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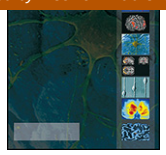

BDNF as an effect modifier for gender effects on pain thresholds in healthy subjects

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ABSTRACT

BDNF is an important marker of neuronal plasticity. It has also been associated with pain processing. Increased BDNF levels are observed in chronic pain syndromes. In order to understand the role of BDNF associated with other factors such as gender on experimental pain we aimed to determine whether experimental heat or pressure pain threshold is correlated with brain derived neurotrophic factor (BDNF) level, gender and age. Heat pain threshold and pressure pain threshold were measured in 49 healthy volunteers (27 females). The multivariate linear regression models (on heat and pressure pain thresholds) revealed a significant effect of gender ($p = 0.001$ for both models), serum BDNF ($p < 0.004$ for both models) and interaction between BDNF and gender (<0.001 for both models). In fact, when adjusting for BDNF levels and age, heat and pressure pain thresholds were significantly reduced in women as compared to men ($p < 0.001$ for both models). These effects were not observed when gender was analyzed alone. These finding suggests that experimental heat and pressure pain threshold is gender-related and BDNF dependent. In fact BDNF has a facilitatory effect on pain threshold in females but has an opposite effect in males; supporting the notion that BDNF is an effect modifier of the gender effects on pain threshold in healthy subjects.

1. Introduction

Brain derived neurotrophic factor (BDNF) is an important marker and modulator of neural activity and NMDA receptor dependent neuronal plasticity in ascending and descending pain transmission pathways [23]. In animals, BDNF and its TrkB receptor are increased in models of bladder inflammation and nerve injury [29,45]. In humans, increased BDNF levels are associated with pain in chronic pancreatitis [51]. In addition, when neutralizing BDNF by anti-BDNF antibody, or TrkB receptor, mechanical allodynia [50] and thermal hyperalgesia were relieved [8].

Furthermore, pain-related BDNF activity seems to be related to gender. In rats BDNF appears to worsen visceral pain in females while the opposite effect in males is observed [21]. In addition, in previous studies, BDNF was associated with a specific effect on depression-like behavior in rats, suggesting that there may be a sexual dimorphism in BDNF function [26]. Though there is evidence of gender-specific BDNF effects in animals; it remains to be determined whether similar mechanisms are observed in humans specially when associated with pain modulation.

Gender effects of behavioral pain have been extensively documented. It is well known that females experience more pain than male in various pain states [5,15,30]. For example, prevalence of various visceral pain disorders, such as chronic pelvic pain and irritable bowel syndrome, is more prevalent in women than men [5]. Similar effects have been demonstrated for experimental thermal pain [6], electrical pain [44], mechanical pain [16,19], ischemic pain [22] and in some types of chronic pain such as in fibromyalgia [12] and migraine [3,47]. Moreover, gender differences in perceived pain to a standardized painful stimulus show that this pain induces differential cortical activity in mid cingulate cortex [13], prefrontal cortex, insula, and thalamus [31] indicating overall that women show increased activity across brain regions associated with medial pain pathway processing. Because this pathway is

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thought to code for affective/motivational components of the pain experience [33,34], increased activity here supports the notion that gender differences in perceived pain are attributable to an affective (state-sensitive) recoding of pain-related afferent signaling.

In summary, previous evidence consistently supports the idea that BDNF contributes to the development and sustainability of various pain states due to its critical role in plasticity-related processes in pain pathways, both at spinal and supraspinal levels [25]. Given animal data, BDNF may also have differential gender effects in pain processing in humans. We therefore aim to investigate whether there is a gender difference in experimentally induced heat and pressure pain thresholds, and then determine whether these differences are explained by differences in BDNF levels.

2. Subjects and methods

The study protocol was approved by the Ethics Committee at the institution in which the work was carried out and was conducted in accordance with the Declaration of Helsinki. All participants signed an informed consent form. Forty-nine healthy volunteers (27 females) aged from 22 to 40 participated in this study. We excluded subjects with peripheral nervous system diseases or any clinically relevant clinical condition and subjects using medication that could affect the sensory perception, such as neuropsychotropics and analgesic drugs. Finally female subjects were required to have normal menstrual cycles and were not pregnant.

