



Review

The Periaqueductal Gray: From Longitudinal columns to defensive behaviour

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ABSTRACT

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Recent findings on the different functional properties of the neuroanatomical columnar subdivision of the Periaqueductal Gray (PAG) have provided a fundamental understanding for the pathophysiology of panic and anxiety disorder. In this review, we focus mainly on the prominent role of the PAG in defensive behaviour by combining both the behavioural and neuroanatomical data.

We have applied the theoretical model of the "two dimensional defence system" by McNaughton and Corr (2004), and thereby constituted an organizational structure on the neuronal circuitry of the different brain regions in relation with this panic- and fear-like behaviour. It has become clear that the dorsal and lateral PAG are involved in the active emotional coping (fight and flight reaction), whereas the ventrolateral PAG is responsible for the passive emotional coping (quiescence/freezing). In this regard, the PAG and other related brain structures are working in concert with different neurotransmitters providing animals with defensive strategies in response to predatory threats. The functional roles of the PAG in these behaviours as characterized in animals warrants further translational studies in humans which may eventually lead to novel approaches in anxiety- and panic-related disorders.

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1. Introduction

1.1 Background

Panic attack is characterized by an acute moment of intense fear and psychological distress with a sudden onset and rapidly building to a peak level. It is frequently followed by somatic and psychological symptoms associated with a sense of impending danger and an urge to escape from the susceptible risk condition (Griez and Schruers, 1998, Griez, et al., 2001). Besides, panic attack is regarded as the central pathological feature of panic disorder with physical symptoms such as shortness of breath, sweating, palpitations, trembling, feeling of choking, chest pain or discomfort, nausea or abdominal distress, feeling dizzy or unsteady, derealisation, fear of losing control, fear of dying, paresthesia, and chills or hot flushes (American-Psychiatric-Association, 2000). The percentage of lifetime prevalence of panic attack in the United States range 3-5.6% and 1.5-5% for panic disorder (American-Psychiatric-Association, 1998 May); and in Europe, the prevalence has been reported as 1.8% for panic disorder (Goodwin, et al., 2005). This disorder does not only cause functional disability but also affects the quality of life and related socio-economic conditions and interpersonal relationships (Abbar, 1996, Klerman, et al., 1991, Wittchen, 1988). To study the neurobiology underlying panic animal models have been developed in which animal display panic-like behaviour. A widely used and highly reproducible rodent model for panic and related behaviours is the electrical stimulation of the periaqueductal gray (PAG). This technique involves the implantation of an electrode to stimulate specific parts of the PAG. Electrical PAG stimulation evokes a typical escape behaviour, which is also referred as defensive behaviour or panic-like behaviour. This behaviour is characterized by fight, flight & freezing responses involving increased locomotor activity, galloping and jumps (Bandler, et al., 1985, Brandao, et al., 1999, Brandao, et al., 1982, Brandao, et al., 2003). The defensive behaviour has been proposed to reflect aversive responses since they are very similar to escape reactions induced by natural aversive stimuli, such as the exposure to a proximal predator (Blanchard, et al., 1988, Blanchard and Blanchard, 1989, Blanchard and Blanchard, 1989). Interestingly, two types of behaviour can be observed during electrical stimulation of the PAG. It has been found that sub-threshold stimulation resulted in freezing and stimulation at the threshold or above induced escape reaction. Following the post-stimulation period, the animals displayed highly immobile, indicative of fear response (Lim, et al., 2008, Vianna, et al., 2001).

In humans, stimulation of the PAG was originally applied for pain relief (Richardson and Akil, 1977, Richardson and Akil, 1977, Young, et al., 1985). Some studies also reported other findings after PAG stimulation. For example, in 1915 it was described by Brown that the PAG was a site for eliciting vocalization or "laughter" when stimulated with electrical current (Brown, 1915). Twenty years later, Kabat and co-workers demonstrated that PAG stimulation increased the respiratory rate and blood pressure (Kabat, et al., 1935). Many other studies have demonstrated that PAG stimulation produced intense emotional fear sensation, impending death with remarkable autonomic changes as characterized by the typical feature of panic attack (Amano, et al., 1978, Mobbs, et al., 2007, Nashold, et al., 1969). Recently, clinical reports have also shown that the reduction of pain and blood pressure were achieved by stimulating the rostral PAG and this is possibly mediated by a reduction in the central sympathetic system (Green, et al., 2007, Green, et al., 2006).

In the search for novel drugs and surgical therapies in panic disorders the PAG seems to be an interesting target.

In 1990 Depaulis and Bandler provided an overview of the anatomical and neurochemical properties of the PAG and its role in defensive behaviour. In this review, we have applied the theoretical model of the "two dimensional defence system" by McNaughton and Corr (McNaughton and Corr, 2004) and constituted an organizational structure for PAG regions in relation with this defensive behaviour. The two dimensional defensive behaviour is categorized into "defensive direction" and "defensive distance" based on the concept of Gray's "Neuropsychology of Anxiety" (Gray, 1982). The first dimension describes the two systems of defensive behaviour associated particularly with the PAG in controlling defensive avoidance (fear) and defensive approach (anxiety) (See Figure 1 and 2). The second dimension illustrates an important hierarchical neural organization of defensive behaviour with respect to the different levels of neuroanatomical structures.

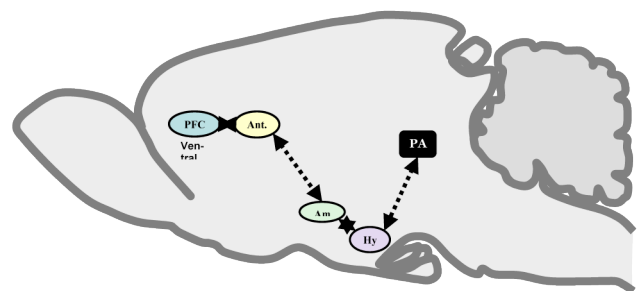


Fig. 1- Schematic illustration of the main upstream connections of the PAG based on the McNaughton and Corr's two-dimensional defence system of "defensive avoidance" for panic and fear behaviour (McNaughton and Corr, 2004). PFC= Prefrontal cortex (ventral stream), Ant. Cg= Anterior Cingulate, Amyg= nuclei of Amygdaloid complex, Hypo=Hypothalamus, and PAG=Periaqueductal Gray.

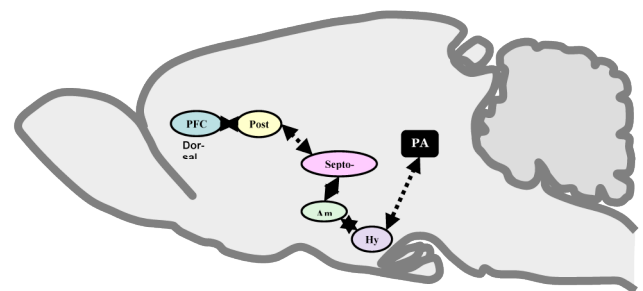


Fig. 2- Schematic illustration of the main upstream connections of the PAG based on the McNaughton and Corr's two dimensional defence system of "defensive approach" for anxiety related behaviour (McNaughton and Corr, 2004). PFC= Prefrontal cortex, Post. Cg= Posterior Cingulate, Amyg= nuclei of Amygdaloid complex, Septo-Hipp= Septo-Hippocampal system, Hypo= Hypothalamus, and PAG= Periaqueductal Gray.

Organisation of the review

We will first describe the intrinsic organization of the PAG including the specific cell types and the anatomical and functional separation into longitudinal columns, followed by a brief description of the regional anatomy in Section 2. In Section 3, the upstream and downstream connections of the PAG and its subdivisions will be discussed in detail. We will mainly focus on the functional and anatomical interconnections.

Subsequently, in Section 4 we will review the neurochemical properties of the PAG including their behaviour correlates. In Section 5, we will discuss the role of the PAG in defensive behaviour in the light of behavioural and neuroanatomical data. Finally, we will discuss the use of animal models in which the PAG is stimulated and how this research may contribute to a promising future in treating neuropsychiatric disorders.

2. Intrinsic organization of the PAG

2.1 Cell types

Examination at the cellular level shows that the PAG consists of diffuse collection of neurons and fibers surrounding the cerebral aqueduct and the reticular formation, which are both important relay centers for ascending and descending sensorimotor pathways to and from the spinal cord (Jordan, et al., 1992, Zieglansberger and Puil, 1973). The morphology of the population of the majority of the neurons in the PAG comprises three major cell types: vertical cells, stellate cells, and horizontal cells (Laemle, 1979). In general, the neurons of the PAG are all relatively small, ranging in size from 8 to 30 μm in diameter. Based on the Golgi-Cos study, these neurons can be divided into three different types, Class I (Ia & Ib), II and III cells (IIIa, IIIb, IIIc, and IIId), according to their size and cytological characteristic (Hamilton, 1973, Hamilton, 1973, Liu and Hamilton, 1980).

(a) Class I (Ia & Ib) neurons are approximately 18 μm in length and 8 μm in diameter. They are the smallest of the three types and have the shape of spindle bipolar neurons with one straight dendritic process. The nucleus is rather large, oval in shape and stains slightly lighter than the surrounding cytoplasm with the Nissl staining. The cells are generally darkly stained and have typically axons projecting from one end of the cell. The nucleus is located in the center and takes up the entire width of the cell. Nissl substances are uniformly distributed and are not found in clumps. Class I neurons are usually found in the medial regions of the PAG. The type Ia is smaller than the type Ib and they are found particularly in the medial PAG.

(b) Class II neurons are approximately 11 μm in length and 12 μm in diameter and are slightly larger with a fusiform or spherical shape. Triangular shaped neurons can also be found and have an apical dendrite that traverses a long distance within the PAG. The nucleus in this cell type is usually irregularly shaped. The axons of these cells are located in the central region, not at a pole as in class I neurons. Class II neurons generally have several dendrites. The Nissl substance is arranged in clumps which are evenly distributed in the cytoplasm. Class II neurons can be found in the area just dorsal to the cerebral aqueduct (the dorsal parts of the PAG) along with a large number of glial cells. (c) Class III (IIIa, IIIb, IIIc, and IIId) neurons are pleomorphic multipolar neurons, approximately 19 μm in length and 15 μm in diameter. They are the largest of the three types with a fusiform, spherical, or triangular shape and have a sparse amount of Nissl substance in their cytoplasm. In Golgi staining, type IIIa has a rhomboid-shaped soma and dichotomically branching dendrites, whereas type IIIb has a spheroidal soma and short axons. Type IIIc has a piriform soma and spiny dendrites, while the type IIId has the largest soma and structure resembles an undifferentiated motor neuron of the CNS. Due to the latter, these cells stain very lightly and usually only axons and dendrites can be identified. The nucleus is large in class III neurons, it is round, centrally located, and darkly staining. These cells (types IIIc and IIId) are found only in the lateral parts of the PAG along with a large number of glial cells. Axons of the types IIIa and IIId with strong branching characteristic are axonal projecting in

nature, whereas the other types are found mostly in the lateral and dorsal parts of the PAG.

