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Serotonergic modulation of pain and analgesic responses: A study in rats with constitutionally altered serotonin transporters

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Abstract

Background: A role of the serotonin (5HT) transporter, a key regulator of serotonergic transmission, in the physiology, pharmacology and genetics of pain responses has been proposed recently. The present study aimed to explore the impact of constitutive differences in the activity of the serotonin transporter, and 5HT homeostasis in general, on the modulation on pain sensitivity and analgesic responses to drugs that utilize 5HT mechanisms.
Methods: A novel genetic animal model, Wistar-Zagreb 5HT rats, obtained by selective breeding of animals for extreme activity of the platelet serotonin transporter was used. As a consequence of breeding, two sublines of this model, termed high-5HT and low-5HT, differ in both central and peripheral serotonin homeostasis. Thermal pain sensitivity of 5HT sublines was assessed at baseline and following administration of analgesic drugs, as determined by paw withdrawal latency to radiant heat stimulation.
Results: Animals from 5HT sublines show differences in both basal pain sensitivity and analgesic responses. Rats with the low-5HT phenotype displayed decreased baseline paw withdrawal latencies (hyperalgesia) in comparison to their high-5HT counterpart (25%; $p < 0.001$). They also showed better analgesic response to acute and prolonged treatment with tramadol ($p = 0.027$) and clomipramine ($p = 0.019$), respectively, whereas administration of fluvoxamine did not produce an analgesic effect in either 5HT subline.
Conclusions: These findings support the idea that functionality of the serotonin transporter is one of the physiological/genetic determinants of individual differences in pain responses and modulation. They also validate Wistar-Zagreb 5HT rats, with constitutionally up-regulated/down-regulated serotonin transporter, as a potential new genetic model for studying serotonergic modulation of pain responses.

1. Introduction

The role of serotonin (5-hydroxytryptamine, 5HT) systems in modulating nociception has long been recognized, and increased activity of brain serotonergic neurons is associated with analgesia and enhanced antinociceptive drug potency (Sommer, 2010; Loyd et al., 2013). Recently, it has become clear that 5HT, acting centrally and/or peripherally, exerts both algesic and analgesic effects, depending upon the site

What's already known about this topic?
- The possibility that functioning of serotonin transporter modulates individual pain response was proposed.

What does this study add?
- Lower efficiency of serotonin transporter is associated with higher pain sensitivity and better analgesic response in rats.
- Results provide evidence that serotonin transporter activity is physiological/genetic determinant of individual differences in pain response.
- Rat sublines with constitutionally up-regulated/down-regulated serotonin transporter may represent a new genetic model for studying serotonergic modulation of pain response.

of action, nature of nociceptive stimuli and subtypes of activated 5HT receptors (Bardin, 2011; Viguier et al., 2013). The possibility that, in addition to 5HT receptors, the serotonin transporter (5HTT) has a role in pain responses has also been proposed (Young et al., 2012).

5HTT is a membrane protein that actively takes released serotonin back to the presynaptic nerve terminal. In addition to being a crucial regulator of serotonergic transmission, 5HTT represents the main target of several classes of psychoactive substances, some of them having significant antinociceptive potency. Based upon the association between human 5HTT gene polymorphisms and pain threshold/modulation (Lindstedt et al., 2011a,b; Palit et al., 2011; Treister et al., 2011), functionality of the *5HTT* gene has been proposed as one of the genetic determinants of pain phenotype. Thus, individuals with the lower 5HTT expression were associated with increased risk of developing chronic pain pathologies (Offenbaecher et al., 1999; Park et al., 2004; Wise et al., 2007). In contrast, they exhibit reduced sensitivity for heat and cold pain (Lindstedt et al., 2011b). As for analgesics, low 5HTT-expressing individuals seem to exhibit a better response to opioid drugs (Kosek et al., 2009).

