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Attenuation of fear-like response by escitalopram treatment after electrical stimulation of the midbrain dorsolateral periaqueductal gray

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ABSTRACT

Electrical stimulation of the dorsolateral periaqueductal gray (dlPAG) has frequently been shown to induce escape and freezing/decreased locomotion responses which mimic panic- and fear-like behaviour. In the present study we tested whether such spontaneous fear-like behaviour could be observed in an open-field test 12 h after dlPAG stimulation. Further, we tested whether this fear-like behaviour could be attenuated by acute or chronic administration of buspirone and escitalopram. Our data demonstrate for the first time that animals showed fear-like behaviour 12 h after dlPAG stimulation, which may possibly reflect panic disorder with anticipatory anxiety/agoraphobic symptoms. Acute and chronic escitalopram, but not buspirone, treatment attenuated the fear-related behaviour. Besides, our data also showed that the stimulation intensities to evoke an escape reaction, a panicogenic response, were significantly higher after chronic buspirone and escitalopram treatment. These results suggest that the fear-like response, which was observed 12 h after dlPAG stimulation, could be considered as a relevant animal model for panic disorder with anticipatory anxiety/agoraphobic symptoms.

Introduction

A panic attack is characterized by an acute moment of intense fear and psychological distress with a sudden onset and rapidly building to a peak level. It is followed by somatic and psychological symptoms associated with a sense of impending danger and an urge to escape from the susceptible risk condition (Griez and Schruers, 1998; Griez et al., 2001; Roy-Byrne et al., 2006). Panic attacks are regarded as the central pathological feature of panic disorder and are characterized by physical symptoms such as shortness of breath, sweating, palpitations, trembling, feeling of choking, chest pain or discomfort, nausea or abdominal distress, feeling dizzy or unsteady, derealisation, fear of losing control, fear of dying, paresthesia, and chills or hot flushes. About two third of panic disorder patients will suffer from agoraphobia: they try to avoid situations in which escape might be difficult or unavailable in the event of having a panic attack or endure these situations with difficulty (American-Psychiatric-Association, 2000; Cassano and Savino, 1993). Agoraphobia develops by associating environmental cues with panic-related cues (Bouton et al., 2001). The percentage of lifetime prevalence of panic attack ranges from 3 to 5.6% (American-Psychiatric-Association, 1998 May). This disorder does not only cause functional disability but also affects the quality of life and related socio-economic conditions and interpersonal relationships (Abbar, 1996; Klerman et al., 1991; Wittchen, 1988).

In clinical laboratory, panic attacks can be provoked by many chemical substances such as sodium lactate infusion (Liebowitz et al., 1984; Liebowitz et al., 1985) or inhalation of carbon dioxide (Esquivel et al., 2009; Overbeek et al., 2005). Besides, it is also shown that electrical stimulation of the human dorsal periaqueductal gray (PAG) produces panic-like symptoms including anxiety, terror, impending death, desire to flee, palpitation, and hyperventilation (Amano et al., 1978; Nashold et al., 1969). In animals, plenty evidence suggests that panic-like responses can be produced by stimulation of the dorsal periaqueductal gray (PAG) (for reviews see, Graeff and Del-Ben, 2008; Schenberg et al., 2001; Vianna et al., 2001). This panic-like behaviour

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of rodent has been characterized by modeling the panic attacks in humans. However, these experimental models only partially mimic the clinical condition of panic attacks in humans insofar that they do not reproduce the anticipatory anxiety/agoraphobic symptoms in the absence of dlPAG stimulation. Thus, previous studies suggested that in dlPAG-evoked behaviour model panic attacks, there are no studies showing a long lasting effect after the dlPAG stimulation even though some studies have already addressed this question (Broiz et al., 2008; Oliveira et al., 2007; Vianna et al., 2001). Since panic attacks in humans usually lead to long lasting effects (Bouton et al., 2001), the face validity of panic disorder with anticipatory anxiety/agoraphobic symptoms (i.e., post-stimulation induced fear) may be improved on these features of dlPAG stimulation.

In our previous studies, we have shown that electrical stimulation of the dlPAG in rats leads to vigorous running and jumping in an open-field arena (Lim et al., 2008a; Lim et al., 2009). Qualitative observations indicated that when stimulated rats were placed in the same open-field arena again after 12 h of escape behaviour, they became highly immobile and showed head scanning, putatively indicative of anticipatory anxiety/agoraphobic behaviour. At present, animal studies have shown that the administration of selective serotonin reuptake inhibitors (SSRIs, e.g. fluoxetine, citalopram, escitalopram, and paroxetine) is able to attenuate or increase the threshold for escape behaviour induced by the dorsal regions of PAG stimulation in rats (Hogg et al., 2006; Schenberg et al., 2002; Vargas and Schenberg, 2001; Zanoveli et al., 2005). In the first part of the present study, we recorded the behaviour of the subjects after having received dlPAG stimulation with parameters evoking panic-like behaviour in the open-field arena. Subsequently, the effects of acute and chronic escitalopram treatment on fear behaviour were tested in the same group of animals. We expected that this SSRI, but not buspirone, would attenuate the fear response induced by dlPAG stimulation based on its efficacy in the treatment of panic disorder in humans. The aim of the present study was to investigate this phenomenon in more detail in an attempt to validate a rat model for panic disorder. We hypothesize that the panic-like response followed by fear-like behaviour 12 h after electrical stimulation of the dlPAG is a model of anticipatory anxiety/agoraphobia in humans, since it can be influenced by the same pharmacological intervention as in humans. The pharmacological treatment of choice for panic disorder is SSRI, such as escitalopram (Hogg et al., 2006; Hollander and Simeon, 2003). Buspirone on the other hand, a classical 5-HT_{1A} receptor agonist, is effective in the treatment of generalized anxiety disorder but not for panic disorder (Ninan, 2004). Furthermore, studies making use of human experimental models for panic have demonstrated a modulatory role of serotonin in panic (Schruers and Griez, 2004; Schruers et al., 2000; Schruers et al., 2002).

Material and methods

Subjects

Subjects were male albino Wistar rats ($N = 40$, 12 weeks old, bred and housed at the Central Animal Facility of Maastricht University, Maastricht, the Netherlands). Rats had an average body weight of 300–350 g at the time of surgery. They were housed individually in standard cages on sawdust bedding in an air-ventilated room (about 20 °C) under a 12/12-h reversed light/dark cycle. Food, standard laboratory chow (Hopefarms, Woerden, the Netherlands), and water were available *ad libitum*. This study was approved by the Animal Experiments and Ethics Committee of Maastricht University, Maastricht, the Netherlands.

Experimental groups

Rats were randomly assigned to one of the following six experimental groups: A. dlPAG stimulation and treatment with saline (STIM-SAL, n = 7); B. dlPAG stimulation and treatment with buspirone (STIM-BUSP, n = 7); C. dlPAG stimulation and treatment with escitalopram (STIM-ESCIT n = 7); D. dlPAG sham surgery and saline treatment (SHAM-SAL, n = 6); E. dlPAG sham surgery and buspirone treatment (SHAM-BUSP, n = 6); and F. dlPAG sham surgery and escitalopram treatment (SHAM-ESCIT, n = 7).

