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FUNCTIONAL MAPPING OF THE PROSENCEPHALIC SYSTEMS INVOLVED IN ORGANIZING PREDATORY BEHAVIOR IN RATS

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Abstract—The study of the neural basis of predatory behavior has been largely neglected over the recent years. Using an ethologically based approach, we presently delineate the prosencephalic systems mobilized during predation by examining Fos immunoreactivity in rats performing insect hunting. These results were further compared with those obtained from animals killed after the early nocturnal surge of food ingestion. First, predatory behavior was associated with a distinct Fos up-regulation in the ventrolateral caudoputamen at intermediate rostro-caudal levels, suggesting a possible candidate to organize the stereotyped sequence of actions seen during insect hunting. Insect predation also presented conspicuous mobilization of a neural network formed by a distinct amygdalar circuit (i.e. the postpiriform-transition area, the anterior part of cortical nucleus, anterior part of basomedial nucleus, posterior part of basolateral nucleus, and medial part of central nucleus) and affiliated sites in the bed nuclei of the stria terminalis (i.e. the rhomboid nucleus) and in the hypothalamus (i.e. the parasubthalamic nucleus). Accordingly, this network is likely to encode prey-related motivational values, such as prey's odor and taste, and to influence autonomic and motor control accompanying predatory eating. Notably, regular food intake was also associated with a relatively weak Fos up-regulation in this network. However, during regular surge of food intake, we observed a much larger mobilization in hypothalamic sites related to the homeostatic control of eating, namely, the arcuate nucleus and autonomic parts of the paraventricular nucleus. Overall, the present findings suggest potential neural systems involved in integrating prey-related motivational values and in organizing the stereotyped sequences of action seen during predation. Moreover, the comparison with regular food intake contrasts putative neural mechanisms controlling predatory related eating vs. regular food intake. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: aggression, feeding behavior, amygdala, hypothalamus, basal ganglia.

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Abbreviations: ABC, avidin–biotin complex; BST, bed nuclei of the stria terminalis; Fos-ir, Fos immunoreactive; fx, fornix; GPl, globus pallidus, lateral segment; MCH, melanin-concentrating hormone.

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Predatory hunting has been regarded as an innate behavioral response seemingly critical for the animals' survival (Eisenberg and Leyhausen, 1972). Most of our knowledge regarding the neural basis of this behavior derives from studies done in the 1960s and 1970s, based on the use of lesion and electrical stimulation methods in cats and rodents. These studies suggest that the organization of predatory attack depends upon sites along the length of the lateral hypothalamus and is mediated by a descending limb of the medial forebrain bundle passing through the ventral tegmental area to the ventral mesencephalic and pontine tegmentum (Egger and Flynn, 1963; Sheard and Flynn, 1967; Chi and Flynn, 1971; Bandler et al., 1972; Berntson, 1972, 1973; Proshansky et al., 1974).

However, due to methodological constraints, these studies were limited in terms of providing a clear definition of the neural systems underlying predatory behavior that occurs under natural conditions. This seems to be particularly true for the studies on rodents, where, depending on the intensity of stimulation, a variety of aggressive responses—ranging from defensive to quiet biting attack—could be evoked from what had been defined as the hypothalamic attack area (see Siegel et al., 1999).

An ethologically based approach for studying predatory behavior was attempted with the mouse-killing paradigm, largely explored by Karli and colleagues (Vergnes and Karli, 1963, 1972; Chaurand et al., 1972; Vergnes, 1975). Unfortunately, this paradigm presents serious limitations constraining its use. Animals need to be food deprived for a few days to present mouse-killing behavior, which will be expressed only by a small percentage of rats (around 16%; Vergnes, 1975). Moreover, the confrontation with a live mouse is frequently associated with overt defensive reactions, such as freezing and flight; therefore, part of the attack episodes may be, in fact, related to defensive behavior.

To circumvent these problems, insect hunting appears as an ideal condition to investigate predatory behavior in rats. In this paradigm, roaches have been chosen as suitable prey, since they are relatively innocuous and easily overcome; likewise, they do not seem to induce appreciable defensive reactions in rats. In addition, considering the voracity that the rats present to consume the roaches, they are supposedly very palatable with potentially high hedonic value. Remarkably, in a recent work, we were able to demonstrate that insect predation and exposure to a natural predator induce an opposite activation pattern of the periaqueductal gray, reflecting, perhaps, the diverse motivational drives underlying each of these responses (Comoli et al., 2003). Importantly, as confirmed for several

different rat strains tested, cockroach predation has been shown to be vividly expressed by all individuals (Rebouças and Schmidek, 1997).

In the present study, we attempted to delineate the prosencephalic sites involved in the integration of innate predatory responses by examining Fos immunoreactivity in the prosencephalon of rats performing insect hunting. To differentiate the Fos increase likely to be related to food intake, the results were further compared with those obtained from animals killed after the early nocturnal surge of food ingestion.

EXPERIMENTAL PROCEDURES

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 light/dark cycle) in the animal quarters, and had free access to water and standard laboratory diet (Nutrilab CR1; Nuvital Nutrientes, Ribeirão Preto, SP, Brazil). Experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996) and the University of São Paulo's Institute of Biomedical Sciences Committee for Ethics and Animal Care in Experimental Research. In the present study, we attempted to minimize the number of animals used and their suffering.

One week before the experimental procedures, animals were individually housed into a Plexiglas cage (50×35×16 cm), and were handled repeatedly by the same investigator who conducted the behavioral tests. Two experimental groups were tested between 15:00 and 16:00 h. One of these 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. The other group of animals ($n=5$) served as controls; they were housed and handled in the same way as the animals that performed insect predation, but were left undisturbed in the cage during the test period. A third experimental group ($n=5$) was tested during the first hour of the dark period, between 18:00 and 19:00 h, when the animals were highly active and presented the early surge of food intake.

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% paraformaldehyde 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. One series of sections was processed for immunohistochemistry with anti-Fos antiserum raised in rabbit (Ab-5; Calbiochem, San Diego, CA, USA; lot D09803) at a dilution of 1:10,000. The primary antiserum was localized using a variation of the avidin–biotin complex system (ABC; Hsu and Raine, 1981). In brief, sections were incubated for 90 min at room temperature in a solution of biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA), and then placed in the mixed avidin–biotin–horseradish peroxidase 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 (Sigma, St. Louis, MO, USA) 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 (pH 7.4). Sections were mounted on gelatin-coated slides, and then dehydrated and coverslipped with DPX (Sigma). An adjacent series was always stained with Thionin to serve as a reference series for cytoarchitectonic purposes.

