sciendo

222 ENDOCRINE REGULATIONS, Vol. 52, No. 4, 222–238, 2018

doi:10.2478/enr-2018-0027

Periaqueductal gray and emotions: the complexity of the problem and the light at the end of the tunnel, the magnetic resonance imaging

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The periaqueductal gray (PAG) is less referred in relationship with emotions than other parts of the brain (e.g. cortex, thalamus, amygdala), most probably because of the difficulty to reach and manipulate this small and deeply lying structure. After defining how to evaluate emotions, we have reviewed the literature and summarized data of the PAG contribution to the feeling of emotions focusing on the behavioral and neurochemical considerations. In humans, emotions can be characterized by three main domains: the physiological changes, the communicative expressions, and the subjective experiences. In animals, the physiological changes can mainly be studied. Indeed, early studies have considered the PAG as an important center of the emotions-related autonomic and motoric processes. However, in vivo imaging have changed our view by highlighting the PAG as a significant player in emotions-related cognitive processes. The PAG lies on the crossroad of networks important in the regulation of emotions and therefore it should not be neglected. In vivo imaging represents a good tool for studying this structure in living organism and may reveal new information about its role beyond its importance in the neurovegetative regulation.

Key words: PAG, fear, pain, in vivo, magnetic resonance imaging

The periaqueductal gray (PAG) is a small deeply located mesencephalic brain structure, surrounding the aqueduct of Sylvius (Figure 1). It is located at the crossroads of different neural circuits regulating the autonomic functions. It is also involved in different behaviors such as social (O'Connell and Hofmann 2011), maternal (Noriuchi et al. 2008), aggressive (Gregg and Siegel 2001), and sexual ones (Holstege and Huynh 2011). PAG roles have been studied mainly by observing the neurovegetative responses to its activation or inhibition, showing its involvement in vocalizations (Jurgens 1994), micturition (Takasaki et al. 2010; Stone et al. 2011), modulation of the respiratory (Farmer et al. 2014; Faull et al. 2015), and cardiac functions (Xavier et al. 2014). These latter responses are often measured to evaluate emotions in animals (Menant et al. 2016a), serving as the first evidence for the role of the PAG in the regulation of emotions (Bandler and Shipley 1994; Bandler et al. 2000). However, the question arose whether the PAG – beside its well-known role in the neural circuit of pain – may also contribute to the emotional component of the pain (Price 2002). The use of in vivo Magnetic Resonance Imaging (MRI) methods have widened our view (Linnman and Borsook 2013) on the PAG complex involvement besides the emotional motoric response also in the emotional-cognitive processes (Mobbs et al. 2007; Wager et al. 2009; Buhle et al. 2013).

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Before examining the emotions-related literature of the PAG, we will first define what the emotions are.

From the definition of emotions toward its neuronal network

Throughout the literature, several definitions and theoretical concepts have been debated and proposed for emotions that have been described by numerous terms (Bindra 1970; Leventhal and Scherer 1987; Leventhal and Patrick-Miller 2000). Some of them have used the term emotional *qualia* (subjective experiences), based on the human perception and feeling (Russell and Mahrabian 1977; McNaughton 1989). Defined as a reaction to a particular event, similar emotional expression has been reported throughout species and described it as an evolutionary adaptive process (Darwin 1872). Some consider that primary and secondary emotions can be distinguished. ***Primary emotions***, which are fear, joy, disgust, sadness, surprise, and anger, are innate and occur in the whole animal kingdom (Leventhal and Scherer 1987). ***Secondary emotions***, which need "a level of complex, conscious reasoning" are learned and appear specifically in humans (Leventhal and Scherer 1987). Emotions can be described with their several components, which are bound and correlated with each other and specific to the emotional *qualia* (Dantzer 2002a; Mauss and Robinson 2009). The three main components used to define the emotions in humans include physiological changes, communicative expressions and subjective experiences, requiring cognitive processes (Figure 2) (Dantzer 2002a).

Several theories have been proposed to describe the emotional processes (from perception to emotions), which differed by the level of the cognitive process integration (Figure 3). According to the ***James-Lange theory***, after perceiving a stimulus that somehow affects the person, disturbing physiological changes such as palpitations, shortness of breath, anxiety, etc. (also called arousal) occur (Cannon 1927). The acknowledgment of these symptoms at cortical level determines an emotional *qualia* (Schachter and Singer 1962). Thus, recognition of physical changes are the emotions. On the contrary, according to the ***Cannon-Bard theory*** (also known as thalamic theory of emotions) (Cannon 1927), the triggering event induces a nerve impulse, which goes directly to the thalamus, where the message divides. One part goes to cortex leading to subjective feelings, while the other part goes to the hypothalamus and triggers neurovegetative physical responses. Thus, interpretation of emotions at cortical level and bodily changes occur simultaneously (Schachter and Singer 1962). The two factors theory of emotions (also known as ***Schachter-Singer theory*** or appraisal theory) emphasize the importance of the cognitive process (Schachter and Singer 1962). More precisely, all the

