European Journal of Neuroscience

Neurobiology of pain, interoception and emotional response: lessons from nerve growth factor-dependent neurons

Yasuhiro Indo

Department of Pediatrics, Kumamoto University Hospital, Honjo 1-1-1, Chuou-ku, Kumamoto 860-8556, Japan

Keywords: congenital insensitivity to pain with anhidrosis, hereditary sensory and autonomic neuropathy, *NTRK1*, polymodal receptor, TrkA

Correspondence: Dr Y. Indo, as above.

E-mail: yindo@kumamoto-u.ac.jp

Running title: Neurobiology of pain and NGF-dependent neurons

Abstract

Although nerve growth factor (NGF) is a well-known neurotrophic factor, it also acts as a mediator of pain, itch and inflammation. Congenital insensitivity to pain with anhidrosis (CIPA) is an autosomal recessive genetic disorder caused by loss-of-function mutations in *NTRK1*, the gene encoding a receptor tyrosine kinase for NGF, TrkA. Mutations in *NTRK1* cause the selective loss of NGF-dependent neurons in otherwise intact systems. NGF-dependent primary afferents are thinly myelinated Aδ or unmyelinated C-fibers that are dependent on the NGF–TrkA system during development. In CIPA, the lack of pain and the presence of anhidrosis (inability to sweat) are due to the absence of both NGF-dependent primary afferents and sympathetic postganglionic neurons, respectively. These peripheral neurons form an interface between the nervous system and the 'body-proper' and play essential roles in the interoception and sympathetic regulation of various tissues or organs. Patients with CIPA also show mental retardation and characteristic behaviors and are probably neuron-deficient within the brain. However, the functions of NGF-dependent neurons in the brain are controversial, both in animal and human studies. This review focuses on various brain regions that express TrkA mRNA, based on data from the *Allen Human Brain Atlas*, and discusses putative neuronal networks related to these brain regions in humans. A better understanding the distribution of NGF-dependent neurons in the brain will provide a framework for further studies to investigate pain, interoception and emotional responses. Furthermore, strategies targeting the molecular mechanisms through which the NGF–TrkA system functions may provide hope for the development of novel analgesics.

Introduction

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. However, the ability to feel pain is important in that it protects us and maintains bodily homeostasis. Various types of congenital insensitivity to pain provide opportunities to consider the physiology of pain and its underlying mechanisms in humans. Children born without the ability to feel pain are unable to prevent injuries from innumerable small and occasionally large physical threats (Melzack & Wall, 1982). They have severely reduced quality of life due to multiple injuries and often die young. Thus, the mediation of pain by the nervous system is considered to be an integral part of the body's defense and homeostatic control.

Nerve growth factor (NGF) is a prototype neurotrophin and the first growth factor to be identified [(Levi-Montalcini, 1987); see also (Cattaneo, 2013) for historical perspective]. NGF plays pivotal roles in controlling the survival and differentiation of the nervous system during embryonic and early postnatal stages (Reichardt, 2006). Genetic defects of NGF–TrkA signal transduction in animals lead to the failure of survival of various NGF-dependent neurons, as these are not maintained due to apoptosis during development (Crowley *et al.*, 1994; Smeyne *et al.*, 1994). NGF-dependent neurons are defined as neurons in the peripheral nervous system (PNS) or central nervous system (CNS) that are dependent on the NGF–TrkA system during development. NGF thus supports the survival of various NGF-dependent neurons, including nociceptive neurons, autonomic sympathetic neurons and some neurons of the CNS.

NGF is also a peripheral pain mediator, particularly in inflammatory pain states (for a review, see Pezet & McMahon, 2006). NGF also alters the response properties of itch-signaling neurons (Ikoma *et al.*, 2006). The NGF–TrkA system is involved in acute inflammation and contributes to peripheral sensitization to pain or itch. Thus, NGF plays critical roles as a significant mediator and modulator of pain, itch and inflammation (Indo, 2010).

NGF-dependent neurons in the PNS include primary nociceptive afferent neurons and sympathetic postganglionic neurons. NGF-dependent primary afferent neurons with thin fibers (NGF-dependent primary afferents) are defined

as primary afferent neurons with a small-diameter, thinly myelinated Aδ-fibers, or unmyelinated C-fibers that depend on the NGF–TrkA system during development (Indo, 2010). These neurons not only detect noxious stimuli but also transmit sensation from the body's interior; this is known as interoceptive sense (Craig, 2002, 2009). NGF-dependent primary afferents are also referred to as 'interoceptive polymodal receptors' (Indo, 2009). NGF-dependent primary afferents are thus responsible for both nociceptive and homeostatic afferent pathways.

Autonomic sympathetic postganglionic neurons are involved in the regulation of various tissues or internal organs, including skin appendages. The sympathetic nervous system enables unconscious maintenance of bodily homeostasis, including the regulation of blood pressure and body temperature. In contrast, emotion is a strong feeling characterized by various complex physical reactions closely related to activities of the sympathetic nervous system. The 'fight-or-flight' response illustrates a strong emotional state associated with an excitation of the sympathetic nervous system. Thus, the sympathetic nervous system underlies both homeostasis and emotional responses.

NGF-dependent primary afferents (or interoceptive polymodal receptors) report the physiological status of various tissues or organs to the brain, which subsequently maintains homeostasis of the whole body along with autonomic, neuroendocrine and behavioral mechanisms (Craig, 2002, 2009; Roosterman *et al.*, 2006).

Congenital insensitivity to pain with anhidrosis (CIPA) is an autosomal recessive genetic disorder due to loss-of-function mutations in *NTRK1,* the gene encoding TrkA. TrkA is a receptor tyrosine kinase for NGF (Indo *et al.*, 1996; Indo, 2001, 2012). CIPA is also known as hereditary sensory and autonomic neuropathy type IV (Axelrod *et al.*, 2006). Defects in NGF–TrkA signal transduction cause a lack of NGF-dependent neurons in patients with CIPA, leading to characteristic phenotypes, including insensitivity to pain and anhidrosis (the inability to sweat). In CIPA, the lack of pain and the presence of anhidrosis are due to the absence of NGF-dependent primary afferents and sympathetic postganglionic neurons, respectively. Patients with CIPA are mentally retarded and show characteristic behaviors. However, the mechanisms

that produce the CIPA phenotype remain to be characterized.

It has been shown in animal studies that neurons in several brain regions express TrkA mRNA (Gibbs & Pfaff, 1994; Sobreviela *et al.*, 1994; Holtzman *et al.*, 1995). Some of these neurons are probably NGF-dependent neurons, as they are absent in TrkA gene-knockout mice (Smeyne *et al.*, 1994; Fagan *et al.*, 1997). Studies in gene-knockout or conditional gene-knockout mice have suggested functions for some TrkA-expressing neurons in the brain (Muller *et al.*, 2012; Sanchez-Ortiz *et al.*, 2012). However, in contrast to those in the PNS, most NGF-dependent neurons in the brain remain uncharacterized.

Traditional animal studies on pain using electrophysiology, pharmacology and/or molecular biology have yielded valuable insights into the molecular basis of pain perception (Basbaum *et al.*, 2009; Mogil, 2009). In addition, lesion experiments in animals and observations in brain-damaged humans have been utilized to study the functions of specific parts of the nervous system. Together, these approaches have revealed an association between the normal function of the lesioned or damaged structures and the symptoms that ensue. However, the interpretation of such associations may not be straightforward, as a lesion may destroy not only neurons originating in that area but also fibers that are passing through the area.

Genetic studies using gene-knockout animals can be an alternative approach to understand the function of neurons related to a specific gene and complement lesion experiments in animals. Nevertheless, animal studies inevitably leave room for speculation whenever the results of such studies are compared to those seen in human studies. A rare human genetic disorder associated with loss of specific neurons as a result of loss-of-function gene mutations can provide opportunities to explore the normal functioning of those neurons in humans. CIPA is considered to be such a rare genetic disorder. Loss-of-function mutations in the human *NTRK1* gene cause the selective loss of a defined cell population, i.e. NGF-dependent neurons, in the nervous system. This gives us a rare opportunity to analyze *in vivo* the functions of NGF-dependent neurons and the neural circuits in which they are located, in what is an otherwise intact system.

Recently, the complete transcriptional architecture of the human brain has

been characterized and reported. This provides important information for understanding the impact of genetic disorders on different functional circuits within various brain regions (Hawrylycz *et al.*, 2012). It would also provide an opportunity to understand the putative effects of *NTRK1* mutations on the brain, and may therefore enable us to elucidate the neuronal networks that are directly or indirectly connected to peripheral NGF-dependent neurons in humans. It is conceivable that neurons of central circuits expressing TrkA mRNA play an important role in pain and 'emotion' arising from the pain system. However, the TrkA-positive neurons may contribute to other brain functions that are not related to the pain system. In either case, it would be intriguing to conduct neuroanatomical studies of TrkA mRNA-expressing neurons in human brains and to investigate their connectivity in known neuronal systems of the CNS. These studies may provide some clues to further investigate and characterize putative neuronal networks or pathways underlying the neuronal basis of pain, interoception and emotional responses in humans. Alternatively, they may reveal unique functions of TrkA mRNA-expressing neurons in the brain that are currently unknown.

This review briefly summarizes the function of NGF-dependent neurons in the PNS and the pathophysiology of CIPA. I subsequently discuss brain regions that express TrkA mRNA, according to data from the *Allen Human Brain Atlas* (Hawrylycz *et al.*, 2012). Further, this review focuses on the putative functions of neurons in brain regions that express TrkA mRNA and discusses the putative neuronal networks related to pain, interoception and emotional responses.

NGF-dependent neurons in the PNS

The NGF-dependent neurons in the PNS, including NGF-dependent primary afferents and sympathetic postganglionic neurons, are considered to form an interface between the nervous system and the body-proper (Fig. 1) (Indo, 2012). The body-proper refers to the organism minus the neural tissues (the central and peripheral components of the nervous system; Damasio, 1994). Thus, these peripheral neurons mediate the reciprocal communication between the brain and the body-proper.

NGF-dependent primary afferents include two classes of neurons: peptidergic and nonpeptidergic. Nonpeptidergic neurons initially express TrkA, but later switch off TrkA and express Ret, another receptor tyrosine kinase for glial cell-derived neurotrophic factor (GDNF) (Silos-Santiago *et al.*, 1995; Molliver *et al.*, 1997; Snider & McMahon, 1998). Both peptidergic and nonpeptidergic neurons are thus considered to be NGF-dependent neurons. NGF-dependent primary afferents innervate all tissues of the body, including skin, muscle, joints, teeth, blood vessels and visceral tissue. The cell bodies of NGF-dependent primary afferents are located either in the dorsal root ganglia alongside the spinal cord or in the trigeminal ganglion (Indo, 2010). A subset of primary afferent neurons in the glossopharyngeal nerve and the vagus nerve are most likely NGF-dependent neurons and transmit visceral afferent information to the brain from the head and neck, and from the thoracic and abdominal cavities, respectively.

NGF-dependent primary afferents also mediate transmission of pain and itch, as well as playing essential roles in interoception (Indo, 2010). NGF-dependent primary afferents terminate in lamina I of the spinal dorsal horn and trigeminal nucleus and conduct information on numerous types of physiological conditions through intervening pathways (such as the spinothalamic tract) to the brain. NGF-dependent primary afferents probably include primary pruriceptive neurons, which were recently characterized in mice (Mishra & Hoon, 2013), as well as primary somatosensory neurons responsible for allergic host defenses, in which anticipatory responses are elicited to promote the avoidance of suboptimal environments (Palm *et al.*, 2012). The latter study has proposed that allergic reactivity may provide an important defense mechanism to protect the host from noxious environmental factors. Neutral environmental stimuli perceived through visual, olfactory and gustatory systems can be temporally associated with the stimulation of somatosensory pathways, resulting in Pavlovian conditioning of neutral cues (such as sight, smell and taste) with the antigen-specific response to allergens (Palm *et al.*, 2012). These somatosensory pathways probably involve NGF-dependent primary afferents. Thus, NGF-dependent primary afferents constitute a part of the homeostatic afferent pathways carrying information on the physiological

status of all tissues of the body; they are fundamental components of the interoceptive pathway (Craig, 2002, 2009).