The relative strength of expression of Fos immunoreactivity was evaluated by an observer without the knowledge of the experimental status using a semiquantitative rating scale derived from the mean values of Fos labeling density. In all animals of each experimental group, these measurements were taken from the prosencephalic regions, which were individually outlined at a selected level, and the Fos-labeled cells and the outlined area were quantified using a Nikon Eclipse E600 microscope (Nikon, Japan) (10× magnification) equipped with a Spot digital camera (Diagnostic Instruments, Inc., Sterling Heights, MI, USA) interfaced to an image analysis system (Image-Pro Plus; Media Cybernetics, Silver Spring, MD, USA).

To provide an independent assessment of the validity of these ratings, counts of the number of Fos immunoreactive (Fos-ir) neurons as a function of experimental status were generated for selected cell groups by using the 10× objective of a Nikon Eclipse E600 microscope equipped with a camera lucida. These were performed by counting all Fos-ir nuclei in a complete series of sections (where the sections were 120 μm apart) through the structures of interest, as defined in adjoining Thionin-stained series. The extrapolating estimated counts were obtained by using the method of Abercrombie (1946) that takes into account the crude count of number of Fos-ir nuclei seen in the sections, the thickness of the sections and the average length of the Fos-ir nuclei. These data were analyzed by a multivariate analysis of variance (one way MANOVA, where we treated cell counting in each selected region as dependent variables, and the experimental groups as the between-group independent variable), followed by multiple comparisons using the Tukey HSD test. The significant level was set at 5%. All the values are expressed as mean±S.E.M.

The figures were prepared for publication by using the Adobe Photoshop (version 4.0; Adobe Systems, Mountain View, CA, USA) for photomicrographs and Adobe Illustrator (version 7.0; Adobe Systems) for line drawings. Only sharpness, contrast, and brightness were adjusted. Unless otherwise indicated, parcellation of the prosencephalic regions follows Swanson (1992).

RESULTS

Before commenting on our experimental data, we shall consider the behaviors displayed by the different experimental groups. The rats exposed to the cockroaches showed a marked predatory behavior, immediately after the prey were introduced into the home cage. At first, the animals sniffed vigorously around the cage, and, as the prey object was located, the rats 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 had been 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 tested took less than 40 min (28.64±3.39 min) to consume the five roaches. In the group of animals killed after the early nocturnal surge of food ingestion, after the lights were turned off, the rats remained very active and consumed up to 4 g of chow during the first hour of the dark phase. Conversely, in the control group—rats killed before the early nocturnal surge of food ingestion and not allowed to hunt roaches—the animals were poorly active during the observation period, and consumed practically no food.

Table 1 summarizes the relative strength of Fos induction seen in all experimental groups. As shown in Table 1, Fos expression in the control rats, killed before the onset of the dark period and not allowed to perform predatory hunting, was low in most regions of the brain. The few areas in which substantial immunolabeling was observed have been previously identified as sites of constitutive Fos protein expression in nonmanipulated rats (Herdegen et al., 1995; Li and Sawchenko, 1998). Therefore, the following account will be mostly focused on the other experimental groups.

In the isocortex, the animals that hunted roaches displayed a striking Fos expression in the visceral and gustatory areas, in primary somatosensory areas corresponding to the body parts more directly involved in locating, catching, and killing the prey (i.e. vibrissae, nose, mouth and upper limb), as well as in the supplemental somatosensory area. Additionally, in this experimental group, a substantial increase in Fos immunolabeling was also found in a number of other visceral-related areas, including the agranular insular, infralimbic and prelimbic areas, as well as in particular association areas, such as the ectorhinal and ventral temporal association areas (Table 1). On the other hand, the animals killed after the early nocturnal surge of food ingestion presented a more widespread mobilization of the isocortex, where most of the motor, sensory, visceral and association areas displayed a large number of Fos-ir cells (Table 1).

Both the animals killed after performing insect predation and those killed after the early nocturnal surge of food ingestion presented a large mobilization of olfactory regions, where high levels of Fos expression were particularly found in the anterior olfactory nucleus and rostral part of the piriform cortex (Table 1). Furthermore, predatory hunting, but not the other experimental conditions, was also associated with a significant increase in Fos immunolabeling in the postpiriform-transition area, mostly distributed throughout the rostral two-thirds of this olfactory region (Table 2; Fig. 1B).

Predatory hunting was also associated with a substantial increase in Fos levels in a particular group of amygdalar nuclei, namely, the anterior part of cortical nucleus, anterior part of basomedial nucleus, posterior part of basolateral nucleus, and medial part of central nucleus (Tables 1 and 2; Fig. 1A, B). We have also observed that these amygdalar cell groups also up-regulate Fos expression, though clearly less intensely, in the animals killed after the early surge of food ingestion (Tables 1 and 2; Fig. 1C, D). Moreover, both experimental conditions also induced comparable increase in Fos levels in other amygdalar sites, including the medial nucleus, nucleus of the lateral olfactory tract, and the anterior basolateral nucleus (Tables 1 and 2; Fig. 1).

Both the animals that performed insect hunting and those that had regular surge of food ingestion up-regulate Fos expression in a number of regions in the anterior division of the bed nuclei of the stria terminalis (BST; Table 1). In the BST, compared with the other experimental groups, predatory hunting was associated with a distinct increase in Fos expression in the rhomboid nucleus and the rostral levels of the anterolateral area (Table 2, Fig. 2A). For the rest of the septal region, only a relatively sparse Fos immunolabeling was observed in all experimental groups (Table 1).

As opposed to the other experimental groups, in animals that had their first predatory hunting experience, a large increase in the number of Fos-ir cells was seen in the ventrolateral part of the caudoputamen at intermediate rostro-caudal levels (Table 1; Fig. 3A), which seems to correspond to the striatal region controlling oral and forepaw motor functions (see Dickson et al., 1994). Additionally, predatory hunting was also associated with increased Fos levels in the caudal part of the caudoputamen (Table 1; Fig. 3B). Conversely, both the animals killed after the first hour of the dark period and those killed after performing insect predation up-regulated Fos expression in the nucleus accumbens (Table 1 and 2). Of particular relevance, compared with the other experimental groups, the animals killed after the onset of the dark period presented a significantly larger number of Fos-ir cells both in the shell and the core regions of the nucleus (Table 2).

As shown in Table 1, in the dorsal thalamus, both experimental groups (i.e. the animals killed after the first hour of the dark period and those killed after performing insect predation) presented marked increase in Fos levels in elements of the midline, intralaminar and anterior groups, as well as in the intermediodorsal nucleus. In the midline group, increased Fos immunolabeling was mostly found in the paraventricular and rhomboid nuclei, while, in the anterior group, a conspicuous up-regulation in Fos levels was observed in the anteromedial, anterodorsal, interanteromedial and interanterodorsal nuclei. Most of the dorsal thalamic sites shown here to up-regulate Fos expression appear to be more mobilized in animals during the first hour of the dark period, which coincides with the early nocturnal peak of activity when the animals are particularly alert.

