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ORIGINAL INVESTIGATION

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Acute effects of clomipramine and fluoxetine on dorsal periaqueductal grey-evoked unconditioned defensive behaviours of the rat

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Abstract *Rationale*: Several antidepressants attenuate conditioned escape behaviours reinforced by the terminus of an electrical stimulus applied to the dorsal periaqueductal grey (DPAG). *Objective*: The present study examined whether the antidepressant and antipanic drugs clomipramine (CLM) and fluoxetine (FLX) also attenuate the DPAG-evoked unconditioned defensive behaviours. *Methods*: Rats with electrodes in the DPAG were electrically stimulated in the absence of any treatment or 30 min after injections of CLM, FLX or saline. Threshold functions of cumulative response frequencies were fitted through the logistic model and compared using likelihood ratio coincidence tests. *Results*: CLM produced non-linear effects on galloping, for which median thresholds (I_{50}) were significantly increased (19±2%) or decreased (–22±2%) with 5 mg/kg and 10 mg/kg, respectively, or did not change with 20 mg/kg. The latter dose further increased the I_{50} of micturition (38±1%) and decreased the defecation output (–33±15%). FLX significantly increased the I_{50} of immobility (22±2%) and galloping (25±3%) with 1 mg/kg and 5 mg/kg, respectively. Moreover, corresponding doses either decreased the maximum output (–25±13%) or increased the I_{50} (56±11%) of defecation. Saline was ineffective. *Conclusions*: While the attenuation of defecation and micturition by 20 mg/kg CLM suggests a peripheral antimuscarinic action, CLM non-linear effects on galloping were most likely due to its differential action on monoaminergic and cholinergic central mechanisms. In contrast, the attenuation of immobility, galloping and defecation by low doses of FLX suggests a serotonin-mediated antiaversive action. Finally, CLM and FLX acute effects on DPAG-evoked unconditioned galloping response were strikingly similar to those reported for DPAG-evoked shuttle-box conditioned escape.

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Introduction

Electrical stimulation of dorsal periaqueductal grey matter (DPAG) has putative aversive properties because rats readily learn to avoid or switch-off the stimulus. Electrical and chemical stimulations of DPAG also produce unconditioned defensive behaviours. Thus, either a freezing behaviour characterised by a tense immobile display, exophthalmus, vibrissae paralysis and/or defecation and micturition, or a flight behaviour made up of trotting, galloping and jumping responses is brought about by electrical or chemical stimulation of DPAG (Sudré et al. 1993; Bittencourt et al. 2000; Schenberg et al. 2000; Vargas et al. 2000). Although the stimulation of DPAG has been formerly proposed as an anxiety model in operant procedures (Schenberg and Graeff 1978; Graeff 1981), DPAG stimulation in healthy humans produces sensations, visceral responses and neurological symptoms remarkably similar to clinical panic attacks (Nashold et al. 1969). Therefore, DPAG stimulation has also been proposed as a model of panic attacks (Gentil 1988; Deakin and Graeff 1991). As a matter of fact, pharmacological evidence in the rat supports the panic-like nature of DPAG stimulation. Thus, while the acute administration of drugs that are effective in panic therapy (panicolytics) – such as the selective serotonin (5-HT) re-uptake inhibitors (SSRIs), fluoxetine (FLX), fluvoxamine and sertraline or the high-potency benzodiazepines alprazolam and clonazepam – attenuated the DPAG-evoked shuttle-box conditioned escape, drugs that are known to precipitate panic attacks (panicogenics) – such as yohimbine, caffeine and cholecystokinin receptor agonists – had a facilitatory effect (Jenck et al. 1990, 1995, 1996, 1998). However, the clinically effective panicolytics imipramine and clomipramine (CLM) were ineffective in the DPAG-evoked shuttle-box escape (Jenck et al. 1990). In contrast, recent studies from our

laboratory showed that the administration of CLM at a time course and dose regimen (5 mg/kg and 10 mg/kg/day, 21 days) not much different from those observed in panic therapy significantly increased the thresholds of DPAG-evoked immobility (24%), trotting (138%), galloping (75%), jumping (45%) and micturition (87%). In addition, the 21-day administration of FLX (1 mg/kg/day) virtually abolished galloping without changing the remaining responses (Vargas and Schenberg 2001).

There are no studies, however, evaluating the acute effects of panicolytics on unconditioned defensive behaviours produced by electrical stimulation of DPAG. Accordingly, the present study employed the threshold logistic analysis (Sudré et al. 1993; Schenberg et al. 1990, 2000) to assess the acute effects of the clinically effective panicolytics CLM and FLX on DPAG-evoked innate defensive behaviours.

Materials and methods

Animals

Male albino Wistar rats (200–250 g) were housed in individual glass-walled cages with food and water ad libitum and a natural light/dark cycle.

Surgery and brain electrodes

Rats were anaesthetised with 400 mg/kg (i.p.) chloral hydrate (Isofar, Rio de Janeiro, Brazil) supplemented by the s.c. infiltration of scalp with 1% lidocaine plus 0.005% epinephrine (Cristália, São Paulo, Brazil). Thereafter, rats were fixed on a stereotaxic apparatus (David Kopf, Tujunga, Calif.) and wrapped with a cloth to avoid surgical hypothermia. With the skull horizontal between bregma and lambda, the bone over the lambda was abraded with the aid of a drill and removed with thin forceps to expose the sinus. Monopolar stainless-steel electrodes (o.d. 200 µm), insulated throughout except at the cross-section of the tip, were then inserted in the right or left dorsal midbrain through a small dura incision just by the sinus. Whenever necessary, the sinus was gently pushed with the electrode itself so as to allow its penetration to the aimed site. The electrode was anchored to the skull by means of a U-shaped, stainless-steel clip, three small screws and dental resin. In addition, the bone was covered with a thin layer of cyanoacrylate methylester glue (Super-Bond, São Paulo, Brazil) just before pouring the dental resin to hold the pieces together. These procedures aided in keeping the electrodes in place for a 30-day period or so allowing the chronic treatment with CLM and FLX reported elsewhere (Vargas and Schenberg 2001).