Sympathetic postganglionic neurons, whose cell bodies are located in the sympathetic ganglia, are also NGF-dependent neurons. In the skin, these neurons are involved in the regulation of blood vessels and lymphatic function, as well as in the regulation of skin appendages, including sweat glands, apocrine glands and hair follicles (Roosterman *et al.*, 2006). The sympathetic nervous system plays critical roles in maintaining homeostasis of body temperature by regulating sweat gland functions and vasoconstriction. Sweating is particularly important for the regulation of body temperature in humans. Sympathetic postganglionic neurons also regulate many other target organs and tissues in the body and constitute a part of the homeostatic efferent pathways.

In addition, NGF-dependent neurons play critical roles in mediating cross-talk between the three ‘super-systems’: the brain, and immune and endocrine systems (Indo, 2012). The brain and immune system are essential for homeostatic regulation and survival (Elenkov *et al.*, 2000). The endocrine system coordinates and controls complex responses of the brain and immune system. For instance, various triggering factors, including immune and hormonal activity and mast cell activation, stimulate NGF-dependent primary afferents (interoceptive polymodal receptors) directly or indirectly. Upon stimulation, these neurons, as well as sympathetic postganglionic neurons, influence inflammation by secreting pro-inflammatory or anti-inflammatory substances at the sites of inflammation (Indo, 2010). The term ‘neurogenic inflammation’ refers to signs of inflammation (e.g., tumor, rubor, calor and dolor) that develop upon neuronal activation and the consequent release of a neuronal mediator (Holzer, 1998). Axon reflex is an efferent function of the NGF-dependent primary afferents (Indo, 2010). Patients with CIPA lack the axon reflex responsible for neurogenic inflammation. This suggests that neurogenic inflammation does not occur without NGF-dependent neurons. In accordance with the concept of ‘super-systems’, NGF-dependent neurons in the PNS are considered to form communication routes between the brain, and immune and endocrine systems.

Together, NGF-dependent neurons in the PNS constitute homeostatic afferent and efferent pathways. In response to the interoceptive polymodal

inputs through NGF-dependent primary afferents, the brain regulates various functions of target organs and tissues through autonomic sympathetic postganglionic neurons. Thus, NGF-dependent neurons in the PNS play critical roles in the neural networks responsible for interoception and homeostasis.

In humans (as well as in many other animals), systemic responses of the sympathetic nervous system often accompany emotional responses. Activation of the various target tissues or organs through sympathetic outflows and integrated feedback from the entire body through NGF-dependent primary afferents probably contribute to emotional responses. Emotion is a strong feeling characterized by various complex reactions, with both mental and physical manifestations closely related to activities of the sympathetic nervous system. The ‘fight-or-flight’ response illustrates a strong emotional state associated with an extreme excitation of the sympathetic nervous system. However, most emotional responses in daily life occur with varying degrees at a subconscious level.

The autonomic sympathetic nervous system is crucial for achieving the appropriate modification of physical parameters in the body that generate bodily states characterizing certain emotions. A sympathetic skin response (SSR) has been used as an index for monitoring bodily state. A definition of the term ‘emotion’ is ‘a collection of changes occurring in both brain and body, usually prompted by a particular mental content’ (Damasio, 1994: p270 in Penguin Books 2005). ‘Feeling’ is the perception of those changes (Damasio, 1994: p270 in Penguin Books 2005). Interoceptive polymodal receptors, conveying visceral signals and signals from the body’s internal milieu to the brain, play critical roles in ‘feelings’ (Damasio, 2003).

Emotional responses contribute to the prevention of danger or trauma. When humans are exposed to danger or trauma, the stimuli or contexts associated with the danger or trauma become learned triggers that unleash emotional responses (LeDoux, 1996, 2000). These emotional experiences subsequently induce the ‘fear conditioning’ by pairing the stimuli or contexts with danger or trauma. The pathophysiology of CIPA strongly suggests that NGF-dependent neurons in the PNS are indispensable for emotional responses.

When taken together, the evidence suggests that NGF-dependent neurons

in the PNS play essential roles in ‘emotions and feelings’, as well as in the interoception (including pain) and homeostasis of the body.

Congenital insensitivity to pain with anhidrosis

Loss-of-function mutations in *NTRK1* lead to an absence of functional TrkA protein in patients with CIPA (Indo *et al.*, 1996; Indo, 2001, 2012). Defects in NGF–TrkA signal transduction cause the failure of various NGF-dependent neurons to survive, due to developmental apoptosis (Indo, 2002). As a result, patients with CIPA lack all NGF-dependent neurons, including NGF-dependent primary afferents and sympathetic postganglionic neurons. They consequently lack pain and itch sensations and neurogenic inflammation as well as sympathetic functions (Indo, 2010).

Patients with CIPA lack all pain sensations, including visceral pain (Indo, 2002). Touch, vibration and position senses are normal. Motor functions are also normal, although repeated trauma can induce secondary dysfunction in the motor system. Patients with CIPA tend to develop hyperthermia in hot environments due to their inability to sweat. They also tend to suffer from hypothermia in cold environments. Clinical and behavioral studies also suggest that patients with CIPA lack interoception and sympathetic regulation of various target tissues, including internal organs (Indo, 2009). For instance, patients with CIPA show symptoms characteristic of an absence of sympathetic innervation of the head: dry skin (anhidrosis), constricted pupils (miosis) and drooping of the upper eyelid (ptosis) (Indo, 2002). The ensuing symptoms can be understood based on the effects of sympathetic fibers on the skin and the eye. Dry skin is caused by lack of sweat secretion. Miosis and slight ptosis are due to paralysis of the pupillary dilator and the smooth tarsal muscle, respectively. This constellation of symptoms is known as ‘bilateral Horner’s syndrome’.

Patients with CIPA have variable degrees of mental retardation and exhibit learning deficits. Hyperactivity and emotional lability are common. Affected children show defects in conceptual thinking, abstract reasoning and social behavior and exhibit symptoms of moderate to severe emotional disturbance (Swanson, 1963; Pinsky & DiGeorge, 1966). Behaviors of children with CIPA are

often characterized as labile, hyperactive and erratic. Assessments of cognitive and adaptive behavior have suggested that many children with CIPA exhibit mental retardation (or learning disabilities) and symptoms of severe attention-deficit-hyperactivity disorder (Levy Erez *et al.*, 2010).

In an autopsy study, the tract of Lissauer (dorsolateral fasciculus) could not be identified at any level of the spinal cord in a patient with CIPA (Swanson *et al.*, 1965). The spinothalamic tracts could not be specifically identified, but the lateral and ventral columns of the spinal cord appeared normal. It is likely that patients with CIPA lack some neurons in the brain. However, in the above case, no obvious gross abnormalities of the brain were recognized (Swanson *et al.*, 1965). The corresponding gene-knockout mice lack basal forebrain cholinergic neurons (BFCNs) and striatal cholinergic neurons (Smeyne *et al.*, 1994). Neither BFCNs nor striatal cholinergic neurons mature fully in knockout mice in the absence of NGF–TrkA signaling (Fagan *et al.*, 1997). These studies have indicated that BFCNs and striatal cholinergic neurons are NGF-dependent neurons in the rodent. Taken together, it is therefore conceivable that mental retardation and characteristic behaviors observed in patients with CIPA may be related to defects of BFCNs and other NGF-dependent neurons in the brain.

Regions that express TrkA mRNA in the human brain

In patients with Alzheimer's disease, a decrease in TrkA mRNA is observed in BFCNs (Boissiere *et al.*, 1997; Mufson *et al.*, 2003). Growing evidence suggests that an imbalance in the expression of NGF and TrkA is a crucial factor underlying BFCN dysfunction in Alzheimer's disease (Mufson *et al.*, 2008). A Phase I clinical trial has been undertaken to examine the utility of *ex vivo* NGF gene therapy for Alzheimer's disease. This trial has shown promise and warrants additional clinical trials (Tuszynski *et al.*, 2005). In addition, significant down-regulation of TrkA expression has been demonstrated during the development of Alzheimer's disease (Ginsberg *et al.*, 2006). This suggests that the dysfunction of TrkA-expressing neurons in the brain may be related to the deterioration of cognitive or other functions observed in patients with Alzheimer's disease. Thus, it would be interesting to analyze the location of TrkA-expressing

neurons in putative neural network in humans. In this regard, a comprehensive expression profile of *NTRK1* in the human brain would be informative, as it may provide clues to the localization of TrkA mRNA expressing neurons and the neuronal circuits in which they are embedded.

Recently, the complete transcriptional architecture of the human brain has been characterized and reported, providing important information for understanding the impact of genetic disorders on different brain regions and on various functional circuits (Hawrylycz *et al.*, 2012). The *Allen Human Brain Atlas* is a multimodal atlas of gene expression anatomy comprising a comprehensive 'all genes-all structures' array-based dataset of gene expression and complementary *in situ* hybridization gene expression studies targeting selected genes in specific brain regions (Shen *et al.*, 2012). The *Atlas* includes data-mining resources that enable researchers to uncover connections between structure, function and molecular biology. Thus, the *Atlas* integrates structure, function and gene expression data to accelerate basic and clinical research of the human brain in normal and disease states. Due to normal variations in brain size and morphology, the number of samples per structure varies across brains. Microarray heat-map displays of z-score or $\log_2$ expression values allow simultaneous visualization of multiple genes and probes across all structures and across brains.

Data on *NTRK1* expression profile in various brain regions have been retrieved from a publicly available online resource of gene expression information of the human brain (*Allen Human Brain Atlas*: http://human.brain-map.org/). From this whole brain microarray data, various brain regions have been selected where the expression z-score of *NTRK1* is higher than 1.0 with two probes (A_23_P34804 and A_24_P265506) in at least one of the brains of six donors. Thus, brain regions possessing a relatively high expression of TrkA mRNA have been selected and are shown in Table 1 (Supporting information Table S1 is also available).

Human brain regions or structures that show relatively high expression of TrkA mRNA are as follows: the basal forebrain (the septal nuclei and the substantia innominate); the striatum [the body and tail of the caudate nucleus, the nucleus accumbens (NAc) and the putamen]; the hypothalamus (the lateral

hypothalamic area, the posterior hypothalamic area and the tuberomammillary nucleus); the cerebellar nuclei (the dentate nucleus and the globose nucleus); the basal part of the pons (the pontine nuclei); the pontine tegmentum [the abducens nucleus, the central gray of the pons, the facial motor nucleus, the nucleus subceruleus, the pontine raphe nucleus, the paramedian pontine reticular formation (PPRF) and the trigeminal nuclei]; the myelencephalon (the arcuate nucleus of the medulla, the cochlear nuclei, the cuneate nucleus, the gracile nucleus, the hypoglossal nucleus, the gigantocellular group, the lateral medullary reticular group, the raphe nuclei of the medulla and the vestibular nuclei).

Functions of neurons in brain regions that express TrkA mRNA

In the following description, general information on the structure and function of the human brain and the nomenclature used are based on a textbook (Brodal, 2010) and on NeuroNames (Bowden, 2002, http://braininfo.rprc.washington.edu/), respectively.

The basal forebrain (septal nuclei and substantia innominate)

The septal nuclei and substantia innominate belong to the basal forebrain. The basal forebrain contains four partially overlapping cell groups (the medial septal nucleus, the nucleus of the vertical limb of the diagonal band, the nucleus of the horizontal limb of the diagonal band and the nucleus basalis of Meynert), where cholinergic and non-cholinergic neurons are intermingled with each other (Mesulam *et al.*, 1983; Mesulam, 2004). The term ‘nucleus basalis’ is used to designate the cholinergic as well as non-cholinergic components in this nucleus. According to the description in the *Allen Human Brain Atlas*, the substantia innominate includes the nucleus basalis of Meynert, the nucleus of the vertical limb of the diagonal band, the nucleus of the horizontal limb of the diagonal band, the nucleus of the anterior commissure, the nucleus of stria terminalis and the olfactory tubercle.

In the human brain, BFCNs provide the major cholinergic innervation to the hippocampus, amygdala and neocortex (Mesulam, 2004). The septal nuclei and the diagonal band send fibers primary to the hippocampal formation, whereas the basal nucleus projects to the rest of the cerebral cortex and the amygdala (Mesulam *et al.*, 1983; Mesulam, 2004). The septal nuclei and the diagonal band of Broca are particularly important for memory (presumably because of their connections with the hippocampus), whereas the basal nucleus is more concerned with maintaining and perhaps focusing attention (Brodal, 2010). The basal nucleus provides the major source of cholinergic input to the amygdala, which is involved in mediating the influences of emotional arousal and stress on learning and memory. The cholinergic activation of the amygdala by the basal nucleus probably contributes to the modulation of memory consolidation (McGaugh *et al.*, 2002). Consistent with this, recent studies have indicated a role for cholinergic neurons in attention and memory mechanisms (McGaugh *et al.*, 2002; Mesulam, 2004; Sarter *et al.*, 2005, 2006; Hasselmo & Sarter, 2011).

