Repeated oral administration of capsaicin increases anxiety-like behaviours with prolonged stress-response in rats

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This study was conducted to examine the psycho-emotional effects of repeated oral exposure to capsaicin, the principal active component of chili peppers. Each rat received 1 mL of 0.02% capsaicin into its oral cavity daily, and was subjected to behavioural tests following 10 daily administrations of capsaicin. Stereotypy counts and rostral grooming were significantly increased, and caudal grooming decreased, in capsaicin-treated rats during the ambulatory activity test. In elevated plus maze test, not only the time spent in open arms but also the percent arm entry into open arms was reduced in capsaicin-treated rats compared with control rats. In forced swim test, although swimming duration was decreased, struggling increased in the capsaicin group, immobility duration did not differ between the groups. Repeated oral capsaicin did not affect the basal levels of plasma corticosterone; however, the stress-induced elevation of plasma corticosterone was prolonged in capsaicin treated rats. Oral capsaicin exposure significantly increased c-Fos expression not only in the nucleus tractus of solitarius but also in the paraventricular nucleus. Results suggest that repeated oral exposure to capsaicin increases anxiety-like behaviours in rats, and dysfunction of the hypothalamic-pituitary-adrenal axis may play a role in its pathophysiology.

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1. Introduction

Pharmacologic doses of capsaicin evoke burning and painful sensations when capsaicin interacts with its receptors located at sensory nerve endings (Bevan and Szolcsányi 1990; Wood 1993). Capsaicin receptor, transient receptor potential vanilloid type 1 (TRPV1), is a nonselective cation channel, predominantly expressed in a subset of primary sensory neurons with A-*d* and C fibres (Guo *et al.* 1999), which plays a key role in the detection of noxious painful stimuli (Caterina *et al.* 1997; Tominaga *et al.* 1998; Hayes *et al.* 2000).

Although the effects of capsaicin on sensory nerve endings are well known, direct effects of capsaicin on taste receptor cells have been poorly understood. *In vitro* study has suggested that capsaicin may enhance or modify taste perception directly in taste receptor cells, possibly, in mediation of its receptor TRPV1 (Park *et al.* 2003). In human studies, capsaicin suppressed responses to sweet, bitter and umami, but not sour and salty stimuli (Prescott and Stevenson 1995; Simons *et al.* 2002; Green and Hayes 2003; Green and Schullery 2003). TRPV1 mRNA expression and its immuno-positive nerve fibres have been demonstrated in the taste buds (Ishida *et al.*

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2002; Kido *et al.* 2003; Liu and Simon 2001). We have recently demonstrated that TRPV1 receptors are co-localized with sweet or bitter receptors in taste sensing cells of the circumvallate papillae of rats (Moon *et al.* 2010) and human (Jahng *et al.* 2010), and that oral exposure to capsaicin in rats results in increased consumption of sweet solutions with decreased expression of sweet receptors in the circumvallate papillae (Gu *et al.* 2009).

The role in the brain of TRPV1, previously thought to only function in sensory neurons, is also emerging (for reviews, see Di Marzo *et al.* 2008; Starowicz *et al.* 2008; Moreira *et al.* 2012). TRPV1 mRNA and immunoreactivity are found throughout the whole neuroaxis, including the cortex, hypothalamus, cerebellum and basal ganglia (Mezey *et al.* 2000; Szabo *et al.* 2002). Immunohistochemical localization of TRPV1 receptors was also observed in the prefrontal cortex, nucleus accumbens, amygdala and hippocampus (Micale *et al.* 2009). Localization of TRPV1 in those brain regions suggests its implication in the control of psycho-motor activities. Indeed, systemic injections of the prototypical TRPV1 agonist capsaicin and various analogs inhibited ambulation, stereotypic behaviour and activity in the open field test in rats (Di Marzo *et al.* 2001a). Also, it has been suggested that the brain TRPV1 participates in anxiety- and depression-like behaviours (Di Marzo *et al.* 2001a; Kasckow *et al.* 2004; Marsch *et al.* 2007; Micale *et al.* 2009). Anxiety-like behaviours were decreased in TRPV1 receptor-deficient mice with reduced long-term potentiation in the hippocampus (Marsch et al. 2007), and systemic capsaicin caused anxiogenic effects in rats (Rubino *et al.* 2008). Intraperitoneal injection of TRPV1 agonists including capsaicin increases depression-like behaviours in rats (Di Marzo *et al.* 2001a; Kasckow *et al.* 2004).

Despite the adverse psycho-emotional effects of capsaicin injection reported as above, many Korean peoples generally believe that hot spicy foods improve negative mood state. In this study, we have examined if oral administration of capsaicin, the principle active component of chili peppers, improves the psycho-emotional behaviours of rats, contrary to its adverse injection effects. Rats received daily oral administration of capsaicin at an edible dose (Park *et al.* 2004), and then were subjected to the ambulatory activity test, the elevated plus maze test to assess anxiety-like behaviours, and the forced swim test to assess depression-like behaviours.