Brain stimulation

Rats were stimulated in a cylindrical Plexiglass open-field apparatus of 60-cm wall height and diameter. The screening sessions were carried out 4–10 days after surgery. The rats were connected to a constant current sine-wave stimulator and placed into the open field, where they remained undisturbed for 15 min to get used to the environment and reduce spontaneous activity. Following this period, stepwise increasing stimuli (0–55 µA, 60 Hz, 1 min, a.c.) were presented at 5-min intervals. A light cable and mercury swivel allowed the free movement of the rat during brain stimulation. In each trial, the intensity was increased by 5 µA until the rat showed the jumping response. All experiments were carried out in a sound-attenuated, temperature-controlled room (23–25°C).

Drug treatment

Only rats that exhibited jumps with peak-to-peak intensities below 55 µA were selected for drug treatments. CLM (clomipramine hydrochloride, Sigma, St. Louis, Mo.) and FLX (fluoxetine hydrochloride, Eli Lilly, São Paulo, Brazil) were dissolved in saline (0.9% NaCl) and distilled water, respectively. CLM (5, 10 and 20 mg/kg, i.p., n=28, 18 and 18, respectively) and FLX (1 mg/kg and 5 mg/kg, i.p., n=20 and 22, respectively) were administered 30 min before the onset of stimulation sessions. Controls were similarly treated with saline (n=24). Drug sedative effects on open-field ambulatory activity were evaluated in separate rats (n=10 per group) similarly treated with saline, FLX (1 mg/kg and 5 mg/kg) or CLM (5 mg/kg and 10 mg/kg).

Behavioural recordings

The rat behavioural output was rated according to a previously made ethogram (Schenberg et al. 2000). The following responses were recorded: sleeping, resting, grooming, rearing, walking, tense immobility, trotting, galloping, jumping, exophthalmus (eyeball protrusion and wide opened eyelids), defecation and micturition. Furthermore, vibrissae paralysis (mystacioplegia) was also observed during the freezing behaviour. Behaviours were recorded in a binary way, as emitted or not, irrespective of their frequency or duration in a single-stimulation trial. In order to determine the response threshold curves, only the “threshold responses”, i.e. those emitted with the minimally effective current, were subjected to statistical analysis. Trotting and galloping were analysed either separately or merged as the “running” response. Moreover, because defecation and micturition also occur spontaneously, unbiased fitting of their stimulus-dependent output was performed, discarding the responses emitted during 0- (sham), 5- and 10-µA stimulation trials, supposedly, due to the rat exploratory activity.

Evaluation of drug sedative effects

Drug sedative effects were assessed in separate groups by means of a custom-built infrared-based actometer. The actometer was made up of an anti-burglary domestic system (Infraset, São Paulo, Brazil) in which the time-constant circuit was modified to allow a faster resetting. The actometer had a 360° monitoring radius and was placed 50 cm above the floor of the open field. The sensor was sensitive to a 12-cm mean displacement of the rat and filtered slow stretching responses, scratching, grooming and small movements of the paws and head. Accordingly, records denote the ambulatory activity only (walking and rearing). Open-field sessions were carried out between 0800 hours and 1500 hours in a sound-attenuated, temperature-controlled room (23–25°C). Naive rats were injected (i.p) with saline, FLX or CLM and placed after 30 min into the open field. Cumulative ambulatory scores of 10-, 20- and 30-min recording periods (i.e., 40, 50 and 60 min after drug injection) were subjected to statistical analysis.

Histology

At the end of experiments, brains were sectioned in a freezing microtome (60-µm sections) and stained with neutral red. Stimulation sites were plotted on diagrams of the rat brain atlas of Paxinos and Watson (1986).

Statistical analysis

Behavioural items were recorded in a binary way, as emitted or not, irrespective of their frequency or duration in a single stimulation trial. Response threshold curves were obtained by logistic fitting of accumulated response frequencies. Significant regression on stimulus intensity was assessed using Wald’s chi-square test. Drug effects were assessed according to a within-subject design. Pre-

and post-drug threshold curves of each group were modelled by means of indicator variables and compared for either location (triggering level) or parallelism (responsiveness) using likelihood-ratio coincidence tests. Behaviour triggering level was represented by estimates of median intensity (I_{50}±SE). Behavioural responsiveness was represented by regression curvature (β±SE), i.e. the parameter that governs the rate of change in response probability as a function of stimulus change. Finally, whenever a low response output precluded the logistic fitting, maximum accumulated frequencies (P_{max}, maximum response output) were compared using odds ratio (ψ) and Pearson's χ^2 analyses. A comprehensive description of our original approach to the threshold logistic analysis of intracranially induced behaviours can be found elsewhere (Schenberg et al. 2000).

Drug sedative effects on open-field ambulatory behaviour were evaluated using repeated-measures analysis of variance (ANOVA) followed by planned contrasts (1 d.f.) for time × group interactions. Pairwise multiple comparisons were considered significant at Bonferroni's 5% level. All statistical analyses were performed using the SAS software (Statistical Analysis Systems, N.C.).

Results

Stimulated sites

Histology was performed in 96 of 130 rats. The remaining rats were lost throughout the ensuing long-term treatment with CLM and FLX (Vargas and Schenberg 2001). Electrodes within the dorsomedial and dorsolateral columns of DPAG or just bordering it comprised 78%. The remaining electrodes were localised in the deep collicular layers (intermediate layer 7%, deep grey/white layers 15%). According to the current parcellation of the PAG (P. Carrive, personal communication), most sites were distributed throughout the intermediate (53) and caudal (29) DPAG or adjoining deep collicular layer (bregma AP coordinates: –6.04 mm to –7.3 mm). Moreover, seven electrodes were localised in the rostral DPAG (–5.8 mm; Fig. 1). Stimulation of these sites yielded significant regressions for defensive responses only. Pooled pre-drug sessions (n=130) showed the following threshold hierarchy: immobility < exophthalmus < jumping < trotting < galloping < micturition < defecation (Table 1). Distribution of sites within the DPAG and deep collicular layers did not differ among drug groups (χ^2=1.2, d.f.=2, P<0.54).