TrkA gene-knockout mice lack BFCNs (Smeyne *et al.*, 1994). These neurons do not mature fully in the absence of NGF–TrkA signaling (Fagan *et al.*, 1997). A recent study on a forebrain-specific conditional TrkA knockout mouse line has demonstrated that TrkA has a key role in establishing the basal forebrain cholinergic circuitry (Sanchez-Ortiz *et al.*, 2012). In addition, the anatomical and physiological deficits caused by a lack of TrkA signaling in BFCNs selectively impact cognitive activity. Another study has also confirmed that NGF–TrkA signaling supports the survival of a small proportion of cholinergic neurons during development (Muller *et al.*, 2012). However, in contrast to the former study, the latter suggests that NGF–TrkA signaling is not required for trophic support or connectivity of the remaining BFCNs. Moreover, behavioral analysis of young adult and intermediate-age mice lacking NGF–TrkA signaling has demonstrated that this signaling is dispensable for both attention behavior and various aspects of learning and memory (Muller *et al.*, 2012). The discrepancy between these two studies may be attributed to a difference in the genetic backgrounds of the mice, or to a difference in the conditional knockout strategies used to ablate a target gene, with different neuronal promoters targeted in the two studies. These studies may also suggest that there is a structural and

functional heterogeneity among the population of BFCNs.

It is likely that patients with CIPA lack these cholinergic neurons. Assessments of cognitive and adaptive behavior have suggested that many children with CIPA exhibit mental retardation (or learning disabilities) and symptoms of severe attention deficit hyperactivity disorder (Levy Erez *et al.*, 2010). Thus, at least some symptoms related to cognitive and adaptive behaviors in CIPA may be caused by the role of cholinergic neurons in attention and memory mechanisms.

The striatum (body of the caudate nucleus, tail of the caudate nucleus, NAc and putamen)

The basal ganglia consist of evolutionarily conserved motor nuclei that form recurrent circuits critical for motor planning (Kreitzer, 2009). An intriguing hypothesis suggests that the vertebrate basal ganglia have evolved as a centralized selection device, specialized to resolve conflicts over access to limited motor and cognitive resources (Redgrave *et al.*, 1999). Recent studies have indicated that the basal ganglia also play a role in cognitive functions (Grahn *et al.*, 2008). Further, the most ventral parts of the basal ganglia contribute to the control of motivation and emotions (Grahn *et al.*, 2008; Kreitzer, 2009).

The striatum is composed of the caudate nucleus and putamen. The ventral striatum is the ventral conjunction of the caudate and putamen that merges into and includes the NAc and striatal portions of the olfactory tubercle (Zhou *et al.*, 2002). The striatum receives three major sources of afferents: the cerebral cortex, the intralaminar thalamic nuclei and dopamine-containing cell groups in the mesencephalon (Kreitzer, 2009). In addition, quantitatively minor afferents to the striatum come from the serotonergic raphe nuclei in the brain stem, which probably targets cholinergic interneurons (Bonsi *et al.*, 2007). Serotonin released by serotonergic fibers originating in the raphe nuclei has a potent excitatory effect on striatal cholinergic interneurons.

The striatum is the primary input nucleus of the basal ganglia and a key neural substrate for procedural learning and memory (Kreitzer, 2009).

Acetylcholine is released into the extracellular space by cholinergic interneurons, which constitute approximately 2% of striatal neurons. Cholinergic mechanisms in the striatum may contribute to the acquisition of learned movements and to other forms of learning (Zhou *et al.*, 2002). *In vivo*, cholinergic interneurons exhibit tonic low-frequency activity that is transiently inhibited in response to visual or auditory cues associated with movement tasks, suggesting that this pause in cholinergic interneuron firing may be associated with behaviorally significant cues (Kreitzer, 2009). The tonically active cholinergic neurons respond (i.e., pause) to unexpected conditioned stimuli that predict rewards, but they also respond to unexpected noxious airpuffs and other unexpected stimuli (Zhou *et al.*, 2002). The pause response of the cholinergic neurons may signal the start of an important event sequence. The pause appears to require coordinated synaptic inputs from both the substantia nigra compacta and intralaminar thalamic nuclei, although the precise mechanisms have yet to be determined (Kreitzer, 2009).

The ventral striatum is composed of the NAc and portions of the olfactory tubercle. It is thought to be phylogenetically older than other parts of the striatum and is primarily related to the limbic structures. The NAc receives afferents from the hippocampal formation, the amygdala, the orbitofrontal cortex and parts of the temporal lobe (Zhou *et al.*, 2002). The NAc also sends efferent fibers to the hypothalamus, the mesencephalic reticular formation and the pedunculopontine nucleus (Brodal, 2010). The NAc projects to the BFCNs, providing a pathway for the NAc to affect cortical arousal, attention and cognitive function (Zhou *et al.*, 2002). The NAc, an important component of the mesolimbic dopaminergic reward system, is also implicated in pain modulation, such as pain-induced analgesia (Gear *et al.*, 1999).

The NAc also receives dopaminergic fibers from the ventral tegmental area (VTA) and sends reciprocal efferent fibers to the VTA (Zhou *et al.*, 2002). Recently, a study on VTA neurons, combining optogenetics with structural imaging and electrophysiology, has been reported in mice (Brown *et al.*, 2012). This study showed that GABA (γ-aminobutyric acid)-releasing neurons of the VTA that project to the NAc inhibit accumbal cholinergic interneurons to enhance stimulus-outcome learning (Brown *et al.*, 2012). The VTA and NAc are essential

for learning about environmental stimuli associated with motivationally relevant outcomes. The task of signaling such events, both rewarding and aversive, from the VTA to the NAc has largely been ascribed to dopaminergic neurons. However, this intriguing study has shown that forcing accumbal cholinergic interneurons to pause in behaving mice enhances discrimination of a motivationally important stimulus that had been associated with an aversive outcome (Brown *et al.*, 2012). These results indicate that VTA GABA projection neurons, through their selective targeting of accumbal cholinergic interneurons, provide a novel route through which the VTA communicates saliency to the NAc.

Considering that the ventral striatum is a phylogenetically conserved structure, these VTA GABA projection neurons may therefore have similar functions in humans. We learn such conditioned aversive behaviors based on the emotional impact elicited when experiencing various pains. These behaviors are components in the physiology of pain and are critical for our survival. Thus, the connections of the NAc probably play an important role for behavior that is governed by emotions.

TrkA gene-knockout mice lack striatal cholinergic neurons (Smeyne *et al.*, 1994). These neurons do not mature fully in the absence of NGF–TrkA signaling (Fagan *et al.*, 1997). In humans, the striatum also contains cholinergic neurons that express TrkA mRNA and a decrease in TrkA mRNA is also observed in the ventral striatum and in the putamen of patients with Alzheimer’s disease (Boissiere *et al.*, 1997).

Patients with CIPA probably lack these cholinergic neurons, although histological studies on the brains of patients have not yet been reported. Patients with CIPA do not show primary motor deficits, although they can suffer from secondary motor dysfunction associated with traumatic injuries. This may suggest a functional significance of the striatal cholinergic neurons in other neuronal function(s) that are not directly related to motor control. Thus, mental retardation (or learning disabilities) and characteristic behaviors (such as symptoms of severe attention deficit hyperactivity disorder) observed in patients with CIPA may be related to defects of NGF-dependent neurons in the striatum.

In summary, TrkA-expressing neurons in the basal ganglia and in particular the striatum may contribute to cognitive and emotional processing in addition to

motor control.

The hypothalamus (lateral hypothalamic area, posterior hypothalamic area, and tuberomammillary nucleus)

A central task of the hypothalamus is to coordinate autonomic, endocrine and somatic motor responses to behavior that is appropriate for the immediate needs of the body; its overall aim is to maintain bodily homeostasis (Brodal, 2010). Distinct sets of neuronal populations in the hypothalamus innervate sympathetic preganglionic neurons. Neurons in the lateral hypothalamus that diffusely project to the cerebral cortex are likely to be important in arousal, and electrical stimulation of the lateral hypothalamus elicits a ‘defense reaction’ that has been described by Cannon (Saper, 2002).

The hypothalamus receives information about ambient temperature from thermoreceptors in the skin and initiates peripheral responses to increase heat production or heat loss. NGF-dependent primary afferents and sympathetic postganglionic neurons play critical roles in thermoreception and peripheral responses, respectively. Because patients with CIPA lack these neurons, they tend to develop hyperthermia in hot environments and hypothermia in cold environments. In this regard, the posterior hypothalamus is considered to be important in controlling shivering (Nagashima *et al.*, 2000).

In humans, the tuberomammillary nucleus of the hypothalamus contains approximately 64 000 histamine-producing neurons that innervate all of the major parts of the cerebrum, cerebellum, posterior pituitary and spinal cord (Haas & Panula, 2003). Neurons of the tuberomammillary nucleus in mammals send projections to the cerebellar cortex, including the Purkinje cell and the granular cell layers (Schweighofer *et al.*, 2004). These histaminergic systems in the brain hold a key position in the regulation of basic bodily functions, including the sleep–waking cycle, energy and endocrine homeostasis and synaptic plasticity and learning (Haas & Panula, 2003).

Thus, some neurons in the human hypothalamus express TrkA mRNA. In addition, an animal study has indicated that TrkA-immunoreactive neurons are observed in the periventricular hypothalamus (Sobreviela *et al.*, 1994).

Hypothalamic neurons expressing the TrkA receptor are likely to perform a critical physiological function.

The cerebellar nuclei (dentate nuclei and globose nucleus) and the basal part of the pons (pontine nuclei)

As a general rule, the cerebellum sends signals to the same regions from which it receives afferents. Studies in primates have revealed that the regions of the cerebellar cortex that receive input from the primary cerebral motor cortex (M1) are the same as those that project to M1 (Kelly & Strick, 2003). Similarly, the regions of the cerebellar cortex that receive input from cerebral area 46 are the same as those that project to area 46 (Middleton & Strick, 2001; Kelly & Strick, 2003). These studies suggest that multiple closed-loop circuits represent a fundamental architectural feature of cerebro-cerebellar interactions (Kelly & Strick, 2003).

Cortico-ponto-cerebellar projections form part of a closed loop system with the cerebral cortex, in which the cerebellum reciprocates projections to the cerebral cortex through the thalamus (Ramnani, 2006). The afferents to the pontine nuclei arise primarily in the cerebral cortex, forming the corticopontine tract. The pontine nuclei project to the cerebellum. In humans, the largest number of cerebellar afferent fibers arises in the pontine nuclei (Brodal, 2010). The pontine nuclei are believed to process information from the cerebral cortex and forward it to the cerebellar cortex.

The fundamental unit of information processing in the cerebellar cortex is the Purkinje cell, which integrates information from two primary pre-cerebellar relay stations: the pontine nuclei and the inferior olive (Ramnani, 2006). Purkinje cells in the cerebellar hemispheres project to the dentate nucleus, whereas Purkinje cells in the intermediate zone project to the interposed nuclei, including the globose nucleus (Brodal, 2010). Signals from the cerebellar hemispheres pass primarily to the motor cortex through the intracerebellar nuclei and the thalamus and there also exists anatomical and physiological evidence of connections from the dentate nucleus (through the thalamus) to the dorsolateral prefrontal cortex (Ramnani, 2006). In humans, the ventral dentate

(interconnected with the prefrontal cortex) is larger than the dorsal dentate (interconnected with the motor cortex). Such connections may suggest the importance of cerebellar influences on cognitive tasks. Indeed, the largest contribution of cortico-pontine fibers comes not from the cortical motor area but from the frontal cortex in the human brain. This suggests that the cerebellum has a more important role in processing information from the prefrontal cortex: an area in which neurons code information at a more abstract level than in the cortical motor areas. There is also anatomical and physiological evidence of connections from the dentate nucleus (through the thalamus) to the dorsolateral prefrontal cortex (Ramnani, 2006).

The concept of internal models in the cerebellum proposes that, through a learning process, the cerebellum forms an internal model to reproduce the dynamics of a body part (Ito, 2008). According to this concept, the intricate neuronal circuitry of the cerebellum encodes an internal model, which is formed and adjusted as a movement is repeated. The internal model ultimately helps the brain to perform the movement precisely, without the need to refer to feedback from the moving part. Recent studies have indicated that the cerebellum may also encode internal models that reproduce the essential properties of mental representations in the cerebral cortex (Ito, 2008). The internal model hypothesis for the control of mental activities predicts that there should be co-activation of the cerebellar hemisphere with the prefrontal and temporo-parietal cortices during the performance of mental tasks, a hypothesis that has been confirmed by recent neuroimaging studies (Ito, 2008).

