Predatory hunting and exposure to a live predator induce opposite patterns of Fos immunoreactivity in the PAG

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Abstract

Considering the periaqueductal gray’s (PAG) general roles in mediating motivational responses, in the present study, we compared the Fos expression pattern in the PAG induced by innate behaviors underlain by opposite motivational drivers, in rats, namely, insect predation and defensive behavior evoked by the confrontation with a live predator (a cat). Exposure to the predator was associated with a striking Fos expression in the PAG, where, at rostral levels, an intense Fos expression was found largely distributed in the dorsomedial and dorsolateral regions, whereas, at caudal levels, Fos-labeled cells tended to be mostly found in the lateral and ventrolateral columns, as well as in the dorsal raphe nucleus. Quite the opposite, insect predation was associated with increased Fos expression predominantly in the rostral two thirds of the lateral PAG, where the majority of the Fos-immunoreactive cells were found at the oculomotor nucleus levels. Remarkably, both exposure to the cat and insect predation upregulated Fos expression in the supraoculomotor region and the laterodorsal tegmental nucleus. Overall, the present results clearly suggest that the PAG activation pattern appears to reflect, at least partly, the animal’s motivational status. It is well established that the PAG is critical for the expression of defensive responses, and, considering the present findings, it will be important to investigate how the PAG contributes to the expression of the predatory behavior, as well.

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1. Introduction

The periaqueductal gray (PAG) is known to play an important role in the modulation of nociceptive sensory transmission [10,43], regulation of the cardiovascular system [20,41], and in the expression of a variety of behaviors, including defensive [21], sexual [52], maternal [40], and feeding behaviors [57,61]. Throughout the past decades, however, two themes have largely dominated research on PAG, namely, inhibition of nociception, and the integration of behavioral responses to threatening or stressful stimuli (see [4]).

The pioneering studies of Hunsperger [34] led to the widely accepted view that the PAG is critical for the expression of defensive behavior. It was first found that lesions centered in the PAG significantly impair spontaneous defensive behavior in cats confronted with a dog, resulting in passive animals that were easily handled, and rarely, if ever, showed defensive responses [34]. Subsequent studies largely corroborated and significantly extended this idea, and showed that PAG stimulation may induce a pattern of somatomotor and autonomic responses reminiscent of the behavior of animals facing natural threats [2,25,35]. In fact, we had shown that exposure to a live predator was associated with a striking activation of the PAG, where Fos expression was particularly abundant in the rostral two thirds of the PAG in the dorsomedial and dorso-
lateral regions; moreover, in the caudal PAG, a more widespread activation was observed [15].

Considering the PAG’s general roles in mediating motivational responses, it would be important, at this point, to examine how the PAG participates in an opposite behavioral circumstance, where the animal, instead of confronting a live predator, plays the predator’s role, and is actually engaged in prey hunting.

Similar to the defensive behavior, predatory hunting has been regarded as an innate behavioral response seemingly critical for the animals’ survival [26]. Previous studies have shown that pathways involved in the expression of defensive and predatory behaviors may diverge at brainstem levels. Thus, while defensive responses appear to be abolished by central gray lesions, predatory responses are said to be eliminated by lesions of the ventral midbrain and pontine reticular formation. Conversely, predatory attack can be elicited by electrical stimulation of the mesencephalic and pontine reticular formation. Lesions of the mesencephalic and pontine tegmentum [9], and appears to be facilitated by electrical stimulation of the midbrain reticular formation [55].

Notably, Karli and colleagues, using the mouse-killing behavioral paradigm, showed that, in rats, extensive PAG lesions facilitated predatory attack, whereas stimulation of the dorsal PAG, known to elicit defensive behavior, inhibited the expression of interspecific aggressive responses [22]. These results suggest that neural systems involved in eliciting defensive responses may inhibit the outcome of predatory attack; however, they do not rule out the possibility that certain PAG regions may indeed facilitate the occurrence of the predatory attack itself or other behaviors likely to be expressed in the predatory hunting context, such as the behavioral responses involved in the prey searching.

