ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Research paper Acta Neurobiol Exp 2010, 70: 56–66

The relationship between pain sensitivity and conditioned fear response in rats

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It might seem obvious that pain sensitivity would predict individual, inborn susceptibilities to aversive stimuli and the strength of fear-conditioned responses. Such relationships are based on the assumption that there is a close association between fear-evoked behavioral reactions and the responses to painful, aversive stimuli. However, this problem has not been systematically studied. To this end, we investigated the relationship between pain sensitivity in two pain tests (the 'tail-flick' and 'flinch-jump' tests) and a conditioned, fear-evoked, freezing response in rats. The results show that there was no correlation between: (1) the conditioned (associative) and the novelty-evoked (non-specific stress-related) fear response and (2) individual differences in pain threshold and fear responses. Furthermore, factor analysis did not group freezing in the conditioned fear test, individual footshock sensibility, or 'tail-flick' reaction to painful stimuli together. These results indicate that pain sensitivity and conditioned emotional responses to pain are not directly correlated.

Key words: pain sensitivity, fear conditioning, individual reactivity, rats

INTRODUCTION

In this study, we challenged the assumption of a close association between the fear-evoked freezing response and shock reactivity. In other words, we tested the hypothesis that the more intensely the unconditioned stimulus is perceived (as painful), the longer the duration of the freezing response. Pain has both physical and affective properties that engage limbic and reticular systems (Werka 1980, Smith et al. 1997, Hebert et al. 1999, Nakagawa et al. 2003, Tanimoto et al. 2003, Lehner et al. 2004, Kasicki et al. 2009). Therefore, it seems reasonable to assume that the sensitivity to pain should predict the affective appreciation of pain. This concept has both clinical and experimental implications. First, it is important for understanding psychopathological mechanisms involved in such disorders such as posttraumatic stress disorder and fibromyalgia. Secondly, it is useful in interpreting the results from animal models of affective pathology that use aversive, painful stimulation. It has been also suggested that the reaction to pain can help to predict the inborn sensibility to the stress (Burns 2006, Xu et al. 2006). Our search in the PUBMED database covering this topic with the key words 'pain perception',' pain threshold', 'conditioned fear' and 'freezing' yielded a very limited number of publications that addressed the influence of pain perception on the strength of affective or associative responses.

We investigated the relationship between pain sensitivity and a conditioned, fear-evoked, freezing response in rats with the use of two pain tests. The term 'pain' is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Part of the survival value of pain is intimately associated with learning. Pain induces a single event learning, the memory of which can last for life (Apkarian 2008). Conditioned fear responses may be related to complex associative mechanisms: processing of the shock experience and consolidation, retrieval, or extinction of aversive memories. However, it may be difficult to clearly determine which of these mechanisms is directly

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Received 18 March 2009, accepted 22 March 2010

responsible for the association (Borta and Schwarting 2005). A question about emotional modality of pain is important because when a perceived threat to an organism becomes greater, the changes in pain sensitivity lead to enhanced defensive mechanisms as pain increases (Miczek et al. 1982, Apkarian et al. 2004).

In our previous study (Lehner et al. 2006) with rats assigned to a low- and to a high- sensitivity group in the 'flinch-jump' pain test according to their sensitivity to the 'flinch' footshock (the electric stimulus used for fear conditioning after the 'flinch-jump' test was the same for the whole population, 0.7 mA), we found that the animals that were more vulnerable to pain showed significantly more freezing behavior in the conditioned fear test. We hypothesized that response to pain may determine other patterns of emotional behavior, reflecting different activation thresholds of brain structures that control anxiety, e.g., the prefrontal and secondary motor cortices. On the opposite, Werka (1980) reported that the latencies of escape responses in control cats, consisting of a bar-press reaction terminating the unsignalled and individually adjusted shocks, did not correlated with footshock intensity. The author concluded that the instrumental responses evoked by painful stimuli are not determined only by pain perception, but rather by changes in the evaluation of biologic value of external pain evoking stimuli.