The role of 5HTT in nociceptive response was documented also in animals. Mice with a genetically inactivated *5HTT* gene have reduced thermal hyperalgesia in neuropathic and inflammatory pain models (Vogel et al., 2003; Palm et al., 2008; Chen et al., 2011) and show decreased opioid-induced analgesia (Fox et al., 2009). In the present study, we have examined the role of constitutional functionality of 5HTT protein in the modulation of pain behaviour using another genetic animal model, Wistar-Zagreb 5HT rats, that has been developed by selective breeding of animals for extreme values of two platelet 5HT parameters: granular 5HT level and activity of 5HT transporter (Cicin-Sain et al., 1995, 2005). Since 5HTT proteins on platelet and neuronal membranes are encoded by the same gene, two sublines of this model (termed high-5HT and low-5HT sublines) differ in both peripheral and central 5HT homeostasis, as shown by neurochemical and pharmacological studies. Thus, animals from the high-5HT subline have higher 5HT levels in blood and several body organs, higher brain extracellular 5HT concentration, especially after pharmacological challenges, higher brain 5HT turnover and *in vivo* recovery of hippocampal 5HT, in comparison with animals from the low-5HT subline. A battery of behavioural tests also gave confirmation of differential serotonergic phenotype of 5HT sublines (Cicin-Sain and Jernej, 2010 and references herein). We hypothesized that constitutional alterations in functionality of 5HT systems would also result in differences in pain sensitivity between 5HT sublines and in responses to analgesic drugs (fluvoxamine, citalopram, tramadol) that use 5HT mechanisms.

2. Methods

2.1 Animals

Studies were performed on 5HT sublines of Wistar-Zagreb 5HT rats developed by selective breeding for extreme values of serotonin levels and activity of serotonin uptake in platelets at the Rudjer Boskovic Institute (Zagreb, Croatia). All experiments were approved by the institutional and national (Ministry of Agriculture, Republic of Croatia) ethical committees and were conducted in accordance with the ILAR Guide for the Care and Use of Laboratory Animals and Croatian animal protection law (NN 135/06 and 37/13).

Development of 5HT sublines has been described previously (Jernej and Cicin-Sain, 1990; Cicin-Sain et al., 1995; Jernej et al., 1999a). Briefly, male and female rats with the highest and lowest values of platelet 5HT parameters, respectively, were mated together to generate high-5HT and low-5HT sublines. Divergence of mean values of platelet 5HT parameters stabilized after 5–6 generations at about 70% (low-5HT subline) and 150% (high-5HT subline) of the mean value of the starting population. Selective breeding was restarted *ab ovo* several times during the past decade with essentially the same dynamic of divergence and final range of differences between sublines, confirming reproducibility of the selection process. Different levels of 5HT in platelets from 5HT sublines are a consequence of inherited differences in the maximal velocities of 5HT uptake from the surrounding plasma into platelets (Cicin-Sain et al., 2005),

reflecting differences in the number of membrane 5HT transporters between 5HT sublines. Analogous differences are present also at the level of functional 5HT transporter protein in the plasma membrane (Hranilovic et al., 2001) and 5HTT mRNA level (Jernej et al., 1999a).

In the present study, male animals from F13 generation of selective breeding, aged approximately 4 months, were used. They were housed three per cage under controlled conditions of temperature (23 ± 2 °C), humidity (55 ± 10%) and light cycle (12 h light/12 h dark) with food (Mucedola, Milan, Italy) and water *ad libitum*.

2.2 Biochemical determinations

Platelet 5HT parameters were determined in all offspring of F13 generation of high-5HT and low-5HT sublines at 5–6 weeks of age, and animals differing approximately twofold in their platelet serotonin level and uptake were selected for further experiments. Methods for repetitive rat blood sampling, preparation of platelet-rich plasma and determination of platelet serotonin level (spectrophotofluorimetrically) and platelet serotonin uptake (radiochemically) were described previously (Jernej et al., 1988, 1999b).

2.3 Behavioural testing

Pain sensitivity of animals was assessed by measuring the paw withdrawal latency (PWL) to the radiant heat using the Hargreaves apparatus (Ugo Basile, Italy). Each rat was used on two occasions: the first day (5-min stay in the apparatus) served to adapt animals to the testing device and 24 h later, tests for baseline pain sensitivity were performed. After the 5-min adaptation in the apparatus, a radiant heat source (intensity: 235 mW/cm^2; glass plate temperature: 50 °C) was focused on the plantar surface of the hindpaw. Time from the activation of the heat source to the paw withdrawal was measured automatically with the cut-off of 30 s. Each test consisted of at least two presentations of each hindpaw with the 5-min interval between trials on the same paw. There were no side-dependent variations in the response, so individual PWL values were determined as the mean of measurements on both paws.