A schematic representation of the time-line of the experiment is shown in Fig. 1. All animals were handled regularly in order to habituate them to being picked up and reduce stress during behavioural testing.

Surgical procedures

A detailed description of the surgical procedure has been reported previously (Temel et al., 2007). In brief, the rats were anesthetized throughout the entire procedure using a combination of ketamine (90 mg/kg) and xylazine (10 mg/kg) injected subcutaneously (s.c.). Rats were placed in a stereotactic apparatus (Stoelting, Wood Dale, USA; model 51653). After making a burr hole in the skull, rats received implantation of the electrode at the level of the dlPAG (coordinates from Bregma: anteroposterior, −7.6; mediolateral, +0.7; and ventral, −4.8; approached with a coronal angle of 10°) (Paxinos and Watson, 1998). A construction of one gold-plated needle-like electrode with an inner wire of a platinum–iridium combination (Technomed, Beek, the Netherlands) with a tip diameter of 50 μm and a shaft diameter of 250 μm, was employed in this experiment (Temel et al., 2007; Temel et al., 2004). The electrodes were fixed in position using dental cement (Heraeus Kulzer, Hanau, Germany). After the operation, rats were injected with Temgesic (0.1 mg/kg, s.c.) and allowed to recover for two weeks.

Deep brain stimulation

All animals in the dlPAG stimulation group underwent a stimulation session in their home cages in order to determine the level of the escape threshold. The stimulation amplitudes were gradually increased until the escape behaviour (i.e. rigorously running with aimless direction) was observed. The stimulation duration was 15 s followed by a stimulation-off period of 45 s. After this the cable

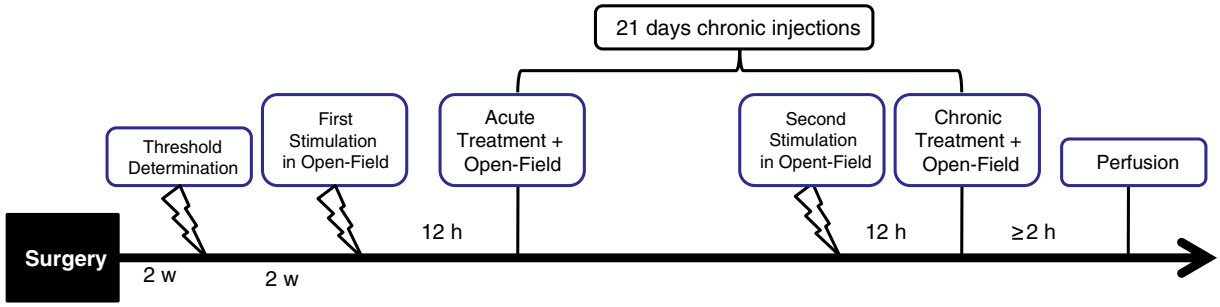

Fig. 1. A schematic representation of the time-line of the experiments (w = weeks and h = hours).

was removed. In this study, the criteria of 2 consecutive positive response of escape reaction were required to determine the escape threshold. The stimulation frequency was set at 50 Hz, and pulse width at 0.1 ms based on previous experience (Lim et al., 2009). Animals which required stimulation intensities above 100 μA were discarded from analysis. The intensities were increased in a stepwise manner until an escape response was observed. A World Precision Instruments (WPI) digital stimulator (DS8000, WPI, Berlin, Germany) and a stimulus isolator (DLS100, WPI, Berlin, Germany) were used to deliver the stimuli. Real time verification of the parameters applied during stimulation was obtained using a digital oscilloscope (Agilent 54622D oscilloscope, Agilent Technologies, Amstelveen, the Netherlands) as reported earlier (Temel et al., 2004). After the threshold determination session, all rats were given a recovery period of two weeks. The sham animals were similarly connected to the stimulator but no current was delivered to the dlPAG.

Drug administration

Escitalopram oxalate (ESCIT) (H. Lundbeck A/S, Copenhagen, Denmark) and Buspirone hydrochloride (BUSP) (TOCRIS, Cookson Inc., Missouri, USA) were dissolved in saline 0.9% NaCl. ESCIT (10 mg/kg) and BUSP (3 mg/kg) were injected (s.c. in a volume of 1 ml/kg) 60 min and 120 min before behavioural testing, respectively. The doses of ESCIT (Lugenbiel et al., 2009; Sanchez et al., 2003) and BUSP (Kakui et al., 2009; Lim et al., 2008b; Tunnicliff et al., 1992) were chosen based on its effective pharmacological profile of previous experimental studies. A week before the actual experimental testing, all animals received 1 ml saline injection 3 times on alternating days in order to habituate the animals to the injection procedure. A single-dose acute treatment was performed before the first open-field test. After recording the behaviour, the subjects entered the chronic experiment in order to test its efficacy of long-term treatment as the maximal effects of these drugs usually take place after a chronic phase (Assie et al., 2006; Bondi et al., 2008; Burghardt et al., 2004; Sato et al., 2008). They received 21 daily injections of ESCIT, BUSP, or SAL. Before the animals were tested the second time in the open-field, the final drug administration was performed 60 min (ESCIT/SAL) or 120 min (BUSP) before testing, similar to the acute experiment. The injection-test intervals were chosen on basis of previous studies.

Behavioural testing

The apparatus consisted of an enclosed open-field (square: 100 cm × 100 cm, and height: 40 cm), clear Plexiglas box with an open top and a dark floor (Lim et al., 2008c). All open-field testing was conducted in the same room in a dimly lit condition from 08:00 till 10:00 h. On the first day of stimulation in the open-field, rats were placed in the open-field arena and connected with the external stimulator through externalized leads. The electrical stimulation was performed based on the previously determined escape threshold level and started 1 min after the rat was placed in the open-field arena. Once the escape reaction was evoked, the cable connected to the rat was detached and the animal was left in the open-field for approximately 30 s. The electrical stimulation for escape reaction was carried out from 20:00 till 22:00 h.

After 12 h of escape behaviour rats were placed again in the open-field arena. The behaviour of each rat was recorded using an automated system consisting of a camera connected to a computer with the Ethovision tracking software (Ethovision, Noldus Information Technology, Wageningen, the Netherlands). The software will automatically calculate and analyze data on the position of the animal relative to its open-field, such as the locomotion/distance moved, velocity, and time spent in the corner of the open-field. A trial was stopped after 5 min and the rat was removed from the arena. Fear or freezing behaviour was defined by a decreased locomotion (immobility) and increased duration of time spent in the corner of the open-field. Escape behaviour was characterized by rigorous and aimlessly running within the open-field arena.