CLM effects

CLM produced non-linear effects on galloping, for which the thresholds were either increased (ΔI_{50}=19±1.8%, χ^2=7.5, d.f.=1, P<0.006), decreased (ΔI_{50}=–22±2.1%, χ^2=6.1, d.f.=1, P<0.01) or did not change with 5, 10 and 20 mg/kg, respectively (Fig. 2). Thresholds of micturition were increased by 20 mg/kg CLM (ΔI_{50}=38±1.1%, χ^2=3.6, d.f.=1, P<0.05). This dose also reduced the maximum output of defecation. Indeed, the defecation pre-drug probability was six times higher than that of rats treated with 20 mg/kg CLM (ψ=6.4±5.6, χ^2=4.9, d.f.=1, P<0.02). Immobility, exophthalmus, trotting, running and jumping responses did not change following CLM injections.

FLX effects

FLX produced significant increases in the thresholds of immobility (ΔI_{50}=22±2.1%, χ^2=9.4, d.f.=1, P<0.002) and galloping (ΔI_{50}=25±2.6%, χ^2=12.0, d.f.=1, P<0.0005) with 1 mg/kg and 5 mg/kg, respectively (Fig. 3). Defecation was also attenuated by 1 mg/kg and 5 mg/kg FLX which caused a fivefold decrease in output probability (ψ=4.8±4.2, χ^2=3.6, d.f.=1, P<0.05) or a significant increase in I_{50} (ΔI_{50}=56±11.4%, χ^2=4.6, d.f.=1, P<0.03), respectively. No changes were observed in exophthalmus, trotting, running, micturition and jumping responses.

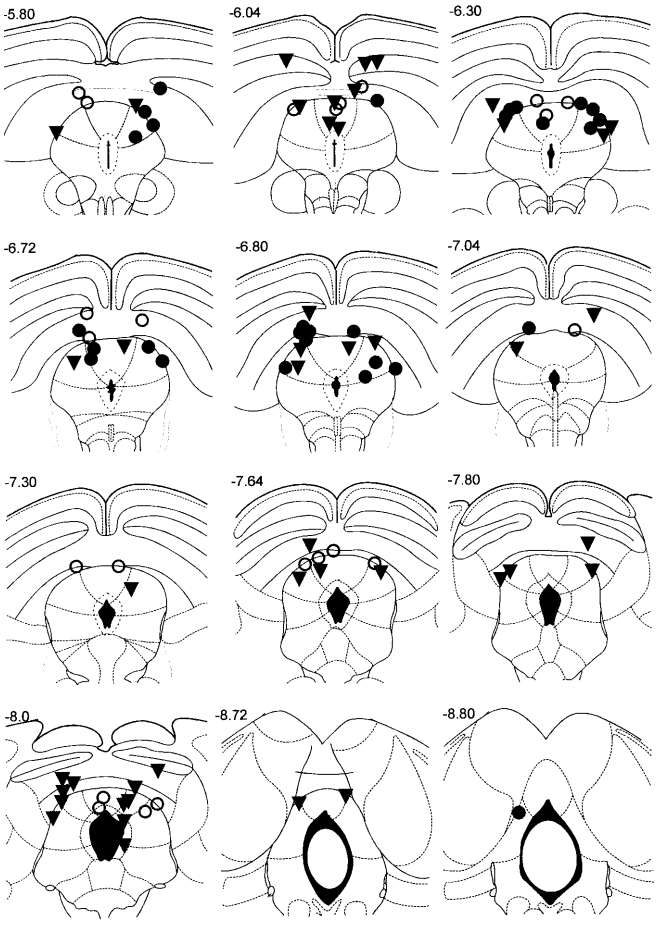

Fig. 1 Brain sites in which stimulation during pre-drug sessions elicited the jumping response with intensities below 55 μA (60 Hz, 1 min, a.c.). *Plates* represent the coronal sections of the rat brain atlas of Paxinos and Watson (1986). Numbers are the rostro-caudal coordinates in relation to bregma (mm). Clomipramine (*filled inverted triangle*), fluoxetine (*filled circle*) and saline (open circle) groups

Table 1 Rat defensive profile of dorsal periaqueductal gray stimulation in pooled pre-drug sessions (n=130). I_{50} median intensity (the estimate of triggering level, i.e. the intensity in which a given response has the higher frequency), β curvature parameter (the estimate of responsiveness, i.e. the rate of change in response probability in function of stimulus change), P_{max} maximum response output (the estimate of stimulus efficacy, i.e. the maximum accumulated frequency). Responses were sorted according to their median intensities

Response	I_{50}±SE (μA)	β±SE	P_{max}±SE (%)
Immobility	25.6±0.2	11.2±0.7	95±2
Exophthalmus	27.0±0.2	13.7±0.8	100±0
Jumping	34.2±0.2	14.3±0.8	100±0
Trotting	37.4±0.3	8.1±0.5	72±4
Galloping	44.8±0.4	8.0±0.6	62±4
Micturition	46.9±0.8	6.1±0.6	46±4
Defecation	56.7±1.3	6.1±0.6	35±4