2.2 Longitudinal columns

The most frequently used subdivision system of the PAG is the separation into four major longitudinal columns according to their cytoarchitectural features (Figs. 3a, 3b, 3c, 3d, and 3e; and Fig. 4). These subdivisions are cell-rich and serve as distinct anatomical modules for specific functions. They are generally classified as the dorsomedial- (dmPAG), dorsolateral- (dlPAG), lateral- (lPAG) and ventrolateral (vlPAG) PAG (Bandler and Shipley, 1994, Carrive and Bandler, 1991, Herrera, et al., 1987, Olszewki and Baxter, 1954). The functions of these major subdivisions are discussed later. In addition, the intermediate part of the lPAG (ilPAG), which is a minor subdivision, has been found to contain dense NOS-staining fibers without NOS-staining neurons. This region in particular is thought to play a role in the modulation of cardiovascular functions including systemic arterial blood pressure and heart rate (Wang, et al., 2001)

The dlPAG has a wedge-shaped form located most part in the rostral and intermediate two thirds region of the PAG. The dmPAG is located medially to the dorsolateral columns and is separated by an imaginary midline. The regions of lPAG and vlPAG columns are located in between the anterior boundaries of the dlPAG and the border of dorsal raphe nucleus (DRN). The boundary between the lateral and ventrolateral columns has been inferred from functional and connectional studies. There is no known specific histochemical marker that can be used to distinguish lPAG from vlPAG columns. However, a constant and useful identifying feature of the vlPAG is the presence of a number of prominent blood vessels (Bandler and Keay, 1996, Bandler and Shipley, 1994, Bandler and Tork, 1987, Clement, et al., 1996).

1.3 Regional anatomy of the PAG

The PAG is located inferiorly to the reticular formation and its inferior border is bounded by the tegmentum (back of the pons), the nucleus fastigius of the cerebellum and medulla's reticular area. Ventral to the PAG, close to the midline, is located the oculomotor nucleus (and associated Edinger-Westphal nucleus), and the medial longitudinal fasciculus. The medial lemniscus is located at anterior-laterally of the PAG and more lateral to the medial longitudinal fasciculus. Further ventrally lies the large red nucleus (nucleus ruber) and close to the red nucleus is the substantia nigra (SN) (Herrera, et al., 1987).

3. Functional connections of the PAG

The PAG receives input from and projects to a variety of higher and lower centers. The organization of the projections differs per subdivision. Most of the data presented here are derived from rodent and non-human primate studies. In general, the connections of the PAG share three different characteristics. Firstly, monosynaptic connections (e.g. from the vlPAG neurons to the locus coeruleus) (Bajic, et al., 2000). Secondly, these connections are implicated only in the mediation of somatic defensive behaviour such as flight, fight and freezing responses. The third characteristic is that the connections are unequally distributed between PAG subdivisions (Vianna and Brandao, 2003).

3.1 Upstream connections

The PAG is interconnected with several upstream brain regions. The majority of the fibers leaving the PAG usually terminate in the parabrachial nuclei, reticular formation, trigeminal motor nucleus and nucleus ambiguus. The main afferents are

from the amygdala, nucleus stria terminalis, hypothalamus, midline thalamus, periventricular gray, the dorsolateral and ventrolateral midbrain tegmentum (Jurgens and Pratt, 1979). In addition, several structures in the CNS receive dopaminergic inputs from the PAG, including the lateral bed nucleus of the stria terminalis, central amygdaloid nucleus (Hasue and Shammah-Lagnado, 2002), nucleus accumbens (Stratford and Wirtshafter, 1990), striatum (Descarries, et al., 1986), lateral habenula (Li, et al., 1993), hippocampus (Pohle, et al., 1984), magnocellular basal forebrain (Semba, et al., 1988), lateral septum and medial prefrontal cortex (Stratford and Wirtshafter, 1990). Different subdivisions are connected with different brain regions. These will be discussed in more detail in the next paragraphs.

3.1.1 Amygdala

The amygdala sends broad projections to the rostral midbrain including the PAG, the deep layers of the superior colliculus, and the lateral mesencephalic reticular formation (Fendt, et al., 1994, Meloni and Davis, 1999). It has been suggested that amygdala and the PAG, together with the medial hypothalamus, constitute an integrated circuitry in the brain that commands defensive behaviour and elaborates aversive emotional and motivational states (McNaughton and Corr, 2004). The amygdala's function is most likely to make distinction and response to the various stimulus inputs from the environment and then sending message to the PAG according to the degree of threat occurring within an organism. The PAG would be in charge of selecting, organizing and commanding the appropriate behaviour and neurovegetative defensive reactions (Fanselow, 1991).

Studies using electrical stimulation to evoke defensive behaviours in awake animals have led to the conclusion that a pathway originating in the amygdala, projecting to the hypothalamus and from there to the PAG is essential for the expression of these responses (Clemente and Chase, 1973, Fonberg, 1972, Kaada, 1967, Siegel and Edinger, 1981).

The central amygdaloid nucleus has an essential link in the pathway mediating the autonomic and behaviour symptoms of conditioned fear. It is possible to disrupt the conditioned fear responses by destroying areas to which the central amygdaloid nucleus project. Studies over the past years have provided detailed descriptions of the efferent targets of the central nucleus in a number of mammalian species (Cassell, et al., 1986, Price and Amaral, 1981, Saper, 1979, Schwaber, et al., 1982, van der Kooy, et al., 1984). It has been shown that lesions of the lateral hypothalamic area interfere with autonomic, but not behaviour conditioned responses; whereas lesions of the midbrain dorsal PAG region interfere with behaviour, but not autonomic conditioned responses. However, lesions of the amygdala in the fear-conditioning pathway disrupt both the behaviour and autonomic responses (Iwata, et al., 1986, LeDoux, et al., 1984). Therefore, it was suggested that the pathways mediating autonomic and conditioned fear responses diverge after the amygdala with projections to the lateral hypothalamic area and PAG.

Further evidence for an amygdala-PAG interaction is derived from studies in which experimental lesions of the rostral dorsal PAG, a target of the central amygdaloid nucleus, disrupted the conditioned freezing response, but did not affect the arterial pressure conditioned response (LeDoux, 1994). In contrast, neither the freezing nor arterial pressure responses were affected by ibotenic acid lesions of the rostral PAG. The observation that lesions of the rostral PAG disrupts behaviour and cardiovascular conditioned responses indicate that the projection from the forebrain mediating the behaviour

conditioned responses passes through, but does not synapse in, the rostral PAG. Nevertheless, injection of ibotenic acid into the caudal PAG region has significantly reduced the duration of conditioned freezing but did not affect the conditioned arterial pressure response. Thus, the intrinsic neurons in caudal PAG contribute to the conditioned freezing, but not to the conditioned arterial pressure response (LeDoux, 1994, LeDoux, et al., 1988).

The midbrain PAG region possesses locomotor functions and receives projections from various limbic forebrain areas, including the central amygdala, and projects to

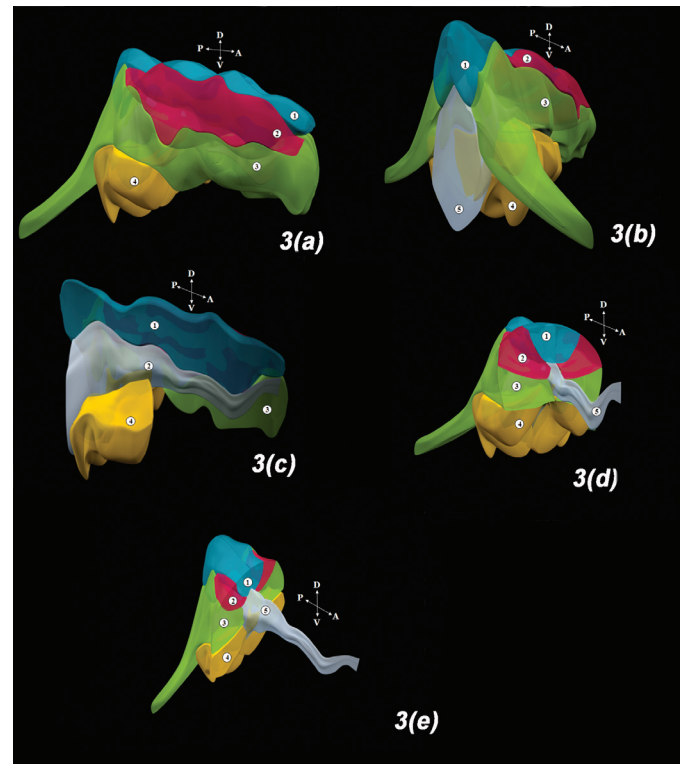


Fig. 3- A: lateral view, B: posterior-oblique view, C: internal sagittal view, D: coronal section view – rostral PAG, and E: coronal section view. These 3-D reconstructions of the subdivisions of the PAG longitudinal columns were created based on the 1998 George Paxinos and Charles Watson rat's Atlas 4th Edition (from Bregma -4.80mm till -8.80mm) using the Computer Aided Design (CAD) Environment software (Ontario, Canada) according to the anatomical and functional differentiation. Abbreviations: 1= dorso-medial PAG, 2= dorsolateral PAG, 3=lateral PAG, 4= ventrolateral PAG, and 5= aqueduct. A= anterior, P= posterior, D= dorsal, and V= ventral.

spinal motor neurons. PAG has been considered as an interface between the limbic forebrain and spinal cord in the expression of fear behaviour. Lesions of the PAG would disconnect spinal motor systems from the amygdala. Processes mediated by the amygdala would thus be deprived of access to final common pathways controlling emotional behaviour when neurons in the caudal PAG are destroyed (Swanson, et al., 1984).

Immunohistochemistry experiments have confirmed the chemical nature of PAG projections to the central amygdaloid nucleus. Robust projections from the PAG and DRN to the lateral bed nucleus of the stria terminalis, central amygdaloid nucleus have been documented with

retrograde (Rizvi, et al., 1991, Shammah-Lagnado, et al., 2001) and anterograde tracing techniques (Cameron, et al., 1995, Cameron, et al., 1995, Rizvi, et al., 1991, Vertes, 1991). Some of these projecting neurons were found to be cholecystikinin- (Seroogy and Fallon, 1989), serotonin (5-HT)- (Li, et al., 1990), vasoactive intestinal polypeptide- (Kozicz, et al., 1998, Petit, et al., 1995) and TH-immunoreactive (A10dc group). The latter mainly contributes to the dopaminergic innervation of the central amygdaloid nucleus. In addition, these small TH-immunoreactive periaqueductal neurons are frequently colocalised with cholecystikinin (Seroogy and Fallon, 1989). The amygdala and PAG receive their input mainly from the innervation of 5-HT-containing fibers originating from the DRN. The axons that project to the amygdala follow the DRN-forebrain tract, while those that go to the PAG run through the DRN-periventricular tract. Most of the nerve fibers that originate in the DRN are thin and have small varicosities that make preferential contact with 5-HT_{2A/2C} receptors (Azmitia and Segal, 1978, Mamounas, et al., 1991, McTavish and Heel, 1992).

The different parts of the midbrain are involved in mediating different measures of conditioned fear, namely fear-potentiated startle and freezing, both of which are dependent on the amygdala. The study by Zhao and co-workers showed that fear-potentiated startle in rats is mediated by neurons in the deep layers of the superior colliculus/deep mesencephalic nucleus of the rostral midbrain through the glutamate non-NMDA receptors whereas the dIPAG was responsible for freezing (Zhao and Davis, 2004). In another study, it was shown that microinjection of glutamate in the dorsal PAG area produced freezing behaviour (Krieger and Graeff, 1985) and microinjection of GABA receptor blockers or GABA inhibitors of glutamic acid decarboxylase (GAD) produced fear-like behaviour with a delay of action about 7 min (Brandao, et al., 1982, Brandao, et al., 1986, Schmitt, et al., 1985). Lesions of the ventral PAG disrupted the conditioned freezing response but did not change the dorsal PAG electrical stimulation or chemical stimulation-induced freezing and escape responses (Vianna, et al., 2001, Vianna, et al., 2001). These findings suggest that freezing and escape responses induced by dorsal PAG stimulation do not depend on the integrity of the ventral PAG.

