WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 1, 284-293. Research Article ISSN 2277– 7105

DESIGN OF A VALIDATION OF CAUDAL ANALGESIMETRY IN EXPERIMENTAL PHARMACOLOGY

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Article Received on
15 Nov. 2017,

Revised on 05 Dec. 2017,
Accepted on 25 Dec. 2017

DOI: 10.20959/wjpr20181-10544

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ABSTRACT

Caudal analgesia is one of the most used tests in recent years to evaluate the reflex response to nociceptive stimulation in the animal model. The aim of our work was to study the different parameters involved in the analgesic effect of a pharmacological substance when performing a tail-flick test. The device used is a Tail-flick meter LE 7106 PANLAB (part of the Harvard Bioscience Family Spain), our animal model was composed of "wistar" rats and "swiss" mice. The analgesic substance used for the control lot was acetylsalicylic acid; the dose for the intraperitoneal administration was 20 mg / kg and 100 mg / kg for the oral administration. Based on our results, there is species-specificity and sex difference for rats. Conversely, there is no sex variability for mice when it comes to response time. The mode of administration of the reference drug for peripheral analgesia does not reach the nociceptive center for the orally administered aspirin group; whereas for the intraperitoneal administered aspirin group, there is a significant difference in the focus 50, 60 and 70 compared to the control groups.

KEYWORDS: Peripheral analgesia, tail-flick, thermal stimulation, aspirin, rats, mice.

BACKGROUND

Pain is defined by the International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience arising from actual or potential tissue damage, or described in terms of such damage", it is further characterized by an emotional state that drives action.[1] Faced with the polymorphism of the pain described by man as a sensation, the absence of verbal communication is an unavoidable obstacle to evaluate the pain in the

animal, it is estimated only by behavioral studies and the examination of his reactions.[2] According to different experimental parameters, the tail-flick latency method can be used to determine the essential level of nociception, and measure the analgesic efficacy of the pharmacological agents. This is a valuable method because of its simplicity, reproducibility, relatively small variation and the minimal need for the device. It is one of the most used tests to evaluate reflex response to nociceptive stimulation.[3] The test is used by focusing a powerful light beam (thermal radiation) on the tail and measuring the withdrawal time of the tail which is also called the response-time.

The objective of our work is to study the various parameters involved in the analgesic effect of a pharmacological substance when performing a Tail flick test.

MATERIAL AND METHODS

1- Device

The device used is an analgesiometer LE 7106 PANLAB (part of the Harvard Bioscience Family Spain), it consists of a stimulus unit (containing a halogen lamp for the Heat Stimulus) and an electronic Control Unit.

The system can be used for rats and mice of different sizes and weights of between 20 g and 400 g. The animals can be put in contention cages to stabilize them and they can be placed on the platform of the stimulus unit. A remote foot-switch controls the test start/stop allowing rapid hands-free experiments. The instrument gives an accuracy of 0.01 seconds for reaction time measurements and a resolution of 0.1 seconds. A stimulus cut-off time is set to 20 seconds by default to avoid tissue damage. The stimulus is provided by a halogen lamp (100W-12V) set on a pivoting support. The lamp has 10 intensities of heat to cover an ascending temperature of 40 C° to 220 C°.

2- Animals

In our study, we identified consanguineous “Wistar” rats (specific pathogen-free) and whose weight was between 105 g and 280 g, and "Swiss" mice that weighed between 14 and 37 g for mice. Twenty-four rats and eight mice were separated into eight lots and divided into contention cages “Fig. 1” and each cage contained the same sex (n = 4) with free access to water and food. The temperature of the accommodation rooms was 22.6 ± 2.5°C (minimum 19.2°C, maximum 28.5°C) and the relative humidity was 68.4 ± 7.9% (minimum: 53.6%, maximum: 87.3%). Each animal is used in a vigilant state and only once per test.

Figure 1: Distribution of animals in cages.