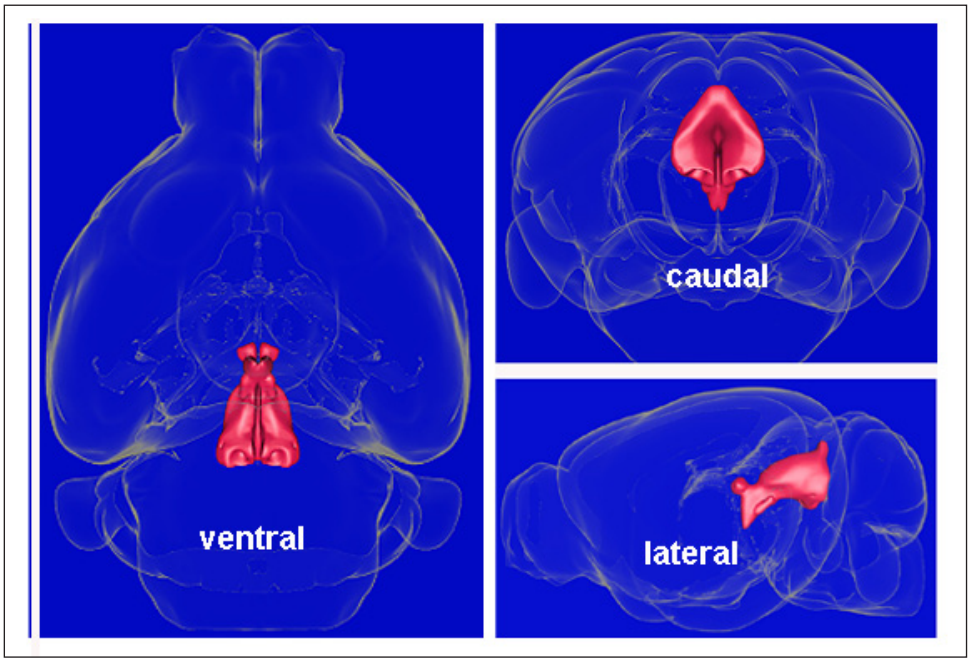

Figure 1. 3D presentations of the periaqueductal gray (PAG) in the mouse brain (red) generated with the help of https://scalablebrainatlas.incf.org/composer/.

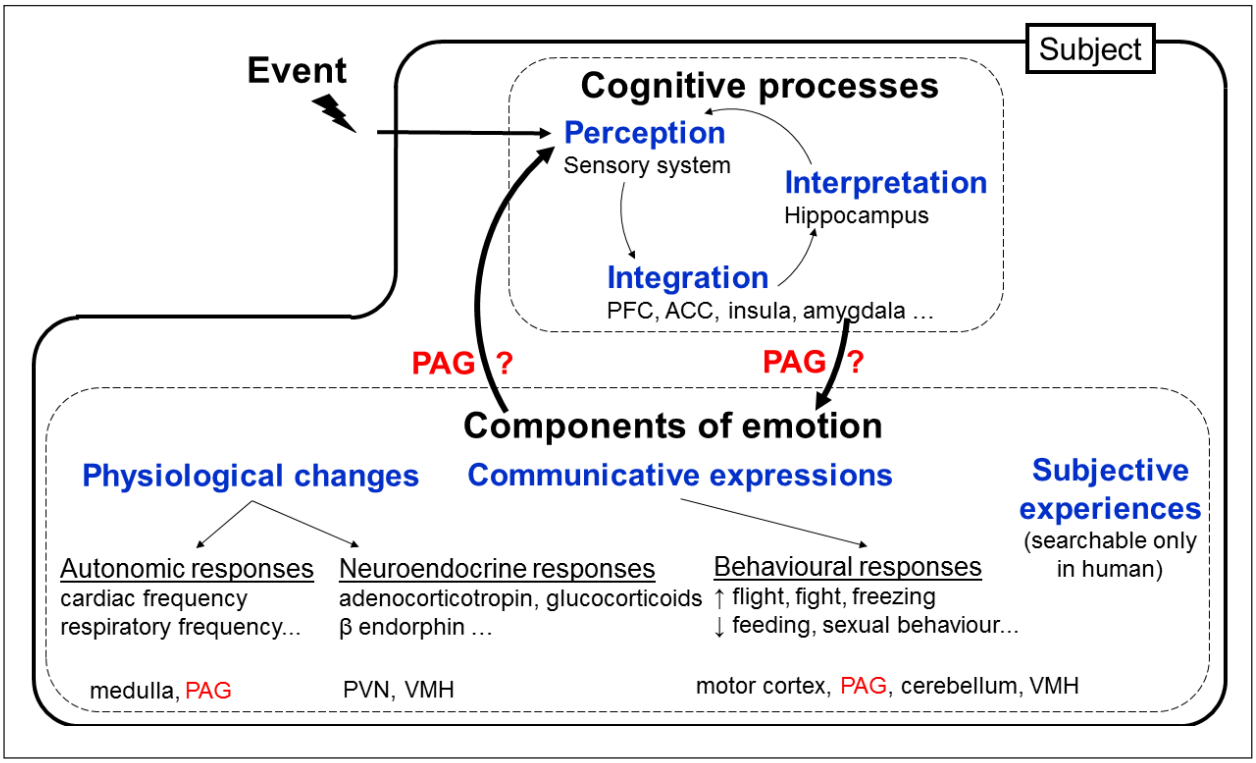

Figure 2. Schematic representation of the emotions circuit in non-human animals from the event perceived by the subject which trigger the loop of the cognitive-bodily changes. The distinct cognitive processes are mediated by specific brain structures which interact with each other and induce specific bodily changes which also influence the cognitive processes. Abbreviations: ACC – anterior cingulate cortex; LH – lateral hypothalamus; PAG – periaqueductal gray; PFC – prefrontal cortex; PVN – paraventricular nucleus of the hypothalamus; VMH – ventromedial hypothalamic nucleus.