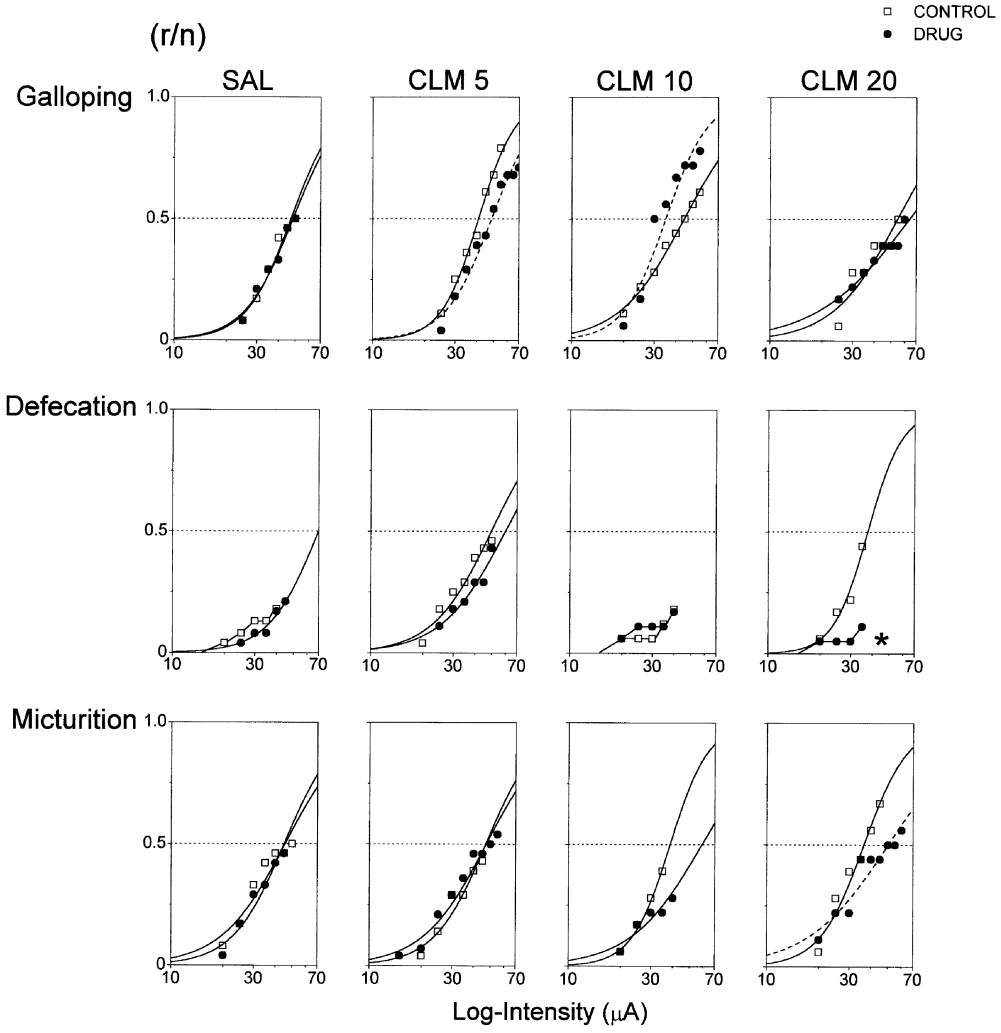

Fig. 2 Acute effects of clomipramine (*CLM* 5, 10 and 20 mg/kg, i.p.) and saline (*SAL* 0.9% NaCl, i.p.) on the thresholds and maximum output of galloping, defecation and micturition evoked by electrical stimulation of dorsal periaqueductal grey. *Sigmoidal curves* represent the best-fitting logistic function of accumulated response frequencies. *Line-plus-symbol graphs* represent data that did not achieve a significant logistic fitting (*r* responders, *n* number of stimulated rats). *Dashed curves* (–-) and *asterisks* (*), $P<0.05$, represent curve location and maximum output significantly different from pre-drug controls, respectively (likelihood ratio coincident tests and maximum output Pearson's χ^2 as appropriate)

Saline effects

Whatever the regression parameter (I_{50}, β, P_{max}), acute injections of saline did not change any DPAG-evoked defensive behaviour.

Drug sedative effects

Compared with pre-drug controls, CLM-, FLX- and saline-treated rats showed a reduced open-field activity in drug sessions carried out the day after (Fig. 4). Nevertheless, while the open-field activity of rats treated with 5 mg/kg CLM or 1 mg/kg and 5 mg/kg FLX did not differ from saline-treated ones, 10 mg/kg CLM significantly reduced the ambulatory activity at 20-min and 30-min recording periods (20-min activity $F_{1,27}=6.2$, $P<0.02$; 30-min activity: $F_{1,27}=7.8$, $P<0.008$; Fig. 4).

Discussion

Compared with chronically treated rats (Vargas and Schenberg 2001), the acute administration of CLM produced fewer and lesser effects on DPAG-evoked defensive behaviours. Hence, while galloping was attenuated by a non-sedative dose of CLM (5 mg/kg), this response was facilitated following the administration of a manifestly depressant dose (10 mg/kg). Accordingly, CLM attenuation of galloping did not correlate with its sedative properties, supporting a specific anti-aversive action instead. However, CLM (10 mg/kg) facilitation of galloping may be associated with the intensification of anxiety and panic attacks seen in treatment onset (Ramos et al. 1993). The neurochemical mechanisms underlying this "first-dose effect" remain obscure. However, besides their 5-HT and noradrenaline reuptake inhibitory actions, tricyclic antidepressants have long been known for their muscarinic blocking activity and respective side effects, such as constipation, urinary retention, blurred vision and dry mouth (Atkinson and Ladinsky 1971; Richelson and Divinetz-Romero 1977; Snyder and Yamamura 1977; Blackwell et al. 1978; Cusack et al. 1994). Moreover, rather than peripherally, imipramine seems to exert its anti-enuretic effect via a supraspinal antimuscarinic action (Sohn and Kim 1997). Thus, CLM non-linear effects on galloping were most probably due to drug or metabolite differential effects on serotonergic, adrenergic and cholinergic synapses. Indeed, it has long been reported that scopolamine has a pro-aversive effect, further decreasing the operant lever pressing which was suppressed by punishment (Miczek 1973).

