



Review

Modulation of defensive behavior by periaqueductal gray NMDA/glycine-B receptor

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Abstract

Glutamate (GLU) associated with glycine, act as co-transmitter at the *N*-methyl-D-aspartate/glycine-B (NMDA/GLY_B) receptor. Dorsal periaqueductal gray (dPAG) neurons express NMDA/GLY_B receptors suggesting a GLU physiological role in mediating the responses elicited by stimulation of this area. Immunohistochemical data provided evidence of a possible correlation among elevated plus-maze (EPM), fear-like defensive behavior, and dPAG activity. The present data show that whereas the NMDA/GLY_B receptor agonists increased the open-arm avoidance responses in the EPM, the antagonists had the opposite effects. Microinjection of NMDA/GLY_B receptor agonists within the dPAG during test sessions in the EPM resulted in an enduring learned fear response detected in the retest. Therefore, in addition to the proposed role for the dPAG in panic attacks (escape), these findings suggest that the dPAG can also participate in more subtle anxiety-like behaviors. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Defensive behavior; Periaqueductal gray; Elevated plus-maze; Fear; Anxiety; *N*-methyl-D-aspartate/glycine-B receptor; Excitatory amino acid; Glutamate; Electrical stimulation; Blood pressure response; Rats

Contents

1. Introduction	697
2. Periaqueductal gray	698
3. The elevated plus-maze as a model to study defensive behavior	698
4. Excitatory amino acid receptors and the defensive system	699
5. Modulation of defensive behavior by periaqueductal gray EAA neurotransmission	701
5.1. Non-NMDA receptors	701
5.2. NMDA receptor complex	702
5.2.1. GLY _B site receptor	703
6. Concluding remarks	705
Acknowledgements	706
References	706

1. Introduction

Defensive behaviors are the reactions of an organism to actual or potential dangers. The conservative nature of defensive behaviors throughout mammalian species has been stressed by many authors [1–6] suggesting the maintenance of ‘hard-wired’ neural circuits responsible for species-specific defensive responses. Two measures can

often be used when studying defensive responses: (1) the motor responses, represented by postures and acts towards the *external milieu*; and (2) the neurovegetative responses, represented by various mechanisms of adaptation or preparedness of the *internal milieu* to cope with the behavioral response.

Studies carried out by Blanchard and Blanchard [7,8], contributed greatly to the current knowledge about the defensive behavior in rodents. Using wild rats, they measured the intensity of defensive behaviors against the experimenter and suggested the concept of defensive

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distance as a key parameter controlling the defensive reactions from freezing to flight in response to an approaching predator, or to defensive threat and attack when preys are driven into a corner. Using cats and rats, these authors developed another model based on a potential threat instead (predatory odor, post-cat presentation). They observed that rats previously exposed to a cat present a different set of defensive behaviors when reintroduced in the same environment, however, lacking the predator. These responses included freezing, risk assessment (head dipping, scanning, rearing, stretch attending postures), and the suppression of ongoing behavior (such as eating or drinking in deprived subjects) [9].

The neural substrates of these behaviors remain unclear. Adams [10] suggested the existence of a brain defensive motivational system, responsible for the integration of different stimuli and the motor patterning that executes the appropriate responses. Gray's behavioral inhibition system [11] and Graeff's brain aversive system [12] sought to define a specific set of brain structures responsible for both passive (inhibitory) or active forms of defensive behaviors, respectively. More recently, Gray and McNaughton [13] incorporated both propositions, Blanchard's defensive distance and the new concept of defensive direction (approach/avoidance), in a single defense system. These authors suggested that parallel pathways are activated when the threat source should be avoided (including the anterior cingulate, the amygdala, the medial hypothalamus and the periaqueductal gray (PAG)) or approached (including the posterior cingulate and the septo-hippocampal system) [13]. Indeed, after the pioneer experiments of Hunsperger and collaborators [14,15] showing that the defensive behaviors in the cat could be elicited by electrical stimulation of PAG, a wealth of evidence supported the PAG as a key structure controlling defensive behavior [4,7,10,16–19]. The motivational aversive properties of PAG [16,20–28] and the autonomic activation component [29] further confirmed and extended these previous findings.

The understanding of PAG role in fear-like defensive behaviors requires, however, the knowledge of its transmitters and respective functions. A general overview of the cellular communications between neurons within the PAG revealed the following neurotransmitter systems: (1) monoamines, acetylcholine and histamine; (2) amino acids; (3) peptides; and (4) simple gases (nitric oxide and carbon monoxide) [30]. These neurotransmitter systems are involved in inhibition of nociception, behavioral responses to threatening or stressful stimuli, and cardiovascular adjustments; a spectrum of functions critical for the animal's survival [31–34].

With this in mind, this review summarizes our results on the role of PAG excitatory amino acid (EAA) neurotransmission in fear-evoked behavior in rats using electrical or chemical stimulation together with appropriate experimental procedures.

2. Periaqueductal gray

There is strong evidence to suggest that the PAG, part of the limbic midbrain area, is an important integrative portion of the neuroaxis able to control the motivational state of an animal [10,24,35,36]. In the 1960s, the aversive motivational feature of the PAG shown in rats by Olds & Olds [17], the deep analgesia after stimulation of the PAG in rats shown by Reynolds [37] and the reports of intense fear or panic, associated with autonomic changes in human patients undergoing neurosurgery, reported by Nashold et al. [38], contributed to our understanding of the functional relevance of the PAG. Those findings corroborate studies in different animal species that systematically demonstrated that the electrical stimulation of a midbrain area (including the PAG) could evoke well-coordinated defensive reactions [15,21,22,39–41]. Since electrical stimulation is unable to discriminate between axons of passage and cell bodies [42], Goodchild et al. [43] suggested that the technique of microinjection of EAA could provide a way to overcome this problem, since EAA selectively excite cell bodies (and their dendritic processes).

In 1991, Bandler and colleagues [18] suggested that anatomical and functional specificity could be expressed in the form of longitudinally arranged neuronal columns that extend for varying distances along the rostrocaudal axis of the PAG. According to these authors, anatomical and functional evidences suggest four longitudinal columns within the PAG, namely, dorsolateral, dorsomedial, lateral and ventrolateral. These columns bear distinct features, mainly related to their immunoreactivity, receptor densities, connections and functions [31,44]. The great diversity of connections with different motor, sensory, limbic, and autonomic structures, may underlie the distinct functions attributed to the PAG [45]. A wealth of data collected over the years have associated the PAG with five major functions: (1) autonomic control; (2) lordosis reflex; (3) vocalization; (4) pain modulation; and (5) emotions related to fear and anxiety [32].

In several animal species, electrical or chemical stimulation of the dorsal portion of the PAG (dPAG; chiefly dorsolateral PAG) elicits flight or defensive postures [12,24], together with neurovegetative responses, mainly consequent of sympathetic activation [22,34,46]. Since these responses can be attenuated by anxiolytic benzodiazepines, a possible relation to fear or anxiety has been proposed [12,22,47].

3. The elevated plus-maze as a model to study defensive behavior

The elevated plus-maze (EPM) test is the most convenient animal model of anxiety. Based on Montgomery's earlier observations that rats had aversion to maze open arms (OA) [48], Handley and Mithani [49] developed an elevated X-maze made up of symmetrical pairs of OA and

Table 1
Possible sites for drug action on NMDA receptor complex (AG, agonist; AT, antagonist)

Site	Mode of action	Endogenous ligand	Drugs
NR1-subunit	Glycine binding site	AG: glycine, D-serine; AT: kynurenic acid ^a	AG: D-cycloserine ^b ; AT: HA966 ^b , 7CIKYN
NR2-subunit	Glutamate binding site Non-competitive antagonist	AG: L-glutamate; L-aspartate	AG: NMDA; AT: AP5, AP7 Ifenprodil ^c , haloperidol, alcohol ^d , trichloroethanol ^d
Ion channel	Uncompetitive blocking at open state channel		Ketamine, MK-801 phencylidine, memantine, amantadine
Modulators	Inhibition Potentiation	Mg ²⁺ ; Zn ²⁺ H ⁺ ; dynorphin A oxidizing agents Polyamines; histamine; reducing agents; arachidonic acid	
Uptake mechanisms	Inhibition	L-Glutamate Glycine	Dihidroksinate, DL-threo-β-benzyloxyaspartate Glycylododecylamide; sarcosine

^a Antagonist at glutamate binding sites at high doses.

^b Partial agonist.

^c Increase potency of protons to block NMDA receptor.

^d Only in high concentrations.

enclosed arms. In this model, the OA approach–avoidance responses have been interpreted as conflict-like behaviors brought about by concurrent exploratory and fear drives. Handley and Mithani [49] showed that this type of maze was sensitive to anxiolytic and anxiogenic drugs and therefore the effects of these drugs should be assessed in terms of preference for the OA as simple ratios (OA/total-arm entries percentage). These predictions were subsequently confirmed (for a review see Ref. [50]), and validated for both rats and mice [51,52].

Blanchard and Blanchard [3] introduced the etho-experimental analysis of behavior combining laboratory-controlled natural aversive stimuli and ethological analysis of behavior. These authors also coined the term ‘risk assessment’ to define the conflict-like behaviors exhibited by animals during the cost/benefit analysis of an aversive situation. The combination of ethological procedures, particularly, of risk assessment measures, with EPM analysis [53,54] validated the model to anxiolytic compounds that differs from benzodiazepines in their mode of action [50].

A notable feature related to the EPM is that it is a fear-eliciting task, per se, providing predictive and face validity as an animal model of anxiety [50,55]. The main arguments for this are (1) aversion to the OA [51]; (2) learned fear without habituation [56,57]; (3) increased plasma corticosterone [51,58]; and (4) analgesia blocked by anxiolytics [59]. Immunohistochemical data provided evidence of a possible correlation among EPM-evoked behaviors, fear/defensive behaviors, and brain activated structures. Increased dorsolateral PAG Fos expression was detected after the exposure to the EPM [60], a predator [61], the odor of a predator [62], an aversive ultrasonic sound [63], during fear-potentiated startle [64] or as a result of systemic injections of anxiogenic drugs [65]. Defensive postures (e.g. crouch, flat back approach) and acts (freezing, risk assessment, avoidance), detected in the above mentioned paradigms suggested that the EPM ethopharmacological analysis can contribute to unravel the role of

dPAG neurotransmitter systems in the modulation of defensive behavior.

