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The medial hypothalamic defensive system: Hodological organization and functional implications

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Abstract

The hypothalamus is a relatively small division of the vertebrate forebrain that plays especially important roles in neural mechanisms assuring homeostasis, defense, and reproduction. Previous studies from our laboratory have suggested a distinct circuit in the medial hypothalamic zone as critically involved in the organization of innate defensive behavior. Thus, after exposure to a natural predator known to elicit innate defensive responses, increased Fos levels in the medial zone of the hypothalamus have been found restricted to the anterior hypothalamic nucleus, dorsomedial part of the ventromedial nucleus, and dorsal premammillary nucleus (PMd). Previous anatomical studies have shown that these Fos-responsive cell groups in the medial hypothalamus are interconnected in a distinct neural system, in which the PMd appears to be a critical element for the expression of defensive responses elicited by the presence of a predator. The purpose of this review is to provide an overview of what is currently known about the functional and hodological organization of this hypothalamic circuit subserving defensive responses.

Keywords: Defensive behavior; Hypothalamus; Septal area; Amygdala; Periaqueductal gray; Anterior hypothalamic nucleus; Ventromedial hypothalamic nucleus; Dorsal premammillary nucleus; Dorsomedial hypothalamic nucleus

1. Introduction

The hypothalamus is responsible for integrating various endocrine, autonomic, and behavioral responses that guarantee the survival of both the individual and the species. Broadly speaking, these responses are involved in regulating metabolism, providing an adequate supply of nutrients and water from the environment, allowing for the generation and care of offspring, and defending the animal from predators and other threats.

The seminal work of Bard (1928) and Hess and Brugger (1943) led to the widely accepted view that the hypothalamus plays an especially important role in the expression of defensive behavior. In an elegant series of experiments with decorticate cats displaying "sham rage" attacks, Bard (1928) localized the most critical area for the expression of defensive responses to the caudal half of the hypothalamus, and Wheatley (1944) later used electrolytic lesions to narrow the area even further to the region of the ventromedial and dorsomedial nuclei. A different approach was pioneered by Hess and Brugger (1943) who electrically stimulated various points throughout the diencephalon, and identified the perifornical region of the lateral hypothalamic area as a key site for eliciting integrated defensive responses. This was confirmed and extended by Hunsperger and colleagues who showed that electrical stimulation along a continuous pathway including the amygdala, stria terminalis, bed nucleus of the stria terminalis, perifornical region of the lateral hypothalamic area, and periaqueductal gray elicits defensive behavior (Hunsperger, 1956; Fernandez de Molina and Hunsperger, 1962). Furthermore, they provided strong evidence indicating that this is a hierarchically organized circuit. For example, responses to amygdalar or hypothalamic stimulation were abolished by lesions of the periaqueductal gray (PAG), while responses could still be elicited from the latter following lesions in the perifornical zone or amygdala (Hunsperger, 1956; Fernandez de Molina and Hunsperger, 1962). They also reported that, following adequate post-surgery recovery times, lesions centered in the perifornical

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zone of the hypothalamus did not significantly impair spontaneous defensive behavior of cats confronted with dog, while lesions of the PAG resulted in passive animals that were easily handled and rarely, if ever, showed defensive behavior (Hunsperger, 1956).

Hypothalamic regions from which defensive behavior can be elicited were subsequently expanded to include the medial zone, since a number of studies have shown that electrical stimulation of this region induces a pattern of somatomotor and autonomic responses that resembles the behavior of animals facing natural threats (Lipp and Hunsperger, 1978; Azevedo et al., 1980; Brutus et al., 1985; Fuchs et al., 1985; Yardley and Hilton, 1986; Lammers et al., 1988). The same reactions are elicited by microinjections of subtoxic doses of excitatory amino acids (Silveira and Graeff, 1992) and drugs which decrease GABAergic neurotransmission (Di Scala et al., 1984; Schmitt et al., 1985; Brandão et al. 1986; Milani and Graeff, 1987), indicating that this region contains groups of neurons commanding defensive behavior that are tonically inhibited by GABAergic neurotransmission. The defense reaction induced by the stimulation of the medial hypothalamus is characterized by coordinated rapid locomotions interspersed with well-directed attempts to escape (Di Scala et al., 1984; Brandão et al., 1986; Milani and Graeff, 1987; Silveira and Graeff, 1992), which contrasts with the explosive behavioral reaction evoked from the PAG, characterized by sudden running bouts and aimless vertical jumps (Di Scala et al., 1984; Bandler et al., 1985).

However, in contrast to the results reported by Hunsperger (1956), with lesions in the perifornical zone of the hypothalamus, small cell body-specific chemical lesions placed in caudal regions of the medial hypothalamic zone, encompassing the dorsal premammillary nucleus (PMd) in particular, significantly impair the expression of spontaneous defensive behavior of animals confronted with a predator, suggesting that this region is essential for the expression of behavioral responses to environmental threats (Canteras et al., 1997). The purpose of this review is to provide an overview of what is currently known about the organization of the hypothalamic circuitry subserving defensive responses.