stimuli induce the same arousal and it is differentiated only by its cognitive appraisal. Based on these concepts and theories, emotions can be defined as a mental process caused by an acute event that induces intense, sudden and transient responses in our body, which appear simultaneously and have relevant aim for the organism (adapted from Kirouac 1998). The last mentioned appraisal theory integrates the three emotional components (see Figure 2) and is widely adapted in studies with non-human species (Boissy et al. 2007; Veissier and Boissy 2007). Thus, for evaluating experimentally-evoked emotions of an animal, the usual approach is to expose it to a standardized event that triggers emotions (Dantzer 2002b). Because of the lack of access to its subjective component, emotions are evaluated through physiological responses such as neurovegetative and neuroendocrine changes, behavioral responses and brain activities. For example, the exposure to a predator in non-human animals increases the cardiac and respiratory frequencies, enhances sympathetic activity, increases the levels of adrenocorticotropin and glucocorticoids (known as stress hormones), inhibit non-defensive behaviors (*e.g.* feeding behavior, sexual behavior) and induce flight or fight behavior or freezing (Apfelbach et al. 2005). These different kinds of emotional responses result from complex mental processes that could be summarized in three steps: the *perception*, the *reaction* and the *cognitive control* (Figure 2) under the control of the neural circuit of emotions (Menant 2016a). The *perception* of the particular and acute event is due to the sensory cortex such as the visual cortex (Furl 2015). The *reaction* is firstly due to *integration and interpretation* of the particular and acute event depending on the subject (age, gender, life story etc.) (Menant 2016a), which includes, for example, the prefrontal cortex (PFC), anterior cingulate cortex (ACC), insula and amygdala (Roxo et al. 2011). Then, the reaction results in *autonomic and endocrine responses*, under the control of the medulla (Bodnar 2012) and cerebellum (Snider and Maiti 1976), and the hypothalamic structures such as the lateral hypothalamic area (LH) and the paraventricular (PVN) and ventromedial (VMH) nuclei (Bodnar 2012). Fi-

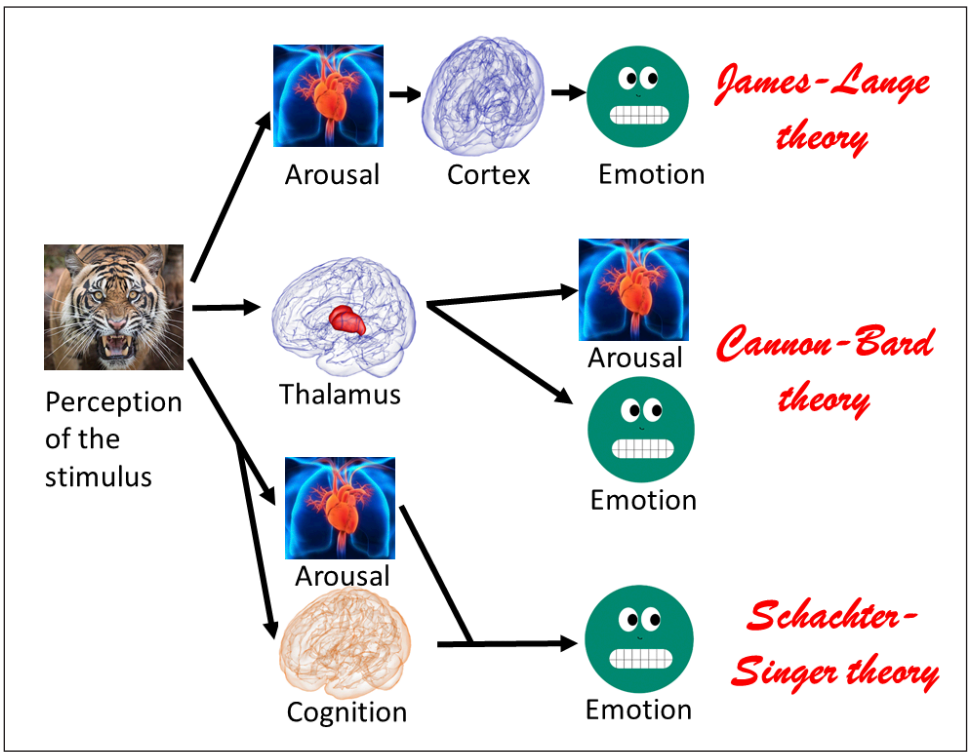

Figure 3. Comparison of the different theories for the generation of emotions. According to the James-Lange theory the perception of physical symptoms induces the feeling of different emotions at cortical level. According to the Cannon-Bard theory (also known as thalamic theory) interpretation of emotions at cortical level and physical changes occur simultaneously with the involvement of thalamus as an important relay site. The two-factor theory of emotions (also known as Schachter-Singer theory or appraisal theory) says that the feeling of emotions is the result of the perception of physical changes in our body in combination with our judgement of the situation based upon our past and present cognitive processes.

nally, the *cognitive control* is the step of reappraisal of the particular and acute event as well as the emotional responses, which can lead to the cessation of emotional reaction. This phase is under the control of higher brain structures such as the frontal cortex, the amygdala or the insula (Figure 2).

Examining emotions. In the experimental context, brain circuit of emotions can be studied in humans exposed to visual stimuli with different emotional valences (Paradiso et al. 1999) or in animals submitted to spontaneous acute and particular situation (Guesdon et al. 2015). It can also be studied in a conditioned situations (Phelps and LeDoux 2005). The principle is based on the fear from receiving a painful stimulus. Indeed, in fear conditioning, animals associate a conditioned stimulus (context or other neutral stimulus such as light, sound etc.) with an unconditioned stimulus (nociceptive stimulus such as footshock) to induce a conditioned response. By this way, it is possible to induce reproducible emotional responses in the animal subjected to the conditioned stimulus. Using this experimental model, different strategies have been developed to identify and study the neuronal network of emotions, which overlaps with that of pain (Garcia-Larrea and Peyron 2013). In all of these experimental approaches the PAG has been identified as a player in the neuronal network of emotions. It is even noted that it is mainly implicated in negative emotional context (fear, panic, aggressive) (Bandler and Shipley 1994; Bandler et al. 2000; Vianna and Brandao 2003). In positive emotions the role of the PAG is mainly associated with its involvement in the vocal communication in accordance with the social context (Kyuhou and Gemba 1998) and could be inferred from its involvement in specific social behaviors (Lonstein et al. 1998; Adolphs 2001; Noriuchi et al. 2008; Shepherd and Freiwald 2018).

