HYPERALGESIA-TYPE RESPONSE REVEALS NO DIFFERENCE IN PAIN-RELATED BEHAVIOR BETWEEN WISTAR AND SPRAGUE-DAWLEY RATS

Katarina Vukojević*, Sanja Lovrić-Kojundžić, Damir Sapunar

Department of Anatomy, Histology and Embryology, Faculty of Medicine,
University of Split, Šoltanska 2, 21 000 Split, Croatia

* Corresponing Author

Abstract

The experience of pain is variable among certain cultures, ethnical groups and among individuals. This variability can be explained by environmental influence, genetic predisposition and plasticity of the existing neuronal pathways. The purpose of this study was to examine a strain-related difference in pain sensitivity between Wistar and Sprague-Dawley rats strains and if there was a difference, could it be overcomes with the robust test. Mechanical sensitivity e.g. existence of paw withdrawal and complex hyperalgesia-type response after needle stimuli has been measured. Both hindpaws (middle, medial and lateral part) were stimulated randomly in appropriate intervals. The results did not demonstrate statistically significant strain difference in pain sensitivity, except in the lateral part of the hindpaw where Sprague-Dawley rats were more sensitive. This data emphasize the importance of selecting a robust behavior test that will be used in investigation of peripheral nerve injury and in neuropathic pain research.

KEY WORDS: neuropathic pain, rat strain, strain differences, pain models, hyperalgesia, pain behavior

Introduction

According to accepted definition, pain is an unpleasant sensory and emotional experience (1). In comparison to other physical sensations, pain is characterized by significant subjectivity and complexity; emotional components contribute the most to this complexity. Complexity of pain sensation is the main obstacle in pain mechanism research because emotional experience is difficult to measure. A high level of variability in response to painful stimuli can be observed among certain cultures, ethnic groups, and individuals (2,3,4). There are numbers of possible reasons for this high variability in pain response, such as the plasticity of the existing neuronal pathways, genetic predisposition and environmental influences. Neural synapses are still forming after birth and traumatic surgical events during early childhood could induce hypersensitivity to pain later in life (5,6). Genetic factors underlie the considerable variation in pain sensitivity (7). The recent study showed that catechol-O-methyltransferase

(COMT) polymorphism influenced human experience of pain and may underlie interindividual differences in the adaptation and responses to pain (8). The elementary function of pain is to act as a defensive mechanism, which warns us about diverse events and protects us from personal injury. Nociceptive or physiological pain sensation is induced by strong stimuli. Contrary to this physiological pain, neuropathic pain lasts long after the injury and does not respond to usual analgesic therapy. Nerve injury in a great number of cases produces neuropathic pain, which is reflected by a high sensibility of pain stimuli (hyperalgesia), spontaneous pain and with receptivity to those stimuli that in normal situations would not produce pain (allodynia) (9). The relatively slow progress in pain research is due to the lack of good experimental animal models that can completely replicate the painful medical conditions found in human neuropathic pain. Over recent years few experimental neuropathic animal pain models have been developed (7,10,11,12,13). These models differ significantly in view of produced symptomatology and, as a result, could represent different groups of neuropathic patients (14). All these models are based on the injury in whole or a part of the sciatic nerve, because it is well-defined innervation area. Further, the term "pain" in the context of animal research should be more exactly interpreted as a "response indicative of an unpleasant experience". In experimental animals it is not possible to measure emotional components of pain, therefore it is necessary to speculate on animal painful experience. Therefore, experimental neuropathic pain models are necessary to characterize certain behaviors that should indicate the sensation of pain in animals (15). In humans, the measured response to high-intensity stimulus is a reported pain experience; whereas in experimental animals, a secondary behavioral indicator is observed. This secondary indicator can be seen in this research, as the induction of paw withdrawal to mechanical stimulus, or complex hyperalgesic reaction that involves long withdrawal latency, paw licking and vocalization. This second one is shown to be the best indicator of pain related behavior (16). Different strains of rats are often used to elucidate the pathogenesis of neuropathic pain, and due to the inadequate test procedures researchers often reported strain-related differences in baseline pain sensitivity, which could significantly interfere with the interpretation of results on neuropathic pain research (16). Hyperalgesia type-response is shown to be the robust test with high sensibility and specificity (16). Our hypothesis was that using such a robust test we could overcome false differences between rat strains. Regional differences in three parts of hindpaw (medial, middle and lateral) were also investigated in order to exclude dermatome variability. The aim of this study was to determine difference in pain sensation between Wistar and Sprague-Dawley rat strains by means of tests that were recently described as best in estimation of pain related behavior (16).