2. Overview of the organization of hypothalamic systems

The hypothalamus is composed of three distinct longitudinal zones (periventricular, medial, and lateral), divided into four rostro-caudal levels or regions (preoptic, anterior, tuberal, and mammillary). The periventricular zone (Fig. 1A) contains most of the neuroendocrine motor neurons and sends important inputs to the autonomic motor system as

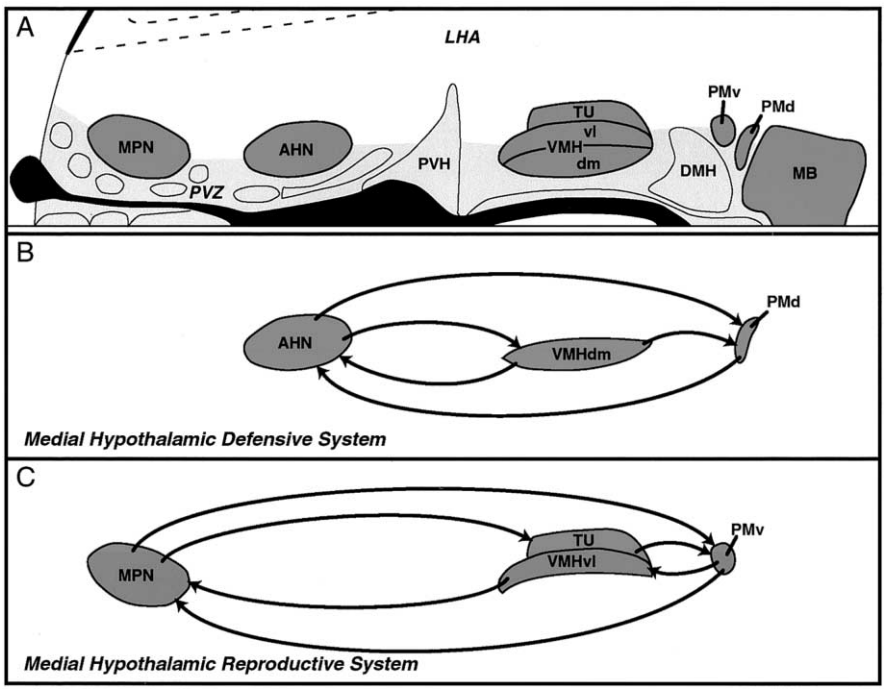

Fig. 1. (A) A schematic horizontal view of the rat brain to show the major subdivisions of the hypothalamus. (B,C) The organization of major direct connections between the components of the medial hypothalamic defensive (B) and reproductive (C) systems. See text for details. Abbreviations: AHN — anterior hypothalamic nucleus; DMH — dorsomedial hypothalamic nucleus; LHA — lateral hypothalamic area; MB — mammillary body; MPN — medial preoptic nucleus; PMd — dorsal premammillary nucleus; PMv — ventral premammillary nucleus; PVH — paraventricular hypothalamic nucleus; PVZ — periventricular hypothalamic zone; TU — tuberal nucleus; VMH, vl, dm — entromedial hypothalamic nucleus, ventrolateral part, dorsomedial part.

well (Swanson, 1987). Moreover, it also contains a complex neural network composed of a number of hypothalamic sites, including the median preoptic, anteroventral periventricular, anterodorsal preoptic, anteroventral preoptic, parastrial, suprachiasmatic, and dorsomedial nuclei, which, by analogy to the motor systems, are thought to represent premotor elements (perhaps acting like "motor" pattern generators) for the neuroendocrine and autonomic output (Thompson, 1997; Thompson and Swanson, 1998; Thompson et al., 1996).

The medial hypothalamic zone consists of a series of well-defined cell groups and is thought to play an important role in the initiation of specific motivated behaviors. A comprehensive analysis of the medial hypothalamic zone axonal projections with the *Phaseolus vulgaris*—leucoagglutinin technique indicates that the anterior hypothalamic nucleus (AHN), dorsomedial part of the ventromedial nucleus (VMHdm), and PMd are highly interconnected (Fig. 1B), and they are segregated from another medial zone circuit that includes the medial preoptic, ventrolateral part of the ventromedial, tuberal, and ventral premammillary nuclei (Fig. 1C) (Simerly and Swanson, 1988; Canteras and Swanson, 1992; Canteras et al., 1992, 1994; Risold et al., 1994). As we shall discuss below, the former hypothalamic circuit is involved in integrating innate defensive responses to environmental threats (Fig. 1B, medial hypothalamic defensive system), and the latter forms part of the sexually dimorphic circuit mediating reproductive and social agonistic behaviors (Fig. 1C, medial hypothalamic reproductive system) (Kollack-Walker and Newman, 1995; Coolen et al., 1996; Canteras et al., 1997).

The mammillary body represents another component of the medial zone of the hypothalamus, and consists of the medial and lateral mammillary nuclei. Differently from other medial hypothalamic sites, the mammillary body is intimately related to the hippocampus, on one hand, and with the anterior thalamic nuclei, on the other, and does not seem to be particularly involved in the initiation of specific motivated behaviors (Swanson, 1987). Instead, it seems particularly involved in subserving other neural functions like spatial working memory (Sziklas and Petrides, 1998) and navigation (Stackman and Taube, 1998).