Histological processing

Two hours after the final testing of the rats in the open-field, rats were placed under generalized anesthesia with Nembutal (75 mg/kg) and perfused transcardially with Tyrode (0.1 M) and fixative solution containing paraformaldehyde, picric acid and glutaraldehyde in phosphate buffer (pH 7.6). The rat brains have been post-fixed for 2 h. Hereafter, the brains were cryoprotected by overnight sucrose treatment. Brain tissue was then quickly frozen with CO_2 and stored at −80 °C. Subsequently, the brains were cut serially (10 series) on a cryostat (MICROM, Walldorf, Germany) into 30 μm frontal sections and again stored at −80 °C. One series of sections per animal was stained with standard hematoxylin–eosin (Merck, Darmstadt, Germany) to examine the localization of the electrode tips.

Statistical analysis

All data are presented as mean ± S.E.M and were analyzed using three-way repeated measures using Treatment and Stimulation as between-subjects factors, and Time (acute and chronic treatment) as within-subject factor. A Duncan post hoc test was used to analyze group differences per time point in more detail. All p-values <0.05 were considered significant.

Results

Histological evaluation of the electrode localization

In all rats, except for four, the electrode tips were located within the dlPAG. One rat in the STIM-SAL group, one rat in the STIM-ESCIT group, and two rats in the SHAM-ESCIT group, the electrode was placed outside the dlPAG. The final group sizes were six animals per group for SHAM-SAL, SHAM-BUSP, STIM-SAL, and STIM-ESCIT groups, and seven animals for groups STIM-BUSP, and SHAM-ESCIT.

The localization of the electrode tip in the dlPAG is illustrated in Fig. 2. Except for the electrode trajectory, we observed no additional tissue damage due to repeated stimulation.

Levels of escape threshold

The intensity of stimulation current applied to the dlPAG of the animals was based on the lowest threshold to induce an escape response. There were no group differences before acute treatment ($F(2,17) = 0.02$, n.s.; see Fig. 3). Remarkably, when rats were stimulated for the second time in the open-field (see Fig. 3), rats from the STIM-BUSP and STIM-ESCIT groups did not show an escape response at the intensity as determined at the first stimulation session. The stimulation intensities to evoke escape behaviour were significantly higher when the threshold was determined for the second time (see Fig. 1) for animals in the STIM-BUSP and STIM-ESCIT groups ($F(2,17) = 26.07$, $p<0.001$).

Open-field behaviour 12 h after dlPAG stimulation

There was no effect of Time on the measure distance moved in the open-field ($F(1,33) = 0.07$; see Figs. 4A/5A). Also, no Time with Treatment/Stimulation interactions were found (F's <1.42, n.s.), indicating that the effects were independent of the time of treatment (acute vs chronic treatment). There was a significant decrease in the distance moved of the rats when the animals were placed in the open-field 12 h after dlPAG stimulation ($F(1,33) = 16.30$, $p<0.01$; see Fig. 4). In addition, there was a Stimulation by Treatment interaction

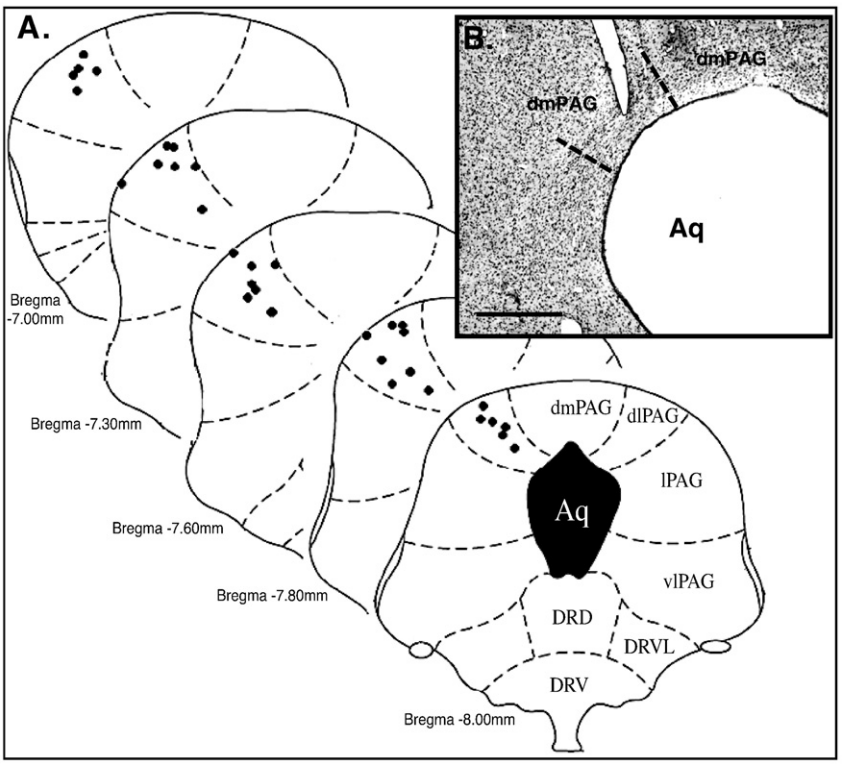

Fig. 2. The histological picture shows a representative low-power photomicrograph of a 30 μm-thick coronal section from the brain of a rat subjected to stereotactic implantation of concentric bipolar electrode to stimulate the dlPAG (B, scale bar = 250 μm) is shown. A, Schematic representation of the electrode sites in the dlPAG according to the Paxinos and Watson atlas (Paxinos and Watson, 1998), respectively. The symbol (●) indicates the electrode localization in the dlPAG. Abbreviations: Aq = aqueduct of Sylvius; dlPAG = dorsolateral periaqueductal gray; dmPAG = dorsomedial periaqueductal gray; lPAG = lateral periaqueductal gray; vlPAG = ventrolateral periaqueductal gray; DRD = dorsal raphe nucleus, dorsal part; DRVL = dorsal raphe nucleus, ventrolateral part; and DRV = dorsal raphe nucleus, ventral part.

($F(2,33) = 4.83$, $p<0.05$), indicating that independent of the duration of treatment the ESCIT-treated rats moved a longer distance in the stimulated animals but not in the sham animals. This was confirmed with additional post hoc analysis.