4. Excitatory amino acid receptors and the defensive system

Glutamate (GLU) is the main excitatory transmitter in the central nervous system [66,67]. GLU receptors are broadly classified as ionotropic and metabotropic. Ionotropic GLU receptor subtypes, further classified according to their specific agonists as α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), kainate (KAIN), and N-methyl-D-aspartate (NMDA), are composed of tetra- or pentameric, heteromeric, subunits forming a central pore with selective conductance to sodium and calcium ions [68]. Recently, a delta (or orphan) receptor was also described [68].

It has been shown that the characteristics of receptor activation for different EAA neurotransmitters include the fact that all of them are activated by endogenous GLU, but with different kinetic properties [69]. Under high GLU concentrations, AMPA and KAIN receptors are rapidly activated and because of low-affinity binding sites, they seem to inactivate quickly. In contrast, GLU activates NMDA receptors with a high affinity, and the kinetics of activation and inactivation of the receptor are much slower than for AMPA or KAIN receptors [69,70]. It has also been shown that metabotropic GLU receptor can potentiate NMDA receptor activation [71]. The NMDA is the most studied GLU receptor subtype, and has many endogenous modulators, including polyamines (spermine and spermidine), histamine, cations (Zn²⁺; H⁺; Mg²⁺), arachidonic acid and dynorphin (Table 1) [69]. Because the NMDA receptor exhibits a voltage-dependent Mg²⁺ blockage in its inactivated state, activation requires the partial depolarization of the membrane to remove the Mg²⁺ ions. GLU activation of NMDA receptor also requires the binding of glycine (GLY) to the strychnine-insensitive GLY_B site. GLY acts thus as an

Table 2
Effects of GLY_B site receptor acting compounds on defensive behavior

Drug, classification	Experimental procedure, behavioral effect	Reference
ACPC ^a ; 7CIKYN ^b	EPM; anxiolytic-like	[97]
KYN ^b (dPAG)	EPM; anxiolytic-like	[123]
HA966 ^b	Conflict test, social interaction, EPM; anxiolytic-like	[96]
5,7-diCl-KYN ^b	Separation-induced vocalization in rat pups; anxiolytic-like	[94]
ACPC ^a	Separation-induced vocalization in rat pups; anxiolytic-like	[159]
ACPC ^a , DCS ^a	Fear-potentiated startle; anxiolytic-like	[95]
5,7-diCl-KYN ^b	Conflict test, social interaction, EPM; anxiolytic-like	[160]
GLY	Forebrain-elicited vocalization; facilitatory effects	[161]
ACPC ^a , DCS ^a , 7CIKYN ^b	Step-down; prolonged latencies	[162]
GLY	Step-down; reversed the ACPC, DCS, 7CIKYN effects	[162]
HA966 ^a ; 7CIKYN ^b (dPAG)	EPM; anxiolytic-like	[85]
5,7-diCl-KYN ^b	Vogel conflict test; anxiolytic-like	[92]
MDL 102,288 ^b	Separation-induced vocalization in rat pups; anxiolytic-like	[98]
GLY; DSER (dPAG)	EPM; anxiogenic-like	[124]
ACPC ^a	Conflict test; anxiolytic-like	[163]
MDL-105,519 ^b	Separation-induced vocalization in rat pups; anxiolytic-like	[164]
DCS ^a	EPM; antagonism of ethanol induced anxiolytic effect	[165]
DCS ^a	EPM; anxiolytic-like	[166]
L-701,324 ^b	EPM, punished drinking test; anxiolytic-like	[167]
GLY (caudal dPAG)	EPM; anxiogenic-like	[134]
HA966 ^a (dPAG)	EPM; anxiolytic-like	[134]
GLY (caudal dPAG)	EPM; reversed HA966 anxiolytic-like effect	[134]

^a Partial GLY_B agonists: ACPC, 1-aminocyclopropanecarboxylic acid; DCS, D-cycloserine.

^b GLY_B antagonists: 5,7-diCl-KYN, 5,7-dichlorokynurenic acid; L-701,324, 7-chloro-4-hydroxy-3-(3-phenoxy)phenyl-2(1H)-quinoline; MDL-102,288, 5,7-dichloro-1,4-dihydro-4-[[[4-[(methoxycarbonyl)amino]phenyl]sulfonyl]imino]-2-quinolinecarboxylic acid; MDL-105,519, (E)-3-(2-phenyl-2-carboxyethenyl)-4,6-dichloro-1H-indole-2-carboxylic acid.

essential co-agonist [72,73]. These unique features make the NMDA a multiple target for the action of drugs, including agonists, competitive antagonists, uncompetitive blockers (that block the ion channel in the open-state) and non-competitive antagonists (that block the ion channel in the resting state).

Therefore, a functional NMDA receptor should combine a NR1 plus at least one NR2 subunit, which express the GLY and GLU recognition sites, respectively. Receptors containing the NR2A subunit exhibit a 10-fold reduction in their affinity for GLY when compared to other NR2 subunits (for detailed review see Refs. [67,69,74]). GLU and GLY are kept at low extracellular concentrations by means of specific uptake mechanisms [74–76].

It is well established that the NMDA receptor complex is involved in many functional processes. These include learning and memory [77], neural development and synaptic plasticity [78], neural injury after ischemia or hypoglycemia [79]; epilepsy and other chronic neurodegenerative disorders [80,81], drug dependence and tolerance [82,83], neuropathic pain [84], and anxiety and depression [85,86].

In the last two decades, behavioral studies have drawn attention to the participation of NMDA receptors in the mediation of defensive behavior. In 1980, Wenger [87] and Brandão et al. [88] showed that systemic administration of phencyclidine (PCP) and ketamine (KET) facilitate punished response in pigeons. At that time, there was no information on the precise sites of action of such compounds, however, the discovery that either PCP or

KET block NMDA receptor activation [69,89], renewed interest in these compounds and their analogues, and since then many studies have confirmed the role of this class of drugs in defensive behavior.

Systemic injections of NMDA receptor antagonists, acting either as NMDA binding site antagonists, or uncompetitive channel blockers, suggest an anti-aversive profile for these compounds, as shown in conflict models, EPM, separation-induced ultrasonic vocalizations and the anxiety/defense test battery [90–94]. In addition, systemic injections of GLY_B receptor antagonists or partial agonists have also shown anxiolytic-like effects (Table 2) [95–98].

Since the early 1980s, exogenous EAA have been applied directly into neural areas of various animal species to elicit specific cardiovascular and behavioral responses related to emotional-aversive reactions [99]. The dPAG has moderate to high GLU-binding site density [100], suggesting that EAA neurotransmission might play a physiological role in mediating the dPAG-evoked behavioral and autonomic responses. After the first report of elicited defensive ‘rage’ following microinjection of GLU within dPAG of freely moving cats and mydriasis, retraction of the ears, vocalization and hissing in head restrained cat [99], a growing body of evidence has extended these observations to other species [101], as well as to the cardiovascular component of the defense reaction [102,103]. This approach may be a useful tool in establishing a complete framework of the endogenous neurotransmitter systems involved in the execution of defensive behavior.

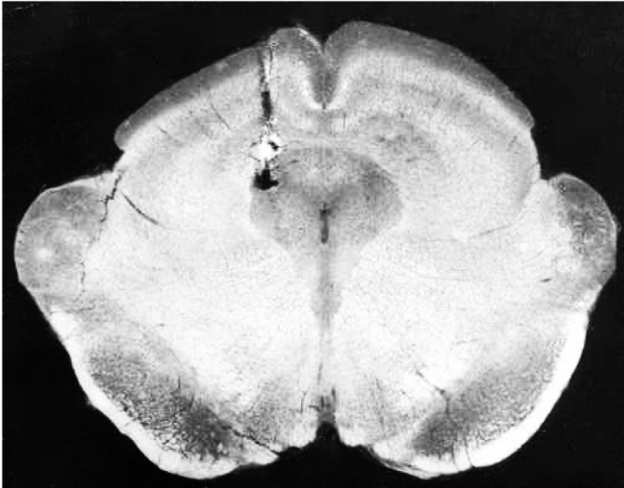


Fig. 1. Photograph of a non-stained frontal section of the midbrain of a rat showing a chemitrode tract within rostral dPAG.

5. Modulation of defensive behavior by periaqueductal gray EAA neurotransmission

5.1. Non-NMDA receptors

In 1985, we began to investigate the effects of EAA antagonists on the defense reaction elicited by GLU, both microinjected within the dPAG (Fig. 1) of freely moving rats [104]. GLU elicited defensive behavior (flight or freezing behavior) associated with an increase in blood pressure (BP). These responses were blocked by L-glutamic acid diethyl ester (GDEE, Fig. 2), a compound that was previously shown to inhibit the effects of GLU, but not NMDA or KAIN, on cat spinal neurons [105,106]. Indeed, KAIN-induced defensive behavior and BP increases were unaffected by GDEE pre-treatment (Fig. 3).

In order to test the physiological significance of GLU mediation of defensive behavior within dPAG, another

group of rats was implanted with an electrode within the dorsomedial hypothalamus (DMH) and a chemitrode (electrode plus guide-cannula) within the dPAG. The dPAG-evoked BP responses and defensive behaviors were both inhibited by GDEE (40 nmol) applied to the stimulation site (Fig. 4, lower panel). Moreover, the microinjection of GDEE into the dPAG blocked the behavioral and BP defensive responses elicited by electrical stimulation of the DMH (Fig. 4, upper panel). The latter data provided the first evidence of a hypothalamic glutamatergic projection to the dPAG [107]. This result was further supported by both neuroanatomical [108] and behavioral findings [109]. Moreover, although the microinjection into the dPAG of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), an AMPA receptor selective antagonist, did not change the hypothalamus-evoked defense reaction [109], recent results using the EPM task revealed an increased OA activity following the intra-periaqueductal injection of CNQX or GDEE within the dPAG suggesting an anxiolytic-like effect [110]. Altogether, these results suggest that AMPA receptors mediate the dPAG-evoked defensive behavior of the rat.