The presence of paw licking behaviour following withdrawal of the paw was also recorded. It was scored as 1 (licking the paw after its withdrawal) or 0 (paw withdrawal alone) for each trial and response was averaged for each animal.

Testing conditions were standardized as much as possible according to literature recommendations (Mogil, 2007). Behavioural testing was performed between 11:00 a.m. and 2:00 p.m., in a separate room adjacent to the colony room, with minimal background noise. Three animals (cage-mates) at a time were placed in the test apparatus, one in each of the three boxes, and after each animal, the apparatus was cleaned with 70% ethanol to eliminate any residual odours. In all experiments, animals from high-5HT and low-5HT sublines were evaluated alternately.

2.4 Drugs and drug administration

To compare 5HT sublines for their nociceptive response, we used drugs shown to be effective in the treatment of various pain conditions and whose nociceptive actions are known to include (potentiate) 5HT systems: tramadol (opioid drug with serotonergic/noradrenergic effects), clomipramine (tricyclic antidepressant with non-selective effects on serotonin/noradrenalin re-uptake) and fluvoxamine (selective serotonin re-uptake inhibitor). Drug doses that were shown to be effective in thermal nociceptive tests were selected according to the literature (Rojas-Corrales et al., 2003; Schreiber and Pick, 2006; Xie et al., 2008). The drug effect on the nociceptive response was examined after acute (tramadol, fluvoxamine) or prolonged (clomipramine) drug treatment. The effect of tramadol (Belupo, Koprivnica, Croatia) and fluvoxamine (Sigma-Aldrich Chemie Gmbh, Munich, Germany) was measured 25 and 30 min after a single intraperitoneal (i.p.) injection at doses of 20 and 10 mg/kg, respectively. Individual PWL values were calculated as the mean of all trials on both paws, and group means were compared with the baseline values of the respective animal groups. Clomipramine (Sigma-Aldrich) was administered by i.p. injections for 21 days, in a daily dose of 15 mg/kg, and the effect of treatment was obtained after the last injection by comparison of PWL values in treated groups with values of respective control groups receiving saline. All drugs were dissolved in 0.9% saline immediately before administration and applied in a volume of 0.5 mL/100g of body weight.

2.5 Skin temperature measurements

To exclude the possible influence of skin temperature on the paw withdrawal or the paw lick (Hole and Tjolsen, 1993), hindpaw skin temperature of animals was measured by means of thermography. The animal was gently grabbed around the chest area, the plantar surface of the hindpaw was exposed and thermographic images of the region of interest were obtained by the highly sensitive infrared thermal imaging camera (T335, FLIR Systems, Inc., Wilsonville, OR, USA). ThermoMED software (Kolaric et al., 2006) was used for thermal analysis and image presentation of temperature values of surfaces inside the thermographic scan (Supporting Information Fig. S1).

2.6 Statistical analysis

Data were analysed using GraphPad Prism, version 5.00 for Windows (GraphPad Software, San Diego, CA, USA; http://www.graphpad.com). The normality of data distribution was checked using D'Agostino & Pearson omnibus normality test and homogeneity of variances by Bartlett's test. Data were further analysed with Student's two-tailed unpaired *t*-test with Welch's correction if variances were significantly different. Categorical variables were analysed using the Fischer's exact test. The relationship between behavioural and biochemical measures was evaluated by the Pearson correlation

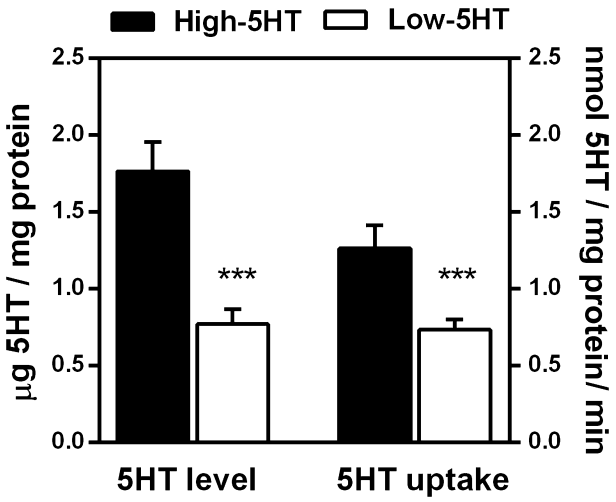

Figure 1 Platelet serotonin (5HT) level, expressed as μg 5HT/mg platelet protein, and velocity of platelet serotonin uptake, expressed as nmol 5HT/mg platelet protein/min, in male animals from high-5HT and low-5HT sublines. Means ± SD, $n = 21$/group, $^{***}p < 0.0001$, Student's two-tailed *t*-test.

coefficients. Results are expressed as individual values or group means ± SD. Differences were considered statistically significant if $p < 0.05$.

