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Αγαπητοί φίλοι και μέλη της Ε.Ε.Β.Ε.Ζ.Ε.,

Στις 28 Σεπτεμβρίου - 09 Οκτωβρίου 2020 πραγματοποιήθηκε το έκτο σεμινάριο LAS EU Functions Course στο Εργαστήριο Έρευνας Παθήσεων Μυοσκελετικού Συστήματος (ΕΕΠΜΣ) της Ιατρικής Σχολής (Νοσοκομείο ΚΑΤ). Η διοργάνωση ήταν επιτυχής με βάση την αξιολόγηση που υπέβαλαν οι συμμετέχοντες.

Τον Σεπτέμβριο 2020 ορίστηκαν και ανακοινώθηκαν τα μέλη που θα απαρτίζουν την Εθνική Επιτροπή για την ευζωία των ζώων που χρησιμοποιούνται για επιστημονικούς σκοπούς για τα επόμενα δύο έτη. Η σύσταση αναφέρεται στην αριθ. 45/236079/18.9. 2020 απόφαση του Υπουργού Αγροτικής Ανάπτυξης και Τροφίμων.

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APPLIED REFINEMENT RESEARCH: MEASURING AFFECTIVE STATE IN LABORATORY MICE

This summary is based on a recently published article: Jirkof, P., Rudeck, J., & Lewejohann, L. (2019). Assessing Affective State in Laboratory Rodents to Promote Animal Welfare—What Is the Progress in Applied Refinement Research?. Animals, 9(12), 1026.

Minimization of suffering is the key aim of refinement research and modern animal welfare concepts highlight the importance of promoting positive welfare states in animals. Reliable assessments of affective states, as well as the knowledge of how to elicit positive states, are central to this concept. In this overview, emerging methods to assess affective states in laboratory mice are presented.

Today, it is assumed that all mammals, and probably all vertebrates, have at least some capacity for primal affective states [1-3]. Affective states with their positive or negative valence have the potential to reflect enhanced or compromised overall quality of life. Even tough, it is a great challenge to measure affective states, considerable progress has recently been made in methods that allow also quantification [4]. The importance of the detection of negative affective states is long established in the laboratory animal science community. Recently, there has been a growing awareness of the need to promote also positive affective states in animals for human use. As a result, the importance of positive affective state assessment, also in mice, the most widely used laboratory species, was noticed [4, 5].

1. Physiological and Clinical Parameters

Parameters like body weight changes, general health and immune system status, reproduction success, or changes in hormone levels or heart rate have been used for decades to assess the impact of husbandry and experimental procedures like blood collection techniques or experimentally induced disease states on the well-being of mice. Even though these kinds of parameters played an important role in assessing the negative impact of conditions and procedures and have been essential in the refinement of animal experimentation, they are not necessarily useful for the assessment of positive welfare [6] and are, therefore, of little help in promoting positive affective states in animals.

2. Behavioral Observations

The assessment of affective states based on behavioral measurements include the non-invasive observation of natural and spontaneously occurring behavior in the animals' familiar environment or testing the animal under conditions that induce certain behaviors. One would expect that the performance of certain behaviors (e.g., nest building) is pleasurable in itself, promotes biological functioning, and hints to a state where all other important needs of an animal are met. Other, sometimes aberrant, behaviors (e.g., stereotypies) may indicate reduced well-being. Nevertheless, the relative importance of many behaviors is not known [7, 8], therefore linking the display of behavioral patterns with affective states of an animal is, in many cases, still speculative. In the following, the most often used behavioral approaches are discussed shortly.

Although mice predominantly rely on olfactory, tactile, and auditory cues in social interaction, body posture and facial expression have been proven to be useful for welfare assessment. The detection of pain by using the pain grimace scale [9] has received much attention for the use in routine pain assessment [10–12]. While grimace scales are suitable in detecting painful states; their use in non-painful experimental conditions has not yet been sufficiently investigated.