3.1.2 Hypothalamus

The hypothalamic projections to the PAG can be divided into four subregions: anterior hypothalamus, the paraventricular nucleus, the posterior hypothalamus and the lateral hypothalamus (Fig. 5a, and 5b). Bandler and colleagues showed in 1982 & 1985 that electrical stimulation of the medial hypothalamus evoked defensive behaviour or "defensive rage" whereas stimulation with excitatory amino acid failed to evoke such behaviour (Bandler, 1982, Bandler and McCulloch, 1984). In the PAG, both electrical and chemical stimulation evokes defensive behaviour. Sun and Guyenet reported that both electrical and chemical stimulation of the lateral hypothalamus increased the systemic arterial pressure, increased lumbar sympathetic nerve discharge, and produced excitation in sympathetic premotor neurons in the rostral ventral lateral medulla (Sun and Guyenet, 1986). They also provided evidence that the descending pathway from the lateral hypothalamus was glutamatergic. This finding was contradicted by Hilton and Redfern as there was no sympathetic arousal as observed in the cardiovascular defensive response evoked by electrical or chemical stimulation of the hypothalamus (Hilton and Redfern, 1986). Furthermore, Hilton and associates demonstrated that sympathoexcitatory responses characteristic of the

defence reaction were readily evoked by both electrical and chemical PAG stimulation (Hilton and Redfern, 1986, Yardley and Hilton, 1986). Electrical stimulation in animals of the areas from which defensive behaviour is evoked produced a pattern of sympathetic excitation characteristically evoked by threatening stimuli. Thus, electrical stimulation of sites in the

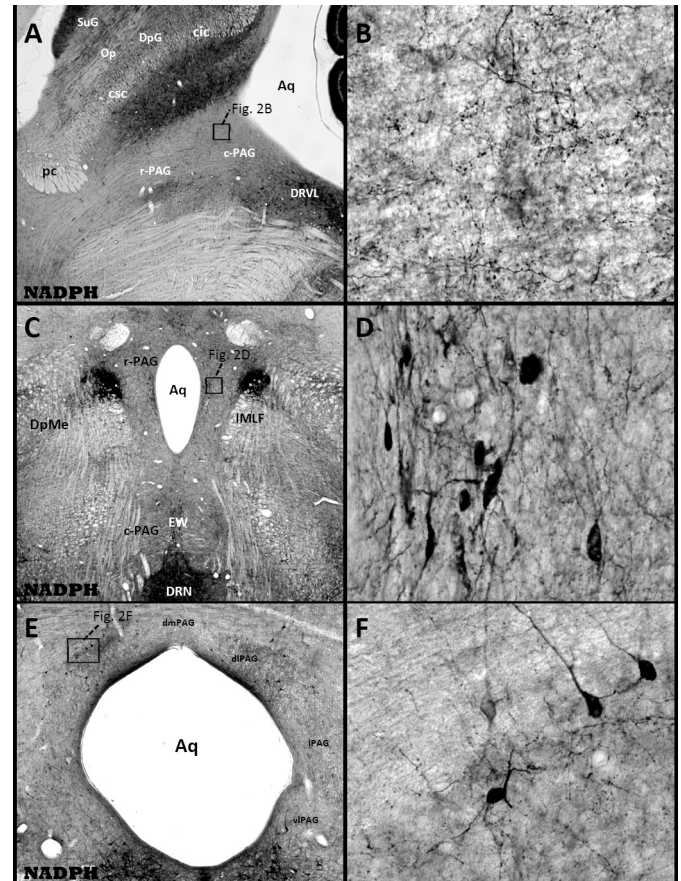


Fig. 4- Bright-field photomicrographs showing a low-magnification overview of the PAG in sagittal section (Fig. 4A), horizontal section (Fig. 4C), and coronal section (Fig. 4E), stained by NADPH-diaphorase histochemistry and visualized with DAB-immunohistochemistry in the normal rat brain. The detailed information of the boxed areas in A, C, and E are shown in 40X high-magnification photomicrographs of B, D, and F, respectively. Abbreviations: Aq= Aqueduct, cic= commissure of the inferior colliculus, csc= commissure of the superior colliculus, c-PAG= caudal periaqueductal gray, dIPAG= dorso-lateral periaqueductal gray, dmPAG= dorsomedial periaqueductal gray, DpG= deep gray layer of the superior colliculus, DpMe= deep mesencephalic nucleus, DRN= dorsal raphe nucleus, DRVL= dorsal raphe ventrolateral, EW= Edinger Westphal nucleus, IMLF= interstitial nucleus of the medial longitudinal fasciculus, IPAG= lateral periaqueductal gray, NADPH= nicotinamide adenine dinucleotide phosphate-oxidase, 5-HT= 5-hydroxytryptamine, Op= optic nerve layer of the superior colliculus, pc= posterior commissure, r-PAG= rostral periaqueductal gray, and SuG= superficial gray layer of the superior colliculus. vIPAG= ventrolateral periaqueductal gray.

amygdala, hypothalamus, and PAG evoke cardiovascular and defensive responses (Hilton, 1975, Hilton and Zbrozyna, 1963, Thornton, et al., 2002, Yang and Coote, 1998).

3.1.2.1 Anterior Hypothalamus

Neurons located in the medial area of the anterior hypothalamus project strongly to the vIPAG (Snowball, et al., 2000) as well as the dIPAG (Semenenko and Lumb, 1992), the dorsal and superior central raphe nuclei in the pontine tegmentum, to the nucleus raphe magnus (NRM), nucleus raphe pallidus (NRP), to the ventral part of the upper medullary medial tegmental field via the medial forebrain bundle, and the dorsal longitudinal fasciculus (Holstege, 1987). Preoptic regions are considered to be part of the anterior hypothalamus. The median preoptic neurons are activated by warmth exposure and project to the rostral PAG. These neurons influence thermoregulatory skin vasodilatation. It has been shown that activation of rostral PAG neurons produced skin vasodilatation, and activation of neurons slightly caudal to this region suppressed vasodilatation in response to local warming of the preoptic area (Zhang, et al., 1995). These findings also suggested that it is quite probable that the rostral PAG and caudal PAG play some role in thermoregulation during environmental thermal stimuli as well as during local preoptic warming.

3.1.2.2 Medial Hypothalamus

The medial hypothalamus sends axons to the dIPAG of the midbrain via the dorsal longitudinal fasciculus (Arnault and Roger, 1987). Electrical stimulation of this area produces affective aggression, and lesions of the PAG can subsequently disrupt this behaviour (Canteras, et al., 2001). Immunohistochemical analysis revealed the presence of 5-HT axons and preterminals throughout the PAG, and in particular, in its dorsolateral aspect which receives major inputs from the medial hypothalamus. This projection has been implicated in its defensive rage behaviour and is mediated by 5-HT_{1A} and 5-HT_{2/1C} receptors (Shaikh, et al., 1997).

3.1.2.3 Paraventricular Hypothalamic Nucleus

The paraventricular hypothalamic nucleus (PVH) projects to the caudal brainstem and spinal cord via the medial forebrain bundle, the mammillotegmental tract and the dorsal longitudinal fasciculus. The fibers continue through the lateral part of the mesencephalon and upper pons, along this way, some fibers project to the nucleus raphe magnus, rostral nucleus raphe pallidus and adjoining reticular formation and specific parts of the medullary lateral tegmental field (Holstege, 1987). The PVH contains a large number of neurotransmitter substances such as oxytocin, vasopressin, somatostatin, dopamine, met-enkephalin, leu-enkephalin, neurotensin, cholecystokinin, dynorphin, substance P, glucagon, renin and corticotrophin releasing factor (Swanson and Sawchenko, 1983). The latter provides a clear evidence of neuroactive substances involved in the PVH-caudal brainstem or spinal pathway (Hermes, et al., 1988). More importantly, the retrograde study of the transportation of the neuroanatomical tracer cholera toxin-b from both the autonomic and endocrine components of the PVH showed neuronal activation and projection to the visceromotor (infralimbic) cortex, median preoptic nucleus, ventromedial preoptic area, bed nucleus of the stria terminalis, parabrachial nucleus, vIPAG, ventrolateral medulla, and nucleus of the solitary tract (Elmqvist and Saper, 1996).

3.1.2.4 Lateral Hypothalamic area

Tracing and autoradiographic studies showed that the

lateral hypothalamus sends fibers to the vIPAG (Behbehani, et al., 1988) and IPAG (Bianchi, et al., 1998), the cuneiform nucleus, parabrachial nuclei, nucleus Kolliker-Fuse, nucleus subcoeruleus, the locus coeruleus, the caudal pontine and medullary medial reticular formation (Holstege, 1988). Anatomical studies using injection of anterograde tracer Phaseolus vulgaris leucoagglutinin into the lateral hypothalamus showed an extensive projection to the ventromedial and the vIPAG and a less dense projection to the medial and dorsal parts of this region (Behbehani, et al., 1988). There was a strong correlation between the response of PAG cells to electrical stimulation and injection of glutamic acid into the lateral hypothalamus and their response to pressure-injected neurotensin (Behbehani, 1995).

3.1.3 Thalamus

The topographic organization studies by Velayos & Reinoso clearly revealed the brainstem afferents of the mediodorsal (MD) thalamic nucleus (Velayos and Reinoso-Suarez, 1982). After injection of the retrograde tracer horseradish peroxidase (HRP) in the medial part of the MD thalamic nucleus, labelled neurons were observed at the level of the interpeduncular nucleus, ventral tegmental area and SN. Injections of HRP in the intermediate part of the mediodorsal nucleus revealed labelled cells in the interpeduncular nucleus, SN, dorsal and central superior raphe nuclei, dorsal tegmental nucleus and coeruleus complex. After injections in the lateral part of MD thalamic nucleus, labelled neurons were observed in the deep layers of the superior colliculus, lateral and vIPAG, oral paramedian pontine reticular tegmentum, interpeduncular nucleus, SN, locus coeruleus, dorsal tegmental nucleus, cuneiform area, and the mesencephalic reticular formation (Velayos and Reinoso-Suarez, 1982). Krout and Loewy observed that PAG projections to the thalamus are predominantly from the IPAG and vIPAG, innervating medial and intralaminar thalamic nuclei (Krout, et al., 2002, Krout and Loewy, 2000). In addition the anterograde and retrograde lectin tracing techniques showed that the rostral reticular thalamic nucleus received inputs not only from the cingulate, orbital and infralimbic cortices, but also from the vIPAG, mesencephalic reticular formation, laterodorsal tegmental nucleus, pedunculopontine nucleus, medial prepectum and ventral tegmental area (Cornwall, et al., 1990).