Although the application of KAIN in the dPAG elicits behavioral [111] and BP defensive responses (Fig. 3) [107], suggesting a pro-aversive effect, the microinjection of KAIN into the dPAG also decreases the expression of fear-potentiated startle, suggesting an anti-aversive effect [26,112]. Because the magnitude of the startle reflex is directly related to the degree of freezing, Walker et al. [113] suggested that dPAG-evoked active behaviors, such as trotting, galloping and jumping [15,114–116], reduce the magnitude of freezing behavior and, consequently, the startle reflex [26]. Therefore, the role of KAIN receptors within the dPAG in defensive behaviors cannot be excluded.

Finally, it was recently shown that the GLU metabotropic agonist, *trans*-(1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylate, microinjected within the dPAG, elicits defensive behaviors characterized by jumps towards the top of the cage, gallops, and darting suggesting the participation of

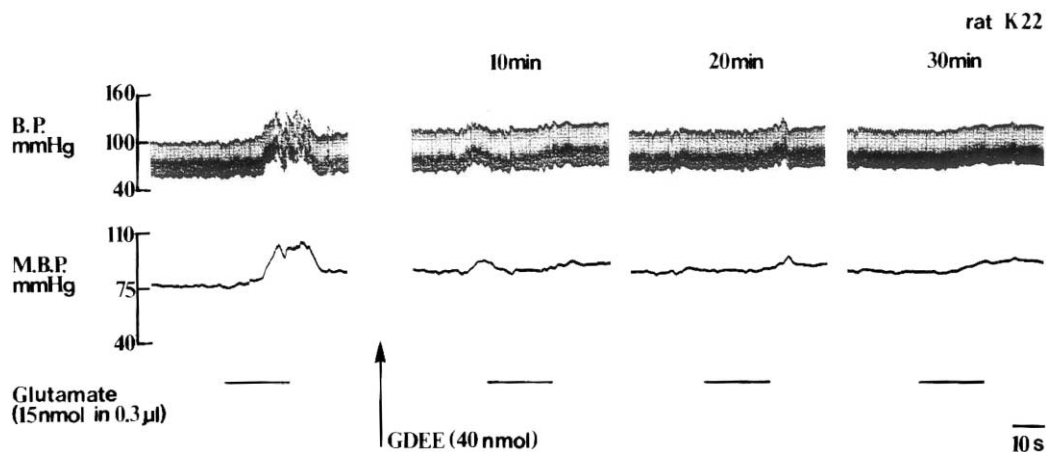


Fig. 2. A typical chart showing a reduction of the mean arterial blood pressure increases elicited by glutamate (15 nmol) following GDEE (40 nmol) treatment, both applied within dPAG. 10, 20 and 30 min represent times after GDEE treatment.

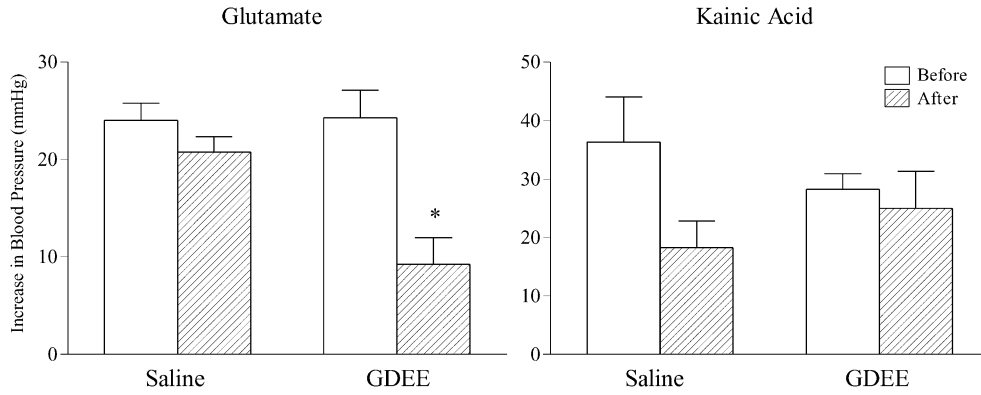


Fig. 3. Anti-aversive effects of GDEE (40 nmol, 0.3 μ l) applied within dPAG of freely moving Wistar rats. Defensive behavior and blood pressure rise elicited by GLU (left panel) was reduced after GDEE. KAIN induced defensive behavior and increase in blood pressure within dPAG (right panel) was not blocked by GDEE. \square Before treatment; ▨ after treatment. * $p < 0.05$, significantly different from the control group.

metabotropic GLU receptors in the mediation of defensive behavior elicited in the dPAG [115].

5.2. NMDA receptor complex

In 1990, we began to target our studies towards the role of the NMDA/GLY_B receptor complex within the dPAG, using a range of different experimental approaches. In anaesthetized rats with a cannula inserted in the dPAG, pre-treatment with the NMDA antagonist, 2-amino-7-

phosphonoheptanoic acid (AP7) reduced the BP responses evoked by GLU [117]. An anxiolytic-like effect of AP7-injection within the dPAG was shown in rats, detected by increased OA activity in the EPM [118]. In cats, the microinjection of the non-selective NMDA/GLY_B receptor antagonist, kynurenic acid (KYN) or AP7 into the dPAG blocked the defensive reaction induced by electrical stimulation of DMH [109,119]. Studies from different laboratories have also shown that the microinjection of AP7 within the dPAG blocked defensive analgesia induced by

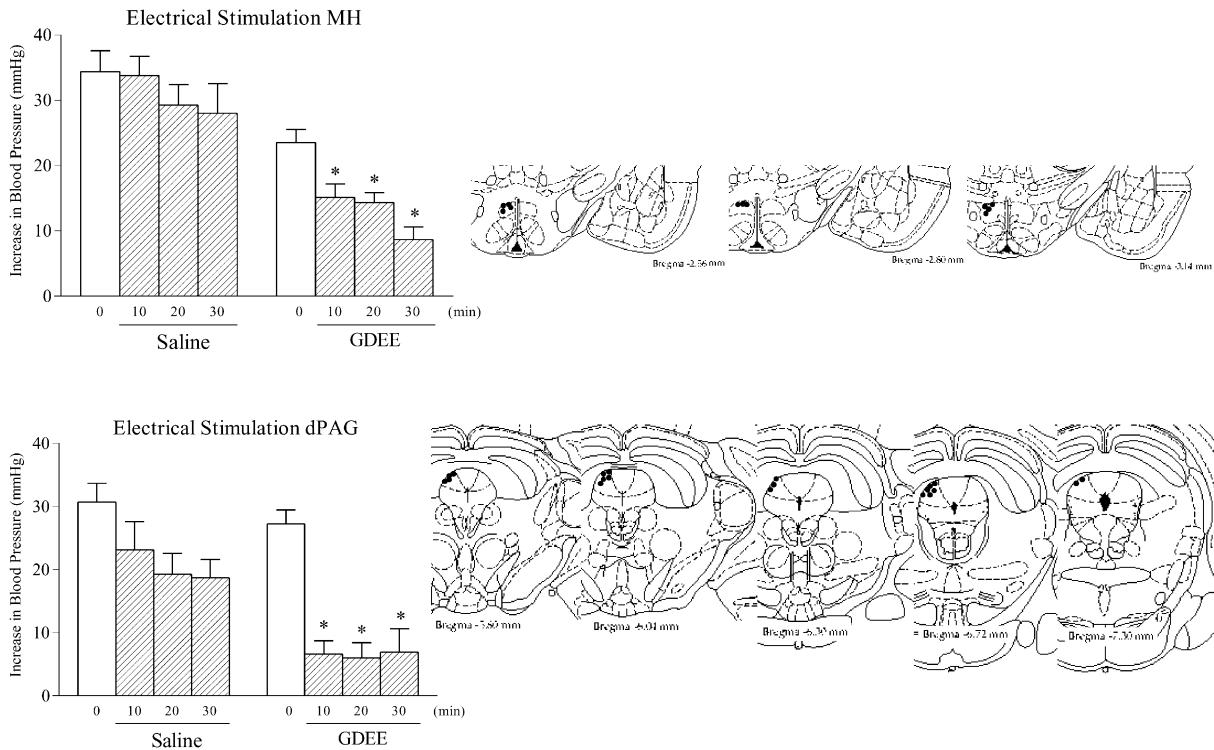


Fig. 4. Anti-aversive effects of GDEE (40 nmol, 0.3 μ l) applied within dPAG of freely moving Wistar rats. Defensive behavior and blood pressure rise elicited by electrical stimulation of the dPAG (lower panel) and electrical stimulation of medial hypothalamus (upper panel) were reduced after GDEE. \square Before treatment; ▨ after treatment. * $p < 0.05$, significantly different from the control group. On the right side, diagrams of coronal diencephalon (upper panel) and midbrain (lower panel) redrawn from Ref. [158] showing electrical stimulation or microinjection sites labeled by Evans Blue. Numbers represent distance in mm from bregma.

Table 3
Compilation of studies showing PAG rostrocaudal differences ((↑) increased)

Experimental condition	Rostrocaudal differences	Reference
Defense reaction induced by GLU	More effective—caudal PAG	[126]
Behavioral observation—freely moving rat	Forward avoidance—caudal PAG	[101]
PAG afferent from central nucleus of amygdala—anterograde tracing study	↑ Labeled neurons—rostral PAG	[127]
PAG efferents to medial pre-optic area—retrograde tracing study	↑ Labeled neurons—rostral PAG	[128]
PAG efferents to medulla—retrograde tracing study	↑ Labeled neurons—caudal PAG	[129]
Fos-ir ^a after non-intentional stimulus	↑ Expression—caudal PAG	[130]
Fos-ir ^a expression after somatosensory stimuli	↑ Expression—caudal PAG	[131]
Estrogen receptor immunoreactive neurons	More reactive—caudal PAG	[132]
Androgen and estrogen receptor distribution	↑ Concentration—caudal PAG	[133]
GLY effects in the EPM	Anxiogenic effect—caudal PAG	[134]

^a Fos-ir, Fos-immunoreactivity.

either an injection of NMDA in the PAG of rats [120] or by social conflict in mice [121]. Since AP7 is a GLU competitive antagonist, these results suggest a modulatory role for compound ligands on the NR2 subunit of the NMDA receptor complex in defensive behavior.