With regard to pain, when a subject receives an unexpected painful heat stimulus on the left hand, the hippocampus and the most lateral part of the cerebellum get activated simultaneously with the superior frontal and superior parietal gyri (Ploghaus *et al.*, 2000). This study implicates the cerebellum in associative learning relating to pain, which represents an important behavior.

The cerebellum and basal ganglia receive many projections from various regions of the cerebral cortex. The striatum and pontine nuclei are input stages of the basal ganglia and the cerebellum, respectively. It has been a longstanding question as to how and where cerebellar circuits interact with basal ganglia circuits. A study in macaques has found a disynaptic pathway that links an

output stage of the cerebellum, the dentate nucleus, with an input stage of the basal ganglia, the striatum (Hoshi *et al.*, 2005). This disynaptic pathway is probably mediated by intralaminar nuclei and/or ventroanterior/ventrolateral thalamus. This pathway thus enables the output stage of cerebellar processing to have a direct influence over the input stage of processing within the basal ganglia.

The cerebellum also receives a third type of afferent that originates in the hypothalamus or in other brainstem structures, which contains various amines or neuropeptides (Schweighofer *et al.*, 2004; Ito, 2008). The projection to the cerebellum from the tuberomammillary nucleus in the hypothalamus is described above. The pontine nuclei also receive connections from parts of the hypothalamus and limbic structures, notably the mammillary bodies and the cingulate gyrus and these connections may form the basis of cerebellar contributions to certain cognitive tasks (Brodal, 2010). In addition, corticopontine connections from limbic structures may contribute to the ability of motivation and emotions to influence movements.

It is likely that patients with CIPA lack some neurons in the pontine nuclei and cerebellar nuclei that express TrkA mRNA. However, histological studies on the brains of CIPA patients have not yet been reported. Patients with CIPA do not show apparent neurological signs that indicate deficits in cerebellar motor function and some patients with CIPA can learn to handle an electrical wheelchair skillfully if necessary (Indo Y., unpublished observations). This kind of handling is usually difficult for patients who suffer from neurovascular diseases in the cerebellum. This suggests that neurons expressing TrkA mRNA in the pontine or cerebellar nuclei may have a physiological function not directly related to motor control. In addition, mental retardation (or learning disabilities) and characteristic behaviors observed in patients with CIPA may be caused by defects of putative NGF-dependent neurons in these brain regions. Thus, putative NGF-dependent neurons that form part of the cortico-cerebellar loop system may provide a clue to understand the currently unknown functions of this system.

The pontine tegmentum (abducens nucleus, central gray of the pons, facial motor nucleus, nucleus subceruleus, pontine raphe nucleus, PPRF and trigeminal nuclei)

Abducens nucleus

The abducens nucleus contains the motoneurons that innervate the ipsilateral lateral rectus muscle and so-called internuclear neurons, which project onto the medial rectus motoneurons of the opposite side (Brodal, 2010). Expression of the three Trk receptors, including TrkA, TrkB and TrkC, has been reported in the oculomotor system of the adult cat (Benitez-Temino *et al.*, 2004). The three receptors are present in all neuronal populations investigated, including abducens motoneurons and internuclear neurons, medial rectus motoneurons of the oculomotor nucleus and the trochlear motoneurons. Two or three Trk receptors are likely to colocalize in a large number of neurons, suggesting that the adequate maintenance of these neurons in the adult may depend on several neurotrophins. The expression of multiple Trk receptors suggests that their associated neurotrophins exert an influence on the normal operation of the oculomotor circuitry. Studies have indicated that the multiple Trk receptors on individual neutrons may act in concert with each other to regulate distinct functions of the oculomotor circuitry (Benitez-Temino *et al.*, 2004).

With regard to NGF–TrkA system, the expression of TrkA receptor in feline or human extraocular motoneurons suggests a role for NGF in their normal operation. However, neurological examinations have not revealed abnormal findings on the ocular motor system in patients with CIPA. Thus, the NGF–TrkA system may regulate multiple aspects of neuronal physiology during the normal operation of these oculomotor neurons.

Central gray of the pons (or pontine central gray)

A lesion study showed that connections between the pontine central gray and the ventromedial hypothalamus are essential for the display (lordosis behavior) of sexual receptivity in female rats (Hennessey *et al.*, 1990). Recent studies

have indicated that brain stem circuits in the periaqueductal gray and the pontine micturition center play critical roles in mediating reflex micturition (Griffiths & Fowler, 2013; de Groat & Wickens, 2013). The most important afferents for initiating micturition are those passing in the pelvic nerve to the sacral spinal cord. These afferents are small myelinated (Aδ) and unmyelinated (C) fibers, which convey information from receptors in the bladder wall to second-order neurons in the spinal cord (de Groat & Wickens, 2013).

Functional brain imaging has identified many forebrain regions that respond with altered neuronal activity to bladder filling or voiding and which therefore form part of the brain–bladder control network. These regions may constitute part of a general 'homeostatic afferent brain network' that governs the processing of sensation and generates appropriate outputs for many different organ systems (Griffiths & Fowler, 2013).

It is not clear whether the expression of TrkA mRNA in central gray of the pons is related to micturition in adult humans. Patients with CIPA seem to be able to control micturition. They usually do not have symptoms related to micturition, such as urinary incontinence, except for those who have suffered from traumatic spinal cord injuries. The possible functions of TrkA-expressing neurons in central gray of the pons are interesting.

Facial motor nucleus

The facial motor nucleus of a human volunteer expresses *NTRK1*. This appears to be unexpected, because spinal motor neurons that innervate skeletal striated muscles are not NGF-dependent neurons. However, facial expressions of humans, as well as animals, often reveal emotions. Facial expressions of emotions, such as sorrow and pleasure, arise independent of conscious will (Brodal, 2010). Furthermore, it is known that lesions of the pyramidal tract do not abolish spontaneous facial expressions. Thus, exploring the expression of *NTRK1* in the facial nucleus appears to be interesting.

Nucleus subceruleus

The brainstem noradrenergic (and adrenergic) centers include several groups (A1–A7) of neurons. The A6 noradrenergic center is located in the nucleus ceruleus of the dorsolateral pontine tegmentum, while the A6 subceruleus is situated in the ill-defined nucleus subceruleus (Naidich *et al.*, 2009).

The nucleus subceruleus may be related to the generation of rapid eye movement (REM) sleep (Simon *et al.*, 2012). It receives glutamatergic input, which may be involved in the activation of neurons during REM sleep. In contrast, spinal motoneurons are inhibited by the brainstem during REM sleep. This inhibition, known as muscle atonia, is most likely exerted by noradrenergic neurons close to the locus coeruleus (the subceruleus).

With regard to REM sleep, the microinjection of NGF into the rostral pontine tegmentum of adult cats rapidly induces long-lasting episodes of REM sleep and TrkA receptors are present in neurons located in mesopontine regions (Yamuy *et al.*, 2000). NGF may thus modulate the electrical activity of neurons in the rostral pontine tegmentum that are responsible for the generation of REM sleep. However, it is not certain whether these neurons include those within the nucleus subceruleus. From this point of view, further investigation into sleep in patents with CIPA is intriguing.

Pontine raphe nucleus

Recent studies have suggested the presence of anatomical and functional diversities among the serotonergic systems that innervate forebrains, which are therefore involved in the control of physiological and behavioral responses, including the control of emotional states (Hale & Lowry, 2011). An immunological study in cats has revealed that TrkA-immunoreactive neurons exist in the pontine raphe nucleus (Yamuy *et al.*, 2000). The raphe nuclei contain primarily serotonergic neurons and are located within the reticular formation. The serotonergic neuron clusters in the brainstem may be divided into two groups, rostral and caudal, on the basis of their distribution and primary projections (Hornung, 2003). The rostral group is confined to the mesencephalon and rostral

pons, with major projections to the forebrain. The caudal group extends from the caudal pons to the caudal portion of the medulla oblongata, with major projections to the caudal brainstem and spinal cord.

The pontine raphe nucleus probably belongs to the rostral group and can be observed between the decussation of the superior cerebellar peduncle and the medial longitudinal fasciculus (Shibata *et al.*, 2012). The pontine raphe nucleus sends serotonergic fibers to the cerebellar hemisphere through the middle cerebellar peduncle (Naidich *et al.*, 2009). The functional properties of serotonergic neurons within the pontine raphe nucleus remain unclear. It remains unclear whether TrkA-expressing neurons in the pontine raphe nucleus are serotonergic or not.

Paramedian pontine reticular formation

An immunological study has revealed that TrkA-immunoreactive neurons exist in the PPRF of cats (Yamuy *et al.*, 2000). The PPRF, located close to the abducens nucleus on each side, refers to a functionally defined area in the pontine reticular formation that is involved in the coordination of conjugate horizontal eye movements (Brodal, 2010). Several types of neurons that show horizontal saccade-related activity are found in the PPRF and medulla (Sparks, 2002). The PPRF sends fibers to the abducens and oculomotor nuclei and coordinates their activities. The PPRF receives signals directly and indirectly from the vestibular nuclei, the superior colliculus and the frontal eye field (Brodal, 2010). Clinically, neurological examinations of patients with CIPA have not revealed deficits of horizontal eye movements.

Trigeminal nuclei

The trigeminal nuclei of the pontine tegmentum probably include the principal sensory trigeminal nucleus, which receives thick myelinated fibers (Aβ) of the trigeminal nerve from the skin (Brodal, 2010). These fibers are considered to convey touch sensations from orofacial regions. Animal studies have revealed that the principal sensory nucleus of the trigeminal nucleus contains TrkA mRNA

in rats (Gibbs & Pfaff, 1994). TrkA-immunoreactive neurons are also present in the mesencephalic trigeminal nucleus of rats (Sobreviela *et al.*, 1994) and cats (Yamuy *et al.*, 2000).

Despite this progress, these studies have not revealed whether the spinal trigeminal nucleus contains TrkA-expressing neurons. The spinal tract of the trigeminal nerve is joined by somatic afferent fibers that have followed the glossopharyngeal and vagus nerves peripherally. The spinal trigeminal nucleus also receives sensory fibers from the intermediate nerves as well as the glossopharyngeal and vagus nerves (Naidich *et al.*, 2009). The spinal tract continues down into the upper cervical segments and corresponds to the zona terminalis (bundle of Lissauer) in the cord (Brodal, 2010). Fibers of the spinal tract of the trigeminal nerve are believed to convey sensations of pain and temperature from the orofacial regions.

Patients with CIPA lack all pain sensations, including visceral pain, although their touch sensations seem to be intact. Thus, the function of TrkA mRNA expressing neurons in the trigeminal nuclei of the pontine tegmentum is intriguing in this regard.

The myelencephalon (arcuate nucleus of the medulla, cochlear nuclei, cuneate nucleus, gracile nucleus, hypoglossal nucleus, gigantocellular group, lateral medullary reticular group, raphe nuclei of the medulla and vestibular nuclei)

Arcuate nucleus of the medulla

The arcuate nucleus of the medulla belongs to the pontine nuclei (Naidich *et al.*, 2009). The pontine nuclei are described above.

Cochlear nuclei

Two cochlear nuclei, the dorsal and ventral nuclei, receive fibers of the cochlea nerves. From the cochlear nuclei, auditory signals are transmitted to the inferior colliculus through the lateral lemniscus (Brodal, 2010). The reticular formation

receives collaterals from the ascending auditory pathways and such connections mediate the sudden muscle activity provoked by a strong, unexpected sound; that is, a startle response (Brodal, 2010). This leads to the movement of the head and eyes and even the body, in the direction of an unexpected sound and often accompanies emotional responses of the body.

Both TrkA mRNA and TrKA protein have been detected within cells located in the cochlear nucleus of rats (Gibbs & Pfaff, 1994; Burette *et al.*, 1997). In addition, neurons of the human cochlear nuclei express TrkA mRNA. Patients with CIPA do not have apparent deficits in the auditory system, because they are capable of learning a language. The physiological function of TrkA-expressing neurons in the cochlear nuclei is interesting.

Cuneate nucleus and gracile nucleus

The dorsal column nuclei, the gracile and the cuneate, are secondary sensory neurons located in the medulla. The dorsal column–medial lemniscus pathway is important for the perception of touch, pressure, vibration and kinesthesia, but it is of primary importance for the discriminatory aspects of sensation (Brodal, 2010).