3- Substances

The analgesic substance used for the control lot was acetylsalicylic acid, the dose for the intraperitoneal administration was 20 mg / kg[4] and 100 mg / kg for the oral administration.[5] The administration of the analgesic substance was extemporaneous. Measurement of analgesic activity was performed at 10 and 30 minutes after administration of aspirin respectively intraperitoneal and oral.

4- Study design

The tests were performed at the same time (at 14:00) and by the same operator. The different parameters involved in our study were: the control lot, and the way of administration of the analgesic substance. The parameters identified in our study were, the time lapse between the beginning of the experiment and the tail withdrawal reflex which is the response-time "Fig. 2".

Figure 2: Realization of the test in rats.

A tail withdrawal time equal to or greater than 6 seconds indicates an elevation of the pain threshold in our animal model; in other words, an analgesic action.[6]

5- Statistical analysis

All data were presented as mean ± Standard error of Mean (S.E.M) and analyzed by Student's t-test. SPSS 17.0 software was used for the statistical study. A value of $p < 0.05$ was considered statistically significant.

RESULTS

1. Response time to focus 10, 20 and 30

The response times of the different groups at focus 10, 20 and 30 were greater than 20 seconds.

2. Analysis of the response time according to the species

The response time of rats and mice decreases with increasing focus. There is a significant difference between the response time of female mice compared to the response time of female rats for all focus with $p < 0.05$ "Fig.3".

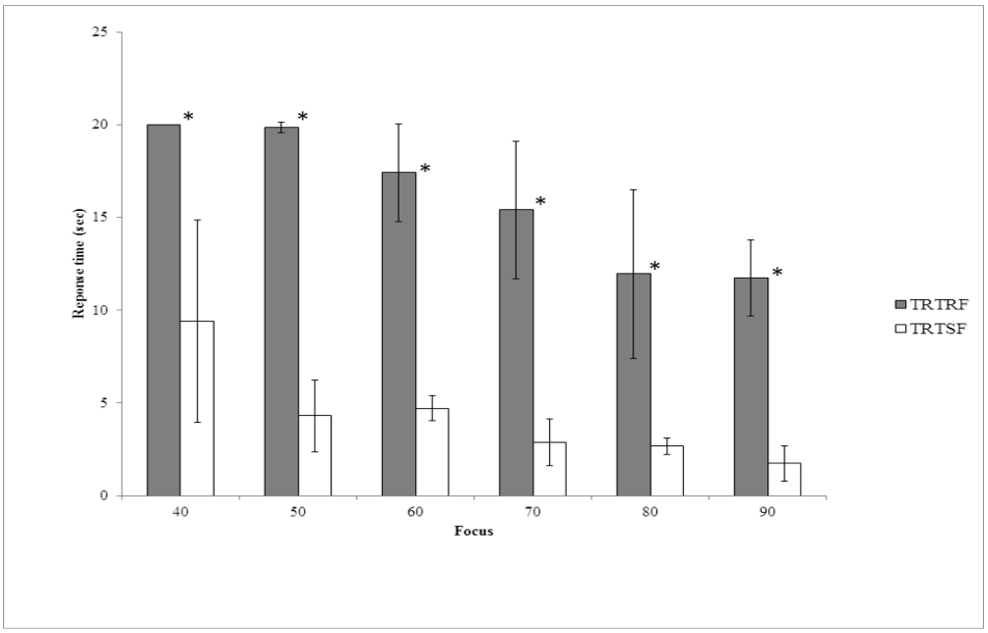

Figure 3: Comparison between of response time of the control group of female rats (TRTRF) and the control group female mice (TRTSF) for different focus. The results are expressed as mean ± S.E.M., n = 4; * p <0.05.

For the response times of male mice and male rats, a significant difference was observed at focus 40 and 90 with $p < 0.05$ "Fig. 4".