3. Results

3.1 Platelet 5HT measures

Values of platelet 5HT measures in male animals used for the measurement of basal nociception are shown in Fig. 1. Animals from high-5HT and low-5HT groups differed by approximately twofold in platelet serotonin level and velocity of platelet serotonin uptake.

3.2 Basal nociception

Individual baseline values for heat sensitivity in animals from the 5HT sublines are shown in Fig. 2. The low-5HT animals respond more quickly to the thermal stimulus than the high-5HT animals (basal nociception: PWL, high-5HT: 16.1 ± 3.51 s; low-5HT: 12.6 ± 2.67 s; $p < 0.001$). Correlation analysis showed positive relationship ($r = 0.494$; $p = 0.0009$; $n = 42$) between platelet 5HT level and PWL (Fig. 3), indicating physiological interrelation between these two measures. The number of trials in which paw licking occurred, expressed in percentage of all trials, was approximately 40% and did not differ between 5HT sublines.

3.3 Hindpaw skin temperature

No differences in the hindpaw skin temperature were observed between animals from the 5HT sublines (high-5HT: 27.8 ± 2.36 °C; low-5HT: 28.8 ± 2.61 °C), indicating that variations in skin temperature did not influence the thermal tests. Representative infrared thermographic images of the hindpaw skin from high-5HT and low-5HT rats are given in Supporting Information Fig. S1.

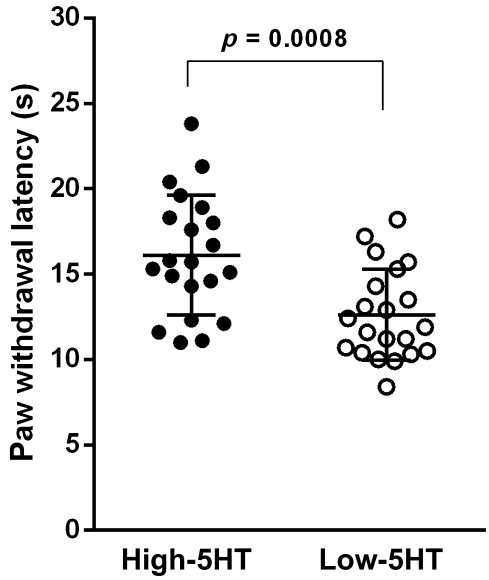

Figure 2 Individual baseline paw withdrawal latency (in seconds) in response to radiant heat in male animals from the high-5HT and low-5HT sublines. Mean values ± SD are also indicated. $n = 21$/group. *p* obtained by Student's two-tailed *t*-test.

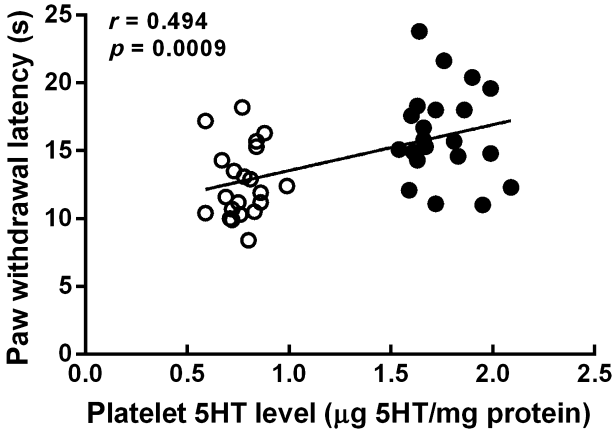

Figure 3 Correlation of paw withdrawal latency (in seconds) and platelet serotonin level (μg 5HT/mg platelet protein) in animals from high-5HT (solid circles) and low-5HT (open circles) sublines. *r* = coefficient of correlation. $n = 42$.