In the ventral thalamus, all experimental groups presented a large number of Fos-ir cells in the intergeniculate leaflet and the ventral part of the lateral geniculate complex. Conversely, in the rostral zona incerta, a significant increase in Fos levels was more restricted to the animals killed after predatory hunting and those killed after the early nocturnal surge of food intake (Table 1).

In the hypothalamus, both the animals that performed predatory hunting and those that had regular food intake presented a significant increase in the number of Fos-ir cells in the magnocellular cell groups, mostly distributed to the supraoptic nucleus, and also, to a lesser degree, to the rostral two-thirds of the magnocellular parts of the paraventricular nucleus (Table 1). Notably, compared with predatory hunting, regular surge of food ingestion appears to be associated with a significantly larger mobilization of these magnocellular cell groups (Table 2). Similar figures were also found for the preautonomic parts of the paraventricular nucleus, where both experimental conditions induced a clear increase in Fos levels, which were significantly higher in the group of animals killed after the regular surge of food intake (Table 2, Fig. 4A, C). Likewise, both experimental conditions were also associated with in-

Table 1. Strength and distribution of Fos expression[a]

	Experimental groups		
	Control	Surge of food intake	Predatory hunting
Isocortex			
Motor areas			
Primary motor area	−	+++	+
Secondary motor area	+	+++	+
Agranular insular area			
Dorsal part	+	++++	++
Ventral part	+	+++	++
Posterior part	+	+++	++
Anterior cingulate areas	−	+++++	+
Auditory areas	+	+++	+
Ectorhinal area	+	++	+++
Gustatory area	+	+++++	+++++
Infralimbic area	+	+++	++
Perirhinal area	−	+	+
Posterior parietal association areas	+	+++++	+
Prelimbic area	+	+++	++
Retrosplenial area	−	+++++	−
Somatosensory areas			
Primary somatosensory area			
Barrel field	−	+++	+++++
Lower limb	+	+++	+
Mouth	−	++++	+++++
Nose	−	+++++	+++++
Trunk	+	++	+
Upper limb	+	+++++	+++++
Suplemental somatosensory area	+	+++++	+++++
Ventral temporal association areas	+	+++	++
Visceral area	+	+++++	+++++
Visual areas	+	+++++	+
Olfactory cortex			
Anterior olfactory nucleus	−	++++	++++
Olfactory tubercle	−	+	+
Piriform area			
Rostral part	+	+++	+++
Caudal part	−	+	+
Endopiriform nucleus	+	+	+
Postpiriform transition area	−	−	+
Hippocampal formation			
Entorhinal area			
Lateral part	−	+	+
Medial part	−	+	−
Presubiculum	−	−	−
Postsubiculum	−	−	−
Parasubiculum	−	−	−
Subiculum	−	−	−
Ammon's horn	−	−	−
Dentate gyrus	−	−	−
Amygdala			
Nucleus of the lateral olfactory tract	+	++	++
Medial nucleus	+	++	++
Cortical nucleus			
Anterior part	+	+++	+++++
Posterior part	+	+	+
Central nucleus			
Medial part	−	+	+++
Lateral part	+	+	+
Lateral nucleus	+	+	+
Basolateral nucleus			
Anterior part	+	++	++
Posterior part	−	++	+++
Basomedial nucleus			
Anterior part	+	++	+++
Posterior part	−	−	−
Posterior nucleus	−	−	−
Septal region			
Lateral nucleus			
Dorsal part	−	+	+
Intermediate part	−	+	−
Ventral part	+	+	+
Septofimbrial nucleus	−	−	−
Triangular nucleus	−	+	−
Medial nucleus/n. diagonal band	−	−	−
Bed nuclei of the stria terminalis			
Anterior division			
Anterodorsal area	−	+	+
Anterolateral area	−	+	+++
Anteroventral area	−	+	+
Oval nucleus	−	+	+
Rhomboid nucleus	−	−	+++
Dorsomedial nucleus	−	−	−
Dorsolateral nucleus	−	−	−
Fusiform nucleus	−	+	+
Magnocellular nucleus	−	−	−
Posterior division			
Principal nucleus	−	−	−
Interfascicular nucleus	−	+	+
Transverse nucleus	−	−	−
Basal ganglia			
Caudoputamen			
Anterior part	−	−	−
Intermediate part			
Dorsal	−	−	+
Ventrolateral	−	−	+++
Posterior part	−	−	+++
Nucleus accumbens			
Shell region	+	++++	++
Core region	+	+++	++
Globus pallidus	−	−	−
Thalamus			
Epithalamus			
Lateral habenula	−	+	−
Medial habenula	−	−	−
Dorsal thalamus			
Midline group			
Paraventricular nucleus	++	++++	+++
Parataenial nucleus	+	+	+
Nucleus reuniens	−	++	+
Rhomboid nucleus	+	+++++	+++++

Table 1. Continued

	Experimental groups		
	Control	Surge of food intake	Predatory hunting
Anterior group			
Anteroventral nucleus	−	+	+
Anteromedial nucleus	+	++++	++
Anteromedial nucleus	−	++++	++
Interanteromedial nucleus	+	+++++	++
Interanterodorsal nucleus	++	++++	++++
Medial group			
Mediodorsal nucleus	−	+	−
Intermediodorsal nucleus	−	+++	++
Lateral group	−	−	−
Ventral group			
Ventral anterior	−	−	−
Ventral medial	−	−	−
Ventral posterior	−	−	−
Gustatory nucleus	−	−	−
Posterior complex	−	−	−
Geniculate group			
Medial geniculate nucleus	−	−	−
Lateral geniculate nucleus	−	−	−
Intralaminar group	−	+++	+++
Ventral thalamus			
Reticular nucleus	−	−	−
Intergeniculate leaflet	+++++	+++++	+++++
Lateral geniculate complex, ventral part	++	+++	+++
Zona incerta			
Rostral	+	++++	+++
Intermediate	−	+	+
Caudal	−	+	+
Hypothalamus			
Periventricular zone			
Median preoptic nucleus	+	++++	+++
Anteroventral periventricular nucleus	+	+	+
Suprachiasmatic nucleus	+++++	+++++	+++++
Supraoptic nucleus	−	+++++	+++
Paraventricular nucleus			
Autonomic part	+	+++++	++
Parvicellular part	+	+++++	+
Magnocellular part	+	++++	++
Arcuate nucleus	−	+++++	++
Posterior periventricular nucleus	−	+	−
Medial zone			
Anteroventral preoptic nucleus	+	++++	+++
Anterodorsal preoptic nucleus	+	++	++
Parastrial nucleus	+	++	++
Medial preoptic nucleus	+	+	−
Anterior hypothalamic nucleus	+	++	+
Retrochiasmatic area	+	++	+
Ventromedial nucleus	−	+	+
Dorsomedial nucleus	+	++	++
Tuberomammillary nucleus	−	+++++	+
Premammillary nuclei	−	+	−
Supramammillary nucleus	+	++	+
Posterior hypothalamic nucleus	−	++	+
Lateral zone			
Lateral preoptic area	+	+	+
Lateral hypothalamic area			
Anterior	−	+	−
Tuberal	+	++	++
Parasubthalamic nucleus	−	++	+++