In the present work, in order to start addressing this issue, we first examined the pattern of Fos immunoreactivity in the PAG of rats that predated cockroaches. Cockroach predation was presently chosen as the behavioral paradigm to explore predatory behavior in the rat, since it is vividly expressed by all individuals, a fact confirmed for all the different strains tested [46]. Moreover, roaches are relatively innocuous and easily overcome, and do not seem to induce appreciable defensive reactions in rats. In contrast to insect predation, however, the mouse-killing behavioral paradigm, widely used in the past to study the neural basis of predatory behavior in rats, presents serious limitations constraining its use in the present study. Thus, mouse-killing behavior is likely to be expressed by only a limited number of individuals (see [58]), and the confrontation with a live mouse appears to induce overt defensive reactions in a significant number of rats (WR Schmidek, personal communication).

The results on the PAG activation pattern in animals that performed predatory hunting were further compared with those from another series of experiments where the distribution of Fos immunoreactivity was analyzed in the PAG of rats confronted with a live predator.

Overall, the present results clearly indicate that insect predation and exposure to a natural predator induce an opposite activation pattern of the PAG, perhaps reflecting the diverse motivational drives underlying each one of these responses.

2. Materials and methods

Adult male Wistar rats (n = 15), weighing about 250 g and obtained from the local breeding facilities, were used in the present study. The animals were kept under controlled temperature (23 °C) and illumination (12-h cycle) in the animal quarters, and had free access to water and standard laboratory diet (Purina). Conditions of animal housing and all experimental procedures were conducted under institutional guidelines, and in accordance with NIH guidelines on animal care.

One week before the experimental procedures, animals were individually housed into a plexiglass cage (50 × 35 × 16 cm) with a wire-meshed cover and front wall, and were handled repeatedly by the same investigator that conducted the behavioral tests. Five animals were then exposed to a live predator by means of the placement of an adult female cat (2.5 kg) in close contact with the home cage for 10 min. Another group of animals (n = 5) was induced to hunt by a simultaneous introduction, into the home cage, of five mature intact cockroaches (Periplaneta americana) raised for this purpose in our laboratory. A third group of rats (n = 5) served as controls; they were housed and handled in the same way as the animals of the other experimental groups, but were neither induced to predate nor exposed to the cat. All the behavioral tests were performed between 17:00 and 18:00 h, and recorded with a video camera.

Ninety minutes after the behavioral tests, each animal was deeply anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and perfused transcardially with a solution of 4.0% para-formaldehyde in 0.1 M phosphate buffer at pH 7.4; the brains were removed and left overnight in a solution of 20% sucrose in 0.1 M phosphate buffer at 4 °C. The brains were then frozen, and four series of 30 μm sections were cut with a sliding microtome in the frontal plane and collected from the caudal thalamus to the rostral levels of the pontine central gray. One complete series of sections was processed for immunohistochemistry with anti-Fos serum raised in rabbit (Ab-5, Oncogene Science, lot # D09803) at a dilution of 1:10000. The primary antiserum was localized using a variation of the avidin–biotin complex system (ABC; [33]). In brief, sections were incubated for 90 min at
room temperature in a solution of biotinylated goat anti-rabbit IgG (Vector Laboratories), and then placed in the mixed avidin-biotin-horseradish peroxidase (HRP) complex solution (ABC Elite Kit; Vector Laboratories) for the same period of time. The peroxidase complex was visualized by a 10-min exposure to a chromogen solution containing 0.02% 3,3’ diaminobenzidine tetrahydrochloride (DAB) with 0.3% nickel–ammonium sulfate in 0.05 M Tris–buffer (pH 7.6), followed by incubation for 10 min in chromogen solution with hydrogen peroxide (1:3000) to produce a blue–black product. The reaction was stopped by extensive washing in potassium phosphate-buffered saline (KPBS; pH 7.4). Sections were mounted on gelatin-coated slides, and then dehydrated and covered-slipped with DPX.