The aim of the present article is to summarize the data supporting the role the PAG in emotions, including the nociceptive component of pain and

its neurochemical factors. It should be noted that the role of the PAG could be reduced to stereotype responses and dependent on the descending projections to the brainstem and spinal cord (Motta et al. 2017) in accordance with its role in emotions-related motoric and neurovegetative responses. However, this point of view may need to be reevaluated based on the in vivo imaging studies describing the PAG activation in several complex cognitive processes related to emotions (Maddock et al. 2003; Noriuchi et al. 2008; Motta et al. 2017). Therefore, we aimed to pay a special attention to understand how MRI studies changed our view about the role of the PAG in emotions from being a passive executive brain area to being an active player in the appraisal.

The periaqueductal gray (PAG) in emotions

Emotions-related motoric and neurovegetative responses to PAG manipulations. First evidence of the motoric role of the midbrain area including the PAG has been described in cats by lesions altering the facio-vocal activity (Kelly et al. 1946), inducing mutism (Skultety 1958; Adametz and O'Leary 1959; Randall 1964) and increasing the hunting postures (Randall 1964). In another study, stimulation of the PAG decreased the blood pressure, induced bradycardia, and panting followed by apnoea in monkeys and cats (Turner 1954). In humans, studies have shown that the PAG stimulation could induce several physiological responses and subjective negatives experiences such as diffuse pain, the urge to urinate, fear (Nashold and Slaughter 1969), nausea, fright, piloerection (Hosobuchi 1987), distress, anxiety, and weeping (Tasker 1982), and feelings of apprehension (Young et al. 1985).

All the authors concluded that the variation of responses depended on the size and localization of the lesion or stimulation site inside the PAG. This observation is supported by complex anatomical and functional organization of this structure reported in several species (Menant et al. 2016b) and was the basic promise of the role of the PAG in coping style (Bandler and Shipley 1994; Bandler et al. 2000; Dampney 2018).

The PAG and negative emotions. The involvement of the PAG in negative emotions has been described in numerous reviews in relationship with functional effects (Bandler et al. 2000; Keay and Bandler 2002; Benarroch 2012) or neuroanatomical organization (Menant 2016a) and will be rapidly overviewed in this part in correlation with the PAG subdivisions (Figure 4, middle part). Tachography studies using MRI supported the presence of four subdivisions in the human PAG similarly to the findings in animals (Ezra et al. 2015). The development of imaging techniques makes identification of functional subregion easier and lead to the conclusion that each subregion of the PAG may, both in human and non-human subjects, participate in distinct functional circuitry (Satpute et al. 2013).

In the *dorsal part of the PAG* (dPAG, including the dorsal and dorsolateral subdivisions), injections of excitatory amino acids (EAA), as the main stimulatory neurotransmitter, increased locomotion in rats (Morgan et al. 1998) and escape behavior in cats (Zhang et al. 1990) or decreased the tonic immobility duration in guinea pigs (Coutinho and Menescal-de-Oliveira 2010) (Figure 4, left side). This stimulation also induced deep and rapid breathing in cats (Subramanian et al. 2008), or modifications in some autonomic responses like decrease of the temperature in body extremities of rats (de Menezes et al. 2006). However, injection of EAA or deep brain stimulation in the dPAG had no effect on the mean arterial pressure in rats (de Menezes et al. 2006) and on the heart rate in humans (Pereira et al. 2010). As a summary, activation of the dPAG is sufficient to induce emotions-related autonomic and behavioral responses, however, the necessity of these subdivisions are not that clear. Indeed, although stimulation of the dPAG decreased the tonic immobility duration in guinea pigs (Coutinho and Menescal-de-Oliveira 2010) the lesion of this area did not modify the expression of freezing behavior in fear conditioned rats (Leman et al. 2003). Moreover, although the dPAG lesion decreased the basal blood pressure (Schenberg et al. 1995; Sampaio et al. 1999), its stimulation had no effect on the arterial pressure (de Menezes et al. 2006).

In the *ventral part of the PAG* (vPAG; including the lateral and ventrolateral subdivisions), stimulation and lesion induced opposite fear reactions. In more details, injections of EAA increased immobility in cats (Zhang et al. 1990), guinea pigs (Monassi et al. 1999; Coutinho and Menescal-de-Oliveira 2010) and rats (Morgan et al. 1998; Morgan and Carrive 2001), and lesions of the vPAG decreased freezing behaviour in rats exposed to a cat (De Oca et al. 1998) (Figure 4, left side). These results confirmed the sufficient and necessary involvement of the vPAG in fear responses. Moreover, injections of EAA in the vPAG induced abnormal pattern of breathing (apnoea), thought to be associated with emotions-related vocalizations in cats such as mew and hisses (Subramanian et al. 2008). This stimulation also modulated heart rate in