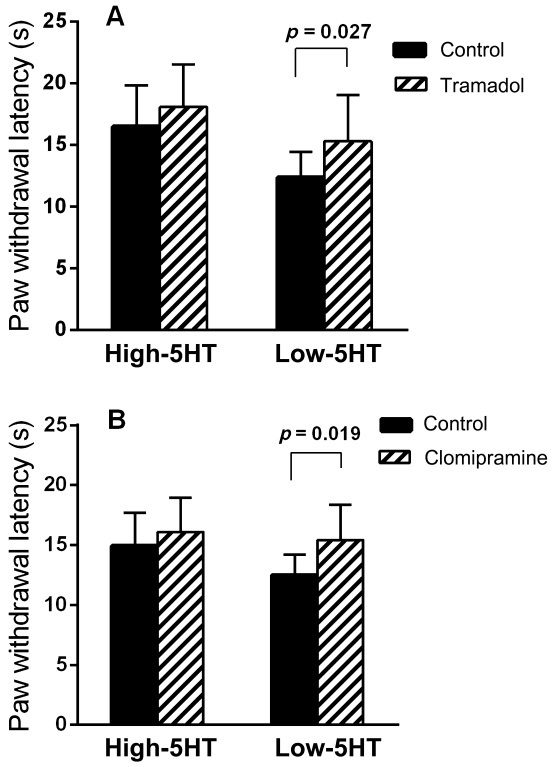

Figure 4 Paw withdrawal latency (in seconds) to heat after systemic treatment of male rats from the high-5HT and low-5HT sublines with tramadol (20 mg/kg, n = 12/group (A), and clomipramine (15 mg/kg, n = 9/group (B) or saline (n = 9–12/group). Each column represents group mean ± SD. p obtained by Student's two-tailed t-test.

3.4 Pharmacological studies

The thermal sensitivity of animals from 5HT sublines following administration of drugs that use 5HT mechanisms in their analgesic actions (selective or mixed-action) is shown in Fig. 4.

Acute administration of tramadol (opioid receptor agonist and serotonin/noradrenalin re-uptake inhibitor) led to the significant elevation of PWL values to the thermal stimulus in treated animals from the low-5HT subline, whereas in the high-5HT subline, only a tendency to increased PWL values could be demonstrated, as compared to the corresponding controls (low-5HT: 24% differences, $p = 0.027$; high-5HT: 10% differences, non-significant) (Fig. 4A). Essentially the same results were obtained following the prolonged administration of clomipramine (non-selective serotonin and noradrenaline re-uptake inhibitor) (Fig. 4B). Increase of PWL values in clomipramine-treated animals was of similar magnitude as for tramadol treatment and differences in the pain sensitivity between treated and control groups were significant again only in animals from the low-5HT subline ($p = 0.019$) (Fig. 4). Administration of fluvoxamine (selective serotonin re-uptake inhibitor) did not produce any changes in PWL to the thermal stimulus. There was no effect on licking behaviour following paw withdrawal regardless the 5HT subline (data not shown).

4. Discussion

The possibility that pain behaviour is modulated by the endogenous efficiency of the 5HTT (Vogel et al., 2003; Palm et al., 2008; Hansen et al., 2011) prompted us to investigate this interrelationship in a novel genetic rat model consisting of two sublines with constitutional differences in platelet 5HTT activity. As mentioned in the Introduction, animals from the high-5HT subline are regarded as hyperserotonergic in relation to the low-5HT subline and vice versa (Cicin-Sain and Jernej, 2010). In a preliminary study exploring pain sensitivity to mechanical stimuli in 5HT sublines, we have observed that the low-5HT rats showed tendency (25% difference, non-significant) to lower basal pain threshold to tail pressure as measured by analgesy-meter (Tvrdeic et al., 2004). In line with these results, the present study showed that animals from the low-5HT subline are more sensitive to thermal stimuli at baseline, and that they respond better to analgesic drugs that use 5HT as part of their mechanism of action.

The mechanisms accounting for the observed differences are not clear at present. Serotonin has complex modulatory roles in the physiological control of pain messages: in the periphery, it reinforces pain, whereas in the central nervous system, it exerts primarily inhibitory, but also excitatory, effects on the nociception (Bardin, 2011; Loyd et al., 2013; Viguier et al., 2013). In our 5HT sublines, the entire 5HT homeostasis is changed due to lifelong alteration of 5HTT, and we could speculate that the regulation of their nociceptive systems may be tuned somehow to a different level, both centrally and/or peripherally, leading to the observed differences in the pain threshold. Generally, central 5HT acts as an activator of descending pain inhibitory system, so a relative deficit in 5HT system activity in the low-5HT animals, in comparison with their counterparts, may facilitate transmission of pain stimuli and vice versa, increased 5HT tone in the high-5HT animals may led to higher activation of this system and could be pain protective in physiological conditions (as observed in this study).