2. Materials and methods

2.1 *Animals*

Male Sprague–Dawley rats (200–250 g, Samtako Bio, Osan, Korea) were individually housed and maintained in a specific pathogen-free (SPF) barrier zone with the constantly-controlled temperature (22±1°C) and humidity (55%) on a 12 h light–dark cycle (lights on at 07:00 h) in the Seoul National University Animal Facility Breeding Colony. Rats had *ad libitum* access to standard rodent chow (Purina Rodent Chow, Purina Co., Seoul, South Korea) and tap water, and were habituated in the animal colony at least for a week before experiments began. Animals were cared for according to The Guide for Animal Experiments 2000, edited by the Korean Academy of Medical Sciences, which is consistent with the NIH Guideline Guide for the Care and Use of Laboratory Animals 1996 revised. All animal protocols were approved by the Committee for the Care and Use of Laboratory Animals at Seoul National University.

2.2 *Drug treatments*

Rats were gently handled by the experimenter (JY Kim) daily for several days prior to the experiment to minimize undesirable effects. Each rat received 1 mL of capsaicin (0.02% suspension in water, Sigma Co., St Louis, MO, USA) or water drop by drop into its oral cavity daily by the same experimenter. Capsaicin dose, 0.02% (approximately 655 μM), used in this study was decided according to previous report by Park *et al.* (2004). It has been reported that oral capsaicin at 320 μM concentration effectively interacts with other taste stimuli (Green and Schullery 2003) and 1% of capsaicin application to skin induces a burning pain in human (Jones *et al.* 2004). Rats showed aversive responses, such as chin rubs, paw wipes and head shakes after each capsaicin treatment, which disappeared within 5 min. Oral administration of capsaicin or water continued until the end of the whole experiment (24 capsaicin administrations total/rat). The behavioural sessions were followed after the 10th daily administration of capsaicin, and each behavioural session was performed 30 min after the oral exposure to capsaicin (n=8 in each group).

2.3 *Ambulatory activity*

Rats were subjected to the ambulatory test on day 11. On each trial, the rat was placed in the center of the activity chamber (43.2 cm in length, 42.2 cm in width, and 30.5 cm in height, MED Associates, VT, USA), a transparent acryl chamber equipped with two horizontal planes of 16 infrared photocell-detector pairs placed in x, y dimension, spaced 2.5 cm apart, and its ambulatory activity was monitored by the computerized system for 30 min. Light condition of the test room was maintained in the same intensity with animal rooms under day-light condition. Ambulatory activity was measured as the total counts of beam interruptions in the horizontal sensor during each consecutive 5 min session. Stereotypic activities, such as body sway, head weaving, grooming and rearing were measured as the total counts of

beam interruptions in the vertical sensor. Defecation activity, number of fecal boli, during the ambulation test of each rat was scored as well. Grooming activity was further analysed; i.e. forepaw and head grooming was considered as rostral grooming, and body, legs, and tail/genital grooming as caudal grooming (Kalueff *et al.* 2007). The activity chamber was cleaned with 70% ethanol after each use to eliminate any olfactory cues of the previously tested rat.

2.4 *Elevated plus maze*

Rats were subjected to the behavioural assessment in an elevated plus maze on day 14, a plus shaped acryl maze with two opposite open arms (50 cm in length and 10 cm in width) and two opposite closed arms (50 cm in length, 10 cm in width, and 31 cm in height), extending out from a central platform (10 cm × 10 cm). The whole apparatus was elevated 50 cm above the floor. The test procedure was followed as previously described (Daniels *et al.* 2004). Each rat was placed in the centre of the maze facing one of the open arms, and then allowed to explore the open or closed arms of the maze for 5 min. The time spent in the different arms was recorded, respectively. Four paws had to be inside the entrance line to each arm, which signalled the start of the time spent in the specific arm, and then the end time was recorded when all four paws were outside the line again. The maze was cleaned with 70% ethanol after each test to prevent influences of the previously tested rat.

2.5 *Forced swim test*

Rats were subjected to the forced swim test on day 16 according to the method previously described (Porsolt *et al.* 1977). Each rat was allowed to swim in a glass cylinder (54 cm in height and 24 cm in diameter) filled with water in 40 cm of depth (23–25°C) for 5 min. All test sessions were recorded by a video camera from the side of the cylinder. Duration of rat's immobility in the water was scored from videotapes by a trained observer who was blinded to the experimental conditions. Immobility was defined as the state in which rats were judged to be making only the movements necessary to keep their head above the surface. Swimming was defined as the state in which rats were judged to be making active swimming motions more than necessary to merely maintain its head above water, and struggling to be climbing, usually directed against the walls.

After the end session of the swim test, rats were allowed to rest in their home cages for a week to minimize any effects of previous stress, and then subjected to a restraint stress for the plasma corticosterone assay.