3. Medial hypothalamic zone and defensive behavior

As previously mentioned, it is well known that either electrical or chemical stimulation of the medial hypothalamic zone may produce a pattern of somatomotor and autonomic responses that resembles the behavior of animals facing natural threats. Unfortunately, using this experimental approach, it is virtually impossible to have a precise delineation of the effective site of stimulation. In addition, this methodology does not allow to determine which medial hypothalamic sites are in fact implicated in modulating innate defensive responses under natural conditions.

Therefore, in order to delineate the hypothalamic circuits putatively involved in the integration of such responses, we started by examining Fos immunoreactivity in the hypothalamus of rats displaying defensive behavior during exposure to a cat, a natural predator (Canteras et al., 1997). It is well established that Fos-like protein expression is a sensitive cellular marker for neuronal activation induced by a variety of stimuli (Morgan and Curran, 1991). Direct exposure to the predator induced freezing responses ("postencounter defense") as well as episodes of vigorous running and jumping ("circa strike defense") in rats (see Blanchard et al.,1989), which, compared to control animals, presented upregulation of Fos expression in specific hypothalamic sites. Notably, in the medial hypothalamus, increased Fos levels were largely restricted to the circuit formed by the AHN, VMHdm, and PMd, and this last nucleus presented the most striking increase in Fos levels in the hypothalamus (Canteras et al., 1997). In addition, many Fos-immunoreactive neurons were found in other parts of the hypothalamus, including the perifornical region, dorsomedial nucleus, and lateral preoptic area.

In view of the overwhelming activation of the PMd during the predatory encounter, we next placed bilateral ibotenic acid lesions in this hypothalamic site to investigate its potential role in the expression of defensive responses. Remarkably, cell body-specific chemical lesions therein virtually eliminated the expression of escape and freezing responses during the predatory encounter (Canteras et al., 1997). Although the day-to-day behavior of these lesioned animals has not been examined systematically, the most obvious qualitative difference as compared to other animals was their docility and paucity of vocalization when handled (Canteras et al., 1997). Corroborating these findings, other studies have shown that either electrical stimulation (Yardley and Hilton, 1986) or microinjections of the GABA antagonist, bicuculline methiodide (Di Scala et al., 1984), into the region of the PMd in fact induce a pattern of somatomotor and autonomic responses resembling the behavior of animals facing natural threats. Therefore, putting together anatomical and functional findings, it was possible to bring into focus a distinct medial hypothalamic system critical for the expression of innate defensive behavior.

4. Neural systems related to the medial hypothalamic defensive system and their putative roles in innate defensive behavior

4.1. Neural inputs to the medial hypothalamic defensive system

As summarized in Fig. 2A, the medial hypothalamic defensive system (MHZ defensive system) receives inputs from widely distributed areas in the forebrain and, to a