Taking these facts into consideration, in the present study we tested the hypothesis that the magnitude of a conditioned fear response (with electric footshock used as unconditioned stimulus) is directly related to animals' reactivity to the footshock. In order to address this issue, we measured the pain threshold in the 'flinch-jump' and 'tail flick' tests in the rat. We also investigated the animal's response to the fear of novelty and its behavior in the conditioned fear test (a freezing response to the aversive stimulus), with footshock intensity used for conditioning adjusted in each animal to match the difference in the strength of an aversive stimulation. The two tests we used measured different aspects of pain perception. The 'flinch-jump' test is under the control of supraspinal and cortical structures. The 'flinch' response indicates the threshold of perception, and the 'jump' response expresses a more emotionally driven reaction (Evans 1961). The tail flick test measures direct activation of a monosynaptic reflex pathway, i.e., the peripheral pain processing in the spinal cord (Akil and Mayer 1972). For deeper analysis of data, multivariate statistical techniques were used, and the results of behavioral tests were analyzed by factor analysis.

METHODS

Subjects

The experiment was performed with a cohort of 70 male Wistar rats. The rats (body weight of 180–200 g), purchased from a licensed breeder, were housed under standard laboratory conditions with a 12-h light/dark cycle (lights on at 07:00 AM), a constant temperature of 21 ± 2°C and 70% humidity. The animals were kept 2 per cage in translucent, polycarbonate cages with bedding (43 × 27 × 19 cm, w/l/h). The experiments were performed in accordance with the European Communities Council Directive, established on 24 November 1986 (96/609 EEC). The local Committee for Animal Care and Use at Warsaw Medical University approved all experimental procedures using animal subjects, according to Polish Law (14/2006).

Scheme of experiments

The rats were well adapted to the environmental conditions of the vivarium.

The following groups were used: rats were assigned to 4 groups, labeled C, n=10 (controls), F, n=30 (footshocked), Cn, n=15 (controls, tested for novelty) and Fn, n=15 (footshocked, tested for novelty). C, F and Fn rats were measured for flinch-jump threshold, whereas Cn rats remained in their home cages without testing. Ten days thereafter (Day 11 after flinch-jump measurements) rats were placed individually in a fear conditioning box using a computerized fear-conditioning system (TSE, Bad Homburg Germany), but did not receive electric footshocks. C and F rats remained there 5 min undisturbed in order to habituate to the experimental situation, under constant white noise conditions (65 dB). Cn and Fn rats were measured during 10 min for the duration of novelty-produced freezing. After completion of this testing the Cn group terminated the experiment. On Day 12, F and Fn rats received fear conditioning training, and C rats remained undisturbed. On Day 13, all rats were placed again in the fear conditioning box to assess the duration of contextual fear-produced freezing. Finally, on Day 21, pain sensitivity to noxious heat was determined in Fn rats with the tail-flick method. For more details, see Table I.

It should be underlined, that different experimental groups (F, Fn and Cn) were used to better control experimental variables: the effect of a preexposure of rats to the flinch-jump testing on their subsequent spontaneous freezing responses and performance in the contextual fear conditioning test using a computerized fear-conditioning system, the influence a short term habituation to the conditioning cage, without a subsequent contextual fear conditioning, on adaptation of rat spontaneous freezing behavior (C group).

Group F and C

Day 0: After habituation, the rats were subjected to the 'flinch-jump' test of pain threshold.

Days 1–10: All animals remained undisturbed in home cages for 10 days. Days 11–13: Animals in the footshocked group (Group F) were subjected to the conditioned fear test, while the control group (group C) was placed in the conditioning box only. The F group was subjected to fear-conditioned training, with individually matched aversive stimulation. The value of the footshock used for conditioning was selected on an individual basis, depending on the 'flinch' response in the 'flinch-jump' test ('flinch' + 30%; e.g., if an animal had a 'flinch' response to 0.35 mA, the intensity of the footshock used for conditioning would be 0.45 mA). The 'flinch' values for all rats ranged from 0.1 mA to 0.75 mA, and the footshock values ranged from 0.13 mA to 0.98 mA. We arbitrarily chose a 30% increase in the stimulus intensity to avoid high intensities that could cause a 'ceiling-effect' or aversive stimulation that was too weak (the majority of unconditioned stimulus values varied between 0.33 to 0.6 mA). The C group was not fear conditioned, and the rats were exposed to the experimental box only on days 1, 2, and 3 of the fear-conditioning test.

