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Innovation reports

StressMatic: Bridging innovation and reliability in animal models of stress

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ABSTRACT

Preclinical research involving animal models of stress exposure typically rely on traditional manual protocols, which are laborious and time-consuming and may compromise reproducibility and the effective translation of findings into clinical applications. StressMatic is an automated stress exposure system (auCMS), designed to improve the standardization and reproducibility of stress-induction methodologies. The auCMS demonstrated consistent efficacy, with animals subjected to automated stressors displaying similar responses to those exposed to conventional manual methods, thus confirming its validity as a reliable tool. While some stressors still require human involvement, the automation of key processes has markedly enhanced efficiency and minimized operational time. This innovative approach reduces the introduction of human error, increases precision, and standardizes experimental workflows, resulting in a more robust preclinical research platform. By streamlining repetitive tasks, the auCMS promotes adaptability in experimental design, particularly in the study of mood disorders. Ultimately, this automated protocol not only enhances the reliability of pharmaceutical screening processes but also strengthens the drug discovery pipeline, facilitating deeper insights into behavioral outcomes and informing therapeutic strategies.

1. Introduction

Technological evolution is inevitable and requires dynamic approaches, innovation and strategy, with the aim of vastly impacting organizational processes.

Preclinical research studies are mostly limited to academic centers where many experimental procedures remain heavily reliant upon the individual researcher manually carrying out protocols at the research bench. However, manual processes have a lot of limitations, in terms of reproducibility, costs and time expenditure.

Digital transformation has revolutionized manual processes, as automation has allowed an increase in production capacity and the replacement of tasks that required a dedicated operator. Processes automation can introduce reproducibility improvements, augment researcher efficiency, enhance data accuracy and reduce downstream costs. However, high-tech robotic solutions might represent a huge financial effort for academia, thus, the cost-effectiveness of these novel solutions must be taken into consideration when thinking about implementing automation in laboratory research. As such, automation suitable for preclinical research labs requires more flexible, modular and cheaper strategies, by combining engineering and biological expertise together to design innovative prototypes and solutions.

In Neuroscience research, specifically in the context of Major Depression Disorder (MDD), several preclinical models of disease have been implemented to understand the potential of novel pharmaceutical antidepressant compounds. The generation of valid and effective animal models of depression has been a challenging task for many researchers in the field. Robust models should comply with specific validation criteria: (a) face validity, whether the model mimics the core symptoms of the disease, (b) construct validity, which considers the theoretical rational behind the features observed in the clinics, and (c) predictive validity, which evaluates its correlation with treatment efficiency in a clinical

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setting (Gururajan et al., 2019; Hao et al., 2019).

Animal models of stress are commonly used to mimic MDD and validate novel therapeutic targets for future clinical trials (Planchez et al., 2019; Kolasa and Faron-Górecka, 2023). These are the most reliable models because they allow to evaluate the effects of a drug on an organism, as an integrated system while meeting those three validation criteria. Furthermore, the unpredictable chronic mild stress (uCMS) model serves as a useful tool for antidepressant drug screening, supporting the investigation of drug mechanisms, treatment resistance and the underlying biology of the disease (Nollet, 2021). It also triggers cellular and molecular changes that are important for understanding the neurobiological aspects of MDD, thus representing the idyllic model (Patrício et al., 2015; Logan et al., 2015).

To induce depressive-like symptoms in rodents, researchers commonly implement the uCMS protocol exposure, where animals are subjected to a series of mild stressors over an extended period, typically lasting 4–8 weeks, in an unpredictable sequence. These stressors involve changes in light/dark cycles, in living conditions, and in feeding or drinking routines. The intensity or duration of stress exposure may be gradually increased during the protocol.

Although the uCMS model has shown its strong translational potential, it has faced criticism for being difficult to reproduce (Argyropoulos and Nutt, 1997). Studies highlight challenges in replicating the protocol specific conditions, with variations in methods, animal facilities environment, and individual animal susceptibility (Markov and Novosadova, 2022). Additionally, the implementation of this model is highly intensive and time-demanding for the operator.