The construction of nests is common in most rodent species, and the motivation and ability to perform nest building persists in laboratory mice. Nest building performance, observed in the home cage, has proved to be a valuable tool to assess brain damage and neurodegenerative diseases but also painful and depression-like states [13]. When using nest building behavior, one has to consider the important thermoregulatory implications this behavior has for mice. While the reduction of nest complexity might hint to negative affective states, complex nests could also be a result or unfavorable temperature conditions. This effect might be exacerbated, in single housed or sick animals that might have a need for higher ambient temperature. No studies exist that analyze whether high nest building activity under standard facility temperatures and social housing conditions are indicative of neutral or positive affective states.

Mice dig burrows under natural conditions and this behavior is also found in laboratory strains. In burrowing tests, the willingness to remove items from artificial burrows are analyzed [13]. The test was initially developed to monitor the progression of neurodegenerative diseases [15]. As burrowing activity decreases in many conditions that are likely accompanied by negative affective states, the behavior has been subsequently used to assess the impact and refinement of housing and experimental conditions. Burrowing behavior is known to be reduced due to post-surgical pain [16] or anesthesia [17]. The recovery of burrowing has been used as a confirmation of the beneficial effects of experimental refinements of surgical techniques [21] or improvement of post-surgical housing conditions [19, 20]. Additionally, burrowing has been used to monitor apathy in Parkinson's disease models [21], stress induced anhedonia [22] and depressive-like behaviors in models for major depressive disorder [23]. It is, therefore, likely that a decline of burrowing behavior is indicative of negative affective states. Nevertheless, most strains show a high level of burrowing activity under standard laboratory conditions, and it is, unclear if the burrowing test could discriminate between neutral and positive affective states.

Another natural behavior that is used as an indicator of well-being is grooming behavior. In the course of the grooming transfer test, a nontoxic, fluorescence oil is applied to the forehead or the mouse, and the speed at which the fur is cleaned is being measured. This method has been used to evaluate analgesic efficacy after surgery [14].

3. Apparatus Based Behavioral Test Paradigms

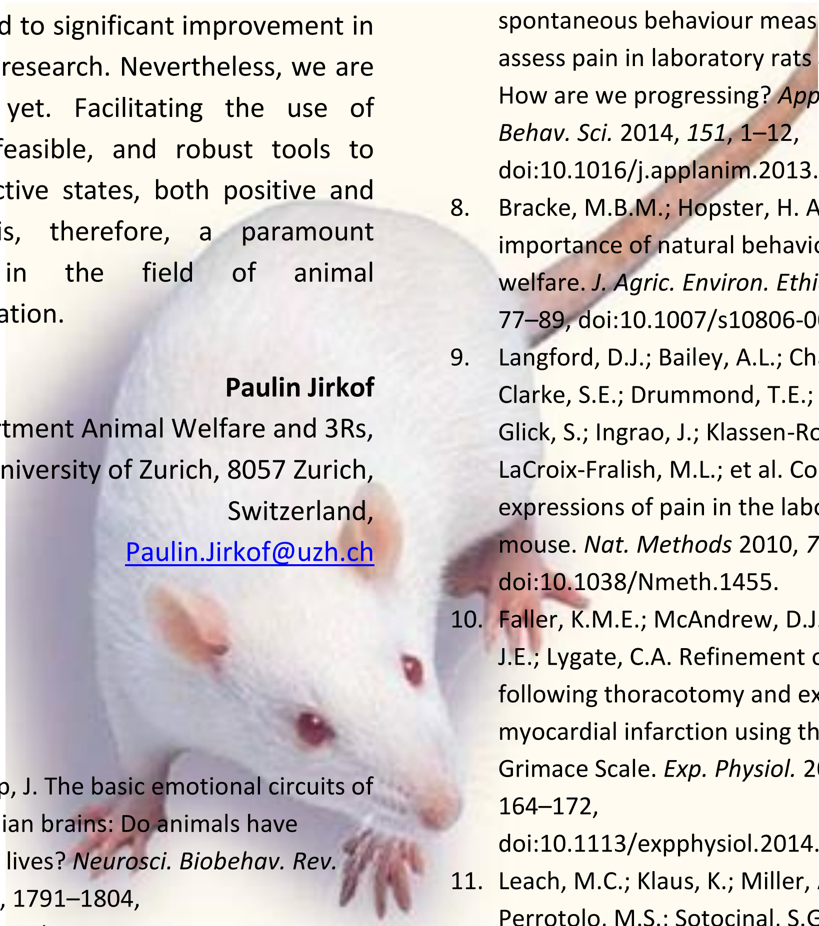
Typical tests for anxiety-related behavior are based on a conflict between approach and avoidance. Commonly used tests are the elevated plus-maze, the elevated zero maze, the black/white box, open field test or the free exploration tests [17, 25]. Although these tests overlap in measuring anxiety related behavior, the different test designs relate differently to components influencing the affective state (e.g., exploration, novelty-induced anxiety, activity [26]). Therefore, it has been recommended to not rely on a single test to assess anxiety related behavior [26,27]. These tests have been utilized to answer applied research questions ranging from assessing the impacts of housing conditions to evaluating the severity of experimental procedures.