Group Fn and Cn

In this part of the experiment, the following groups were used: the Fn group consisted of fear-conditioned rats; the Cn group consisted of control rats that were novelty-exposed only and not subjected to the 'flinch-jump' test. For more details, see Table I.

The animals were well adapted to the vivarium.

Day 0: After habituation, the Fn group was subjected to the 'flinch-jump' test of pain threshold. The Cn group was left undisturbed. Days 1–10: All animals remained undisturbed in their home cages for 10 days. Days 11–13: The Fn group was subjected to fear-conditioned training with individually matched aversive stimulation as described above. The Cn group was tested on day 11 only in the novel context of the fear-conditioning box (i.e., the freezing response to novelty was measured for 10 min). For more details, see Table I. Days 14–20: All animals remained undisturbed in their home cages for 7 days. Day 21: The Fn group was subjected to the 'tail-flick' test of pain threshold (Table I).

Table I

Summary of experimental procedures

Animal groups	Experimental days				
	0	11	12	13	21
C	Flinch-jump	Habituation	Habituation	Contextual freezing	x
F	Flinch-jump	Habituation	Fear conditioning	Contextual freezing	x
Cn	x	Novelty freezing	x	x	x
Fn	Flinch-jump	Novelty freezing	Fear conditioning	Contextual freezing	Tail flick

(C) controls; (F) footshocked; [(Cn) and (Fn)] controls and footshocked, respectively, tested on Day 11 for magnitude of novelty-produced freezing (Novelty freezing); (Flinch-jump) testing flinch-jump threshold; (Fear conditioning) fear conditioning to electric footshock; (Contextual freezing) testing to contextual fear stimulus; (x) not applicable.

Experimental procedures

The 'flinch-jump' test procedure

The test was performed in footshock boxes made of Plexiglas (30 × 30 × 60 cm, w/l/h), with a grid floor made of stainless steel bars wired to a shock generator, as described previously (Lehner et al. 2004). The naïve rats were placed individually into the box. Shocks were delivered to the grid floor of the test box through a shock generator. After a 3-min period of habituation to the test box, shock titrations continued to increase in a stepwise manner (0.05 mA, 0.05–1.2 mA range), depending upon the responsiveness of the rat. The 'flinch' threshold was defined as the lowest shock intensity that elicited a detectable response. The 'jump' threshold was defined as the lowest shock intensity that elicited simultaneous removal of at least three paws (both hindpaws) from the grid. To avoid foot damage, the cut-off was 1.2 mA. In this way, the 'flinch' and 'jump' thresholds in mA were defined for each rat. The time gap between shocks was 10 s, and each animal was tested only once.

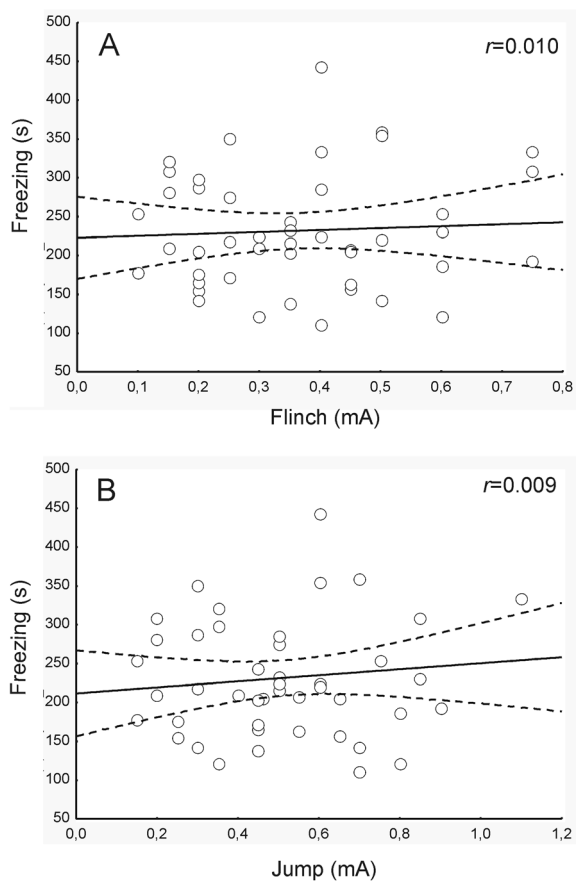

Fig. 1. (A) Lack of significant correlation between flinch and freezing (r=0.057, P=0.71). (B) Lack of significant correlation between jump and freezing (r=0.121, P=0.42). Groups F and Fn: n=45.