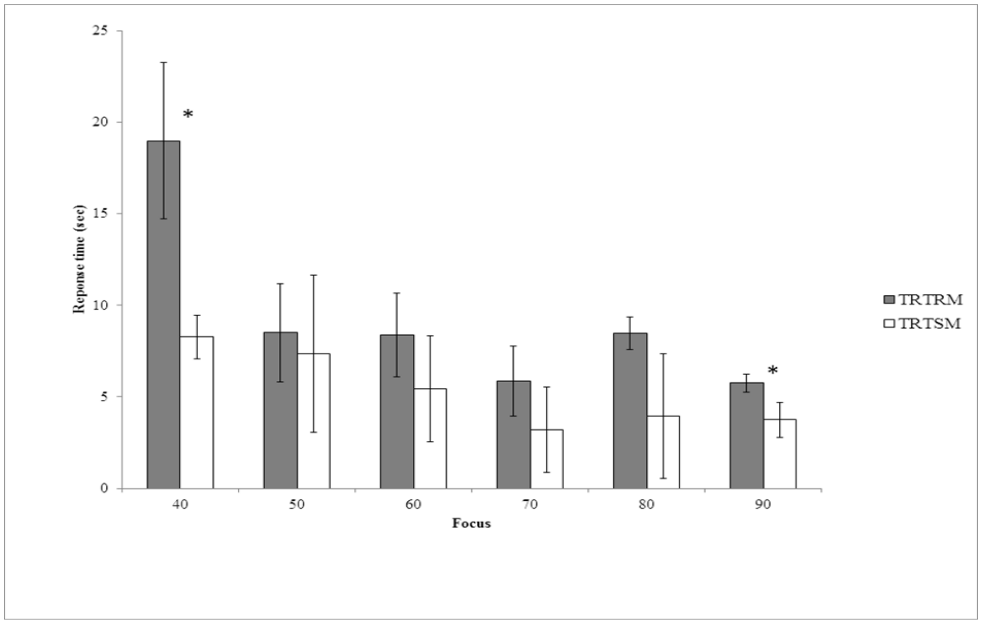

Figure 4: Comparison between response time of the control group of male rats (TRTRM) and the control group male mice (TRTSM) for different focus. The results are expressed as mean ± S.E.M., n = 4; * p <0.05.

3. Latency analysis by sex

3.a. Mice

The response time of the referenced lot of male and female mice decreases by increasing the focus without there being a significant difference between the two groups "Fig. 5".

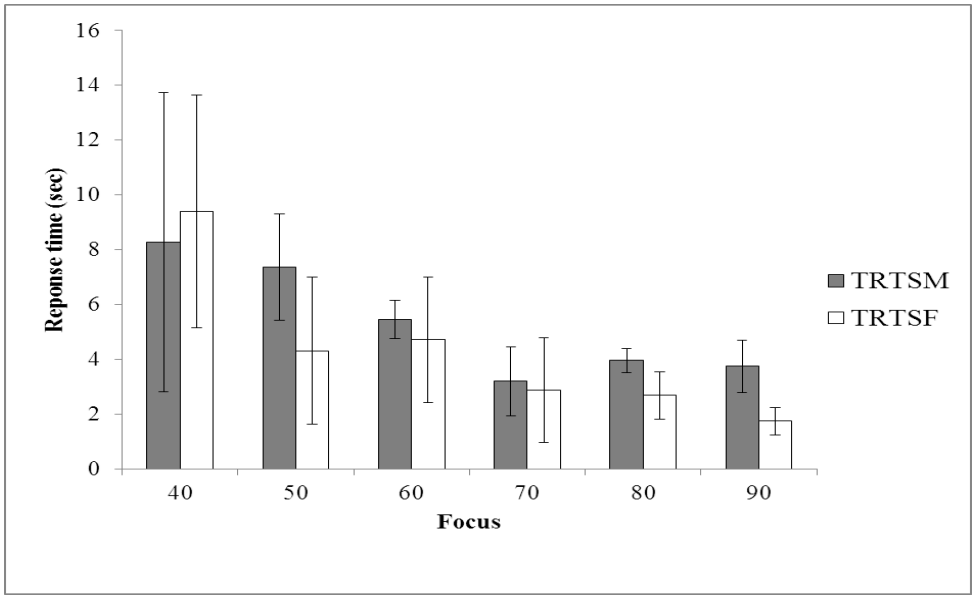

Figure 5: Comparison between response time of the control group female mice (TRTSF) and the control group male mice (TRTSM) for different focus. The results are expressed as mean ± S.E.M., n = 4; * p <0.05.