Animal studies have indicated that some neurons of the dorsal column nuclei project to a region of the ventral pontine reticular formation that contains neurons involved in pain processing, cardiovascular regulation, respiratory control and arousal (Van Bockstaele *et al.*, 1993). This region of the ventral pontine reticular formation receives inputs from a variety of nuclei involved in somatosensory, auditory and autonomic function and may contribute to the integration of exteroceptive and interoceptive sensory inputs.

The dorsal columns also contain some descending axons that form synaptic contacts in the dorsal horn of rats (particularly in lamina V, containing neurons excited by signals from nociceptors) (Masson *et al.*, 1991). Thus, the dorsal column nuclei are part of a neuronal network that, by way of descending connections, controls the flow of sensory information from the spinal cord. The projection of the dorsal column nuclei to the brainstem reticular formation may

contribute to the integration of exteroceptive and introceptive sensory inputs, while the projection of dorasal column nuclei to the spinal cord may be involved in coordinating these sensory inputs at the level of spinal cord.

A recent study has characterized motor control ability in patients with CIPA. The grip force during the object grasp–lift–holding task is significantly greater in patients with CIPA than in control subjects, albeit with less reproducibility and greater fluctuation in the acceleration of the object (Kawashima *et al.*, 2012). Moreover, some patients show an absence of temporal coupling between the grip and load force. This is the first study to characterize motor control ability in patients with CIPA and has suggested that anticipatory modulation of grip force is partially impaired in such patients. Alternatively, this impairment of grip force may be related to an aberrant integration of exteroceptive (touch) and interoceptive (pain) sensory inputs in the CNS.

It is remains uncertain whether neurons of dorsal column nuclei express TrkA receptor in rodents. Furthermore, the function of neurons expressing TrkA mRNA in human dorsal column nuclei is not known.

Hypoglossal nucleus

The hypoglossal nucleus consists of the cell bodies of the motor fibers that form the hypoglossal nerve. The hypoglossal nerve is the motor nerve of the tongue. Reflex movements of the tongue occur in swallowing (and vomiting) and are activated through the brainstem reflex centers located in the reticular formation (Brodal, 2010). Expression of TrkA receptor in the hypoglossal nucleus has not been described in animal studies and the function of hypoglossal neurons expressing TrkA mRNA in humans is not known.

Brainstem neurons in the perihypoglossal regions are considered to relay information from the inner ear and vestibular apparatus to the cerebellum and tectum. Neurons of the prepositus hypoglossal nucleus are responsive to NGF in rats (Sukhov *et al.*, 1997), and animal studies have indicated that these neurons express TrkA mRNA (Gibbs & Pfaff, 1994; Holtzman *et al.*, 1995) or TrkA protein (Sobreviela *et al.*, 1994; Holtzman *et al.*, 1995; Sukhov *et al.*, 1997). However,

data on the expression of TrkA mRNA in the prepositus hypoglossal nucleus are not available in the *Allen Human Brain Atlas*.

The reticular formation (gigantocellular group, lateral medullary reticular group and raphe nuclei of the medulla)

In the pons and medulla, the medial two-thirds of the reticular formation consist of many large cells and the lateral one-third contains almost exclusively small cells. In general, the lateral part receives inputs, whereas the medial part is efferent (executive) (Brodal, 2010). The efferents convey the influence of the reticular formation to higher centers, such as the thalamus and lower centers, such as the spinal cord.

According to NeuroNames, the medullary reticular formation consists of the central medullary reticular group and the lateral medullary reticular group (Bowden, 2002, http://braininfo.rprc.washington.edu/). The former group includes the gigantocellular reticular nucleus, the lateral paragigantocellular reticular nucleus, the dorsal paragigantocellular reticular nucleus and the ventral reticular nucleus. The latter group includes the lateral reticular nucleus and the parvicellular reticular nucleus.

The raphe nuclei together form a narrow plate of neurons in the midline of the medulla and are considered part of the reticular formation (Brodal, 2010). As described above for the pontine nuclei, serotonergic neuron clusters may be allocated, on the basis of their distribution and primary projections, into two groups: rostral and caudal. The caudal group, including the raphe magnus nucleus (RMg), raphe obscurus nucleus (ROb), raphe pallidus nucleus (RPa) and parts of the adjacent lateral reticular formation, extends from the caudal pons to the caudal portion of the medulla oblongata, with major projections to the caudal brainstem and spinal cord (Hornung, 2003).

The efferent projections of the caudal group terminate in the visceral and somatic motor nuclei and in the lateral reticular formation in the brainstem. They descend through two parallel pathways in the spinal cord (Hornung, 2003). The dorso-lateral pathway originates primarily in the RMg and terminates in the dorsal horn. The ventro-medial pathway originates in part in the ROb and RPa

and terminates in the intermediate and ventral horn (Hornung, 2003). The RMg is involved in descending control of the transmission of nociceptive messages (Fields *et al.*, 2006).

The afferent projections to the anterior part of the caudal group (RMg, rostral RPa, and parts of the adjacent lateral reticular formation) originate in several hypothalamic nuclei, the dorsolateral periaqueductal gray, the central nucleus of the amygdala, the bed nucleus of the stria terminalis and the medullary reticular formation (Hornung, 2003). RMg and rostral RPa also receive direct catecholaminergic inputs. There are also converging inputs onto RMg, ROb and RPa from visceral sensory afferents and ventrolateral periaqueductal gray matter (PAG) (Hornung, 2003).

Recent studies have shown that noxious thermal stimuli activate serotonergic neurons in the lateral paragigantocellular reticular (LPGi) and the RMg nuclei in rats (Gau *et al.*, 2009; Gau *et al.*, 2013). Serotonergic neurons of the LPGi are responsible for inhibition of the cardiac baroreflex induced by strong thermal noxious stimuli in rats (Gau *et al.*, 2009). The LPGi serotonergic neurons respond to noxious mechanical stimulation (such as pinch), but do not respond to light mechanical or innocuous thermal stimulation (Gau *et al.*, 2013). Similarly, non-serotonergic neurons of the LPGi and RMg also respond to noxious thermal stimuli, but not to innocuous thermal stimulation. Serotonergic neurons of the LPGi and RMg are probably involved in descending control of nociceptive messages. The LPGi and RMg receive major afferent projections from the dorsal PAG and the ventral PAG, respectively. According to the different roles that the ventral PAG and dorsal PAG play in nociceptive responses, it has been suggested that the LPGi serotonergic neurons are key players in both analgesia and cardiac vascular activation, characterizing an active defense reaction induced by strong noxious stimuli. In contrast, the RMg serotonergic neurons may intervene mostly in circumstances other than acute nociception, such as intense fear or inescapable pain, which induce passive coping behavior with immobility, decreased cardiac activity and strong analgesia (Gau *et al.*, 2013). Thus, it is likely that LPGi and RMg serotonergic cells play an important but contrasting role in the serotonin-mediated modulation of the cardiac baroreflex and transmission of nociceptive messages occurring under intense

noxious conditions.

It is also known that descending projection neurons from the raphe nuclei of the medulla to the spinal cord are a mixed population of serotonergic and non-serotonergic neurons (Hornung, 2003). This dual projection probably contributes to complementary functions. Similarly, a mixed population of serotonergic and non-serotonergic neurons in the LPGi and RMg may contribute to complementary functions.

In humans, neurons of gigantocellular and lateral medullary groups, as well as the raphe nuclei of the medulla, express TrkA mRNA. The gigantocellular and perigigantocellular neurons in the medullary reticular formation express TrkA mRNA in rats (Gibbs & Pfaff, 1994). A previous study of dual-stained sections from rats has revealed that 45%of the serotonergic neurons of the raphe nuclei coexpress TrkA immunoreactivity (Sobreviela *et al.*, 1994). The majority of these dual-labeled neurons are seen in the median raphe nucleus, ROb, and RMg.

The reticular formation, including the raphe nuclei, receives all kinds of sensory information through the collaterals of secondary sensory neurons (Brodal, 2010). Thus, signals from a vast array of receptors can influence neurons of the reticular formation. Whenever a receptor is stimulated, the signals reach not only the cortical areas important for the perception of the stimulus but also the reticular formation. Efferent connections of the reticular formation reach most parts of the CNS (from the spinal cord to the cerebral cortex), while afferents bring diverse sensory information. Thus, the reticular formation seems to be built for integration and attends primarily to tasks involving the nervous system and the organism as a whole (Brodal, 2010). These tasks are probably important for homeostatic control.

Thus, it is likely that TrkA-expressing neurons in the gigantocellular and lateral medullary groups, as well as in the raphe nuclei of the medulla, contribute to homeostatic bodily control in humans.

Vestibular nuclei

Afferents from the vestibular apparatus in the inner ear end in the vestibular nuclei in the upper medulla and lower pons. From there, signals flow in three

primary directions: to the spinal cord, to neuronal groups controlling eye movements and to the cerebellum (Brodal, 2010). These connections chiefly control automatic responses aimed at maintaining an upright position and at fixation of visual targets. The vestibular nuclei also receive afferents from other parts of the CNS, including the spinal cord, the reticular formation, certain mesencephalon and the cerebellum (Brodal, 2010). The vestibular nuclei receive signals from the cerebral cortex, most of which are indirect through the reticular formation but some of which are direct.

The expression of three Trk receptors (TrkA, TrkB and TrkC) has been observed in the oculomotor system of the adult cat (Benitez-Temino *et al.*, 2004), as described above. In addition, this study revealed that the three receptors are present in the vestibular nuclei and the prepositus hypoglossi nuclei. A further study in rats has also reported that the vestibular nuclear complex expresses three forms of the Trk receptors. Approximately one-third of each type of Trk-immunoreactive neuron expressed Fos protein, a maker for vestibular activation, following sinusoidal acceleration along either the anterior–posterior or interaural axis in the horizontal plane (Zhang *et al.*, 2005). The study has speculated that most otolith-related central vestibular neurons of adult rats coexpress at least two Trk receptors and that Trk receptors and their cognate neurotrophins may function in these neurons to modulate gravity-related spatial information during horizontal head movements.

With regard to gravity-related spatial information, a vestibular reflex originating in the otolith organs and other body graviceptors modulates sympathetic activity during changes in posture with regard to gravity. A human study has indicated that a vestibulosympathetic reflex, probably originating primarily in the otolith organs, contributes to blood pressure maintenance during forward linear acceleration (Kaufmann *et al.*, 2002).

In patients with CIPA, automatic responses related to vestibular functions, such as controlling eye movements and bodily postures, seem to be intact. However, clinical neurological studies evaluating the vestibular function of patients with CIPA have not yet been reported. A recent study on walk using two-dimensional video gait analyses has revealed that young patients with CIPA walk faster, with a longer step length and higher heel contact angular velocity,

than young control participants (Zhang *et al.*, 2013). The study has suggested that their data may explain the frequency of lower extremity injuries in patients with CIPA. Furthermore, their study may also suggest a deviation in vestibular function in patients with CIPA as compared to control subjects.

Vestibular information seems to be processed in a distributed network that integrates several modes of sensory information related to extrapersonal space and the body. This distributed vestibular network is connected to and overlaps with other networks, notably those that control movements and also networks related to pain, attention, motivation and emotions (Brodal, 2010). The impact of the NGF–TrkA system on the functions of vestibular nuclei awaits further studies.

Putative neuronal networks related to pain, interoception and emotional responses

Primates have a distinct image of interoceptive input (Craig, 2002, 2009). NGF-dependent primary afferents, including interoceptive polymodal receptors, convey various types of information from the body to the brain through the lamina I spinothalamocortical pathways (Indo, 2009, 2010, 2012). Such information is subsequently conveyed to the insula through the thalamus or brainstem sites of homeostatic integration. Subsequently, the insula sends signals to regions such as the ventral (or ventromedial) prefrontal cortex and the medial prefrontal cortex (or anterior cingulate cortex) (Damasio, 1994, 2003; Craig, 2002, 2009).