2.6 *Plasma corticosterone assay*

Rats were placed in a restraint box for 2 h, in which rats were able to move their four limbs but not to change their body orientation. Tail blood was collected at 0, 20, 60 and 120 min time points during the restraint period, and centrifuged at 2,000 rpm for 20 min. The plasma samples were frozen in liquid nitrogen, and stored at −80°C until used for the assay. Plasma levels of corticosterone were determined by radioimmunoassay using ^{125}I-labelled Coat-A-Count kit (Siemens, CA, USA). The sensitivity of the assay was 5.7ng/mL. The intra-assay coefficient of variation was 4–12.2%.

Rats were placed in the test room at least 2 h prior to each test to minimize unwanted stress effects, and all behavioural assessments were performed between 9:00 AM and 12:00 PM of the day to avoid the influences of circadian variances.

2.7 *c-Fos immunohistochemistry*

One hour after a single oral exposure to capsaicin or water (n=4 in each group), rats were anesthetized with over-doses of sodium pentobarbital (Hallym Pharmaceutical Co., Seoul, Korea) and transcardially perfused first with heparinized isotonic saline and then with 4% paraformaldehyde (Merck Co., Damstadt, Germany) in 0.1 M sodium phosphate buffer. Brains were rapidly dissected out, blocked, post-fixed for 2 h, and then transferred into 30% sucrose (Sigma Co., MO, USA) overnight for cryoprotection. Forty-micron coronal sections were cut on a freezing, sliding microtome (HM440E, Microm Co., Germany). Alternate sections were collected throughout the rostro-caudal extent of the NTS (between bregma −3.2 mm and −14.3 mm) and the hypothalamic PVN (between bregma −1.3 mm and −2.1 mm). The coordinates were based on Paxinos and Watson (2005). Immunohistochemistry was performed with standard DAB reaction using commercial ABC kit (Vectastain Elite Kit, Vector Laboratories, CA, USA) as previously described (Jahng *et al.* 2004). Polyclonal rabbit anti-c-Fos antibodies (1:20000 dilution, Calbiochem, Darmstadt, Germany) were used as primary antibodies, and biotinylated anti-rabbit IgG (1:200 dilution, Vector Laboratories, CA, USA) as secondary. Immunostained sections were mounted in an anatomical order onto gelatin-coated slides from 0.05 M phosphate buffer, air-dried, dehydrated through a graded ethanol to xylene, and cover-slipped with Permount.

2.8 *Quantitative and statistical analyses*

The number of c-Fos immune-positive nuclei in each section was blind-counted by hand after digitizing the immune-stained sections in 720 × 540 micron images using an Olympus BX-50 microscope (Olympus Co., Tokyo, Japan)

and Leica image analysis system (Leica Microsystems, Wetzlar, Germany). Cells containing a distinct brown dot were counted as c-Fos-positive cells. The number of cells in two sections from the PVN region (closest sections to bregma −1.88 mm) from each brain was averaged. The NTS was divided into two subregions: caudal (ventral and caudal to the area postrema) and intermediate (abutting the forth ventricle). Each of these two subregions was represented by four sections of the NTS sections collected from each rat. Cell counts for all sections within each region of each rat were averaged per section, and the individual mean counts for each region averaged across rats by region within experimental groups.

All data was analysed by one- or two-way (corticosterone data; treatment × time) analysis of variance (ANOVA), and preplanned comparisons with the controls were performed by *post hoc* Fisher's PLSD test when necessary, using StatView software (Abacus, Berkeley, CA). Values are presented by mean±S.E. For all comparisons, the level of significance was set at $P \leq 0.05$.

3. Results

Repeated oral exposure to capsaicin (1 mL of 0.02% capsaicin suspended in water daily) did not affect daily food or water intake (figure 1). Body weight gain of capsaicin-treated rats did not differ from control rats.

Each rat was placed in the activity chamber 30 min after the oral exposure to capsaicin on day 11 (after the 11th oral capsaicin exposure) and their ambulatory activities were recorded for 30 min (figure 2). Ambulatory counts, the total counts of beam interruptions in the horizontal sensor, and the travelled distance were gradually decreased during the test session both in capsaicin-treated and control rats, with no differences between the groups (figure 2). Centre zone activity, the time spent in the centre zone and defecation activity during the activity test were not significantly reduced in capsaicin rats, compared to control rats (figure 3A and B). However, stereotypy counts, the total counts of beam interruptions in the vertical sensor, were significantly increased in capsaicin rats [$F(1,14)=5.516$, $P=0.034$] compared with control rats (figure 3C). The number of rostral grooming was markedly increased [$F(1,14)=14.952$, $P=0.0017$], and caudal grooming decreased [$F(1,14)=15.250$, $P=0.0016$], in capsaicin rats compared with control rats (figure 3D).