For female mice, the response time was less than 6 seconds from focus 50. For male mice, the response time was less than 6 seconds from focus 60 (Table 1).

Table 1: Reponse time for groups male mice (TRTSM), female mice (TRTSF), male rats (TRTRM) and female rats (TRTRF). The results are expressed as mean ± S.E.M., n = 4.

	Focus 40	Focus 50	Focus 60	Focus 70	Focus 80	Focus 90
TRTSF	9,4 ± 5,46	4,31 ± 1,93	4,71 ± 0,69	2,88 ± 1,26	2,68 ± 0,44	1,75 ± 0,95
TRTSM	8,27 ± 4,25	7,35 ± 2,69	5,46 ± 2,28	3,19 ± 1,91	3,95 ± 0,86	3,75 ± 0,5
TRTRF	20 ± 0	19,85 ± 0,28	17,42 ± 2,62	15,41 ± 3,72	11,95 ± 4,55	11,75 ± 2,06
TRTRM	18,99 ± 1,17	8,5 ± 4,31	8,37 ± 2,89	5,84 ± 2,34	8,47 ± 3,40	5,75 ± 0,95

3.b. Rats

The response time of the reference lots decreased with increasing focus, a significant difference between the female and male rats groups was observed at focus 50, 60, 70 and 90 with $p < 0.05$ “Fig. 6”.

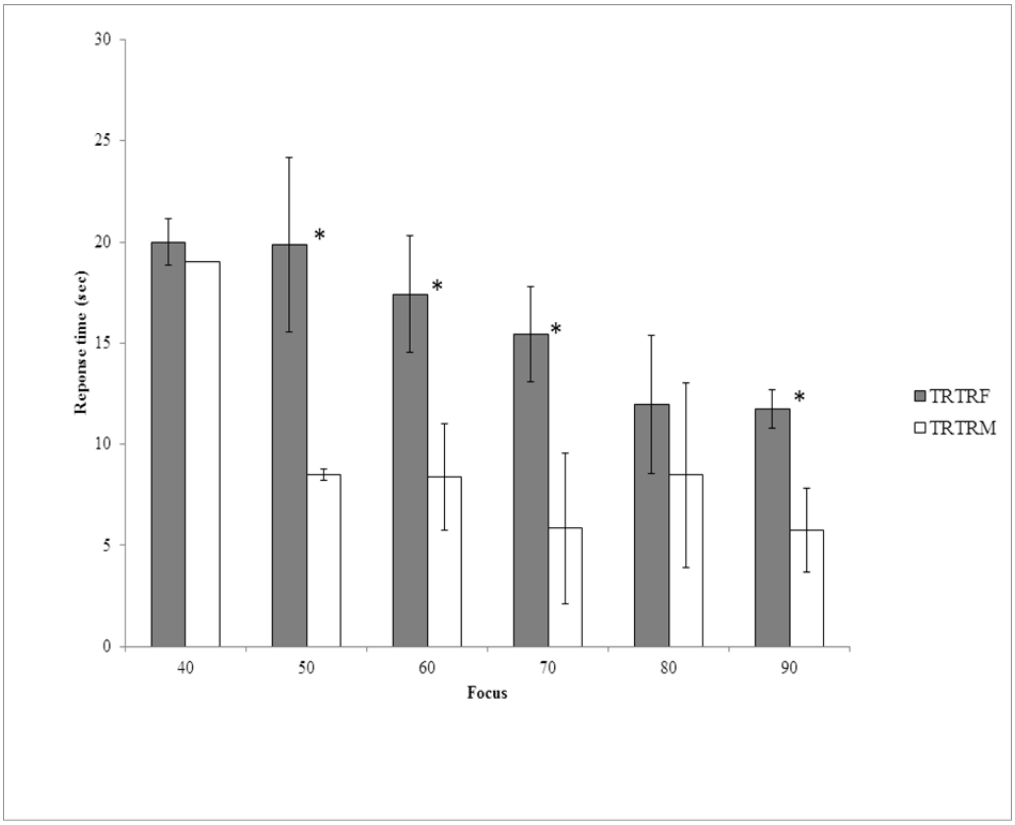

Figure 6: Comparaison between response time of the control group of female rats (TRTRF) and control group of male rats (TRTRM) for different focus. The results are expressed as mean ± S.E.M., n = 4; * p <0.05.