The same information from the NGF-dependent primary afferents is also conveyed to many other parts of the CNS (Indo, 2009). These include the PAG, the hypothalamus and the amygdala. These neuronal structures or nuclei are important for the coordination of autonomic, endocrine and somatic responses that optimize our total response to physical and mental challenges. Autonomic responses are also an intrinsic part of the emotional process (Saper, 2002). In particular, systemic responses of the sympathetic nervous system often accompany emotional responses. When humans are exposed to danger or trauma, stimuli or contexts associated with the danger or trauma become

learned triggers that unleash emotional responses, the ‘fight-or-flight’ response (LeDoux, 1996). Several regions of the brainstem are involved in the control of autonomic processes to maintain homeostasis of the body. These brainstem centers are conceptually divided into two sites: the brainstem homeostatic integration (BHI) site and the brainstem homeostatic motor (BHM) site. Both sites probably include various neurons scattered within and interconnected between the midbrain, pons and medulla oblongata. The anatomical substrates of the BHI and BHM sites are probably groups of neurons in the reticular formation that are formed by extensive networks of interconnected neurons.

NGF-dependent primary afferents and sympathetic postganglionic neurons constitute homeostatic afferent and efferent pathways, respectively, and provide the basis for somato-autonomic reflex arcs (Indo, 2009). NGF-dependent neurons in the PNS form an interface between the nervous system and the body-proper and constitute a neuronal network for maintaining homeostasis (Fig. 2). Here, the ‘body-proper’ means the organism minus its neural tissues (the central and peripheral components of the nervous system) (Damasio, 1994).

In the homeostatic afferent pathway, many fibers of the lamina I spinothalamic tract, which mediates interoceptive signals, send collaterals to the reticular formation, presumably to the homeostatic integration regions (Craig, 2002). Collaterals of the ascending axons from the sensory (spinal) trigeminal nucleus supply the same kind of information from the face. Visceral sensory signals reach the reticular formation through collaterals of ascending fibers from the nucleus of the solitary tract, which receives afferents from the glossopharyngeal nerve and the vagus nerve. Visual, auditory and vestibular signals also reach the reticular formation. In addition, the reticular formation is influenced by sensory cranial nerve nuclei and other brainstem nuclei, such as the PAG, the superior colliculus and the vestibular nuclei (Brodal, 2010). Thus, signals from virtually all kinds of receptors can influence neurons of the reticular formation. Whenever a receptor is stimulated, the signals reach not only the cortical areas important for the perception of the stimulus but also the reticular formation. The ascending reticular connections are of particular importance for the general level of activity of the cerebral cortex, which, in turn, affects consciousness and attention (Brodal, 2010).

In the homeostatic efferent pathway, sympathetic preganglionic neurons are located in the intermediolateral cell columns of the spinal cord and transmit efferent outputs from the hypothalamus to sympathetic postganglionic neurons (Morrison & Nakamura, 2011). Fibers from the hypothalamus also end in the BHM site and serve to coordinate the activity of different peripheral parts of the autonomic system (Kerman, 2008; Morrison & Nakamura, 2011). The BHM site is also connected to various brain regions and influences their activities. Limbic structures, notably the amygdala, also send fibers to the reticular formation (Aggleton, 2000). Such connections probably mediate emotional effects on autonomic and somatic motor functions. The PAG is related to the suppression of nociceptive signals. It also helps initiate defensive reactions to external threats or other forms of stress (LeDoux, 1996). Efferent connections from the PAG to the reticular formation initiate the coordinated alterations of circulation and respiration, pain perception, and automatic movements in response to threatening or novel stimuli. Corticoreticular fibers arise primarily from the cortical areas that give rise to the pyramidal tract and end predominantly in the regions of reticular formation that send axons to the spinal cord (Brodal, 2010). This corticoreticulospinal pathway is of special importance for the control of voluntary and automatic movements. The reticular formation receives afferents from the cerebellum and this pathway is important for the cerebellar influence on motor neurons and the autonomic nervous system (Brodal, 2010).

Thus, the BHM site can be affected by virtually all other parts of the CNS. Its function is probably related to premotor networks that organize several complex behaviors, including the control of body posture, orientation of the head and body toward external stimuli, control of eye movements, as well as coordination of autonomic nervous system activity.

In addition to the BHI and BHM sites, there are many other neuronal structures in the brain situated between the homeostatic afferent and efferent pathways (Fig. 2). These neuronal structures are involved in an array of brain functions. For instance, the amygdala, which contributes to emotional responses, receives inputs from a wide range of levels of cognitive processing and sends outputs toward various regions of the brain (LeDoux, 1996, 2002; Aggleton, 2000). The hypothalamus, with closely linked structures in the brainstem and

amygdala, acts directly on the internal environment through its control of the endocrine system and autonomic nervous system. The autonomic nervous system is essential for adaptive behavior and the control of internal bodily state.

Thus, many neurons or neuronal pathways located in various regions of the brain contribute to homeostatic afferent inputs, from the body to the brain, or to efferent outputs, from the brain to the body. These neuronal pathways are believed to underlie the response to pain, interoception, homeostasis and emotion.

In rats, neurons in the interpeduncular nucleus (IPN) contain TrkA protein and mRNA (Gibbs & Pfaff, 1994; Sobreviela *et al.*, 1994; Holtzman *et al.*, 1995). The IPN receives the retroflex fasciculus from the habenula (Fig. 2). Numerous brain circuits serving diverse functions are considered to share the axial anatomy of the habenula–IPN. These brain circuits may be involved in a variety of brain functions and behaviors, including nociception, learning and memory, motor activity, sexual and maternal behavior, stress, affective states (anxiety, depression and reward phenomena), sleep, and eating and drinking behavior (Klemm, 2004). Unfortunately, data on the expression of TrkA mRNA in the IPN was not available in the *Allen Human Brain Atlas* at the time of writing.

The habenula is a phylogenetically old brain structure that is present in virtually all vertebrate species (Hikosaka, 2010). It is therefore worthwhile discussing the habenula here. The habenula consists of two distinct nuclei, the medial (MHb) and lateral (LHb) habenular nuclei. Both nuclei receive afferent connections primarily through the stria medullaris, while they project output pathways through the retroflex fasciculus (Lecourtier & Kelly, 2007). The MHb extends projections almost exclusively to the IPN through the fasciculus retroflexus, and cholinergic inputs to the MHb seem to activate the habenulo-interpeduncular pathway (Fowler *et al.*, 2011). The habenulo-interpeduncular tract regulates avoidance of noxious substances, triggering an inhibitory motivational signal. Thus, the habenula is a node of a reciprocal route for communication between the limbic and extrapyramidal systems and probably plays important roles in learning, memory and attention.

A previous study has demonstrated that electrical stimulation of the tooth pulp, regarded as a noxious stimulus, induces expression of the c-fos protein

(making neuronal excitation) in the LHb of the cat diencephalon, suggesting that LHb neurons may contribute to the modulation of nociception (Matsumoto *et al.*, 1994). Recent studies have indicated that the habenula plays a critical role in behavioral choices through its effects on neuromodulator systems, in particular the dopaminergic and serotonergic systems (Hikosaka, 2010). The habenula receives afferent projections primarily through the stria medullaris from the limbic system, including the septum, the diagonal band of Broca, the lateral preoptic area and the lateral hypothalamus as well as from the basal ganglia, including the striatum and the globus pallidus. The habenula sends efferent projections through the fasciculus retroflexus (also known as the habenula–interpeduncular tract) to the midbrain areas involved in the release of dopamine (the substantia nigra pars compacta and ventral tegmental area) and serotonin (the median raphe nucleus and dorsal raphe nucleus). Recent studies have revealed that the habenula is involved in the processing of aversive information, such as pain, stress and failure (Matsumoto & Hikosaka, 2007, 2009 a,b). Animals may fight or escape from aversive events, but they often stop moving (freeze) before acting. Hikosaka has proposed that the primary function of the habenula is to suppress motor activity under such adverse conditions (Hikosaka, 2010). Thus, the habenula may act as a node to link the forebrain to the midbrain regions involved in regulating emotional behaviors, thereby providing a fundamental mechanism for both survival and decision-making.

Further studies have reported that the LHb is involved in aversion-associated behaviors (Lammel *et al.*, 2012) and in the rodent learned helplessness model of depression (Li *et al.*, 2011). A recent study has used a newly developed quantitative method for the continuous assessment and control of active responses to behavioral challenge, synchronized with single-unit electrophysiology and optogenetics in freely moving rats (Warden *et al.*, 2012). This study has demonstrated that some neurons of the medial prefrontal cortex project to the brainstem dorsal raphe or lateral habenula. Furthermore, selective activation of the medial prefrontal cortical cells projecting to these neuronal structures induces a profound, rapid and reversible effect on selection of the active behavioral state. These results may contribute to understanding the neuronal circuitry underlying action selection and motivation in behavior.

Organisms interact with their external or internal environments through various afferent (sensory) and efferent (motor) mechanisms (Cameron, 2009). Human functional neuroimaging can provide insights into the whole-brain systems that regulate internal bodily functions through the autonomic nervous system. The feedback influences of changes in internal bodily states on neural systems are believed to support emotions and behaviors (Critchley *et al.*, 2011).

Studies using functional magnetic resonance imaging (fMRI) in healthy subjects have demonstrated signal changes in multiple brain sites during an autonomic response in humans. One of these studies has analyzed the cold pressor test in healthy subjects (Harper *et al.*, 2000). Submersion of the forearm in ice-cold water usually causes an increase in blood pressure as an autonomic response. The peripheral neural input and output in this autonomic reflex are provided by NGF-dependent primary afferents in the skin and sympathetic postganglionic neurons, respectively (Fig. 2). Pressor challenges elicit changes of signal intensity in various brain regions: medial and orbital prefrontal cortex; anterior cingulate cortex; midline and medial thalamus, particularly caudally; hypothalamus; midbrain; ventral and dorsal pons; the temporal lobe, including amygdala, hippocampal formation, and adjacent perirhinal and entorhinal cortical regions; insular cortex; and cerebellum (Harper *et al.*, 2000).

Another study has analyzed the SSR which is an autonomic response in humans and is one index of autonomic arousal that reflects sympathetic tone (Critchley *et al.*, 2000). SSR can be used as an indirect measure of attention, cognitive effort or emotional arousal. A fMRI study in healthy subjects has demonstrated signal changes in multiple brain sites that are associated with activity corresponding to the generation and afferent representation of discrete SSR events (Critchley *et al.*, 2000). Regions that covaried with increased SSR include the right orbitofrontal cortex, right anterior insula, left lingual gyrus, right fusiform gyrus and left cerebellum. At a less stringent level of significance, bilateral medial prefrontal cortex (Brodmann's area 10) and right inferior parietal lobule are likely to covary with SSR. Thus, various brain regions implicated in emotion and attention are involved in the generation and representation of peripheral SCR responses.

A further study has revealed that in humans, rostral medullary raphe neurons in the ventral midline of the medulla, immediately caudal to the pons, are selectively activated in response to a thermoregulatory challenge, pointing to the location of thermoregulatory neurons (McAllen *et al.*, 2006). These neurons, homologous to those of the RPa in rodents, may be responsible for cold-defense and mediating both the cutaneous vasoconstriction and thermogenic responses to ambient cooling (probably by means of sympathetic drive). Thus, it is likely that these raphe neurons act as a synaptic relay in homeostatic efferent pathways for cold defense (McAllen *et al.*, 2006).

With regard to the thermoregulation of the body, a neurobiological study in rats has revealed a thermosensory pathway that triggers physiological heat-defense responses to elevated environmental temperature (Nakamura & Morrison, 2010). Using *in vivo* electrophysiological and anatomical approaches, it has been found that neurons in the dorsal part of the lateral parabrachial nucleus transmit cutaneous warm signals from spinal somatosensory neurons directly to the thermoregulatory command center, the preoptic area. These neurons are located adjacent to another group of neurons that mediate cutaneous cool signaling to the preoptic area. This warm sensory pathway is required to elicit autonomic heat-defense responses, such as cutaneous vasodilation, to skin-warming challenges. These studies in rodents, together with fMRI studies in humans, contribute to our understanding of the homeostatic neuronal circuits responsible for maintaining body temperature during environmental temperature challenges.

The primary thermoregulatory effectors are the cutaneous blood vessels for control of heat loss, the brown adipose tissue and skeletal muscle for thermogenesis, and various species-dependent mechanisms such as sweating, panting and saliva spreading for evaporative heat loss (Morrison & Nakamura, 2011). The activation of these effectors is regulated by parallel but distinct effector-specific and core efferent pathways within the CNS that are influenced by shared cutaneous thermal afferent signals (Morrison & Nakamura, 2011).

