entries into each zone. Because the difference of anxiety level between female and male rodent in the EPM task was reported previously [4–6], therefore, to test this novel EP task, two group mice coming from littermates that containing seven male and 10 female C57/BL6J mice were used. The anxiety level difference between these two groups was examined by OF, EPM, and EP task, respectively. In the OF task, the animals were placed at the center of the OF, and the total travel distance of animals was recorded during 3 min to assess animal spontaneous activity [7,8]. In EPM task, the animals were placed at the center of the EPM. The entries into and the time spent in the

closed arm, open arm, and center (intersection of the four arms) of EPM were recorded during 3 min [9,10]. In the EP task, the animals were placed at the center zone. Then, the entries into and the time spent in each zone of EP were recorded during 3 min.

During the test duration, all the animals explored freely on the EP, and no animal slipped down. Statistical analysis showed that neither OF task (Figure 2A') nor EP task (Figure 2A'') found the difference of spontaneous activity between the two group mice, which indicated that the EP task can be used to assess animal locomotor activity. Further investigations showed that no significant difference of time spent in each zone of EPM was found between the two groups (Figure 2B-B'). However, EPM task showed that slightly more entries into open arm were found in male group compared with female group (Figure 2B-B''), which indicated the slightly higher anxiety level of female mice in EPM. Next, we examined the anxiety difference of the above two group mice using EP, as shown in Figure 2C-C', although no difference of time spent in the center zone between the two groups was found, but female group spent significant less time in the outer zone ($*P < 0.05$) and spent significant more time in the middle zone ($*P < 0.05$), which indicated the significantly higher anxiety level of female mice. Further analysis showed that more entries into the center zone ($*P < 0.05$) and middle zone ($P = 0.054$) were found in female group, which further indicated the higher level of female in the EP task. Thus, compared with EPM task, the

improved EP task displayed higher sensitivity in examining animal anxiety difference. Additionally, the EP task can combine OF and EPM into one to simultaneously assess rodent locomotor activity and anxiety.

In summary, we developed a novel EP task, which displayed equivalent sensitivity to OF in assessing rodent locomotor activity and improved sensitivity in assessing rodent anxiety level compared with EPM. Additionally, the EP task can combine OF and EPM into one for simultaneously assessing rodent spontaneous activity and anxiety level, which may greatly simplify experimental procedure and save much more time for researchers.

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Conflict of Interest

The authors declare no conflict of interest.

References

- Steimer T. Animal models of anxiety disorders in rats and mice: Some conceptual issues. *Dialogues Clin Neurosci* 2012;**13**:495–506.
- Haller J, Alicki M. Current animal models of anxiety, anxiety disorders, and anxiolytic drugs. *Curr Opin Psychiatry* 2012;**25**:59–64.
- Cryan JF, Sweeney FF. The age of anxiety: Role of animal models of anxiolytic action in drug discovery. *Br J Pharmacol* 2011;**164**:1129–1161.
- Johnston AL, File SE. Sex differences in animal tests of anxiety. *Physiol Behav* 1991;**49**:245–250.
- Weintraub A, Singaravelu J, Bhatnagar S. Enduring and sex-specific effects of adolescent social isolation in rats on adult stress reactivity. *Brain Res* 2010;**1343**:83–92.
- Simpson J, Ryan C, Curley A, et al. Sex differences in baseline and drug-induced behavioural responses in classical behavioural tests. *Prog Neuropsychopharmacol Biol Psychiatry* 2012;**37**:227–236.
- Alfieri JA, Pino NS, Igaz LM. Reversible behavioral phenotypes in a conditional mouse model of TDP-43 proteinopathies. *J Neurosci* 2014;**34**:15244–15259.
- Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *Eur J Pharmacol* 2003;**463**:3–33.
- Arantes R, Tejada J, Bosco GG, et al. Mathematical methods to model rodent behavior in the elevated plus-maze. *J Neurosci Methods* 2013;**220**:141–148.
- Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: A novel test of anxiety in the rat. *Pharmacol Biochem Behav* 1986;**24**:525–529.