Uncompetitive blockers of the NMDA receptor complex (Table 2) have been shown to reduce defensive behavior in various experimental situations. Systemic injection of 5-methyl-10,11-dihydro-5H-dibenzo[*a,d*]cyclohepten-5,10-imine hydrogen maleate (MK801) in male rats submitted to the anxiety/defense battery revealed an anti-predator defensiveness effect [122]. The behavioral profile presented by the rats included increased transits and eating during cat presentation and decreased orientation to and proxemic avoidance of the cat compartment in the post-cat period. Moreover, KET microinjection within the dPAG has been found to increase OA activity in an EPM (Schmitt and Carobrez, unpublished data). Overall, the above evidence, along with the fact that the PAG has a moderate density of radiolabeled binding sites for PCP receptors [89], strengthen the role of NMDA mediation in defensive behavior elicited within the PAG and suggests that drug action within the channel pore, as is the case for uncompetitive blockers of the NMDA receptor, might be a useful tool to explore the nature of this mediation.

5.2.1. GLY_B site receptor

The role of GLY-binding site in the NMDA receptor complex in defensive behavior has been also studied in our laboratory over the last 10 years. Accordingly, KYN microinjected within dPAG of rats submitted to the EPM task exhibited anxiolytic effects [123]. Further evidence for the role of the GLY_B site within the dPAG in defensive behavior came with two subsequent studies also using the EPM and microinjections of compounds directly within the dPAG. Whereas the first study showed that 7-chlorokynurenate (7CIKYN) and (±)-3-amino-1-hydroxy-2-pyrrolidone (HA966) reduced the fear-like behavior of rats stimulated in the dPAG or exposed to the EPM [85], the second study showed that the administration of GLY or D-serine (DSER) increased the aversion to the OA of the

EPM, suggesting an anxiogenic-like action of the agonist [124]. The interaction of the activation of dPAG GLY_B receptors and classical anxiolytic drugs was carried out microinjecting either GLY or 7CIKYN in the dPAG of rats systemically treated with diazepam or the anxiogenic drug, pentylenetetrazole (PTZ) [125]. GLY abolished the increase in OA activity observed in control animals treated with peripheral injections of diazepam and intra-periaqueductal injections of artificial cerebrospinal fluid (CSF). Notably, the failure of GLY to block sedative effects at higher doses of diazepam (unpublished results), supports the selective involvement of dPAG GLY_B site in the anxiolytic effect of the benzodiazepine. On the other hand, microinjection of 7CIKYN within dPAG quite remarkably reversed the anxiogenic effects of systemic PTZ, obtained in control rats microinjected with CSF. The seemingly fearless PTZ/7CIKYN-treated rats showed a dose-related increase in OA activity but not exploratory activity, suggesting a selective anxiolytic-like effect [125]. Altogether, the above results suggest that the NMDA/GLY_B site within the dPAG participates in the mediation of defensive behavior.

In addition, literature review reports a differential participation of rostral and caudal areas of the dPAG in the mediation of defensive behavior [101,126]. Moreover, these areas also differ with respect to their neuroanatomical connections [127–129], Fos immunoreactivity after distinct experimental procedures [130,131] and hormone receptors [132,133] (Table 3).

Accordingly, the differential participation of rostral and caudal areas of dPAG in the anxiogenic effect of GLY was recently appraised in our laboratory [134]. Data showed that the microinjection of the GLY_B antagonist, HA966 produced an anxiolytic-like increase in OA activity in all levels of dPAG (rostral, AP = −5.8 mm; intermediate, AP = −6.7 mm; caudal, AP = −7.6 mm). However, GLY produced an anxiogenic-like reduction in OA activity only when applied to the caudal level of dPAG [134]. Either the differential distribution of NMDA receptor subunits along the rostrocaudal extent of dPAG, resulting in a differential affinity of GLY for its binding site [135,136], or the saturation of GLY_B binding sites in the rostral dPAG, making it

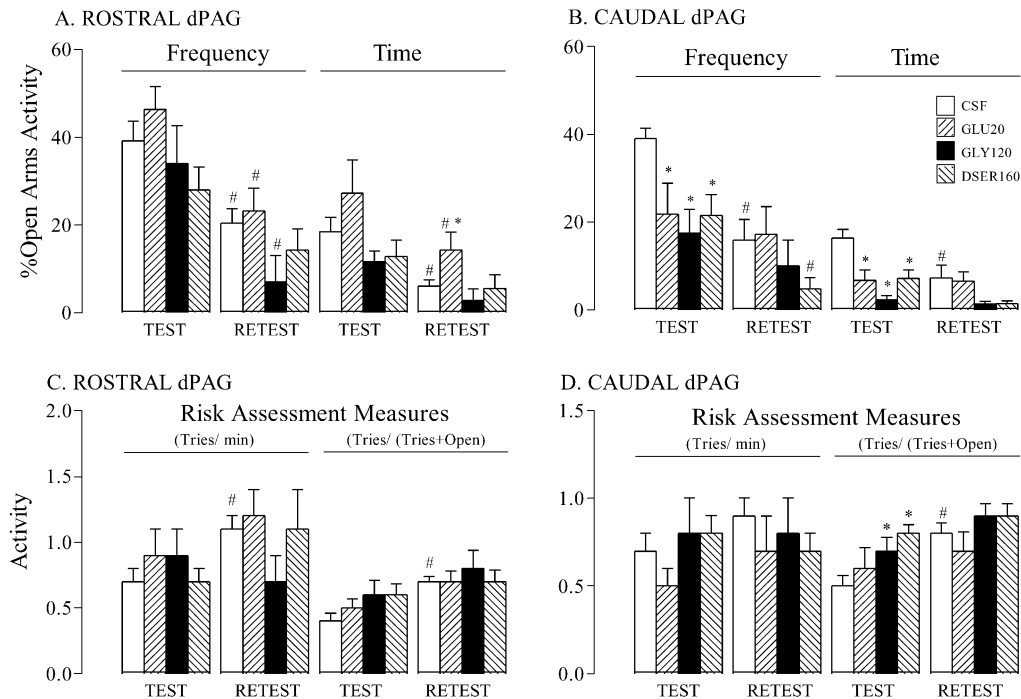


Fig. 5. Effects of GLU (20 nmol; $n = 9$, rostral; $n = 11$, caudal), GLY (120 nmol; $n = 7$, rostral; $n = 7$, caudal) and D-serine (DSER; 160 nmol; $n = 11$, rostral; $n = 13$, caudal) microinjected ($0.3 \mu\text{l}$) within the rostral (A and C) or caudal (B and D) dPAG of rats 10 min before the test in an EPM. Retest was performed 24 h later without drugs. Columns represent the mean \pm SEM percentage of frequency and time spent on the arms of the EPM (A and B) and of risk assessment measures (C and D, see text for definitions). CSF: cerebrospinal fluid. * $p < 0.05$, significantly different from the CSF group. # $p < 0.05$, significantly different from previous test values.

unresponsive to exogenous GLY [137,138], could explain these data. Indeed, although the interaction of NMDA receptor subunits are not fully understood [139], different combinations of NR1 and NR2 subunits can result in differences in sensitivity to exogenous GLY.

Electrophysiological studies have shown the following rank order for relative affinities of GLY for the GLY_B site: NR1/NR2D > NR1/NR2C > NR1/NR2B > NR1/NR2A [135,139,140]. Therefore, a greater distribution of NMDA receptors composed of NR1/NR2A subunits should reduce the final effect due to exogenous GLY. Studies addressing the relative affinity of GLY_B receptor antagonists to NMDA/ GLY_B receptor subunits are not conclusive [74] and therefore suggest that they might bind with similar affinities to all the subunit combinations.

Literature is controversial about the presumed saturation of GLY_B binding sites in vivo [69,74,138,141]. Eventually, GLY concentrations in the synaptic cleft can be reduced below saturation levels ($< 1 \mu\text{M}$) by highly effective active transporters [142–144]. Moreover, binding at the GLY_B site can be regulated by endogenous modulators, such as KYN, that block the GLY_B receptor, or zinc, that reduces the affinity of GLY [74]. Therefore, it remains to be established whether there is a specific condition that induce GLY transporters to reduce GLY synaptic concentrations below saturation levels and thus modulate defensive behaviors.

In order to confirm and extend this study, our laboratory evaluated the rostrocaudal behavioral mapping of NMDA/

GLY_B receptors within the dPAG, using the EPM. Rats received CSF, GLU (20 nmol), GLY (120 nmol), or DSER (160 nmol) each microinjected within rostral (-5.8 mm) or caudal (-7.6 mm) dPAG. Thereupon, the animals were submitted to the test session in the EPM for 5 min. Twenty-four hours later the animals were retested in the EPM for 5 min.

In addition to the OA activity, the frequency of attempts to reach the OAs were recorded as tries [57,145]. The number of tries was combined with traditional measures to calculate two risk assessment measures: (1) the number of tries per min; and (2) the index of success (values close to zero) or failure (values close to 1) to enter the OAs.

Data obtained after rostral dPAG microinjections of CSF, GLU, GLY and DSER are shown in Fig. 5(A) and (C). When the compounds were injected within the rostral dPAG, before the test session, neither OA (Fig. 5(A)) nor risk assessment (Fig. 5(C)) activities were affected. A reduction in OA activity on retest (Fig. 5(A)) is consistent with previous findings showing that a 5 min previous maze experience increases fear behavior in the EPM-task [56,57,146]. An increased risk assessment behavior for control rats on retest (Fig. 5(C)) showed an increased evaluation of the apparatus in spite of the increased fear, suggesting that there is no habituation to the fear elicited by the maze.