In contrast to the complete absence of effects of saline, FLX caused modest but significant attenuation of DPAG-evoked defensive behaviours. The attenuation of galloping by a non-sedative dose of FLX (5 mg/kg) supports the 5-HT inhibitory modulation of flight. Indeed, iontophoretic application of 5-HT predominantly inhibited single neuron activity in the dorsolateral and lateral sectors of PAG (Lovick 1994). Moreover, these inhibitory actions were potentiated by the iontophoretic application of a SSRI panicolytic, paroxetine, supporting the FLX inhibitory modulation of PAG (Lovick 1994). Because galloping was attenuated to a much greater extent following a 21-day administration of 1 mg/kg/day FLX

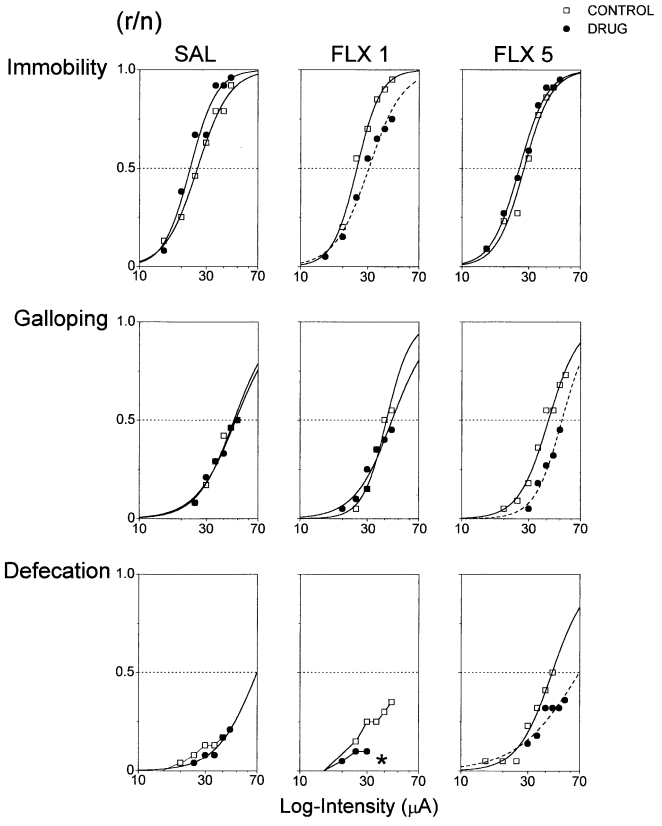

Fig. 3 Acute effects of fluoxetine (*FLX* 1 mg/kg and 5 mg/kg, i.p.) and saline (*SAL* 0.9% NaCl, i.p.) on the thresholds and maximum output of immobility, galloping and defecation evoked by electrical stimulation of dorsal periaqueductal grey. *Sigmoidal curves* represent the best-fitting logistic function of accumulated response frequencies. *Line-plus-symbol graphs* represent data that did not achieve a significant logistic fitting (*r* responders, *n* number of stimulated rats). *Dashed curves* (---) and *asterisks* (*), $P<0.05$, represent curve location and maximum output significantly different from pre-drug controls, respectively (likelihood ratio coincident tests and maximum output Pearson's χ^2 as appropriate)

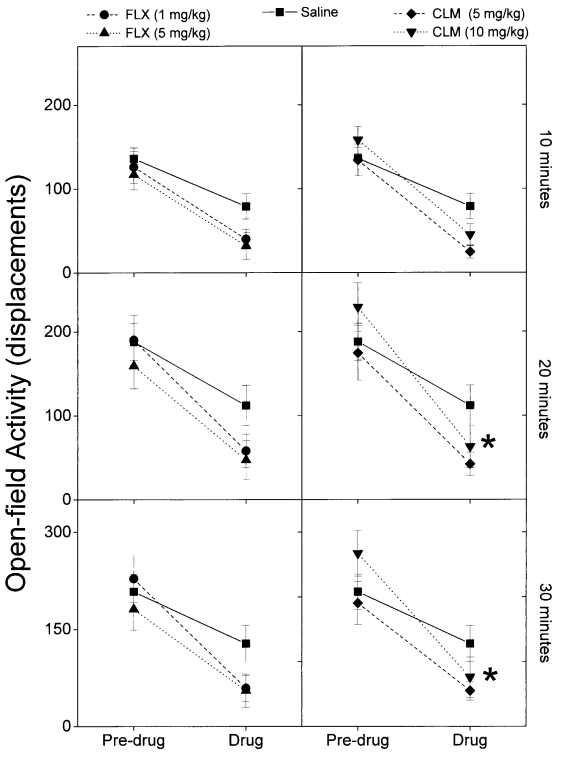

Fig. 4 Fluoxetine (*FLX* 1 mg/kg and 5 mg/kg, i.p.) and clomipramine (*CLM* 5 mg/kg and 10 mg/kg, i.p.) acute effects on open-field exploratory activity. Pre-drug sessions were performed the day before drug testing. Activity was recorded 10, 20 and 30 min after session onset. *$P<0.05$, significantly different from saline-treated rats (repeated-measures analysis of variance followed by planned contrasts for time × group interactions)

(Vargas and Schenberg 2001), both short- and long-term mechanisms seem to be involved in FLX inhibitory effects on galloping. Alternatively, galloping attenuation could have been a by-product of a facilitation of freezing brought about by the enhancement of 5-HT transmission in the "behavioural inhibition system" (Gray 1991). Yet, instead of a facilitation, immobility was attenuated or did not change after acute administration of 1 mg/kg and 5 mg/kg FLX, respectively. Therefore, 5-HT seems to inhibit both freezing and flight behaviours. Immobility attenuation, however, wanes following the chronic administration of FLX (Vargas and Schenberg 2001). Finally, attenuation of galloping but not immobility following the administration of 5 mg/kg FLX suggests a complex interplay of DPAG-evoked somatic defensive behaviours. Apparently, galloping attenuation with the higher dose of FLX shifted the defensive repertoire towards immobility and trotting, compensating an eventual attenuation of the latter responses. As a matter of fact, a similar interplay of galloping and trotting was also observed following the chronic treatment with FLX (Vargas and Schenberg 2001). In any event, FLX seems to downgrade the defensive repertoire from flight to freezing and thence to non-defensive behaviours.