2.1. Dependent variables

The dependent variables of interest were heat and pressure pain thresholds. To assess thermal threshold, we determined the quantitative heat pain thresholds using the method of limits on a computer Peltier based device thermode, size 30 mm × 30 mm. It was attached to the skin on the ventral aspect of the subjects' midforearm. After a trial-run to familiarize the volunteers with the procedures, we performed the test series. Subjects were always assessed by the same researcher, who systematically read the instructions and explained the standardized experimental procedure using a modified previously published protocol [37] for quantitative sensory testing (QST) assessments.

The baseline temperature was set initially at 32 °C and was increased at a rate of 1 °C/s, to a maximum at 52 °C. The participant was asked to press a button as quickly as possible when stimulation became warm or painful. Three assessments were taken with an interstimulus interval of 40 s [42] and thresholds were calculated by taking the average temperature of the three assessments. The position of the thermode was altered slightly between trials (although it remained on the left ventral forearm) to prevent either sensitization or response suppression of cutaneous heat nociceptors.

Pressure pain detection thresholds (PPDT) values were quantified using a Fisher's pressure algometer with a surface area of 1.0 cm^2 (Pain Diagnostics and Thermography, Great Neck, NY). Prior to the test trial, the subject was instructed to verbally report perception of the pain onset. During the testing session, the tip of the algometer was applied over the tibial surface. The pressure was gradually increased at a rate of 1 kgf/s. The average values of PPT in kgf/cm^2 (lb/cm^2) of three successive readings taken at intervals of 3–5 min were used as outcome.

Subjects were asked to say 'stop' immediately when a discernible sensation of pain, distinct from pressure or discomfort, was felt. At this point, the experimenter immediately retracted the algometer [7]. The digital display continued to show the value of pressure applied at the moment the algometer was retracted.

Table 1
Characteristics of the sample ($n = 49$).

	Male ($n = 22$)	Female ($n = 27$)	p
Age (years)	25.40 ± 5.37	27 ± 7.53	0.18
State-anxiety at baseline	20.98 ± 4.11	20.00 ± 4.13	0.55
Trait-anxiety	18.55 ± 3.96	18.57 ± 4.70	0.91
Depressive symptoms on Beck inventory	3.35 ± 2.94	3.11 ± 2.99	0.91

2.2. Independent variables

(a) The blood level of BDNF was one of the independent variables of interest. Blood samples were collected from all subjects before the experiment. Blood samples were centrifuged in plastic tubes for 10 min at 4500 × *g* at 4 °C, and serum was stored at −80 °C for hormone assays. Serum BDNF was determined using a *ChemiKine* BDNF Sandwich ELISA Kit, CYT306, Chemicon/Millipore, Billerica, MA, USA. The lower detection limits of the kits is 7.8 pg/mL for BDNF. The other main independent variable was gender. We also analyzed the effects of age in our model.
(b) Depressive symptoms were assessed by the Beck Depression Inventory (BDI) [10].
(c) Anxiety was measured with the refined version of the rash analysis of the State-Trait Anxiety Inventory (STAI), adapted to Brazilian Portuguese [18].

2.3. Statistical analysis

To compare the baseline parameters between genders we use *t* test for independent sample. Stepwise multiple linear regression models were performed with heat and pressure pain thresholds as dependent variables. An interaction term for gender *vs.* BDNF was used to assess a possible effect modifier of serum BDNF on the relationship between gender and pain threshold. In order to ensure normally distributed data, we did log transformation for BDNF. Data were analyzed using STATA software (11.0 version, College Station, TX, USA). *P* values less than 0.05 (2-tailed) were considered significant.

3. Results

Forty nine subjects participated in this study (27 females), see baseline demographic and psychological characteristics in Table 1. There were no statistically significant differences in the baseline variables between males and females.

Interestingly, when analyzing experimental pain in females and males, there was a small non-significant difference between groups. The mean ± standard deviation of pressure pain threshold in males and females was 6.60 ± 1.93 and 6.38 ± 1.97, respectively; while for heat pain threshold, it was 43.03 ± 3.08 and 42.25 ± 3.80, respectively (Table 2).