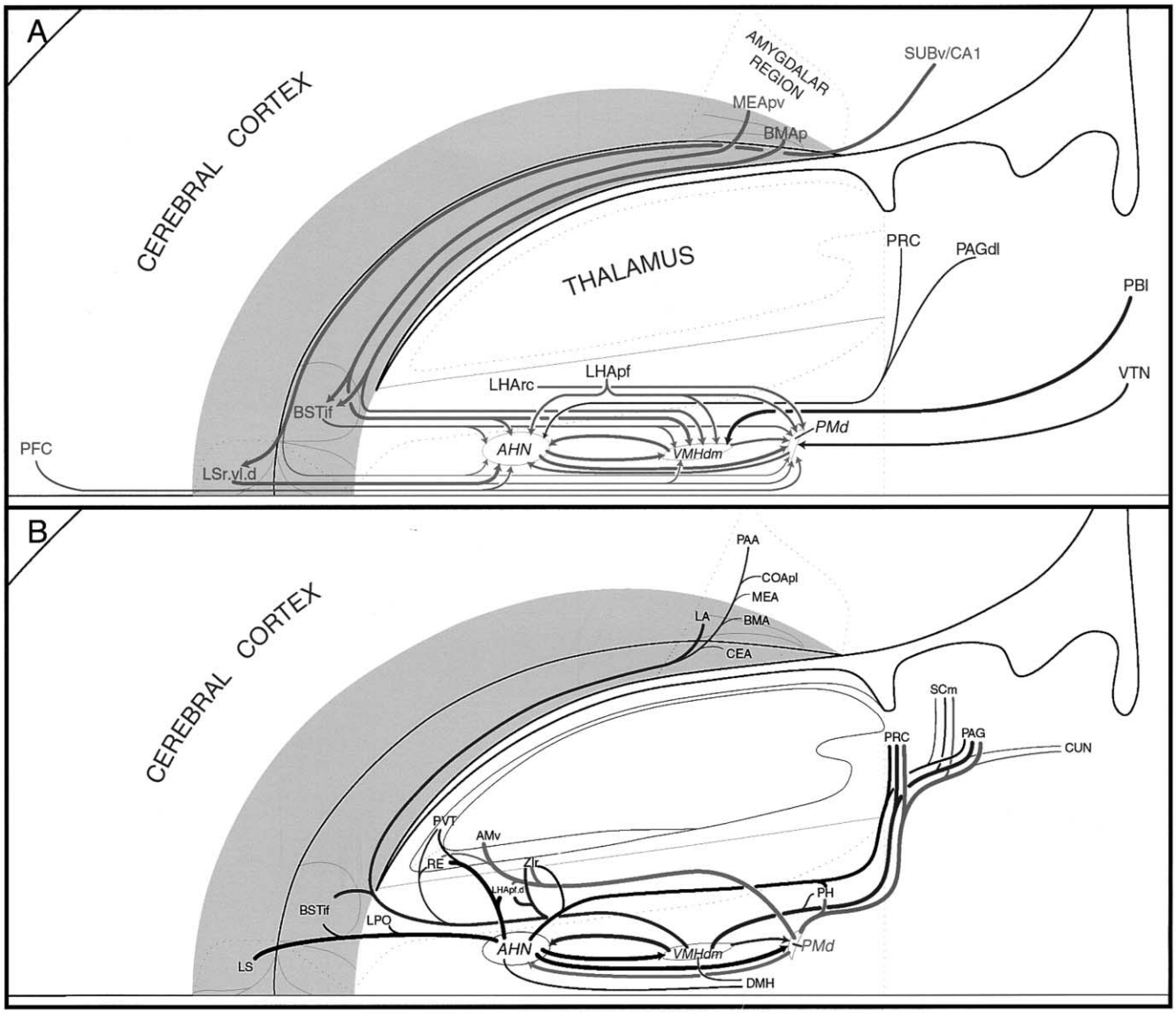

Fig. 2. (A) Neural inputs to the medial hypothalamic defensive system — primary sources of telencephalic, hypothalamic, and brainstem inputs to the elements of the medial hypothalamic defensive system. (B) Neural outputs of the medial hypothalamic defensive system — the general organization of projections from the anterior hypothalamic nucleus dorsomedial part of the ventromedial nucleus and dorsal premammillary nucleus. The magnitude of each pathway represented in (A) and (B) is roughly proportional to the thickness of line representing it. See text for details. Abbreviations: AHN — anterior hypothalamic nucleus; AMv — anteromedial thalamic nucleus, ventral part; BMA, p — basomedial amygdalar nucleus, posterior part; BSTif — bed nuclei of the stria terminalis, interfascicular nucleus; CA1—field CA1, Ammon's horn; CEA—central amygdalar nucleus; COApl — cortical amygdalar nucleus, posterolateral part; CUN — cuneiform nucleus; LA — lateral amygdalar nucleus; LHApf — lateral hypothalamic area, perifornical region; LHApfd — dorsomedial rostral perifornical region of the lateral hypothalamic area; LHArc — lateral hypothalamic area, retinoceptive region; LPO — lateral preoptic area; LS — lateral amygdalar nucleus; LS — lateral septal nucleus; LSrvld — lateral septal nucleus, rostral part, ventrolateral zone, dorsal region; MEApv — medial amygdalar nucleus, posteroventral part; PAA — piriform amygdaloid area; PAGdl — periaqueductal gray, dorsolateral part; PBl — parabrachial nucleus, lateral part; PFC — prefrontal cortex; PH — posterior hypothalamic nucleus; PMd — dorsal premammillary nucleus; PRC — precommissural nucleus; PVT — paraventricular thalamic nucleus; RE — nucleus reuniens; SCm — superior colliculus, medial region; SUBv — subiculum, ventral part; VMHdm — ventromedial hypothalamic nucleus, dorsomedial part; VTN — ventral tegmental nucleus; ZIr — zona incerta, rostral part.

lesser extent, from the brainstem as well. The major telencephalic sources of inputs to the MHZ defensive system are the posteroventral part of the medial amygdalar nucleus, the posterior part of the basomedial amygdalar nucleus, the lateral septal nucleus, and the interfascicular nucleus of the bed nuclei of the stria terminalis (BST). In addition, the infralimbic and prelimbic areas of the prefrontal cortex are also known to provide direct inputs to this hypothalamic system. The MHZ defensive circuit also receives significant intrahypothalamic inputs from specific regions of the lateral hypothalamic area, including the retinoceptive and perifornical regions. In contrast, only a

few sites in the brainstem appear to provide appreciable projections to this hypothalamic system, namely the precommissural nucleus, dorsolateral part of the periaqueductal gray, superior lateral subnucleus of the parabrachial area, and the ventral tegmental nucleus.

A number of studies have shown that the infralimbic and prelimbic areas of the prefrontal cortex provide a moderate projection to the AHN and PMd (Brittain, 1988; Comoli et al., 2000). Behavioral studies corroborate the idea that the medial prefrontal cortical areas exert a marked influence on the expression of defensive responses (Siegel and Chabora, 1971; Siegel et al., 1974), and the projection to these components of the MHZ defensive system may well represent a direct path for this control. Moreover, it is important to bear in mind that the medial prefrontal cortex may also provide massive inputs to the main brainstem sites targeted by the MHZ defensive system, namely the precommissural nucleus and the dorsolateral part of the periaqueductal gray (PAGdl) (Wyss and Sripanidkulchai, 1984; Sesack et al., 1989; Canteras and Goto, 1999a; Floyd et al., 2000).

