Home Cage-Based Long-Term Monitoring of Fear in Mice: Novel Approach to Determine Individual Differences in Risk Assessment and Avoidance

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Introduction

Behavioral phenotyping is indispensible for biomedical research. Fear conditioning has been one of the most successful behavior assays in behavioral neuroscience, to investigate the neural systems and molecular basis of various aspects of emotional learning across a wide range of species [1]. Dysfunction of the fear circuits is assumed to underlie mechanisms of affective disorders and is frequently investigated in rodent models. However, many currently used behavior assays, including classical fear learning tests are geared for higher throughput. Thus, they have short test duration and require frequent human interference, inducing considerable data variation, interpretational ambiguity and limited translational value. Except for few studies employing novel approaches [e.g. 2], there is a general lack of progress in behavioral neuroscience for measuring and interpreting behavioral responses of laboratory animals particularly under ethologically valid conditions [3].

Material and Methods

In view of the above-mentioned limitations, we developed a fully automated home cage system (DualCage; see *HomeCage*[Plus], Biobserve, St. Augustin, Germany) consisting of a safe home compartment (HC) coupled to a risk-prone test compartment (TC) separated by a controllable door as in passive avoidance experiments [4]. This approach allowed the investigation of conditioned contextual fear responses and concomitant behavioral changes in mice from baseline behavior including circadian activity, via fear acquisition, consolidation with post-shock activity assessment, and retention to extinction determined by TC re-exploration. Behavioral responses were based on deliberate choice, motivated exclusively by novelty-seeking and were monitored over several days without human intervention. This approach may help to avoid the negative consequences of unspecific stressors on behavioral responses. A separation between HC and TC is necessary for exploiting novelty-seeking behavior and decision-making at distinct times. The resolution of the system is demonstrated by comparing the performance of male mice of the two closely related substrains C57BL/6J (6J) and C57BL/6N (6N). Compared to 6J mice, 6N mice show stronger fear responses in fear conditioning followed by delayed extinction [e.g. 5], which was reconfirmed by us in classical fear conditioning. When a mouse had fully entered the TC, the door was closed for inescapable shock exposure. Mice of different groups were subjected to various levels of negative reinforcement based on the number (0, 1, 3 or 5) of 2-s foot shocks (0.7 mA, scrambled) presented 30 s after their first TC visit to determine the

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impact of reinforcement levels on retention performance. After the training session, the door was opened and mice returned to their HC. Different levels of reinforcement were used because classical fear conditioning studies based on freezing reported an increase of fear suggesting a simple form of learning, whereas complex forms of learning show an impairment with increasing arousal ("stress") as indicated by the Yerkes-Dodson law [reviewed in 6]. Since passive avoidance and contextual fear conditioning are both hippocampus-dependent learning tasks [4,7], the lack of fear learning impairment in classical fear conditioning by higher levels of reinforcement is surprising [6]. Twenty-four hours after training, contextual fear memory and its extinction were assessed for 48 h based on deliberate TC revisits and re-exploration. This included the analysis of the body posture of mice based on 3-point tracking to assess stretch-attend postures as an unambiguous sign of risk assessment in the face of threat [8]. The study was approved by the animal research committee of the VU University Amsterdam and conducted according to Dutch regulations in compliance with the European Council Directive (86/609/EEC).

Results and Discussion

Comparison of circadian activity between the two mouse substrains identifies a significant difference in locomotor activity that went unnoticed in classical tests [e.g., 5]. 6N mice showed an increased latency to re-enter the TC suggesting increased fear compared to 6J mice. These long retention transfer latencies (several hours) indicate long-lasting fear response, and thus emphasize the importance of extended time scales for behavioral monitoring. TC visits were predominantly confined to the dark phase. Considerable inter-individual performance variation within both isogenic substrains suggests epigenetic contributions to individual differences and highlight different, i.e. active or passive, coping styles [9]. Sub-populations of mice that never re-entered the TC constitute a clear model of post-traumatic stress disorder. In contrast to classical fear conditioning and passive avoidance results, there was no difference in fear extinction in 6J versus 6N mice that re-entered the TC. This suggests that unspecific arousal ("stress") in classical behavior tests impairs extinction learning in 6N mice, since extinction of conditioned heart rate responses in the home cage under conditions of reduced unspecific "stress" also failed to show differences between the two substrains [5].

Conclusions

This novel approach improves ethological and face validity while reducing interpretational ambiguity of emotional states in models of fear learning, memory and anxiety-related disorders. This is accomplished by exploiting automated behavioral measures of high translational value such as risk-assessment and avoidance - core endophenotypes of affective disorders. This novel approach is thus a highly valuable asset for extending and rectifying interpretations based on classical fear behavior tests. Our approach is currently being extended to other mouse strains including mutants and pharmacological intervention studies, for further validation.

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References

1. LeDoux, J.E. (2000). Emotion circuits in the brain. *Annual Reviews of Neuroscience* **23**, 155-184.

2. Fonio, E., Benjamini, Y., Golani, I. (2009). Freedom of movement and the stability of its unfolding in free exploration of mice. *Proceeding of the National Academy of Sciences USA* **106**, 21335-21340.

3. Spruijt, B.M., Peters, S.M., de Heer, R.C., Pothuizen, H.H.J., van der Harst, J.E. (2014). Reproducibility and relevance of future behavioral sciences should benefit from a cross fertilization of past recommendations and today's technology: "Back to the future". *Journal of Neuroscience Methods* (available online at http://dx.doi.org/10.1016/j.jneumeth.2014.03.001).

4. Baarendse, P.J.J., van Grootheest, G., Jansen, R.F., Pieneman, A.W., Ögren, S.O., Verhage, M., Stiedl, O. (2008). Differential involvement of the dorsal hippocampus in passive avoidance in C57BL/6J and DBA/2J mice. *Hippocampus* **18**, 11-19.

5. Stiedl, O., Radulovic, J., Lohmann, R., Palve, M., Birkenfeld, K., Kammermeier, J., Sananbenesi, F., Spiess, J. (1999). Strain and substrain differences in context- and tone-dependent fear conditioning. *Behavioural Brain Research* **104**, 1-12.

6. Diamond, D.M., Campbell, A.M., Park, C.R., Halonen, J., Zoladz, P.R. (2007). The temporal dynamics model of emotional memory processing: a synthesis on the neurobiological basis of stress-induced amnesia, flashbulb and traumatic memories, and the Yerkes-Dodson law. *Neural Plasticity* **2007**, 60803.

7. Stiedl, O., Birkenfeld, K., Palve, M., Spiess, J. (2000). Impairment of conditioned context- but not tone-dependent fear of C57BL/6J mice by the NMDA receptor antagonist APV. *Behavioural Brain Research* **116**, 157-168.

8. Yang, M., Augustsson, H., Markham, C.M., Hubbard, D.T., Webster, D., Wall, P.M., Blanchard, R.J., Blanchard, D.C. (2004). The rat exposure test: a model of mouse defensive behaviors. *Physiology & Behavior* **81**, 465-473.

9. Koolhaas, J.M., de Boer, S.F., Buwalda, B., van Reen, K. (2007). Individual variation in coping with stress: a multidimensional approach of ultimate and proximate mechanisms. *Brain, Behavior & Evolution* **70**, 218-226.