

## Introduction

- ❖ 1 in 4 Australians suffer from an anxiety disorder or from depression. Anxiety and depression can often be comorbid (occur at the same time).<sup>1</sup>
  - ❖ Reduced brain serotonin transporter (5-HTT) protein levels have been associated clinically with sufferers of both anxiety disorders<sup>2</sup> and depression.<sup>3</sup>
  - ❖ Mice that have no brain 5-HTT protein (5-HTT<sup>-/-</sup> mice) display comorbid increases in both anxiety-like<sup>4</sup> and depressive-like behaviour.<sup>5</sup>
  - ❖ Environmental enrichment paradigms can therapeutically reduce increased anxiety-like and depression-like behaviours in mice.<sup>6</sup>
- **Study aim:** to determine the therapeutic potential of environmental enrichment to 5-HTT<sup>-/-</sup> mice.
- **Study hypothesis:** environmental enrichment will therapeutically restore the comorbid increases in 5-HTT<sup>-/-</sup> mice anxiety-like and depression-like behaviour.

## Methodology

- ❖ The presence or absence of 5-HTT protein was our first independent variable, so we obtained 5-HTT<sup>-/-</sup> and littermate control (5-HTT<sup>+/+</sup>) mice from the mouse colony we established at the Florey. The use of littermates kept all other brain proteins in 5-HTT<sup>-/-</sup> and 5-HTT<sup>+/+</sup> mice nearly identical, which was essential for our conclusions.
- ❖ The second independent variable was the housing condition (Figure 1). At adult age, equal numbers of 5-HTT<sup>-/-</sup> and 5-HTT<sup>+/+</sup> mice were assigned to live in environmental enrichment cages or to remain living in standard housing. Environmental enrichment (EE) cages were constructed by arranging numerous objects with different shapes and textures in complex configurations. The objects and configuration nature were changed weekly to supply novelty to the mice.
- ❖ There were two dependent variables, anxiety-like and depression-like behaviour, measured after 2 weeks of EE using the elevated-plus maze (EPM) and after 3 weeks of EE using the forced swim test (FST) (Figure 2). These tests were chosen because both have strong predictive validity, since drugs that reduce anxiety and depression in humans when given to mice reduce anxiety-like and depression-like behavior on the EPM and the FST respectively.



Standard Housing Env. Enrichment (EE)