The projection from the lateral septal nucleus to the MHZ defensive circuit arises predominantly from the dorsal region of the ventrolateral zone of the rostral part of the nucleus (LSrvld), which provides a strikingly dense projection to the ANH, in addition to a moderate innervation to the PMd and capsular region of the VMHdm (Risold and Swanson, 1997; Comoli et al., 2000). In the present context, it is important to note that one of the syndromes associated with lesions of the lateral septal nucleus is the hyperdefensiveness referred to as ''septal rage'' (Albert and Chew, 1980). Interestingly, it has been noted that ''septal rage'' is mainly observed after lesion involving the LSrvld (Albert and Chew, 1980). As with other regions of the lateral septal nucleus, the LSrvld contains a large population of GABAergic neurons that in turn are likely to provide inhibitory inputs to circuits mediating defensive reactions. Risold and Swanson (1997) have shown that the LSrvld receives inputs from intermediate regions of field CA1 and subiculum, which may also provide modest direct inputs to the MHZ defensive system, particularly to the AHN and PMd (Van Groen and Wyss, 1990; Comoli et al., 2000). Taken as a whole, this evidence supports the idea that the MHZ defensive system is modulated by a specific septohippocampal domain.

The MHZ defensive system also receives strong inputs from the amygdala, arising from the posteroventral part of the medial and posterior part of the basomedial amygdalar nuclei. The amygdalar region is known to be critically involved in the expression of innate defensive responses during encounters with a predator (Blanchard and Blanchard, 1972). The posteroventral part of the medial amygdalar nucleus provides a strikingly dense projection to the VMHdm as well as substantial inputs to the AHN (Canteras et al., 1995). Recent findings in the rat indicate that the posteroventral part of the medial amygdalar nucleus presents a very strong and selective activation following exposure to cat odor, suggesting that this particular region of the ''vomeronasal amygdala'' is involved in pheromone-like processing of predator's odor (Dielenberg et al., 2001). The core region of the ventromedial nucleus receives a substantial input from the posterior part of the basomedial amygdalar nucleus (Petrovich et al., 1996), which receives massive inputs from the lateral amygdalar nucleus, and therefore is likely to integrate olfactory, insular, prefrontal, and temporal cortical processing (for references, see Swanson and Petrovich, 1998).

Amygdalar inputs to the MHZ defensive system may also be relayed through the interfascicular nucleus of the BST, which innervates all components of this hypothalamic system (Comoli et al., 2000; NS Canteras, unpublished observations). It has been shown that the BST seems to modulate the unconditioned startle reflex (Walker and Davis, 1997), although its particular roles in the context of other innate defensive responses, such as exposure to a natural threat, remain to be investigated. In addition, it is important to emphasize that intermediate regions of field CA1 and the subiculum, included in the septohippocampal domain involved in modulating the MHZ defensive system, may also serve as an important way station for several amygdalar cell groups (i.e., lateral, posterior basolateral, and posterior basomedial nuclei) to influence the MHZ defensive system (Petrovich et al., 1996; Pikkarainen et al., 1999).

Apart from the intrinsic connectivity, the elements of the MHZ defensive system may also be targeted by other hypothalamic sites. We have recently shown that the PMd appears to be densely innervated by a region of the lateral hypothalamic area located immediately dorsal to the supraoptic nucleus, heavily targeted by the lateral component of the retinohypothalamic tract (Comoli et al., 2000). According to Leak and Moore (1997), the appearance of retinal ganglion cells projecting to this particular region of the lateral hypothalamic area is similar to those projecting to the suprachiasmatic nucleus (type III ganglion cells), and, therefore, these cells are likely to convey information about environmental light and darkness. Interestingly, in the context of defensive responses, it seems plausible to suggest that different behavioral strategies might be expressed depending on the environmental luminescence. For example, instead of flight behaviors, freezing immobility seems to be particularly effective as a camouflage in darkness, where freezing greatly reduces prey visibility and noise generation, which is a major cue for sound-locating birds of prey such as the barn owl (Konishi, 1995). Moreover, it seems reasonable to believe that this path may, at least in part, mediate the well-documented anxiogenic profile induced by high levels of environmental luminescence (File, 1980; File and Hyde, 1978; Crawley, 1981).

All elements of the MHZ defensive system receive a substantial innervation from a region located just ventral to the fornix at caudal levels of the AHN, which was included in the perifornical site first observed by Hess and Brugger (1943) and later confirmed by Hunsperger (1956) as a

key hypothalamic site for eliciting integrated defensive responses in the cat. This region is known to receive inputs from lateral parabrachial regions involved in transmitting noxious stimuli (Bester et al., 1997) and shares strong bidirectional connections with the dorsolateral zone of the rostral part of the lateral septal nucleus (LSrdl) (Risold and Swanson, 1997), which, as discussed for the LSrvld, also seems to be involved in septal lesions that are most effective in producing the hyperdefensiveness state referred to as ''septal rage'' (Albert and Chew, 1980). As noted above, this part of the perifornical region has been associated with defensive behavior, where electrical or chemical stimulation evokes either attack or escape responses (for references, see Roeling et al., 1994). Interestingly, after electrical stimulation of this region, Roberts and Nagel (1996), by using ^{14}C-deoxyglucose autoradiographs, found increased metabolic activity in all elements of the MHZ defensive system and in the lateral septum particularly correlated with escape but not with attack responses.