Materials and Methods

All animal procedures were in accordance with the regulations of the Animal Care Committee, Faculty of Medicine, University of Split. In this study we used male outbred rats (150 – 250 g) of the Wistar (n=52) and Sprague-Dawley (n=28) strain. Animals were obtained from Vivarium for experimental animals, University of Split, Croatia. The rats were housed in individual plastic cages in temperature-controlled environment, maintained on a 12:12 h, light-dark cycle. Before testing, the animals were habituated to the environment in which the testing was performed. The plastic cages sized 10 x 25 x 30 cm were put on a table with a metal, mesh-wire surface (3 x 3 mm) to allow access to the plantar side of the paw. Hindpaw stimulation was induced with a 22 gauge spinal needle when all four paws were on the wire floor. The needle was applied with pressure adequate to indent but not to penetrate the plantar skin. The test was conducted on both hindpaws. Each of the three areas of both hindpaws (medial, middle and lateral part) was stimulated five times, separated by at least five seconds. The withdrawal response was registered as positive if the paw was removed. Positive hyperalgesia-type reaction was noted if animal showed long-term withdrawal, vocalization, paw licking and guarding of the paw (10). All data were expressed as probability of positive response (the number of positive responses was divided with the total number of stimuli). Our sensory testing paradigm has been validated by previous study (16). The data are presented as mean and standard deviation with a confidence interval of 95 percent. The different strains were tested with non-parametric Mann-Whitney U test after the Kolmogorov-Smirnov test, which indicated that the data are not normally distributed. Multivariate analysis of variance (MANOVA) was also used with repeated measures to assess the inter-reaction of within-subject variables and between-subjects factors. This was done to see if the variability between groups was higher than variability within groups. Significance was accepted at $p<0,05$.

RESULTS

REGIONAL DIFFERENCES IN PAIN SENSATION BETWEEN STRAINS

In hyperalgesia-type reactions for the lateral part of the hindpaw a statistically significant difference (Mann-Whitney, p=0,029) was observed. The mean and standard deviation for Wistar rats were 0,03±0,09 (95 % CI=0,00-0,05), and for the Sprague-Dawley were 0,09±0,13 (95% CI=0,04-0,14) (Figure 1A). This difference indicates greater pain sensitivity in Sprague-Dawley in comparison to Wistar rats. Statistically significant differences were not found in the medial and middle part of the hindpaw between strains. In withdrawal type reactions also no statistically significant difference was found for the total hindpaws (data not shown).

TOTAL WITHDRAWAL AND TOTAL HYPERALGESIA

The mean and standard deviation of total withdrawal upon mechanical stimulus for Wistar rats were 0,56±0,25 (95%CI=0,49-0,63) and for Sprague-Dawley rats were 0,53±0,31 (95%CI=0,41-0,65). Existing differences between these strains were not statistically significant (Mann-Whitney, p=0,836) (Figure 1B). When measuring the total hyperalgesia-type reaction in all three parts of the hindpaws upon mechanical stimulus, the mean and standard deviation for Wistar rats were 0,03±0,07 (95%CI=0,01-0,05) and Sprague-Dawley rats were 0.06±0.09 (95%CI=0,02-0,09). The difference between the strains was not statistically significant (Mann-Whitney, p=0,105) (Figure 1C).

Data are presented as mean, and rely on the sum of data for left and right hindpaws. The difference in pain sensitivity between strains is statistically significant (Mann-Whitney) and it is indicated by lines connecting the bars for two strains. Total withdrawal (B) and hyperalgesia-type response (C) upon mechanical stimulus with needle in Wistar (WI) and Sprague-Dawley (SD). In both strains data for all three parts of the hindpaws (medial, middle and lateral) was taken as the mean. Differences between strains were not statistically significant. The vertical bar represents the total data range. The data show probability of the total sum responses from left and right hindpaws. The data for individual analysis of the left and right hindpaw and the differences between investigated rats' strains did not statistically differ from the sum of data.

MULTIVARIATE ANALYSIS

Statistical comparisons were also performed with multivariate analysis of variance (MANOVA) using repeated measures to validate the results. Within-subject variables were withdrawals and hyperalgesia of medial, middle and lateral parts of the hindpaw and between-subject factor was Wistar and Sprague-Dawley rat strain. With Wilks Lambda, it was confirmed that the inter-reaction among within-subject variables and between-subject factors was not statistically significant (Wilks λ=0,869, F(6, 68)=1,705, p=0,133) (Figure 2A). We used the total withdrawals and total hyperalgesia for within-subject variables and between-subject factor were rat strains again. However, this inter-reaction was not statistically significant (Wilks λ=0,950, F(2, 72)=1,902, p=0,157) (Figure 2B).