Asking the animals themselves for their preferences is a straightforward approach in animal welfare research [27] that has been applied frequently in studies on the refinement of housing conditions. Preference tests help to learn about the view and motivation of the animals. There are several methods available like mazes or shuttle boxes for conditioned place preference tasks [27]. Notably, animals do not necessarily choose what is best for their health and fitness, nor can it be concluded from the preference for a certain good that the animal suffers if this item is not provided [29]. Nevertheless, preference testing is an animal centric strategy to evaluate what an animal wants. In most preference tests, only two items are tested against each other. It has been proposed that by a combination of multiple binary choices, a ranking can be done [29]. Another promising approach to estimating the value of items from an animal's point of view is to charge a price for resources [28]. These approaches test preference strength by limiting access through barriers that need to be overcome [30] or by letting the animals work by means of lever pressing [31]. The amount of work the animal is willing to "pay" is taken as a measure for the strength of the preference. So far, only few robust data for ranked preferences are available.

The cognitive judgement bias (CJB) test relies on associative learning, combining a stimulus at one end of the scale with a reward and a punishment at the other. The cognitive bias is tested after training is completed by measuring the response to ambiguous stimuli that lie between the previously learned associations. Positive affective states predispose an optimistic response (the ambiguous stimulus is more likely interpreted as if it predicts a reward), while negative affective states lead to a pessimistic expectation [32]. There were many attempts to adapt this test for mice, but, as of yet, there is no protocol that would allow repeated testing in mice without facing a considerable dropout rate and lengthy training. Nevertheless, CJB tests are a promising instrument for measuring affective states when methodological problems can be overcome.

Conclusions

Most of the applied approaches focus on capturing short-lasting affective states in direct relation to experimental procedures. However, the promotion and evaluation of animal welfare should be understood as an approach that concerns the whole life of an animal. It would, therefore, be desirable to monitor affective states over long periods. Thus, long-term observations and assessment criteria should be further promoted. Many of the measures relate to assessing negative affective states (e.g., pain grimace scale), and often, the absence of obvious indicators for negative affective states is interpreted as being positive. This, however, falls short in truly assessing positive affective states and can be seen as a lost opportunity in current refinement research. Moreover, some of the indicators for positive affective states rather indicate functioning normal behavior than necessarily being indicative of positive affective states. Arising methods for the automated assessment of behavior and facial expressions and the integration of artificial intelligence using novel deep learning approaches for observation and data analysis might fill this gap and lead to significant improvement in refinement research. Nevertheless, we are not there yet. Facilitating the use of validated, feasible, and robust tools to assess affective states, both positive and negative, is, therefore, a paramount objective in the field of animal experimentation.