To address these limitations and reproducibility challenges, the project herein presented (StressMatic) had the objective of designing and validating an automated protocol for uCMS exposure (auCMS) in rodents, grounded in the original (manual) uCMS protocol.

2. Project description

2.1. Development and validation of an automated prototype

In a collaboration between the company Bn'ML – Behavioral & Molecular Lab and academic partners, a multidisciplinary team composed by an industrial designer, a mechanical engineer, an electrotechnical and computer engineer, as well as neuroscience researchers joined forces to the ideation and concretization (including construction, optimization and implementation) of the new automated prototype.

2.2. Manual uCMS protocol

Paul Willner's uCMS protocol (Willner, 2016a, 2016b), the most well-characterized and validated rodent model of depression, includes a wide range of stressors such as confinement in a space that is smaller than usual, tilted cage (≈45°), damp bed, overnight illumination, food deprivation followed by inaccessible food, so that animals can smell but cannot reach the food, water deprivation followed by exposure to an empty bottle, reversed light/dark cycle, exposure to strobe lights, overcrowding by placing more animals than usual in a cage, cage switch, and startle noise. For 6 weeks, rodents are randomly and continuously exposed to these mild stressors.

The protocol typically involves 4 stressors during the day and one stressor at night.

A key factor for the successful implementation of the uCMS protocol resides in its unpredictability, together with the conjugation of different stressors. Two simultaneous stressors are used both during the day and at night, including water deprivation with overnight illumination, food deprivation with tilted cage, tilted cage with reversed light/dark cycle, food deprivation with wet bed, and water deprivation with strobe lights.

Importantly, when uCMS protocol is performed, sex and aged-matched controls are handled by the experimenters every week throughout the 6-week protocol to promote habituation.

This manual process of changing stressor every 2–4 hours during the day, every day during the 6-week protocol is very time-consuming and prone to human error, and requires highly trained, specialized and qualified investigators.

2.3. auCMS prototype development

The novel equipment was designed to automate the uCMS (auCMS), allowing all cages to undergo the stressors simultaneously and almost without human intervention. The equipment structure was modeled based on a traditional cage rack, but with an automated version connected to a computer to allow the upload of scheduled protocols, including specific stressors and light settings (Fig. 1). The system is designed to hold 24 cages (2 animals per cage), guaranteeing the manipulation of 48 animals at once.

2.3.1. auCMS protocol optimization

The stress protocol is uploaded to the computer that is connected to the automated rack and can be run for a specified period (6 weeks in the case of uCMS). The stressors can be categorized as totally automated (TA), partially automated (PA) and non-automated (NA), as detailed in Table 1. TA are uniquely performed in an automated manner, without the intervention of the operator. PA stressors were optimized to represent a more user-friendly version than the one employed in the manual method. NA stressors are those that depend exclusively on the intervention of the experimenter and were kept as defined in the manual protocol.

The optimization of the automated rack was a gradual process and progressed in a dynamic manner. The current status of each stressor is described as below:

Totally automated stressors (TA):

- Tilted Cage
  In the original protocol (uCMS), cages are usually placed on a grid on the floor or on a table to allow tilting. The automated equipment includes a motor system for applying stressors such as the tilted-cage setup (Fig. 2, a. and b.). The angle was pre-programmed to 45°.
- Damp Bedding
  In the manual protocol (uCMS), water is added to cages individually. In the automated rack, however, water is supplied evenly and simultaneously to all cages through a system of pipes connected to the cage lids (Fig. 2, c. and d.), at a programmable rate. Optimizing this system required finding the right tube diameter and material. A 16 mm low-density polyethylene tube was chosen for equal water distribution and safety. Clamps were used to prevent leaks. The system also supplies air via the same tubes, with the aim of drying the tubes after water use, preventing the growth of bacteria and fungi. The final structure divides the system into two sections (1st/2nd and 3rd/4th lines) to minimize water volume errors. Mini taps near the tube outlets allow water flow to be adjusted (Fig. 2, c. and d.). Before the beginning of a protocol, the system is pre-tested, and the mini taps adjusted to ensure equal water volumes, which requires priming the tubes.
- Light-dependent stressors
  Several stressors, including overnight illumination, inverted light/dark cycle, exposure to strobe lights, are light dependent. Each type of stressor is programmed individually in the software, as they are often combined with other stressors (e.g., inverted light cycle with tilted cage). Additionally, a set of two strobe lights were added to the system (Fig. 2, e., f. and g.).
- Shaking – additional stressor
  The motorized system was also designed for shaking. Although this is not usually a stressor from the uCMS protocol, it was added to expand the rack's capabilities. During equipment testing, uneven shaking speeds were observed across rack lines. After investigation, the problem was traced to a mechanical flaw, the cam not rotating