3.1.4 Raphe nuclei and reticular formation

The median raphe nucleus and the DRN are localized in the midbrain and contain cell bodies of 5-HT neurons that project to the forebrain and midbrain including the PAG (Azmitia and Segal, 1978). The connection of raphe nuclei with the PAG has been shown to play a role in the pathophysiology of generalized anxiety disorder and panic disorder (Abrams, et al., 2004, Chaouloff, 2000). In this respect, the activation of the DRN-periventricular 5-HT pathway, which also innervates the dorsal PAG, inhibited the fight or flight reactions in response to proximal danger, behaviour reactions that have been related to panic (Blanchard, et al., 2001, Blanchard, et al., 2003, Graeff, 2002, McNaughton and Gray, 2000). Furthermore, the neural connectivity of raphe nuclei and PAG has been investigated using the elevated T-maze, the inhibitory avoidance and one-way escape (Pobbe and Zangrossi, 2005). This study showed that, intra-DRN injection of the 5-HT_{1A} receptor antagonist WAY-100635 had consistently affected the performance of these two behaviours. On the other hand, the dorsal PAG local administration of WAY-100635 had by itself no effect, but blocked the effect of intra-DRN injection of WAY-100635 on one-way escape in the elevated T-maze. In contrast to WAY-100635,

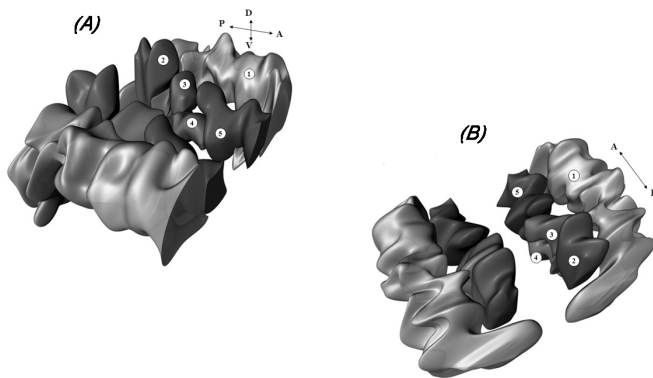


Fig. 5- A: Superior oblique view, from anterior to posterior direction, and B: Superior oblique view, from posterior to anterior direction. These 3-D reconstructions of the subdivision of the hypothalamus were created based on the 1998 George Paxinos and Charles Watson rat's Atlas 4th Edition (from Bregma -1.30mm till -4.80mm) using the Computer Aided Design (CAD) Environment software (Ontario, Canada). Abbreviations: 1=, Lateral hypothalamus, 2= Posterior hypothalamus, 3= Dorsomedial hypothalamic nucleus, 4= Ventromedial hypothalamic nucleus, and 5= Anterior hypothalamic nucleus. A=anterior, P=posterior, D=dorsal, and V=ventral.

intra-dorsal PAG injection of the 5-HT_{2A} receptor antagonist ketanserin blocked all behaviour effects of intra-DRN injection of WAY-100635 (Pobbe and Zangrossi, 2005). A retrograde labelling study from the nucleus raphe magnus and adjoining reticular formation showed considerable numbers of labelled neurons in the lateral PAG, vIPAG, dmPAG, except for the dIPAG (Mantyh, 1983, Mantyh, 1983). At the caudal pontine levels, the PAG fibers gradually shift ventrally and medially to terminate in the ventral part of the medullary medial reticular formation and in the nucleus raphe magnus (Holstege, 1988, Jurgens and Pratt, 1979). The caudal vIPAG and the ventrally adjoining mesencephalic tegmentum send fibers to the nucleus raphe pallidus. The neurons in the dmPAG projects strongly to the medially located nucleus raphe magnus while the lateral PAG and the laterally adjoining mesencephalic tegmentum project mainly laterally to the ventral part of the caudal pontine and upper medullary medial reticular formation with virtually no projections to the nucleus raphe magnus (Holstege, 1988).

3.1.5 Substantia nigra and ventral tegmental area

The connection between the SN/ventral tegmental area and the vIPAG has been identified as the pathway to modulate cardiovascular physiology and nociception (Kirouac and Pittman, 2000). This functional link has been demonstrated by stimulation studies of the SN/ventral tegmental area which produced significant cardiovascular responses and pain modulation that were mediated by a pathway to the vIPAG and DRN (Altier and Stewart, 1998, Kirouac and Pittman, 2000). However, there is no evidence at present that antinociception elicited by activation of neurons in the SN/ventral tegmental area is mediated by a projection to the vIPAG. A retrograde tracing study has clearly shown that the vIPAG receives substantial input from the SN/ventral tegmental area (Coffield, et al., 2004, Comoli, et al., 2003). This projection does not appear to use dopamine as neurotransmitter, but is thought to be GABAergic (Kirouac, et al., 2004).

3.1.6 Cortical connections

A number of cortical projections in the medial prefrontal areas such as the infralimbic, prelimbic, anterior cingulate, and precentral areas and lateral areas along the rhinal sulcus (the anterior and posterior insular areas, and the perirhinal areas) project axons that terminate focally within the rostrocaudal columns of the PAG (Bandler, et al., 1985, Illing and Graybiel, 1986, Neafsey, et al., 1986, Shipley, et al., 1991). The neural projections from the prefrontal cortical region to the PAG arise primarily from the medial prefrontal areas 25, 32, and 10m, anterior cingulate, and dorsomedial areas 24b and 9, selected orbital areas 14c, 13a, lai, 12o, and caudal 12l, and ventrolateral area 6v (An, et al., 1998). In addition, the caudal parts of the prefrontal cortex also project to the PAG. These projections come from the posterior cingulate cortex, the dorsal dysgranular, and granular parts of the temporal polar cortex, the ventral insula, and the dorsal bank of the superior temporal sulcus. The projections from the medial prefrontal areas 10m, 25, and 32 terminate predominantly in the bilateral dIPAG columns. Fibers from orbital areas 13a, lai, 12o, and caudal 12l terminate primarily in the vIPAG column, whereas fibers from dorsomedial areas 9 and 24b terminate mainly in the lateral column (An, et al., 1998).

Several studies have shown that the medial prefrontal network is subdivided into three main circuits (An, et al., 1998, Bernard and Bandler, 1998, Ongur, et al., 1998). These three subdivisions are based on the projections to the specific columns of the PAG. The first circuit originates from the orbital areas within the medial prefrontal network (areas 13a, lai, 12o and caudal 12l) and project selectively to the vIPAG and to the lateral hypothalamus. The second circuit runs from the medial wall areas (10m, 25, and 32) to the dIPAG and to the medial and lateral hypothalamus. The third circuit has its origin at the level of the dorsomedial areas 24b and 9 and project predominantly to the IPAG, with additional projections to the dIPAG, vIPAG and to the dorsal hypothalamus (An, et al., 1998, Bernard and Bandler, 1998, Ongur, et al., 1998).

In summary, (1) dorsal PAG: receives information from the caudodorsal part of the medial wall which includes anterior cingulate dorsal, anterior cingulate ventral, and the caudal parts of the prelimbic area and infralimbic area; (2) vIPAG: (a) projections from the rostroventral part of the medial wall specifically rostral portions of prelimbic and infralimbic areas, (b) from the adjacent medial orbital cortex which includes medial orbital, ventral orbital, and the medial part of ventrolateral orbital, and (c) from the lateral orbital and agranular insular cortical areas including dorsolateral orbital cortex, dorsal agranular insular cortex, and posterior agranular insular cortex; (3) IPAG: receives input from the rostral prelimbic cortex, caudal prelimbic cortex, anterior cingulate dorsal, dorsolateral orbital cortex, and dorsal agranular insular cortex; (4) dIPAG, vIPAG, dmPAG IPAG: all receive projections from the perirhinal cortex.

The medial prefrontal wall region is divided into the infralimbic, prelimbic, and medial frontal polar areas. The infralimbic area (IL) corresponds to Brodmann area 25. The prelimbic area (PL) corresponds to Brodmann area 32 and possesses a well-defined lamination. The frontal polar area (FP) occupies medial (FPm) and lateral (FPI) parts of the frontal pole. The dorsomedial convexity includes anterior cingulate and precentral areas. The anterior cingulate cortex, which corresponds to Brodmann area 24, can be distinguished from the prelimbic cortex by a broader and less densely packed layer V. The anterior cingulate cortex was further divided into dorsal (ACd) and ventral (ACv) divisions. The precentral

area is divided into precentral medial (PrCm) and precentral lateral (PrCl) areas. Although the whole of the orbital region is agranular, it can be divided into medial orbital (MO), ventral orbital (VO), ventrolateral orbital (VLO), lateral orbital (LO) and dorsolateral orbital (DLO) areas. Agranular insular cortex lies more caudally, superficial to the claustrum. It was subdivided into dorsal (Ald), ventral (Alv) and posterior (Alp) areas. Dysgranular (DI) and granular (GI) insular cortices were also distinguished (Krettek and Price, 1977, Krettek and Price, 1977, Ray and Price, 1992).

In the rat, primary motor areas of forelimbs, hind limbs and trunk project exclusively to the IPAG and vIPAG, while primary auditory and secondary visual cortex innervate the dIPAG. Perirhinal, anterior cingulate and agranular lateral retrosplenial cortex project to the dIPAG. The vIPAG receives projection from medial, ventral, dorsal and ventrolateral cortex, dorsolateral orbital and posterior insular agranular cortex. The prelimbic and infralimbic cortex are connected to rostroventral portion of vIPAG and dorsocaudal portion of dIPAG (Vianna and Brandao, 2003).

3.2 Downstream connections

3.2.1 Pons and medulla oblongata

The caudal PAG neurons send projections to the nucleus retroambiguus, a structure located in the lateral portion of the most caudal part of the medulla oblongata. This structure in turn projects to the motor neurons innervating the pharynx, soft palate, larynx, intercostals and abdominal muscles; which may account for the vocalization responses during lateral PAG stimulation in animals (Holstege, 1987, Jurgens and Ploog, 1970).

The PAG projections to the pontine lateral tegmentum or paralemniscal cell group is considered to mediate the antinociceptive action, which does not completely disappear after blocking the nucleus raphe magnus and adjacent reticular formation (Guimaraes and Prado, 1999). This finding corresponds with dorsal PAG stimulation induced analgesia after physiological blockage of the nucleus raphe magnus and adjacent reticular formation (Gebhart, et al., 1983, Guimaraes and Prado, 1999, Sandkuhler and Gebhart, 1984).

The rostroventrolateral medulla (RVLM) receives input from neurons from the raphe obscurus, raphe pallidus, raphe magnus, DRN, and also from the ventrolateral, lateral and ventral regions of the PAG, as shown by a double-labelling study with Cholera toxin subunit B (Bago, et al., 2002). Cholera toxin B positive neurons were located in all PAG regions and these cells were also 5-HT-positive. The majority of the 5-HT containing cells are located in the vIPAG, a few in the IPAG and ventral PAG, and none in the dorsolateral and dorsal PAG regions (Bago, et al., 2002). However, the attenuation of the cardiovascular components of a defence response evoked from the dorsal PAG, which involves 5-HT in the rostroventrolateral medulla neurons, could probably be initiated via a direct or indirect pathway from the vIPAG (Lovick, 1992). Furthermore, the sympathetic components of an active defence response are also mediated through the rostroventrolateral medulla during the activation of dorsal PAG neurons (Lovick, 1991). The hypotensive response evoked by the sympathoexcitatory region of rostroventrolateral medulla neurons has been shown to receive some projections from the neurons located in the vIPAG (Carrive and Bandler, 1991, Chen and Aston-Jones, 1995).

The vIPAG-rostroventrolateral medulla pathway is also involved in the mediation of the sympatho-inhibition during the response to severe haemorrhage. This sympatho-inhibitory response to haemorrhage depends upon the

integrity of neurons in the caudal midline raphe neurons, which may be activated during haemorrhage by input from the vIPAG (Henderson, et al., 1998). In addition, 5-HT projections to the rostroventrolateral medulla play a modulatory role at sympatho-excitatory neurons via the baroreceptor, and chemoreceptor (Miyawaki, et al., 2001).

