European Neuropsychopharmacology 96 (2025) 15–16

Contents lists available at ScienceDirect

European Neuropsychopharmacology

journal homepage: www.sciencedirect.com/journal/european-neuropsychopharmacology

Attention to the quality of methods is critical for valuable research: some examples relevant for animal models of psychiatric disorders

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The quality of scientific research is based on at least three main premises: relevant hypotheses, appropriate experimental designs, and rigorous methodology. Unfortunately, the full description of the methodology is declining in the main body of manuscripts, particularly those from high-impact journals. Although this problem can apparently be solved with a detailed methods description in supplementary materials, not all readers download and read those materials. In addition, relevant details are lacking even in those additional materials. I would like to emphasize it, using a few examples that concern animal models in psychiatry.

Exposure to stress is known to play a major role in pathological processes in animals and humans, and considerable attention has been paid to the involvement of the hypothalamus-pituitary-adrenal (HPA) axis. This axis is primarily regulated by the paraventricular nucleus of the hypothalamus (PVN) and the release of the corticotropin-releasing factor/hormone (CRF or CRH). This neuropeptide stimulates the synthesis and secretion of ACTH, which, in turn, controls the secretion of glucocorticoids – cortisol in most mammals, corticosterone in rats and mice – by the adrenal cortex. Even considering this very simple framework of the HPA axis, interpretating results from the literature is often problematic for several reasons. First, only a minority of papers report plasma levels of ACTH in addition to corticosterone or cortisol levels. This omission becomes a major problem when analyzing responses to relatively severe stressors, particularly in laboratory animals. The maximum adrenal capability to secrete corticosterone is achieved with levels of ACTH that are half of those achieved after severe stressors, meaning that corticosterone levels no longer accurately reflect actual ACTH release (see Armario et al., 2020). In addition, there are other critical factors further complicating interpretation. Values of corticosterone using appropriate techniques (classically radioimmunoassays) should be around 10–20 ng/ml in the first light on hours and 100–150 ng/ml at lights off in male rats, with females displaying considerably higher levels (Spencer and Deak, 2017; Armario et al., 2023). However, most published papers report significantly higher basal values, often exceeding 50 ng/ml at lights on in males. This is mainly due to all the procedures associated with blood sampling, including transporting the cages and handling the animals (Armario et al., 2023). This is conceptually critical because we erroneously interpret them as reflecting basal levels to establish parallelism with human data. For instance, depression in humans is considered to be associated with hypercortisolism. Therefore, the induction of depression-like behaviors in animals after exposure to chronic stress, a putative animal model of depression, should be paralleled by elevated basal levels of corticosterone. However, exposure to chronic stress can sensitize the response of the HPA axis to mild stressors (Belda et al. 2015), and thus the differences between controls and chronically stressed animals might reflect a differential response to mild stressors rather than actual alterations of basal corticosterone. Moreover, in the last decades, classical radioimmunoassay methods have been replaced for commercial enzyme-like immunoassays (ELISA) kits for measuring hormone levels. However, in our experience, some of these kits do not provide reliable results, and this can lead to false or aberrant measurements. It should be mandatory for any commercial assay kit to be previously validated. This includes demonstrating parallelism between serial plasma dilutions and the standard curve, achieving a high recovery (around 100 %) of the amount of standards added to the samples and confirming that measured values fall within the expected biological range, using quality papers as a reference. If the kit eventually works, it is not much more expensive to have done these validations before assaying the relevant samples. If the kit does not work, we will preserve the samples and avoid publishing erroneous data. It is easy to see how far we are from these premises when reading published reports.

To demonstrate behavioral alterations in animals reminiscent of those observed in psychiatric disorders, researchers rely on tests that

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https://doi.org/10.1016/j.euroneuro.2025.04.001
Received 11 March 2025; Received in revised form 18 April 2025; Accepted 23 April 2025
Available online 22 May 2025

presumably measure for instance anxiety, depression, attention, or cognitive processes (Gentcturk and Unal, 2024). The extent to which the behaviors evaluated parallel those observed in psychiatric patients is an extremely relevant issue that is beyond the scope of the present comments (Ennaceur and Chazot, 2016; Stupart et al., 2023). Instead, I will focus on how insufficient description of methods can compromise the interpretation of behavioral results. It is of note that even if the animals are habituated to handling and other laboratory routines, they can be aroused and/or stressed by the procedures, which can affect animals behavior during testing. Importantly, if the experimental groups differ in the reactivity to these procedures (more sensitive or more resilient), the tests would evaluate state rather than stable trait characteristics. Thus, to interpret the results appropriately, we need to know some relevant methodological details.