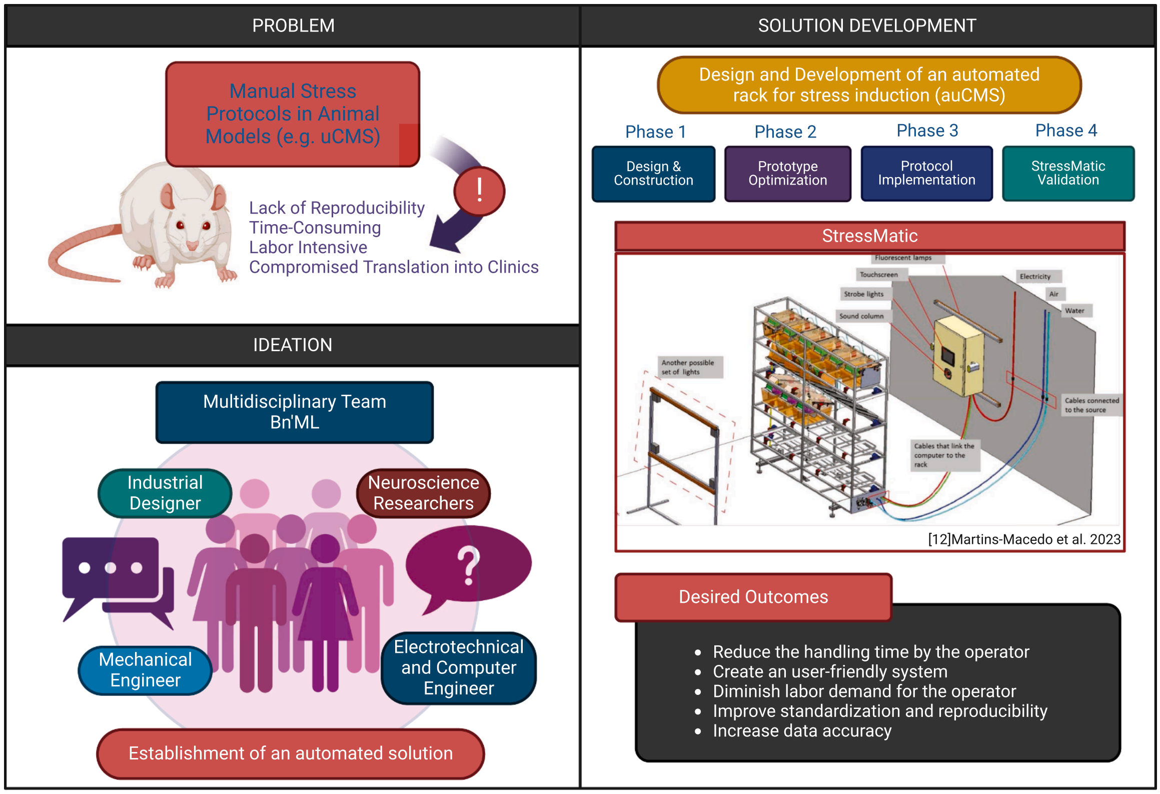

Fig. 1. StressMatic Project conceptualization.

Table 1
Categories of stressors of the automated rack.