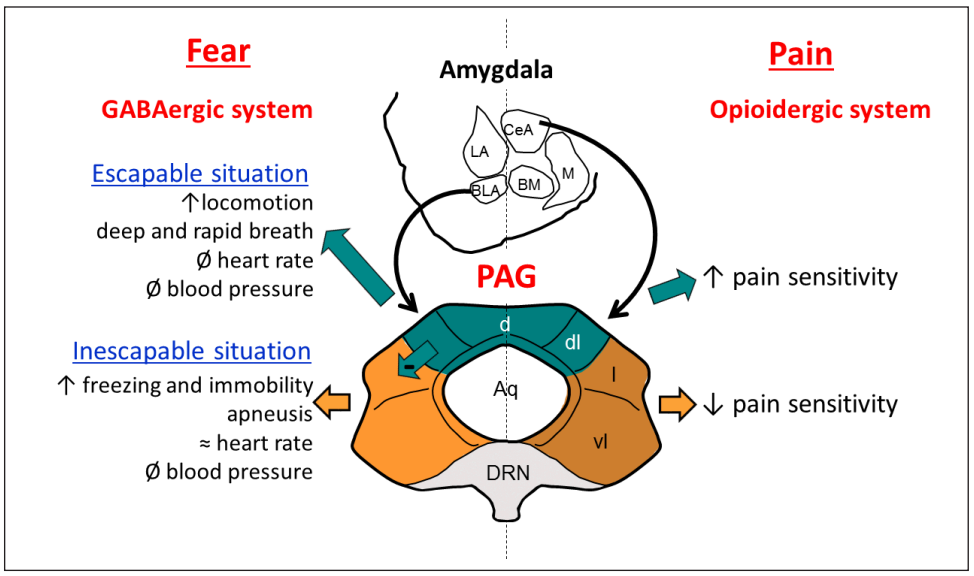

Figure 4. Schematic representation of the responses induced by the stimulation of the periaqueductal gray (PAG) subdivisions and its functional connections with the amygdala nucleus in fear and pain based upon rat brain. Some fear responses induced by the PAG is mediate by the GABAergic system from the basolateral nucleus of the amygdala, whereas some pain responses induced by the PAG is mediate by the opioidergic system from the central amygdalar nucleus. Abbreviations: Aq – Aqueduc of Sylvius; BLA – basolateral amygdalar nucleus; BM – basomedial amygdalar nucleus; CeA – central amygdalar nucleus; LA – lateral amygdalar nucleus; M – medial amygdalar nucleus; d – dorsal PAG; l – lateral PAG; dl – dorsolateral PAG; vl – ventrolateral PAG; DRN – dorsal raphe nucleus.

humans (Pereira et al. 2010) and rats (Morgan and Carrive 2001; Walker and Carrive 2003) without modifications of the mean blood pressure (Morgan and Carrive 2001).

The importance of the neuronal connections between the different subdivisions of the PAG has also been studied in fear. Although lesion of the ventral parts decreased the duration of freezing in fear conditioned test (De Oca et al. 1998), it did not influence the triggering and duration of the freezing behavior, respectively, after electrical and chemical (gamma-aminobutyric acid (GABA) receptor antagonist) stimulation of the dPAG (Vianna et al. 2001). This result suggests that – despite existing anatomical connections (Jansen et al. 1998) – the vPAG does not influence the freezing behavior regulated by the dorsal parts. However, lesion of the dorsal parts increased the duration of the freezing in fear conditioned test and innate fear, suggesting that this part could inhibit the freezing behaviors through controlling the ventral parts (De Oca et al. 1998).

For studying the activation of specific PAG subdivisions, the technique of c-Fos (an immediate early gene, whose presence indicates cellular activation) immunochemistry was also used. Activation of the PAG during the fear conditioning supported its involvement in negative feelings. Later freezing behavior in a previously fear associated context requires learning and memory. In this regard, the re-exposure of the rats to the context, where footshock was previously applied, induced c-Fos expression in their PAG, more precisely in the vPAG (Carrive et al. 1997), providing some support for the involvement of the PAG in emotions-related cognitive processes. Moreover, in fear conditioning test, rats expressed less freezing behaviors when they were lesioned in the vPAG after or before the training period (De Oca et al. 1998). Because the vPAG-lesioned rats are more sensitive to pain (Figure 4) and able to express freezing behavior in fear situations (exposition to a cat or to a new environment) (De Oca et al. 1998), the vPAG might be necessary for retrieval during learned fear. That is not the case for the dPAG, as rats lesioned in the dPAG before the training period expressed more freezing in the footshock associated context, but also when they were exposed to a cat (De Oca et al. 1998). Thus, the dPAG could regulate fear during innate negative events and not during memory recall. The different subdivisions of the PAG participate in different neuronal circuits supported by their specific anatomical connections with the rest of the brain (Menant 2016a), which can differ between species (Menant et al. 2018).

Altogether, activation of the dPAG induces active responses, whereas the vPAG is responsible for immobility (Figure 4). As proposed by Bandler and co-workers (Bandler and Shipley 1994; Bandler et al. 2000; Benarroch 2012) the dPAG induces active coping strategies associated to escapable context and the vPAG is responsible for the passive coping strategies associated to unescapable context.

The PAG and pain. Emotions and pain are closely linked. Indeed, "pain contains emotional feelings" and the emotional context could influence the intensity of the pain sensation (Price 2002). The PAG plays a main role in the descending pain modulatory pathway since it receives projections from cortices (cingulate and insular), amygdala and hypothalamus and modulates the ascending pain transmission through its projections to the rostral ventromedial medulla (RVM) (Basbaum and Fields 1984; Loyd and Murphy 2009). As for emotional responses, the PAG subdivisions rather than the PAG as a whole have to be studied. Indeed, in rats, lesions of the dPAG increased the pain threshold, whereas lesions of the ventral parts decreased it after tonic, muscle pain-inducing intramuscular saline injection (Lei et al. 2014). In rodents, studies showed higher c-Fos expression in the vPAG after induction of a persistent (cutaneous and deep somatic) pain than after an intermittent (cutaneous) pain event. On the contrary, c-Fos expression was higher in the dPAG after induction of an intermittent than a persistent pain (Benarroch 2012); these two events being associated to an escapable and an inescapable situation, respectively (Figure 4, right side). At first glance, the PAG implication in pain modulation could be only due to descending information to the medulla and spinal cord.