Contextual fear-conditioning test procedure

The fear-conditioning experiment was performed using a computerized fear-conditioning system (TSE, Bad Homburg Germany), as described previously (Maciejak et al. 2003). The fear-conditioning test was conducted in the other cage than the 'flinch-jump' test. Fear conditioning was carried out in the experimental cage equipped with the grid floor (36 × 21 × 20 cm, w/l/h) under constant white noise conditions (65 dB). The experiment was performed on three consecutive days in the same testing box and experimental chamber. On the first day, the animals (group F, C) were placed separately in a conditioning box and left undisturbed for 5 min, allowing adaptation to experimental conditions (habituation to novel context). The other animals (group Fn and Cn) were exposed for 10 min to the same box (i.e., to a

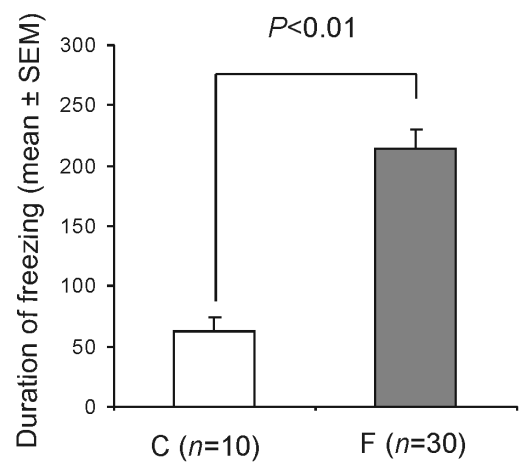

Fig. 2. Duration of freezing in control (C, n=10) and fear conditioned (F, n=30) rats. Both groups were tested in the flinch-jump test, and 10 days later were exposed during 3 consecutive days to the experimental procedure. On Day 1, both groups remained for 5 min in the conditioning box for adaptation. On Day 2, F rats were placed into the conditioning apparatus for 10 min, and from the second minute they were given a series of electric footshocks through the metal grids of the floor. Controls remained in their home cages. On Day 3 both groups were placed for 10 minutes in the conditioning box without footshocks to score the duration of immobility. The data from this last session are shown in the graph as means ± SEM.

novel context) during the first day of a contextual fear-conditioning test, and their spontaneous, novelty-induced freezing behavior was recorded. The following day, during a session, after 2 min of habituation, each animal from the appropriate group (group F and Fn) received three footshocks (footshock intensity was individually matched) for 1 s and repeated every 60 s; and left undisturbed for an additional 5 min in the experimental cage (Table I). On the third day, the freezing response of rats was examined for 10-min in the testing box, without any further stimulation (group F, C, and Fn).

The freezing behavior was measured by a photobeam system (10 Hz detection rate) controlled by the fear-conditioning system, under constant white noise conditions. Photobeams were spaced 1.3 and 2.5 cm in the direction of the x-axis and the y-axis, respectively. The absolute duration of inactivity was defined as no interruption of any photobeam over 5 s. This was then summed for the entire 10-min experimental session (total time of freezing). Automated measurement of the freezing response has been used by our group and others, and it has been pharmacologically validated using various clinically effective and experimental anxiolytic and anxiogenic agents (Maciejak et al. 2003, Szyndler et al. 2005). The computerized method, based on the latency between photobeam interruption, is a reliable scoring criterion in rodents. Furthermore, computer values obtained during contextual fear-conditioning tests showed high correlation with hand-scored freezing, where r values ranged from 0.87 to 0.94 (Valentinuzzi et al. 1998, Takahashi 2004).

