The caudal prethalamus: Inhibitory switchboard for behavioral control?

Alex Fratzl[1,2], Sonja B. Hofer[1,*]

[1]Sainsbury Wellcome Centre for Neural Circuits and Behavior, University College London, London, United Kingdom

[*]Lead contact, correspondence to s.hofer@ucl.ac.uk

[2]Present address: Institute of Molecular and Clinical Ophthalmology Basel, 4031 Basel, Switzerland

Summary

Prethalamic nuclei in the mammalian brain include the zona incerta, the ventral lateral geniculate nucleus and the intergeniculate leaflet, which provide long-range inhibition to many targets in the midbrain, hindbrain and thalamus. These nuclei in the caudal prethalamus can integrate sensory and non-sensory information and together exert powerful inhibitory control over a wide range of brain functions and behaviors that encompass most aspects of the behavioral repertoire of mammals, including sleep, circadian rhythms, feeding, drinking, predator avoidance and exploration. In this perspective we highlight the evidence for this wide-ranging control and lay out the hypothesis that one role of caudal prethalamic nuclei may be that of a behavioral switchboard that - depending on the sensory input, the behavioral context, and the state of the animal - can promote a behavioral strategy and suppress alternative, competing behaviors by modulating inhibitory drive onto diverse target areas.

In Brief

In this perspective, Fratzl and Hofer review the main nuclei in the caudal prethalamus and propose that given their wide-ranging inhibitory control over diverse brain functions, these brain regions may act together as a behavioural switchboard.

Keywords

Prethalamus, ventral lateral geniculate nucleus, zona incerta, intergeniculate leaflet, long-range inhibition, inhibitory control, behavioral control, sensory processing, ventral thalamus

The caudal prethalamus, a GABAergic system well poised to control behavior

Nuclei of the prethalamus

The prethalamus is composed of a collection of predominately GABAergic nuclei, formerly known as "ventral thalamus" or subthalamus (Puelles and Martinez, 2013). The prethalamic nuclei are located between thalamus, midbrain and hypothalamus (**Figure 1**), and share a common developmental origin different to the thalamus (former 'dorsal thalamus') (Nakagawa, 2019). The prethalamus comprises four to five distinct regions, including the thalamic reticular nucleus (TRN), the zona incerta (ZI), the ventral lateral geniculate nucleus (vLGN), and the intergeniculate leaflet (IGL) (**Figure 1**). The small subgeniculate nucleus, situated between vLGN and ZI, may constitute a further prethalamic nucleus (Delaunay et al., 2009), but it is unknown if its connectivity or function are distinct from the surrounding areas. We have adopted here the terminology used in rodents, however, the exact nomenclature of prethalamic areas can vary between species. In cats, the IGL is considered a subdivision of the vLGN (Nakamura and Itoh, 2004), while in primates, the IGL and vLGN together form the pregeniculate nucleus (Livingston and Mustari, 2000; Pinato et al., 2009). Moreover, the relative size, location and level of cell differentiation of prethalamic areas varies across species (Niimi et al., 1963). For instance, while vLGN and dorsal LGN (dLGN) are of comparable size in the mouse, the LGN is significantly larger than the pregeniculate nucleus in primates (Niimi et al., 1963).

Historically, the subthalamic nucleus (STN) has been grouped together with prethalamic nuclei into the subthalamus. However, because the STN is of different developmental origin, contains mainly glutamatergic neurons and is now believed to form part of the hypothalamus (Barbier and Risold, 2021), it should be considered separately. The TRN shares a common origin with the other prethalamic nuclei, however, it acquires a strikingly different connectivity profile later in development. The TRN receives glutamatergic input exclusively from the cerebrum (mainly neocortical layer 6 neurons) and thalamocortical projection cells, and thalamic neurons are the only recipients of TRN output. In contrast, the remainder of the prethalamus integrates signals from diverse brain areas, including from the sensory periphery and the midbrain, and shows widespread projection patterns in the fore-, mid- and hindbrain (**Figure 2**, Harrington, 1997; Mitrofanis, 2005; Monavarfeshani et al., 2017). The TRN therefore seems to constitute a distinct functional entity which has been reviewed in detail elsewhere (for review see e.g. Halassa and Acsády, 2016; Crabtree, 2018; Wolff et al., 2021), and thus will not be discussed here. This review is focused on the ZI, vLGN and IGL, the nuclei constituting the caudal part of the prethalamus. Note that for simplicity, for the remainder of this review, the term caudal prethalamus specifically refers to ZI, vLGN and IGL.

Connectivity with brain areas in the fore-, mid- and hindbrain

The caudal prethalamus holds a special position within the mammalian brain, situated at the crossroad of most major systems (**Figure 2**). It receives direct input from the sensory periphery and prominent projections from layer 5 neurons across most of the dorsal cerebral cortex. Moreover, it is strongly and bidirectionally connected with many areas in the midbrain, as well as with the hypothalamus and the cerebellum via the pons. In addition, caudal prethalamic nuclei project to the medulla and the spinal cord and innervate specifically higher-order nuclei in the thalamus (Shammah-Lagnado et al., 1985; Harrington, 1997; Moore et al., 2000; Kolmac and Mitrofanis, 2000; Livingston and Mustari, 2000; Mitrofanis, 2005; Urbain and Deschênes, 2007; Monavarfeshani et al., 2017), through which they can affect neocortical processing (Liu et al., 2015; Weitz et al., 2019), and ZI even sends sparse projections directly to neocortical areas (Lin et al., 1990; Nicolelis et al., 1992, 1995; Lin et al., 1997). Taken together, its prominent connectivity with most subsections of the mammalian brain and the inhibitory nature of the majority of these projections (Harrington, 1997; Mitrofanis, 2005; Monavarfeshani et al., 2017), predict that the caudal prethalamus is well poised to exert widespread control over different brain functions. And indeed, caudal prethalamic areas have been shown to not only play a role in sensory processing, but to significantly influence almost all aspects of the behavioral repertoire of mammals, ranging from sleep to exploration, and from various defensive to appetitive behaviors, such as hunting, feeding and drinking (**Figure 3**). The following sections will highlight the evidence for this wide-ranging control over sensory and different behavioral functions, with a particular focus on rodent literature.