[a] Ratings reflect the density of positively labeled cells (−, 0-50 cells/mm^2; +, 51-150 cells/mm^2; ++, 151-250 cells/mm^2; +++, 251-350 cells/mm^2; ++++, 351-450 cells/mm^2; +++++>451 cells/mm^2).

creased mobilization of the arcuate nucleus, which presented a significantly larger number of Fos-ir cells in the animals killed after the early nocturnal surge of food ingestion (Table 2, Fig. 4B, D). On the other hand, only the group of animals killed after the onset of the dark period, but not the other experimental groups, presented a conspicuous increase in Fos expression in the parvicellular parts of the paraventricular nucleus, where the Fos-ir cells tended to be mostly distributed to the region containing corticotrophin-releasing hormone cells (Table 2, Fig. 4A, C; see Swanson, 1987).

As shown in Table 1, both experimental conditions were also associated with increased Fos expression in elements of the periventricular and medial hypothalamic zones, namely, the median, anteroventral, and anterodorsal preoptic nuclei, as well as the parastrial and dorsomedial nuclei, thought to be related to a complex visceromotor pattern generator network involved in controlling hypothalamic neuroendocrine motoneuron pools and preautonomic parts of the paraventricular nucleus (Thompson and Swanson, 2003). Moreover, in the medial hypothalamic zone, the animals killed after the onset of the dark period also presented a striking increase in Fos expression in the tuberomammillary nucleus (Table 1).

In the present study, we also quantified the Fos expression in the intermediate hypothalamic area (see Geeraedts et al., 1990), which coincides with the hypothalamic attack area as defined on functional studies using chemical or electrical stimulation in the rat (see Siegel et al., 1999). According to our analysis, however, no significant difference in the Fos-ir cell counting was found among the different experimental conditions (Table 2).

In the lateral hypothalamus, both the animals killed after predatory hunting and those killed after the early nocturnal surge of food intake presented a significant increase in the number of Fos-ir cells located dorsolaterally to the fornix, at tuberal levels, in a region known to contain neurons expressing melanin-concentrating hormone (MCH) or orexins (Bitten-

Table 2. Estimated number of Fos-ir neurons in selected prosencephalic groups[a]

	Experimental groups		
	Control	Surge of food intake	Predatory hunting
Olfactory cortex			
Postpiriform transition area	101.4±14.13	91.2±13.68^{ns}	243.8±26.15*,†
Amygdala			
N. of the lateral olfactory tract	51.2±5.94	189±11.75*	207.8±17.36^{*,ns}
Medial nucleus	77.6±10,91	205±10.19*	200.8±20.59^{*,ns}
Cortical nucleus, anterior part	54.4±10,61	239.6±27,14*	463.8±37,62*,†
Basomedial nucleus, anterior part	118.4±18.55	325.2±40.29*	636.8±47.28*,†
Central nucleus			
Medial part	77±12.72	178.8±10.11*	443.6±35.32*,†
Lateral part	106.4±12.29	100.8±10.2^{ns}	87.2±11.23^{ns,ns}
Basolateral nucleus			
Anterior part	144±18.22	318.8±27.25*	286.8±18.4^{*,ns}
Posterior part	41.6±5.51	92.8±7.98*	149.8±14.76*,†
Lateral nucleus	93.2±8.02	97.4±11.42^{ns}	87.8±8.5^{ns,ns}
Septal region			
Bed nuclei of the stria terminalis			
Anterolateral area	21.2±2.9	38±3.78*	62±5.73*,†
Rhomboid nucleus	3.2±0.96	5.4±1.28^{ns}	27±2.91*,†
Basal ganglia			
Nucleus accumbens			
Shell region	115.4±21.09	861.6±43.61*	333.2±28.58*,†
Core region	174±25.94	599.6±40.67*	379±35.93*,†
Hypothalamus			
Supraoptic nucleus	22.8±4.26	252±24.59*	143.8±24.52*,†
Paraventricular nucleus			
Magnocellular part[b]	7.8±2.71	168.6±15.25*	57.6±7.24*,†
Parvicellular part[c]	16.2±3.4	272.6±18.8*	19.8±2.92^{ns,†}
Autonomic part[d]	18.2±2.76	94±6.04*	51±8.35*,†
Arcuate nucleus	35.4±7.14	630.2±56.68*	146.4±15.65*,†
Intermediate hypothalamic area	92.4±5.67	109.8±4.02^{ns}	99.2±10.27^{ns,ns}
Lateral area, tuberal region	97.6±7.85	471.6±32.82*	495.2±36.42^{*,ns}
Parasubthalamic nucleus	18.8±3.76	89.6±7.45*	160.4±13.54*,†

[a] Values are mean (±SEM) corrected (Abercrombie, 1946) counts of the number of Fos-ir neurons in complete series of sections through the indicated structures.

[b] Counts from the lateral and medial zones of the posterior magnocellular part.

[c] Counts from the anterior parvicellular, dorsal zone of the medial parvicellular, and periventricular parts.

[d] Counts from the dorsal parvicellular, ventral zone of the medial parvicellular, and lateral parcivellular parts.

* Differs significantly from control, $P<0.05$, ^{ns} $P>0.05$.

† Differs significantly from the experimental group of animals killed after the early nocturnal surge of food ingestion, $P<0.05$, ^{ns} $P>0.05$.

court et al., 1992; Sakurai et al., 1998; Table 2). Finally, a distinct cluster of Fos-ir neurons surrounding the medial edge of the subthalamic nucleus was particularly evident in animals that performed predatory hunting (Fig. 2B). This region corresponds to the parasubthalamic nucleus and also presented a smaller increase in Fos expression in the animals killed after the early nocturnal surge of food intake (Table 2).

DISCUSSION

In the present study, we outlined the prosencephalic sites seemingly involved in organizing predatory hunting, and compared these results with those obtained from animals that had had a regular food intake during the onset of the night cycle. In both situations, the animals were fully alert and ingested nutrients, either prey or regular laboratory chow. The present experimental approach enabled us to compare the neural control of homeostatic and prey-induced eating, in addition to suggesting the putative neural sites underlying the stereotyped sequence of actions seen during hunting procedures.