The 'tail-flick' test procedure

This test was performed using the Tail-Flick Unit (UGO BASILE) and measured the sensitivity of the tail to a noxious, thermal stimulus. On the three consecutive days prior to testing, rats were placed on the apparatus surface for 15 min and immobilized by

Table II

Correlation analysis		
Parameters	Pearson's coefficient (r)	P-value
Flinch/novelty freezing response	(+) 0.09	0.75
Jump/novelty freezing response	(-) 0.34	0.22
Flinch/conditioned freezing response	(-) 0.01	0.66
Jump/conditioned freezing response	(-) 0.02	0.52
Individual response footshock intensity/conditioned freezing	(-) 0.13	0.69
Conditioned freezing response/novelty freezing duration	(-) 0.07	0.78
Flinch/tail flick	(-) 0.16	0.57
Jump/tail flick	(-) 0.19	0.49
Tail flick/novelty freezing response	(+) 0.38	0.15
Tail flick/conditioned freezing response	(-) 0.04	0.88

The data originate from the Cn group (n=15). Novelty freezing response was measured in the conditioning box without previous fear conditioning and aversive stimulation (i.e., the spontaneous, novelty-induced freezing was evaluated). Conditioned freezing response was measured the next day after the administration of footshocks in the same contextual situation.

hand to habituate the animals to the 'tail-flick' apparatus and procedure (handling). The ambient temperature of the room was maintained at 24 ± 0.5ºC. Withdrawal of the tail activated the photocell and determined the response latency (0.1 s accuracy). The heat stimulus was produced by infrared energy (IR) consisting of a 50W source bulb (the IR energy, 1 mW for 1 s corresponds to 1 mJ; the thermal nociceptive stimuli were expressed in mW/cm^2). The light intensity was adjusted in a group of 7 rats, not used in the experimentation. Two trials with a light source intensity of 65 IR were performed at 3 min inter-trial intervals in order to achieve a 'tail-flick' latency of 3–5 s. Two days thereafter, the test was performed twice for each rat, each rat separated by 45 min long interval. The radiant heat stimulus from the 50W bulb was focused 4–7 cm from the distal end of the tail. The time interval between the onset of tail heating and the withdrawal response was measured electronically. In the absence of a response, the tail heating was terminated after 15 s to prevent tissue damage. 'Tail-flick' latency for each rat was calculated as the average of two consecutive measurements (modified according D'Amour and Smith 1941, Tseng and Collins 1995, Choi et al. 2000, Cecchi et al. 2008).

Data analysis

The data are shown as means ± SEM. The data on the novelty-induced freezing and freezing response after fear-conditioning were analyzed by Student's *t*-test (Figs 2 and 3). A probability value of $P<0.05$ was considered significant in this study. For the correlation analysis, Pearson's coefficient was calculated (Fig. 1, Table II). The results of behavioral tests were assessed by factor analysis, using principal component analysis (PCA) with an orthogonal rotation (varimax). The number of factors was selected using the root curve analysis. The factors for eigenvalues >1.0 were taken into consideration. The contribution of behavioral variables to each factor is referred to as factor loading. Only factor loading values that were higher than 0.7 (or lower than −0.7) were considered significant (Table III). The statistical package Statistica for Windows, Release 7 (StatSoft Inc., USA) was used for statistical calculations.

RESULTS

The Student's *t*-test revealed a significant difference in the freezing responses between the C (control) and