Remarkably, whereas the attenuation of defecation and micturition by 20 mg/kg CLM could be due to a peripheral cholinergic blockade, DPAG-evoked defecation was also attenuated by FLX, which is devoid of any antimuscarinic action (Cusack et al. 1994). It is worth noting that exogenous 5-HT sensitises normal peristalsis and facilitates stress- and corticotropin releasing factor (CRF)-induced defecation via 5-$HT_{3/4}$ bowel receptors (Miyata et al. 1992, 1998; Sanger et al. 1998). Accordingly, 5-HT has been proposed as a key sensitising agent in the aetiology of irritable bowel syndrome, a functional gastrointestinal disorder (Sanger 1996). In addition, central 5-HT_{1A} receptors seem also to stimulate normal peristalsis (Croci et al. 1995). Consequently, FLX attenuation of DPAG-evoked defecation was most probably a specific anti-aversive action at DPAG properly or its efferent pathways. Indeed, recent transneuronal track tracing studies with pseudorabies virus suggested that colonic motility is chiefly controlled by Barrington's nucleus, formerly considered solely as a micturition centre, but also by the lateral PAG and spinally projecting locus coeruleus CRF neurons (Monnikes et al. 1994; Valentino et al. 2000). In turn, the PAG is known to project to Barrington's nucleus and locus coeruleus rostromedial dendrites (Valentino et al. 1994; Luppi et al. 1995; Blok and Holstege 1996). These data give neuroanatomical support to the elicitation of defecation and micturition by electrical and chemical stimulation of PAG (Schenberg et al. 1990, 2000; Sudré et al. 1993; Bittencourt et al. 2000; Vargas et al. 2000; Vargas and Schenberg 2001). Further, 5-HT inhibits the PAG and locus coeruleus neuron activity, supporting the FLX inhibitory modulation of DPAG-evoked defecation (Shiekhattar and Aston-Jones 1993; Lovick 1994). More importantly, given the high co-morbidity of panic disorder and irritable bowel syndrome (Lydiard et al. 1994; Lydiard and Falsetti 1999), FLX attenuation of DPAG-evoked defecation is likely to underlie the reported effectiveness of antipanic agents on concomitant panic and irritable bowel disorders (Lydiard et al. 1986).

Antidepressant effects confirmed previous studies with DPAG-evoked shuttle-box escape behaviour. In particular, while the shuttle-

box escape was attenuated by SSRIs, FLX (10–32 mg/kg), sertraline (1–10 mg/kg) and fluvoxamine (10–22 mg/kg), no threshold changes were observed following 10-mg/kg and 22-mg/kg doses of the non-selective tricyclic antidepressants imipramine and CLM (Jenck et al. 1990). Likewise, in the present study, while galloping was attenuated by a non-sedative dose of FLX (5 mg/kg), it was facilitated or did not change in the same dose range of CLM that failed in attenuating the DPAG-evoked shuttle-box escape. Notably, CLM had no effects on running (merging of trotting and galloping), the behaviour most likely recorded in the shuttle-box escape paradigm. The striking similarity of antidepressant actions on innate and shuttle-box escape behaviours thus supports the unconditioned nature of the latter behaviour. Consequently, instead of a "conditioning", DPAG-evoked shuttle-box escape would be more akin to a stimulus titration procedure.

The neurochemistry of DPAG-evoked innate behaviours and their complex interplay remains unclear. Indeed, most studies on this issue were carried out with the shuttle-box escape procedure and provide information on running responses only. However, as far as the 5-HT transmission is concerned, it is noteworthy that intra-periaqueductal injection of both 5-HT and the SSRI zimelidine produced antiaversive effects (Schütz et al. 1985). Administration of 5-HT receptor agonists and antagonists yielded, however, conflicting results. Therefore, while the systemic injection of these drugs suggested a pro-aversive role of both 5-HT_{1A} and 5-HT_{2A} receptor subtypes, the local injection of corresponding drugs led to opposite results (Jenck et al. 1989; Nogueira and Graeff 1995). Thus, either the loss of drug specificity at high concentrations attained by intracerebral injections or the different sites of action of systemically and locally injected drugs could underlie these conflicting data. Indeed, DPAG-evoked unconditioned flight in the open-field was either enhanced or attenuated by the 5-HT_{1A} selective agonist, 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OHDPAT), depending on the route of administration, systemic or local, respectively (Beckett and Mardsen 1997).

It is also noteworthy that the predator-elicited flight behaviour was potentiated by acute administration of imipramine (5, 10 and 15 mg/kg), which either reduced the avoidance distance or increased the flight speed (Griebel et al. 1995, 1996; Blanchard et al. 1997). A similar effect on galloping was observed in the present study after the administration of a 10-mg/kg dose of CLM, which both reduced the triggering level and increased the responsiveness (parameters akin to "avoidance distance" and "flight speed", respectively). However, in contrast to the present data, the predator-elicited flight behaviour was facilitated by 5 mg/kg and 10 mg/kg FLX (Griebel et al. 1996; Blanchard et al. 1997). Therefore, albeit similar, the substrates of DPAG-evoked and predator-elicited flight behaviours seem not to be identical. However, in contrast to the lack of effects of the higher doses of CLM (>10 mg/kg) in both DPAG-evoked innate and shuttle-box escape behaviours, CLM (15 mg/kg) attenuated the bar-pressing decremental escape in which rats learn to titrate the intensity of DPAG stimulation (Kiser et al. 1978). Decremental escape was further potentiated by para-chlorophenylalanine and depressed by 5-hydroxytryptophan, supporting the 5-HT mediation of CLM attenuating effects (Kiser and Lebovitz 1975; Kiser et al. 1978). Therefore, CLM differential effects on DPAG-evoked, predator-elicited and bar-pressing escape behaviours suggest the involvement of multiple 5-HT systems controlling these responses.