Only a few sites in the brainstem appear to provide direct inputs to the MHZ defensive system. Previous anatomical studies have shown that the AHN receives a moderate projection from the precommissural nucleus and PAGdl (Cameron et al., 1995a; Canteras and Goto, 1999a), which, as we shall discuss below, represent the main brainstem targets of the MHZ defensive system. It is widely accepted that the PAGdl can be differentiated from other parts of the PAG on both morphological and functional grounds, and, as we shall consider below, appears to integrate neural information particularly related to ''psychological stressors,'' such as the presence of a natural predator. The PAGdl, in turn, triggers ''active emotional coping responses,'' which include increased somatomotor activity (e.g., freezing, fight or flight), hyperreactivity, hypertension, tachycardia, and a nonopioid-mediated analgesia (for references, see Floyd et al., 2000). Thus, this pathway from the PAGdl to the MHZ defensive system is likely to convey critical feedback information related to the outcome of defensive responses to a given psychological stressor.

Previous anatomical and functional studies have shown that the VMHdm is heavily targeted by lateral parabrachial sites involved in transmitting noxious stimuli (Bester et al., 1997)—a fact of obvious relevance in the context of the initiation of defensive responses. Finally, a number of anatomical studies have reported that the PMd receives a massive projection from the ventral tegmental nucleus (Shibata, 1987; Comoli et al., 2000). In sharp contrast to the main forebrain sources of inputs to the PMd, which provide a strong bilateral projection to the nucleus, the projection from the ventral tegmental nucleus is essentially ipsilateral (Shibata, 1987; Comoli et al., 2000). Very little is currently known about the functions of the ventral tegmental nucleus itself, although considering its intimate methodological relationship with the medial mammillary nucleus, it seems reasonable to suppose that this brainstem site is in a position to convey information from a neural system involved in spatial working memory (see Sziklas and Petrides, 1998), which may be of particular relevance for an effect of attentional mechanisms on the selection of appropriate escape strategies.

4.2. Neural outputs from the medial hypothalamic defensive system

As summarized in Fig. 2B, the main telencephalic targets of the elements of the MHZ defensive system are the lateral septal nucleus, the interfascicular nucleus of the BST, and the lateral amygdalar nucleus. The projections to the lateral septal nucleus arise chiefly from the AHN, which is known to integrate most of the septohippocampal inputs to this MHZ system, and are directed to the dorsal region of the ventrolateral zone of the rostral part of the lateral septal nucleus (LSrvld) and to the dorsolateral zone of the rostral part of the nucleus (LSrdl) (Risold et al., 1994), both of which are considered to be integral parts of the specific septohippocampal domain likely to be involved in modulating innate defensive responses to environmental threats. In contrast to those just discussed for the lateral septal nucleus, the projections to the interfascicular nucleus of the BST and to the amygdala arise chiefly from the VMHdm (Canteras et al., 1994), which, as previously shown, integrates most of the amygdalar inputs to the MHZ defensive system. Similarly to the LSrvld, the interfascicular nucleus of the BST also projects to all elements of the MHZ hypothalamic system. Moreover, the main amygdalar targets of the VMHdm (e.g., the lateral nucleus, the piriform–amygdaloid area, and posterior lateral part of the cortical nucleus) are known to provide substantial projections to the posterior part of the basomedial nucleus (see Swanson and Petrovich, 1998), another source of innervation to the MHZ defensive system. Taken together, this evidence suggests that these outputs from the MHZ defensive system may serve as feedback loops, perhaps providing critical information for an adequate telencephalic modulation of innate defensive responses.

In the dorsal thalamus, the nucleus reuniens and the ventral part of the anteromedial nucleus receive a dense innervation from the MHZ defensive system. The AHN contributes with a considerable innervation to the rostral parts of the nucleus reuniens, which also receives significant, but less dense, inputs from the VMHdm and PMd (Canteras and Swanson, 1992; Canteras et al., 1994; Risold et al., 1994). The nucleus reuniens represents the major source of thalamic projections to the hippocampal formation, and is thought to play a key role in modulating transmission through the hippocampal system, (Herkenham, 1978; Wouterlood et al., 1990; Risold et al., 1997). In the present context, it is important to consider the potential roles of this path in emotion-related learning and memory, especially if we consider the critical role of the hippocampal formation in neural mechanisms related to short-term memory and the more permanent consolidation of particular events in other

regions of the cortex (for references, see Swanson et al., 1987). The PMd provides a massive projection to the ventral anteromedial thalamic nucleus, which, in turn, projects to the lateral retrosplenial area thought to be involved in modulating the eye and head movements associated with attentional mechanisms (Risold and Swanson, 1995).