The caudal prethalamus exerts extensive control over diverse brain functions

The role of caudal prethalamus in sensory processing

<u>IGL and vLGN in visual processing</u>

The caudal prethalamus is organized in sensory domains (Harrington, 1997; Mitrofanis, 2005) and is one of the major recipients of direct visual and somatosensory information from the periphery in the mammalian brain (Shammah-Lagnado et al., 1985; Harrington, 1997; Kolmac et al., 1998; Kolmac and Mitrofanis, 2000; Livingston and Mustari, 2000; Shaw and Mitrofanis, 2002; Mitrofanis, 2005; Simpson et al., 2008; Hammer et al., 2014; Monavarfeshani et al., 2017). Auditory input seems less pronounced (Mitrofanis, 2002, 2005), even though caudal prethalamic nuclei can regulate sound-evoked behavior (Chou et al., 2018; Venkataraman et al., 2019; Hormigo et al., 2020; Venkataraman et al., 2021). As demonstrated in many vertebrate species, the IGL and vLGN are densely innervated by retinal axons

(Harrington, 1997; Kolmac and Mitrofanis, 2000; Livingston and Mustari, 2000; Hammer et al., 2014; Monavarfeshani et al., 2017). In many species, the vLGN has two parts, an internal and an external division, and retinal axons only project to the external, lateral part (Harrington, 1997; Monavarfeshani et al., 2017). Non-image-forming retinal ganglion cells such as intrinsically photosensitive- or cadherin-3-positive cells (Do and Yau, 2010) have previously been reported to form a large fraction of the retinal input to IGL and vLGN (Hattar et al., 2006; Osterhout et al., 2011; Monavarfeshani et al., 2017), however, more recent evidence points towards greater diversity in the RGC types innervating vLGN and IGL (Rivlin-Etzion et al., 2011; Hammer et al., 2014; Monavarfeshani et al., 2017). Neurons in the vLGN and IGL respond to changes in overall luminance, but many vLGN neurons also exhibit diverse spatial receptive fields and show a clear retinotopic organization (Spear et al., 1977; Sumitomo et al., 1979; Nagata and Hayashi, 1984; Holcombe and Guillery, 1984; Harrington, 1997; Pienaar et al., 2018; Ciftcioglu et al., 2020). However, receptive fields in vLGN are significantly larger than those of dLGN neurons, and to date it is unclear to what degree visual response properties in vLGN are driven by retinal input or inherited from the prominent projections from visual cortex layer 5 neurons or the superior colliculus (Spear et al., 1977; Sumitomo et al., 1979; Bourassa and Deschênes, 1995; Harrington, 1997; Livingston and Mustari, 2000; Monavarfeshani et al., 2017; Ciftcioglu et al., 2020).

Early lesion studies in rats indicated that vLGN (and potentially the very lateral part of the ZI) is critical for discriminating different levels of luminance, but not for discriminating stimulus orientation (Legg and Cowey, 1977a, 1977b; Legg, 1979), however, the roles of vLGN and IGL in visual processing remain unclear. The vLGN innervates thalamocortical neurons in the pulvinar, the higher-order thalamic nucleus of the visual system, also known as lateral posterior (LP) nucleus in rodents (Moore et al., 2000; Blot et al., 2021; Fratzl et al., 2021). These vLGN projections would be well positioned to regulate visual processing and visual information flow in the cerebral cortex by inhibiting thalamocortical interactions (Zhou et al., 2016; Beltramo and Scanziani, 2019; Blot et al., 2021). vLGN and IGL also project to the superior colliculus (SC), and vLGN in particular has a strong inhibitory influence on visual processing in the SC (Fratzl et al., 2021). vLGN receives significant motor-related and vestibular input and may therefore play a role in visuo-motor integration, for instance to distinguish self-induced from externally generated visual motion or to suppress saccade-induced visual signals (Magnin et al., 1974; Livingston and Fedder, 2003). IGL, and to a lesser degree vLGN, exhibit commissural projections to their contralateral counterparts, which may facilitate the detection of interocular differences in luminance (Pienaar et al., 2018).

ZI in somatosensory processing

The main prethalamic recipient of somatosensory information is the ventral part of the ZI (Shammah-Lagnado et al., 1985; Harrington, 1997; Kolmac et al., 1998; Shaw and Mitrofanis, 2002; Mitrofanis,

2005; Urbain and Deschênes, 2007; Simpson et al., 2008). While lemniscal and paralemniscal pathways from the medulla target different nuclei in the thalamus - the ventral posterior complex (VP) and the posterior nucleus (PO), respectively (Petersen, 2007) - these somatosensory pathways seem to converge within the zona incerta (Simpson et al., 2008). Zona incerta neurons respond to whisker deflections and other somatosensory stimuli (Nicolelis et al., 1992; Urbain and Deschênes, 2007; Zhao et al., 2019) and are organized in somatotopic maps (Nicolelis et al., 1992; Mitrofanis and Mikuletic, 1999). Interestingly, similar to visual responses in vLGN, receptive fields of zona incerta neurons are larger than those of thalamic neurons (Nicolelis et al., 1992). The role of the ZI in sensory processing is not fully established, but it was proposed to gate information flow to the cortex via projections from the ventral ZI to PO thalamus (Trageser and Keller, 2004; Lavallée et al., 2005; Trageser et al., 2006; Urbain and Deschênes, 2007; Escudero and Nuñez, 2019). For instance, cholinergic activation suppresses activity in ZI (Trageser et al., 2006), while inactivation of ZI increases whisker responses in PO (Trageser and Keller, 2004; Lavallée et al., 2005; Escudero and Nuñez, 2019), establishing a potential mechanism for internal state-dependent regulation of somatosensory signals in PO (Trageser et al., 2006). Furthermore, the ZI has been linked to the processing of pain (**Figure 3A**, Masri et al., 2009; Petronilho et al., 2012; Moon and Park, 2017; Hu et al., 2019; Wang et al., 2020; Lu et al., 2021). ZI stimulation has been shown to reduce mechanical and thermal pain in mice, rats and humans (Petronilho et al., 2012; Hu et al., 2019; Wang et al., 2020; Lu et al., 2021), while suppression of ZI activity increases pain perception (Moon and Park, 2017; Wang et al., 2020). Moreover, spinal cord lesions resulting in central pain syndrome in rats reduce evoked and spontaneous activity in ZI neurons, and lead to an increase in PO activity (Masri et al., 2009). In addition, the vLGN has recently been implicated in nociceptive processing through its projections to the periaqueductal gray (PAG) (Hu et al., 2022). Taken together, there is strong evidence that caudal prethalamic circuits can regulate thalamocortical interactions through their inhibitory influence on higher-order sensory thalamus. This influence is paralleled by inhibitory projections from the TRN. While both of these inhibitory pathways likely play important roles in sensory processing, the exact differences in their function remain to be explored (Halassa and Acsády, 2016).