In line with 5HT receptor influences on pain modulation, the observed difference in pain threshold between 5HT sublines might be due to differential

adaptation of 5HT receptors, specifically 5HT1A/1B subtypes that mediates inhibitory control of heat-evoked nociceptive message (Kayser et al., 2007), but also other subtypes involved in the regulation of normal nociception (Loyd et al., 2013). Our previous studies indicate that 5HT1A receptors are differentially regulated in WZ-5HT sublines upon behavioural stimulation (Bordukalo-Niksic et al., 2010).

In contrast to PWL, the extent of licking behaviour after paw withdrawal did not differ between 5HT sublines. Since PWL to thermal stimuli targets mainly spinal mechanisms (Kayser et al., 2007), and paw lick/flinch response depends mainly upon supraspinal mechanisms (Dennis et al., 1980), these results are compatible with the assumption that 5HT systems might have different impacts in processing various behavioural manifestations associated with pain (Palit et al., 2011).

As to peripheral effects, in the model of transient pain used here, it is not very likely that endogenous differences in 5HT level contribute to differences in pain sensitivity between 5HT sublines. It is known that 5HT released from platelets following initial cell injury activates nociceptors and exerts proalgesic effects acting directly or through facilitation of other peripheral pain mediators (Sommer, 2010; Loyd et al., 2013). However, a brief, high intensity thermal stimulus, as applied here, induces little tissue damage (Dray, 1995) and, although some local 5HT release has been shown in both primary and secondary areas after exposure to a radiant heat (Sasaki et al., 2006), increase in tissue 5HT in these conditions is considerably lower than after pain conditions producing peripheral inflammation (Nakajima et al., 2009). 5HT, rapidly released during inflammation, sensitizes afferent nerve fibres contributing thus to hyperalgesia and nerve injury (Sommer, 2004). In 5HTT knockout mice, thermal hyperalgesia was reduced in inflammatory model of persistent pain (intraplantar injection of complete Freund's adjuvant) and could be re-established by the injection of 5HT (Palm et al., 2008). Therefore, it might be expected that in our 5HTsublines, differing twofold in the platelet 5HT levels, modulation of analgesia by peripheral 5HT mechanisms come more into play under inflammation-induced platelet release, but this remains to be investigated in future studies. The possibility that 5HT, as a vasoactive molecule, differentially affects surface skin temperature and, through effect secondary to this, thermal pain perception, was excluded by thermographic measuring of hindpaw skin temperature.

It has previously been shown that individual differences in the locomotor activity and/or emotionality could influence some of the responses in nociceptive tests (Rhudy and Meagher, 2000; Sommer, 2004; Kayser et al., 2007) and this possibility should also be considered in 5HT sublines. In previous behavioural studies (open field, elevated plus maze, zero maze, rotarod, Morris water maze), we have obtained several measures that can be used as a locomotor index of high-5HT and low-5HT animals. They mostly indicate lack of significant differences in locomotion between 5HT sublines, but there are also some measures (e.g., speed) where the low-5HT animals performed better (Bordukalo-Niksic et al., 2010; Cicin-Sain and Jernej, 2010). As to emotionality, we have previously shown that high-5HT rats display more anxiety-related behaviour across several tests (Hranilovic et al., 2005; Bordukalo-Niksic et al., 2010), so potential interlink of pain and anxiety, as a confounding factor, cannot be excluded. A number of studies suggest that negative affective states such as anxiety are associated with enhanced pain and pain perception threshold in humans, although opposite results were also reported (Rhudy and Meagher, 2000; Creech et al., 2011). Rats bred for high anxiety display lower sensitivity to thermal pain as compared to those bred for low anxiety (Jochum et al., 2007), and our findings are compatible with this report. In this context, it is noteworthy that our Wistar-Zagreb 5HT rat model might provide a new experimental tool for studies on 5HT modulation of the interrelation between pain perception and other 5HT-related behaviours, such is emotionality.

