

## **Role of environment and experimenter in reproducibility of behavioral studies with laboratory mice**

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Behavioral phenotyping of mice has received a great deal of attention during the past three decades. However, there is still a pressing need to understand the variability caused by environmental and biological factors, human interference, and poorly standardized experimental protocols. The inconsistency of results is often attributed to the inter-individual difference between the experimenters and environmental conditions. The present work aims to dissect the combined influence of the experimenter and the environment on the detection of behavioral traits in two inbred strains most commonly used in behavioral genetics due to their contrasting phenotypes, the C57BL/6J and DBA/2J mice. To this purpose, the elevated O-maze, the open field with object, the accelerating rotarod and the Barnes maze tests were performed by two experimenters in two diverse laboratory environments. Our findings confirm the well-characterized behavioral differences between these strains in exploratory behavior, motor performance, learning and memory. Moreover, the results demonstrate how the experimenter and the environment influence the behavioral tests with a variable-dependent effect, often with mutually exclusive contributions. In this context, our study highlights how both the experimenter and the environment can have an impact on the strain effect size without altering the direction of the conclusions. Importantly, the general agreement on the results is reached by converging evidence from multiple measures addressing the same trait. In conclusion, the present work elucidates the contribution of both the experimenter and the laboratory environment in the intricate field of reproducibility in mouse behavioral phenotyping.