There was an effect of Time on the measure time spent in the corner areas of the open-field ($F(1,33) = 11.38$, $p<0.01$; see Figs. 4B/5B), indicating that overall the rats spent more time in the corner areas after chronic treatment. However, no Time with Treatment/Stimulation interactions were found (F's<0.92, n.s.). There was a clear increase in the time spent in the corner areas when the animals were placed in the open-field 12 h after dlPAG stimulation ($F(1,33) = 14.43$, $p<0.01$)

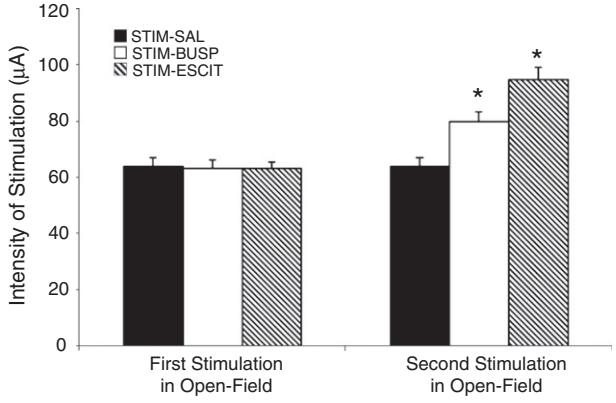

Fig. 3. This figure shows the current intensities (μA) necessary to induce escape behaviour. Data represent means ± S.E.M. Indication: *significant difference from the STIM-SAL group during the second stimulation in the open-field. Abbreviations: STIM-SAL, dlPAG stimulation and treatment with saline; STIM-BUSP, dlPAG stimulation and treatment with buspirone; and STIM-ESCIT, dlPAG stimulation and treatment with escitalopram.

after stimulation effect of stimulation. Further, there was a clear Stimulation by Treatment interaction ($F(2,33) = 10.12$, $p<0.01$), indicating that independent of the duration of treatment ESCIT reduced the time in the corner squares in the stimulated animals but not in the sham animals. This was confirmed with additional post hoc analysis.

For the measure mean velocity a similar picture emerged as for the measure distance moved (see Figs. 4C/5C). No effect of Time and the associated interactions Treatment and Stimulation were found (F's<1.45, n.s.), indicating that the effects were independent of the time of treatment (acute vs chronic treatment). However, stimulation decreased the mean velocity of movements ($F(1,33) = 16.57$, $p<0.01$). The Treatment by Stimulation interaction effect ($F(2,33) = 4.81$, $p<0.05$) again showed that ESCIT increased the mean velocity in the stimulated animals but not in the sham animals. These findings were supported by the post hoc analyses.

Discussion

In this study, electrical stimulation of the dlPAG evoked escape reactions followed by decreased locomotion/freezing response when animals were placed back into the open-field 12 h later. These findings indicate that dlPAG stimulation induces long lasting (at least 12 h) fear which seems to be associated with the open-field environment. Further, it was shown that ESCIT, but not BUSP, treatment was effective in attenuating the fear-like behaviour in both the acute and chronic treatment conditions. Interestingly, the stimulation intensities to evoke escape behaviour were increased after chronic treatment with ESCIT and BUSP, indicating that chronic BUSP treatment could be beneficial to increase the threshold for a panic attack. Altogether, our data show that dlPAG stimulation induces behavioural and physiological changes which were observed

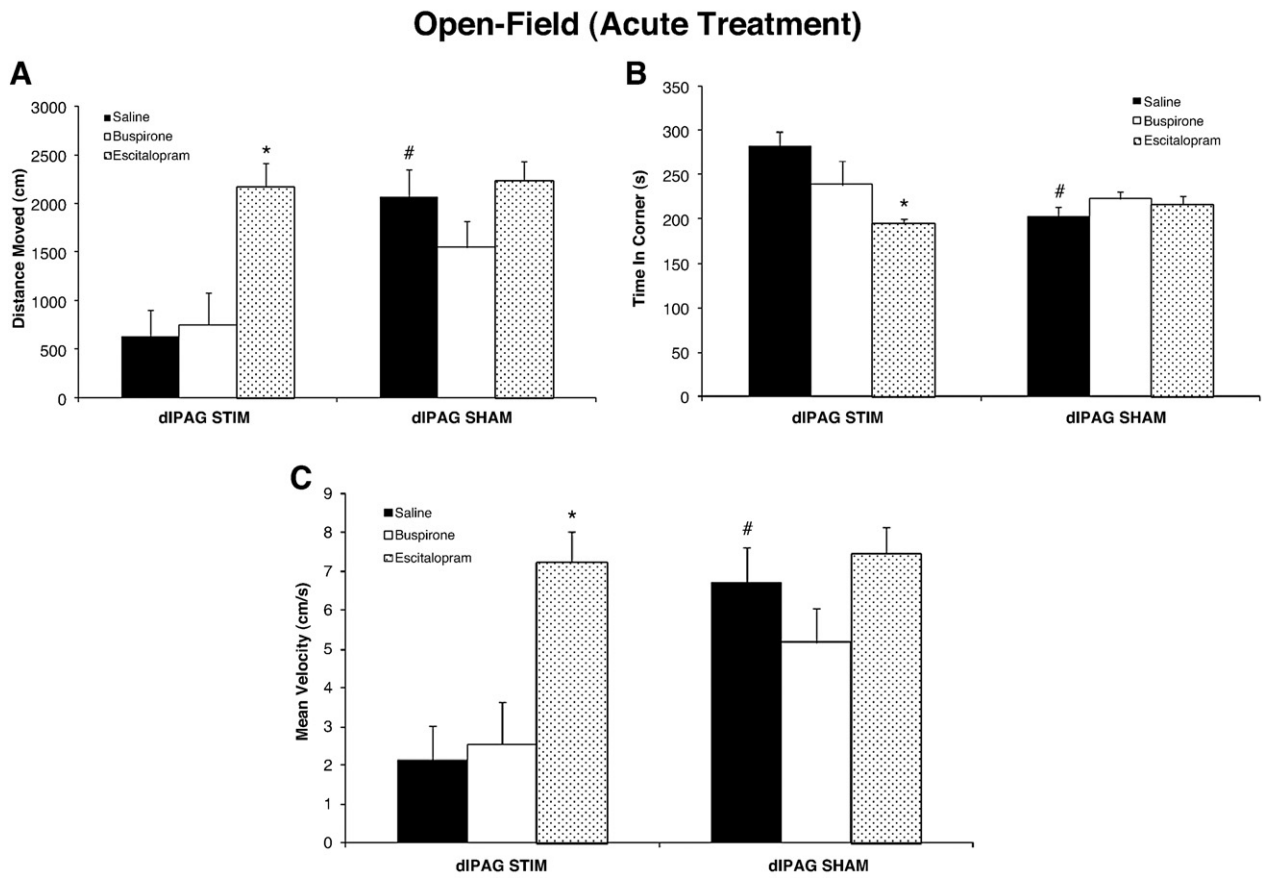

Fig. 4. A set of bar graphs showing the behavioural data of acute treatment with saline, buspirone, and escitalopram for the dlPAG stimulation, and dlPAG sham rats. The data of distance moved (A), time spent in the corner zones (B), and mean velocity (C) in the open-field are expressed as means ± S.E.M. Indication: *significant difference from the STIM-SAL and STIM-BUSP, and #significant difference from the STIM-SAL.

12 h after stimulation. Moreover, the observed freezing behaviour/decreased locomotion in the open-field can be considered to model internally generated fear.