Evaporative heat loss for the control of body temperature is a species-dependent mechanism. Sweating is essential for body temperature control in humans. Recurrent febrile episodes due to anhidrosis are one of the

characteristic features observed in patients with CIPA. Because patients with CIPA lack peripheral neurons that are essential for both thermal reception and effector functions, they show hyperthermia (recurrent febrile episodes) in hot environments and hypothermia in cold environments. These symptoms are due to a lack of NGF-dependent primary afferents and sympathetic postganglionic neurons. Thus, NGF-dependent primary afferents and sympathetic postganglionic neurons are considered to be thermal receptors and thermal effectors, respectively.

Studies using fMRI in humans also provide information on the sensory or affective components of pain. The latter is considered an emotional aspect of pain and is often difficult to study in rodents. In a human study, volunteers who experienced a painful stimulus compared it to the feeling elicited when they observe a signal indicating that their loved one is receiving a similar pain stimulus (Singer *et al.*, 2004). This procedure enabled the measurement of pain-related brain activation (known as ‘pain matrix’). The bilateral anterior insula cortex (AIC), rostral anterior cingulate cortex (ACC), brainstem and cerebellum were activated when subjects experienced pain and also when they received a signal that a loved one was experiencing pain, while activity in the posterior insula/secondary somatosensory cortex, the sensorimotor cortex and the caudal ACC was specific to subjects receiving pain. Together, these structures probably contribute to a part of the pain network in the human brain that forms a subjective representation of feelings that allows us to predict the effects of emotional stimuli with respect to the self. In addition, it serves as the neural basis for our ability to understand the emotional importance of a particular stimulus for another person and to predict its likely associated consequences (Singer *et al.*, 2004). These findings suggest that we use representations reflecting our own emotional responses to pain to understand how others experience pain (Singer, 2006).

In general, two brain regions, the insula and ACC, have been suggested to provide a subjective representation of internal body and subjective states (Damasio, 1994, 2003; Craig, 2002). Craig (2002) proposed that the posterior insula cortex is important for forming a primary interoceptive representation of the physiological condition of the body.

The ACC seems to be related to various domains of brain function and is implicated in a wide range of conditions and behaviors, although controversy surrounds its function. The ACC has also been proposed to participate in the willed control of behavior, with the potential to translate intentions to actions (Paus, 2001). According to this proposal, the structural and functional organization of the primate ACC have three key elements: motor channels, which provide access to skeletomotor and oculomotor output systems as well as vocalizations; extensive connections with the lateral prefrontal cortex, which provide access to the cognitive apparatus of neocortical areas; and afferents from the midline thalamus and the brainstem, which provide a strong modulatory influence reflecting the arousal state of the organism. Thus, the ACC probably plays critical roles in the behavioral control of various domains, including motor control, cognition and the arousal/drive state of the organism. All of these domains are likely contribute to all aspects of pain.

Craig (2009) has also suggested that the AIC is involved in the re-representation of interoception and he offers one possible basis for its involvement in all subjective feelings in the body as well as perhaps emotional awareness. He regards the AIC as the probable site for awareness on the basis of its afferent representation of feelings from the body and the ACC as the probable site for the initiation of behaviors. Thus, the AIC and ACC may have a fundamental role in Damasio's 'somatic marker' hypothesis (Damasio, 1994; Craig, 2009).

Human fMRI studies, together with neurobiological studies in animals, have indicated that the experience of pain is associated with activity in a distributed cortical and subcortical network. It is likely that when this network enters a state of synchronized activity, we experience unpleasant emotional and bodily states. This network is probably involved in interoception as well as emotional responses. NGF-dependent neurons in the PNS mediate reciprocal communication between the brain and the body-proper and contribute to interoception and emotional responses. Most information coming from the body through NGF-dependent primary afferents is conducted to the brain unconsciously; the brain is able to maintain homeostasis through feedback mechanisms for which autonomic sympathetic neurons are indispensable. This

is well illustrated by (unconscious) homeostatic control of body temperature. However, when stimulus intensities reach noxious ranges, NGF-dependent neurons are excited vigorously, provoking pain and defense responses as well as emotional responses. Putative NGF-dependent neurons distributed in the CNS are connected through various intervening neurons to the NGF-dependent neurons in the PNS. Thus, it is also likely that NGF-dependent neurons in the CNS contribute to this network for interoception and emotional responses.

Conclusions

The complex pain network that contributes to interoception and emotional responses has several characteristic properties, including its pronounced plasticity and strong learning capacity. Pain is a strong stimulus for learning, as we learn immediately learn to avoid what has previously led to tissue injuries as well as what has threatened to do so. The pathophysiology of CIPA strongly suggests that NGF-dependent neurons in the PNS are essential for such a learning process. NGF-dependent neurons in the CNS may also contribute to the plasticity and learning properties of the complex pain network. Thus, the putative existence of NGF-dependent neurons in the CNS will provide a framework for further studies to investigate pain, interoception and emotional responses. Furthermore, strategies to target the molecular mechanisms of the NGF–TrkA system may offer a prospect for the development of novel analgesics.

Supporting Information

Additional supporting information can be found in the online version of this article:
Table S1. Expression profile of *NTRK1* in various brain regions

Acknowledgements

This work was supported in part by the Japan Society for the Promotion of Science (JSPS) (KAKENHI) Grand-in-Aid fro Scientific Research and by the

Ministry of Health, Labour and Welfare: Health and Labour Science Research Grants (Research on Intractable Diseases). The author reports no conflict of interest.

Abbreviations

ACC, anterior cingulate cortex; AIC, anterior insula cortex; BFCNs, basal forebrain cholinergic neurons; BHI, brainstem homeostatic integration; BHM, brainstem homeostatic motor; CIPA, congenital insensitivity to pain with anhidrosis; CNS, central nervous system; fMRI, functional magnetic resonance imaging; GABA, γ-aminobutyric acid; GDNF, glial cell-derived neurotrophic factor; IPN, interpeduncular nucleus; LHb, lateral habenular nucleus; LPGi, lateral paragigantocellular reticular nucleus; M1, primary cerebral motor cortex; MHb, medial habenular nucleus; NAc: nucleus accumbens; NGF, nerve growth factor; NGF-dependent primary afferents, NGF-dependent primary afferent neurons with thin fibers; PAG, periaqueductal gray matter; PNS: peripheral nervous system; PPRF, paramedian pontine reticular formation; REM, rapid eye movement; RMg, raphe magnus nucleus; ROb, raphe obscurus nucleus; RPa, raphe pallidus nucleus; SSR, sympathetic skin response; VTA, ventral tegmental area.

Figure legends

Fig. 1. Nerve growth factor (NGF)-dependent neurons mediate reciprocal communication between the brain and the body-proper. NGF-dependent neurons constitute a part of the neural network for interoception and homeostasis and play important roles in emotions and adaptive behaviors. The diagram is a schematic presentation of the transmission signals that occur between the body-proper and the brain through NGF-dependent neurons, including NGF-dependent primary afferents (P) (interoceptive polymodal receptors) and sympathetic postganglionic neurons (S). The body-proper refers to the organism minus the neural tissues (the central and peripheral components of the nervous system). NGF-dependent primary afferents are dorsal root ganglia (DRG) neurons or trigeminal ganglia (TG) neurons with free nerve endings. The trigeminal nerve carries sensory information from the face, sinuses, teeth and the anterior portion of the oral cavity. Axons of the trigeminal nerve ganglion cells that process pain and temperature sensations terminate in the spinal nucleus of the trigeminal nerve. A subset of neurons in the glossopharyngeal nerve and the vagus nerve are most likely NGF-dependent neurons (not shown). APG, autonomic preganglionic neurons; DRG, dorsal root ganglia; SG, sympathetic ganglion; STT, spinothalamic tract. This figure has been reproduced, with permission, from Indo (2012).

Fig. 2. A putative neuronal network for interoception, homeostasis and emotional response. The diagram is a schematic presentation of the transmission signals between the body-proper and the brain through NGF-dependent neurons, including the NGF-dependent primary afferent neurons (interoceptive polymodal receptors), sympathetic postganglionic neurons, the basal forebrain cholinergic neurons (BFCNs) and the striatal cholinergic neurons. The body-proper means the organism minus the neural tissues (the central and peripheral components of the nervous system). Asterisks indicate neurons or neural structures expressing TrkA mRNA in humans. TrkA mRNA-expressing neurons, not shown here, include those of the cerebellar nuclei, pontine nuclei, and various nuclei in the pontine tegmentum and myelencephalon (see Table 1). Several regions of the

brainstem are involved in the control of autonomic processes to maintain homeostasis of the body. These brainstem centers are conceptually divided into two sites: the brainstem homeostatic integration (BHI) site and the brainstem homeostatic motor (BHM) site. Both sites probably include various neurons of the reticular formation and others that are scattered and interconnected in the brainstem. Bidirectional arrows indicate interconnected regions. Some connections have been omitted for clarity. The axon reflex is an efferent function of the interoceptive polymodal receptors, contributing to local defensive reactions in the body (Indo, 2010). Limbic sensory and prefrontal cortices include insula/somatosensory cortices and ventral and medial prefrontal cortices, respectively. They have interconnections with one another and connections with various other neural structures (Damasio, 1994; LeDoux, 1996, 2002). BFCNs send widespread projections to widespread areas of cortex and subcortical nuclei. The hypothalamus also controls endocrine responses. Together, these NGF-dependent neurons constitute part of a putative neuronal network for interoception and homeostasis and probably play important roles in emotion and adaptive behavior. NTS, nucleus tractus solitarius; VMpo, posterior portion of ventral medial nucleus (Blomqvist *et al.*, 2000); VMb, basal portion of ventral medial nucleus (Blomqvist *et al.*, 2000); MTL, medial temporal lobe memory system (LeDoux, 2002); PAG, periaqueductal central gray; PFC, prefrontal cortex; IPN, interpeduncular nucleus.

References

Aggleton, J.P. (2000) *The Amygdala: A Functional Analysis*. Oxford University Press, New York.

Axelrod, F.B., Chelimsky, G.G. & Weese-Mayer, D.E. (2006) Pediatric autonomic disorders. *Pediatrics*, **118**, 309-321.

Basbaum, A.I., Bautista, D.M., Scherrer, G. & Julius, D. (2009) Cellular and molecular mechanisms of pain. *Cell*, **139**, 267-284.

Benitez-Temino, B., Morcuende, S., Mentis, G.Z., de la Cruz, R.R. & Pastor, A.M. (2004) Expression of Trk receptors in the oculomotor system of the adult cat. *J. Comp. Neurol.*, **473**, 538-552.

Blomqvist, A., Zhang, E.T. & Craig, A.D. (2000) Cytoarchitectonic and immunohistochemical characterization of a specific pain and temperature relay, the posterior portion of the ventral medial nucleus, in the human thalamus. *Brain*, **123**, 601-619.

Boissiere, F., Faucheux, B., Ruberg, M., Agid, Y. & Hirsch, E.C. (1997) Decreased TrkA gene expression in cholinergic neurons of the striatum and basal forebrain of patients with Alzheimer's disease. *Exp. Neurol.*, **145**, 245-252.

Bonsi, P., Cuomo, D., Ding, J., Sciamanna, G., Ulrich, S., Tscherter, A., Bernardi, G., Surmeier, D.J. & Pisani, A. (2007) Endogenous serotonin excites striatal cholinergic interneurons via the activation of 5-HT 2C, 5-HT6 and 5-HT7 serotonin receptors: implications for extrapyramidal side effects of serotonin reuptake inhibitors. *Neuropsychopharmacol.*, **32**, 1840-1854.

Bowden, D. M. (2002) NeuroNames. BrainInfo, National Primate Research Center, University of Washington,1991-present. Available http://braininfo.rprc.washington.edu/.

Brodal, P. (2010) *The Central Nervous System: Structure and Function,* 4th Edn. Oxford University Press, New York.

Brown, M.T., Tan, K.R., O'Connor, E.C., Nikonenko, I., Muller, D. & Luscher, C. (2012) Ventral tegmental area GABA projections pause accumbal cholinergic interneurons to enhance associative learning. *Nature*, **492**, 452-456.

Burette, A., Jalenques, I. & Romand, R. (1997) Neurotrophin receptor immunostaining in the rat ventral cochlear nucleus. *Brain Res.*, **776**, 10-23.

Cameron, O.G. (2009) Visceral brain-body information transfer. *NeuroImage*, **47**, 787-794.

Cattaneo, A. (2013) Immunosympathectomy as the first phenotypic knockout with antibodies. *Proc. Natl Acad. Sci. U S A*, **110**, 4877-4885.

Craig, A.D. (2002) How do you feel? Interoception: the sense of the physiological condition of the body. *Nat. Rev. Neurosci.*, **3**, 655-666.