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ΕΠΙΣΤΗΜΟΝΙΚΕΣ ΕΚΔΗΛΩΣΕΙΣ

Τον Οκτώβριο 2020 θα πραγματοποιηθεί το e-Learning Course **'Recognition of pain, suffering and distress and its application in the evaluation of severity of the procedures' (species specific: mice and rats)** foreseen within the Erasmus+ HERMES Project. Για περισσότερες πληροφορίες επισκεφθείτε την ιστοσελίδα https://www.hermes4las.eu/call-for-applications-e-learning-course/?fbclid=IwAR15A2MOSsme6eIlYp3qSSTmF2A6Jn0XlEtGM0WQKMcTD2viOrKR-Vqc08w.

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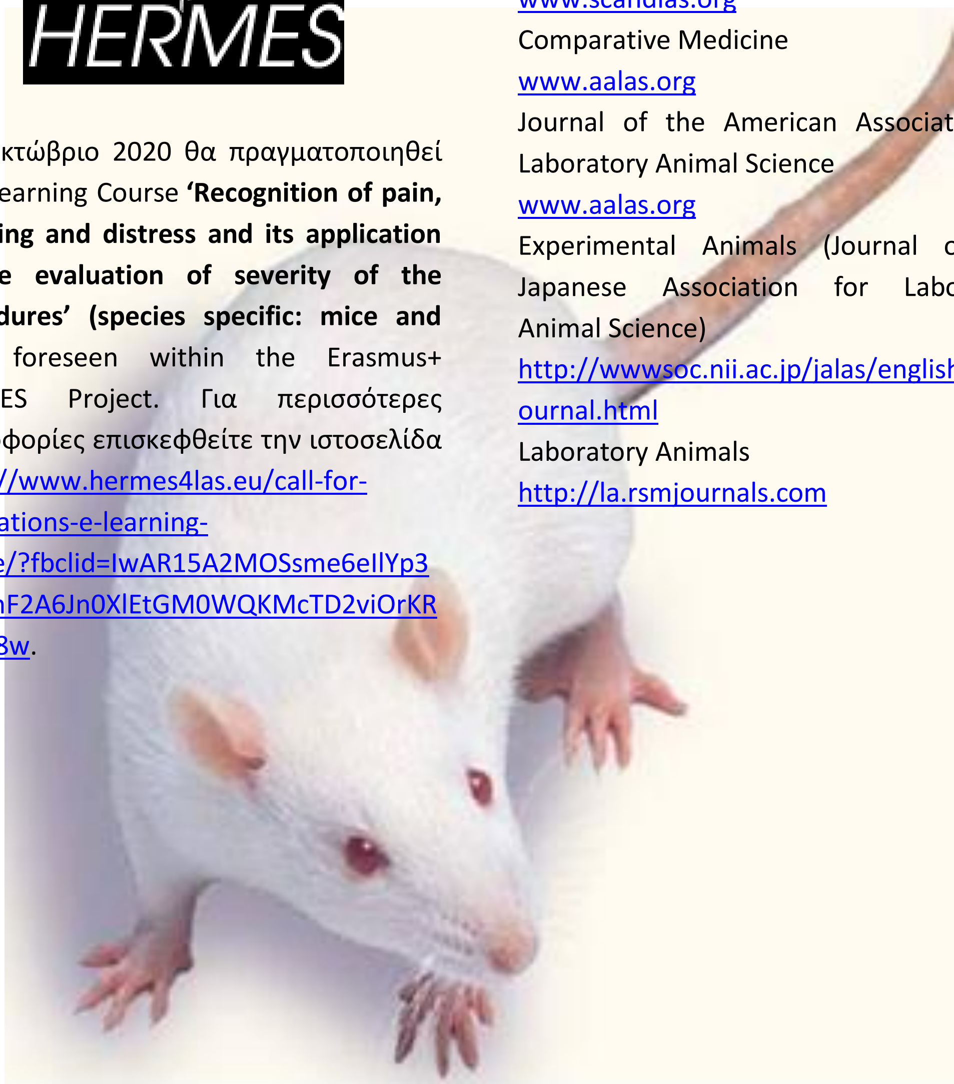

ΕΠΙΜΕΛΕΙΑ ΣΥΝΤΑΞΗΣ:

Βασίλειος Ντάφης

Αναστασία Τσιγκοτζίδου