The pharmacology of defence reaction has long been hindered by the lack of an appropriate approach for measuring the intracranially induced innate behaviours. Nevertheless, the present and recent studies (Schenberg et al. 2000; Vargas and Schenberg 2001) showed that the threshold logistic analysis can provide accurate information about drug effects on the triggering level (I_{50}), responsiveness (β) and maximum output (P_{max}) of DPAG-evoked defensive behaviours. The neuronal mechanisms controlling each variable are not understood thus far; however, either distinct receptors or ion channels of the same or different neuron types could underlie eventual changes in these parameters (Schenberg et al. 2000). More importantly, the method herein employed was sensitive to behaviour-specific drug effects that are often masked in operant analysis of single active tasks such as bar-pressing or shuttle-box escape. Finally, the threshold logistic analysis detected effects of CLM and FLX doses within the true clinical range. In this regard, it should be stressed that the thresholds of jumping of the present study were lower than those of trotting and galloping. The stringent criterion employed in rat screening for drug treatments, i.e. selection of rats that jumped with less than 55 μA, most probably biased the sample defensive profile towards the high-threshold, drug-resistant jumping behaviour. Indeed, immobility, trotting, galloping and jumping are usually elicited with increasing stimuli when rats are pooled on either a plain neuroanatomical basis (Bittencourt et al. 2000) or through a more flexible criterion, i.e. presentation of any flight response, such as trotting, galloping or jumping, with less than 50 μA (Sudré et al. 1993). Thus, the rather stringent criterion of rat selection should be considered in the appraisal of present drug effects.

In conclusion, in contrast to the CLM non-linear effects on galloping, FLX attenuation of immobility and galloping with non-sedative doses suggests a 5-HT antiaversive action similar to that reported for FLX and other SSRIs on DPAG-evoked shuttle-box escape behaviour. Moreover, while the attenuation of defecation and micturition by the higher dose of CLM suggests a peripheral antimuscarinic effect, FLX attenuation of defecation supports a 5-HT-mediated central effect.

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References

Atkinson J, Ladinsky H (1972) A quantitative study of the anticholinergic action of several tricyclic antidepressants on the rat isolated fundal strip. Br J Pharmacol 45:519–524

Beckett S, Marsden CA (1997) The effect of central and systemic injection of the 5-HT_{1A} receptor agonist 8-OHDPAT and the 5-HT_{1A} receptor antagonist WAY100635 on periaqueductal grey-induced defence behaviour. J Psychopharmacol 11:35–40

Bittencourt AS, Carobrez AP, Schenberg LC (2000) Intrinsic properties of lateral and dorsolateral columns of periaqueductal gray matter. Soc Neurosci Abstr 18:2257

Blackwell B, Stefopoulos A, Enders P, Kuzma R, Adolphe A (1978) Anticholinergic activity of two tricyclic antidepressants. Am J Psychiatry 135:722–724

Blanchard RJ, Griebel G, Henrie JA, Blanchard DC (1997) Differentiation of anxiolytic and panicolytic drugs by effects on rat and mouse defense test batteries. Neurosci Biobehav Rev 21:783–789

Blok BF, Holstege G (1996) The neuronal control of micturition and its relation to the emotional motor system. In: Holstege G, Bandler R, Saper CB (eds) The emotional motor system. Elsevier, Amsterdam, pp 113–126

Croci T, Landi M, Bianchetti A, Manara L (1995) Drug-induced defaecation in rats: role of central 5-HT1A receptors. Br J Pharmacol 115:203–209

Cusack B, Nelson A, Richelson E (1994). Binding of antidepressants to human brain receptors: focus on newer generation compounds. Psychopharmacology 114:559–565

Deakin JFW, Graeff FG (1991) 5-HT and mechanisms of defence. J Psychopharmacol 5:305–315

Gentil V (1988) The aversive system, 5-HT and panic attacks. In: Simon P, Soubrié P, Wildlocher D (eds) Selected models of anxiety, depression and psychosis. Karger AG, Basel, pp 142–145

Graeff FG (1981) Minor tranquilizers and brain defense systems. Braz J Med Biol Res 14:239–265

Gray JA (1991) The psychology of fear and stress, 2nd edn. Cambridge University Press, Cambridge

Griebel G, Blanchard DC, Agnes RS, Blanchard RJ (1995) Differential modulation of antipredator defensive behavior in Swiss- Webster mice following acute or chronic administration of imipramine and fluoxetine. Psychopharmacology 120:57–66

Griebel G, Blanchard DC, Blanchard RJ (1996) Predator-elicited flight responses in Swiss-Webster mice: an experimental model of panic attacks. Prog Neuropsychopharmacol Biol Psychiatry 20:185–205

Jenck F, Broekkamp CLE, Van Delft AML (1989) Effects of serotonin receptor antagonists on PAG stimulation induced aversion: different contributions of $5HT_1$, $5HT_2$ and $5HT_3$ receptors. Psychopharmacology 97:489–495

Jenck F, Broekkamp CLE, Van Delft AML (1990) The effect of antidepressants on aversive periaqueductal gray stimulation in rats. Eur J Pharmacol 177:201–204

Jenck F, Moreau JL, Martin JR (1995) Dorsal periaqueductal grayinduced aversion as a simulation of panic anxiety: elements of face and predictive validity. Psychiatry Res 57:181–191

Jenck F, Martin JR, Moreau JL (1996) Behavioral effects of CCKB receptor ligands in a validated simulation of panic anxiety in rats. Eur J Neuropsychopharmacol 6:291–298

Jenck F, Moreau JL, Berendsen HH, Boes M, Broekkamp CL, Martin JR, Wichmann J, Van Delft AM (1998) Antiaversive effects of $5HT_{2C}$ receptor agonists and fluoxetine in a model of panic-like anxiety in rats. Eur Neuropsychopharmacol 8:161–168