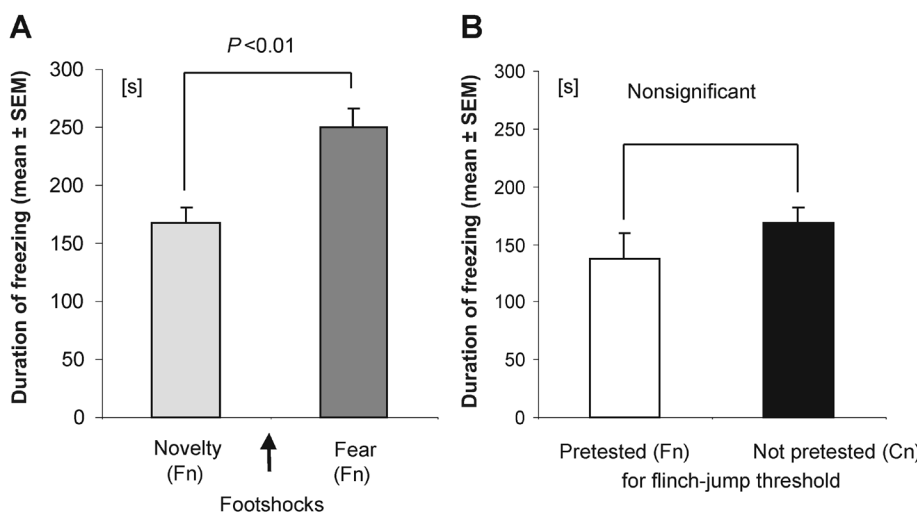

Fig. 3. (A) Duration of freezing in control rats tested for the effect of novelty (the Fn group, *n*=15, in Table I). The rats were given the flinch-jump test, and 10 days later were exposed during 3 consecutive days to the experimental procedure. On Day 1 the rats were screened in the conditioning box during 10 min without footshocking for the duration of novelty-produced immobility. On Day 2, the same rats were put into the conditioning apparatus for 10 min, and starting from the second minute, they were given a series of electric footshocks through the metal grids of the floor. On day 3, the rats were placed for 10 minutes in the conditioning box without footshocking to score the duration of fear-produced immobility. (B) Duration of freezing in rats exposed to novelty for 10 minutes 10 days after the flinch-jump test (Fn group, *n*=15 in Table I) compared to rats that were not given the flinch-jump test (Cn group, *n*=15 in Table I).

F (fear-conditioned) groups, i.e., the effect of conditioning alone (t_{38}=5.22, P<0.01, Fig. 2). A significant difference between the spontaneous freezing responses to the novel context measured on the first day in the conditioning box (Fn group, the first day, novelty freezing), and the conditioned freezing response measured on the third day of fear conditioning (Fn group, contextual freezing, third day, t_{28}=2.75, P<0.01, Fig. 3A) was also found. Student's *t*-test did not show a significant difference in spontaneous freezing responses to the novel context measured on the first day in the conditioning box, between the Fn and Cn groups (t_{28}=1.19, P=0.24, Fig. 3B). It is noteworthy that novelty freezing (Fn group, first day, Fig. 3A) was much stronger than freezing of control rats (i.e., after 3 days of habituation to the experimental box) on the third day of the fear-conditioning test (C group, Fig. 2). This was probably due to better habituation by the C group to experimental conditions.

Correlation analysis showed no significant correlation between 'flinch' or 'jump' and duration of freezing (Fig.1A, B), and 'flinch', 'jump', 'tail-flick', novelty-induced freezing and conditioned freezing responses, or between freezing duration and individual footshock intensity during conditioning training (Fn group, Table II). Similarly, in the F group, statistical analysis did not reveal any correlation between duration of a freezing response and individually matched footshock intensity (r=−0.02, P=0.92). The results of the factor analysis are shown in Table III. Factor analysis allowed for extraction of three independent factors representing 35.3%, 26.3% and 25.3% of the total variance. Factor 1 (eigenvalue =1.41) represented freezing to novelty and the 'tail-flick' response. Factor 2 (eigenvalue =1.05) represented the individual footshock intensity, determined individually for each rat depending on the 'flinch' response in the 'flinch-jump' test. Factor 3 (eigenvalue =1.09) represented a freezing response recorded during exposure to the fear-conditioning test. It is noteworthy that factor analysis did not group freezing and footshock intensity parameters with the same factor (Table III).

DISCUSSION

The most important result of the present study is that pain reactivity does not predict rat behavior in the conditioned fear test (i.e., freezing response to the fear-evoking context). Our results did not show a direct relationship between the conditioned (associative,

Table III

Multifactor analysis

Analyzed parameters	Factor 1	Factor 2	Factor 3
	Eigenvalue (% of total variance)		
	1.41 (35.3)	1.05 (26.3)	1.9 (25.3)
individual footshock intensity	−0.023	−0.963*	−0.053
novelty freezing response (1–10 min)	0.857*	0.208	0.144
conditioned freezing response (1–10 min)	0.013	0.047	0.987*
tail flick	0.803*	0.318	−0.141

* Loadings higher than 0.7 (or lower than −0.7)

The data originate from the Fn group (n=15). Freezing responses were evaluated in the conditioned fear test. Footshock intensity was established separately for each rat. For more details see Methods section.