The role of the caudal prethalamus in the regulation of sleep and circadian rhythms

IGL and circadian rhythms

One of the best characterized functions of caudal prethalamic circuits is the influence of the IGL on circadian rhythms through its projections to the hypothalamic suprachiasmatic nucleus (SCN) (Harrington, 1997; Gooley and Saper, 2005; Morin, 2013, **Figure 3B**). The SCN endogenously drives circadian rhythms, including the sleep-wake cycle in mammals by integrating photic and nonphotic time cues to generate circadian output signals (Gooley and Saper, 2005; Morin, 2013). While the SCN

receives direct visual input from the retina, it is also heavily innervated by GABAergic axons from retinorecipient IGL neurons (Harrington, 1997; Moore et al., 2000; Morin, 2013). Lesions of IGL neurons or their projections to the SCN induce a variety of phenotypes, including delayed or altered re-entrainment of the circadian rhythm after a change in the photoperiod (Harrington, 1997; Edelstein and Amir, 1999; Gall et al., 2013). In a diurnal rodent IGL lesions even induced a dramatic behavioral shift towards a night-active locomotor pattern (Gall et al., 2013). Interestingly, IGL lesions have a particularly strong effect on nonphotic circadian entrainment (Johnson et al., 1988; Biello et al., 1991; Meyer et al., 1993; Harrington, 1997; Lewandowski and Usarek, 2002; Smith et al., 2015; Fernandez et al., 2020): For instance, bilateral IGL lesions strongly disrupt rhythmic locomotor activity in complete darkness (Lewandowski and Usarek, 2002) and prevent serotonin-induced changes in the circadian rhythm (Smith et al., 2015). Most SCN-projecting IGL neurons co-release neuropeptide Y (NPY), which has been proposed to counteract photic effects on circadian rhythms mediated by the direct, glutamatergic retino-hypothalamic pathway (Gooley and Saper, 2005; Morin, 2013). Consistent with this model, NPY+ neurons in IGL control non-light entrained feeding rhythms: restricting the access to food to specific time periods in complete darkness leads to robust food-anticipatory activity in normal animals, but blocking synaptic transmission of NPY+ IGL projections in the SCN strongly reduced this time-locked activity (Fernandez et al., 2020), likely by disrupting pacemaker properties of the SCN. Together, the above studies indicate that the IGL integrates both visual and non-visual signals to modulate circadian rhythms through the SCN.

IGL and ZI regulate sleep

Caudal prethalamic circuits have also been studied in the context of sleep (**Figure 3B**). Early studies in cats suggested that the ZI can influence sleep and sleep-wake cycles (Starzl et al., 1951; Naquet et al., 1966, but see Jurkowlaniec et al., 1990). Indeed, ZI neurons in rodents are differentially engaged during different phases of the sleep-wake cycle (Koyama et al., 2003; Blanco-Centurion et al., 2021), and a specific cell type in the ventral ZI, Lim-Homeobox-6 (Lhx6)-expressing neurons, has been found to bidirectionally regulate sleep time in adult mice. (Liu et al., 2017). Moreover, the selective deletion of these neurons during development leads to both REM and NREM sleep impairments. Lhx6-positive neurons are activated by sleep pressure and directly inhibit wake-active hypocretin and GABAergic cells in the lateral hypothalamus, indicating an important role of the incertal Lhx6 population in sleep regulation (Liu et al., 2017). A recent study found that IGL can also influence sleep: lesions of IGL GABAergic neurons attenuate the sleep-promoting effect of light exposure in mice (Shi et al., 2020), suggesting a more general engagement of caudal prethalamic circuits during sleep regulation. Caudal prethalamic circuits have also been found to affect other autonomic functions such as temperature control, the estrous cycle and ovulation (MacKenzie et al., 1984; James et al., 1987; Jirikowski et al., 1988; Spencer et al., 1988; Jirikowski et al., 1991; Kiyohara et al., 1995; Siddiqui et al., 2000).

The caudal prethalamus controls defensive behaviors

A growing body of recent literature in mice links the caudal prethalamus to the regulation of defensive behaviors (**Figure 3C**), such as reactions to imminent threat. Several studies have demonstrated that optogenetic activation of GABAergic neurons in the ZI attenuates reactions to auditory stimuli perceived as threats (Chou et al., 2018; Venkataraman et al., 2019; Hormigo et al., 2020; Venkataraman et al., 2021). Conversely, suppression of GABAergic ZI neurons amplifies fear-related reactions such as freezing and escape in response to innately threatening, as well as negatively conditioned auditory stimuli (Chou et al., 2018; Venkataraman et al., 2019; Hormigo et al., 2020). Several different ZI pathways have been implicated in these effects, including ZI projections to the PAG and the mesencephalic locomotor region (MLR) in the midbrain, as well as to the thalamic nucleus reuniens (Chou et al., 2018; Hormigo et al., 2020; Venkataraman et al., 2021). More recently, the vLGN has been established as a critical control hub for defensive behaviors, in particular for visually evoked fear responses. Activation of GABAergic vLGN neurons abolishes escape from (Fratzl et al., 2021), and strongly attenuates freezing (Salay and Huberman, 2021) in response to innately threatening visual stimuli - looming black spots that mimic an approaching aerial predator (Yilmaz and Meister, 2013; Branco and Redgrave, 2020). Conversely, inactivation of GABAergic vLGN neurons increases fear responses to visual threats (Fratzl et al., 2021; Salay and Huberman, 2021). This control over defensive reactions seems to at least in part be mediated by vLGN projections to the medial SC and their suppressive effect on SC activity (Evans et al., 2018; Fratzl et al., 2021). Interestingly, the vLGN has a modality-specific influence on SC activity and behavior: it mainly inhibits visually-responsive, but not sound-responsive neurons in the deeper layers of SC, which likely explains a stronger effect on visual- than on sound-evoked defensive reactions when manipulating the vLGN-to-SC pathway (Fratzl et al., 2021). Different circuits in the caudal prethalamus may therefore regulate fear reactions to imminent threats of different modalities.