However, using electrophysiological approach, the existence of reciprocal and short-latency interactions between the PAG and the sensory thalamus was reported, which modified the pain perception in male patients treated by deep brain stimulation (Wu et al. 2014). Human MRI studies confirmed the existence of ascending projections from the PAG using probabilistic tachography (Ezra et al. 2015). The additional knowledge obtained with this method was that despite the similarities in the columnar structure of the PAG there appears to be different patterns of cortical connectivity between humans and non-human animals. We recently described specific connections between the PAG areas and the rest of the brains in sheep that was different from other mammals (Menant et al. 2018). These differences could lead to alteration in the coping style, specific for each species (prey, predator, territorial, gregarious etc.), considering the neuroethology of the cognitive function as it was proposed for friendship (Brent et al. 2014). Nevertheless, PAG subdivisions have afferent and efferent connections with PFC, cingulate cortex (Kyuhou and Gemba 1998) and amygdala (Hopkins and Holstege 1978; LeDoux et al. 1988; Oka et al. 2008), which are known to be involved in memory processes; and in an emotional context there is a correlation between the activation of all of these brain regions. This supports Schenberg's idea expressed during the symposium of the "Brazilian Society of Neuroscience and Behavior", that "in the PAG there seems to be great overlapping systems" (Blanchard et al. 2001).

Motta et al. (2017) described three distinct ascending neuronal networks from the PAG which might have a role in risk assessment, fear learning or motivational drive for appetitive seeking. In the later case the expressed behavior depended on social context, which suggest the PAG's involvement in positive emotions.

The PAG and social behavior: involvement in positive emotions? As seen in the introduction, the PAG is involved in the emission of vocalization in several animal species (Jurgens 1994), some of these vocalizations being specific to socio-emotional contexts of the behaviors (Jurgens 1994; Davis et al. 1996; Kyuhou and Gemba 1998). The involvement of the PAG in social behavior changes along the rostrocaudal axes rather than between ventral and dorsal parts (Satpute et al. 2013). For example, in guinea pigs, the electrical stimulation of the rostral part of the PAG induced "low whistle", known to be a separation call in this species, and the stimulation of the caudal part induced "purr", known to be a mating call (Kyuhou and Gemba 1998), both vocalizations associated with social contexts. In lactating rats, the electrolytic lesion of the caudal PAG modified several postures with socio-emotional behavioral consequences: decreased fear responses associated with increased attacks against male intruder; reduced sexual proceptivity and receptivity; and lead to ineffective nursing posture for milking ejection (Lonstein and Stern 1998; Lonstein et al. 1998). Using c-Fos immunohistochemistry activation of the caudal part of the PAG varied in accordance with the expression of maternal behaviors (suckling, kyphosis) (Lonstein and Stern 1997). Human electrophysiological measurements – recording differences between infant vocal- and sound control-stimulation from the PAG – suggested that this structure allows rapid propagation of information with emotional valence (in this study

from infant cues) through cortical and subcortical brain regions (Parsons et al. 2017). Because all these behavioral responses are necessary to establish social relationship with others (Levy 2002; Sebe et al. 2008; Gaudin et al. 2018), and because these social bonds are associated with positive emotional state, we suppose that the involvement of the PAG in emotions is not restricted to negative emotions.

Emotions-related neurochemical composition of the PAG

It is essential to consider the neurochemical factors insight the PAG subdivisions.

The result of the chemical stimulations suggests that the **glutamatergic** system mediates the freezing responses associated to the dPAG (Vianna et al. 2001).

On the other hand, local **serotonin** (5-HT) (Clements et al. 1985) injections into the vPAG decreased nociception (Bartsch et al. 2004), whereas injections into the dPAG decreased defensive behavior in rats (Beckett and Marsden 1997; Sela et al. 2011; Campos et al. 2013) and anxiety-like behaviors in mice (Nunes-de-Souza et al. 2011). We can assume that these specific roles of the subdivisions maybe due to different inputs from emotionally relevant brain areas such as the hypothalamic nuclei (Saavedra et al. 1974), amygdala (Asan et al. 2013; Bombardi 2014) or PFC (de Almeida et al. 2008), rather than the divergent localization of receptors seen in the case of the opioidergic system.

Indeed, the deviating role of the PAG subdivisions in regulation of pain has been supported by the divergent presence of **opioids** such as β-endorphin (Pesini et al. 2001) and their receptors (μ opioid receptor) (Moskowitz and Goodman 1985; Monteillet-Agius et al. 1998; Abbadie et al. 2000). The transcutaneous electrical nerve stimulation (TENS) is known to reduce pain sensitivity through the opioidergic system. Rats have been used to study the pain threshold after induction of knee joint inflammation in combination with reversible functional activation of the dPAG or vPAG. The results showed that during TENS the pain threshold of the injured rats is similar to the control rats when the dPAG is inactivated, but is lower when the vPAG is inactivated (DeSantana et al. 2009). In line with this idea, rats, which responded less to heat stimulation, expressed more β-endorphin in the vPAG (Laprairie and Murphy 2009). These results support the notion that analgesia is mediate by the opioidergic system in the vPAG (Monteillet-Agius et al. 1998; Abbadie et al. 2000; Wiedenmayer and Barr 2000) (Figure 4), that is consistent with its high μ-receptor density in rats (Monteillet-Agius et al. 1998; Abbadie et al. 2000). Moreover, the opioid system of the PAG is also deeply involved in maternal behavior, since morphine injection in the rostral PAG disrupts maternal care in nulliparous rats (Moura et al. 2010), and dams exhibit different pattern of opioid receptors' expression in comparison with multiparous rats (Teodorov et al. 2011).