Data obtained on subjects microinjected within the caudal dPAG revealed a different set of results and are shown in

Fig. 5(B) and (D). Injection of GLU, GLY or DSER reduced OA activity in the test session (Fig. 5(B)) when compared to the CSF group. In the retest, there was a further reduction in OA activity for CSF control rats, confirming data previously shown, and for DSER, whereas for GLU or GLY groups no effect was observed (Fig. 5(B)). Analysis of both risk assessment measures in the test session after treatment showed that only the risk index was increased by GLY or DSER. In the retest session, the CSF group was found to be increased, similar to data previously shown in subjects microinjected within the rostral dPAG (Fig. 5(D)).

The results presented here, showed that GLU, GLY and DSER, when microinjected within two different portions along the rostrocaudal axis, might affect behavioral responses in a different fashion. The consistent finding is that all three drugs used have shown anxiogenic-like effects restricted to the caudal dPAG, confirming previous results [134].

Taken together, since NMDA/GLY_B compounds applied within the dPAG can modify the behavioral responses detected in the EPM, including risk assessment and OA avoidance, a possible participation of this brain structure in more subtle defensive behavior cannot be excluded. Therefore, it is tempting to speculate that activation of NMDA/GLY_B receptors within dPAG can also produce anxiety-like phenomena, related or not to panic syndrome, which has been associated within the dPAG [47,147–149].

6. Concluding remarks

The results reported and discussed in this review strongly suggest the participation of EAA neurotransmission in dPAG mediation of defensive behavior. The main arguments underlying this assertion include (1) induction of behavioral and neurovegetative components of defensive behavior by the main EAA agonist subtypes microinjected within the dPAG; (2) modification of spontaneous defensive behavior by EAA antagonists or blockers applied within the dPAG; (3) reduction of GLU- or electrically induced defensive behaviors by EAA receptor antagonists; and (4) after substantial sampling, reliability of results obtained using different experimental approaches.

Based on these findings, the presence of all EAA receptors within the dPAG [100] suggest that endogenous GLU would act initially at AMPA and KAIN receptors which possess rapid activation and inactivation times, further depolarizing the membrane and removing the Mg²⁺ block from the NMDA ion channel, therefore allowing the slower activation of the NMDA receptor complex, which could not be potentiated by GLU action on metabotropic binding sites.

These properties of the NMDA receptor are thought to be essential for many types of synaptic plasticity including those related to memory formation, regulation of movement and in influencing the receptive field size of sensory neurons

in the visual cortex [150]. Recently, LTP [28] and kindling [151] of PAG have been demonstrated, both phenomena associated with neural plasticity. All of these features can be related to increased arousal and learned avoidance in defensive animals and therefore fit well with the proposed role of EAA receptors in mediating defensive behavior.

In our work, the study of the role of the NMDA receptor in defense has been approached mostly using the EPM task and GLY_B receptor acting compounds microinjected within the dPAG. The reason for using the EPM was largely discussed in Section 3. Furthermore, the reason for using GLY_B receptor acting compounds was based on specific properties related to this receptor site within the dPAG, such as (1) being part of the NMDA receptor complex, which as discussed earlier could underlie physiological processes related to defensive behavior; (2) presence as endogenous compounds with both agonist (GLY and DSER) and antagonist (KYN) actions; (3) increased defensive behavior in rats, without the jumping or wild running behavior obtained when using NMDA or GLU, therefore allowing its use in the EPM task; (4) literature data showing its participation underlying the mode of action of antidepressants [152–154] and drugs of abuse such as opioids [155], cocaine [156], and ethanol [157].

A role of the dPAG in anxiety and, specifically, in panic attacks has been proposed [147–149]. The flight behavior or jumping that follows dPAG stimulation [4,114], as well as the reports from human patients undergoing neurosurgery [38,150] give support to this view. However, the experiments discussed earlier cannot rule out the importance of the dPAG in a more subtle defensive behavior, such as risk assessment or OA avoidance, detected when using the EPM. Microinjections of GLY and DSER within the dPAG were both capable of eliciting a well-coordinated increase in avoidance response to the OA of the maze.

In a more integrated view of the hierarchical defense system, as proposed by some authors [12,13,147], a high activation of the dPAG, elicited by electrical stimulation or high doses of GLU (above 40 nmol), would lead to panic-like behavior overtaking the control of the whole defense system. On the other hand, low activation of the dPAG elicited by GLY, DSER or GLU (20 nmol) was able to increase OA avoidance not only in the test but also in the retest session in the EPM. The EPM experience and the drug microinjection within dPAG during the test session resulted in a long-lasting effect detected in the retest, suggesting that the dPAG can influence the acquisition of learned fear response, a more attenuated expression of the defensive behavior. Although the hierarchical view of the defensive system [12,13] predicted only undirected escape responses for the dPAG, the results discussed earlier suggest also that this brain area could participate in more oriented and cognitive defensive responses. It remains to be determined if the activation of GLY_B receptors in the dPAG could also be related to anticipatory anxiety intrinsically related to the panic syndrome.

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References

- [1] Darwin C. The expression of emotions in man and animals. London: Murray, 1872.
- [2] Panksepp J. Toward a general psychobiological theory of emotions. *Behav Brain Sci* 1982;5:407–67.
- [3] Blanchard DC, Blanchard RJ. Ethoexperimental approaches to the biology of emotion. *Ann Rev Psychol* 1988;39:43–68.
- [4] Graeff FG. Role of 5HT in defense behavior and anxiety. *Rev Neurosci* 1993;4:181–211.
- [5] Nesse RM. Proximate and evolutionary studies of anxiety, stress and depression: synergy at the interface. *Neurosci Biobehav Rev* 1999;23:895–903.
- [6] Parmigiani S, Palanza P, Rodgers J, Ferrari PF. Selection, evolution of behavior and animal models in behavioral neuroscience. *Neurosci Biobehav Rev* 1999;23:957–70.
- [7] Blanchard RJ, Blanchard DC. Attack and defense in rodents as ethoexperimental models for the study of emotion. *Prog Neuropsychopharmacol Biol Psych* 1989;13:13–4.
- [8] Blanchard DC, Blanchard RJ. Behavioral correlates of chronic dominance–subordination relationships of male rats in a seminatural situation. *Neurosci Biobehav Rev* 1990;14:455–62.
- [9] Blanchard DC, Blanchard RJ, Tom P, Rodgers RJ. Diazepam changes risk assessment in an anxiety/defense test battery. *Psychopharmacology* 1990;101(4):511–8.
- [10] Adams DB. Brain mechanisms for offense, defense and submission. *Behav Brain Sci* 1979;2:201–41.
- [11] Gray JA. The neuropsychology of anxiety: an enquiry into the functions of the septo-hippocampal system. Oxford: Oxford University Press, 1982.
- [12] Graeff FG. Minor tranquilizers and brain defense systems. *Brazilian J Med Biol Res* 1981;14:239–65.
- [13] Gray JA, McNaughton N. The neuropsychology of anxiety. 2nd ed. New York: Oxford University Press, 2000.
- [14] Fernandez de Molina A, Hunsperger RW. Central representation of affective reaction in forebrain and brainstem: electrical stimulation of amygdala, stria terminalis and adjacent structures. *J Physiol* 1959;145:251–65.
- [15] Hunsperger RW. Affektraktionen auf elektrische reizung im hirn-stamm der katze. *Helv Physiol Pharmacol Acta* 1956;14:70–92.
- [16] Hilton SM. Ways of viewing the central nervous control of the circulation—old and new. *Brain Res* 1975;87(2–3):213–9.
- [17] Olds ME, Olds J. Approach escape interactions in the rat brain. *Am J Physiol* 1962;203:803–10.
- [18] Schenberg LC, De Aguiar JC, Graeff FG. GABA modulation of the defense reaction induced by brain electrical stimulation. *Physiol Behav* 1983;31(4):429–37.
- [19] Bandler R, Carrive P, Depaulis A. Introduction—emerging principles of organization of the midbrain periaqueductal gray matter. In: Depaulis A, Bandler R, editors. *The midbrain periaqueductal gray matter*, New York: Plenum Press, 1991. p. 1–8.
- [20] Fernandez de Molina A, Hunsperger RW. Organization of the subcortical system governing defense and flight reaction in the cat. *J Physiol* 1962;160:200–13.
- [21] Skultety FM. Stimulation of periaqueductal gray and hypothalamus. *Arch Neurol Psychiat* 1963;8:608–20.
- [22] Schenberg LC, Graeff FG. Role of the periaqueductal gray substance in the antianxiety action of benzodiazepines. *Pharmacol Biochem Behav* 1978;9:287–95.
- [23] Blanchard DC, Williams G, Lee EMC, Blanchard RJ. Taming of wild *Rattus norvegicus* by lesions of the mesencephalic central gray. *Physiol Psychol* 1981;9:157–63.
- [24] Bandler R. Brain mechanisms of aggression as revealed by electrical and chemical stimulation: suggestion of a central role for the midbrain periaqueductal grey region. In: Epstein A, Morrison A, editors. *Progress in psychobiology and physiological psychology*, New York: Academic Press, 1988. p. 67–154.
- [25] Fanselow MS, DeCola JP, De Oca BM, Landeira-Fernandez J. Ventral and dorsolateral regions of the midbrain periaqueductal gray (PAG) control different stages of defensive behavior: dorsolateral PAG lesions enhance the defensive freezing produced by massed and immediate shock. *Aggres Behav* 1995;21:63–77.
- [26] Walker DL, Davis M. Involvement of the dorsal periaqueductal gray in the loss of fear-potentiated startle accompanying high footshock training. *Behav Neurosci* 1997;111:692–702.
- [27] Amoranpanth P, Nader K, Ledoux JE. Lesions of periaqueductal gray dissociate-conditioned freezing from conditioned suppression behavior in rats. *Learn Mem* 1999;6:491–9.
- [28] Adamec R. Does long term potentiation in periaqueductal gray (PAG) mediate lasting changes in rodent anxiety-like behavior (ALB) produced by predator stress? Effects of low frequency stimulation (LFS) of PAG on place preference and changes in ALB produced by predator stress. *Behav Brain Res* 2001;120(2):111–35.
- [29] Abrahams VC, Hilton SM, Zbrozyna AW. Active muscle vasodilatation produced by stimulation of the brainstem: its significance in the defense reaction. *J Physiol* 1960;154:491–513.
- [30] Beitz AJ. Periaqueductal gray. In: Paxinos G, editor. *The rat nervous system*, 2nd ed. San Diego: Academic Press, 1995. p. 173–82.
- [31] Bandler R, Shipley MT. Columnar organization in the midbrain periaqueductal gray: modules for emotional expression? *Trends Neurosci* 1994;17(9):379–89.
- [32] Behbehani MM. Functional characteristics of the midbrain periaqueductal gray. *Prog Neurobiol* 1995;46:575–605.
- [33] Hilton SM, Redfern WS. A search for brain stem cell groups integrating the defence reaction in the rat. *J Physiol* 1986;378:213–28.
- [34] Carobrez AP, Schenberg LC, Graeff FG. Neuroeffector mechanisms of the defense reaction in the rat. *Physiol Behav* 1983;31(4):439–44.
- [35] Nauta WJH. Hippocampal projections and related neural pathways to the midbrain in the cat brain. *Brain* 1958;81:319–40.
- [36] Mantyh PW. The midbrain periaqueductal gray in the rat, cat, and monkey: a Nissl, Weil, and Golgi analysis. *J Comp Neurol* 1982;204:349–63.
- [37] Reynolds DV. Surgery in the rat during electrical analgesia by focal brain stimulation. *Science* 1969;161:444–5.
- [38] Nashold BS, Wilson WP, Slaughter DG. Sensation evoked by stimulation in the midbrain of man. *J Neurosurg* 1969;30:14–24.
- [39] Edwards SB, Flynn JP. Corticospinal control of striking in centrally elicited attack behavior. *Brain Res* 1972;41(1):51–65.
- [40] Bovier P, Broekkamp CL, Lloyd KG. Enhancing GABAergic transmission reverses the aversive state in rats induced by electrical stimulation of the periaqueductal grey region. *Brain Res* 1982;248(2):313–20.
- [41] Mos J, Kruk MR, Van Der Poel AM, Meelis W. Aggressive behavior induced by electrical stimulation in the midbrain central gray of male rats. *Aggres Behav* 1982;8:261–84.
- [42] Ranck Jr. JB. Which elements are excited in electrical stimulation of mammalian central nervous system: a review. *Brain Res* 1975;98(3):417–40.
- [43] Goodchild AK, Dampney RAL, Bandler R. A method for evoking physiological responses by stimulation of cell bodies, but not axons