Kiser RS, Lebovitz RM (1975) Monoaminergic mechanisms in aversive brain stimulation. Physiol Behav 15:47–53

Kiser RS, German DC, Lebovitz RM (1978) Serotonergic reduction of dorsal central gray area stimulation-produced aversion. Pharmacol Biochem Behav 9:27–31

Lovick TA (1994) Influence of the dorsal and median raphe nuclei on neurons in the periaqueductal gray matter: role of 5-hydroxytryptamine. Neuroscience 59:993–1000

Luppi PH, Aston-Jones G, Akaoka H, Chouvet G, Jouvet M (1995) Afferent projections to the rat locus coeruleus demonstrated by retrograde and anterograde tracing with cholera-toxin B subunit and Phaseolus vulgaris leucoagglutinin. Neuroscience 65:119–160

Lydiard RB, Falsetti SA (1999) Experience with anxiety and depression treatment studies: implications for designing irritable bowel syndrome clinical trials. Am J Med 107:65S–73S

Lydiard RB, Laraia MT, Howell EF, Ballenger JC (1986) Can panic disorder present as irritable bowel syndrome? J Clin Psychiatry 47:470–473

Lydiard RB, Greenwald S, Weissman MM, Johnson J, Drossman DA, Ballenger JC (1994) Panic disorder and gastrointestinal symptoms: findings from the NIMH Epidemiologic Catchment Area project. Am J Psychiatry 151:64–70

Miczek KA (1973) Effects of scopolamine, amphetamine and chlordiazepoxide on punishment. Psychopharmacologia 28: 373–389

Miyata K, Kamato T, Nishida A, Ito H, Yuki H, Yamano M, Tsutsumi R, Katsuyama Y, Honda K (1992) Role of the serotonin$_3$ receptor in stress-induced defecation. J Pharmacol Exp Ther 261:297–303

Miyata K, Ito H, Fukudo S (1998) Involvement of the 5-HT_3 receptor in CRH-induced defecation in rats. Am J Physiol 274:G827–G831

Monnikes H, Schmidt BG, Tebbe J, Bauer C, Tache Y (1994) Microinfusion of corticotropin releasing factor into the locus coeruleus/subcoeruleus nuclei stimulates colonic motor function in rats. Brain Res 644:101–108

Nashold BS, Wilson WP, Slaughter DG (1969) Sensations evoked by stimulation in the midbrain of man. J Neurosurg 30:14–24

Nogueira RL, Graeff FG (1995) Role of 5-HT receptor subtypes in the modulation of dorsal periaqueductal gray generated aversion. Pharmacol Biochem Behav 52:1–6

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, 2nd edn. San Diego, Academic Press

Ramos RT, Gentil V, Gorenstein C (1993) Clomipramine and initial worsening in panic disorder: beyond the "jitteriness syndrome". J Psychopharmacol 7:265–269

Richelson E, Divinetz-Romero S (1977) Blockade by psychotropic drugs of the muscarinic acetylcholine receptor in cultured nerve cells. Biol Psychiatry 12:771–785

Sanger GJ (1996) 5-Hydroxytryptamine and functional bowel disorders. Neurogastroenterol Motil 8:319–331

Sanger GJ, Banner SE, Smith MI, Wardle KA (1998) SB-207266: 5-HT_4 receptor antagonism in human isolated gut and prevention of 5-HT-evoked sensitization of peristalsis and increased defaecation in animal models. Neurogastroenterol Motil 10: 271–279

Schenberg LC, Graeff FG (1978) Role of the periaqueductal gray substance in the antianxiety action of benzodiazepines. Pharmacol Biochem Behav 9:287–295

Schenberg LC, Costa MB, Borges PCL, Castro MFS (1990) Logistic analysis of the defense reaction induced by electrical stimulation of the rat mesencephalic tectum. Neurosci Biobehav Rev 14:473–479

Schenberg LC, Marçal LPA, Seeberger F, Barros MR, Sudré ECM (2000) L-type calcium channels selectively control the defensive behaviors induced by electrical stimulation of dorsal periaqueductal gray and overlying collicular layers. Behav Brain Res 111:175–185

Schütz MTB, Aguiar JC, Graeff FG (1985) Anti-aversive role of serotonin in the dorsal periaqueductal grey matter. Psychopharmacology 85:340–345

Shiekhattar R, Aston-Jones G (1993) Sensory responsiveness of brain noradrenergic neurons is modulated by endogenous brain serotonin. Brain Res 623:72–76

Snyder SH, Yamamura HI (1977) Antidepressants and the muscarinic acetylcholine receptor. Arch Gen Psychiatry 34: 236–239

Sohn UD, Kim CY (1997) Suppression of the rat micturition reflex by imipramine. J Auton Pharmacol 17:35–41

Sudré ECM, Barros MR, Sudré GN, Schenberg LC (1993) Thresholds of electrically induced defence reaction of the rat: short- and long-term adaptation mechanisms. Behav Brain Res 58:141–154

Valentino RJ, Page ME, Luppi PH, Zhu Y, Van Bockstaele E, Aston-Jones G (1994) Evidence for widespread afferents to Barrington's nucleus, a brainstem region rich in corticotropin-releasing hormone neurons. Neuroscience 62:125–143

Valentino RJ, Kosboth M, Colflesh M, Miselis RR (2000) Transneuronal labeling from the rat distal colon: anatomic evidence for regulation of distal colon function by a pontine corticotropin-releasing factor system. J Comp Neurol 417:399–414

Vargas LC, Schenberg LC (2001) Long-term effects of clomipramine and fluoxetine on dorsal periaqueductal gray-evoked innate defensive behaviours of the rat. Psychopharmacology 155: 260–268

Vargas LC, Marques TA, Schenberg LC (2000) Micturition and defensive behaviors are controlled by distinct neural networks within the dorsal periaqueductal gray and deep gray layer of the superior colliculus of the rat. Neurosci Lett 280:45–48