First, when animals are housed in groups, it is almost impossible to find information about how the different animals of a cage are processed. Second, behavioral tests are usually conducted outside the animal housing room. Therefore, we need to move all the cages to the experimental room for some time before testing or to move each cage/animal individually from the housing to the testing room. Yet, most studies do not describe how long the animals wait before testing, or how they are maintained while waiting to be tested. Moreover, it is difficult to know how sudden noise associated with opening the cages, handling the animals and other routines can affect performance. Presumably, some tests such as the open-field and the elevated plus maze are more sensitive to these perturbations than others (e.g., forced swim) and the effects might be strain-dependent (e.g., Rodgers and Cole, 1993;Izidio et al., 2005). Third, in some papers, fortunately a minority, animals are exposed to various tests on the same day and the interval between tests is not indicated. Previous exposure to one test could affect performance in the next (e.g., Darwish et al., 2001), hence complicating the interpretation of the results. While evaluating the same animals in different paradigms is essential for enhancing the validity of animal models of psychiatric disorders or to test the neuropsychopharmacological profile of drugs, they should not be conducted consecutively on the same day (Erkizia-Santamaría et al., 2025). A relevant example is the chronic unpredictable stress (CUS) model, the most widely used animal model of depression, originally described by Katz et al. (1981). The most relevant behavioral characteristic of this model at the time was the lack of response of chronically stressed animals to the stimulating effect (in terms of activity in a novel environment) of prior exposure to low-intensity acute stress. The differences were not evident in the absence of this prior exposure to acute stress. The contribution of uncontrolled factors in animal facilities has been suggested to impair the reproducibility of the CUS model (Markov and Novosadova, 2022)

The improvement of animal models of vulnerability to psychiatric disorders or the refinement of the behavioral paradigms to assess relevant phenotypes is a challenging task (Harro et al., 2023). However, we can easily contribute to a better understanding of experimental results and their meaning by reporting methodological details more thoroughly. Thus, I encourage referees to always demand detailed information about these methodological aspects when evaluating a manuscript. These criticisms and refinements would not only help authors to find more appropriate analytical techniques and experimental procedures in their future research, but also greatly contribute to the quality of the manuscripts and the validity of the results regarding animal models of psychiatric disorders.

Declaration of competing interest

The author declarse no competing financial interest or potential conflict of interest.

References

Armario, A., Belda, X., Gagliano, H., Fuentes, S., Molina, P., Serrano, S., Nadal, R., 2023. Differential hypothalamic-pituitary-adrenal response to stress among rat strains: methodological considerations and relevance for neuropsychiatric research. Curr. Neuropharmacol. 21, 1906–1923.
Armario, A., Labad, J., Nadal, R., 2020. Focusing attention on biological markers of acute stressor intensity: empirical evidence and limitations. Neurosci. Biobehav. Rev. 111, 95–103.
Belda, X., Fuentes, S., Daviu, N., Nada, l.R., Armario, A., 2015. Stress-induced sensitization: the hypothalamic-pituitary-adrenal axis and beyond. Stress. 18, 269–279.
Darwish, M., Korányi, L., Nyakas, C., Almeida, O.F., 2001. Exposure to a novel stimulus reduces anxiety level in adult and aging rats. Physiol. Behav. 72, 403–407.
Ennaceur, A., Chazot, P.L., 2016. Preclinical animal anxiety research - flaws and prejudices. Pharmacol. Res. Perspect. 4, e00223.
Erkizia-Santamaria, I., Meana, J.J., Ortega, J.E., 2025. Preclinical drug development: seeking to improve the reproducibility of results from behavioural experiments in animals. Eur. Neuropsychopharmacol. 95, 2–3.
Gencturk, S., Unal, G., 2024. Rodent tests of depression and anxiety: construct validity and translational relevance. Cogn. Affect. Behav. Neurosci. 24, 191–224.
Harro, J., 2023. Psychiatric vulnerability in animal models. Eur. Neuropsychopharmacol. 66, 28–29.
Izídio, G.S., Lopes, D.M., Spricigo Jr, L., Ramos, A., 2005. Common variations in the pretest environment influence genotypic comparisons in models of anxiety. Genes. Brain Behav. 4, 412–419.
Katz, R.J., Roth, K.A., Carroll, B.J., 1981. Acute and chronic stress effects on open field activity in the rat: implications for a model of depression. Neurosci. Biobehav. Rev. 5, 247–251.
Markov, D.D., Novosadova, E.V., 2022. Chronic unpredictable mild stress model of depression: possible sources of poor reproducibility and latent variables. Biology (Basel) 11, 1621.
Rodgers, R.J., Cole, J.C., 1993. Influence of social isolation, gender, strain, and prior novelty on plus-maze behaviour in mice. Physiol. Behav. 54, 729–736.
Spencer, R.L., Deak, T., 2017. A users guide to HPA axis research. Physiol. Behav. 178, 43–65.
Stupart, O., Robbins, T.W., Dalley, J.W., 2023. The wrong tools for the right job: a critical meta-analysis of traditional tests to assess behavioural impacts of maternal separation. Psychopharmacology (Berl) 240, 2239–2256.