However, both ZI and vLGN have also been implicated in regulating defensive behavior more generally, even in the absence of imminent threat. Suppressing activity of GABAergic vLGN neurons increases risk-avoidance behaviors and reduces exploration of exposed spaces in the open field test, the elevated plus maze and other aversive environments (Fratzl et al., 2021; Salay and Huberman, 2021). Blocking synaptic transmission of neurons in the ZI produces similar results in these essays (Zhou et al., 2018, 2021), while different ZI cell types may have opposing influences on anxiety-related behavior (Li et al., 2021). Fear generalization has been linked to reduced activity in the ZI and, consistently, activation of GABAergic ZI neurons decreased generalized fear responses after fear conditioning (Venkataraman et al., 2019), potentially through projections to the thalamic nucleus reuniens (Venkataraman et al., 2021). Activating these ZI projections strongly reduced freezing in

response to a neutral, unconditioned stimulus (CS-), but had much less effect on freezing responses to a negatively conditioned stimulus (CS+). This indicates that the ZI to nucleus reuniens pathway is likely important for generalized anxiety responses, while the learned conditioned response to a negatively conditioned auditory stimulus may involve different circuits (Venkataraman et al., 2021).

Intriguingly, while most studies report suppression of defensive behavior when activity in vLGN or ZI is high, specific subcircuits in these nuclei seem to have the opposite effect: activation of parvalbumin-positive neurons in the ventral ZI has been reported to enhance sound-evoked escape behavior through a pathway to PO thalamus (Wang et al., 2019) and glutamatergic vLGN neurons as well as vLGN projections to nucleus reuniens increase threat-evoked freezing when activated (Salay and Huberman, 2021). These studies emphasize the complexity of interactions between different circuits and cell types in the caudal prethalamus in mediating divergent behavioral functions.

The caudal prethalamus drives appetitive behavior and motivational state

Caudal prethalamic circuits can regulate animals' motivational state and different types of appetitive behaviors, in particular in relation to water and food consumption (**Figure 3D**). Electrical stimulation of the ZI in rats causes increased water intake (Huang and Mogenson, 1972), while ZI lesions, in particular of the rostral and medial part, strongly inhibit water intake (Walsh and Grossman, 1973, 1976; Evered and Mogenson, 1976, 1977; Brown and Grossman, 1980; Mok and Mogenson, 1986) and sodium appetite (Walsh and Grossman, 1977). Local infusions of D2 but not D1 dopamine receptor agonists into the medial ZI reduced water intake (Tonelli and Chiaraviglio, 1995), indicating that dopaminergic signaling could affect water ingestion via ZI pathways. Moreover, the ZI receives signals from osmoreceptors and other receptors that affect water intake (Mok and Mogenson, 1986, 1987), emphasizing its critical role in the control of thirst and drinking.

Similarly, the ZI is thought to regulate food intake and food seeking behaviors. Lesions of the rostral ZI in rats impair feeding in response to cellular glucoprivation (Brown and Grossman, 1980), and optogenetic stimulation of GABAergic neurons in the rostral ZI of mice leads to rapid binge-like eating, in particular of high-fat food, and body weight gain (Zhang and van den Pol, 2017), likely through projections to the paraventricular thalamus (PVT) (Zhang and van den Pol, 2017). Increased serotonergic signaling inhibits food intake and disinhibits PVT neurons by reducing GABAergic synaptic transmission from the ZI (Ye and Zhang, 2021). This serotonergic mechanism is disrupted after a chronic high-fat–high-sucrose diet in mice, suggesting a role of ZI circuits in obesity (Ye and Zhang, 2021).

The ZI has also been linked to hunting and exploration (**Figure 3E**). GABAergic neurons in the anterior medial sector of the ZI can integrate prey-related sensory signals, in particular from the SC (Shang et al., 2019; Zhao et al., 2019). Activating these ZI neurons in mice induces a strong appetitive motivational drive which also promotes hunting and attack of prey, while ZI neuron suppression impairs hunting and strongly reduces free-reward consumption (Zhao et al., 2019). The drive to hunt and attack prey may be specifically mediated by ZI projections to the PAG. However, interestingly, medial ZI neurons, and their projections to PAG have also been shown to be important for non-food related exploratory and novelty-seeking behavior (Ahmadlou et al., 2021). Specifically, tachykinin-1-expressing ZI neurons in mice bidirectionally regulate motivational drive to investigate novel objects and unknown conspecifics. Furthermore, signals related to novelty and novelty-seeking have also been found in the primate ZI, and effects of manipulating ZI activity in primates indicate that the ZI regulates gaze shifts, in particular those related to novelty-seeking (Ogasawara et al., 2021).

Neurons in vLGN and IGL have also been linked to regulation of motivational drive through their projections to the lateral habenula (LHb). Activating the GABAergic vLGN/IGL input to the LHb alleviates depressive-like symptoms in mice by reducing LHb activity (Huang et al., 2019). Interestingly, the pathway from the retina to the LHb via the vLGN/IGL appears to underlie the antidepressant effects of light exposure in mice (Golden et al., 2005; Huang et al., 2019). Taken together, strong evidence indicates that the caudal prethalamus integrates external sensory cues with internal processes to drive different aspects of motivational state, most notably relating to exploratory, hunting, drinking and feeding behaviors.

The role of the caudal prethalamus in motor behaviors

The caudal prethalamus is highly interconnected with sensorimotor centers such as the superior colliculus, the PAG, the pontine nuclei, and the cerebellum, and has thus classically been associated with motor and sensorimotor functions (Graybiel, 1974; Harrington, 1997; Moore et al., 2000; Mitrofanis, 2005; Monavarfeshani et al., 2017; Nakamura, 2018; Kebschull et al., 2020). For instance, vLGN receives significant motor-related and vestibular input (Graybiel, 1974; Zimny et al., 1986; Vaudano and Legg, 1992; Harrington, 1997; Kolmac and Mitrofanis, 2000; Livingston and Mustari, 2000; Monavarfeshani et al., 2017; Nakamura, 2018), and saccade-related responses have been found in both vLGN and ZI of cats and primates (Magnin et al., 1974; Hikosaka and Wurtz, 1983; Ma, 1996; Livingston and Fedder, 2003; Ogasawara et al., 2021). Such signals may constitute motor efference copies, important for predicting the sensory consequences of a motor action and suppressing the self-induced sensory feedback, for instance saccade-induced visual motion (Magnin et al., 1974; Livingston and Fedder, 2003). Alternatively, motor-related signals implicate ZI circuits in directing motor output (Ma, 1996; Ogasawara et al., 2021). Support for this idea comes from a study mapping neuronal activity