Figure 1. Housing conditions

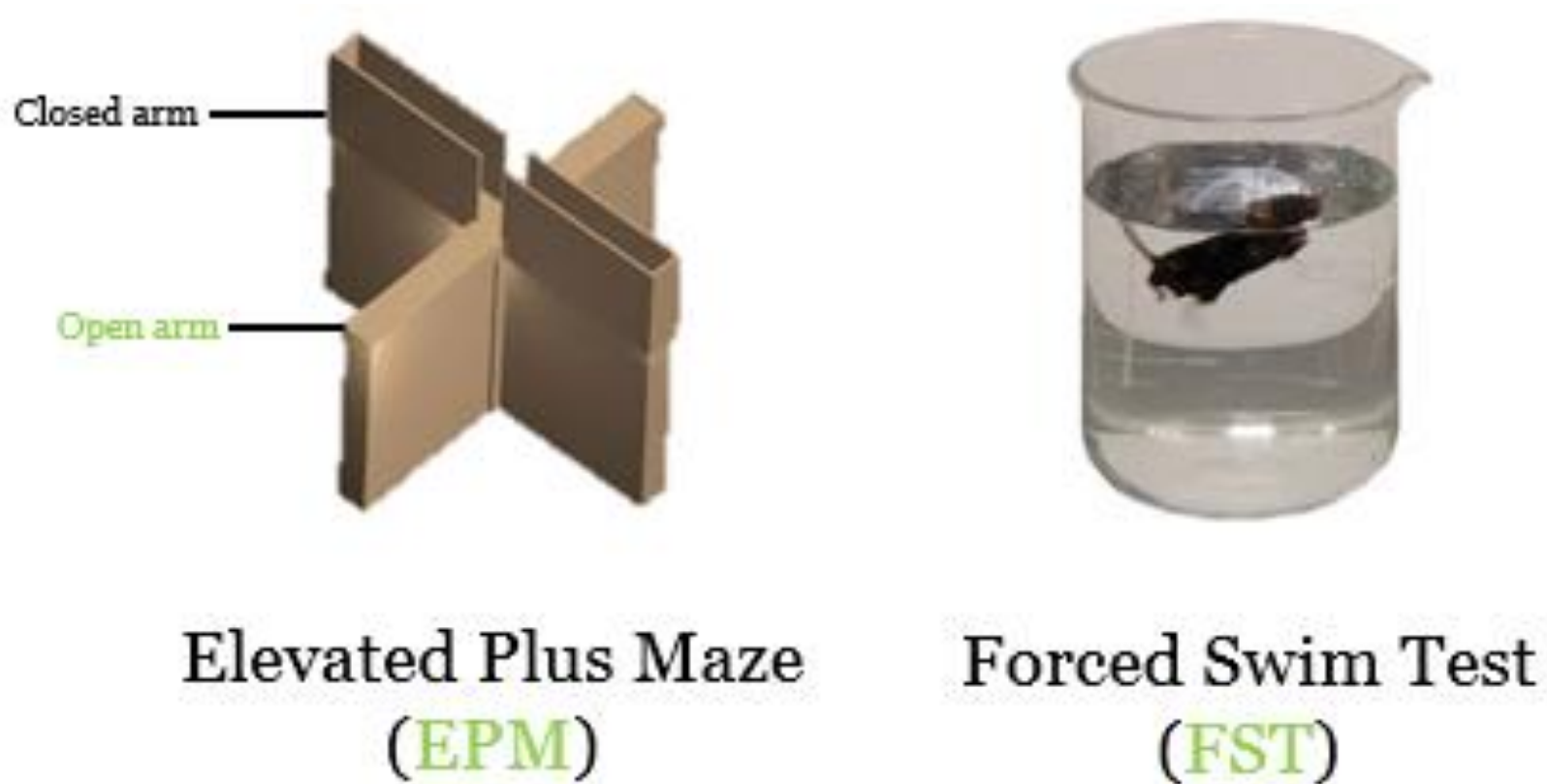


Figure 2. Behavioural testing paradigms.

## Results

- ❖ The EPM puts mice in a conflict situation between their need to be safe and their need to explore new places (Figure 2).
  - ❖ The maze is “solved” by mice spending time in the less safe open arms.
  - ❖ Mice that display anxiety-like behaviour are less able to solve the maze. Thus they spend a smaller percentage (%) of time in the open arms of the maze.
  - ❖ Therefore the main measure of anxiety-like behaviour on the EPM is % time in open arms over the 5 min test period (Figure 3).
- 5-HTT<sup>-/-</sup> mice in standard housing conditions had significantly increased anxiety-like behaviour compared to 5-HTT<sup>+/+</sup> control mice (Figure 3).
- When housed in an enriched environment, there were no significant differences in anxiety-like behaviour between 5-HTT<sup>-/-</sup> mice and 5-HTT<sup>+/+</sup> control mice (Figure 3).