We then fitted a regression model to assess the relationship of pain, gender and BDNF for both models. These models revealed a significant effect of gender ($p = 0.001$ for both models), serum BDNF ($p < 0.004$ for both models) and interaction between BDNF and gender (<0.001 for both models). In fact, when adjusting for BDNF levels and age, heat and pressure pain thresholds were significantly reduced in women as compared to men ($p < 0.001$ for both models). Age was not significant in both models.

4. Discussion

The purpose of this study was to investigate whether pain modulation (experimental heat and pressure pain thresholds) is gender

Table 2
Multivariate linear regression of the interaction between BDNF and pain thresholds by gender ($n = 47$).

Parameter	β	t	p	95% CI
(A) Dependent variable: heat pain threshold				
Serum BDNF ng (log)	−16.40	5.38	0.004	(−27.27 to −5.54)
Gender	−10.67	2.89	0.001	(−16.52 to −4.83)
Age (years)	0.02	0.22	0.828	(−0.12 to 0.15)
Interaction	11.63	3.24	0.001	(5.09 to 18.16)
Heat pain threshold *vs.* serum BDNF (log) by gender				
Serum BDNF ng (log) in male ($n = 22$)	−4.78	2.24	0.04	(−9.48 to −0.09)
Serum BDNF ng (log) in female ($n = 27$)	6.82	2.17	0.004	(2.33 to 11.30)
(B) Dependent variable: pressure pain threshold				
Serum BDNF ng (log)	−10.36	−3.42	0.001	(−16.47 to −4.24)
Gender	−6.07	−3.72	0.001	(−9.36 to −2.78)
Age (years)	−0.02	−0.04	0.97	(−0.08 to 0.08)
Interaction	5.93	1.82	0.0001	(3.24 to 10.60)
Pressure pain threshold *vs.* serum BDNF (log) by gender				
Serum BDNF ng (log) in male	−3.43	−1.34	0.02	(−6.25 to −0.60)
Serum BDNF ng (log) in female	3.49	1.18	0.007	(1.07 to 5.91)

dependent when controlled for BDNF levels. As expected, women experienced more pain than men when exposed to painful stimulations and adjusted to BDNF levels. These findings suggest that the level of serum BDNF is an important effect modifier for the effects of gender on experimental pain. Higher levels of BDNF were correlated with higher pain thresholds in females and in inversely in males. It is important to emphasize that these data were obtained in healthy volunteers using an experimentally controlled acute pain stimulus.

The present findings support the notion that BDNF levels may be a neurobiological mechanism underlying the gender-related differences in pain thresholds. BDNF has emerged as a key mediator of synaptic plasticity, neuronal connectivity and dendritic arborization [20,24]. Together with other biological factors (ex. neurotransmitters, hormones and other neurotrophins), BDNF orchestrates mechanisms of neuronal plasticity and survival. However, in our cross-sectional design, our data only allow us to hypothesize this cause–effect relationship. Further studies are needed to elucidate the possible mechanism implicated in this response and to clarify the issue about the gender-specific relationship of BDNF on pain thresholds.

Accordingly, the effect of gender in the relationship between BDNF and pain threshold can be explained by the estrogen levels, which regulates the increase in BDNF mRNA in areas associated with nociceptive sensory processing such as hippocampus, cerebral cortex and spinal cord [2]. Therefore the relationship between gender and BDNF on pain threshold observed in this study may indirectly confirm the role of the regulatory effect of estrogen in sensitization of central nervous system centers associated with nociceptive sensory processing. This hypothesis is plausible, because the level of BDNF is regulated by the level of estrogen, which has co-analgesic effect. Furthermore estrogen regulates opioid receptor density in several pain-related areas (PAG, parabrachial nucleus (PBn)); that is significantly lower in female rats during proestrus (higher plasma estrogen) compared with diestrus and metestrus phases or male rats [4]. In fact, short-term treatment with estrogen in ovariectomized rats decreases the number of opioid binding sites in the brain, decreasing the analgesic effects of morphine [32]. In addition, estrogen modulates the potency of morphine by influencing G protein coupling [35].

In response to sustained pain, men had larger magnitudes of μ-opioid system activation than women in the anterior thalamus, ventral basal ganglia, and amygdala [52]. This evidence demonstrates that at equivalent levels of pain or stimulus, men and women differ in the magnitude and direction of response of the μ-opioid system across a wide neural network. Thus, opioid response to pain might explain the opposite effect of BDNF in the relationship between genders and pain threshold.