In order to delineate the PAG columns, another series of sections was processed for immunohistochemistry with a monoclonal antiserum directed against brain nitric oxide synthase raised in mouse (Sigma) at a dilution of 1:1000. The antigen–antibody complex was localized by using a variation of the avidin-biotin complex system (ABC; [28]), with a commercially available kit (ABC Elite Kit; Vector Laboratories). The sections were mounted on gelatin-coated slides and then treated with osmium tetroxide to enhance visibility of the reaction product. Slides were then dehydrated and covered-slipped with DPX. A third series was stained with thionin to serve as a reference series for cytoarchitectonic purposes. The PAG columns (dorsomedial, dorsolateral, lateral, and ventrolateral) were delineated using the guidelines described by Carrive et al. [19].

Fos-like immunoreactive cells were detected with the X10 objective of a Nikon Eclipse E600 microscope equipped with a camera lucida. For a cell to be considered as expressing Fos-like immunoreactivity, the nucleus of the neurons had to be of appropriate size (ranging approximately from 8 to 15 μm) and shape (oval or round), show the characteristic blue–black staining of oxidized DAB-Ni, and be distinct from the background at magnification of X10. For each animal, at six distinct rostrocaudal levels of the PAG, Fos-positive cells were plotted and counted from two adjacent sections (120 μm apart). For each experimental group (five animals in each group), the counts were then divided by the number of counted sections to provide a mean cell count per slice for each PAG column in each of the six rostrocaudal segments. The data were analyzed by a multivariate analysis of variance (one-way MANOVA, where we treated Fos-like immunoreactive cell counts in each different PAG region as dependent variables, and the experimental groups as the between-groups independent variable), followed by planned comparisons. The significant level was set at 5%. All the values are expressed as mean ± S.E.M.

3. Results

The simultaneous introduction of five cockroaches into the animals’ home cage induced a marked predatory behavior. Thus, at first, the animals sniffed vigorously around the cage, and, as the prey object was located, the predator rushed toward the roaches and tried to seize them. The prey capture was assisted by pinning the prey to the substrate with the forepaws, or grasping the prey with the forepaws either simultaneously or shortly after the killing bite was administered toward the prey’s head. The killed roaches were then taken to one corner of the cage, where the rats started eating them voraciously. All the animals observed in the present study took less than 40 min (28.64 ± 3.39 min) to consume the five roaches placed into their home cages.

In order to describe our results, we followed the PAG parcellation originally proposed by Bandler et al. [1] and recently revised by Carrive et al. [19]. This parcellation is based on anatomical, neurochemical, and functional data, and divides the PAG into dorsomedial, dorsolateral, lateral, and ventrolateral columns, in addition to a ventromedial portion containing the supraoculomotor region, as well as the oculomotor, dorsal raphe, and laterodorsal tegmental nuclei, usually considered on functional and anatomical grounds as separable from the rest of the PAG. The animals that hunted the roaches displayed a similar pattern of Fos immunoreactivity along the rostrocaudal axis of the PAG (Fig. 1A–F, Fig. 5D). Thus, compared with the other experimental groups, insect predation induced a significantly larger increase in Fos expression in the rostral two thirds of the lateral column of the PAG (all \( F_{1,12} \geq 37.198, \) all \( P < 0.001 \)) (Fig. 4), where the majority of the labeled cells were found at the oculomotor nucleus levels (Fig. 1A–D, Figs. 4 and 5D). Interestingly, at these levels, Fos-immunoreactive cells tended to be clustered in the outer half of the lateral column (Fig. 1B, C, Fig. 5D), which appears to correspond, at least in part, to a region known to present a distinct acetylcholinesterase-positive neuropil [51]. Moreover, compared with the control group, the animals that preformed predatory hunting presented a significant increase in the number of Fos-immunostained cells in the supraoculomotor region (all \( F_{1,12} \geq 5,835, \) all \( P < 0.05 \)) (Fig. 1B, C, Fig. 3B, C, Fig. 5A, D) and in the laterodorsal tegmental nucleus (\( F_{1,12} \geq 100,148, \) \( P < 0.001 \)) (Fig. 1F, Fig. 3F), whereas, in the other PAG sites, the number of Fos-immunoreactive cells found for the animals that hunted the roaches did not significantly differ from the one found for the control group (Fig. 4).