acquired) and the novelty-evoked (spontaneous, non-specific, related to the stress of the experimental procedure) fear responses or between individual differences in pain reactivity and fear responses. Furthermore, the factor analysis did not group freezing-conditioned fear responses, individual footshock intensities or 'tail-flicks' with the same factor. Moreover, these data (absence of differences between the Fn and Cn group responses to novelty exposure, Fig. 3B) indicate that the 11-day delay following the 'flinch-jump' test was sufficient to cancel the previous stress experience on rat behavior in the fear-conditioning test. In the 'tail-flick' test, it was possible to directly estimate animals' sensitivity to pain, in contrast to the responsiveness to the aversive and unpleasant, but not obviously painful, electric stimulation. Absence of any correlation between 'flinch' and 'tail-flick' responses might indicate that the behavioral pain expression is not a reliable indication of pain perception. The 'flinch-jump' test is under the control of supraspinal and cortical structures (with the response indicating the threshold of perception), while the 'tail-flick' test measures direct activation of a monosynaptic reflex pathway, i.e., the peripheral pain processing in the spinal cord. The lack of correlation between the results of both tests indicates a differential involvement of emotional and perceptual components for the elaboration of these two behavioral responses. Additionally, pain sensibility did not correlate with innate, novelty-evoked (unconditioned) fear responses. There was also no correlation between conditioned and novelty-evoked fear responses (Table II); i.e., the response to novelty did not predict the animal's reaction to the conditioned, fear-evoking context. Similarly, Werka (1980) found that instrumental response latencies in cats (bar-press escape responses from unsignalled footshock), were not directly related to the aversive stimuli intensity, indicating that also instrumental responses are determined by changes in the interpretation of biological value of external pain evoking stimuli.

The surprising lack of significant correlation between pain reactivity and fear conditioning is counterintuitive, given the physiological and psychological nature of pain stimulation. It is noteworthy that the 'flinch-jump' test measured innate aversion to painful stimuli at the sensorimotor level (shock reactivity), and that the expression of a specific pain response did not directly relate to the intensity of the stimulus. These data suggest that the central processes controlling pain sensing and anxiety are not directly interrelated (Melzack and Wall 1965). There are also clinical data indicating that the pain perception in chronic back pain and fibromyalgia depends on emotions (Flor et al. 2002, Schneider et al. 2004). In experiments on Pavlovian conditioning both the chronic back pain patients and the healthy controls showed higher skin conductance values to the aversive than the pleasant stimuli, suggesting that the aversive stimuli elicited more arousal in both groups (Schneider et al. 2004). The other data allowed for interpretation that conditioning effects might have been due to enhanced attention than operant conditioning and individual learning may have very variable components (Lousberg et al 1996, Flor et al. 2002). On the whole, it seems that experience of an acute pain does not in every conditions have a direct relationship to the intensity of noxious stimuli. That is probably because the nociceptive stimuli have several properties, i.e. (1) a sensory modality, (2) they evoke an emotional reaction, (3) they transmit information about the changes in the environment. It is conceivable, therefore, that even weak painful stimuli may be sufficient to evoke an efficient and rapid reaction in the threatening situation (Werka 1980).

It is now clear that the most important structure involved in the control of affective stimuli is the amygdala. This coordinates the processing of conditioned and unconditioned fear, in particular, sensory input from descending projections of the periaquaductal grey, which controls both spinal and trigeminal dorsal horn nociceptive transmission (nucleus raphe magnus and the paragigantocelullar nucleus) (Lovick 2008). Moreover, it has been suggested that emotional learning can take place without the involvement of the higher processing centers of the brain and can be controlled directly by the thalamo-amygdala pathway without involvement of the neocortex (Ledoux 1998, Wunsch et al. 2003). However, the majority of data indicate that in the case of conditioned responses, higher brain centers, including the frontal cortex, play an integrative role. There is much evidence coming from the preclinical and clinical studies supporting this conclusion (Petrovic et al. 1999, Witting et al. 2006, Zhuo 2006, Lehner et al. 2008b, Klucken et al. 2009). The ability to process aversive associations of fearful or painful events is important for normal behavior and its mechanisms seem to involve the interaction between the medial prefrontal cortex, basal ganglia and amygdala (Apkarian 2008).