across large parts of the brain during a task in which mice needed to turn a wheel either to the left or to the right in response to different visual stimuli (Steinmetz et al., 2019). The study found that ZI contains the highest proportion of neurons of any recorded brain region encoding the directional choice of the animal prior to action initiation. Moreover, electrical stimulation of the ZI induces motor responses in cats (Kaelber and Smith, 1979; Murer and Pazo, 1993), while lesions or inhibition of ZI in rats has been shown to lead to motor deficits (Mogenson et al., 1985; Edwards and Isaacs, 1991). Together, these data suggest that ZI may be important for generating a selected motor output, possibly through interactions with the SC and the midbrain reticular nucleus (Mitrofanis, 2005; Inagaki et al., 2022). An important role of the ZI in motor function has also been proposed in humans (Ossowska, 2020). An early study suggested that interference with ZI signaling pathways can improve muscle rigidity and tremor in Parkinson's disease patients (Mundinger, 1965). While the mechanistic role of the ZI in motor control remains unclear, recent findings have confirmed these results and established the ZI as a promising therapeutic target for deep brain stimulation in Parkinson's disease (Plaha et al., 2006; Ossowska, 2020).

The role of the caudal prethalamus in learned behaviors

While most evidence links the caudal prethalamus to instinctive behaviors and various autonomous functions, its circuits may also influence learned behaviors and learning itself. A pathway from the retina to the thalamic nucleus reuniens via the vLGN/IGL has been described as critical for the beneficial effects of light therapy on spatial memory (Huang et al., 2021): daily bright-light treatment significantly improved performance of mice in spatial memory tasks, and the activity of vLGN/IGL neurons projecting to the nucleus reuniens was necessary to observe these beneficial effects of light treatment, while activating reuniens-projecting vLGN/IGL neurons improved spatial memory (Huang et al., 2021).

Furthermore, specifically PV+ neurons in the ventral ZI have been shown to be important for the acquisition and remote recall of conditioned fear memory in mice (Zhou et al., 2018). Blocking ZI synaptic transmission not only increased general anxiety, but also impaired the acquisition of conditioned fear responses. Interestingly, blocking ZI transmission after successful fear conditioning also strongly affected conditioned fear responses later on, indicating a role of the ZI for memory retention or recall (Zhou et al., 2018). Moreover, in two further studies, ZI activation robustly suppressed both freezing responses and active avoidance responses after fear conditioning and suppressing ZI instead of presenting the conditioned stimulus could trigger the avoidance response in conditioned mice, but not in naive animals (Hormigo et al., 2020; Venkataraman et al., 2021). Caudal prethalamic circuits, and in particular the ZI, can therefore significantly influence acquired defensive behaviors.

Interestingly, caudal prethalamic areas are prominently interconnected with the cerebellum, which has been studied extensively in the context of learning (Zeeuw and Brinke, 2015). Both ZI and vLGN directly project to the pontine nuclei (Harrington, 1997; Kolmac et al., 1998; Moore et al., 2000; Kolmac and Mitrofanis, 2000; Mitrofanis, 2005; Halverson and Freeman, 2010; Monavarfeshani et al., 2017; Fratzl et al., 2021), the main source of mossy fiber input to the cerebellum (Kratochwil et al., 2017). In turn, the cerebellum projects back to ZI and vLGN through the deep cerebellar nuclei: the ZI receives direct input from the anterior interposed and the dentate nucleus (Mitrofanis and deFonseka, 2001; Kebschull et al., 2020), while the vLGN is specifically innervated by the posterior part of the interposed nucleus (Graybiel, 1974; Zimny et al., 1986; Vaudano and Legg, 1992; Nakamura, 2018). The function of these cerebellum-prethalamus loops is still unclear, but the vLGN-cerebellar pathway may be important for visual eye-blink conditioning (**Figure 3F**, Koutalidis et al., 1988; Halverson et al., 2009; Halverson and Freeman, 2010; Steinmetz et al., 2013; Kashef et al., 2014). Muscimol inactivation of rat vLGN strongly reduces the conditioned eye-blink response to a visual conditioned stimulus (CS), but not to a somatosensory CS (Steinmetz et al., 2013). Using electrical stimulation of vLGN in rats as CS is sufficient for the acquisition of conditioned eye blink responses to both vLGN stimulation and a visual stimulus (Halverson et al., 2009), indicating a shared neural pathway, likely through the pontine nuclei (Halverson and Freeman, 2010). The visual information necessary for eye-blink conditioning may thus at least in part be relayed to the cerebellum via the vLGN. Electrophysiological recordings showed that neural activity in vLGN in response to a visual stimulus normally adapts rapidly, but remains high and does not adapt when this stimulus becomes conditioned, and vLGN responses to the CS are strongest in trials with eye-blink response (Kashef et al., 2014). vLGN activity may therefore stabilize sensory representations relevant for future actions through cerebellar feedback to vLGN from the interposed cerebellar nucleus (Clark et al., 1997; Kashef et al., 2014). The function of the parallel ZI-pons-cerebellum loop has not been investigated but could potentially be similarly involved in learning associations between other sensory modalities. More generally, neuronal activity in both ZI and vLGN has been shown to be modulated by experience and learning (Kashef et al., 2014; Chou et al., 2018; Fratzl et al., 2021), indicating that caudal prethalamic pathways play a role in mediating experience-dependent changes in behavior.

The caudal prethalamus, an inhibitory control hub?