In the group of animals that confronted the predator, during the 10 min of direct exposure, all the five animals remained expressing a robust freezing response most of the time (8.18 ± 0.55 min), and, compared with the other...
experimental groups, presented, in several specific PAG sites, a marked increase in Fos immunoreactivity (Fig. 2A–F, Fig. 5B). Thus, at the level of nucleus of Darkschewitsch, Fos expression was particularly increased in the dorsomedial column, whereas the lateral PAG contained relatively sparser Fos staining (Fig. 2A). Proceeding caudally, at the oculomotor nucleus levels, a striking Fos expression was found in the dorsomedial

Fig. 1. (A–F) Camera lucida drawings of sections taken through the periaqueductal gray, arranged from rostral (A) to caudal (F), showing the distribution of Fos-immunoreactive cells (black dots) in a rat that predated roaches. The approximate distance from the interaural line is indicated on the top of each figure. For abbreviations, see list. Scale bar = 1 mm.
and dorsolateral PAG, whereas, in sharp contrast, the lateral PAG contained a much smaller number of stained cells (Fig. 2B, C, Fig. 5B). Moreover, at these levels, compared with the control group, a distinct increase in Fos expression was also noted in the supraoculomotor region (all $F_{1,12} \geq 21.682$, all $P < 0.001$) (Fig. 2B, C, Fig. 3B, C, Fig. 5A, B). At the caudal half of the PAG, the dorsomedial and dorsolateral columns of the PAG continued expressing a remarkable number of Fos-stained cells, which, however, tended to show a clear decline toward the caudal end of the PAG (Fig. 2D–F, Fig. 4). Conversely, at these levels, in the lateral PAG, Fos expression increased toward the caudal end (Fig. 2D–F, Fig. 4), where an impressive number of Fos-labeled cells was observed (Fig. 2F). Notably, according to the parcellation proposed by Ruiz-Torner et al. [51], at the caudal end of the PAG, the ventrolateral column encompasses that which

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Fig. 2. (A–F) Camera lucida drawings of sections taken through the periaqueductal gray, arranged from rostral (A) to caudal (F), showing the distribution of Fos-immunoreactive cells (black dots) in a rat exposed to the predator. The approximate distance from the interaural line is indicated on the top of each figure. For abbreviations, see list. Scale bar = 1 mm.
we have presently considered as being the lateral region. Exposure to the predator also induced a robust Fos expression in the rest of the ventrolateral column, which presented a larger number of stained cells toward the caudal pole of the PAG (Fig. 2D–F, Fig. 4). In addition, compared with the control group, at caudal levels of the PAG, a distinct increase in Fos immunoreactivity was also found in the dorsal raphe nucleus (all $F_{1,12} \geq 148.939$, all $P < 0.001$) (Fig. 2D–F, Fig. 3D–F), where the region of the lateral wing contained a particularly high density of immunoreactive cells (Fig. 2E). Finally, at the caudal end of the PAG, compared with the control group, exposure to the predator also induced a significant Fos expression increase in the laterodorsal tegmental nucleus ($F_{1,12} \geq 269.71$, $P < 0.001$) (Fig. 2F, Fig. 3F).

Fig. 3. (A–F) Camera lucida drawings of sections taken through the periaqueductal gray, arranged from rostral (A) to caudal (F), showing the distribution of Fos-immunoreactive cells (black dots) in a control rat. The approximate distance from the interaural line is indicated on the top of each figure. For abbreviations, see list. Scale bar = 1 mm.
4. Discussion

Before discussing our results regarding the pattern of Fos immunoreactivity in the PAG, we should consider some of this technique’s potential limitations. Although Fos protein expression has been used as a sensitive cellular marker for neuronal activation induced by a variety of stimuli [42], it is important to keep in mind that the absence of neuronal Fos expression cannot be interpreted as lack of influence on neuronal activity, especially considering that Fos expression is not induced by the opening of ionotropic channels that do not increase the intracellular levels of second messengers, such as the classical chloride channel [56]. Moreover, absence of Fos staining may also result from an antibody dilution, inadequate to detect small changes in some neuronal populations. Therefore, in the present study, several antibody concentrations were initially screened to determine a dilution resulting in robust Fos staining and low levels of background labeling.