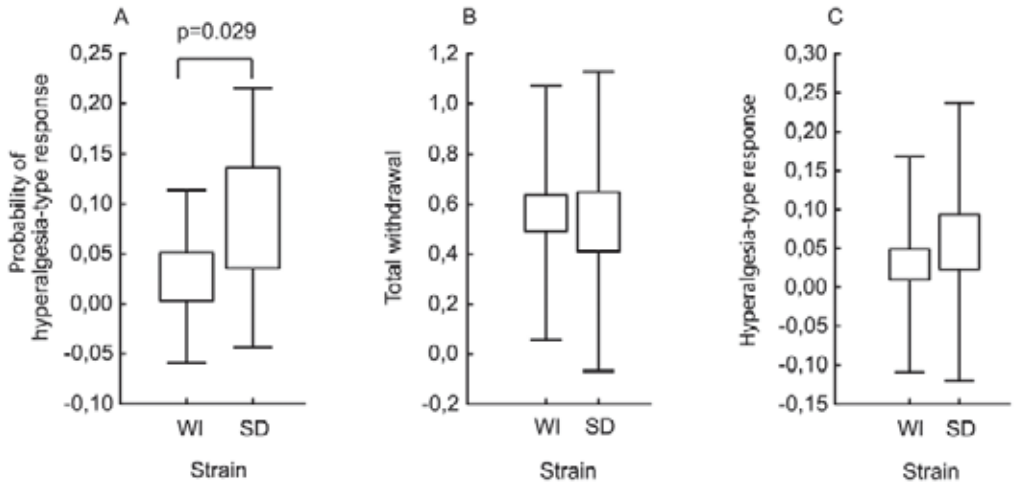
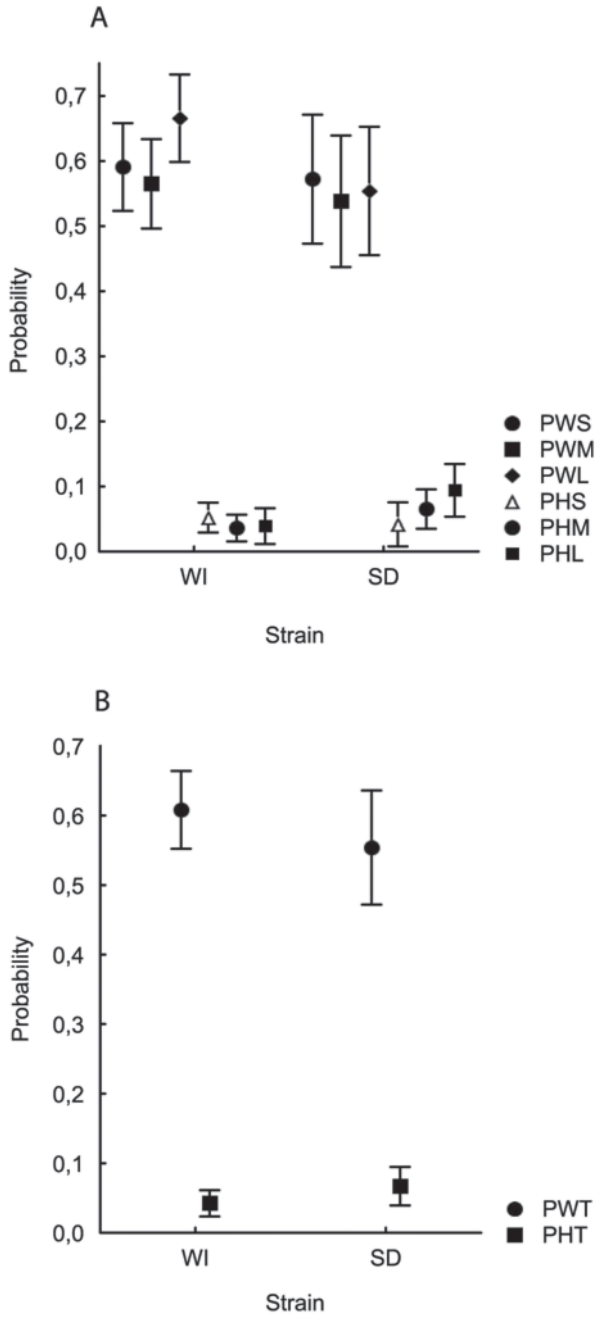


FIGURE 1. The probability of hyperalgesia-type response (A) after mechanical stimulus with needle in lateral part of hindpaw in Wistar (WI) and Sprague-Dawley (SD) rats.