It could be argued that the freezing behaviour observed 12 h after stimulation could be regarded as generalized fear after stimulation. However, before the rats were tested in the open-field no freezing was observed in their home cage. The rats showed a normal behavioural repertoire in their home cage and the freezing behaviour/decreased locomotion was observed as soon as they were placed in the open-field. In the present study we did not test whether the fear was also expressed in another environment. Previous data have shown that DPAG-evoked freezing persists when rats were moved to another context immediately after the stimulation, but was completely absent 24 h later (Vianna et al., 2001). It should be noted that in this latter study relatively small boxes were used in which the testing condition was modified. To what extent the dlPAG stimulation induced fear, as shown in the present study, generalizes to other environment conditions needs to be investigated in further studies.

It was hypothesized that the activation of 5-HT_{1A} receptors in dlPAG produces inhibition of aversive responses, an anti-aversive effects (Brandao et al., 1991). Furthermore, Graeff also suggested that the increase of 5-HT availability within the midbrain decreases the magnitude of panic (Graeff, 2004). Electrophysiological and microdialysis studies have demonstrated that the somatodendritic 5-HT_{1A} receptors become desensitized after prolonged administration of SSRIs (Dawson et al., 2000; Hamon et al., 1990; Invernizzi et al., 1994). This is proposed to lead to the therapeutic effect of ESCIT as antidepressant and panicolytic drug which can be achieved either via the stimulation of 5-HT_{1A} receptors indirectly through increased availability of synaptic 5-HT or possibly mediated by activation of 5-HT_{1A} receptors (De Vry, 1995). The behavioural effect observed herein after panic attacks was in accordance with previous reports showing the panicolytic properties of ESCIT in the electrically stimulated dorsal PAG (Hogg et al., 2006). The latter authors reported that ESCIT significantly reduced the escape- or panic-like behaviour indicating the potential therapeutic efficacy for panic disorders and possibly for fear.

In this study, ESCIT was found to be effective in reducing the fear responses following both after acute and chronic administration. This is in line with previous studies in which acute SSRIs treatment reduced the dlPAG stimulation induced panic-like behaviour (Hogg et al., 2006) and reduced fear responses in an inescapable electric foot-shock paradigm (Hashimoto et al., 1996; Inoue et al., 1996a,b). On the other hand, there is also a study reporting a lack of acute SSRI treatment in a dlPAG stimulation model (Borelli et al., 2004). The effects of acute 5-HT treatment are mixed. For example, it has been reported that acute treatment with SSRIs enhanced auditory fear conditioning in rats (Burghardt et al., 2004). While in human study it was shown that acute administration of L-5-hydroxytryptophan inhibited panic (Schruers et al., 2002). Some possible explanations for these discrepant findings could be related to the different experimental methodologies used and the complex neural mechanism underlying fear circuitry. In view of this, it should be noted that the different processes of fear acquisition (inescapable electric foot-shock or auditory fear conditioning vs the intrinsic generation of fear by dlPAG stimulation) induce a different pattern of fear sensory information and this will also influence the treatment effects.

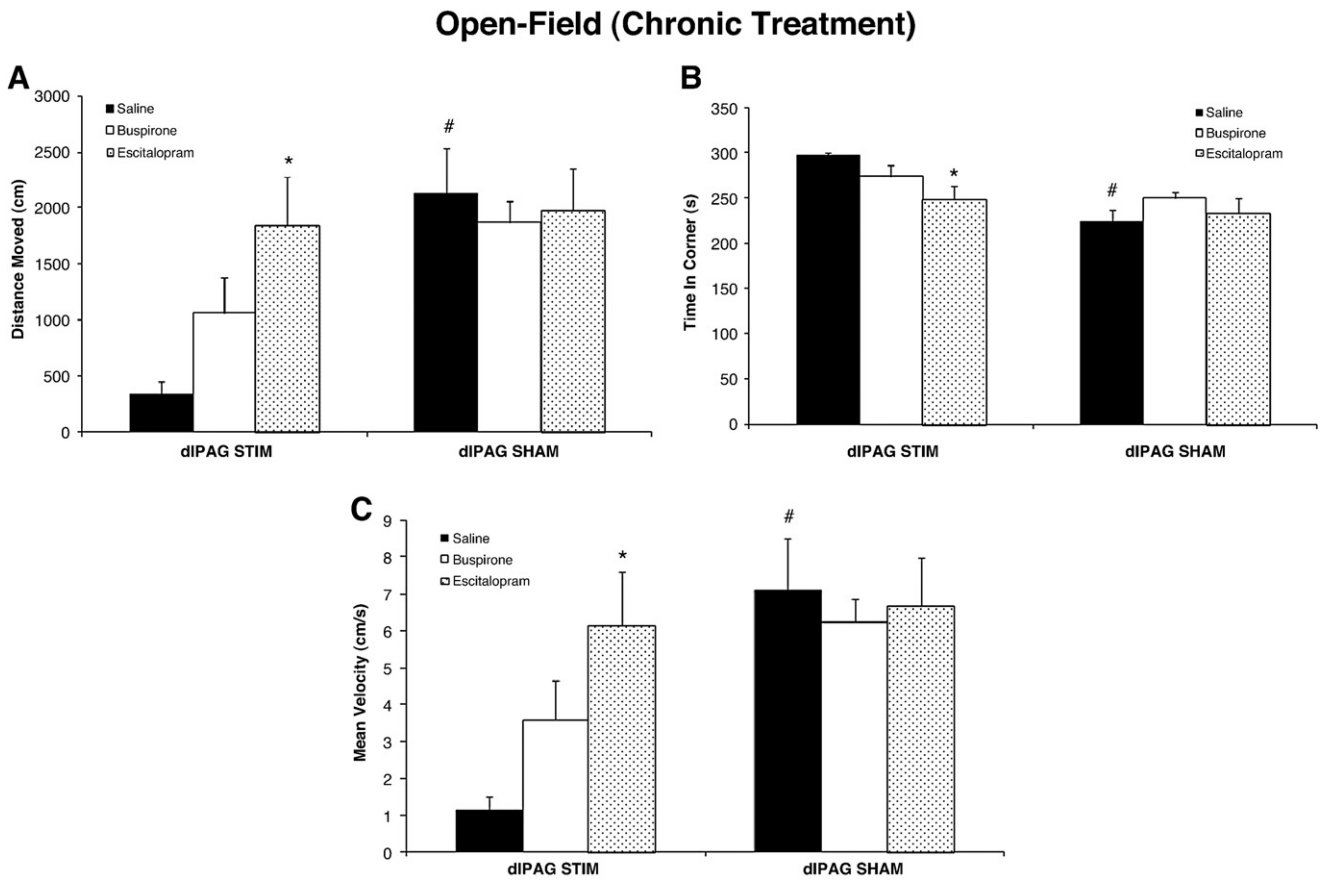

Fig. 5. A set of bar graphs showing the behavioural data of chronic treatment with saline, buspirone, and escitalopram for the dlPAG DBS, and dlPAG sham rats. The data of distance moved (A), time spent in the corner zones (B), and mean velocity (C) in the open-field are expressed as means ± S.E.M. Indication: * and #significant difference from the STIM-SAL.