We have presently shown that different behavioral responses, underlain by opposite motivational drives, such as insect predation and defensive behavior during the confrontation with a live predator, are accompanied by an opposite pattern of mobilization of the PAG columns.

The present results confirm and, in many ways, extend the conclusions drawn by our previous study on the pattern of Fos immunoreactivity in the PAG after the encounter with a live predator [15]. Although the number of Fos-stained cells in the PAG regions of the present experiments was much higher when compared with the figures observed in that previous study, a similar profile in the distribution of Fos immunoreactive cells was presently found along the rostro-caudal extent of the dorsomedial, dorsolateral, lateral, and ventrolateral PAG columns. Thus, similarly to what we had previously reported for animals exposed to a live predator [15], we have presently found, at rostral levels, an intense Fos expression largely distributed in the dorsomedial and dorsolateral regions, whereas, toward the caudal end of the PAG, Fos-labeled cells tended to be more distributed in the ventrolateral and lateral columns.

Interestingly, in the PAG, there is a striking overlap between the distribution of Fos expression induced by confrontation with a live predator and the sites of projection from the dorsal premammillary nucleus plus the dorsomedial part of the ventromedial hypothalamic nucleus [16,17], which are known to represent integral elements of the medial hypothalamic defensive system [18]. Of particular relevance, this medial hypothalamic system receives inputs from specific amygdalar and septohippocampal domains likely to be involved in processing information related to environmental threats, and, in turn, appears to be critical for the adequate expression of innate fear responses [18].

According to the present data, the rostral two-thirds of the dorsolateral column is the most mobilized PAG site during the encounter with the predator. It is well established that stimulation of the dorsal PAG evokes vigorous somatomotor responses, such as running and

![Fig. 4. Frequency histograms show the mean number of Fos-immunoreactive cells per section in all experimental groups (control, insect predation, and exposure to the predator) for the dorsomedial (dm), dorsolateral (dl), lateral (l) and ventrolateral (vl) regions of the PAG. Levels A–F correspond to the rostrocaudal levels depicted in Figs. 1–3. Data are expressed as mean ± S.E.M.](image-url)
jumping, as well as reactive immobility (freezing), accompanied by sympathoexcitation, non-opioid analgesia, and cardiorespiratory changes, characterized by blood pressure and heart rate increase, as well as vasodilatation in hindlimb muscles, along with an increase in the rate and depth of respiration [3,20,41,43].

Apart from receiving inputs from elements of the medial hypothalamic defensive system, the dorsolateral PAG, at the level of the oculomotor and trochlear nuclei, receives substantial projections from the medial regions of the intermediate and deep layer of the superior colliculus, as well as from the cuneiform nucleus, which are known to be critical for transmitting information...
related to visual threats, such as rapidly expanding or looming shadows in the upper visual field [47]. Moreover, the dorsolateral PAG is also densely innervated by elements of the orbitomedial prefrontal cortex, including the caudal parts of the infralimbic and prelimbic areas, dorsal and ventral parts of the anterior cingulate cortex, as well as the perirhinal cortex, thought to be involved in mediating active emotional coping response to psychological stressors [27]. Actually, in addition to exposure to a live predator, a number of other situations which may threaten the animals (e.g. exposure to the plus maze and handling) are also likely to induce a prominent Fos expression upregulation in the rostral part of the dorsolateral PAG (NS Canteras, personal observations). Importantly, however, this PAG region does not seem to be particularly activated by any physical stressor, such as cutaneous and visceral pain (see [35]).