In the ventral thalamus, all the components of the MHZ defensive system provide a substantial projection to the rostral pole of the zona incerta (Canteras and Swanson, 1992; Canteras et al., 1994; Risold et al., 1994). Although a great deal remains to be learned about the connectivity and possible functional roles of this region of the zona incerta, hodologic evidence indicates that it is intimately related to the main brainstem targets of this hypothalamic system (e.g., the precommissural nucleus and the dorsolateral PAG) (Cameron et al., 1995a; Elias and Bittencourt, 1997; Canteras and Goto, 1999a).

The precommissural nucleus and the PAG represent the main brainstem targets of the MHZ defensive system. As originally noted by Hunsperger (1956), the PAG is a key site to organize defensive responses, where lesions result in passive animals that rarely, if ever, show defensive behavior. The projection from the VMHdm and the PMd to the PAG is very dense and presents a clear topography (Canteras and Swanson, 1992; Canteras et al., 1994). Thus, at the level of the nucleus of Darkschewitsch, the dorsomedial part of the PAG receives a massive innervation from the VMHdm in addition to a significant, but sparser, projection from the PMd. At the level of the oculomotor and trochlear nuclei, this latter hypothalamic site provides a strikingly dense projection to the dorsolateral part of the PAG, which, in addition to the dorsomedial PAG, is also considerably targeted by fibers arising from the VMHdm. At caudal levels, axons from the VMHdm spread to project to the dorsomedial, dorsolateral, lateral, and ventrolateral parts of the PAG, whereas those from the PMd continue to provide a relatively circumscribed projection to the dorsolateral PAG. Compared to the other components of the MHZ defensive system, the AHN provides a much lighter projection to the PAG, particularly directed to the rostral dorsomedial and caudal ventrolateral parts of the PAG (Risold et al., 1994). Interestingly, the pattern of projection from the MHZ defensive system to the PAG largely overlaps the pattern of PAG activation of animals exposed to a predator, where Fos expression was mostly seen in the rostral two thirds of the PAG in the dorsomedial and dorsolateral regions, whereas in the caudal PAG a less intense but more widespread activation was observed (Canteras and Goto, 1999b). A similar pattern of PAG activation was also described after administration of drugs known to induce panic in humans (Singewald and Sharp, 2000), differing, however, from that seen after physical stressors (e.g., cutaneous pain, footshock, restraint stress, swim stress, opiate withdraw), which fail to evoke consistent Fos expression within the dorsolateral PAG (Keay and Bandler, 1993; Pezzone et al., 1993; Cullinan et al., 1995; Bellchambers et al., 1998; Li and Sawchenko, 1998). Taken together, this evidence strongly supports the idea that the dorsolateral PAG appears to play a critical role in the PAG for integrating forebrain limbic information related to "psychological stressors" like the presence of a natural predator.

All the elements of the MHZ defensive system provide a particularly dense projection to the precommissural nucleus, which, similarly to PAG regions densely targeted by this system, also presents a dramatic increase in Fos immunoreactivity in animals exposed to a predator (Canteras and Goto, 1999b). Unfortunately, we are not aware of any reports on possible functional roles played by the precommissural nucleus. However, we have found that this nucleus presents a connective pattern similar in many ways to the rostral part of the dorsolateral PAG (Cameron et al., 1995a,b; Canteras and Goto, 1999a) and therefore is likely to share with this latter region a number of integrative functions.

A relatively sparse projection was also observed from the elements of the MHZ defensive system to the cuneiform nucleus and medial regions of intermediate and deep layers of the superior colliculus (Canteras and Swanson, 1992; Canteras et al., 1994; Risold et al., 1994). Notably, these brainstem sites respond to visual-threatening stimuli, such as suddenly expanding shadows in the upper visual field, and, via a projection to the rostral part of the PAGdl, are thought to exert a marked influence on the control of defensive responses (Redgrave and Dean, 1991).

Apart from the intrinsic connectivity, the elements of the MHZ defensive system appear to innervate a number of other hypothalamic sites, including the lateral preoptic area, the dorsomedial rostral perifornical region, the posterior hypothalamic nucleus, and the dorsomedial hypothalamic nucleus (DMH).

The projection from the MHZ system to the lateral preoptic area arises primarily from the AHN (Risold et al., 1994). As previously mentioned, the lateral preoptic area is another hypothalamic site that upregulates Fos expression in animals exposed to a predator. A number of studies indicate that the lateral preoptic area participates in the modulation of somatomotor responses (especially locomotor behavior) and general arousal associated with motivated behavior (Swanson, 1987; Swanson et al., 1984). Therefore, it is reasonable to suggest that this path may contribute, at least in part, to the general behavioral arousal associated with the defensive responses.

All the elements of the MHZ defensive circuit provide a clear projection to the dorsomedial rostral perifornical region, located immediately adjacent to the ventrolateral border of the rostral part of the nucleus reuniens (Canteras and Swanson, 1992; Canteras et al., 1994; Risold et al., 1994). Unfortunately, a great deal remains to be learned about the connectivity and possible functional roles of this particular site of the perifornical region, which also seems to be particularly activated during the exposure to a predator (Canteras et al., 1997).