3.2.2 Spinal Cord

Some neurons in the IPAG send fibers through the ipsilateral ventral funiculus of the cervical spinal cord to terminate in laminae VIII and the adjoining part of VII (Martin, et al., 1979). A few fibers descend ipsilaterally in the lateral funiculus to terminate in the T1-T2 intermediolateral cell column (Holstege, 1988). The neural projection to the medial part of intermediate zone in the cervical cord is involved in the head movement and not in nociception. In addition, the descending IPAG projections to the nucleus raphe pallidus, caudal pontine medial reticular formation continues to the axial muscle motoneurons in the spinal cord (Mouton, et al., 2005). This projection may be responsible for the medullary reticulospinal activation of axial muscle electromyogram (EMG) and lateral vestibulospinal activation of back muscle EMG in the rat after lateral PAG stimulation (Cottingham, et al., 1987, Cottingham and Pfaff, 1987).

4. Neurochemistry of the PAG

4.1 Monoamines and amino acids

4.1.1 Serotonin

In the PAG, as in the other structures of the CNS, 5-HT plays a major role in the regulation of anxiety and panic disorders (Abrams, et al., 2004, Chaouloff, 2000) (Deakin, 1991). This is based on the distribution of 5-HT-immunoreactive fibers and DRN projections to the substantia nigra, striatum, amygdala and frontal cortex (Imai, et al., 1986, Steinbusch, 1981, van der Kooy and Hattori, 1980). 5-HT-like immunoreactive cell bodies have been found in the ventrolateral and ventromedial regions of the caudal PAG and 5-HT-like immunoreactive processes throughout the PAG (Please see Fig. 6) (Clements, et al., 2004). The dorsal PAG also contains 5-HT_{1A} and 5-HT_{2A} receptors (Pobbe and Zangrossi, 2005). Moreover, the DRN has long been considered as a centre for coordination of behaviour activation and modulation of cardiovascular and respiratory activity. In line with this, stimulation of the PAG causes changes of behaviour, cardiovascular and respiratory function. These findings suggest that the effect of PAG stimulation on panic-anxiety, aversion and avoidance behaviour might involve the DRN. Furthermore, the PAG and DRN are interconnected (Fuxe, 1965, Lindvall, et al., 1974, Petrov, et al., 1992, Peyron, et al., 1996, Tanaka, et al., 1994).

Further studies have shown that the nucleus raphe magnus receives not only 5-HT input but also glutamatergic, cholinergic and peptidergic (NT, Substance P, Leu- and Met-enkephalin) from adjacent reticular nucleus cuneiformis and PAG (Basbaum and Fields, 1984, Behbehani and Zemlan, 1986, Richter and Behbehani, 1991). It is important to note that microinjection of the 5-HT₁ receptor agonist (5-methoxy-N,N-dimethyltryptamine; 5-MeODMT) into the dorsal PAG increased the escape threshold, whereas microinjection of 5-HT_{2A} receptor antagonists (metergoline or ketanserin) induced aversive reaction (Schutz, et al., 1985). Interestingly, Nogueira and Graeff showed that intra-PAG administration of the 5-HT_{1A} receptor agonists (8-hydroxy-2-di-n-propylaminotetralin hydrobromide; 8-OH-DPAT and BAY-R-1532) decreased the escape threshold during PAG stimulation (Nogueira and Graeff, 1995). On the other hand, Jenck et al. (1989), Beckett and Marsden (1997) showed the opposite effects with

systemic injection of 5-OH-DPAT (Beckett and Marsden, 1997, Jenck, et al., 1989) in comparison with other neurochemical substances.

4.1.2 Dopamine

Immunohistochemical staining for tyrosine-hydroxylase (TH) revealed that TH-positive neurons can be found throughout the IPAG and vIPAG (See Fig.7) (Han, et al., 2003). These cells are classified microscopically into two groups depending on their size and location, (i) large size cells: TH-positive neurons with 30–40 μ m in diameter, located in the lateral region of the PAG, with a multipolar morphology, and giving rise to abundant TH-positive fibers; and (ii) small size cells: rounded neurons of 10–15 μ m in diameter located adjacent to the aqueduct of Sylvius surface in the vIPAG. Numerous TH-positive fibers are scattered within the vIPAG, with regularly spaced varicosities (asterisks). Both types of cells are thought to be neurons because they have shown to express the NeuN neuronal marker (Flores, et al., 2004). It has been observed that the small TH-immunoreactive PAG neurons contain cholecystokinin (Seroogy, et al., 1989). Large dopaminergic neurons of the PAG appear to participate in the supraspinal modulation of opiate-induced analgesia, but spinal nociceptive pain reactions, as measured by the tail-immersion reaction, seem not to be affected after PAG dopaminergic neuron manipulation. The dopaminergic network of the PAG (See Fig. 8) has been included into the dorsocaudal A10 group as described by Hokfelt (A10dc group, 1984), and it is considered as a dorsal part of the dopaminergic periventricular system (Lindvall, et al., 1974, Moore, et al., 1978, Moore, 1978, Moore and Bloom, 1978)

Both D1 and D2 receptors are expressed by PAG neurons (Martin-Ruiz, et al., 2001) and colocalization of D1 and D2 receptors is rare in the central nervous system (Missale, et al., 1998). However, PAG neurons that are involved in the modulation of opiate-induced analgesia express D1 rather than D2 dopamine receptors. The neuronal pathways underlying these analgesic effects are largely unknown at present.

4.1.3 Noradrenaline

Noradrenaline (NA) belongs to the category of catecholamines and plays an important role in the central cardiovascular regulation. The PAG is densely innervated by catecholamine-containing terminals (Herbert and Saper, 1992) and NA is highly concentrated in the midbrain, including the PAG (Versteeg, et al., 1976, Versteeg, et al., 1976). A study based on the injection of anterograde tracer biotinylated dextran amine (BDA) into the vIPAG found labelled noradrenergic neurons in the dorsolateral and ventrolateral pontine tegmentum. The PAG contains a dense network of adrenergic and noradrenergic fibres which consists of ascending fibres from the caudal and rostral ventrolateral medulla to the ventral and dorsal parts of the PAG (Herbert and Saper, 1992). Microinjection of NA into the rostral, medial and caudal portions of the dorsal PAG evoked pressor responses. The pressor response is based on the mean arterial pressure and heart rate of the rats. The magnitude or degree of the pressor responses varied according to the location of the injection site along the dorsal PAG column. This response is usually accompanied by bradycardia and no gross behaviour change. The pressor response was significantly higher when NA was injected into the rostral portion of the dorsal PAG and decreased when injections were performed at the caudal parts (Pelosi and Correa, 2005). Injections of the NMDA receptor antagonist AP7 into the dorsal PAG induced anti-aversive effects in animal models of anxiety, such as in the elevated plus maze (Guimaraes, et

al., 1991) or the Vogel punished licking test (Molchanov and Guimaraes, 1999). In other studies, microinjection of NMDA into the PAG induced seizures followed by generalized clonic convulsions (N'Gouemo and Faingold, 1999, Peterson, et al., 2000, Raisinghani and Faingold, 2003). The stimulation of the hypothalamus elicits predatory attack in which the mean current strength of predatory attack can be diminished by microinjection of noradrenaline into the dorsal PAG. This indicates that hypothalamus-induced aggressive responses involve beta adrenoceptive mechanisms located in the dorsal PAG (Saha, et al., 2004).

4.1.4 Glutamate

The distribution of glutamate-like immunoreactive neurons has been observed in the PAG with the majority of the stained neurons being in the ventrolateral and dorsal PAG subdivisions. Aspartate-like immunoreactive neurons also exhibit a similar distribution pattern. These neurons are usually fusiform or triangular in shape and predominantly localized in the dorsolateral and ventrolateral subdivisions of the PAG. The numbers of aspartate-like immunoreactive neurons are documented less than the numbers of glutamate-like immunoreactive neurons (Clements, et al., 1987). Immunohistochemical staining methods have shown the presence and distribution of glutamate receptors in cell bodies located in the dorsal parts of the PAG. The glutamatergic system in the PAG has been associated with the expression of defensive rage responses (Beitz, 1989, Carobrez, 2003, Lima, et al., 2007).

The apparent convulsive behaviour was confirmed as seizure activity from EEG recordings (Peterson, et al., 2000). One of the glutamate-receptors that plays an important role in defensive responses is the kainate receptor. These receptors participate in excitatory neurotransmission by activating postsynaptic receptors and also bind to kainate and acts as a cation channel.

They also modulate the release of inhibitory neurotransmitter GABA through a presynaptic mechanism. There are five types of kainate receptor subunits GluR5, GluR6, GluR7, KA1 and KA2, which are similar to AMPA and NMDA receptor subunits. Kainate receptors play a role in both pre- and postsynaptic neurons (Huettnner, 2003). They have a limited distribution in the brain when compared to AMPA and NMDA receptors. In the PAG, prominent mRNA has been found of GluR-A, -B, and NR1. In adult rats, kainate triggers a full range of behaviour and autonomic defensive responses (Bandler and Depaulis, 1988). Administration of kainate receptor antagonist into the PAG induces anxiolytic-like effects in the elevated plus maze (Matheus and Guimaraes, 1997). The pretreatment with AMPA/kainate or NMDA glutamate receptor antagonists also attenuated the defensive reactions (flight reactions characterized by running and jumping) induced by an NO donor in the dIPAG (Moreira, et al., 2004). Injection of kainic acid into the DRN significantly increased the 5-HT release in the amygdala and the dorsal PAG (Adell, et al., 1991). In line with this, Viana and co-workers (Viana, et al., 1997) found that the 5-HT release in both structures enhanced inhibitory avoidance and impaired one-way escape in the elevated T-maze which is in accordance with Deakin & Graeff's suggestion that release of 5-HT in the amygdala enhances conditioned fear (inhibitory avoidance) while the release of 5-HT in the dorsal PAG decreases unconditioned fear (escape) (Deakin, 1991, Viana, et al., 1997).

Local infusion of the non-NMDA, AMPA/kainate glutamate receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(F)-quinoxaline (NBQX) into the dorsal/

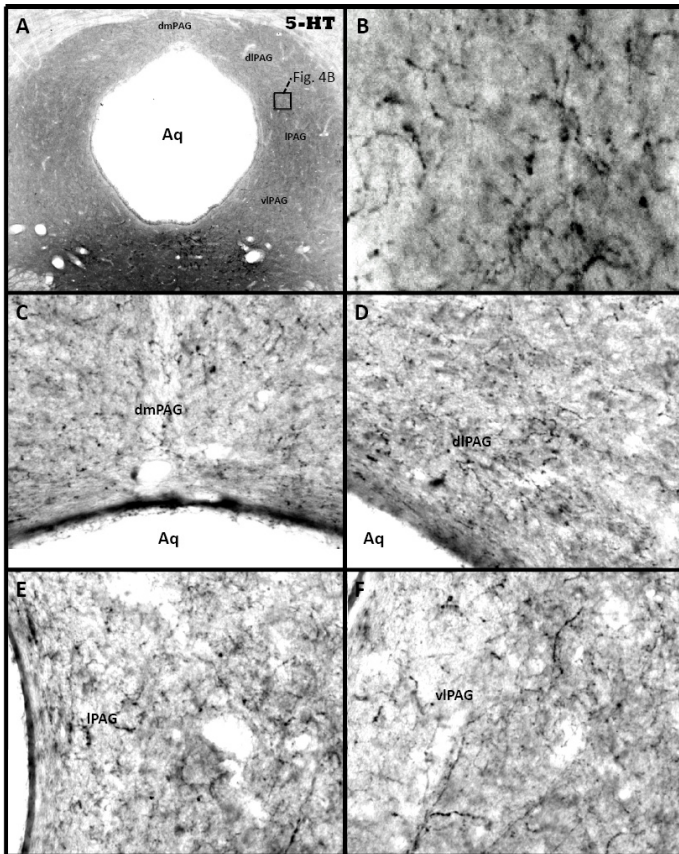


Fig. 6- Bright-field photomicrographs showing a coronal section (Fig. 6A) of a normal rat brain processed for serotonin (5-HT) immunohistochemical staining to illustrate the widespread, diffuse innervation pattern of serotonergic fibers throughout the entire PAG region. Boxed area in Fig. 6A is shown in a 100X high-magnification photomicrograph in Fig. 6B. Illustrative images of 5-HT immunoreactivity in the dmPAG (Fig. 6C), dlPAG (Fig. 6D), IPAG (Fig. 6E), and vlPAG (Fig. 6F) areas are shown with 40X high-magnification.

lateral PAG, the lateral mesencephalic reticular formation, or the superficial superior colliculus did not affect the fear-potentiated startle. But the NBQX tended to reduce contextual freezing when infused into the dorsal/lateral PAG. However, the same dose of NBQX infused into the deep layers of the superior colliculus/deep mesencephalic nucleus (deep SC/DpMe) blocked fear potentiated startle. This suggests that the deep SC/DpMe, but not the ventral PAG, is critical for fear-potentiated startle but not freezing; whereas the PAG, but not the deep SC/DpMe, is critical for freezing (Zhao and Davis, 2004).