In addition, our study shows that the current intensity for the escape threshold was remarkably increased in the groups of STIM-BUSP and STIM-ESCIT. This corroborates previous studies on the acute reduction of aversive response when animals were subjected to the dorsal PAG stimulation using anti-panic agents such as alprazolam, clonazepam, zimelidine (Jenck et al., 1990; Jenck et al., 1995), escitalopram, fluoxetine, and paroxetine (Hogg et al., 2006). It was proposed that the anti-aversive effects were obtained by increasing the extracellular synaptic availability of 5-HT in the brain (Jenck et al., 1995; Schutz et al., 1985) as well as through the activation of 5-HT_{2c} receptors (Jenck et al., 1998). The present study showed that BUSP was able to increase the aversive threshold for escape reaction but did not reduce the freezing behaviour in the open-field testing. It could be argued that the increase in threshold may be due to a more general effect of drug treatment since various types of drugs can change the threshold for inducing a panic-like response. On the other hand, the anti-panic-like effect may be related with a more specific effect of SSRI treatment.

The PAG is considerably rich in 5-HT immunoreactive nerve terminals which mainly come from the serotonin-containing neurons in the dorsal raphe nucleus (Beitz et al., 1986; Lovick et al., 2000). Many studies on the 5-HT_{1A} receptor subtype have indicated that this subsystem plays an important role in the modulation of fear conditioning (Ceglia et al., 2004; Pobbe and Zangrossi, 2005; Quartermain et al., 1993). Recent findings on the 5-HT_{1A} receptor subtype have indicated that this structure is highly involved in the modulatory function underlying generalized anxiety disorder and defensive behaviour (Argyropoulos et al., 2004; Quartermain et al., 1993). Furthermore, our previous results have shown that BUSP treatment induced a different pattern of neuronal activation in the different PAG regions (the rostral dlPAG part was found to be responsive to buspirone treatment but not the caudal dlPAG), indicating a different level of sensitivity of the 5-HT_{1A} receptor in the pathophysiology of anxiety and panic disorder (Lim et al., 2008b). It is clinically well established that BUSP is effective for generalized anxiety disorder but not for panic disorder (Ninan, 2004). Previous experimental evidence has also demonstrated no effects on escape behaviour, the panic-like reaction in the dlPAG stimulated animals (Connor and Davidson, 1998; Poltronieri et al., 2003). However, some opposing results showed that the administration of 5-HT_{1A} agonist into the dorsal PAG was possible to inhibit this escape reaction during the electrical stimulation of this structure (de Bortoli et al., 2006; Nogueira and Graeff, 1995).

Taken together, the long lasting effects of dlPAG stimulation as demonstrated in this study show similarities with the panic disorder anticipatory anxiety/agoraphobic symptoms typical for panic disorder in humans. This fear-like response, in the absence of direct dlPAG stimulation, was attenuated by ESCIT treatment while BUSP was not effective, thereby paralleling pharmacological effects in humans. Further, in addition to the many studies that have shown that dorsal PAG stimulation is an animal model for panic attacks, our results for the first time suggest that the acute panic-like response after dlPAG stimulation followed by fear-like behaviour 12 h later can be considered as a relevant animal model for panic disorder with anticipatory anxiety/agoraphobic symptoms.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.expneurol.2010.08.035.

References

Abbar, M., 1996. Panic disorder and panic attack. Encephale 22 (Spec No 5), 13–18.

Amano, K., Tanikawa, T., Iseki, H., Kawabatake, H., Notani, M., Kawamura, H., Kitamura, K., 1978. Single neuron analysis of the human midbrain tegmentum. Rostral mecencephalic reticulotomy for pain relief. Appl. Neurophysiol. 41, 66–78.

American-Psychiatric-Association, 1998. Practice guideline for the treatment of patients with panic disorder. Work Group on Panic Disorder. Am. J. Psychiatry 155 (5 Suppl), 1–34 (May).

American-Psychiatric-Association, 2000. Diagnostic and Statistical Manual of Mental DisordersFourth ed. American Psychiatric Press, Washington, DC. Text Revision.

Argyropoulos, S.V., Hood, S.D., Adrover, M., Bell, C.J., Rich, A.S., Nash, J.R., Rich, N.C., Witchel, H.J., Nutt, D.J., 2004. Tryptophan depletion reverses the therapeutic effect of selective serotonin reuptake inhibitors in social anxiety disorder. Biol. Psychiatry 56, 503–509.

Assie, M.B., Lomenech, H., Ravailhe, V., Faucillon, V., Newman-Tancredi, A., 2006. Rapid desensitization of somatodendritic 5-HT1A receptors by chronic administration of the high-efficacy 5-HT1A agonist, F13714: a microdialysis study in the rat. Br. J. Pharmacol. 149, 170–178.

Beitz, A.J., Clements, J.R., Mullett, M.A., Ecklund, L.J., 1986. Differential origin of brainstem serotoninergic projections to the midbrain periaqueductal gray and superior colliculus of the rat. J. Comp. Neurol. 250, 498–509.

Bondi, C.O., Rodriguez, G., Gould, G.G., Frazer, A., Morilak, D.A., 2008. Chronic unpredictable stress induces a cognitive deficit and anxiety-like behavior in rats that is prevented by chronic antidepressant drug treatment. Neuropsychopharmacology 33, 320–331.

Borelli, K.G., Nobre, M.J., Brandao, M.L., Coimbra, N.C., 2004. Effects of acute and chronic fluoxetine and diazepam on freezing behavior induced by electrical stimulation of dorsolateral and lateral columns of the periaqueductal gray matter. Pharmacol. Biochem. Behav. 77, 557–566.

Bouton, M.E., Mineka, S., Barlow, D.H., 2001. A modern learning theory perspective on the etiology of panic disorder. Psychol. Rev. 108, 4–32.

Brandao, M.L., Lopez-Garcia, J.A., Graeff, F.G., Roberts, M.H., 1991. Electrophysiological evidence for excitatory 5-HT2 and depressant 5-HT1A receptors on neurones of the rat midbrain tectum. Brain Res. 556, 259–266.

Broiz, A.C., Oliveira, L.C., Brandao, M.L., 2008. Regulation of conditioned and unconditioned fear in rats by 5-HT1A receptors in the dorsal periaqueductal gray. Pharmacol. Biochem. Behav. 89, 76–84.

Burghardt, N.S., Sullivan, G.M., McEwen, B.S., Gorman, J.M., LeDoux, J.E., 2004. The selective serotonin reuptake inhibitor citalopram increases fear after acute treatment but reduces fear with chronic treatment: a comparison with tianeptine. Biol. Psychiatry 55, 1171–1178.

Cassano, G.B., Savino, M., 1993. Symptomatology of panic disorder: an attempt to define the panic-agoraphobic spectrum phenomenology. In: Montgomery, S.A. (Ed.), Psychopharmacology of Panic. Oxford University Press, New York, pp. 38–57.