In order to further assess the anxiety-like behaviours, rats were subjected to an elevated plus maze test 30 min after the oral exposure to capsaicin on day 14 (after the 14th oral capsaicin exposure). Not only the time spent in open arms [$F(1,14)=21.502$, $P=0.0004$] but also the percent arm entry into open arms [$F(1,14)=5.341$, $P=0.0366$] was reduced in capsaicin rats compared with control rats (figure 4A and B). To assess depression-like behaviours, rats were subjected to

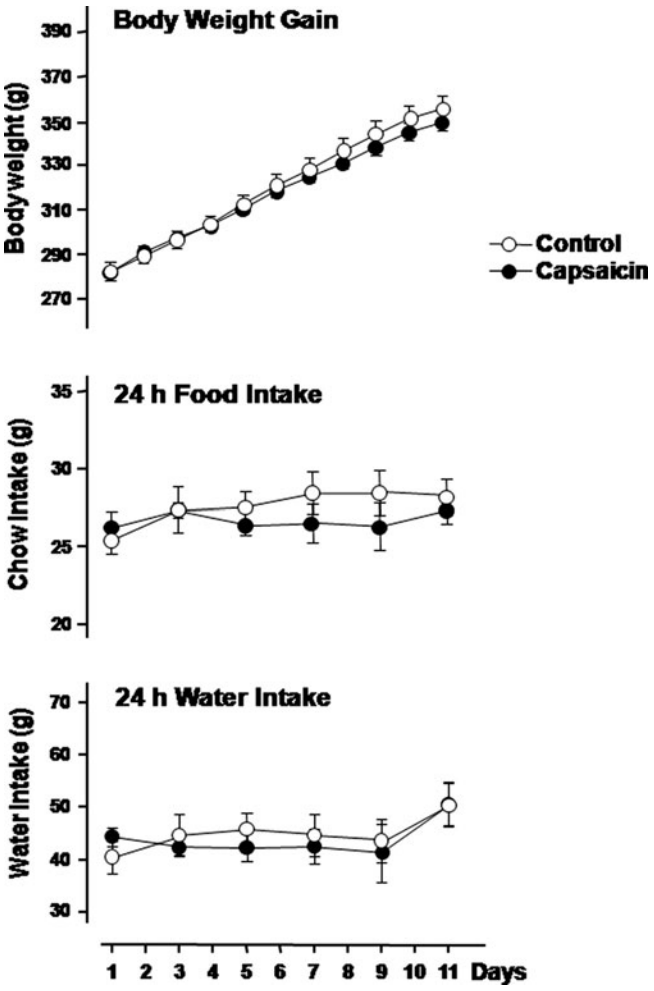

Figure 1. Body weight gain and daily consumption of chow and water. Rats received an oral administration of 1 mL of 0.02% capsaicin or water daily. Data are presented by mean±S.E.

the forced swim test 30 min after the oral exposure to capsaicin on day 17 (after the 17th oral capsaicin exposure). Swimming duration during the 5 min of test session was decreased [$F(1,14)=9.837$, $P=0.0073$], and struggling increased [$F(1,14)=10.921$, $P=0.0052$], in capsaicin rats compared with control rats, but immobility duration did not differ between the groups (figure 4C).

A week after the swim test, rats received 2 h of restraint stress, and the tail blood was collected at 0, 20, 60 and 120 min time points during the restraint session, used for plasma corticosterone assay (figure 5). Analysis of the plasma corticosterone levels by two-way ANOVA showed main effect of restraint time [$F(3, 24)=12.708$, $P < 0.0001$], and significant interaction between treatment and time [$F(3,24)=3.811$, $P=0.023$]. The plasma corticosterone levels of control rats were significantly increased at 20 min after the onset of

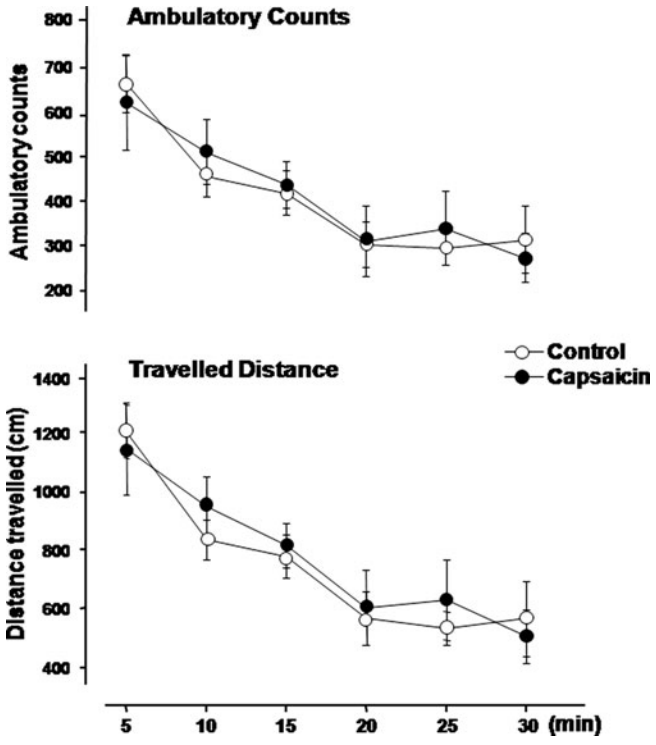

Figure 2. Ambulatory counts and the distance travelled during 30 min of ambulatory activity test, which were scored consecutively at every 5 min session. Rats were placed in the activity chamber 30 min after the 11th oral exposure to capsaicin. Data are presented by mean±S.E.

restraint stress ($P < 0.05$ vs 0 time point) and restored to the basal levels at 60 min after the restraint onset. However, in capsaicin treated rats, the stress-induced corticosterone increase was persisted until 60 min after the onset of restraint (figure 5). Basal levels of corticosterone (0 time point) did not differ between the groups.