4.1.5 GABA

Gamma-amino butyric acid (GABA) is the most ubiquitous inhibitory transmitter with two major subtype receptors, GABA-A and GABA-B receptors. The GABA-A receptor is an ionotropic receptor that gates a chloride channel and GABA-B receptor is metabotropic, activating a second messenger cascade that activates a potassium channel. Immunohistochemical studies have shown that GABAergic neurons are widely distributed throughout the whole brain and are the major source of synaptic inhibition in the central nervous system. The precursor for GABA is glutamate and the

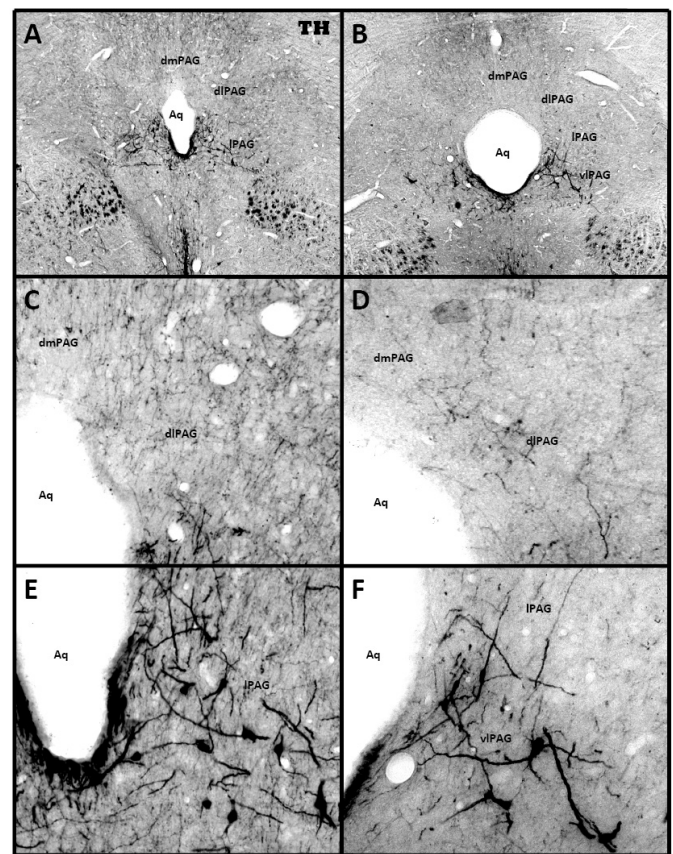


Fig. 7- Bright-field photomicrographs showing a coronal section of the rostral (Fig. 7A) and caudal (Fig. 7B) PAG regions containing tyrosine hydroxylase (TH) visualized with immunohistochemistry to illustrate the specific localization of TH neurons in the IPAG and vlPAG regions. Illustrative images of the rostral and caudal regions are shown with 20X magnification photomicro-graph in C and E; and D and F.

key synthesizing enzyme is glutamic acid decarboxylase (GAD) which is a relatively good marker for GABAergic neurons (Bear, et al., 2001, Brodal, 2004, Kandel, et al., 1995).

The GABA immunoreactive neurons are sparsely scattered throughout the PAG, but high numbers are present particularly in the dorsal and ventrolateral subdivisions (Borelli, et al., 2005, Vianna, et al., 2003). The post-embedding double-immunogold electron microscopic method of labeling provides better visualization of the subcellular morphological structures and permits quantification of the GABA and enkephalin neurotransmitters. This method has shown that there are 247 vesicle-containing axon terminals and 165 dendrites which are classified as GABA in the vlPAG (Renno, et al., 1999). The enkephalin- and/or GABA-labelled terminals in this PAG area contain densely packed round or pleomorphic, small, agranular vesicles and form mainly symmetrical pre- and postsynaptic density contacts with enkephalin-labelled dendrites (Renno, et al., 1999). An autoradiography study has shown that both GABA-A and GABA-B receptors exist in the PAG which indicates that many of the GABA-responsive PAG neurons have both subtypes of GABA receptors (Bowerly, et al., 1987). With regard to this, the next finding by Reichling and associates in 1990, the short intrinsic circuit in the PAG, identified the GABAergic presynaptic fibers in the dlPAG (Reichling and Basbaum, 1990, Tredici, et al., 1983).

Injection of GABA into the midbrain PAG activates medullary neurons that are involved in pain inhibition

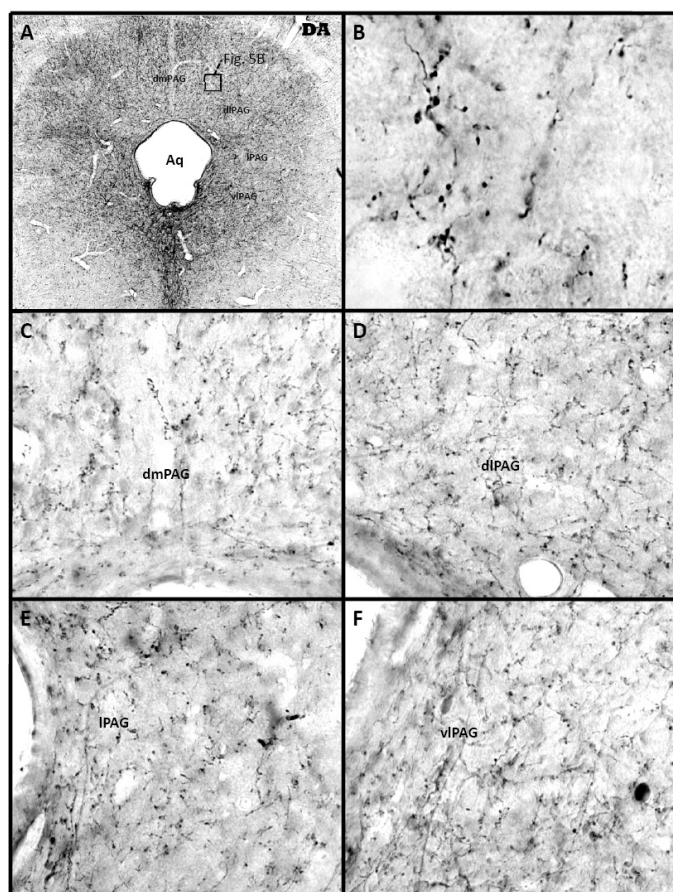


Fig. 8- Bright-field photomicrographs showing a coronal section (Fig. 8A) of a normal rat brain processed for dopamine (DA) immunohistochemical staining to illustrate the widespread, diffuse innervation pattern of dopaminergic fibers throughout the entire PAG region. Boxed area in Fig. 8A is shown in a 100X high-magnification photomicrograph in Fig. 8B. Illustrative images of DA immunoreactivity in the dmPAG (Fig. 8A), dlPAG (Fig. 8B), IPAG (Fig. 8C), and VIPAG (Fig. 8D) areas are shown with 40X high-magnification.

and potentiate morphine-induced analgesia (Levy and Proudfit, 1979, Moreau and Fields, 1986, Morgan, et al., 2003). GABAergic mechanisms in the PAG modulate the descending pain inhibitory system (Behbehani, et al., 1990). Electrophysiological studies have suggested that activation of μ -opioid receptors inhibits GABA neurons, thereby allowing the off-cells of the PAG to activate neurons in the nucleus raphe magnus, ultimately producing analgesia (Fields, et al., 1991). This suggests that GABAergic neurons are involved in the modulation of antinociception effects of opiates at the level of PAG.

4.2 Neuropeptides

4.2.1 Substance P

Substance P is a short-chain neuropeptide that functions as a neurotransmitter. It belongs to the tachykinin neuropeptide family. In the central nervous system, substance P has been associated in the regulation of mood disorders, anxiety, stress, reinforcement, neurogenesis, respiratory rhythm, neurotoxicity, nausea/emesis and pain (Harrison and Geppetti, 2001). Substance P is particularly concentrated in the amygdala and other regions that regulate affective behaviour

and neurochemical responses to stress. The PAG also contains substance P-positive, medium-sized fibers with multiple varicosities (Kramer, et al., 1998, Maubach, et al., 1999, Renoldi and Invernizzi, 2006).

4.2.2 Cholecystokinin

Cholecystokinin (CCK)-immunoreactive fibers are ranged from small to medium size with regular varicosities and its receptors are widely distributed within the gut and the brain (Moran, et al., 1986, Zarbin, et al., 1983). The immunohistochemical studies have shown that the CCK-like immunoreactive fibers and terminals are present throughout the rostrocaudal levels of the PAG and are more heavily distributed in the caudal two-thirds area. It was also demonstrated that a higher concentration of CCK immunoreactivity fibers and terminals was found in the dlPAG as compared with the dmPAG (Liu, et al., 1994).

Recently, systemic administration of CCK₂ receptor agonist has been shown to trigger panic attacks in human (Bourin, et al., 1991, Bradwejn and Koszycki, 2001). This effect seems to involve the dPAG since systemic administration of the latter drug induced Fos immunoreactivity in this area (Singewald and Sharp, 2000). Further evidence for the involvement of CCK in panic is that intra-dPAG or systemic injection of CCK-8s induced escape or panic-like reaction in rats (Zanoveli, et al., 2004). This finding was also consistent with previous reports that intra-dIPAG injection with CCK₂ receptor agonist CCK-4 facilitated escape behaviour when the animals were tested in the elevated T-maze (Bertoglio, et al., 2006, Bertoglio and Zangrossi, 2005) and in the bowl-shape cage (Bertoglio, et al., 2007). On the other hand, the treatment with CCK₂ receptor antagonist LY225910 had significantly inhibited the escape reaction in the elevated T-maze, indicative of panicolytic-like action (Bertoglio and Zangrossi, 2005). In view of these behavioural and immunohistochemical studies, among other factors, they support strongly that the regulatory site for panic-related behaviour is probably located in the dlPAG.

4.2.3. Enkephalin

In 1979, Gramsch and co-workers detected β -endorphin and met-enkephalin in the PAG (Gramsch, et al., 1979). Several investigations showed that enkephalin neurons especially in the vlPAG play a role in pain modulation (Budai and Fields, 1998, Williams, et al., 1995). Enkephalinergic terminals synapse on GABA containing dendrites in the PAG.