Exposure to predator also induced an impressive Fos expression in the ventrolateral PAG. However, in contrast to what has just been said about the dorsal PAG, stimulation of the ventrolateral PAG evokes hyporeactive immobility, sympathoinhibition, blood pressure and heart rate decrease, and opioid-dependent analgesia [3,20,24,41,43], typically found in the recuperative phase of a number of stressful situations. Since our behavioral paradigm prevented direct contact between prey and predator, there were no physical harms to the animals. As far as we can tell, after the encounter, the rats did not seem to present passive emotional coping responses, such as the ‘hyporeactive immobility’ normally seen after the defeat, but instead, continued expressing a strong ‘hypereactive immobility’ (freezing) along with a clear exophthalmos. Therefore, it is quite questionable whether the increased Fos expression in the ventrolateral PAG, observed in the present experiments, should be correlated with passive emotional coping responses. Here, it is noteworthy that a similar increased Fos expression in the ventrolateral PAG has been reported in rats under pentobarbital anesthesia, and which had received chemical stimulation in the dorsolateral PAG [53]. Overall, the evidence supports the idea that the increased Fos expression in the ventrolateral PAG, observed in our experiments, should be viewed as integrating the general pattern of PAG mobilization in response to the presence of a live predator. Although quite speculative, it is plausible to believe that exposure to the predator may induce increased Fos expression in inhibitory interneurons of the ventrolateral PAG. Indeed, many authors have proposed that, during exposure to environmental threats, the dorsal PAG may inhibit the ventrolateral PAG, and some support for this comes from in vitro studies with PAG slices (see [7]).

According to the present results, exposure to predator also induced increased Fos expression in the ventromedial region of the PAG, where a substantial number of immunoreactive cells were found in the supraoculomotor region, as well as in the dorsal raphe and laterodorsal tegmental nuclei. In contrast to the present findings, however, in our previous study examining the pattern of Fos expression in the PAG of rats exposed to a natural predator [15], we have found only a sparse number of Fos-immunoreactive cells in the ventromedial PAG. Since the experimental paradigms used for exposing the prey to the predator were very similar in both studies, we believe that this discrepancy may well be related to the higher sensitivity of the anti-Fos serum used in the present experiments. Corroborating this idea is the fact that, in the present analysis, after confrontation with the predator, the number of Fos-immunoreactive cells found in the dorsomedial and dorsolateral PAG was approximately three times higher than previously reported in our former study.

Several behavioral situations associated with high levels of alertness (e.g. physically aversive foot shock [39], swim stress, and insect predation) are likely to upregulate Fos expression in the supraoculomotor region and the laterodorsal tegmental nucleus. Although a great deal still remains to be learned about the connectivity and putative functional roles of the supraoculomotor region, a number of evidence associates this region with the oculomotor control [59]. Conversely, the laterodorsal tegmental nucleus, which provides extensive cholinergic projections to the forebrain [12], is thought to be particularly involved in modulating global attentive states in response to novel stimuli [37].

As far as the dorsal raphe nucleus is concerned, it is noteworthy that the nucleus also upregulates Fos expression in response to other stressful situations [6,31,35,38,54]. To our knowledge, the particular roles played by the dorsal raphe nucleus during the encounter with a predator have never been previously addressed. However, for the present context, it is important to know that this nucleus appears to mediate, at least partly, the behavioral depression occurring in response to uncontrollable stressors (i.e. inescapable tailshock) [29,30], which may somehow be compared with our experimental paradigm where the animals had no chance of escaping from the predator.

Quite strikingly, an opposite pattern of PAG activation was seen in animals that performed insect predation, thus, corroborating the idea that predatory behavior appears to be favored whenever the neural circuitry underlying defensive responses is not particularly activated (see Section 1). In sharp contrast to what was found for the animals confronted with a live predator, predatory hunting is associated with increased Fos expression predominantly in the rostral two thirds of the lateral PAG, as well as, to a lesser degree, in the
supraoculomotor region and the laterodorsal tegmental nucleus. In these experiments, the majority of the Fos-immunoreactive cells were found at the levels of the oculomotor nucleus, where the labeled neurons tended to be clearly clustered in the outer half of the lateral PAG.

The present observations corroborate the idea that insect hunting performed by rats retains the basic behavioral patterns observed in other forms of predatory behavior in mammals [26]. During insect predation, the animals first tried to locate the prey by sniffing vigorously around the cage, reflecting, perhaps, a vivid foraging behavior; and they eventually caught the roaches, performing the predatory attack itself. Unfortunately, at the moment, it is not possible to ascertain if the pattern of PAG activation observed during the predatory hunting is associated with both foraging and predatory attack, or just one of these behavioral responses. According to preliminary data from our laboratory, increased Fos expression in this same region of the lateral PAG was also found after foraging behavior of animals that were allowed neither to predate insects nor to consume regular chow.