The **GABA**ergic system was studied in relation with the amygdala-PAG pathway to evaluate its importance in emotions (Figure 4, left side). Injection of muscimole (GABA-A receptor agonist) in any amygdala nucleus decreased the time of freezing behavior both after electrical stimulation of the dPAG and exposure to conditioned fear test (Martinez et al. 2006). Moreover, c-Fos expression was increased in all subdivisions of the PAG after injection of muscimole in the basolateral complex of the amygdala (BLA) when the animals were fear conditioned (Rea et al. 2011). These results suggest a functional connection between the PAG and the amygdala, which has anatomical background (Rizvi et al. 1991; Oka et al. 2008; Chiou et al. 2014). This later connection seems to be specific for fear, as injection of muscimole in the BLA did not inhibit the pain response to hindpaw formalin injection (Rea et al. 2011). Although we have to mention that the amygdala-PAG pathway might be also involved in pain in some extent, as in rats, injection of formalin in the hindpaw induced c-Fos expression in both the PAG and central nucleus of the amygdala (CeA), and the electrical stimulation of the CeA increased c-Fos expression in the PAG (Nakamura et al. 2013) (Figure 4, right side). However, as the afore mentioned formalin injection induced a higher density of β-endorphin cells in the vPAG (Nakamura et al. 2013), which was associated to pain reduction (Laprairie and Murphy 2009) we might assume that the pain response may be triggered not by GABAergic, but rather opioidergic pathways.

The **oxytocinergic (OT)** system is present in the PAG, which contains OT-fibres (Swanson and McKellar 1979; Roeling et al. 1993) and OT-receptors (Yoshimura et al. 1993; Freeman et al. 2014). This system – specifically in the caudal PAG – is important for the anxiolytic effect of the motherhood. For example, the injection of an OT-antagonist into the caudal PAG of lactating dams increased anxiety-related behaviors and low dose of OT in stressed dams reduced their anxiety (Figueira et al. 2008). In this specific socio-emotional context of the motherhood, dams have to care for their pups and express defensive behavior

against threats like e.g. predator. In these processes the PAG subdivisions have specific, albeit opposite roles (Sukikara et al. 2010).

In conclusion, as it seems essential to consider the PAG with its anatomical and functional connections for understanding its involvement in emotions, it is also necessary to consider the neurochemical factors of the PAG with special attention to the subdivisions.

Emotions and the PAG: How in vivo imaging changed our view?

Given how we have defined the emotions and the paradigm used to study the neuronal network of emotions in animals, it is easy to understand that the subjective component of emotions cannot be studied due to the absence of verbalization in animals. Even humans are known to lie about their emotions, but at least they could be asked about their feelings. When we do this during in vivo measurements of brain activities, we can have deeper insight into the neuronal network of emotions, which cannot be obtain with any other method. The in vivo imagery also allowed to study the metabolism of brain structures using positron emission tomography (PET) scan, single photon emission computed tomography (SPECT), and magnetic resonance spectroscopy imaging (MRSI). Whereas the PET and SPECT methods required injections of radioactive tracers, MRSI does not. This makes the later method to be "unique tool to probe the biochemistry in vivo providing metabolic information non-invasively" (Osorio-Garcia et al. 2012). The development of high field strengths MRI machines and the design of the radio frequency coil improved the signal sensitivity of the MRSI method and allowed to distinguish small molecules (van der Graaf 2010) such as N-acetyl-aspartate, which is an indicator of brain pathology and pain (Baslow 2002). For example, using the 1H MRSI method in human, subjects with chronic daily headache had higher level of N-acetyl-aspartate-glutamate in the PAG than healthy subjects (Buonanotte et al. 2006), suggesting that the PAG could be involved in pain perception through the glutamatergic system. Animal studies may have suggested the involvement of glutamate in pain perception (Carstens et al. 1990), but for that invasive methods were necessary which might even influence the observed phenomenon. The MRSI method is the first tool, which allows the detection of neurochemical composition of a small structure like the PAG via a completely noninvasive way allowing its extensive use in human studies as well. Moreover, the in vivo imaging is the only approach that allows to study the involvement of the PAG connections in complex cognitive processes occurred in emotions, pain or social behaviors.

The PAG and negative emotions. Watching negative images (Buhle et al. 2013) or anticipating an anxious speech (Wager et al. 2009) the PAG activity was increased in humans in association with increased heart rate confirming its involvement in the emotions-related motoric processes (Wager et al. 2009). However, using functional MRI (fMRI) it has been also shown that the activity of the PAG depends on the strength of the fear-inducing situations suggesting a more complex role. In humans, distal presence of a tarantula, an escapable situation, did not activate the PAG; however, its proximal presence, a non-escapable situation, increased its activity (Mobbs et al. 2007). Whereby, the situation was more inescapable, higher was the activity of the PAG (Mobbs et al. 2007), which reflected the distance of the threat (Mobbs et al. 2010) and the probability of the negative event (Mobbs et al. 2007) judged by cognitive processes involving the PAG.

The impact of the emotional state on force control was evaluated by behavioral responses in the same time as physiological markers and brain activation. Negative emotions facilitated the force control and concluded – in accordance with results of brain activation studies – that right frontal gyrus, amygdala, and PAG are key-regions to mediate this effect (Blakemore et al. 2016). Because the PAG is activated in pain and emotional context, patients were submitted to warm stimulation (low vs. high pain) and negative emotional images (negative vs. neutral). Higher PAG activation has been observed after high vs. low pain and after watching negative vs. neutral images, suggesting the involvement of the PAG in affective processes (Buhle et al. 2013). This idea is supported by brainstem (including PAG) activation during evaluation of pleasant words compared to neutral ones confirmed by fMRI (Maddock et al. 2003).