- of passage, within localized regions of the central nervous system. *J Neurosci Meth* 1982;6:351–63.
- [44] Bandler R, Keay KA. Columnar organization in the midbrain periaqueductal gray and the integration of emotional expression. In: Holstege G, Bandler R, Saper CB, editors. *Progress in brain research*, vol. 107. Amsterdam: Elsevier, 1996. p. 285–300 chapter 17.
- [45] Beitz AJ, Shepard RD. The midbrain periaqueductal gray in the rat. II. A golgi analysis. *J Comp Neurol* 1985;237:460–75.
- [46] Hilton SM. The defence-arousal system and its relevance for circulatory and respiratory control. *J Exp Biol* 1982;100:159–74.
- [47] Graeff FG. Neurotransmitters in the dorsal periaqueductal gray and animal model of panic anxiety. In: Briley M, File SE, editors. *New concepts in anxiety*, London: Macmillan, 1991. p. 288–312.
- [48] Montgomery KC. The relation between fear induced by novelty stimulation and exploratory behavior. *J Comp Neurol* 1955;48:254–60.
- [49] Handley SL, Mithani S. Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of fear-motivated behavior. *Naunyn-Schmiedeberg's Arch Pharmacol* 1984;327:1–5.
- [50] Rodgers RJ, Cole JC. The elevated plus-maze: pharmacology, methodology and ethology. In: Cooper SJ, Hendrie CA, editors. *Ethology and psychopharmacology*, New York: Wiley, 1994. p. 9–44.
- [51] Pellow S, Chopin P, File SE, Briley M. Validation of open/closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Meth* 1985;14:149–67.
- [52] Lister RG. The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology* 1987;92:180–5.
- [53] Cole JC, Rodgers RJ. An ethological analysis of the effects of chloridiazepoxide and bretazenil (Ro 16-6028) in the murine elevated plus-maze. *Behav Pharmacol* 1993;4(6):573–80.
- [54] Cruz AP, Frei F, Graeff FG. Ethopharmacological analysis of rat behavior on the elevated plus-maze. *Pharmacol Biochem Behav* 1994;49(1):171–6.
- [55] Handley SL. 5-Hydroxytryptamine pathways in anxiety and its treatment. *Pharmacol Ther* 1995;66(1):103–48.
- [56] Treit D, Menard J, Royan C. Anxiogenic stimuli in the elevated plus-maze. *Pharmacol Biochem Behav* 1993;44(2):463–9.
- [57] Bertoglio LJ, Carobrez AP. Previous maze experience required to increase open arms avoidance in rats submitted to the elevated plus-maze model of anxiety. *Behav Brain Res* 2000;108(2):197–203.
- [58] Rodgers RJ, Haller J, Holmes A, Halasz J, Walton TJ, Brain PF. Corticosterone response to the plus-maze: high correlation with risk assessment in rats and mice. *Physiol Behav* 1999;68(1–2):47–53.
- [59] Lee C, Rodgers RJ. Antinociceptive effects of elevated plus-maze exposure: influence of opiate receptor manipulations. *Psychopharmacology* 1990;102(4):507–13.
- [60] Silveira MC, Sandner G, Graeff FG. Induction of Fos immunoreactivity in the brain by exposure to the elevated plus-maze. *Behav Brain Res* 1993;56(1):115–8.
- [61] Canteras NS, Goto M. Fos-like immunoreactivity in the periaqueductal gray of rats exposed to a natural predator. *Neuroreport* 1999;10(2):413–8.
- [62] Dielenberg RA, Hunt GE, McGregor IS. When a rat smells a cat: the distribution of Fos immunoreactivity in rat brain following exposure to a predatory odor. *Neuroscience* 2001;104(4):1085–97.
- [63] Beckett SR, Duxon MS, Aspley S, Marsden CA. Central c-fos expression following 20 kHz/ultrasound induced defence behaviour in the rat. *Brain Res Bull* 1997;42(6):421–6.
- [64] Campeau S, Falls WA, Cullinan WE, Helmreich DL, Davis M, Watson SJ. Elicitation and reduction of fear: behavioural and neuroendocrine indices and brain induction of the immediate-early gene c-fos. *Neuroscience* 1997;78(4):1087–104.
- [65] Singewald N, Sharp T. Neuroanatomical targets of anxiogenic drugs in the hindbrain as revealed by Fos immunocytochemistry. *Neuroscience* 2000;98(4):759–70.
- [66] Collingridge GL, Lester RAJ. Excitatory amino acid receptors in the vertebrate central nervous system. *Pharmacol Rev* 1989;40:143–210.
- [67] Ottersen OP, Storm-Mathisen J. *Handbook of chemical neuroanatomy. Glutamate*, vol. 18. Amsterdam: Elsevier, 2000.
- [68] Petralia RS, Rubio ME, Wang YX, Wenthold RJ. Regional and synaptic expression of ionotropic glutamate receptors. In: Ottersen OP, Storm-Mathisen J, editors. *Handbook of chemical neuroanatomy, Glutamate*, vol. 18. Amsterdam: Elsevier, 2000. p. 145–82.
- [69] Dingledine R, Borges K, Bowie D, Traynelis SF. The glutamate receptor ion channels. *Pharmacol Rev* 1999;51(1):7–61.
- [70] Benveniste M, Mayer ML. Kinetic analysis of antagonist action at *N*-methyl-D-aspartic acid receptors. Two binding sites each for glutamate and glycine. *Biophys J* 1991;59(3):560–73.
- [71] Lan J, Skeberdis VA, Jover T, Zheng X, Bennett MV, Zukin RS. Activation of metabotropic glutamate receptor 1 accelerates nmda receptor trafficking. *J Neurosci* 2001;21(16):6058–68.
- [72] Johnson JW, Ascher P. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* 1987;325:529–31.
- [73] Kleckner NW, Dingledine R. Requirement for glycine in activation of NMDA-receptor expressed in *Xenopus* oocyte. *Science* 1988;241:835–7.
- [74] Danysz W, Parsons CG. Glycine and *N*-methyl-D-aspartate receptors: physiological significance and possible therapeutic applications. *Pharmacol Rev* 1998;50:597–664.
- [75] Danbolt NC. Glutamate uptake. *Prog Neurobiol* 2001;65(1):1–105.
- [76] Berger AJ, Dieudonné S, Ascher P. Glycine uptake governs glycine site occupancy at NMDA receptor of excitatory synapses. *J Neurophysiol* 1998;80:3336–40.
- [77] Morris RGM. Synaptic plasticity and learning: selective impairment of learning in rats and blockade of long-term potentiation in vivo by *N*-methyl-D-aspartate receptor antagonist AP5. *J Neurosci* 1989;9:3040–57.
- [78] Cotman CW, Monaghan DT, Ganong AH. Excitatory amino acid neurotransmission: NMDA receptors and Hebb-type synaptic plasticity. *Annu Rev Neurosci* 1988;11:61–80.
- [79] Yenari MA, Tong DC, Albers GW. Glycine antagonists for treatment of ischemic brain injury. In: Terhorst GJ, Korf J, editors. *Clinical pharmacology of cerebral ischemia*, Totowa: Humana Press, 1997. p. 127–51.
- [80] Meldrum B. Possible therapeutic applications of antagonists of excitatory amino acid neurotransmitters. *Clin Sci* 1985;68:113–22.
- [81] Schwartz BL, Hashtroudi S, Herting RL, Schwartz P, Deutsch SI. D-Cycloserine enhances implicit memory in Alzheimer patients. *Neurology* 1996;46:420–4.
- [82] Marek P, Beneliyahu S, Gold M, Liebeskind JC. Excitatory amino acid antagonist (kynurenic acid and MK-801) attenuate the development of morphine tolerance in the rat. *Brain Res* 1991;547:77–81.
- [83] Rossetti ZL, Carboni S. Ethanol withdrawal is associated with increased extracellular glutamate in the rat striatum. *Eur J Pharmacol* 1995;283:177–83.
- [84] Eisenberg E, Pud D. Can patients with chronic neuropathic pain be cured by acute administration of the NMDA receptor antagonist amantadine? *Pain* 1998;74:337–9.
- [85] Matheus MG, Nogueira RL, Carobrez AP, Graeff FG, Guimaraes FS. Anxiolytic effect of glycine antagonists microinjected into the dorsal periaqueductal grey. *Psychopharmacology* 1994;113(3–4):565–9.
- [86] Maes M, Debacker G, Suy E, Minner B. Increased plasma serine concentrations in depression. *Neuropsychobiology* 1995;31:10–15.
- [87] Wenger GR. Effects of phencyclidine and ketamine in pigeons on behavior suppressed by brief electrical shocks. *Pharmacol Biochem Behav* 1980;12(6):865–70.
- [88] Brandão ML, Fontes JC, Graeff FG. Facilitatory effect of ketamine on punished behavior. *Pharmacol Biochem Behav* 1980;13(1):1–4.
- [89] Contreras PC, Quirion R, O'Donohue TL. Autoradiographic distribution of phencyclidine receptors in the rat brain using