Type of Stressor	
TA	Tilted cage (approximately 45°)
	Housing on damp bedding during the night
	Overnight illumination
	Inverted light/dark cycle
	Exposure to strobe lights
	Startle noise
PA	Confinement to a restricted space
	Food deprivation followed by exposure to inaccessible food
NA	Water deprivation followed by exposure to an empty bottle
	Overcrowding
	Cage switch

close to the Teflon board, which was fixed by adding a metallic ring near the spring system (Fig. 2, h.).

- Sound-dependent stressor

A sound system was connected to the rack and integrated into the software to deliver the startle-noise stressor (Fig. 2, i.).

Partially automated stressors (TA):

Partially automated stressors were adapted from the manual protocol to minimize experimenter operation.

- Confinement

In the manual version (uCMS), confinement involves placing three animals in a plastic box. In this modified version, an acrylic insert was designed to reduce the cage space and confine the animals (Fig. 2, j. and k.). This eliminates the need to handle each animal and greatly reduces the time required to apply the stressor. To develop this stressor, the volume of the plastic boxes used in the manual protocol (3 L) was measured, and the new device was built to replicate the same effect.

- Food Deprivation followed by exposure to inaccessible food

In the original protocol, the researcher removes the food from the cages, and after a period of food deprivation, places a container in the cage grid to allow the animals to sniff but not eat the food for an hour. To simplify this process, an accessory was designed for the food deprivation stressor (Fig. 2, l.). The food container allows easy access or restriction simply by adjusting its position.

Non-automated stressors (NA):

Non-automated stressors, which rely entirely on the experimenter's intervention, are not performed by the novel equipment. These include the following stressors: water deprivation, overcrowding and cage switch, as outlined in Table 1, and remain unchanged from the traditional protocol.

3. Impact

3.1. Validation of the auCMS protocol

To evaluate the auCMS as a stress inducer in rats, we assessed its ability to trigger key indicators of depressive-like behavior using several behavioral tests, specifically to evaluate anxiety-, depressive-, anhedonic-like and cognitive dimensions. Indeed, to validate the novel system, we performed a comparison between rodents that were exposed to the manual or to the automated protocols. Additionally, animals that are usually exposed to stress protocols have a disruption in their normal corticosterone serum levels, leading to neural damage. This can be perceived not only in behavioral outcomes, but predominantly in molecular and cellular analyses.

Specifically, we found that the automated stress protocol (auCMS) produced the same behavioral deficits as the manual protocol (uCMS), including anxiety- (EPM and NSF), depressive-like behaviors (FST), anhedonia (SCT and SDT), and cognitive impairments (NOR). Chronic stress led to HPA-axis hyperactivity, disrupting glucocorticoid secretion and altering the circadian rhythm of corticosterone levels. Measuring corticosterone is crucial for evaluating the effectiveness of stress protocols and both automated and manual protocols induced significant

Fig. 2. TA and PA stressors optimization.

changes in corticosterone levels, with a day peak and lower levels at night, confirming successful chronic stress induction in both auCMS and uCMS groups (for detailed results please read our publication (Martins-Maced et al., 2023).

3.2. Implications of StressMatic

The introduction of the automated stress protocol (auCMS) represents a significant improvement to this particular research field in several dimensions.

From the operator point of view, there is a marked reduction in the time needed to execute the different protocols, since most of the stressors no longer need human intervention, or substantially minimize it. From a health and safety perspective, the working load is also significantly reduced, which protects the operator from the physical demands of preparing such protocols and the associated health concerns. It should also be noted that the entire system is very user-friendly, which potentiates and accelerates the learning curve and its implementation.

Furthermore, the application of such automated protocol has a tremendous impact on the scientific validity inherent to its usage. In fact, there is an obvious upgrade in the standardization of protocols, which in turn favors their reproducibility and wide implementation. Consequently, the accuracy and precision of the data obtained is also improved, a fundamental aspect for a successful translation of the preclinical findings into the clinical setting. Importantly, improved reproducibility also impacts animal welfare, by enabling a reduction in the number of animals necessary to achieve statistical and meaningful results.