In contrast to our findings, studies of mice with complete deficiency in 5HTT (5HTT knockout) showed no changes in their baseline heat sensitivity as compared to paired wild-type mice. However, they have attenuated thermal hyperalgesia in neuropathic models (Vogel et al., 2003; Palm et al., 2008; Chen et al., 2011; Hansen et al., 2011), suggesting different roles for 5HT in nociceptive transmission in injured versus naive animals (Vogel et al., 2003). The lack of alterations in baseline pain sensitivity of 5HTT knockout mice, which was unexpected, was proposed to result from compensatory changes of 5HT receptors in response to their reduced 5HT levels (Vogel et al., 2003; Hansen et al., 2011). In contrast to complete loss of 5HTT activity in 5HTT knockout model, our 5HT sublines represent an animal model of physiological extremes in 5HTT activity, which confer serotonergic system changes. A differential net effect on 5HT (and other neurotransmitter systems) is therefore reasonable to expect in these two genetic animal models. It could be assumed that Wistar-Zagreb 5HT rats mimic naturally occurring situations better than knockout mutants, and this is of particular importance when studying naive animals.

Pharmacological experiments explored whether baseline differences in pain sensitivity between 5HT sublines are paralleled by differences in their analgesic response to drugs acting, at least partly, through the inhibition of 5HTT. In contrast to fluvoxamine, which is selective for 5HTT, clomipramine and tramadol have additional mechanisms of action through noradrenergic and/or opioidergic system (Fuller et al., 1978; Claassen, 1983; Driessen and Reimann, 1992). It has been shown in humans that agents that enhance extraneuronal concentrations of both serotonin and noradrenalin are more efficient analgesics than selective serotonin re-uptake inhibitors (Briley and Moret, 2008; Lee and Chen, 2010) and our findings are in line with these reports. There is also a possibility that observed drug effects represent reversal of hyperalgesia in the low-5HT sublines. Notwithstanding, it seems that the intensity of analgesic effect might be modulated by the efficiency of 5HTT. Report on the correlation between analgesic response and baseline pain sensitivity is, to the best of our knowledge, the first one linking these two pain-related phenotypes, and according to our results, 5HTT efficiency might be one mechanism forming the basis of this relationship.

Findings that better responses to drugs that use 5HT mechanisms in their analgesic actions might be associated with lower 5HTT activity are in line with recent clinical study suggesting that individuals with a genotype coding for low 5HTT transcriptional efficacy have greater analgesic response to an exogenous opioid drug than individuals with a genotype coding for high 5HTT expression (Kosek et al., 2009). Our results, although obtained in an animal genetic model, open the possibility that measuring of platelet 5HT level/uptake may have clinical value as an indicator of an individual response to pain treatment.

In summary, the present study provides evidence for the role of 5HTT functionality in modulating pain-related behavior s. Results obtained on a novel genetic rat model indicate that physiologically higher 5HT tone is pain protective (as shown previously in studies using pharmacological increase in 5HT transmission), and that constitutional differences in 5HTT activity might contribute to the individual variability in analgesic response as suggested previously by studies on 5HTTLPR polymorphisms.

Author contributions

L.C-S. designed the study and wrote the manuscript; M.K. managed animal selection and breeding, carried out the experiments and participated in the first version of the manuscript; D.K. performed thermographic measurements and analyses; A.T. contributed to the study design and critically revised the manuscript; and R.S. contributed to animal breeding/selection. All authors discussed the results, commented the manuscript and approved the final version.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Thermographic images of the hindpaw skin from the high-5HT (upper) and low-5HT (lower) rats obtained by a digital infrared camera (T335, FLIR Systems, Inc., USA) are shown. Images on the right side illustrate part of the surface inside the thermographic scan which was analysed. Characteristics of the thermal imaging camera: uncooled focal plane array detector (micro bolometer) with geometric resolution of 76800 pixels per picture (320 × 240); spectral range from 8 to 14 μm; temperature range between –20 and 650 °C; minimum detectable temperature resolution (NETD) < 0.05 °C at 30 °C. FLIR ResearchIR software was used for remote control and transfer of data from the camera to a computer. For thermal analysis and image presentation of temperature values inside the thermographic scan the ThermoMED software (Kolaric et al., 2006) was used.