In our previous study, we demonstrated that rats more vulnerable to pain (HS group with the flinch threshold of <0.45 mA) showed significantly more freezing behavior in the conditioned fear test (i.e., the freezing response after fear conditioning using the same, constant, footshock intensity of 0.7 mA) (Lehner 2006). We recently found, however, that, while a serotonergic lesion of the prefrontal cortex significantly disinhibited HS rat behavior controlled by fear (a freezing response), it left the rat pain threshold intact (Lehner et al. 2008b). Interestingly, the HS animals had also stronger 5-HT- and CRF-related immunostaining in the M2 secondary motor cortical area and increased concentration of GABA in the basolateral nucleus of the amygdala (*in vivo* microdialysis) compared with rats less vulnerable to pain (Lehner et al. 2008a). These data point to the differences in the anatomy and function of the neurotransmitter systems in the examined groups of animals, that control different aspects of rat emotional behavior (Sacchetti et al. 1999). Recent accounts have conceptualized pain as multidimensional, consisting of emotional, sensory and cognitive components. The neuronal circuitries associated with emotion and pain only partially overlap, providing reciprocal influences. The data from imaging studies indicate that a major part of the anterior cingulate seems to be an important anticipatory response region, reflecting nociceptive stimulus parameters, while the insula is involved in nociception and in reflecting perceived stimulus magnitudes independent of the sensory modality (Rainville et al. 1997, Apkarian 2008).

The animal models used here have some limitations. Since testing 'flinch-jump' responses in the 'flinch-jump' test is a continuous process, it is possible that aversive associations are formed between pain and fear responses. This could be due to contacts with the metal grid floor, which was also used in the fear-conditioning test. However, in the 'flinch-jump' test, the rats received different quantities of shock stimulation, depending on their behavior, so the possibility of a long-lasting association is rather small. It is also conceivable that previous sensory experiences could influence sensory and emotional components of subsequent conditioned fear learning. However, such reservation can be ignored because control experiments showed that a 10-day interval between testing was long enough for Fn and Cn animals to express similar spontaneous freezing responses. It is noteworthy that both groups differed in only one procedural aspect - Fn rats were pre-tested in the 'flinch-jump' test, while Cn rats were left undisturbed in their home cages. Another issue deserving comment relates to the fact that the lack of correlation between the flinch-jump measures and the duration of freezing could be explained by the possibility, that the fear behavior, being contaminated with latent inhibition (as a result of preexposure of animals to the contextual situation on Day 1 of the contextual fear conditioning test), was not fully expressed. This phenomenon could play a role, however, it should be stressed that the individual responses of rats to the fear conditioned context remained very strong. It is also noteworthy that a short term habituation to the conditioning cage is a standard part of the experimental procedure to attenuate the inter- and between-groups variability induced by an acute response to novelty, and the preexposure was as short as possible to get rid off the novelty effect.

CONCLUSION

The behavior of rats in two tests of pain reactivity did not correlate with conditioned and unconditioned (novelty-related) fear responses. This indicates that individual differences in fear responses were not directly determined by pain perception or fear of novelty. Conditioned fear responses are probably related to other associative mechanisms, such as processing of the shock experience and consolidation, retrieval, or extinction of aversive memories (Borta and Schwarting 2005). However, it is difficult to clearly determine which of these mechanisms could be responsible for the reported phenomena. These findings may help to better understand the biological correlates relative to an individual's susceptibility to fear.

ACKNOWLEDGEMENTS

The authors want to express their sincere gratitude for the anonymous Referee and the Journal Editor (Professor T. Werka) for their contribution to the final version of the manuscript.

The study was supported by statutory grant No. 5010308050 from the Institute of Psychiatry and Neurology, Warsaw, Poland and grant, No. 0440/B/P01/2009/36 ("The search for the neurobiological mechanisms of extinction of fear responses, and individual pharmacotherapy of anxiety disorders") from the Ministry of Science and Higher Educations,

Warsaw, Poland (M.L). The authors declare that they have no competing financial interests. The authors express their sincere gratitude to Mrs. Ala Biegaj for her excellent technical support.

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