Cell-type and pathway specificity of circuits in ZI, vLGN and lGL

Despite a largely common developmental origin (Puelles and Martinez, 2013; Nakagawa, 2019; Puelles et al., 2021), caudal prethalamic circuits are heterogenous and consist of distinct subregions and cell types (**Figure 4**). Two to five structural layers within vLGN have been described in several

mammalian species including mice, differentiated by cytoarchitecture and molecular marker expression (Niimi et al., 1963; Harrington, 1997; Meng et al., 1998; Livingston and Mustari, 2000; Nakamura, 2018; Sabbagh et al., 2021). The distinct external part of vLGN is retinorecipient, while the internal vLGN does not receive retinal axons and fuses medially with the ZI without clear anatomical border. IGL can partly be distinguished from vLGN by expression of Neuropeptide Y (Harrington, 1997), while vLGN contains several types of inhibitory neurons, including nitric oxide synthase-positive, parvalbumin-positive and somatostatin-positive cells and a small percentage of glutamatergic neurons (Meng et al., 1998; Sabbagh et al., 2021; Fratzl et al., 2021; Salay and Huberman, 2021). IGL neurons can be distinguished from vLGN by their projections to SCN, but if and how the layers and cell types within vLGN differ in their connectivity and function, is still unclear. However, recent work has begun to shine light on specific vLGN pathways. vLGN neurons projecting to the SC and the thalamic nucleus reuniens, and glutamatergic vLGN neurons have differential effects on visual threat responses in mice (Salay and Huberman, 2021), and calcium responses in SC-projecting vLGN neurons are strikingly different to those of the average vLGN population (Fratzl et al., 2021), indicating segregated vLGN pathways with different functions (Moore et al., 2000).

The ZI can be divided into at least four distinct zones (**Figure 4**, Kolmac and Mitrofanis, 1999; Mitrofanis, 2005). The rostral ZI contains somatostatin cells and most of the incertal dopaminergic cells, which are contiguous with dopaminergic cells in the hypothalamus (Wagner et al., 1995; Mitrofanis, 2005). Many of the more visceral ZI functions, such as hormone release, ingestive activity regulation and sleep, may be mediated by the rostral ZI, potentially through its connections with the hypothalamus and the paraventricular thalamus (Walsh and Grossman, 1973, 1976, 1977; Mok and Mogenson, 1986; Tonelli and Chiaraviglio, 1995; Wagner et al., 1995; Mitrofanis, 2005; Liu et al., 2017; Zhang and van den Pol, 2017). However, the rostral ZI likely serves further roles, including the regulation of defensive behaviors, through targets such as the SC and the PAG (Mitrofanis, 2005; Bolton et al., 2015; Essig and Felsen, 2016; Chou et al., 2018). The large central part of the ZI is divided into a dorsal and a ventral zone, with largely non-overlapping populations of nitric-oxide-synthase- and parvalbumin-positive neurons, respectively, and with a population of somatostatin-positive neurons located in the lateral part of the ZI, close to the vLGN. Axons from both the sensory periphery and cortical areas predominantly innervate the ventral ZI, which might contribute to sensory processing through projections to PO thalamus and the SC (Kolmac et al., 1998; Shaw and Mitrofanis, 2002; Mitrofanis, 2005; Simpson et al., 2008). Both parvalbumin- and somatostatin-positive neurons and several different ZI pathways have been implicated in the regulation of different fear-related behaviors (Chou et al., 2018; Zhou et al., 2018; Wang et al., 2019; Hormigo et al., 2020; Li et al., 2021; Venkataraman et al., 2021), for instance, activity of parvalbumin-positive neurons projecting to the thalamic PO can enhance defensive flight reactions (Wang et al., 2019). The dorsal ZI is interconnected with brainstem

areas and projects to the intralaminar thalamus, but its function remains unclear (Kolmac et al., 1998; Power et al., 1999; Mitrofanis, 2005). Food-seeking, hunting and novelty-seeking behaviors have been associated with projections from the medial part of the ZI to the PAG (Shang et al., 2019; Zhao et al., 2019; Ahmadlou et al., 2021). Finally, the caudal ZI, enriched in calretinin- and calbindin-positive neurons (Kolmac and Mitrofanis, 1999; Mitrofanis, 2005; Watson et al., 2014), may be most relevant for motor-related functions (Edwards and Isaacs, 1991; Plaha et al., 2006; Ossowska, 2020).

While much remains unknown about the function of specific cell types, circuits and output pathways of the caudal prethalamus, future studies will likely be able to assign specific behavioral functions to distinct cell types and pathways that can be differentially activated. It is currently unclear if and to what degree different circuits in the prethalamus interact with each other. However, there is evidence for synaptic connectivity within and between caudal prethalamic nuclei (Harrington, 1997; Moore et al., 2000; Mitrofanis, 2005; Urbain and Deschênes, 2007), which may allow these circuits to influence each other. For instance, while an animal is engaged in a specific behavior, such as foraging, interactions in the caudal prethalamus could intrinsically suppress alternative behavioral strategies, such as predator avoidance or sleeping (**Figure 5**).

Information conveyed through caudal prethalamic pathways

Circuits in the ZI, vLGN and IGL can control a wide range of behaviors (**Figure 3**), however, it is much less clear when these pathways are engaged and what information they convey. Since all caudal prethalamic areas show sensory responses (see above section on sensory processing), they were traditionally thought to mainly convey sensory signals. And, indeed, visual information transmitted through IGL and vLGN has been shown to be important for circadian entrainment and visual eye-blink conditioning (Koutalidis et al., 1988; Harrington, 1997; Edelstein and Amir, 1999; Halverson et al., 2009; Gall et al., 2013; Halverson and Freeman, 2010; Steinmetz et al., 2013; Kashef et al., 2014), and can affect functions as diverse as sleep, spatial memory and depression-like behavior (Huang et al., 2019; Shi et al., 2020; Huang et al., 2021). Both the vLGN and ZI respond to potentially threatening sensory stimuli and regulate reactions to these sensory threats (Chou et al., 2018; Zhou et al., 2018; Wang et al., 2019; Venkataraman et al., 2019; Hormigo et al., 2020; Fratzl et al., 2021; Salay and Huberman, 2021; Venkataraman et al., 2021), and ZI circuits have been proposed to convert prey-related sensory signals into appetitive motivational drive (Shang et al., 2019; Zhao et al., 2019). More surprisingly, recent evidence indicates that caudal prethalamic pathways also convey non-sensory information. For instance, the IGL most strongly affects non-photic circadian entrainment, mediates the effect of serotonin on circadian rhythms and controls feeding rhythms in darkness, in the absence of periodic dark-light cycles acting as sensory circadian cue (Johnson et al., 1988; Biello et al., 1991; Meyer et al., 1993; Harrington, 1997; Lewandowski and Usarek, 2002; Smith et al., 2015; Fernandez

et al., 2020). ZI neurons are also activated by non-sensory signals such as sleep pressure (Starzl et al., 1951; Naquet et al., 1966; Koyama et al., 2003; Liu et al., 2017; Blanco-Centurion et al., 2021), while the vLGN receives information about the animal's experience of threat, and activity in both vLGN and ZI are modulated by the perceived level of threat (Chou et al., 2018; Fratzl et al., 2021). Caudal prethalamic circuits can therefore integrate sensory and non-sensory signals and regulate a large proportion of an animal's behavioral repertoire accordingly. The caudal prethalamus could thus act as a switchboard for choosing behavioral responses depending on incoming sensory signals and non-sensory information such as the animal's internal state and knowledge (**Figure 5**).