There may be additional mechanisms to explain this finding. Firstly, other hormones, such as testosterone may also explain these differences as in the case of testosterone, this hormone may protect against the hyperactivity of hypothalamus pituitary–adrenal (HPA) axis [39,49]; which may be deregulated in stress response between men and women [49,50]. Also, pain may activate difference neural circuitry in men and women [11,17,38]. For instance, Naliboff et al. demonstrated that while women showed greater activation in the ventromedial prefrontal cortex, right anterior cingulate cortex, and left amygdala in response to a visceral stimulus; men showed greater activation of the right dorsolateral prefrontal cortex, insula, and dorsal pons/periaqueductal gray [28].

Secondly, one can hypothesize that the gender-specific effects of BDNF on the synaptic activity is at least in part due to the temporal summation of reflex responses to a greater extent than it does withdrawal reflex threshold. This is consistent with current psychophysical and electrophysiological data, which show that women summate more easily than men and thus tend to up-regulate their central processing relays more readily [6,36,40,41,43]. In this scenario, higher BDNF levels would amplify this response and therefore be associated with more pain.

Thirdly, another factor to consider when studying gender differences in withdrawal reflex activity (and pain sensitivity in general) is the cyclical fluctuation of gonadal steroids during the menstrual cycle. Indeed, Tassorelli et al. [48] recently found that pain sensitivity and nociceptive flexion responses increase during the luteal as opposed to the follicular phase of the menstrual cycle. It is possible, therefore, that our female subjects' reflex responses may be different (and comparable to those of men) because they were not all tested during their follicular phase. In this context, BDNF levels could be a surrogate for menstrual hormonal variation.

Fourthly, according to a primate model, BDNF is a selective marker of early adversity in the female gender and a higher level is indicative of abnormal responses manifested as a depression-like state. This increase in BDNF levels early on may contribute to the generation of individual differences in stress neurocircuitry, providing a substrate for altered vulnerability to depressive disorders at adulthood [27]. Maladaptive or repeated activation of stress responsive biological mediators, such as neurotrophins, may have long-term influences on stress sensitivity at adulthood and increase vulnerability for stress-related illnesses, including psychopathology and changes in pain threshold.

Taking into account that the luteal phase was not controlled in this study this is an important limitation of this study. However, it is unlikely that the direction of the correlation between BDNF and pain threshold would be different even if we had controlled menstrual phase. In addition, although a previous study showed correlation between pain threshold an menstrual cycle [9], this relationship has not been confirmed in a subsequent study in which pain threshold was unchanged during both the follicular and luteal phase of the menstrual cycle [19].

Nevertheless, future studies are needed to properly assess the relative impact of fluctuating status variables (ex. hormonal profile) on the context of gender-related differences observed here. Although the design we used is not ideal to infer a direct causal relationship, it permits us investigate this interaction between BDNF and gender in pain threshold in controlled manner in order to exclude the influence of several confounding factors observed in clinical situations, such as baseline pain, psychological factors, and effect of other analgesics. Our design allowed us to investigate this interaction in a semi-objective way, using thermal and pressure stimulus measures as opposed to using primarily subjective scales [1,14]. In summary, in this study, we found that the individual pain thresholds were influenced by gender however when controlled for BDNF. Several factors may explain these findings such as the effects of estrogens on opioid activity, effects of other hormones such as testosterone on pain processing. Finally the effects of gonadal hormones on neuroplasticity including immediate and previous effects that may take place during neural development may explain our results as discussed above.

Other limitations to this study should be considered. First, we found that age was not correlated with pain threshold. This incongruence with previous studies may be explained by the limited age range of our sample as we aimed to recruit a homogeneous sample to reduce other sources of variability. Second, one should consider that a limitation of our data is that the pressure of the thermode was not controlled in any special way. This factor has been discussed to be a potential confounder in similar pain measurement setups [46]. The strong relationships we observed suggest that slight pressure differences would not significantly affect position–temperature relationships.

Overall, these findings show that the level of serum BDNF is correlated with pain threshold when differentiated by gender. Higher levels of BDNF were correlated with higher pain thresholds in females and inversely in males. Further studies should assess additional variables that may help to explain variability in pain threshold across healthy and also chronic pain subjects.

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