The enkephalinergic system in the PAG is also involved in the modulation of hypothalamus-induced predatory attack behaviour (Siegel, et al., 1997). The predatory attack was elicited by electrical stimulation of lateral hypothalamus in the rat and it was completely suppressed by injections of delta-alanine methionine enkephaline in the dorsal PAG (Bhatia, et al., 1997). The injection of delta-alanine methionine enkephaline into the dPAG also completely suppressed the somatomotor components of predatory attack behaviour (Manchanda, et al., 1995). The administration of naloxone facilitated the hypothalamus-induced attack behaviour and cancelled the inhibitory effect of delta-alanine methionine enkephaline (Bhatia, et al., 1997, Brutus and Siegel, 1989, Manchanda, et al., 1995, Weiner, et al., 1991).

4.2.4 Opioid receptors

Opioid peptides are derived from proenkephalin A, and are found in very high levels in the PAG (Pittius, et al., 1984). Opioid peptides act on their receptors. These are (Pfeiffer, et al., 1982, Pfeiffer and Herz, 1982, Pfeiffer, et al., 1982). Opiates typically exert inhibitory effects on neurons in the central nervous system except for PAG neurons (Gent and Wolstencroft, 1976).

Opioid effects in the PAG are mimicked by glutamate receptor agonists including NMDA and electrical stimulation (Jacquet and Squires, 1988, Jensen and Yaksh, 1989) and all drive PAG output. Numbers of neurons expressing c-Fos immunoreactivity after opioid withdrawal in both awake and anesthetized rats were elevated in the lateral and ventrolateral subdivisions of the PAG, predominantly in the caudal areas of the vlPAG (Chieng, et al., 1995).

Stimulation of opioid receptors within the PAG activates the descending inhibitory pathways and suppresses nociception (Bellgowan and Helmstetter, 1998, Rossi, et al., 1994). In addition, vlPAG stimulation in opioid-naive animals with excitatory amino acids produces quiescence, hyporeactivity, hypotension, bradycardia, and opioid-mediated analgesia (Keay, et al., 1997). Many vlPAG neurons that descend to the rostral ventromedial medulla, the excitation of which is thought to be crucial for analgesia, have immunolabelling for μ -opioid receptors (Commons, et al., 2000). The PAG also mediates several behaviour reactions such as freezing and defensive immobility, which are partly under opioid control (Fanselow, et al., 1991). The μ -opioid receptor immunoreactivity is present in both subpopulations of GABAergic vlPAG neurons and vlPAG neurons that project to the rostral ventromedial medulla (Commons, et al., 2000).

The κ -opioid receptor was previously known for its ability to cause strange psychoactive effects which have been called 'hallucinogenic'. Pharmaceutical companies looking for novel analgesics investigated kappa-opioid agonists and discovered that they caused unwanted side effects in humans which made them non-applicable as pain medications. The kappa opioid agonist, enadoline significantly increased measures of sedation, confusion and dizziness, produced visual distortions and feelings of depersonalization, and increased urinary output. The highest dose (160 μ g/70 kg) was not tolerated and led to psychotomimetic effects (Walsh, et al., 2001). When rats are given the kappa opioid receptor agonist U50,488 during the first 3 post-natal weeks, they exhibited an increase in ultrasonic vocalization production and in contrast with adult rats showed no behaviour activation (Carden, et al., 1994, Kehoe and Boylan, 1994). Kappa opioid receptors are also expressed in the PAG as early as the first postnatal week (Kitchen, et al., 1990).

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5. Discussion

5.1 The defence circuit of the PAG

According to the two-dimensional neuropsychology of defence proposed by McNaughton & Corr and in agreement with previous evidence, PAG and its connections control the alterations of circulation, respiration, pain perception, behaviour and automatic movements in response to threatening or novel stimuli (McNaughton and Corr, 2004). As for the PAG efferents, the majority of the fibers leaving the PAG usually terminate in the parabrachial nuclei, reticular formation, trigeminal motor nucleus and nucleus ambiguus. The PAG also receives afferents from the following structures such as the amygdala, nucleus stria terminalis, hypothalamus, thalamus, periventricular gray, the dorsolateral and ventrolateral midbrain tegmentum (Jurgens and Pratt, 1979). Besides, several structures in the CNS receive dopaminergic (TH-ir) inputs from the DR/PAG, including the lateral bed nucleus of the stria terminalis, central amygdaloid nucleus (Hasue and Shammah-Lagnado, 2002), accumbens (Stratford and Wirtshafter, 1990), caudate-putamen (Descarries, et al., 1986), lateral habenula (Li, et al., 1993), hippocampus (Pohle, et al., 1984), magnocellular basal forebrain (Semba, et al., 1988), lateral septum and medial prefrontal cortex (Stratford and Wirtshafter, 1990, Yoshida, et al., 1989).

The medial hypothalamic area, including the anterior hypothalamus, dorsomedial part of the ventromedial hypothalamus (VMHdm), and dorsal preammillary nucleus (PMd), which together form a defensive system (Canteras, 2002, Canteras, et al., 2001), shows a strong activation to conditioned fear (e.g. cat odor, as well as a live cat) (Canteras, et al., 1997, Dielenberg, et al., 2001, Dielenberg, et al., 2001, Dielenberg and McGregor, 2001). The bed nucleus of the stria terminalis, ventral part of the lateral septum, and PAG are also activated during cat odor exposure. Keay & Bandler illustrated the axons of medial prefrontal cortex densely targeted the ventromedial hypothalamic nucleus and anterior hypothalamic area and terminated within the dlPAG (Canteras, 2002, Vianna and Brandao, 2003). On the other hand, the orbital and anterior insular prefrontal cortex areas selectively targeted the lateral hypothalamus and projected only to the vlPAG. As for the IPAG, the dorsomedial prefrontal cortex convexity and anterior cingulate cortex (area 24) firstly project its fiber to the dorsal hypothalamic areas and finally terminated in the IPAG column.

It is interesting to note that the functional significance

of the parallel projection from the prefrontal cortex to the different parts of hypothalamus finally terminate in specific columns of the PAG. Recent data have shown that the amygdala and the PAG, together with the medial hypothalamus, constitute an integrated circuitry in the brain that commands defensive behaviour and elaborates aversive emotional and motivational states. The function of the amygdala would be to synthesize the various stimulus inputs from the environment and then signal to the PAG according to the degree of threat represented to the organism (LeDoux, 1994, LeDoux, et al., 1988, McNaughton and Corr, 2004).

A series of retrograde studies revealed that the projections to the PAG arise predominantly from the medial prefrontal cortex wall and a few selected orbital/anterior insular prefrontal cortex regions (Floyd, et al., 2000, Reep and Winans, 1982). In addition, injections of anterograde tracers into each PAG-projecting prefrontal region revealed distinct columnar patterns in the PAG. The medial prefrontal cortex projects densely to the dIPAG; whereas vIPAG received an exclusive robust input from the orbital and anterior insular areas (12o, 12l, 13a, 14c, lai) and weaker input from the medial and dorsomedial prefrontal areas (Floyd, et al., 2000, Floyd, et al., 2001, Jasmin, et al., 2004). In addition, the dorsomedial prefrontal cortex (areas 9 & 24) was found to project robustly to the IPAG column, which indicates that different types of defensive behaviour are resulting from different columnar organization of the PAG (Gabbott, 2003, Gabbott, et al., 2003, Jasmin, et al., 2004, Yasui, et al., 1991).

In line with McNaughton and Corr's defensive system, Keay and Bandler (2001) divided emotional coping strategies of different types of stress into active and passive strategies. The active coping is regarded as the activation of either the dorsolateral or lateral PAG columns; whereas passive emotional coping strategy is triggered by activation of the ventromedial PAG column. This behaviour was observed during the microinjection of excitatory amino acid into the rostral dIPAG or IPAG which evoked a confrontational defensive reaction, while microinjection at the caudal dIPAG or IPAG evoked a response of escape or flight reaction (Keay and Bandler, 2001). In addition, hypertension and tachycardia were noticed during the rostral dIPAG or IPAG (confrontational defence system) stimulation with decreased blood flow to skeletal muscle and viscera, but increased extracranial flow. Meanwhile, stimulation of the caudal dIPAG and IPAG evoked only the hypertension which was accompanied by increased blood flow to the skeletal muscle but decreased blood circulation in the visceral and oral facial region. In contrast to the stimulation of dIPAG or IPAG, microinjection of excitatory amino acid into the vIPAG produced a passive reaction of quiescence/immobility, decreased vigilance and hyporeactivity with hypotension and bradycardia, not responding to any external environmental stimuli. The similar effect of hypotensive responses without significant heart rate changes was also observed during the stimulation in the dmPAG (See Table 2) (Pajolla and de Aguiar Correa, 2004, Pajolla, et al., 2005).

5.2 Summary

In this review, we have outlined the general neuroanatomy and a neurochemical property of the PAG. The PAG has been considered a key structure to coordinate behaviour in response to threatening stimuli and pain regulation. It has been demonstrated that stimulation of this area in rats initiate different defensive behaviours such as fight, flight and freeze in response to aversive external and internal stimuli. Besides, it also eliminates the perception of pain by stimulation which is called stimulation produced analgesia. Several physiological changes have been observed in the alteration of blood circulation, respiration, pain

perception, autonomic movement and postural adjustment with complex behaviour during and after the stimulation or lesions of the PAG. In addition, the PAG also modulates several biological functions such as reproductive or sexual behaviour, maternal behaviour, vocalization, anxiety, cardiovascular, and respiratory activities. The finding that the PAG is composed of different anatomical columns has contributed to the understanding of its functional differentiation. It consists of several columnar groups of neurons, each differing with regard to their connections. More specifically, it has become clear that the dorsal and lateral PAG are involved in the active emotional coping (fight and flight reaction), whereas the ventrolateral PAG is responsible for the passive emotional coping (quiescence/freezing). Several behaviours are coordinated through these columns by means of efferent connections to the reticular formation, raphe nuclei, cuneiform nucleus, and thalamus. The main afferent connections consist predominantly of projections from the sensory cortex, prefrontal cortex, cingulate cortex, motor cortex, insular cortex via the hypothalamus, amygdala, dorsal and median raphe nuclei. Such connectivity of different neuroanatomical structures presents a picture of neural systems in controlling the fundamental basis for defensive reactions. In view of these, the neuropsychology of defence also requires different interaction of neurochemical properties in concert with their morphological structures to achieve the optimal condition for defensive functions.

5.3 Perspectives

The history of PAG investigation dated almost a century old (See Table 2) and the future research should focus on the neurobiology aspect of its pathogenesis using the state-of-art technology of electrophysiological investigation. The precise relationship between the experimentally induced panic and naturally occurring panic attack share many similar clinical features in humans. However, the exact relationship of the underlying pathogenesis between these two remains largely unknown. Many studies in which the effects of the PAG stimulation have been investigated in combination with psychopharmacological challenge showed that the PAG is involved in panic or anxiety-like behaviour. Thus, electrical stimulation of the PAG in animals would be a promising future direction in the aspect of neuropsychiatric disorders for developing potential panicolytic and anxiolytic drugs. Besides, the anatomical structure and functional roles of the PAG in the animal brain have been studied extensively, whereas the human PAG is still poorly understood. It will be essential to conduct some fundamental investigations on the translational studies from the experimental rodent findings into human subjects.