Rats were sacrificed for c-Fos immunohistochemistry 1 h after a single exposure to oral capsaicin or water. c-Fos immuno-positive nuclei was significantly increased in the PVN [$F(1,6)=7.947$, $P=0.0304$]and the intermediate NTS [$F(1,6)=21.497$, $P=0.0435$] of capsaicin treated rats compared with water-treated control rats (figure 6). c-Fos immunoreactivity in the caudal NTS of capsaicin-treated rats did not differ from control rats.

4. Discussion

Human studies have demonstrated that consumption of red pepper decreases appetite and increases satiety (Yoshioka *et al.* 1999; Westerterp-Plantenga *et al.* 2005), and increases energy expenditure (Yoshioka *et al.* 1998 2001), which is thought to be mediated by increased activity of sympathetic nervous system by capsaicin, the principal active component of red pepper (Watanabe *et al.* 1987). In this study, intra-oral pretreatment with capsaicin at 0.02% concentration did not affect daily consumption of standard chow or water. Furthermore, body weight gain of capsaicin-treated rats did not differ from control rats. These results are in accordance with our previous report (Gu *et al.* 2009), revealing that oral capsaicin at the dose used in this study may not affect ingestive behaviour and/or energy expenditure of rats.

It has been reported that systemic capsaicin decreases dopamine release in the striatum (Hajos *et al.* 1986) and decreases motor activity in rodents (Di Marzo *et al.* 2001b; Lastres-Becker *et al.* 2003), possibly in mediation of the brain TRPV1 receptors. Intraperitoneal capsaicin at 1 mg/kg dose caused a significant reduction in locomotion (Lee *et al.* 2006), and the effective systemic dose of capsaicin on locomotion was as low as 0.1 mg/kg (Di Marzo *et al.* 2001a). However, in this study, repeated oral exposure to capsaicin at daily dose of 0.2 mg/rat did not affect the locomotor activity; i.e. the ambulatory counts, the total counts of beam interruptions in the horizontal sensor, or the distance travelled were not decreased in capsaicin-treated rats. This discrepancy may be due to the differences in administration route and/or treatment duration. That is, rats received a single intraperitoneal injection of capsaicin in the previous study (Di Marzo *et al.* 2001a) but repeated oral administration in this study.

Anxiogenic effects induced by systemic injection with TRPV1 agonists have been reported (for reviews, see Di Marzo *et al.* 2008; Starowicz *et al.* 2008; Moreira *et al.* 2012). In this study, repeated oral administration of capsaicin increased stereotypy counts of rats during the activity test, suggesting increased anxiety-like behaviours. Indeed, rostral grooming was markedly increased, but caudal grooming decreased, in capsaicin-treated rats. Grooming is often seen in animal models of stress and anxiety (Barros *et al.* 1994; Kalueff and Tuohimaa 2005), leading to a long-standing view of grooming as an anxiogenic response (Ferre *et al.* 1995; Nosek *et al.* 2008). It has been reported that highly stressed mice spend significantly more time grooming rostral areas than caudal (Kalueff and Tuohimaa 2004; Kalueff *et al.* 2007). Thus, the current results reveal that repeated oral administration of capsaicin increases stress-induced anxiety-like behaviours in rats. Increased anxiety-like behaviours by repeated oral capsaicin were further confirmed by decreased scores in open arm stay and entry, and increased scores in closed arm stay and entry, during the elevated plus maze test in this study.

It has been shown that alterations in the depression-like states in rodents by chronic stress affect grooming behaviours (Yalcin *et al.* 2007; Kompagne *et al.* 2008; Piato *et al.* 2008), and that intraperitoneal injection of TRPV1 agonists including capsaicin increases depression-like behaviours in