The validity of our observations is obviously constraint by the limitations of the methodology used in the present investigation. Although Fos protein expression has been used as a sensitive cellular marker for neuronal activation induced by a variety of stimuli (Morgan and Curran, 1991), it is important to keep in mind that the absence of neuronal Fos expression cannot be interpreted as lack of influence on neuronal activity. In line with this view, Fos expression is not induced by the opening of ionotropic channels that do not increase the intracellular levels of second messengers (Sheng and Greenberg, 1990); therefore, it provides only a partial view of the mobilized brain systems. With these caveats in mind, we shall proceed discussing our results.

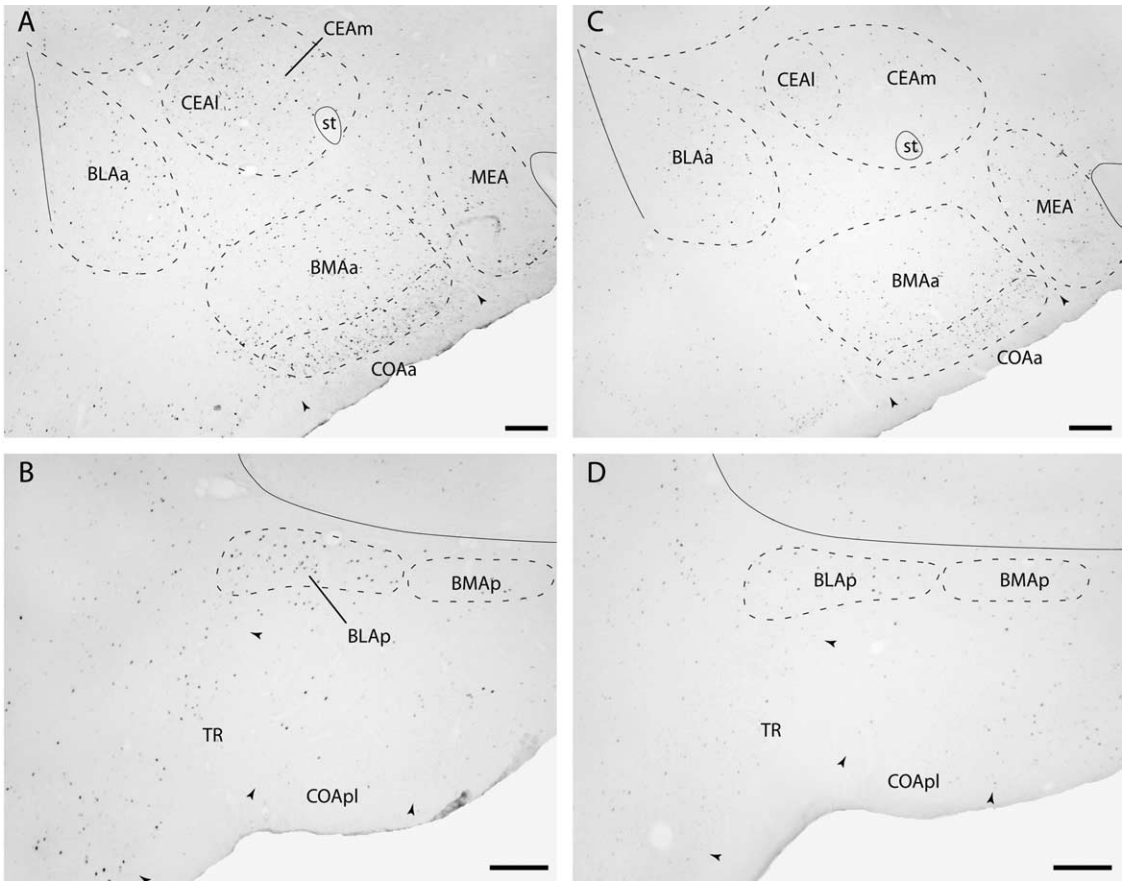

Fig. 1. Photomicrographs of transverse Fos-stained sections of the amygdalar region, at selected rostro-caudal levels (rostral: A, C; caudal: B, D), from a rat that performed predatory hunting (A, B) and from an animal killed after the early nocturnal surge of food ingestion. Left side of the brain. Scale bars=200 μm.

In our experiments, animals that hunted roaches were not particularly hungry since they were not eating the available food pellets by the time the cockroaches were introduced into the cage. Therefore, during insect predation, the prey—a very palatable food with potentially high hedonic value—were seemingly much more powerful than the homeostatic drives to trigger the food intake. Moreover, during insect hunting, the animals displayed a clear stereotyped sequence of actions to capture, kill and ingest the prey.

Abbreviations used in the figures

act	anterior commissure, temporal limb
ARH	arcuate nucleus hypothalamus
BLAa	basolateral nucleus amygdala, anterior part
BLAp	basolateral nucleus amygdala, posterior part
BMAa	basomedial nucleus amygdala, anterior part
BMAp	basomedial nucleus amygdala, posterior part
BSTal	bed nuclei of the stria terminalis, anterolateral area
BSTav	bed nuclei of the stria terminalis, anteroventral area
BSTmg	bed nuclei of the stria terminalis, magnocellular nucleus
BSTp	bed nuclei of the stria terminalis, principal nucleus
BSTrh	bed nuclei of the stria terminalis, rhomboid nucleus
CEAl	central nucleus amygdala, lateral part
CEAm	central nucleus amygdala, medial part
COApl	cortical nucleus amygdala, posterolateral part
CP	caudoputamen
cpd	cerebral peduncle
ec	external capsule
ME	median eminence
MEA	medial nucleus amygdale
PSTH	parasubthalamic nucleus
PVHdp	paraventricular nucleus hypothalamus, dorsal parvicellular part
PVHmpd	paraventricular nucleus hypothalamus, medial parvicellular part, dorsal zone
PVHmpv	paraventricular nucleus hypothalamus, medial parvicellular part, ventral zone
PVHpml	paraventricular nucleus hypothalamus, posterior magnocellular part, lateral zone
PVHpv	paraventricular nucleus hypothalamus, periventricular part
SI	substantia innominata
SNcl	substantia nigra, compact part, lateral region
st	stria terminalis
STN	subthalamic nucleus
TR	postpiriform transition area