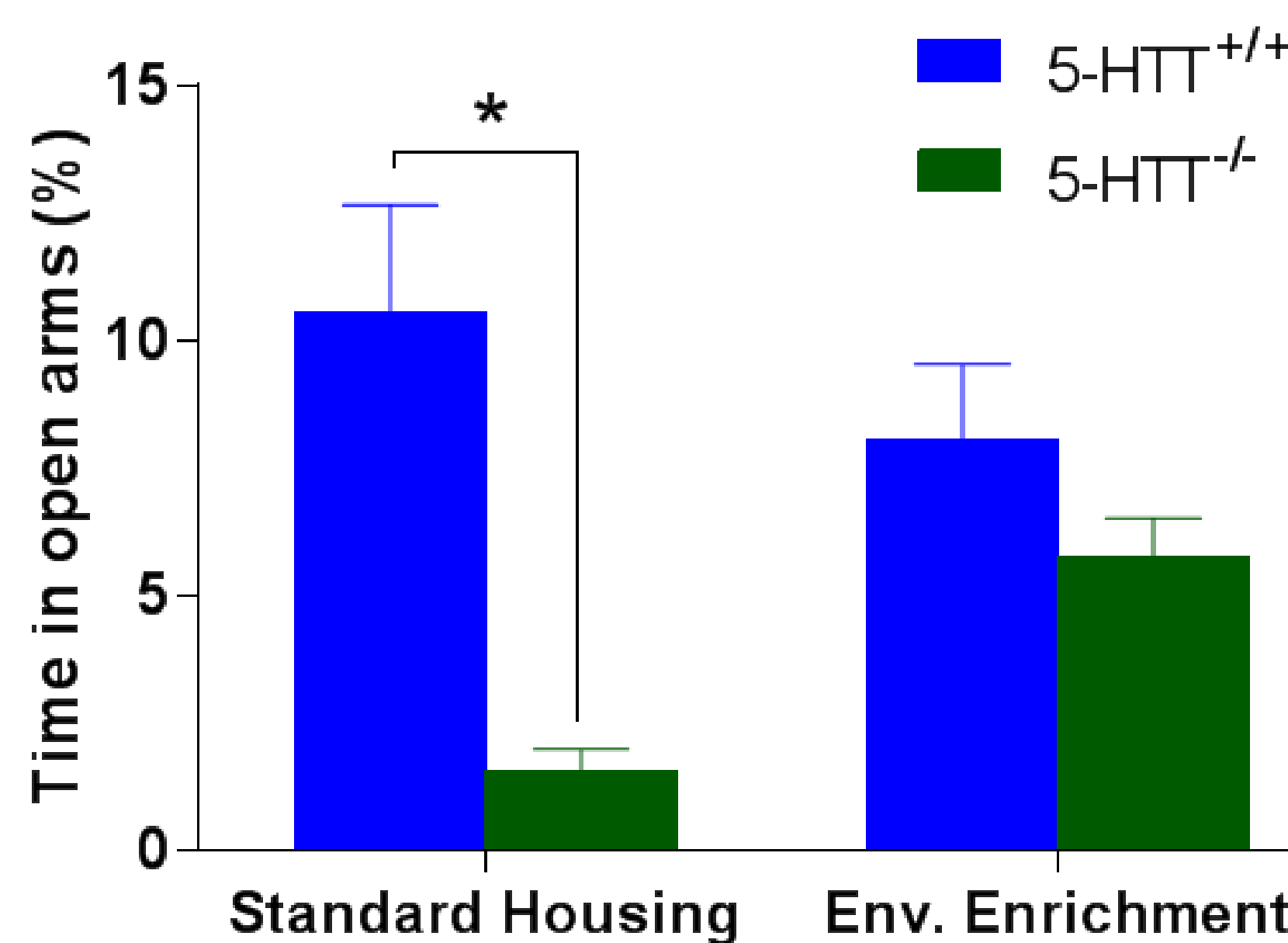


Figure 3: Environmental enrichment reduces anxiety-like behaviour in 5-HTT<sup>-/-</sup> mice. Data is expressed as mean ± SEM; n = 11-15 mice; \* p < 0.05 = statistically significant difference between 5-HTT<sup>-/-</sup> and 5-HTT<sup>+/+</sup> mice.

- ❖ Although mice can swim, it is a stressful experience they will immediately try to escape from. In the FST, mice are forced to swim when escaping is impossible (Figure 2).
  - ❖ Mice that stop attempting to escape become completely immobile and float.
  - ❖ Mice that display depression-like behaviour spend less time attempting to escape. Thus, they spend a higher percentage (%) of their time immobile during the FST.
  - ❖ Therefore the main measure of depression-like behaviour on the FST is % time immobile over the 6 min test period (Figure 4).
- 5-HTT<sup>-/-</sup> mice in standard housing conditions had significantly increased depression-like behaviour compared to 5-HTT<sup>+/+</sup> control mice (Figure 4).
- When housed in an enriched environment, 5-HTT<sup>-/-</sup> mice still had significantly increased depression-like behaviour compared to 5-HTT<sup>+/+</sup> control mice (Figure 4).