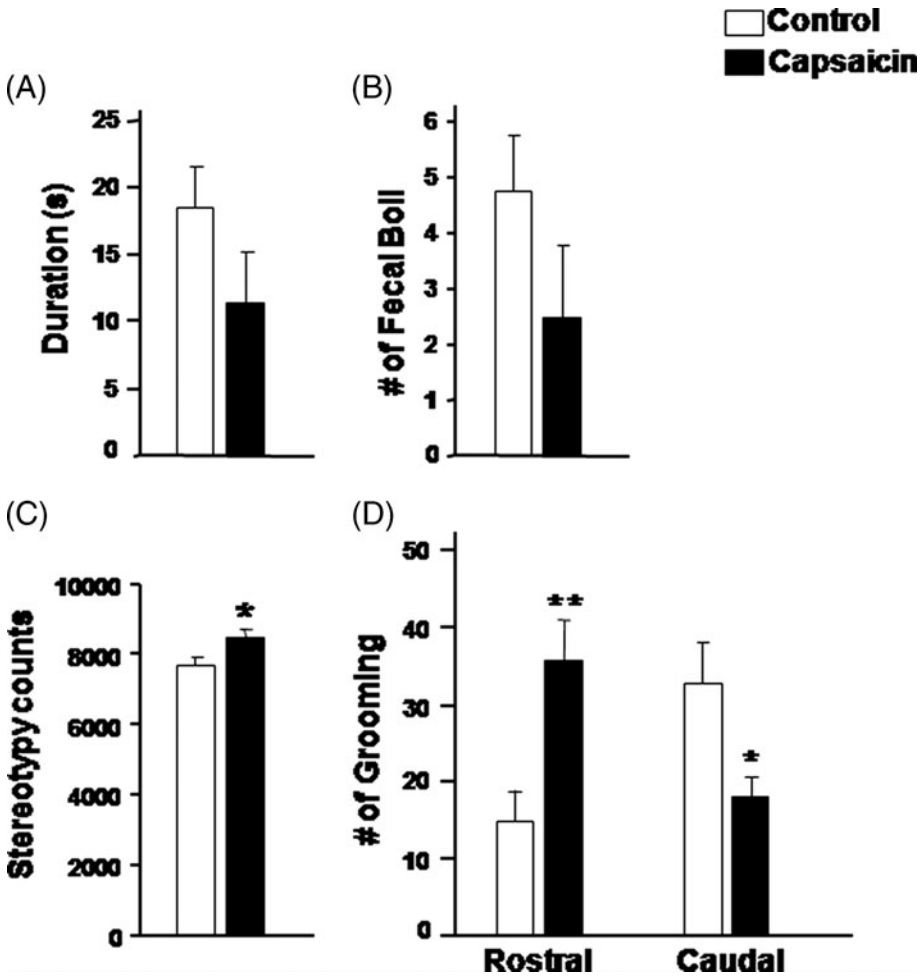

Figure 3. Time spent in the center zone (**A**), defecation activity (**B**), stereotypy counts (**C**) and grooming behaviours (**D**), which were scored during 30 min of activity test. **P*<0.05, ***P*<0.01 vs controls. Data are presented by mean±S.E.

rats (Di Marzo *et al.* 2001a; Kasckow *et al.* 2004). Oral capsaicin affected grooming behaviours of rats as mentioned above, and reduced swimming duration in the forced swim test in this study. It has been reported that reduced swimming and increased immobility during forced swim test represent increased depression-like behaviours in rodents (Ara and Bano 2012; Djordjevic *et al.* 2012). However, immobility duration of capsaicin-treated rats during the swim test did not differ from control rats and the decreased swimming appeared to be mainly due to increased struggling in this study. Thus, it is concluded that repeated oral administration of capsaicin may increase the depression-like states in rats, as indicated by the affected grooming behaviours and reduced swimming, but its depressive effect appears to be subtle; i.e. it did not affect immobility duration in the swim test. Kasckow *et al.* (2004) reported that systemic injections of TRPV1 agonist olvanil increased immobility of rats during the forced swim test, but they did not analyse swimming duration in their study. Recently, Hayase (2012) has reported that intraperitoneal injections of TRPV1 agonists capsaicin and olvanil did not affect either immobility or swimming of naïve mice during forced swim test; however, these produced antidepressant-like effects in nicotine-treated mice, showing increased immobility. Swimming behaviours of nicotine-treated mice were not affected by intraperitoneal injection of TRPV1 agonists. Collectively, behavioural effect of capsaicin in forced swim test is more likely controversial.

In this study, the stress-induced elevation of plasma corticosterone was prolonged in capsaicin-treated rats compared with control rats, suggesting a dysfunction of the HPA axis by repeated oral capsaicin. Dysfunctions of the HPA axis have been implicated in anxiety (Albenidou-Farmaki *et al.* 2008) and depression (Heim *et al.* 2000), and in animal models, stress-induced anxiety- and depression-like behaviours were