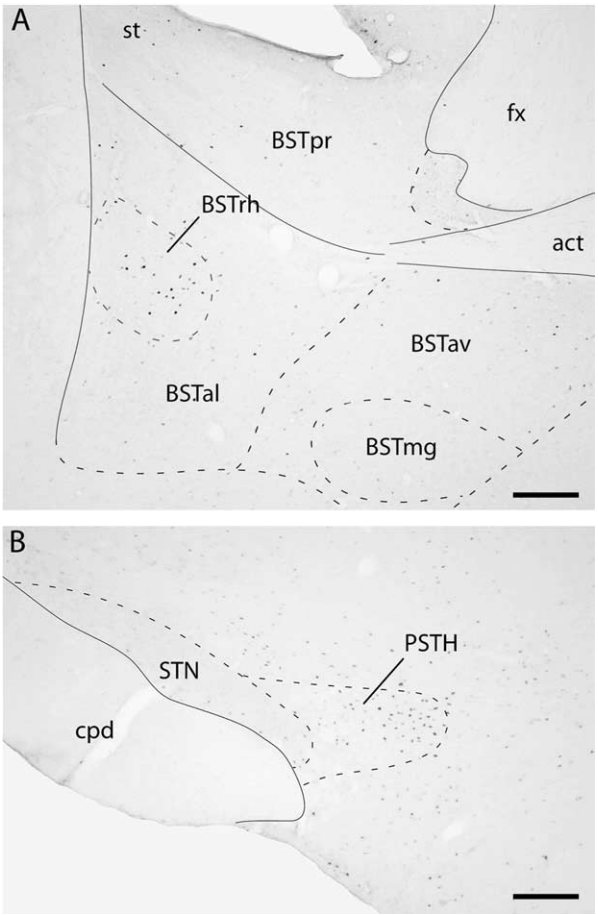

Fig. 2. Photomicrographs of transverse Fos-stained sections of the anterior division of the BST (A) and the parasubthalamic nucleus (B) from a rat that performed predatory hunting. Left side of the brain. Scale bars=200 μm.

Of particular relevance, we found that predatory hunting was associated with a conspicuous Fos expression in a distinct set of amygdalar nuclei (i.e. the anterior part of cortical nucleus, anterior part of basomedial nucleus, posterior part of basolateral nucleus, and medial part of central nucleus). Notably, regular food intake was associated with a relatively weak Fos up-regulation in these regions. In fact, these amygdalar nuclei, along with the postpiriform-transition area (here also shown to be mobilized during insect predation), are highly interconnected and form a distinct circuit involved in integrating taste and olfactory information related to feeding behavior (see Luskin and Price, 1983; Bernard et al., 1993; Petrovich et al., 1996). This amygdalar circuit also receives isocortical information from a number of visceral- and gustatory-related areas, namely, primary gustatory and visceral areas, visceral association areas (i.e. agranular insular region), and infralimbic and prelimbic areas (see McDonald, 1998), all of which are here shown to up-regulate Fos expression during insect predation. Taken together, the data support the view that this amygdalar circuit integrates a wealth of neural paths seemingly involved in relaying information regarding the prey's odor and taste, which may serve as critical motivational values to drive the predatory behavior.

Regarding the functional roles of this amygdalar system, it is well known that the central nucleus—the main output way station of the circuit—is involved in controlling feeding behavior (Minano et al., 1992; Kask and Schioth, 2000). In particular, the nucleus appears to integrate food hedonic values (Glass et al., 2000; Pomonis et al., 2000) and to influence searching and consumption of palatable food (Hitchcott and Phillips, 1998; Pomonis et al., 2000). In line with this view, the larger mobilization of this amygdalar system during insect predation may be reflecting, at least partly, the higher palatability of the prey as compared with regular chow. Moreover, in the context of predatory hunting, it would be important to examine to what extent this amygdalar circuit may influence the animals' drive to search, capture and consume the prey.

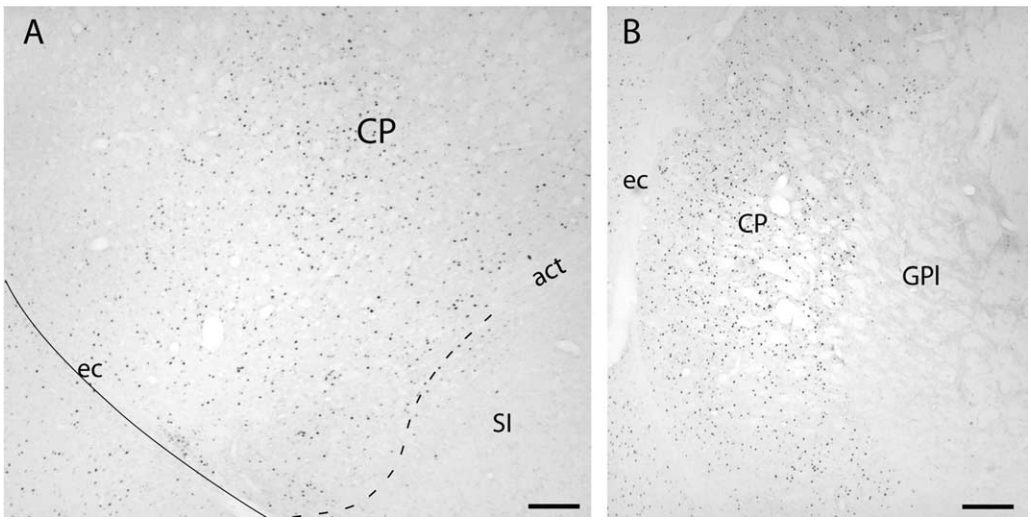

Fig. 3. Photomicrographs of transverse Fos-stained sections of the intermediate (A) and caudal (B) levels of the caudoputamen from a rat that performed predatory hunting. Left side of the brain. Scale bars=200 μm.