The posterior hypothalamic nucleus also receives substantial projections from all elements of the MHZ defensive system (Canteras and Swanson, 1992; Canteras et al., 1994; Risold et al., 1994). Importantly, this hypothalamic site may represent another route for the MHZ defensive system to influence the hippocampal formation. It has been shown that the posterior hypothalamic nucleus projects to a number of subcortical structures with direct inputs to the hippocampal formation, including the supramammillary nucleus, nucleus reuniens, lateral dorsal thalamic nucleus, medial septal nucleus, and nucleus of the diagonal band, and it also provides direct cortical inputs to the entorhinal and perirhinal areas (Vertes et al., 1995). In fact, it has been suggested that the posterior hypothalamic nucleus plays a critical role in mnemonic processes associated with significant emotional events (Vertes et al., 1995). Moreover, several studies have indicated that the posterior hypothalamic nucleus may integrate somatomotor and visceromotor activity related to defensive responses. It has been shown that injections of GABA antagonists into the posterior hypothalamic nucleus of anesthetized rats produce increased heart rate and blood pressure (Di Micco and Abshire, 1987; Di Micco et al., 1986), whereas injections of these same substances into the nucleus of the behaving rat produce a significant increase in locomotor activity, interpreted as a component of escape or flight responses (Shekhar and Di Micco, 1987).

With the exception of the PMd, all other components of the MHZ defensive system send a moderate projection to the DMH (Canteras and Swanson, 1992; Canteras et al., 1994; Risold et al., 1994; Thompson and Swanson, 1998). The DMH appears to be particularly activated by a number of stressful situations (e.g., footshock, restraint stress, swim stress, dehydration, and exposure to a predator) (Cullinan et al., 1995, 1996; Canteras et al., 1997; Li and Sawchenko, 1998; NS Canteras, personal observations), and has been implicated in the behavioral, autonomic, and endocrine responses to acute stressors (Shekhar, 1993; Shekhar and Katner, 1995; Inglefield et al., 1994; File et al., 1999). Previous anatomical studies indicate that the DMH is intimately related to a number of periventricular hypothalamic sites, and receives direct inputs from circumventricular organs, such as the organum vasculosum of the lamina terminalis and the subfornical organ, which are thought to relay information related to many peripheral plasma parameters to the nucleus (Thompson and Swanson, 1998; Thompson et al., 1996). Thus, in the present context, it is important to consider that the DMH is thought to mediate lactate-induced panic-like responses in rats (Shekhar et al., 1996), which have been shown to be completely blocked after tetrodotoxin infusion into the organum vasculosum of the lamina terminalis (Shekhar and Keim, 1997).

The DMH has traditionally been viewed as belonging to the medial zone of the hypothalamus. However, on the basis of recent anatomical analyses, it was concluded that the DMH has little in common with the medial zone and is thus more appropriately considered part of the periventricular zone (Thompson, 1997; Thompson and Swanson, 1998; Thompson et al., 1996). The DMH provides massive projections to the paraventricular hypothalamic nucleus, where particularly dense innervation has been described to the dorsal, lateral, ventral, and forniceal parts, which generate descending projections to the brainstem and spinal cord sites related to the autonomic output control, and to the periventricular and medial parvicellular parts, which contain neurons synthesizing hypophysiotropic somatostatin, corticotropin, and thyrotropin-releasing hormones (see Thompson et al., 1996). The DMH also shares significant bidirectional connections with the median preoptic, parastrial, anteroventral preoptic, anterodorsal preoptic, and anteroventral periventricular nuclei in the preoptic region, which in turn are known to project to parvicellular and/or magnocellular parts of the paraventricular hypothalamic nucleus (Thompson and Swanson, 1998; Thompson et al., 1996). In fact, it has been suggested that the DMH, together with these preoptic regions, forms a complex interconnected network thought to work as "premotor" elements for the neuroendocrine and autonomic output (Thompson, 1997).

Notably, the intrahypothalamic projections of the DMH almost completely avoid all members of the MHZ defensive system, and in contrast to the latter hypothalamic sites, the nucleus provides only a very sparse projection to the PAG (Thompson et al., 1996). Alternatively, a potential route for the DMH to modulate defensive behavioral responses would be the thalamic paraventricular nucleus, which represents the single largest extrahypothalamic target of the DMH (Thompson et al., 1996). Thus, in addition to providing direct inputs to the MHZ defensive system, primarily directed to the VMHdm, the thalamic paraventricular nucleus also sends substantial projections to a number of sources of inputs to this hypothalamic system (e.g., the infralimbic cortex, the lateral septal nucleus, and the posterior part of the basomedial amygdalar nucleus) (Moga et al., 1995). Moreover, the thalamic paraventricular nucleus also projects to a number of other limbic sites, such as the anterior division of the BST, central amygdalar nucleus, and nucleus accumbens, known to influence the outcome of several goal-oriented behaviors.

5. Conclusion

The present analysis helps to delineate the neural circuits apparently involved in the organization of innate defensive behaviors. Clearly, a great deal remains to be learned about the organization and chemistry of the defensive behavior system, as well as the functional role of its various components. However, it now seems likely that systems mediating other classes of partly instinctive goal-oriented behavior (specifically ingestive and reproductive) share similar organizing principles, and it will be important to define

them more precisely and to determine how these systems are interrelated.

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