Forepaw Licking and Jumping as Indexes for Pain Threshold and Pain Tolerance Level during the Hot-plate Test

Kazuhide UCHIDA *, Miki SAKAMOTO *, Eri NAKAGAWA *, Takeshi TATEDA *

Abstract Purpose : When two patterns of behavior, forepaw licking and jumping, were used as criteria for the hot-plate test, a disagreement between the results was reported. We hypothesized that the two behavior patterns represent different indexes for pain sensitivity, pain threshold and pain tolerance level. The aim of the present study was to investigate this hypothesis. Methods : Latency until forepaw licking and jumping was measured in each mouse using the hot-plate test at times between 10:00 and 12:00 h. The latency times were compared for several stimulation intensities (45-65°C). Results : Both reaction times based on forepaw licking and jumping increased as the stimulation intensity decreased (one-way ANOVA, p < 0.05). The jumping response was more sensitive to changes in stimulation intensity than the forepaw licking response. The series of jumping reaction times was longer than the forepaw licking reaction times (two-way ANOVA, p < 0.01). A significant correlation between the licking and jumping latencies was not observed at any stimulation intensity. Conclusion : Forepaw licking and jumping are the recommended indexes for pain threshold and pain tolerance level, respectively, during the hot-plate test.

Key words : Hot-plate test, Pain threshold, Pain tolerance level

I Introduction

The hot-plate test, a method for assessing pain sensitivity, is used extensively in studies of pain involving rodents. The procedure consists of measuring the amount of time that elapses from the start of the stimulus to the appearance of pain behavior, this time is known as the latency or reaction time. Although intensity of the stimulus can be regulated by thermal variations in the platform attached to the measurement device, only a single stimulating intensity (usually in the neighborhood of 55°C) is usually selected in most studies. Many patterns of behavior are observed when rodents are placed on the platform. These patterns can be classified into three categories : sniffing responses (immobile sniff, walking sniff), primary noxious-evoked responses (forepaw licking, hindpaw licking, stamping), and noxious-evoked escape responses (leaning posture, jumping off) 1). Pain behavior is a very important factor in the hot-plate test because it is used as the criterion for deciding the reaction time. Although only one pattern of pain behavior is usually chosen as the decision criterion in almost all studies, no grounds for this selection have been established. When two patterns, forepaw licking and jumping, were used as criteria, a disagreement between the results was reported, despite similar experimental conditions 2). In view of this disagreement, we hypothesized that the two patterns represent different indexes for pain sensitivity, pain thresh-
old and pain tolerance level. The aim of the present study was to investigate this hypothesis. Reaction times based on the two patterns were compared under several stimulating intensities.

II Materials and Methods

Five-week-old male C3H/He strain mice were used in the study. The animals were maintained under a light-dark cycle of 12:12 hours (lights on 06:30–18:30 h) and allowed access to food and water ad libitum.

Latency until forepaw licking and jumping was measured in each animal using the hot-plate test at times between 10:00–12:00 h. The measurement device used was the model 39D hot plate analgesia meter (IITC Inc., CA, USA). The latency was compared under several stimulating intensities (45–65°C) in intact mice. Fifteen mice were assigned to each group. All mice were used only once and never again. In addition, many patterns of behavior were carefully observed before and during the hot-plate test. The observations provided qualitative information regarding the presence and priority of behavior patterns.

The data are expressed as the means ± standard deviation and were statistically analyzed using the Smirnov test for extreme values, one-way and two-way ANOVAs, Duncan’s new multiple range test, and the correlation coefficient test. Differences with probability values of less than 0.05 were considered to be significant.

II Results

Both reaction times based on forepaw licking and jumping increased as the stimulating intensity decreased (one-way ANOVA : p < 0.05, see Fig. 1). The series of reaction times based on jumping was longer than that based on forepaw licking (two-way ANOVA : p < 0.001). When the correlation between the two reaction times based on the different behavior patterns was statistically analyzed, no significant correlations were observed at any of the stimulus temperatures (see Table 1) and the scatter diagram had a roughly elliptical distribution.

General observations of pain behavior showed that forepaw licking occurred more quickly than other noxious-evoked responses. In contrast, jumping was always the slowest of all responses. The appearance of a leaning posture was faster than that of jumping and was also observed in the cages at room temperature. Every mouse did not stamp on the platform.

IV Discussion

Jumping latency was usually longer than licking latency, regardless of changes in stimulation intensity. Hence, the difference between latency times was remarkable (see Fig. 1). The jumping response was more sensitive to change in stimulation intensity than the forepaw licking response because the curved line slope for the jumping latency was sharper than that of the licking latency. The sudden prolong of licking latency at 45°C is not reflects on true pain relief since
forepaws were cooled by leaning posture. We think that low temperature caused the leaning posture. The leaning posture, a common behavior, is observed frequently in cage at room temperature. The licking latency at 45°C was overestimated by the cooling; therefore the true value is lower than found value. The consideration suggests that the slope of jumping latency may be sharper than that of licking latency at lower temperature (44-50°C). The licking and jumping latencies were mutually independent because no correlations between the latencies were observed at any of the stimulation intensities (see Table 1) and an elliptical distribution was obtained in all of the correlating plots. These results indicate that the licking and jumping latencies have dissimilar characteristics.

Pain threshold and pain tolerance level have been defined as the least experience of pain and the greatest level of pain, respectively. In other words, they are lower and upper limits of pain sensitivity. During the hot-plate test, these concepts should correspond to the fastest and slowest noxious–evoked responses. The present results show that forepaw licking and jumping satisfy these conditions. When several patterns of behavior are considered, based on the present and previous observations, leaning posture and stamping appear to be unreliable criteria. The leaning posture is not always a noxious–evoked response; it can be an inquiring behavior, such as a sniff, in some cases. Stamping is not a common behavior in every mouse, and the appearance of this behavior may be related to the frequency of hindpaw licking. Therefore, forepaw licking and jumping are recommended as the indexes for pain threshold and pain tolerance level, respectively, during the hot-plate test. Forepaw licking and jumping have been reported to be primary and escape noxious–evoked responses, respectively. This previous report partially supports our present conclusion.

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References
1) Espejo EF, Mir D: Structure of the rat’s behaviour in the hot plate test. Behav Brain Res 56: 171-176, 1993

<table>
<thead>
<tr>
<th>T(°C)</th>
<th>Regression equation</th>
<th>Correlation coefficient</th>
<th>n</th>
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<td>45</td>
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T: temperature
P: probability
NS: not significant


ホットプレート法における疼痛閾値と疼痛耐性水準の指標としての前肢薦めおよび跳躍

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ホットプレート法における前肢薦めと跳躍までの反応時間を、マウスを用いて各種刺激強度（45〜65℃）で比較した。
両反応時間は刺激強度低下とともに延長したが、その割合は跳躍でより顕著であった。両行動間の比較では、全刺激強度におとど跳躍より反応時間は長かった。各刺激強度における両行動間の反応時間に相関は認められなかった。疼痛行動の観察において、前肢薦めの早く認められ、対照的に跳躍は最も遅く認められた。壁への寄り掛けは跳躍よりも早く、室温のケージ内でも認められた。また、足踏みは必ずしも認められる行動とは言えない。
これらの結果および行動観察より、前肢薦めおよび跳躍はそれぞれホットプレート法における疼痛閾値と疼痛耐性水準の指標になると結論する。

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