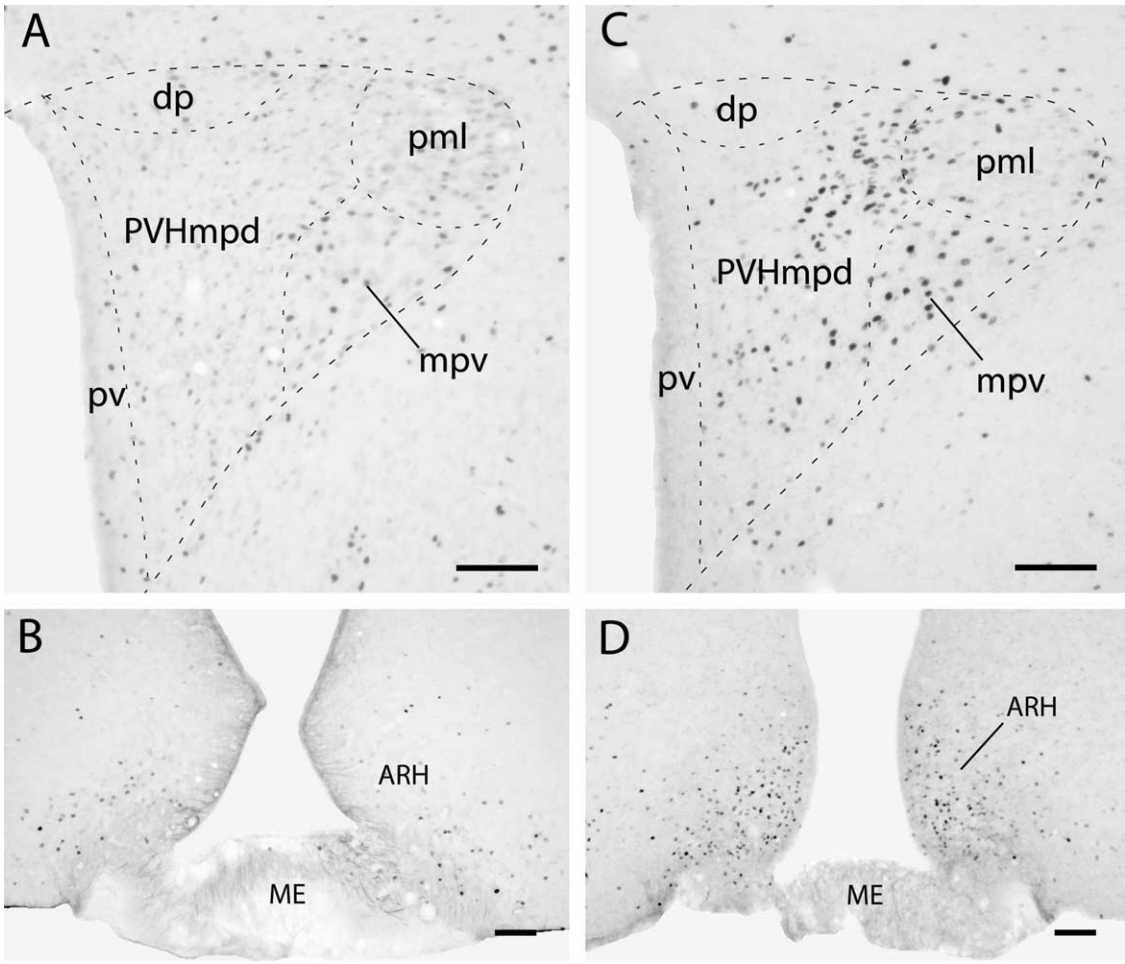

Fig. 4. Photomicrographs of transverse Fos-stained sections of the paraventricular (A, C) and arcuate nucleus (B, D) from a rat that performed predatory hunting (A, B) and from an animal killed after the early nocturnal surge of food ingestion (C, D). Left side of the brain. Scale bars=100 μm.

Curiously, the central nucleus of the amygdala has also long been known as an important amygdalar output site for expression of conditioned fear responses. However, recent experimental data indicate that central amygdalar neurons are not essential for conditioned, nor are they essential for unconditioned fear responses (Koo and Kim, 2003; Li et al., 2004). Indeed, the amygdalar pathway here shown to be mobilized during predatory hunting is distinct from those that mediate innate fear responses to a live predator (see Canteras et al., 2001).

Insect predation was also associated with a significantly larger Fos expression in the anterolateral area and rhomboid nucleus of the BST, and the parasubthalamic nucleus, which represent major projection targets of this amygdalar circuit considered to integrate prey-related motivational values. Anatomical studies have shown that the rhomboid nucleus of the BST and the parasubthalamic nucleus, along with the central amygdalar nucleus, provide inputs to hindbrain control regions involved in influencing the motor output associated with feeding, and in modulating digestive and metabolic responses occurring in both cephalic and consummatory phases of feeding behavior (see Dong and Swanson, 2003; Goto and Swanson, 2004).

Animals that performed predatory hunting also presented a distinct Fos up-regulation in the ventrolateral caudoputamen at intermediate rostro-caudal levels. On functional grounds, there is a wealth of data implicating the intermediate ventrolateral caudoputamen in controlling orofacial movements and forepaw usage accompanying feeding behavior (Dunnett and Iversen, 1982; Pisa, 1988; Pisa and Schranz, 1988; Bakshi and Kelley, 1991; Salamone et al., 1993). Furthermore, injection of low amphetamine doses into this striatal region stimulates feeding in satiated animals (Kelley et al., 1989), and produces conditioned place preference (Baker et al., 1998), suggesting a potential role in reward mechanisms.

As suggested for other forms of stereotyped behaviors, it is also important to consider that the intermediate rostrocaudal levels of ventrolateral caudoputamen may as well be viewed as a possible candidate to organize the stereotyped sequence of actions—action syntax—observed during predatory hunting (see Cromwell and Berridge, 1996). Importantly, the amygdalar circuit here considered as integrating

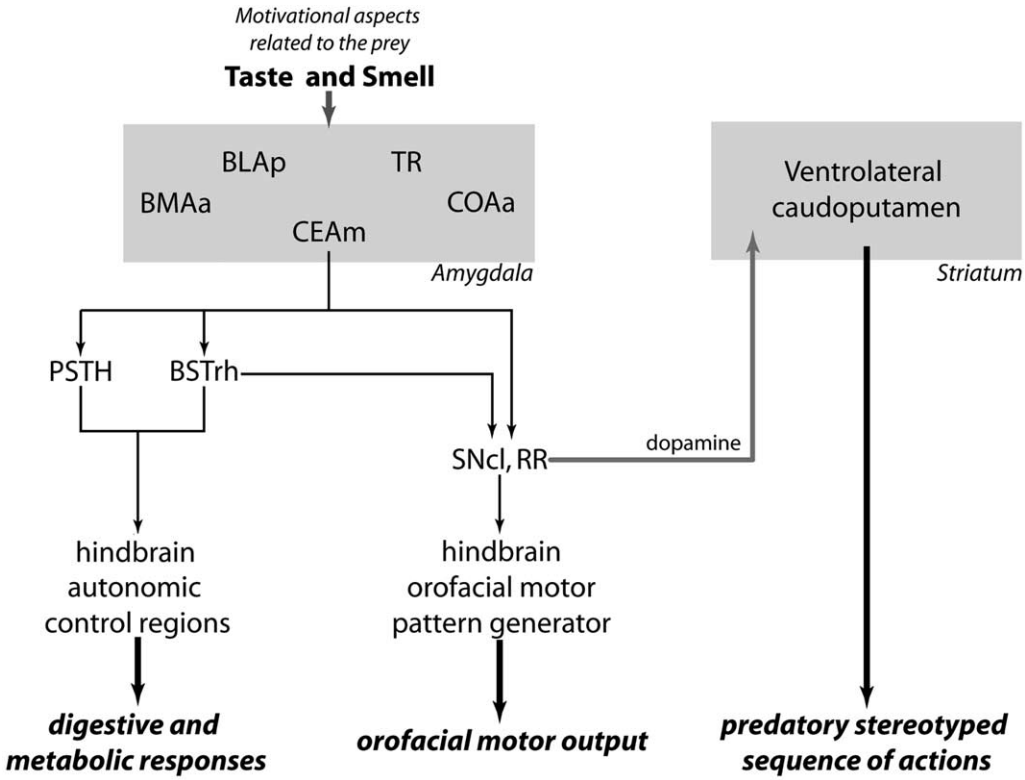

Fig. 5. Schematic diagram showing putative brain systems involved in organizing predatory hunting.

prey-related motivational values, as well as affiliated BST sites, can reach the intermediate ventrolateral caudoputamen via a dopaminergic path from the retrorubral field (Deutch et al., 1988; Dong and Swanson, 2003), and it is also in a position to modulate reward and stereotyped motor responses related to predatory behavior.