For female rats, the response time was greater than 6 seconds for all groups. For male rats, response time was less than 6 seconds for focus 70 and 90 (Table 1).

4- Analysis of the latency time according to the way of administration

The latency of the male reference lots (oral aspirin and intraperitoneal aspirin) decreases with increasing focus "Fig. 7".

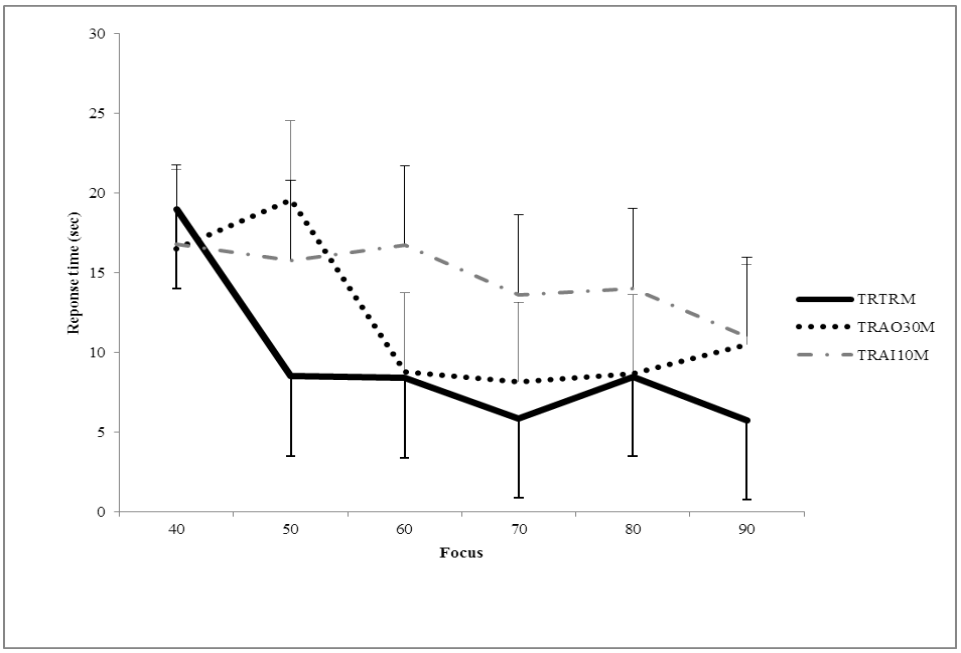

Figure 7: Comparison between response time of the control group of male rats (TRTRM) and male reference lots (oral aspirin (TRAO30M) and intraperitoneal aspirin (TRAI10M).

It was significantly greater than 6 seconds for the intraperitoneal aspirin reference lot with a significant difference compared to the control group in focus at 50, 60 and 70 (Table 2).

Table 2: Reponse time for groups control rats (male (TRTRM), female (TRTRF), reference lots oral aspirin (male (TRAO30M), female (TRAO30F), reference intraperitoneal aspirin (male (TRAI10M), female (TRAI10F)). The results are expressed as mean ± S.E.M., n = 4; * p <0.05.