Further, electrical stimulation of the PAG has been shown to produce analgesic effect and lowering the blood pressure. These findings suggest a possible mechanism in the therapeutic approach to modify the pathological states of neuronal condition as well as their firing patterns. Such potential approach in normalizing the mechanism of dysfunctional neurons and its connectivity may possibly contribute to future neuropsychiatry surgery in terms of normalizing its neuronal firing patterns. Though, many hypotheses have been proposed to explain on how deep brain stimulation exerts its therapeutic effects, but still there is no well-established theories underlying its working mechanism (Lozano and Eltahawy, 2004, Montgomery and Gale, 2007). More importantly, addressing the application of these electrophysiological modulation techniques and a careful assessment during the intervention is required to explore these possible future therapies.

Table 1- Definition of different types of defensive behaviour associated with periaqueductal gray stimulation either by electricity or chemical compound.

Terms	Definition
Affective defense behavior	It is characterized by an aggressive response based on the presence of elements of either fear and/or threat, which may be real or perceived to be a threat stimulus as defined in both human and animal studies. In rodents, the behavioural response includes a flattening of the ears, a shrinking or lowering of the body, drawing in of the head, piloerection, hissing, and pupillary dilatation.
Conditioned fear	Behavioural inhibition with neurovegetative changes such as raised fur, defecation, urination, and teeth clattering which is associated with previous experience of aversive stimulus.
Defecation	Discharge of feces during emotional distress or normal physiological function.
Defensive behavior	Behavioural and neuropsychological reaction in response to fear or anxiety condition with respect to avoidable and unavoidable threat stimuli. It is commonly categorized into flight, fight, and freezing response in the laboratory animal studies.
Defensive rage	It is a form of aggressive behavior that characterized by remarkable sympathetic activation with behavioural signs such as arching of the back, pupillary dilatation, piloerection, retraction of the ears, growling, hissing, and paw striking at a moving object.
Exophthalmus	Eyeball protrusion and wide opened eyelids (contraction of orbital and tarsal sympathetic muscles, respectively). The eyes take on a spherical shape and brilliant appearance suggestive of an increased entrance of light.
Flight	It is a general model of predation defense behaviour to avoid or flee the animals from the attack of predator. In rats, the behaviour comprises the running (trotting or galloping) and/or jumping responses.
Fight	It is a form of aggressive behaviour to defend the animals against any attack from predators that the prey may fight back ferociously. Sometimes, it can be defending for a territory, food, shelter and mates.
Freezing	Most prey animals will avoid fights with predators, in general they will display a posture of tense immobility accompanied by exophthalmus, mystacioplegia and, quite often, defecation and micturition, accompanied by hypertension, hypervigilance and increased autonomic function.
Gallop	Fast running alternating stance and swing movements of anterior and posterior limb pairs.
Grooming	Self-directed behaviors consisting of the repetitive manipulation of the fur of the head and trunk as well as the manipulation and/or licking of the genitals. The rat displays a 'sitting' posture (upright posture on flexed hind limbs).
Immobile behaviour	The animals remain on a location usually with four paws on the ground.
Jumping	Upward leaps directed to the border of the open arena.
Micturition	Discharge of urine during aversive response or normal physiological function.
Mystacioplegia	Paralysis of ongoing vibrissae movements.
Panic-like symptoms	Palpitation, blushing of face and neck and respiratory arrest or hyperventilation, feelings of terror or impending death, and desire to flee. The behavior mimics defensive and escape behavior.
Predatory attack	Aggressive behaviour specifically triggered by the presence of a prey object within the visual field of the predator that consists of a purposeful and goal-directed attack with absence of sympathetic arousal.
Rearing	Upright posture with extended hind limbs and forelegs lean against the wall or an object.
Resting	Quiescent horizontal posture with open or half-open eyes, reduced sniffing activity and muscle relaxation as suggested by the flexion of the limbs and lowering of trunk and tail. The head may be raised and the rat displays a 'sphinx' like posture.
Running	Increased locomotor activity with rapid movement of fore- & hind-limb toward or away from aversive stimuli.
Sleeping	Quiescent horizontal posture with closed eyes, no sniffing activity and overall muscle relaxation as suggested by the flexion of the limbs and lowering of the trunk, head, neck & tail.
Tense immobility	Overall behavioural arrest accompanied by an increased muscle tonus as suggested by the extension of the neck and/or limbs and raising of head, trunk and/or tail. Except for the visible tachypnoea, the rat looks like a 'statue' for periods lasting from 10 s to the end of the stimulus (60 s).
Trotting	Running keeping the same pattern of walking
Walking	Typical rat locomotion showing out phase stance and swing movements of the contralateral limbs.

Table 2- The table presents the major milestones in the history of PAG research. The following dates and events were gathered chronologically from several sources based on the scientific published data dated since 1915.

1915	Brown made the first observation of the central gray in phonation. He stimulated the cut surface of the transected brain stem of rostral PAG and obtained a sound 'resembling laughter'.
1935	Kabat et al. demonstrated that central gray stimulation increases the respiratory rate and blood pressure.
1937	Magoun et al. demonstrated that stimulation of rostral PAG yielded species-specific calls.
1944	Bailey and Davis described the central gray has been implicated in rage and fear reactions.
1954	Olszewski and Baxter subdivided the human PAG into a region adjacent to the mesencephalic aqueduct termed the subnucleus medialis, lateralis and dorsalis.
1954	Delgado et al. stimulated the dorsal PAG with observation of laboratory animals easily learned to switch off the experimental electrical stimulation.
1956	Hunsperger conducted electrical stimulation of the dorsal PAG in laboratory animals which induced defensive reactions, such as vigorous flight or defensive aggression.
1958	Nauta's limbic system-midbrain circuit proposed that limbic system has robust connections with mesencephalic structures such as PAG & tegmentum.
1963	Skultety demonstrated that many autonomic reactions can be elicited by stimulation or lesions of the central gray.
1969	Reynolds discovered that electrical stimulation of the periaqueductal gray induce profound analgesia.
1969	Nashold et al. demonstrated that during stimulation of human central gray produces feeling of intense fear.
1972	Wise et al. elaborated the theoretical model on serotonin with anxiety and expected the facilitation of serotonin in the escape behaviour.
1972	Kiser et al. (1972, 1975, 1978, 1980) showed the different ways of increasing 5-HT activity in the dorsal PAG had an anti-aversive effect, whereas 5-HT depletion resulted in the facilitation of escape from dorsal PAG electrical stimulation.
1973	Nobin and Bjorklund were the first to describe scattered neurons displaying catecholamine fluorescence lying ventrolateral to the aqueduct in the human fetal midbrain. Serotonin-containing cells were first identified in the human ventrolateral and ventral central gray using the Falck-Hillarp histochemical technique.
1973	Hamilton conducted a study on the cytoarchitecture of the PAG subdivisions.
1975	Kiser and Lebovitz demonstrated that the stimulation of the dorsal central gray induces aversive behaviour in animals which might serve as animal model for anxiety.
1977	Jurgens and Muller-Preuss emphasized the importance of direct descending limbic system input to the primate central gray.
1977	Hosobuchi et al., Richardson and Akil showed preliminary results on stimulation of the PAG was effective in reducing intractable pain.
1978	Schenberg & Graeff demonstrated that Benzodiazepines diminishes the aversive behaviour induced by central gray stimulation.
1979	Laemle described the vertical and horizontal cell in central gray, stellate cells with 4 randomly oriented primary dendrites and reported the neuronal axons arise from soma ascend and descend in the central gray.
1979	Holstege mapped the central gray cells projecting to the spinal cord, raphe magnus/ pallidus and adjacent reticular formation of the cat.
1979	Gramsch et al. detected moderate amount of both β -endorphin & met-enkephalin in human central gray.
1979	Sakuma and Pfaff described the central gray has been implicated in sexual behaviour.
1979	Rose demonstrated electrophysiologically the presence of neurons in the primate central gray which respond to genital, rectal, innocuous somatosensory and various forms of noxious stimulation.
1980	Kesner and Calder described the central gray has been implicated in the memory storage.
1981	Hardy and Leichnetz demonstrated that the prefrontal cortex of the monkey projects predominantly to the lateral dorsal subdivision of the central gray.
1981	Morato de Carvalho et al. carried out an experiment using electrical stimulation of the dorsal PAG as a punishing stimulus and showed that the neural substrate of dorsal PAG delivered punishment seems to be different from that of peripherally applied punishment as compared with Graeff 1974 electrical foot shock punishment.

1982	Pfeiffer et al. identified the localization of μ -, δ -, and κ -opiate receptor binding sites in the human PAG and possess the excitatory effects in the periaqueductal gray which play a critical role in the opiate activation of the descending pain modulatory systems as opioid-induced analgesia
1982	Mantyh investigated the hypothalamus with greatest descending input to the primate central gray, described the fusiform neuron in the monkey central gray, multipolar neurons and stellate cells.
1982	Brandao et al. concluded that the GABAergic terminals tonically inhibit the neurons of the dorsal PAG that control defensive behavior and serotonergic fibers seem to exert a phasic inhibition of aversion.
1983	Palacios et al. concluded that serotonin receptors are present in the human central gray.
1983	Pearson et al. described tyrosine hydroxylase immunoreactive fibers in the ventral and ventrolateral PAG.
1984	Cortes et al. identified the human central gray contains a high density of muscarinic receptors especially in the dorsolateral subdivision.
1985	Kirzinger and Jurgens suggested that stimulation of caudolateral involved in vocal motor control.
1986	Takahashi et al. confirmed the distribution of serotonergic neurons.
1987	Pazos et al. identified the serotonin-1 & serotonin-2 receptors are present in human central gray.
1987	Clements et al. identified the distribution of glutamate-like immunoreactive neurons in ventrolateral and dorsal subdivisions of central gray.
1988	Beitz et al. provided the first detailed analysis of the interaction of central gray descending projections with bulbospinal neurons in the rat.
1989	Jenck et al. reported that 5-HT1A-receptor agonist enhanced escape from DPAG electrical stimulation.
1990	Graeff et al. concluded that the amygdala, the medial hypothalamus and the PAG constitute a set of interrelated structures, brain aversive system (BAS) that includes the dorsal PAG (Graeff 1981) act together to produce response suppression, emotional and motivational states.
1994	Bandler & Shipley described the columnar organization in the midbrain periaqueductal gray with 4 subdivisions as the dorsomedial, dorsolateral, lateral and ventrolateral PAG.
1995	Nogueira and Graeff explored the roles of serotonin receptors subtypes 5-HT1A and 5-HT2A/2C in the regulation of aversion in the dorsal PAG.
1996	Reddy et al. identified the neurotransmitter receptors: nicotinic, muscarinic, serotonergic, opioid, and kainate receptors in the middle gestational period of fetal development in the human PAG.
1999	Renno et al. confirmed the colocalization of GABA and enkephalin in the ventrocaudal periaqueductal gray.
1999	Brandao et al. regarded the dPAG contained fear neural substrates, with previous experimental evidence (see Brandao et al., 1982).
2000	Kirouac & Pittman showed a projection from the ventral tegmental area to the periaqueductal gray involved in cardiovascular regulation.
2004	McNaughton and Corr present a two-dimensional neuropsychology of defense: fear/anxiety and defensive distance
2004	Zhao & Davis confirmed that fear-potentiated startle is mediated by neurons in the deep layers of the superior colliculus/ deep mesencephalic nucleus whereas the dorsal/lateral PAG responsible for freezing.
2005	Yoshida et al. concluded that the rostral PAG receives warm signals from the median preoptic nucleus and the caudal PAG receives cold signals from the dorsomedial hypothalamus/dorsal hypothalamic area.
2006	Maekawa et al. confirmed that the ventromedial hypothalamus are connected to the PAG.

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