Neocortical influence on behavior through caudal prethalamic pathways

The prethalamus receives signals from many different sources (**Figure 2**), however, one particularly prominent input stems from the cerebral cortex. While neocortical projections to the thalamic reticular nucleus predominantly originate from layer 6 neurons (Crabtree, 2018), the ZI, vLGN and IGL are specifically innervated by cortical layer 5 neurons, which are thought to constitute the driving output of the cerebral cortex (Groh et al., 2014; Sherman, 2016). In fact, most subcortically-projecting layer 5 neurons in dorsal cortex may form branches in the caudal prethalamus (Bourassa and Deschênes, 1995; Mitrofanis and Mikuletic, 1999; Shaw and Mitrofanis, 2002). The caudal prethalamus receives information from sensory, motor and associative cortical areas, and these projections can strongly influence activity in their prethalamic target areas (Urbain and Deschênes, 2007; Barthó et al., 2007; Escudero and Nuñez, 2019). Diverse signals have been shown to be conveyed by different cortical areas which can both enhance or suppress certain behaviors (Chou et al., 2018; Wang et al., 2019; Ahmadlou et al., 2021). Motor, somatosensory and prefrontal cortical areas affect somatosensory responses in the ZI and could thereby gate sensory information flow to the cortex depending on the animal's motor actions and behavioral context (Trageser and Keller, 2004; Lavallée et al., 2005; Trageser et al., 2006; Escudero and Nuñez, 2019). Moreover, prefrontal cortical projections may carry signals about the motivational drive to investigate and knowledge about potential environmental threats to the medial and rostral ZI, respectively (Chou et al., 2018; Ahmadlou et al., 2021), while projections from temporal cortex have been suggested to convey predictions about novel objects to the ZI to direct novelty-seeking gaze shifts in primates (Ogasawara et al., 2021).

Much is still unknown about what information corticofugal projections from different cortical areas send to the caudal prethalamus. But importantly, these projections provide the cortex with an impactful inhibitory pathway for the control of subcortical processing and behavioral output. Corticofugal projections also directly innervate a large number of subcortical areas where they can target both excitatory and inhibitory neurons (Chen et al., 2018; Doykos et al., 2020). The net influence of these direct projections on their target regions remains to be elucidated, as previous studies in

various species showed a wide range of effect sizes (Wickelgren and Sterling, 1969; Hoffmann and Straschill, 1971; Schiller et al., 1974; Ogasawara et al., 1984; Wang et al., 2010; Zhao et al., 2014; Evans et al., 2018). However, direct corticofugal projections unlikely enable significant suppression of down-stream targets, while inhibitory projections from ZI and vLGN can robustly suppress neuronal activity in these target areas (Trageser and Keller, 2004; Lavallée et al., 2005; Chou et al., 2018; Zhao et al., 2019; Ahmadlou et al., 2021; Fratzl et al., 2021). This provides a route for neocortex to exert strong inhibitory control over behavioral output. At the same time, caudal prethalamic areas can provide feedforward inhibitory pathways to balance direct excitatory input to diverse areas, since anatomical data indicates that numerous brain areas as well as the retina project to many targets both directly, and indirectly through the caudal prethalamus (**Figure 6**).

The caudal prethalamus and the basal ganglia, two complementary inhibitory pathways?

Much more extensively studied than the caudal prethalamus, the basal ganglia represent alternative long-range inhibitory pathways between the neocortex and the mid- and hindbrain. Intriguingly, the basal ganglia output nuclei, such as the substantia nigra pars reticulata (SNr), project to many of the same target areas as caudal prethalamic axons, where both have a strong inhibitory influence (Mink, 1996; Hikosaka et al., 2000; McElvain et al., 2021). Moreover, both caudal prethalamic and basal ganglia output nuclei contain GABAergic neurons with high baseline firing rates and may therefore be able to act like inhibitory gates that need to transiently decrease inhibitory tone onto their targets to allow initiation of different actions (Chevalier and Deniau, 1990; Hikosaka, 2007; Ciftcioglu et al., 2020). What then is the difference between these two inhibitory pathways from the neocortex? The basal ganglia are thought to be involved in a variety of cognitive, emotional and motor-related functions, in particular the initiation and execution of movements (Nelson and Kreitzer, 2014). One proposed role of the basal ganglia is that of a learning machine that re-evaluates cortical input through reinforcement learning and thereby selects future actions for learned behaviors (Cox and Witten, 2019). Information from the cortex thereby travels through several synapses in basal ganglia regions before reaching brainstem targets. Caudal prethalamic circuits provide a more direct and therefore faster influence of neocortical activity on subcortical structures and may be most relevant for acute and rapid behavioral control of instinctive, non-learned actions.

Conclusions and future remarks

The diverse cell types and output pathways of the ZI, vLGN and IGL in the caudal prethalamus can influence most brain systems, including the midbrain, medulla, cerebellum, hypothalamus, thalamus

and even neocortex. Through these pathways, caudal prethalamic nuclei together exert inhibitory control over almost all aspects of the instinctive repertoire of mammals, including sleep, circadian rhythms, feeding, drinking, exploring and staying safe from predators. While there is little data on caudal prethalamic circuits in the context of social behavior, given its extensive behavioral control it is probable that the caudal prethalamus can also affect this aspect of behavior. In addition, the caudal prethalamus can impact learning and learned behavior.