4. Discussion

Preclinical research using animal models of stress exposure has for long been uniquely based on manual protocols, which has often been cited as one of the reasons for the failure to translate results to the clinic (Stanford, 2020). In fact, to the best of our knowledge, besides StressMatic there are no automated protocols in the literature, which highlights the novelty of developing an automated system. In addition, the standardization of the type, duration and sequence of the stressors employed in each protocol is of the utmost importance, however, this standardization is very difficult to achieve in completely manual-based protocols.

In this project, the validation of the auCMS protocol revealed robust results, with stress-exposed animals responding similarly to the manual protocol, which confirmed that the automated rack is a reliable and promising tool for preclinical research. The potential of reproducibility is considerably high, considering that most of the stressors applied can be pre-programmed and executed, without any human intervention. Obviously, one of the main limitations of the present version of the automated rack is that it is not fully automated, as there are some stressors that partially or totally depend on the operator. Significant improvements were made in terms of operationality to the stressors based on "confinement" and "food deprivation", which significantly reduced the time needed to apply those stressors. The stressors dependent on animal manipulation such as "overcrowding" and "cage switch" continue to be manually operated, as it is extremely difficult and expensive to automate procedures that imply moving specific animals between cages. Nevertheless, most stressors applied are now fully automated, and the implementation of such system in the present version is more affordable, having more potential to be universally adopted.

The development of StressMatic opens new interdisciplinary frontiers, merging biology, medicine, and mechanics with digitalization to revolutionize preclinical research. This innovation highlights the potential for future devices to fully automated stress protocols in animal models, which could reduce the number of animals required by improving reproducibility and precision, thus aligning with the principles of the 3Rs (Replacement, Reduction, and Refinement). This reduction in animal use also encourages a more humane approach to preclinical research.

Looking ahead, advances in automation, such as integrating artificial intelligence (AI) and machine learning, could allow for real-time monitoring and adjustment of experimental protocols based on the ongoing behavior and physiology of the animals. By using sensors and cameras, automated systems could detect stress responses or behavioral changes without needing direct human observation. Additionally, AI could assist in analyzing large datasets generated by automated experiments, identifying patterns that may not be obvious in traditional manual setups. This data-driven approach would significantly improve decision-making, allowing researchers to fine-tune experiments based on more accurate, dynamic insights.

Furthermore, as robotic systems become more sophisticated, tasks like animal handling, currently a major limitation in automation, could become fully automated. For example, automated cage switching and handling systems could be implemented, reducing both the labor-intensive nature of these tasks and the stress caused to the animals by human intervention. Such advancements would not only streamline the research process but also minimize animal suffering, leading to a more ethical approach in line with the principles of animal welfare.

By incorporating cutting-edge technologies and robotics, the next generation of automated systems will redefine how animal models are used, promoting more ethical, efficient, and reliable scientific discoveries. These advancements could reduce dependency on animal models altogether, as more predictive and automated systems are developed.

5. Conclusion

With the implementation of StressMatic, it was possible to reduce labor intensity, streamline repetitive tasks and increase productivity, flexibility, and agility. Indeed, the automation of processes allows to minimize human error, heightens accuracy, and standardizes workflows, ultimately resulting in time and cost savings.

Automation will allow to achieve higher consistency and efficiency levels in preclinical research, in particular in research that heavily relies on models of mood disorders and depends on behavioral outcomes. These improvements will enhance the validity, reliability, and precision of data analysis and interpretation, given the refinement of technical processes. The automated rack protocol (auCMS) for inducing stress in rodents represents significant progress towards the global use of such model and enhances the reliability of screening new pharmaceutical compounds and will permit to achieve deeper evidence to inform drug discovery and strengthen the overall research process.

CRediT authorship contribution statement

Joana Martins-Macedo: Data curation, Methodology, Validation, Writing – original draft. **Luísa Pinto:** Conceptualization, Data curation, Supervision, Funding acquisition, Writing – review & editing. **João F. Oliveira:** Conceptualization, Validation, Writing – review & editing. **Eduardo D. Gomes:** Methodology, Validation, Writing – original draft. **Patrícia Patrício:** Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Writing – review & editing.

Conflict of interest

The authors declare no conflict of interest.

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