Insect predation was also associated with increased Fos levels in caudal striatal regions. Notably, caudal striatal regions have been mostly associated with sensory integration (McGeorge and Faull, 1989) and have been shown to modulate orienting responses (Fairley and Marshall, 1986), which are functions seemingly critical during the prey capturing procedures.

The evidence supports the view that predatory hunting relies on a neural network comprised by a particular amygdalar circuit and affiliated BST and hypothalamic sites. We hypothesize, as indicated in Fig. 5, that this network integrates the motivational aspects related to the prey, such as odor and taste, and influences autonomic and motor output accompanying predatory eating. Furthermore, we suggest that the intermediate ventrolateral caudoputamen may control the stereotyped sequence of actions seen during hunting procedures.

It is important to consider that the novelty associated with confronting a live cockroach has supposedly influenced, at least in part, the present results. Indeed, novelty has been associated with increased Fos expression in a number of prosencephalic sites here shown to be mobilized during predatory hunting, such as widespread isocortical areas, caudal caudoputamen, nucleus accumbens (shell and core), posterior part of the BST, and a number of amygdalar nuclei (i.e. the medial, lateral and anterior basolateral nuclei; Badiani et al., 1998; Emmert and Herman, 1999; Day et al., 2001).

Overall, our findings support the idea that predatory behavior should be considered as a particular class of feeding behavior. Therefore, to differentiate the Fos increase associated with the regular feeding, we have also examined animals that had a normal surge food intake during the first hour of the night cycle.

The onset of the dark period coincides with the time of the day when the animals are naturally awake and particularly alert. We have presently found that animals killed after this period presented a diffuse mobilization of the isocortex, as well as a striking Fos expression in a number of thalamic sites and in the tuberomammillary nucleus, known to modulate general levels of arousal (Sherin et al., 1998).

During the onset of the night cycle, the animals also present the early surge of regular food intake. Apparently, this surge of food ingestion was associated with a larger Fos expression in hypothalamic periventricular sites related to the homeostatic control of eating, namely, the arcuate nucleus and autonomic parts of the paraventricular nucleus (see Saper et al., 2002). These findings are consistent with the idea that the mechanisms supporting homeostatic drive for feeding are naturally operant at the onset of the dark period. On the other hand, predatory hunting was associated with a much smaller Fos up-regulation in these periventricular hypothalamic sites. Thus, suggesting that these circuits govern-

ing the homeostatic feeding may be less critical for controlling predatory behavior.

Animals killed after the first hour of the dark period presented a larger Fos expression both in the shell and the core regions of the nucleus accumbens. There is a wealth of experimental data favoring the idea that this mobilization of the nucleus accumbens during the onset of the dark period should be related to the accumbal function in modulating locomotor activity (Campbell et al., 1997; Zahm, 1999), instead of reflecting any possible role of the nucleus in controlling the early nocturnal surge of feeding. Corroborating this view, there is strong evidence indicating that regular food intake does not seem to depend upon the accumbal integrity (Koob et al., 1978). Nevertheless, the shell region of the nucleus, in particular, has been thought to modulate the intake of highly palatable food, and, thus, to be involved in controlling hedonic eating (see Saper et al., 2002). Perhaps, it is also involved in controlling predatory hunting, as well. Unfortunately, our data are inconclusive on this matter, and the role played by the accumbal circuits in controlling insect predation remains to be established.

Both predatory hunting and regular food intake were also associated with Fos up-regulation in hypothalamic magnocellular cell groups, mostly observed in the supraoptic nucleus, and also, to a lesser degree, in the rostral two thirds of the magnocellular parts of the paraventricular nucleus. Apparently, the ingestion of dry chow induced larger increase in the plasma osmolality and accounted for the larger mobilization seen in these magnocellular cell groups after regular surge of food intake. In line with this finding, the ingestion of dry chow was also associated with a larger Fos up-regulation in elements of the visceromotor pattern generator network involved in signalizing plasma osmolality, such as the median and anteroventral preoptic nuclei (see Thompson and Swanson, 2003).

In addition, both the animals killed after the onset of the dark period and those killed after performing predatory hunting presented comparable increase in Fos levels, at tuberal levels, in a region of the lateral hypothalamus that contains cell groups expressing MCH and orexins (also known as hypocretins; Bittencourt et al., 1992; Sakurai et al., 1998). Additionally, we have also observed that both experimental conditions also up-regulate Fos expression in another brain region containing MCH neurons: the rostral zona incerta (Bittencourt et al., 1992). These peptides are thought to play a key role in driving feeding likely to be mediated, at least in part, by enhancing arousal and locomotor activity (Saper et al., 2002). At this point, it would be important to determine to what extent cells expressing orexins or MCH up-regulate Fos expression in our experimental groups, and to see how these peptides modulate predatory hunting and the behavioral events observed during the onset of the dark period.

Finally, we have also quantified Fos expression in the hypothalamic attack area, which has been previously shown to yield a variety of aggressive responses ranging from defensive to quiet biting attack (see Siegel et al., 1999). On anatomical grounds, the hypothalamic attack area almost completely coincides with the intermediate hypothalamic area as delineated by Geeraedts et al. (1990). According to our observations, it does not seem to be particularly mobilized during predatory hunting. Nevertheless, previous findings from our laboratory indicate that this hypothalamic region up-regulates Fos expression during exposure to a predator and social agonistic encounters (Canteras et al., 2001, N. S. Canteras, personal observations), thus suggesting a potential role underlying other forms of aggressive responses, such as defensive attack and social aggression.

Overall, the present study points to important aspects on the neural organization of predatory behavior: first, by suggesting specific neural systems involved in integrating prey-related motivational values and in organizing the stereotyped sequences of action seen during predation; second, by revealing that different neural systems appear to mediate predatory-related eating and regular food intake. We are well aware that there is a great deal of experimental work that needs to be done to prove our hypothesis, which certainly serves as a solid ground for future research on the neural organization of predatory behavior.

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