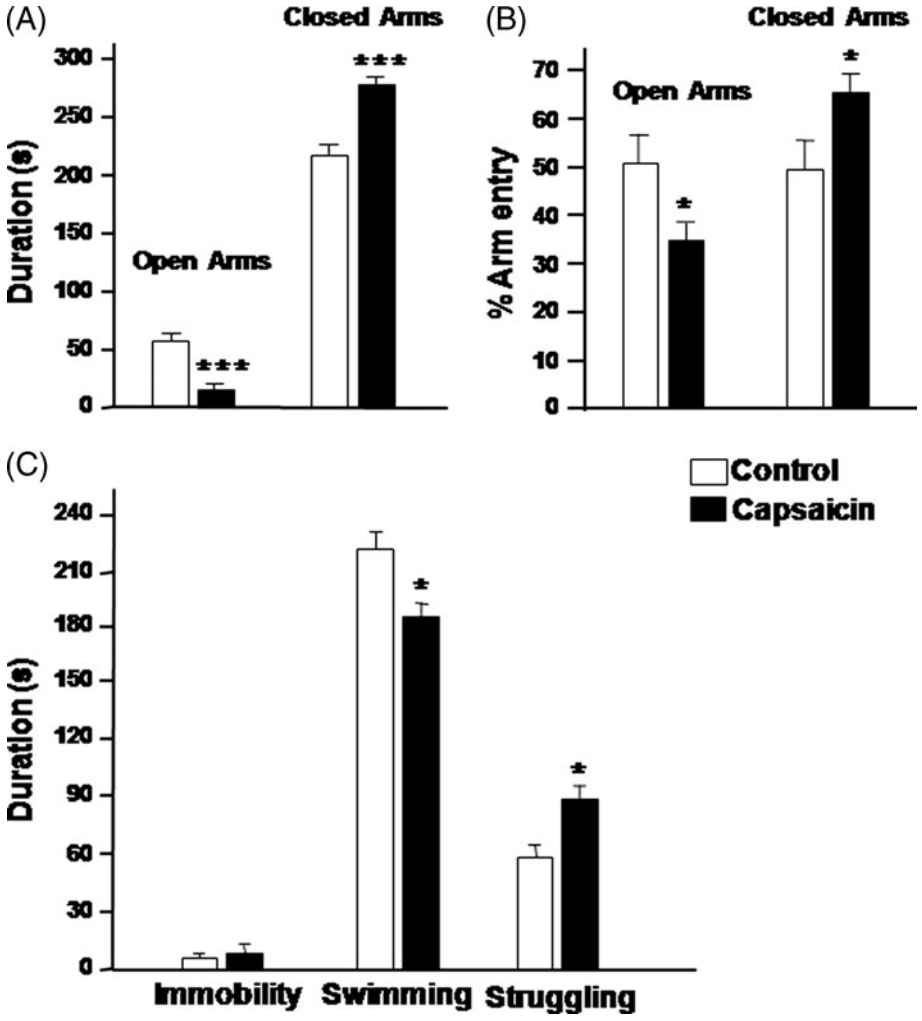

Figure 4. Time spent in each arms (**A**) and percent arm entry (**B**) during elevated plus maze test, and the scores during forced swim test (**C**). Rats were subjected to elevated plus maze test 30 min after the 14th oral exposure to capsaicin, and to forced swim test 30 min after the 17th oral exposure to capsaicin with a 15 min of pre-swim test on the prior day. *P<0.05, ***P<0.001 vs controls. Data are presented by mean±S.E.

associated with the HPA axis dysfunctions (Jahng 2011). Thus, it is likely that the HPA axis dysfunction may be implicated in the pathophysiology of anxiety-like behaviours by repeated oral administration of capsaicin.

Plasma level of glucocorticoids is elevated as a consequence of the HPA axis activation responding to stress, and the hypothalamic paraventricular nucleus (PVN) is located at the center of the HPA axis. Many studies have reported that meal ingestion activates neurons in the PVN and the nucleus tractus of solitarius (NTS), and oral-pharyngeal-esophageal and gastric cues may contribute to meal-induced c-Fos expression, a conventional marker for neuronal activation, in brain regions (Fraser *et al.* 1995; Rinaman *et al.* 1998; Pecoraro and Dallman 2005). The taste information relays to the PVN neurons via the rostral and intermediate NTS (Harrer and Travers 1996; Yamamoto 1998), and the visceral information via the caudal NTS (Tsukamoto and Adachi 1994; Yamamoto 1998; Berthoud and Neuhuber 2000). In this study, we have demonstrated that oral exposure to capsaicin increases c-Fos expression in the PVN and the intermediate NTS, but not in the caudal NTS. These results suggest that the capsaicin-induced oral information relays to the PVN via the intermediate NTS may activate the HPA axis. Further studies should attempt to define the underlying mechanisms by which repeated oral capsaicin affects the HPA axis function, such as c-Fos expression in the PVN and NTS following repeated capsaicin administrations and the PVN expression of corticotropin-releasing hormone after a single and repeated oral capsaicin.