MRI studies in humans were able to separate PAG subregions related to different emotions, similarly as it has been found with other techniques in animals (Satpute et al. 2013). Emotionally aversive images activated the lateral parts of the PAG.

The PAG and pain. Several fMRI studies have reported that physical pain induced PAG activation regardless of the location of the induction site on the body [hand (Fairhurst et al. 2012; Buhle et al. 2013), somatic or visceral (Dunckley et al. 2005)] and the

characteristic of the stimulation [heat (Fairhurst et al. 2012; Buhle et al. 2013), electrical stimulation (Dunckley et al. 2005)].

In the aim to better understand the pain perception (La Cesa et al. 2014) and the management of pain in patients (Yu et al. 2017; Harper et al. 2018), anticipatory phenomenon and placebo effect have been studied and the PAG has been identified as an important player of these complex cognitive processes. Indeed, PAG activity increased not only when subjects received heat stimulation on their hand, but also when they were only warned and not stimulated (Fairhurst et al. 2007). Uncertainty specifically and potently increased pain, which was correlated with the activity of the PAG measured by fMRI (Yoshida et al. 2013). Thus, the PAG was activated during anticipation of a negative event (Roy et al. 2014) suggesting its involvement not only in analgesia, but also in the cognitive processes such as recalling events. The development of placebo effect would require learning and recall processes (Stewart-Williams and Podd 2004). Indeed, pain induction (thermal, shock or laser stimuli) led to placebo effect through modulation of the opioidergic–cholecystokininergic–dopaminergic pain network including the PAG (Benedetti et al. 2011). More precisely, placebo effect increased the PAG activity leading to an increased threshold of pain sensitivity, finally to less pain sensation. The PAG activity was correlated to other brain regions known to be involved in memory or pain such as the hypothalamus (Eippert et al. 2009), thalamus, ACC, insula (Wager et al. 2004; Bingel et al. 2006) and RVM (Eippert et al. 2009). Additionally, using MRI and tachography the functional connection between the above mentioned areas and the PAG was also confirmed (Ezra et al. 2015).

Altogether, in vivo imaging used in humans and interventional approaches performed in animals have demonstrated the importance of the PAG in negative affective contexts (negative emotions or pain). However, whereas animal researches demonstrated the PAG as a relay of cognitive information to the brainstem and spinal cord for stereotyped responses, in vivo imaging allowed considering the PAG as a structure involved in complex cognitive processes transmitting information to cortical levels. Interestingly, these approaches also allowed the reanalysis of the role of the PAG in the social cognition.

The PAG and social cognition. Social cognition could also be approached through examining empathy (Adolphs 2001). Empathy is conceptualized by a behavior, a personality dimension, and an experienced emotions concept (Reynolds and Scott 1999) and could be seen at the interface of emotions and social cognition. Empathy is essential to inhibit aggressive behavior towards others and is seen as a prosocial rather than an antisocial behavior (Decety et al. 2010). In human, children viewing other in painful situation vs. non-painful situation, activation of the whole neuronal circuit of pain including the PAG could be observed (Decety et al. 2008). In adults submitted to similar protocols, introduction of a blame game component influenced the PAG activation (Decety et al. 2010) suggesting that the level of empathy is in correlation with the PAG activation. Because the PAG is also involved in aggressive behaviors (Depaulis et al. 1989; Bandler et al. 2000), it is interesting to report another study performed by Decety et al. (2009) showing others in pain (accidental or intentional), brain activation of adolescent with aggressive conduct disorders compared with healthy controls. All the subjects showed PAG activation after viewing accidental pain vs. no pain, whereas PAG activation did not differ between accidental or intentional pain. Moreover, no difference of PAG activation has been reported between adolescent with aggressive conducted disorders and healthy controls.

Social cognition encompasses different phenomena as social perception, social recognition, social communication, and social behaviors that may involve different brain structures and neuronal networks (Adolphs 2001; Brent et al. 2014). In this context, functional neural network of social communication has been examined by fMRI in macaque monkeys showing the involvement of the PAG (Shepherd and Freiwald 2018). In humans, mothers were exposed to video of their own child (or another one) submitted to a pleasant (playing) or distress (separation) situation. The study revealed activation of the PAG in mother viewing their own child vs. other child, whatever was the context (Noriuchi et al. 2008), indicating that the PAG could be involved in social object recognition or bond attachment. These results – together with other studies on animals showing the involvement of the PAG in maternal behaviors including defense (Moura et al. 2010; Sukikara et al. 2010; Klein et al. 2014; Barba-Muller et al. 2018) – support that the PAG might be viewed as an important brain structure of the caregiving parental brain (Young et al. 2017).

Conclusions

Taken together, the PAG is a brain structure coordinating not only motoric and autonomic neurovegetative outcomes, but it seems that is also involved in

a complex of cognitive processes. This is supported by its extensive anatomical and functional connections. The inclusion of MRI in these studies helped to change our view on the PAG that it is not only a passive executor but also an active participant in cognitive processes.

However, we should be aware of the differences between species and different animal models. Therefore, we have to consider pain, negative and positive emotions, and social cognition from an evolutionary point of view, as it has been proposed in the Research Domain Criteria (RDoC) system (Anderzhanova et al. 2017). Thus, comparison of species is of particular importance to better describe and understand the brain and its role in the cognitive functions, as it has been reviewed in more and more papers [parental brain (Bales 2017); friendship (Brent et al. 2014)]. This requires thorough knowledge of the behavior of different species using ethological approaches and to explore anatomical (Menant et al. 2018) and functional (Najafi et al. 2017) connections of different brain structures with the help of MRI and to develop post-processing tools to get a more comprehensive picture of the various species.

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