Legend:

PWS- probability of withdrawal reaction in middle part of left and right hindpaw;

PWM- probability of withdrawal reaction in medial part of left and right hindpaw;

PWL- probability of withdrawal reaction in lateral part of left and right hindpaw;

PHS- probability of hyperalgesia reaction in middle part of left and right hindpaw;

PHM- probability of hyperalgesia reaction in medial part of left and right hindpaw;

PHL- probability of hyperalgesia reaction in lateral part of left and right hindpaw;

PWT- probability of total withdrawal reaction of left and right hindpaw;

PHT- probability of total hyperalgesia reaction of left and right hindpaw.

FIGURE 2. Multivariate analysis of variance with repeated measures using within-subject variables (A) PWS, PWH, PWL, PHS, PHM, PHL and (B) PWT, PHT, and between-subject factor (WI, SD) shows that inter-reactions are not statistically significant. The vertical bar presents total data range.

Discussion

Our study showed no statistically significant differences in pain sensation between Wistar and Sprague-Dawley rat strain, with the exception of hyperalgesia-type reaction of the lateral part of hindpaws where Sprague-Dawley rats were more pain sensitive to mechanical stimuli. Our findings showed that a robust behavior tests are necessary for better interpretation and comparison of results in different neuropathic pain research. However, we studied only one factor, which should be taken into consideration when planning this kind of research. Upon reviewing literature it was noted that other parameters, such as laboratory conditions, genetics, sex, type of used tests, the surface on which rat stands, may also influence baseline pain sensitivity(17-20). Previous research on pain sensitivity often noted differences in various rat strains. In most cases these difference were attributed to genetic or environmental factors (21). One of the contributing factors is the rat strain. Therefore, it is necessary to determine the strain influence on the outcomes of the experiments (21). Strain differences were often noticed in studies that did not use reliable tests (16). As a result, there is a need for more reliable testing to overcome strain differences and other influencing factors that could affect the research outcome.

In this study, the existence of strain-related differences in pain sensitivity was not confirmed. The reason for this could be sought in quality of withdrawal and hyperalgesia tests. Mechanically stimulated withdrawal tests were shown not to be specific enough (16). Withdrawal was a segmental flexion reflex, connected to tactility and exists despite decerebration, spinal injury or in general anesthesia, which all excludes painful experiences (22). This flexion reflex alone is not decisive enough as a reaction to determine the existence of pain. In humans this reflex may appear significantly under pain thresholds and changes in the flexion reflex do not necessarily indicate pain sensation (23,24). To be more accurate, von Frey withdrawal test may cause itching or tickling rather than a pain sensation (25). However, hyperalgesia types of response, that include prolonged withdrawal, vocalization, paw licking, and paw guarding, could be considered a strong indicators that exclude, as seen in this study, influence of different strains of animals on the outcome of the experiments. This is especially important for neuropathic pain models involving animals, because hyperalgesia-type response can truly be considered a pain response, and can be considered the most important condition for satisfying such models of study. The value of the hyperalgesia test was confirmed by Hogan and al. showing 60 percent sensibility and 90 percent specificity (16). The only statistically significant strain difference was found between lateral parts of hindpaws in hyperalgesia type responses, where Sprague-Dawley rats showed higher pain sensitivity than Wistar rats. This difference could be a result of different dermatome innervation on the lateral part of hindpaws. In rewieving literature this part of the hindpaw often demonstrated higher pain sensitivity because it is the area with hair overgrowth with its own innervation (16). With the development of new neuropathic pain research methods, it has been discovered that some animal models cannot fully show neuropathic pain symptoms. For example, symptoms like the appearance of glow and itching in chronic constriction injury models are more often than pain alone, but these symptoms can not be fully understood as specific response in animal behavior (26). Respectively, behaviors noticed in this model do not necessarily mean pain, they might represent other signs such as paresthesia and dysethesia (27). This became especially important when assessing the efficacy of some analgetics when testing was determined to be ineffective. Considering further the problems in neuropathic pain research the issue of investigator bias must be mentioned. This is especially possible in tests that do not have enough confidence in characterizing certain behaviours as painful. For example, in the Hargreaves box or the von Frey test, where hindpaw withdrawal was noted, the animal could withdraw the paw to walk, groom itself, or make postural adjustments, while some investigators wished to confirm this behavior as response to testing, thus becoming subjective bias (28). According to this, false results in neuropathic pain research may happen due to bias, or inadequate methods. As earlier explained, withdrawal is flexion response under a pain threshold and because of this it is not an appropriate sign of the existence of pain. Neuropathic pain models require development of behavior tests that can reliable recognize differences in pain related behavior between experimental animals.

Conclusion

1. Hyperalgesia-type response reveals no difference in pain-related behavior between Wistar and Sprague-Dawley rat strains.
2. Hyperalgesia-type response is the robust test which could overcome false differences between rat strains.
3. In hyperalgesia reaction, the lateral parts of hindpaws in Sprague-Dawley rats were found to be more sensitive to pain than in the Wistar strain.

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