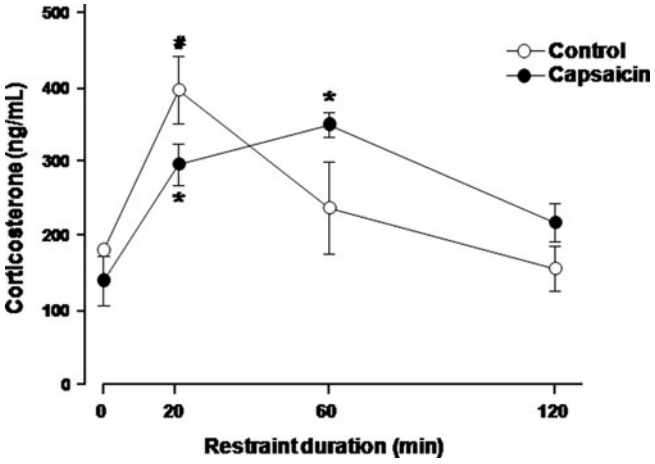

Figure 5. Plasma corticosterone levels during 2 h of restraint session. Rats were subjected to restraint stress following a week of recovery from the forced swim test. Oral treatment of capsaicin or water continued during the recovery period. Rats were placed in the restraint box 30 min after the oral exposure to capsaicin, and tail blood was collected at each time point. *P<0.05 vs 0 time point of capsaicin rats, $^{\#}P$<0.05 vs 0 time point of control rats. Data are presented by mean±S.E.

The capsaicin dose used in this study (0.02%) was based on a prior study, indicating that this dose, when presented in the form of capsaicin-containing food, is voluntarily ingested by rats (Park *et al.* 2004). However, the current study utilized experimenter-controlled administration of 0.02% capsaicin suspended in water, which produced acute aversive reactivity and an anxiogenic profile following repeated administration. It is possible that a different pattern of 'psycho-emotional' effects may have been observed with voluntary ingestion of the same dose mixed with other palatable substances, which is relevant to the typical human form of capsaicin consumption, as mentioned above (it is generally believed that hot spicy foods improve negative mood state). Human study has reported that, in general, there were no correlations between depressive symptoms and either intensity or pleasantness of the sweet, bitter, sour and salty tastants (Scinska *et al.* 2004), suggesting that depressive symptoms may not influence taste reactivity. However, anxiety level was positively correlated with bitter and salt taste thresholds (Heath *et al.* 2006). Thus, it is plausible that aversive property of oral capsaicin dose could have contributed to its anxiogenic potential in this study, and this could be cleared in future studies by the inclusion of additional aversive tastants.

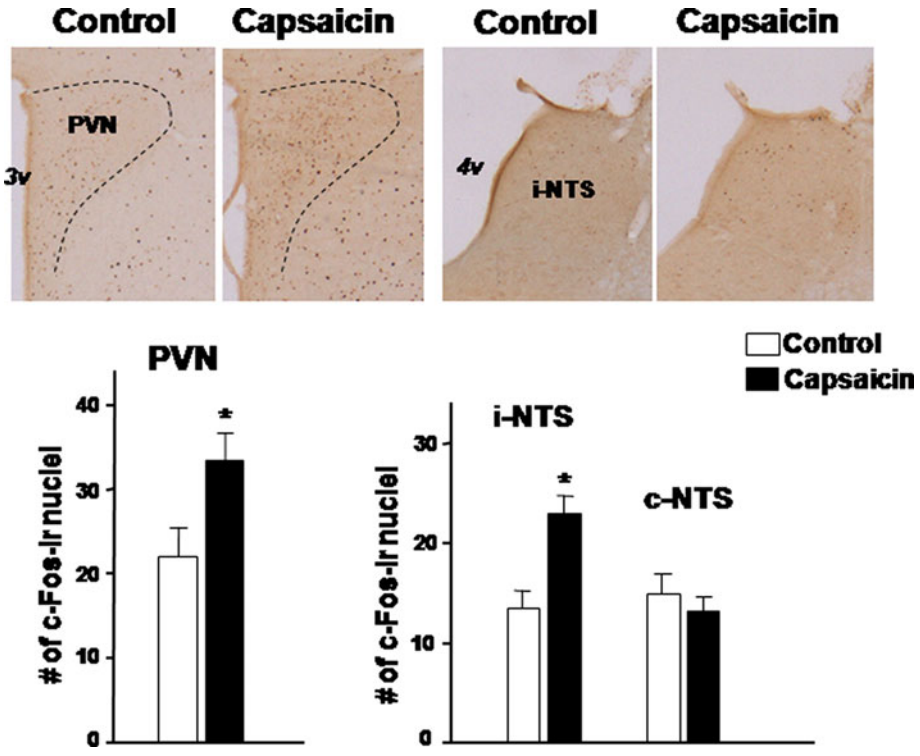

Figure 6. c-Fos immunohistochemistry in the hypothalamic paraventricular nucleus and the brainstem nucleus tractus of solitaries. Rats were transcardially perfused with 4% paraformaldehyde 1 h after a single administration of oral capsaicin. *P<0.05 vs controls, PVN; paraventricular nucleus, 3v; third ventricle, 4v; fourth ventricle, i-NTS; intermediate nucleus tractus of solitaries, c-NTS; caudal nucleus tractus of solitaries. Data are presented by mean±S.E.

In conclusion, repeated oral exposure to capsaicin increased anxiety-like behaviours in rats, which was accompanied by a prolonged elevation of the plasma corticosterone responding to restraint stress. Results suggest that the HPA axis dysfunction may be implicated in the pathophysiology of anxiety-like behaviours by repeated oral capsaicin.

Acknowledgements

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