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Improving Tail Flick Analgesic Test by a Custom-Made Restraining Chamber

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ABSTRACT

The tail flick test is a common method used to evaluate acute pain in animal models. However, there seems to be a technical limitation to the use of this test, deserving a more detailed consideration. The problem is related to the use of physical restraints during the test, which in turn exposes the animal to stress and could therefore undesirably affect the obtained results. In the present report, a newly designed restraining chamber was used to improve the tail flick test regarding the mentioned limitation. Also, the baseline tail flick latency in animals undergoing the test using the classic restrainers was compared to that in those restrained by the newly designed chamber. The results indicated that the baseline tail flick latency was significantly lower in animals restrained by the newly designed box compared to those restrained by the tube-shaped version, which could be attributed to stress-induced analgesia. In conclusion, it is recommended that researchers use boxes similar to the custom-made box used in this study to prevent stress-induced errors when measuring nociceptive thresholds in rats.

Keywords: Tail flick, Restraint, Stress, Analgesia, Rat

1. Introduction

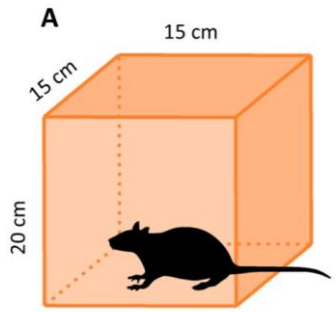
Researchers evaluating analgesic effects of drugs often use the tail flick test in animal models, such as rats and mice. Although widely accepted in the literature, this method is associated with a technical limitation related to the use of physical restraints during the experiment [1-4]. Physical restraint is actually necessary to place the animal's tail adjacent to a noxious stimulus, especially in the radiant heat version of the test. For this purpose, researchers generally use conventional hard Plexiglas tubes as restrainer (Fig.1C). This might impose some degree of stress-induced analgesia (SIA) on animals, which in turn could result in overestimation of baseline sensitivity. Previous studies have shown that exposure to acute stress (restraining the animal for a while) results in a significant increase in tail flick latency [5, 6] as well as in plasma level of corticosterone in rates [7]. It has been reported that high levels of plasma corticosteroids are associated with analgesia induced by short-term stress [8-10]. In this study, a simple box recently designed in our laboratory was used in the tail flick test to minimize the mentioned stress.

2. Methods

As shown in Fig.1A and B, the apparatus used in this study was a rectangular box made of translucent (in this case dark green) Plexiglas with 15×15×20 cm dimensions [11]. In this box, animal's tail could be easily exposed to heat through the gaps located in the inferior parts of the four surrounding dimensions. In contrast to classic tube-shaped restrainers, this box is spacious enough not to expose the animal to physical stress. Thus, the animal is not agitated during the experiment, which seems to be much more ethical from the animal rights viewpoint. Furthermore, it easily gets acclimated to the chamber and its tail could be available for the experimenter. It should be noted that this box could be manually made with a remarkably lower cost than the commercially available restrainers. Therefore, in this study, the baseline tail flick latency was compared between two groups of animals (n=7 per group). Male Wistar rats, weighing 250-300 g, were kept in Plexiglas breeding cages in groups of four animals per cage with woodchip bedding and free access to food and water. Animals were housed in a colony room at a stable temperature with 12-h light/dark cycles (the light period started at 7 am). All experiments were performed according to the ethical guidelines set by the Ethics Committee of Faculty of Medical Sciences, Tarbiat Modares University based on the NIH Guide for the Care and Use of Laboratory Animals. Animals underwent the test using both types of restrainers (classic tube-shaped ones and the newly designed box). A tail flick analgesiometer apparatus (Harvard, Holliston, US) was used for the test. A light beam, generated by a 150 W electrical bulb, was focused on the dorsal part of the animal's tail via a concave mirror located above the bulb. The same heat intensity was adjusted for both experimental groups. Also, a 10 s cut-off time was considered to prevent damage to the animal's tail.

3. Results and discussion

The obtained results indicated that the baseline tail flick latency was significantly lower in animals restrained by the newly designed box than in those restrained by the tube-shaped version (Fig.2). Thus, it seems that a significant degree of SIA is induced in animals prior to the experiment following the application of tube-shaped restrainers. It has been previously shown that restraining the animal could change the analgesic effect of some drugs [12-15]. Undoubtedly, this could adversely affect the final test results, as observed in the phenomenon under study. Therefore, analgesia might be more sensitively measured with lower baseline latencies in animals not exposed to stress. In particular, in studies with low statistical power (i.e. in cases where the investigator needs to reduce the standard error, and/or the expected effect is small), reducing stress is of great importance. With respect to these concerns, authors found the issue worth reporting. By the way, there are other points that should be considered by researchers in assessing thermal nociceptive thresholds in rats. For example, it is highly recommended to avoid working on female subjects because changes in hormonal profile during the estrous cycle adversely affect pain perception [16-18].



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Race	Wistar
Gender	Male
Animals' weight	250-300 g
Age	8-10 weeks
Time of experiment	2-4 pm
Room temperature	20–22 °C
Acclimatization time	2 h

Fig. 1. Schematic (A) and real (B) pictures of the newly designed box for the tail flick test compared to classic tube-shaped restrainers (C). Information associated with variables in the tail flick experiment (D).

In addition, it is more rational to perform nociceptive tests during adulthood because, for example, pain-modulatory brain structures are fully developed during this period, while during adolescence, the maturation of many regulatory pathways is still in process throughout the nervous system [19]. Finally, researchers are strongly advised to choose a fixed time for behavioral assessment of nociceptive responses because it has been shown that changes in circadian rhythm potently affect the results of pain measurement at behavioral level [20].

The new box used in this report could be easily employed in almost all pain studies, especially those assessing the basic mechanisms of pain modulation with an emphasis on nociceptive threshold. Another issue is that researchers currently report changes in pain intensity as normalized values such as the percentage of maximum possible effect (MPE%) [11, 21]. Although this method well indicates the trend of drug effect over time, the final interpretation might be misleading if baseline thresholds are altered by restraint-induced stress. Thus, it is suggested that researchers report changes in baseline thresholds as raw values in their results. This enables the reader to reach a more accurate understanding of basic alterations in cellular responses to painful stimuli.

4. Conclusion

In conclusion, it is recommend that researchers in this field use boxes similar to the custom-made product used in this study to prevent stress-induced errors when measuring nociceptive thresholds in rats.

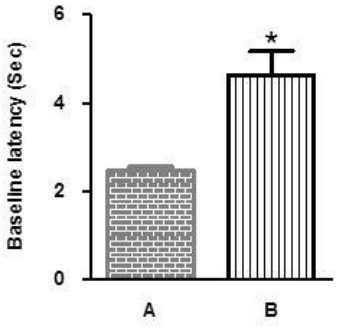

Fig. 2. Comparison of the baseline tail flick latency between the two groups of animals**:** A) restrained by the newly designed box and B) restrained by a commercially available tube-shaped restrainer. Data are expressed as mean ± SEM, *$p < .05$, n=7 per group.

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