- [3H]1-(1-(2-thienyl)cyclohexyl)piperidine ([3H]TCP). *Neurosci Lett* 1986;67(2):101–6.
- [90] Bennett DA, Amrick CL. 2-Amino-7-phosphonoheptanoic acid (AP7) produces discriminative stimuli and anticonflict effects similar to diazepam. *Life Sci* 1986;39(25):2455–61.
- [91] Stephens DN, Meldrum BS, Weidmann R, Schneider C, Grutzner M. Does the excitatory amino acid receptor antagonist 2-APH exhibit anxiolytic activity? *Psychopharmacology* 1986;90(2):166–9.
- [92] Plaznik A, Palejko W, Nazar M, Jessa M. Effects of antagonists at the NMDA receptor complex in two models of anxiety. *Eur Neuropsychopharmacol* 1994;4:503–12.
- [93] Dunn RW, Corbett R, Fielding S. Effects of 5-HT1A receptor agonists and NMDA receptor antagonists in the social interaction test and the elevated plus maze. *Eur J Pharmacol* 1989;169(1):1–10.
- [94] Kehne JH, McCloskey TC, Baron BM, Chi EM, Harrison BL, Whitten JP, Palfreyman MG. NMDA receptor complex antagonists have potential anxiolytic effects as measured with separation-induced ultrasonic vocalizations. *Eur J Pharmacol* 1991;193:283–92.
- [95] Anthony EW, Newins ME. Anxiolytic-like effects of *N*-methyl-D-aspartate-associated glycine receptor ligands in the rat potentiated startle test. *Eur J Pharmacol* 1993;250:317–24.
- [96] Corbett R, Dunn RRW. Effects of HA-966 on conflict, social interaction, and plus-maze behaviors. *Drug Develop Res* 1991;24:201–3.
- [97] Trullas R, Jackson B, Skolnick P. Anxiolytic properties of 1-amino-cyclopropanecarboxylic acid, a ligand at strychnine insensitive glycine receptors. *Pharmacol Biochem Behav* 1989;34:313–6.
- [98] Kehne JH, Baron BM, Harrison BL, McCloskey TC, Palfreyman MG, Poirrot M, Salituro FG, Siegel BW, Slone AL, Vangiersbergen PLM, White HS. MDL-100,458 and MDL-102,288: two potent and selective glycine receptor antagonists with different functional profiles. *Eur J Pharmacol* 1995;284:109–18.
- [99] Bandler R. Induction of rage following microinjections of glutamate into midbrain but not hypothalamus of cats. *Neurosci Lett* 1982;30(2):183–8.
- [100] Albin RL, Makowiec RL, Hollingsworth Z, Dure IV LS, Penney JB, Young AB. Excitatory amino acid binding sites in the periaqueductal gray of the rat. *Neurosci Lett* 1990;118:112–5.
- [101] Bandler R, Depaulis A. Midbrain periaqueductal gray control of defensive behavior in the cat and the rat. In: Depaulis A, Bandler R, editors. *The midbrain periaqueductal gray matter*, New York: Plenum Press, 1991. p. 175–98.
- [102] Krieger JE, Graeff FG. Defensive behavior and hypertension induced by glutamate in the midbrain central gray of the rat. *Brazilian J Med Biol Res* 1985;18:61–7.
- [103] McDougall A, Dampney R, Bandler R. Cardiovascular components of the defence reaction evoked by excitation of neuronal cell bodies in the midbrain periaqueductal grey of the cat. *Neurosci Lett* 1985;60(1):69–75.
- [104] Carobrez AP, Krieger JE, De Aguiar JC, Graeff FG. Antagonism by L-glutamic acid diethyl ester (GDDE) of the defense reaction caused by microinjection of glutamate into the dorsal periaqueductal gray-matter of the rat. *Brazilian J Med Biol Res* 1985;18(5–6):A656.
- [105] Haldeman S, Huffman RD, Marshall KC, McLennan H. The antagonism of the glutamate-induced and synaptic excitations of thalamic neurones. *Brain Res* 1972;39(2):419–25.
- [106] McLennan H, Lodge D. The antagonism of amino acid-induced excitation of spinal neurones in the cat. *Brain Res* 1979;169(1):83–90.
- [107] Graeff FG, Carobrez AP, Silveira MCL. Excitatory amino acids and the brain aversive system. In: Cavalheiro EA, Lehmann J, Turski L, editors. *Neurology and neurobiology*, Frontiers in excitatory amino acids research, vol. 46. New York: Liss, 1988. p. 325–32.
- [108] Beart PM, Nicolopoulos LS, West DC, Headley PM. An excitatory amino acid projection from ventromedial hypothalamus to periaqueductal gray in the rat: autoradiographic and electrophysiological evidence. *Neurosci Lett* 1988;85:205–11.
- [109] Lu CL, Shaikh MB, Siegel A. Role of NMDA receptors in hypothalamic facilitation of feline defensive rage elicited from the midbrain periaqueductal gray. *Brain Res* 1992;581(1):123–32.
- [110] Matheus MG, Guimaraes FS. Antagonism of non-NMDA receptors in the dorsal periaqueductal grey induces anxiolytic effect in the elevated plus maze. *Psychopharmacology* 1997;132(1):14–8.
- [111] Depaulis A, Bandler R, Vergnes M. Characterization of pretentorial periaqueductal gray matter neurons mediating intraspecific defensive behaviors in the rat by microinjections of kainic acid. *Brain Res* 1989;486(1):121–32.
- [112] Fendt M. Expression and conditioned inhibition of fear-potentiated startle after stimulation and blockade of AMPA/kainate and GABA(A) receptors in the dorsal periaqueductal gray. *Brain Res* 2000;880(1–2):1–10.
- [113] Walker DL, Cassella JV, Lee Y, De Lima TC, Davis M. Opposing roles of the amygdala and dorsolateral periaqueductal gray in fear-potentiated startle. *Neurosci Biobehav Rev* 1997;21(6):743–53.
- [114] Sudre EC, de Barros MR, Sudre GN, Schenberg LC. Thresholds of electrically induced defence reaction of the rat: short- and long-term adaptation mechanisms. *Behav Brain Res* 1993;58(1–2):141–54.
- [115] Molchanov ML, Guimaraes FS. Defense reaction induced by a metabotropic glutamate receptor agonist microinjected into the dorsal periaqueductal gray of rats. *Brazilian J Med Biol Res* 1999;32(12):1533–7.
- [116] Bittencourt AS, Carobrez AP, Schenberg LC. Intrinsic properties of lateral and dorsolateral columns of periaqueductal gray matter. *Soc Neurosci Abs* 2000;26:2257.
- [117] Batista-Da-Silva AP, Rae GA, Carobrez AP. Microinjection of D-2-amino-7-phosphonoheptanoate into the dorsal periaqueductal gray matter reduces the pressor response to glutamate injected at the same site. *Brazilian J Med Biol Res* 1990;23(8):705–8.
- [118] Guimaraes FS, Carobrez AP, De Aguiar JC, Graeff FG. Anxiolytic effect in the elevated plus-maze of the NMDA receptor antagonist AP7 microinjected into the dorsal periaqueductal grey. *Psychopharmacology* 1991;103(1):91–4.
- [119] Schubert K, Shaikh MB, Siegel A. NMDA receptors in the midbrain periaqueductal gray mediate hypothalamically evoked hissing behavior in the cat. *Brain Res* 1996;726(1–2):80–90.
- [120] Jacquet YF. The NMDA receptor: central role in pain inhibition in rat periaqueductal gray. *Eur J Pharmacol* 1988;154(3):271–6.
- [121] Siegfried B, de Souza RL. NMDA receptor blockade in the periaqueductal grey prevents stress-induced analgesia in attacked mice. *Eur J Pharmacol* 1989;168(2):239–42.
- [122] Blanchard DC, Blanchard RJ, Carobrez AP, Veniegas R, Rodgers RJ, Shepherd JK. MK-801 produces a reduction in anxiety-related antipredator defensiveness in male and female rats and a gender-dependent increase in locomotor behavior. *Psychopharmacology* 1992;108:352–62.
- [123] Schmitt ML, Graeff FG, Carobrez AP. Anxiolytic effect of kynurenic acid microinjected into the dorsal periaqueductal gray matter of rats placed in the elevated plus-maze test. *Brazilian J Med Biol Res* 1990;23(8):677–9.
- [124] Schmitt ML, Coelho W, Lopes-de-Souza AS, Guimarães FS, Carobrez AP. Anxiogenic-like effect of glycine and D-serine microinjected into dorsal periaqueductal gray matter of rats. *Neurosci Lett* 1995;189:93–6.
- [125] De-Souza MM, Schenberg LC, Carobrez AP. NMDA-coupled periaqueductal gray glycine receptors modulate anxiolytic drug effects on plus-maze performance. *Behav Brain Res* 1998;90:157–65.
- [126] Bandler R, Prineas S, McCulloch T. Further localization of midbrain neurones mediating the defence reaction in the cat by microinjections of excitatory amino acids. *Neurosci Lett* 1985;56(3):311–6.
- [127] Rizvi TA, Ennis M, Behbehani MM, Shipley MT. Connections between the central nucleus of the amygdala and the midbrain periaqueductal gray: topography and reciprocity. *J Comp Neurol* 1991;303(1):121–31.
- [128] Rizvi TA, Ennis M, Shipley MT. Reciprocal connections between