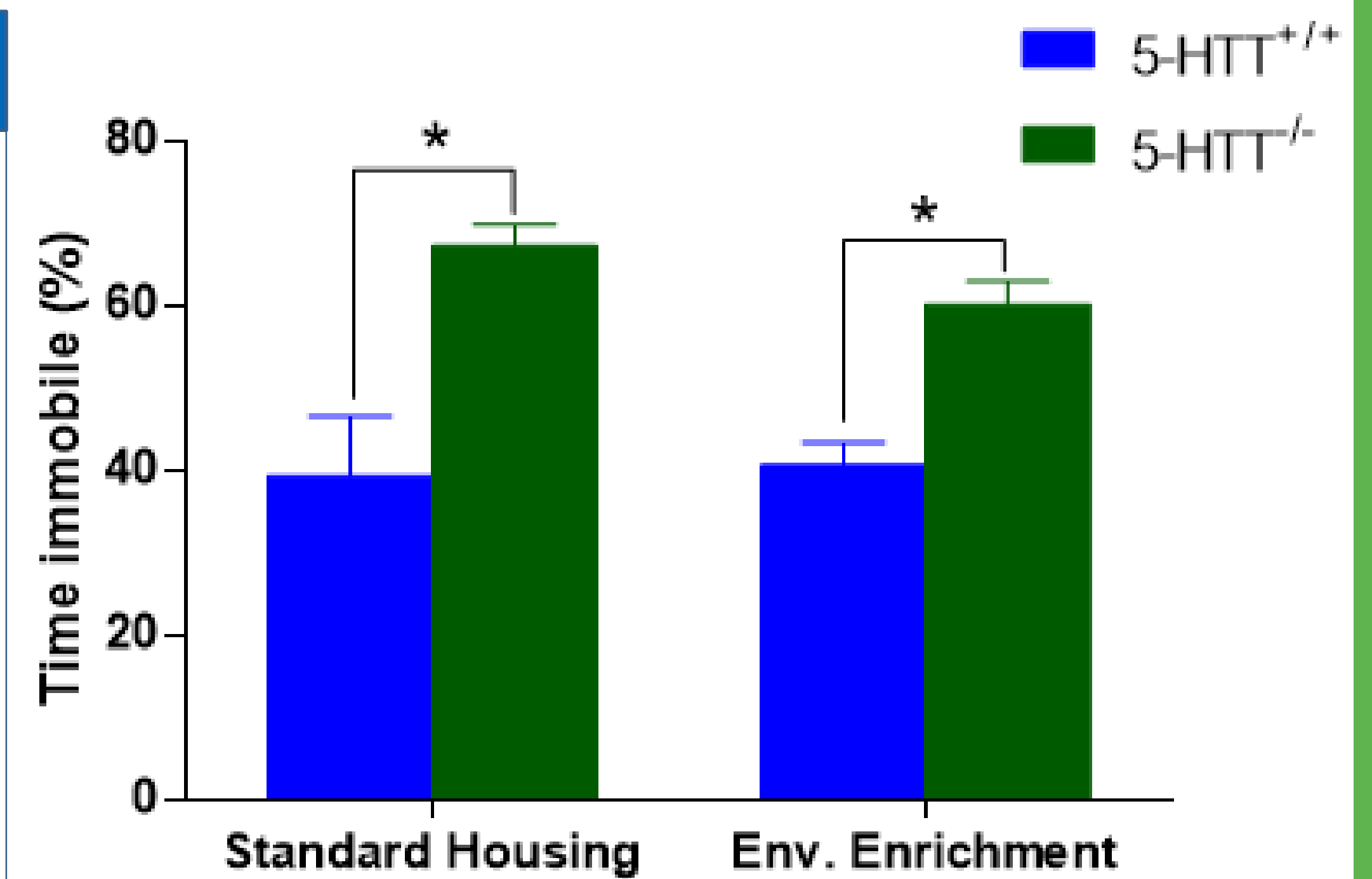


Figure 4: Environmental enrichment does not alter depression-like behaviour in 5-HTT<sup>-/-</sup> mice. Data is expressed as mean ± SEM; n = 5-13 mice; \* p < 0.05 = statistically significant difference between 5-HTT<sup>-/-</sup> and 5-HTT<sup>+/+</sup> mice.

## Discussion

- ❖ 5-HTT<sup>+/+</sup> mice were our control mice. As expected, during the EPM those mice strongly preferred the closed arms, spending at maximum 10% of their time in the exposed open arms. In agreement with previous studies using the FST, control mice spent less than half of their time immobile. Thus we are confident that our experiments measured the behaviour we intended to measure.
- ❖ Standard housed 5-HTT<sup>-/-</sup> mice spent nearly 1/5<sup>th</sup> the time in open arms and spent nearly double the time immobile. This is also in agreement with previous studies using 5-HTT<sup>-/-</sup> mice.<sup>4,5</sup>
- ❖ EE restores the anxiety of the 5-HTT<sup>-/-</sup> mice but unexpectedly did not alter the anxiety of 5-HTT<sup>+/+</sup> mice as we recently published.<sup>7</sup>
- We identified no outliers in this study as the standard error of the mean (SEM) for the four bar plots in both figures is consistently narrow. This is due to the high number of mice in each group (n > 10). In the FST experiment, the low n (5) is restricted to the standard housing 5-HTT<sup>-/-</sup> mice. 5-HTT<sup>+/+</sup> mice and all mice housed in enriched environments had n values > 10.
- ❖ A limitation in the methodology is the reliance on one test for both anxiety- and depression-like behaviour. Additional behavioural tests measuring other aspects of anxiety (place avoidance) or depression (anhedonia) would further strengthen the validity of our conclusions.
- As EE was therapeutic for one behaviour but not the other, our results provide evidence that comorbid anxiety and depression require distinct therapeutic interventions.
- In turn, our results also suggest dissociable origins of comorbid anxiety and depression in the brain.

## Conclusions

- We aimed to determine the therapeutic potential of environmental enrichment to 5-HTT<sup>-/-</sup> mice and found that it reduces their anxiety-like behaviour but does not alter their depression-like behaviour.
- We hypothesized that environmental enrichment would therapeutically reduce both behaviours in 5-HTT<sup>-/-</sup> mice. Instead, we provide preclinical evidence of a therapeutic potential for reducing anxiety but not ameliorating depression symptoms. This has implications for the current use of non-drug based clinical interventions (such as cognitive behavioural therapy) for patients comorbid for both these disorders.

## References

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## Acknowledgements

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