	Focus 40	Focus 50	Focus 60	Focus 70	Focus 80	Focus 90
TRTRM	18,99 ± 1,17	8,5 ± 4,31	8,37 ± 2,89	5,84 ± 2,34	8,47 ± 3,40	5,75 ± 0,95
TRAO30M	16,51 ± 4,03	19,58 ± 0,84 *	8,75 ± 5,09	8,15 ± 3,01	8,65 ± 2,69	10,5 ± 3,51
TRAI10M	16,77 ± 4,07	15,79 ± 5,72 *	16,73 ± 3,56 *	13,62 ± 3,48 *	14,03 ± 3,36	11 ± 4,54
TRTRF	20 ± 0	19,85 ± 0,28	17,42 ± 2,62	15,41 ± 3,72	11,95 ± 4,55	11,75 ± 2,06
TRAO30F	11,65 ± 5,69	10,85 ± 3,41 *	12,59 ± 6,39	7,18 ± 1,45 *	9,75 ± 1,29	8 ± 4
TRAI10F	18,18 ± 2,92 *	16,54 ± 3,14	10,45 ± 4,26	9,18 ± 0,97 *	8,05 ± 1,32	9,25 ± 3,68

DISCUSSION

All the tests of focus 10, 20 and 30 were above 20 seconds because the intensity of the light was below the threshold that could trigger a nociception for our animal model. On the basis of our results, performing the Tail-flick test at focus 10, 20 and 30 for rats and mice would not be recommended. There is a species-specificity for the caudal analgesia test for a focus above or equal to 40. It should be noted that mice are more sensitive than rats; the withdrawal reflex of the tail after a nociceptive stimulus, an exposure to heat in our case, in mice is faster than in rats.

Shaifali Bhalla and al.[7,9] used male mice as a witness to determine analgesic activity, the latency time was measured by the same device as ours in focus 50; it was between 2 and 3 seconds. Beatriz de la Puente and Al.[10] compared the latency of two types of homozygous and heterozygous male mice, no significant difference between the two groups was detected by the same device used in our study; the time of tail withdrawal was between 3 and 4 seconds for both groups. Hong Ruan and al.[11] used as a device (TAILFLICK 7360, UGO Basile, Varese, Italy) to determine the nociception threshold of male mice, the measured latency was 3 seconds for a light intensity of 40. Liga Zvejniece and al.[12] used male mice to determine latency of the control lot measured between 5 and 7 seconds by the Tail-flick apparatus (Model DS20, Hugo Basile, Italy). Tianga Yaya Soro and al.[13] used to determine analgesic activity for female rats, a Tail-flick device (7360, Ugo basile, Comerio, Italy) which is a device consisting of a bulb emitting radiant heat from 55°C to 60°C; they obtained as a result a withdrawal of the tail for a time of between 4 and 6 seconds during control tests.

Our study demonstrated that there is no mice sex variability or difference when it comes to response time for all focus. Conversely, there is a difference in sex for rats. Male rats are more sensitive than female rats to thermal nociception: the response time was less than 6 seconds in focus 70 and 90. The response time of female rats was significantly higher than the one of the males and it was greater than 6 seconds in all types of focus. Thus, female sex rats should not be included in caudal analgesic studies. In a study by S. Stevens Negus and al.[14] that compared nociception tests in a monkey animal model, they report that male monkeys tend to be slightly more sensitive to thermal stimuli than female monkeys in the follicular phase during the menstrual cycle. In addition to the variation of the sex regarding the response to the nociception tests, the age and the weight of the animal would probably have an impact on the response time.[15]

The mode of administration of the referenced drug in the male sex for peripheral analgesia which is aspirin, either orally at the dosage of 100 mg / kg or intraperitoneally at the dosage of 20 mg / kg, achieves the nociceptive center since the response time was greater than 6 seconds for all groups with a significant difference for focus 50, 60 and 70 for the intraperitoneal aspirin group and the focus 50 for the oral aspirin group compared to control groups.

Further Tail-flick studies should be performed by increasing the dosage of aspirin and comparing these doses with controls to normalize peripheral analgesia tests. These doses of analgesics must reach the nociception center and must be comparable to the dose ranges determined in humans.

CONCLUSION

According to different experimental parameters, this technique can be used to determine the basic level of nociception and measure the analgesic efficacy of the pharmacological agents. We must be vigilant when interpreting. Our study has allowed us to shed light on various parameters that might be involved in the results of studies of peripheral analgesia.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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