- the medial preoptic area and the midbrain periaqueductal gray in rat: a WGA-HRP and PHA-L study. *J Comp Neurol* 1992;315(1):1–15.
- [129] Chen S, Aston-Jones G. Extensive projections from the midbrain periaqueductal gray to the caudal ventrolateral medulla: a retrograde and anterograde tracing study in the rat. *Neuroscience* 1996;71(2):443–59.
- [130] Valverde-Navarro AA, Olucha FE, Garcia-Verdugo JM, Hernandez-Gil T, Ruiz-Torner A, Martinez-Soriano F. Distribution of basal-expressed c-fos-like immunoreactive cells of the periaqueductal grey matter of the rat. *Neuroreport* 1996;7(15–17):2749–52.
- [131] Lonstein JS, Stern JM. Somatosensory contributions to c-fos activation within the caudal periaqueductal gray of lactating rats: effects of perioral, rooting, and suckling stimuli from pups. *Horm Behav* 1997;32(3):155–66.
- [132] VanderHorst VG, Schasfoort FC, Meijer E, van Leeuwen FW, Holstege G. Estrogen receptor-alpha-immunoreactive neurons in the periaqueductal gray of the adult ovariectomized female rat. *Neurosci Lett* 1998;240(1):13–6.
- [133] Murphy AZ, Shupnik MA, Hoffman GE. Androgen and estrogen (alpha) receptor distribution in the periaqueductal gray of the male rat. *Horm Behav* 1999;36(2):98–108.
- [134] Teixeira KV, Carobrez AP. Effects of glycine or (+/–)-3-amino-1-hydroxy-2-pyrrolidone microinjections along the rostrocaudal axis of the dorsal periaqueductal gray matter on rats' performance in the elevated plus-maze task. *Behav Neurosci* 1999;113(1):196–203.
- [135] Priestley T, Laughton P, Myers J, Le Bourdelles B, Kerby J, Whiting PJ. Pharmacological properties of recombinant human *N*-methyl-D-aspartate receptors comprising NR1a/NR2A and NR1a/NR2B subunit assemblies expressed in permanently transfected mouse fibroblast cells. *Mol Pharmacol* 1995;48(5):841–8.
- [136] Buller AL, Monaghan DT. Pharmacological heterogeneity of NMDA receptors: characterization of NR1a/NR2D heteromers expressed in *Xenopus* oocytes. *Eur J Pharmacol* 1997;320(1):87–94.
- [137] Fletcher EJ, Lodge D. Glycine reverses antagonism of *N*-methyl-D-aspartate (NMDA) by 1-hydroxy-3-aminopyrrolidone-2 (HA-966) but not by D-2-amino-5-phosphonovalerate (D-AP5) on rat cortical slices. *Eur J Pharmacol* 1988;151(1):161–2.
- [138] Kemp JA, Foster AC, Leeson PD, Priestley T, Tridgett R, Iversen LL, Woodruff GN. 7-Chlorokynurenic acid is a selective antagonist at the glycine modulatory site of the *N*-methyl-D-aspartate receptor complex. *Proc Natl Acad Sci USA* 1988;85(17):6547–50.
- [139] Nahum-Levy R, Lipinski D, Shavit S, Benveniste M. Desensitization of NMDA receptor channels is modulated by glutamate agonists. *Biophys J* 2001;80:2152–66.
- [140] Kutsuwada T, Kashiwabuchi N, Mori H, Sakimura K, Kushiya E, Araki K, Meguro H, Masaki H, Kumanishi T, Arakawa M, Mishina M. Molecular diversity of the NMDA receptor channel. *Nature* 1992;358(6381):36–41.
- [141] Schell MJ, Brady Jr. RO, Molliver ME, Snyder SH. D-serine as a neuromodulator: regional and developmental localizations in rat brain glia resemble NMDA receptors. *J Neurosci* 1997;17(5):1604–15.
- [142] Bergeron R, Meyer TM, Coyle JT, Greene RW. Modulation of *N*-methyl-D-aspartate receptor function by glycine transport. *Proc Natl Acad Sci USA* 1998;95(26):15730–4.
- [143] Zafra F, Gomez J, Olivares L, Aragon C, Gimenez C. Regional distribution and developmental variation of the glycine transporters GLYT1 and GLYT2 in the rat CNS. *Eur J Neurosci* 1995;7(6):1342–52.
- [144] Supplisson S, Bergman C. Control of NMDA receptor activation by a glycine transporter co-expressed in *Xenopus* oocytes. *J Neurosci* 1997;17(12):4580–90.
- [145] Sanson LT, Carobrez AP. Long-lasting inhibitory avoidance acquisition in rats submitted to the elevated T-maze model of anxiety. *Behav Brain Res* 1999;101(1):59–64.
- [146] Rodgers RJ, Shepherd JK. Influence of prior maze experience on behaviour and response to diazepam in the elevated plus-maze and light/dark tests of anxiety in mice. *Psychopharmacology* 1993;113(2):237–42.
- [147] Deakin JFW, Graeff FG. 5-HT and mechanisms of defence. *J Psychopharmacol* 1991;5:305–15.
- [148] Jenck F, Moreau JL, Martin JR. Dorsal periaqueductal gray-induced aversion as a simulation of panic anxiety: elements of face and predictive validity. *Psychiat Res* 1995;57:181–91.
- [149] Vargas LC, Schenberg LC. Long-term effects of clomipramine and fluoxetine on dorsal periaqueductal grey-evoked innate defensive behaviours of the rat. *Psychopharmacology* 2001;155:260–8.
- [150] Wisden W, Seeburg PH, Monyer H. AMPA, kainate and NMDA ionotropic glutamate receptor expression—an in situ hybridization atlas. In: Ottersen OP, Storm-Mathisen J, editors. *Handbook of chemical neuroanatomy, Glutamate*, vol. 18. Amsterdam: Elsevier, 2000. p. 99–143.
- [151] Omori N, Ishimoto T, Mutoh F, Chiba S. Kindling of the midbrain periaqueductal gray in rats. *Brain Res* 2001;903:162–7.
- [152] Skolnick P. Antidepressants for the new millennium. *Eur J Pharmacol* 1999;375:31–40.
- [153] Petrie RXA, Reid IC, Stewart CA. The *N*-methyl-D-aspartate receptor, synaptic plasticity, and depressive disorder. A critical review. *Pharmacol Ther* 2000;87:11–25.
- [154] Popik P, Wróbel M, Nowak G. Chronic treatment with antidepressants affects glycine/NMDA receptor function: behavioral evidence. *Neuropharmacology* 2000;39:2278–87.
- [155] Herman BH, Vocci F, Bridge P. The effects of NMDA receptor antagonists and nitric oxide synthase inhibitors on opioid tolerance and withdrawal: medication development issues for opiate addiction. *Neuropsychopharmacology* 1995;13:269–93.
- [156] Morrow BA, Taylor JR, Roth RH. R-(+)-HA-966, an antagonist for glycine NMDA receptor prevents locomotor sensitization to repeated cocaine exposures. *Brain Res* 1995;673:165–9.
- [157] Hoffman PL. Glutamate receptors in alcohol withdrawal-induced neurotoxicity. *Metab Brain Dis* 1995;10:73–9.
- [158] Paxinos G, Watson C. *The rat brain in stereotaxic coordinates*. 4th ed. San Diego: Academic Press, 1998.
- [159] Winslow JT, Insel TR. Infant rat separation is a sensitive test for novel anxiolytics. *Prog Neuropsychopharmacol Biol Psychiat* 1991;15:745–57.
- [160] Corbett R, Dunn RW. Effects of 5,7-dichlorokynurenic acid on conflict, social interaction and plus maze behaviors. *Neuropharmacology* 1993;32:461–6.
- [161] Jurgens U, Lu CL. The effects of periaqueductally injected transmitter antagonists on forebrain-elicited vocalization in the squirrel monkey. *Eur J Neurosci* 1993;5(6):735–41.
- [162] Faiman CP, Viu E, Skolnick P, Trullas R. Differential effects of compounds that act at strychnine-insensitive glycine receptors in a punishment procedure. *J Pharmacol Exp Ther* 1994;270:528–33.
- [163] Przegalinski E, Tatarczynska E, Derenwesolek A, Chojnackawojcik E. Anticonflict effects of a competitive NMDA receptor antagonist and a partial agonist at strychnine-insensitive glycine receptors. *Pharmacol Biochem Behav* 1996;54:73–7.
- [164] Baron BW, Harrison BL, Kehne JH, Schmidt CJ, Vangiersbergen PLM, White HS, Siegel BW, Senyah Y, McCloskey TC, Fadaye GM, Taylor VL, Murawsky MK, Nyce P, Salituro FG. Pharmacological characterization of MDL-105,519, an NMDA receptor glycine site antagonist. *Eur J Pharmacol* 1997;323:181–92.
- [165] Moraes Ferreira VM, Morato GS. D-cycloserine blocks the effects of ethanol and HA-966 in rats tested in the elevated plus-maze. *Alcohol Clin Exp Res* 1997;21(9):1638–42.
- [166] Karcz-Kubicha M, Jessa M, Nazar M, Plaznik A, Hartmann S, Parsons CG, Danysz W. Anxiolytic activity of glycine-B antagonists and partial agonists: no relation to intrinsic activity in the patch clamp. *Neuropharmacology* 1997;36:1355–67.
- [167] Kotlinska J, Liljequist S. A characterization of anxiolytic like actions induced by the novel NMDA/glycine site antagonist, L-701,324. *Psychopharmacology* 1998;135:175–81.