Ceglia, I., Acconcia, S., Fracasso, C., Colovic, M., Caccia, S., Invernizzi, R.W., 2004. Effects of chronic treatment with escitalopram or citalopram on extracellular 5-HT in the prefrontal cortex of rats: role of 5-HT1A receptors. Br. J. Pharmacol. 142, 469–478.

Connor, K.M., Davidson, J.R., 1998. Generalized anxiety disorder: neurobiological and pharmacotherapeutic perspectives. Biol. Psychiatry 44, 1286–1294.

Dawson, L.A., Nguyen, H.Q., Smith, D.I., Schechter, L.E., 2000. Effects of chronic fluoxetine treatment in the presence and absence of (+/−)pindolol: a microdialysis study. Br. J. Pharmacol. 130, 797–804.

de Bortoli, V.C., Nogueira, R.L., Zangrossi Jr., H., 2006. Effects of fluoxetine and buspirone on the panicolytic-like response induced by the activation of 5-HT1A and 5-HT2A receptors in the rat dorsal periaqueductal gray. Psychopharmacology (Berl.) 183, 422–428.

De Vry, J., 1995. 5-HT1A receptor agonists: recent developments and controversial issues. Psychopharmacology (Berl.) 121, 1–26.

Esquivel, G., Fernandez-Torre, O., Schruers, K.R., Wijnhoven, L.L., Griez, E.J., 2009. The effects of opioid receptor blockade on experimental panic provocation with CO_2. J. Psychopharmacol. 23, 975–978.

Graeff, F.G., 2004. Serotonin, the periaqueductal gray and panic. Neurosci. Biobehav. Rev. 28, 239–259.

Graeff, F.G., Del-Ben, C.M., 2008. Neurobiology of panic disorder: from animal models to brain neuroimaging. Neurosci. Biobehav. Rev. 32, 1326–1335.

Griez, E., Schruers, K., 1998. Experimental pathophysiology of panic. J. Psychosom. Res. 45, 493–503.

Griez, E.J.L., Faravelli, C., Nutt, D., Zohar, J., 2001. Anxiety Disorders: an Introduction to Clinical Management and Research. .

Hamon, M., Gozlan, H., el Mestikawy, S., Emerit, M.B., Bolanos, F., Schechter, L., 1990. The central 5-HT1A receptors: pharmacological, biochemical, functional, and regulatory properties. Ann. N. Y. Acad. Sci. 600, 114–129 (discussion 129–131).

Hashimoto, S., Inoue, T., Koyama, T., 1996. Serotonin reuptake inhibitors reduce conditioned fear stress-induced freezing behavior in rats. Psychopharmacology (Berl.) 123, 182–186.

Hogg, S., Michan, L., Jessa, M., 2006. Prediction of anti-panic properties of escitalopram in the dorsal periaqueductal grey model of panic anxiety. Neuropharmacology 51, 141–145.

Hollander, E., Simeon, D., 2003. Anxiety disorders, Textbook of Clinical Psychiatry, 4th ed. American Psychiatric Publishing, Washington, DC, pp. 543–565.

Inoue, T., Hashimoto, S., Tsuchiya, K., Izumi, T., Ohmori, T., Koyama, T., 1996a. Effect of citalopram, a selective serotonin reuptake inhibitor, on the acquisition of conditioned freezing. Eur. J. Pharmacol. 311, 1–6.

Inoue, T., Tsuchiya, K., Koyama, T., 1996b. Serotonergic activation reduces defensive freezing in the conditioned fear paradigm. Pharmacol. Biochem. Behav. 53, 825–831.

Invernizzi, R., Bramante, M., Samanin, R., 1994. Chronic treatment with citalopram facilitates the effect of a challenge dose on cortical serotonin output: role of presynaptic 5-HT1A receptors. Eur. J. Pharmacol. 260, 243–246.

Jenck, F., Broekkamp, C.L., Van Delft, A.M., 1990. The effect of antidepressants on aversive periaqueductal gray stimulation in rats. Eur. J. Pharmacol. 177, 201–204.

Jenck, F., Moreau, J.L., Martin, J.R., 1995. Dorsal periaqueductal gray-induced aversion as a simulation of panic anxiety: elements of face and predictive validity. Psychiatry Res. 57, 181–191.

Jenck, F., Moreau, J.L., Berendsen, H.H., Boes, M., Broekkamp, C.L., Martin, J.R., Wichmann, J., Van Delft, A.M., 1998. Antiaversive effects of 5HT2C receptor agonists and fluoxetine in a model of panic-like anxiety in rats. Eur. Neuropsychopharmacol. 8, 161–168.

Kakui, N., Yokoyama, F., Yamauchi, M., Kitamura, K., Imanishi, T., Inoue, T., Koyama, T., 2009. Anxiolytic-like profile of mirtazapine in rat conditioned fear stress model: functional significance of 5-hydroxytryptamine 1A receptor and alpha1-adrenergic receptor. Pharmacol. Biochem. Behav. 92, 393–398.

Klerman, G.L., Weissman, M.M., Ouellette, R., Johnson, J., Greenwald, S., 1991. Panic attacks in the community. Social morbidity and health care utilization. JAMA 265, 742–746.

Liebowitz, M.R., Fyer, A.J., Gorman, J.M., Dillon, D., Appleby, I.L., Levy, G., Anderson, S., Levitt, M., Palij, M., Davies, S.O., et al., 1984. Lactate provocation of panic attacks. I. Clinical and behavioral findings. Arch. Gen. Psychiatry 41, 764–770.

Liebowitz, M.R., Gorman, J.M., Fyer, A.J., Levitt, M., Dillon, D., Levy, G., Appleby, I.L., Anderson, S., Palij, M., Davies, S.O., et al., 1985. Lactate provocation of panic attacks. II. Biochemical and physiological findings. Arch. Gen. Psychiatry 42, 709–719.

Lim, L.W., Blokland, A., Visser-Vandewalle, V., Vlamings, R., Sesia, T., Steinbusch, H., Schruers, K., Griez, E., Temel, Y., 2008a. High-frequency stimulation of the dorsolateral periaqueductal gray and ventromedial hypothalamus fails to inhibit panic-like behaviour. Behav. Brain Res. 193, 197–203.

Lim, L.W., Temel, Y., Sesia, T., Vlamings, R., Visser-Vandewalle, V., Steinbusch, H.W., Blokland, A., 2008b. Buspirone induced acute and chronic changes of neural activation in the periaqueductal gray of rats. Neuroscience 155, 164–173.

Lim, L.W., Temel, Y., Visser-Vandewalle, V., Steinbusch, H., Schruers, K., Hameleers, R., Esquivel, G., Griez, E., Blokland, A., 2008c. Effect of buspirone on the behavioral regulation of rats in low versus high anxiety conditions. Arzneimittelforschung 58, 269–276.