Circuits in the caudal prethalamus receive prominent input from neocortical areas, but are also innervated by many subcortical areas, the sensory periphery and neuromodulatory systems (**Figure 2**). Caudal prethalamic nuclei can therefore integrate diverse sensory and non-sensory signals, and control behavior depending on the balance of sensory input, an animal's internal state (e.g. sleep pressure, hunger or fear state) and prior experience. One of the roles of the caudal prethalamus may therefore be that of a switchboard, that - depending on the behavioral context and state of the animal - can select which behavioral strategy to pursue, such as searching for food or avoiding risk of predation (**Figure 5**). Caudal prethalamic pathways could - with a combination of inhibition and disinhibition of specific down-stream areas - enable the chosen behavioral response, while suppressing alternative behaviors in a 'winner-takes-all' fashion. The caudal prethalamus could thereby implement switches in behavioral policies, either via top-down instructions from its inputs, or even intrinsically through intra-areal interactions between different prethalamic circuits that could inhibit or disinhibit each other. Interestingly, the prethalamic TRN has previously also been proposed to act as a switchboard, specifically for thalamocortical processing (Crabtree and Isaac, 2002; Halassa and Acsády, 2016). To test this and other hypotheses of prethalamic function, it will be important to more clearly delineate the different prethalamic circuits and their roles, as well as their interconnectivity. Moreover, we need to gain a better understanding of what information the caudal prethalamus receives, how it translates this information into behavioral influence, and, importantly, how these circuits interact, for instance through simultaneous recordings from different identified cell types in the caudal prethalamus during naturalistic behaviors. The recent advances in tools for targeting, manipulating and recording from specific cell types and circuits in behaving animals will facilitate answering these questions and will shed light on the function of caudal prethalamic circuits in the near future.

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Declaration of interests

The authors declare no competing financial interests.

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1003 # Figure legends

Figure 1. Anatomical location of prethalamic nuclei in the mouse brain.

Schematic depicting prethalamic nuclei in coronal sections 2.8 mm (**A**) 2.2 mm (**B**) and 1.6 mm (**C**) posterior of Bregma. BLA: basolateral amygdala, CTX: cerebral cortex, CP: caudoputamen, dLGN: dorsal lateral geniculate nucleus, HPC: hippocampus, HY: hypothalamus, IGL: intergeniculate leaflet, MB: midbrain, TH: thalamus, TRN: thalamic reticular nucleus, vLGN: ventral lateral geniculate nucleus, ZI: zona incerta.

Figure 2. Caudal prethalamic connectivity with brain areas in the fore-, mid- and hindbrain.

The caudal prethalamus (red) is interconnected with several brain areas in the cortex (purple), thalamus (blue), hypothalamus (yellow), mid- (green), and hindbrain (brown) and receives input from neuromodulators and the sensory periphery (gray).

Figure 3. Caudal prethalamic circuits regulate various brain functions and behaviors.

Different caudal prethalamic circuits are involved in the regulation of various brain functions and behaviors including pain processing (**A**), sleep and circadian rhythms (**B**), defensive behaviors (**C**), feeding and drinking (**D**), hunting and exploration (**E**), and visual eye-blink conditioning (**F**). IGL: intergeniculate leaflet, LHA: lateral hypothalamic area, MLR: mesencephalic locomotor region, PAG: periaqueductal grey, PN: pontine nuclei, PO: posterior nucleus of the thalamus, PVT: paraventricular nucleus of the thalamus, RE: thalamic nucleus reuniens, SC: superior colliculus, SCN: suprachiasmatic nucleus, vLGN: ventral lateral geniculate nucleus, ZI: zona incerta, ZIm: medial ZI, ZIr: rostral ZI, ZIv: ventral ZI.

Figure 4. Cell-type and pathway specificity of caudal prethalamic circuits.

(**A**). Schematic depicting the spatial organization of different cell types within distinct caudal prethalamic subregions. Note that the list of cell types is not exhaustive, and some of these cell types may be overlapping. Organization of ZI cell types according to Kolmac and Mitrofanis, 1999, Mitrofanis, 2005 and Ahmadlou et al., 2021. Organization of vLGN and IGL cell types according to Harrington, 1997; Meng et al., 1998; Sabbagh et al., 2021 and Salay and Huberman, 2021. (**B**) Schematic depicting main (but non-exhaustive) output pathways of different caudal prethalamic subsections to areas in the cortex (purple), thalamus (blue), hypothalamus (yellow), mid- (green), and hindbrain (brown). Cell types: CB: calbindin, ECEL1: endothelin converting enzyme like 1, NOS: nitric oxide synthase, NPY: neuropeptide Y, PENK: proenkephalin, PV: parvalbumin, SST: somatostatin, TAC1: tachykinin precursor 1, TH: tyrosine hydroxylase, VGLUT2: vesicular glutamate transporter 2. Brain regions: CTX: cortical areas, , HY: hypothalamic areas (excluding SCN), IGL: intergeniculate leaflet ILT: intralaminar thalamic nuclei, LD: lateral dorsal nucleus of the thalamus, LP: lateral posterior nucleus of the thalamus, lSC: lateral superior colliculus, MRN: midbrain reticular nucleus, mSC: medial superior colliculus, MY: medulla, PAG: periaqueductal grey, PN: pontine nuclei, PO: posterior nucleus of the thalamus, PRN: pontine reticular nucleus, PTC: pretectal areas, PVT: paraventricular nucleus of the thalamus, RE: nucleus reuniens of the thalamus, RN: red nucleus, SCN: suprachiasmatic nucleus, SCsg: superior colliculus, superficial grey, vLGN: ventral lateral geniculate nucleus, vLGNe: external

vLGN, vLGNi: internal vLGN, ZI: zona incerta, ZIc: caudal ZI, ZId: dorsal ZI, ZIr: rostral ZI, ZIv: ventral ZI.

Figure 5. The caudal prethalamus, an inhibitory switchboard for behavioral control

The caudal prethalamus may act as a switchboard, that - depending on the behavioral context and state of the animal - can regulate which behavioral strategy to pursue. A sensory stimulus (yellow) might trigger escape (orange, **A**) during a state of increased anxiety (red, **A**), whereas the same stimulus could induce hunting behavior (green, **B**) during a state of increased hunger (blue, **B**).

Figure 6. Feedforward inhibition through caudal prethalamic circuits.

Feedforward inhibitory pathways are a common motif of caudal prethalamic circuits and may balance direct excitatory input to diverse areas, such as retinal input to the SCN via the IGL (**A**), retinal input to the SC via the vLGN (**B**), cortical input to the SC via the vLGN (**C**) and cortical input to the PO thalamus via the ZI (**D**). IGL: intergeniculate leaflet, PO: posterior nucleus of the thalamus, SC: superior colliculus, SCN: suprachiasmatic nucleus, vLGN: ventral lateral geniculate nucleus, ZI: zona incerta.