In contrast to the PAG regions mobilized in response to confrontation with the predator, the rostral two-thirds of the lateral PAG do not receive inputs from elements of the medial hypothalamic defensive system. However, similar to several other PAG sites, the portion of lateral PAG mobilized during insect predation is also substantially targeted by a number of medial prefrontal fields (e.g. prelimbic and anterior cingulate areas) [27], which, at the top of the perception-action cycle, are thought to play a critical role in the behavioral planning [28,36]. Moreover, this region of the lateral PAG is also known to receive moderate inputs from the medial part of the central amygdalar nucleus [49], where we have also observed increased Fos expression in response to predatory hunting [23]. Here, it is noteworthy that the central amygdalar nucleus appears to play a key role in the integration of the hedonic value of the aliments [44]; and the nucleus is also known to be involved in the acquisition of conditioned approach responses to palatable food [32], reflecting, perhaps, its potential role in controlling foraging behavior. Furthermore, previous anterograde tract-tracing studies have shown that this particular region of the lateral PAG, mobilized during insect predation, also receives massive inputs from the retrochiasmatic area [48] and the anterior part of the ventromedial hypothalamic nucleus [16]. While the putative functional roles of this latter hypothalamic region remain yet to be investigated, there is experimental evidence indicating that the retrochiasmatic area is intimately related to the hypothalamic circuitry that regulates feeding [60]. Therefore, in addition to the medial prefrontal cortex, the region of the lateral PAG activated during predatory hunting also appears to be influenced by prosencephalic sites seemingly involved in regulating foraging and feeding behaviors. Although feeding and predatory behaviors are likely to be subserved by distinct neural systems (see [5]), it has been shown that food-deprivation facilitates the expression of predatory behavior [58].

The region of the lateral PAG here described as upregulating Fos expression in response to predatory hunting also receives substantial somatosensory input from the cervical levels of the spinal cord and the laminar spinal trigeminal nucleus, thought to convey nociceptive and thermoreceptive information, respectively, from the region of the forelimbs and face [11]. Consistent with the anatomy, functional studies have indicated that physical stressors, such as cutaneous pain, evoke Fos expression within the lateral PAG [35,39]. Considering the experimental paradigm used for insect predation in our study, it is quite unlikely that the increased Fos expression in the lateral PAG, observed in the present experiments, is correlated with any physical injury occurred during confrontation with the roaches. Nevertheless, it is important to note that stimulation of the lateral PAG has been classically associated with increased somatomotor activity, sympathoexcitation, and non-opioid-mediated analgesia [3,20,24,41,43], which, in the context of the predatory hunting, may represent a very adaptive defensive mechanism against potential harms that may occur during the confrontation with the prey.

The efferent projections of this particular region of the lateral PAG mobilized during predatory hunting have yet to be critically analyzed. However, taken the rostral region of the lateral PAG as a whole, previous anatomical studies have shown that it provides extensive descending projections to a number of brainstem sites critically involved in controlling somatomotor and autonomnic responses [14]. Moreover, the rostral region of the lateral PAG has also been shown to project extensively to the lateral hypothalamic area, perhaps reflecting its potential roles in the control of generalized arousal and sensorimotor integration [13]. Notably, this hypothalamic projection also targets a particular region in the lateral hypothalamic area classically known to elicit predatory attack when electrically stimulated (see [50]).

In conclusion, the PAG integrates a vast array of limbic information, likely to reflect the animal’s motivational status; and we have presently shown that behaviors associated with opposite motivational drives, such as defensive and predatory behaviors, evoke a quite distinct PAG activation pattern. On the behavioral side, it is well established that the PAG is critical for the expression of defensive responses, and, considering the present findings, it will be important to investigate how the PAG contributes to the expression of the predatory behavior, as well. Therefore, in the future, we will be able to determine to what extent the PAG influences the
switch of goal-oriented behaviors in response to different motivational drives.

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