Lim, L.W., Temel, Y., Visser-Vandewalle, V., Blokland, A., Steinbusch, H., 2009. Fos immunoreactivity in the rat forebrain induced by electrical stimulation of the dorsolateral periaqueductal gray matter. J. Chem. Neuroanat. 38, 83–96.

Lovick, T.A., Parry, D.M., Stezhka, V.V., Lumb, B.M., 2000. Serotonergic transmission in the periaqueductal gray matter in relation to aversive behaviour: morphological evidence for direct modulatory effects on identified output neurons. Neuroscience 95, 763–772.

Lugenbiel, P., Sartorius, A., Vollmayr, B., Schloss, P., 2009. Creatine transporter expression after antidepressant therapy in rats bred for learned helplessness. World J. Biol. Psychiatry 1–5.

Nashold Jr., B.S., Wilson, W.P., Slaughter, D.G., 1969. Sensations evoked by stimulation in the midbrain of man. J. Neurosurg. 30, 14–24.

Ninan, P.M.S., 2004. Buspirone and gepirone, In: Schatzberg, A.F., Nemeroff, C.B. (Eds.), Textbook of Psychopharmcology, 3rd ed. American Psychiatric Publishing, Washington, DC, pp. 391–404.

Nogueira, R.L., Graeff, F.G., 1995. Role of 5-HT receptor subtypes in the modulation of dorsal periaqueductal gray generated aversion. Pharmacol. Biochem. Behav. 52, 1–6.

Oliveira, L.C., Broiz, A.C., de Macedo, C.E., Landeira-Fernandez, J., Brandao, M.L., 2007. 5-HT2 receptor mechanisms of the dorsal periaqueductal gray in the conditioned and unconditioned fear in rats. Psychopharmacology (Berl.) 191, 253–262.

Overbeek, T., Schruers, K., van Leeuwen, I.D., Klaassen, T., Griez, E., 2005. Experimental affective symptoms in panic disorder patients. Can. J. Psychiatry 50, 175–178.

Paxinos, G., Watson, C., 1998. The Rat Brain in Stereotaxic Coordinates, 4th ed. Academic Press.

Pobbe, R.L., Zangrossi Jr., H., 2005. 5-HT(1A) and 5-HT(2A) receptors in the rat dorsal periaqueductal gray mediate the antipanic-like effect induced by the stimulation of serotonergic neurons in the dorsal raphe nucleus. Psychopharmacology (Berl.) 183, 314–321.

Poltronieri, S.C., Zangrossi Jr., H., de Barros Viana, M., 2003. Antipanic-like effect of serotonin reuptake inhibitors in the elevated T-maze. Behav. Brain Res. 147, 185–192.

Quartermain, D., Clemente, J., Shemer, A., 1993. 5-HT1A agonists disrupt memory of fear conditioning in mice. Biol. Psychiatry 33, 247–254.

Roy-Byrne, P.P., Craske, M.G., Stein, M.B., 2006. Panic disorder. Lancet 368, 1023–1032.

Sanchez, C., Bergqvist, P.B., Brennum, L.T., Gupta, S., Hogg, S., Larsen, A., Wiborg, O., 2003. Escitalopram, the S-(+)-enantiomer of citalopram, is a selective serotonin

reuptake inhibitor with potent effects in animal models predictive of antidepressant and anxiolytic activities. Psychopharmacology (Berl.) 167, 353–362.
Sato, H., Skelin, I., Debonnel, G., Diksic, M., 2008. Chronic buspirone treatment normalizes open field behavior in olfactory bulbectomized rats: assessment with a quantitative autoradiographic evaluation of the 5-HT1A binding sites. Brain Res. Bull. 75, 545–555.
Schenberg, L.C., Bittencourt, A.S., Sudre, E.C., Vargas, L.C., 2001. Modeling panic attacks. Neurosci. Biobehav. Rev. 25, 647–659.
Schenberg, L.C., Capucho, L.B., Vatanabe, R.O., Vargas, L.C., 2002. Acute effects of clomipramine and fluoxetine on dorsal periaqueductal grey-evoked unconditioned defensive behaviours of the rat. Psychopharmacology (Berl.) 159, 138–144.
Schruers, K., Griez, E., 2004. The effects of tianeptine or paroxetine on 35% CO_2 provoked panic in panic disorder. J. Psychopharmacol. 18, 553–558.
Schruers, K., Klaassen, T., Pols, H., Overbeek, T., Deutz, N.E., Griez, E., 2000. Effects of tryptophan depletion on carbon dioxide provoked panic in panic disorder patients. Psychiatry Res. 93, 179–187.
Schruers, K., van Diest, R., Overbeek, T., Griez, E., 2002. Acute L-5-hydroxytryptophan administration inhibits carbon dioxide-induced panic in panic disorder patients. Psychiatry Res. 113, 237–243.
Schutz, M.T., de Aguiar, J.C., Graeff, F.G., 1985. Anti-aversive role of serotonin in the dorsal periaqueductal grey matter. Psychopharmacology (Berl.) 85, 340–345.
Temel, Y., Visser-Vandewalle, V., van der Wolf, M., Spincemaille, G.H., Desbonnet, L., Hoogland, G., Steinbusch, H.W., 2004. Monopolar versus bipolar high frequency stimulation in the rat subthalamic nucleus: differences in histological damage. Neurosci. Lett. 367, 92–96.
Temel, Y., Boothman, L.J., Blokland, A., Magill, P.J., Steinbusch, H.W., Visser-Vandewalle, V., Sharp, T., 2007. Inhibition of 5-HT neuron activity and induction of depressive-like behavior by high-frequency stimulation of the subthalamic nucleus. Proc. Natl. Acad. Sci. U. S. A. 104, 17087–17092.
Tunnicliff, G., Brokaw, J.J., Hausz, J.A., Matheson, G.K., White, G.W., 1992. Influence of repeated treatment with buspirone on central 5-hydroxytryptamine and dopamine synthesis. Neuropharmacology 31, 991–995.
Vargas, L.C., Schenberg, L.C., 2001. Long-term effects of clomipramine and fluoxetine on dorsal periaqueductal grey-evoked innate defensive behaviours of the rat. Psychopharmacology (Berl.) 155, 260–268.
Vianna, D.M., Landeira-Fernandez, J., Brandao, M.L., 2001. Dorsolateral and ventral regions of the periaqueductal gray matter are involved in distinct types of fear. Neurosci. Biobehav. Rev. 25, 711–719.
Wittchen, H.U., 1988. Natural course and spontaneous remissions of untreated anxiety disorders—results of the Munich follow-up study (MFS). In: Hand, I., Wittchen, H.U. (Eds.), Panic and Phobias: 2. Treatments and Variables Affecting Course and Outcome. Springer, Berlin, pp. 3–17.
Zanoveli, J.M., Nogueira, R.L., Zangrossi Jr., H., 2005. Chronic imipramine treatment sensitizes 5-HT1A and 5-HT 2 A receptors in the dorsal periaqueductal gray matter: evidence from the elevated T-maze test of anxiety. Behav. Pharmacol. 16, 543–552.