Craig, A.D. (2009) How do you feel–now? The anterior insula and human awareness. *Nat. Rev. Neurosci.*, **10**, 59-70.

Critchley, H.D., Elliott, R., Mathias, C.J. & Dolan, R.J. (2000) Neural activity relating to generation and representation of galvanic skin conductance responses: a functional magnetic resonance imaging study. *J. Neurosci.*, **20**, 3033-3040.

Critchley, H.D., Nagai, Y., Gray, M.A. & Mathias, C.J. (2011) Dissecting axes of autonomic control in humans: insights from neuroimaging. *Auton. Neurosci.-Basic.*, **161**, 34-42.

Crowley, C., Spencer, S.D., Nishimura, M.C., Chen, K.S., Pitts-Meek, S., Armanini, M.P., Ling, L.H., MacMahon, S.B., Shelton, D.L., Levinson, A.D. & Phillips, H.S. (1994) Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. *Cell*, **76**, 1001-1011.

Damasio, A.R. (1994) *Descartes' Error: Emotion, Reason, and the Human Brain*. Putnam, New York.

Damasio, A.R. (2003) *Looking for Spinoza: Joy, Sorrow, and the Feeling Brain*. Harcourt, Orlando.

Elenkov, I.J., Wilder, R.L., Chrousos, G.P. & Vizi, E.S. (2000) The sympathetic nerve–an integrative interface between two supersystems: the brain and the immune system. *Pharmacol. Rev.*, **52**, 595-638.

Fagan, A.M., Garber, M., Barbacid, M., Silos-Santiago, I. & Holtzman, D.M. (1997) A role for TrkA during maturation of striatal and basal forebrain cholinergic neurons *in vivo*. *J. Neurosci.*, **17**, 7644-7654.

Fields, H.L., Basbaum, A.I. & Heinricher, M.M. (2006) Central nervous system mechanisms of pain modulation. In McMahon, S.B. & Koltzenburg, M. (Eds), *Wall and Melzack's Textbook of Pain*. Elsevier, Philadelphia, pp. 125-142.

Fowler, C.D., Lu, Q., Johnson, P.M., Marks, M.J. & Kenny, P.J. (2011) Habenular α5 nicotinic receptor subunit signalling controls nicotine intake. *Nature*, **471**, 597-601.

Gau, R., Sevoz-Couche, C., Laguzzi, R., Hamon, M. & Bernard, J.F. (2009) Inhibition of cardiac baroreflex by noxious thermal stimuli: a key role for lateral paragigantocellular serotonergic cells. *Pain*, **146**, 315-324.

Gau, R., Sevoz-Couche, C., Hamon, M. & Bernard, J.F. (2013) Noxious stimulation excites serotonergic neurons: a comparison between the lateral paragigantocellular reticular and the raphe magnus nuclei. *Pain*, **154**, 647-659.

Gear, R.W., Aley, K.O. & Levine, J.D. (1999) Pain-induced analgesia mediated by mesolimbic reward circuits. *J. Neurosci.*, **19**, 7175-7181.

Gibbs, R.B. & Pfaff, D.W. (1994) *In situ* hybridization detection of trkA mRNA in brain: distribution, colocalization with p75NGFR and up-regulation by nerve growth factor. *J. Comp. Neurol.*, **341**, 324-339.

Ginsberg, S.D., Che, S., Wuu, J., Counts, S.E. & Mufson, E.J. (2006) Down regulation of trk but not p75NTR gene expression in single cholinergic basal forebrain neurons mark the progression of Alzheimer's disease. *J. Neurochem.*, **97**, 475-487.

Grahn, J.A., Parkinson, J.A. & Owen, A.M. (2008) The cognitive functions of the caudate nucleus. *Prog. Neurobiol.*, **86**, 141-155.

Griffiths, D.J. & Fowler, C.J. (2013) The micturition switch and its forebrain influences. *Acta Physiol.*, **207**, 93-109.

de Groat, W.C. & Wickens, C. (2013) Organization of the neural switching circuitry underlying reflex micturition. *Acta Physiol.*, **207**, 66-84.

Haas, H. & Panula, P. (2003) The role of histamine and the tuberomamillary nucleus in the nervous system. *Nat. Rev. Neurosci.*, **4**, 121-130.

Hale, M.W. & Lowry, C.A. (2011) Functional topography of midbrain and pontine serotonergic systems: implications for synaptic regulation of serotonergic

circuits. *Psychopharmacology*, **213**, 243-264.

Harper, R.M., Bandler, R., Spriggs, D. & Alger, J.R. (2000) Lateralized and widespread brain activation during transient blood pressure elevation revealed by magnetic resonance imaging. *J. Comp. Neurol.*, **417**, 195-204.

Hasselmo, M.E. & Sarter, M. (2011) Modes and models of forebrain cholinergic neuromodulation of cognition. *Neuropsychopharmacol.*, **36**, 52-73.

Hawrylycz, M.J., Lein, E.S., Guillozet-Bongaarts, A.L., Shen, E.H., Ng, L., Miller, J.A., van de Lagemaat, L.N., Smith, K.A., Ebbert, A., Riley, Z.L., Abajian, C., Beckmann, C.F., Bernard, A., Bertagnolli, D., Boe, A.F., Cartagena, P.M., Chakravarty, M.M., Chapin, M., Chong, J., Dalley, R.A., Daly, B.D., Dang, C., Datta, S., Dee, N., Dolbeare, T.A., Faber, V., Feng, D., Fowler, D.R., Goldy, J., Gregor, B.W., Haradon, Z., Haynor, D.R., Hohmann, J.G., Horvath, S., Howard, R.E., Jeromin, A., Jochim, J.M., Kinnunen, M., Lau, C., Lazarz, E.T., Lee, C., Lemon, T.A., Li, L., Li, Y., Morris, J.A., Overly, C.C., Parker, P.D., Parry, S.E., Reding, M., Royall, J.J., Schulkin, J., Sequeira, P.A., Slaughterbeck, C.R., Smith, S.C., Sodt, A.J., Sunkin, S.M., Swanson, B.E., Vawter, M.P., Williams, D., Wohnoutka, P., Zielke, H.R., Geschwind, D.H., Hof, P.R., Smith, S.M., Koch, C., Grant, S.G. & Jones, A.R. (2012) An anatomically comprehensive atlas of the adult human brain transcriptome. *Nature*, **489**, 391-399.

Hennessey, A.C., Camak, L., Gordon, F. & Edwards, D.A. (1990) Connections between the pontine central gray and the ventromedial hypothalamus are essential for lordosis in female rats. *Behav. Neurosci.*, **104**, 477-488.

Hikosaka, O. (2010) The habenula: from stress evasion to value-based decision-making. *Nat. Rev. Neurosci.*, **11**, 503-513.

Holtzman, D.M., Kilbridge, J., Li, Y., Cunningham, E.T., Jr., Lenn, N.J., Clary, D.O., Reichardt, L.F. & Mobley, W.C. (1995) TrkA expression in the CNS: evidence for the existence of several novel NGF-responsive CNS neurons. *J. Neurosci.*, **15**, 1567-1576.

Holzer, P. (1998) Neurogenic vasodilatation and plasma leakage in the skin. *Gen. Pharmacol.*, **30**, 5-11.

Hornung, J.P. (2003) The human raphe nuclei and the serotonergic system. *J.*

Chem. Neuroanat., **26**, 331-343.

Hoshi, E., Tremblay, L., Feger, J., Carras, P.L. & Strick, P.L. (2005) The cerebellum communicates with the basal ganglia. *Nat. Neurosci.*, **8**, 1491-1493.

Ikoma, A., Steinhoff, M., Stander, S., Yosipovitch, G. & Schmelz, M. (2006) The neurobiology of itch. *Nat. Rev. Neurosci.*, **7**, 535-547.

Indo, Y. (2001) Molecular basis of congenital insensitivity to pain with anhidrosis (CIPA): mutations and polymorphisms in TRKA (NTRK1) gene encoding the receptor tyrosine kinase for nerve growth factor. *Hum. Mutat.*, **18**, 462-471.

Indo, Y. (2002) Genetics of congenital insensitivity to pain with anhidrosis (CIPA) or hereditary sensory and autonomic neuropathy type IV. Clinical, biological and molecular aspects of mutations in TRKA(NTRK1) gene encoding the receptor tyrosine kinase for nerve growth factor. *Clin. Auton. Res.*, **12 Suppl 1**, 20-32.

Indo, Y. (2009) Nerve growth factor, interoception, and sympathetic neuron: lesson from congenital insensitivity to pain with anhidrosis. *Auton. Neurosci.-Basic.*, **147**, 3-8.

Indo, Y. (2010) Nerve growth factor, pain, itch and inflammation: lessons from congenital insensitivity to pain with anhidrosis. *Expert Rev. Neurother.*, **10**, 1707-1724.

Indo, Y. (2012) Nerve growth factor and the physiology of pain: lessons from congenital insensitivity to pain with anhidrosis. *Clin. Genet.*, **82**, 341-350.

Indo, Y., Tsuruta, M., Hayashida, Y., Karim, M.A., Ohta, K., Kawano, T., Mitsubuchi, H., Tonoki, H., Awaya, Y. & Matsuda, I. (1996) Mutations in the TRKA/NGF receptor gene in patients with congenital insensitivity to pain with anhidrosis. *Nat. Genet.*, **13**, 485-488.

Ito, M. (2008) Control of mental activities by internal models in the cerebellum. *Nat. Rev. Neurosci.*, **9**, 304-313.

Kaufmann, H., Biaggioni, I., Voustianiouk, A., Diedrich, A., Costa, F., Clarke, R., Gizzi, M., Raphan, T. & Cohen, B. (2002) Vestibular control of sympathetic activity. An otolith-sympathetic reflex in humans. *Exp. Brain Res.*, **143**, 463-469.

Kawashima, N., Abe, M.O., Iwaya, T. & Haga, N. (2012) Abnormal capacity for grip force control in patients with congenital insensitivity to pain. *Exp. Brain Res.*, **218**, 579-588.

Kelly, R.M. & Strick, P.L. (2003) Cerebellar loops with motor cortex and prefrontal cortex of a nonhuman primate. *J. Neurosci.*, **23**, 8432-8444.

Kerman, I.A. (2008) Organization of brain somatomotor-sympathetic circuits. *Exp. Brain Res.*, **187**, 1-16.

Klemm, W.R. (2004) Habenular and interpeduncularis nuclei: shared components in multiple-function networks. *Med. Sci. Monit.*, **10**, RA261-RA273.

Kreitzer, A.C. (2009) Physiology and pharmacology of striatal neurons. *Annu. Rev. Neurosci.*, **32**, 127-147.

Lammel, S., Lim, B.K., Ran, C., Huang, K.W., Betley, M.J., Tye, K.M., Deisseroth, K. & Malenka, R.C. (2012) Input-specific control of reward and aversion in the ventral tegmental area. *Nature*, **491**, 212-217.

Lecourtier, L. & Kelly, P.H. (2007) A conductor hidden in the orchestra? Role of the habenular complex in monoamine transmission and cognition. *Neurosci. Biobehav. R.*, **31**, 658-672.

LeDoux, J.E. (1996) *The Emotional Brain: The Mysterious Underpinnings of Emotinal Life*. Simon & Schuster, New York.

LeDoux, J.E. (2000) Emotion circuits in the brain. *Annu. Rev. Neurosci.*, **23**, 155-184.

LeDoux, J.E. (2002) *Synaptic Self: How Our Brains Become Who We Are*. Viking, New York.

Levi-Montalcini, R. (1987) The nerve growth factor: thirty-five years later. *EMBO J.*, **6**, 1145-1154.

Levy Erez, D., Levy, J., Friger, M., Aharoni-Mayer, Y., Cohen-Iluz, M. & Goldstein, E. (2010) Assessment of cognitive and adaptive behaviour among individuals with congenital insensitivity to pain and anhidrosis. *Dev. Med. Child Neurol.*, **52**, 559-562.

Li, B., Piriz, J., Mirrione, M., Chung, C., Proulx, C.D., Schulz, D., Henn, F. & Malinow, R. (2011) Synaptic potentiation onto habenula neurons in the learned helplessness model of depression. *Nature*, **470**, 535-539.

Masson, R.L., Jr., Sparkes, M.L. & Ritz, L.A. (1991) Descending projections to the rat sacrocaudal spinal cord. *J. Comp. Neurol.*, **307**, 120-130.

Matsumoto, M. & Hikosaka, O. (2007) Lateral habenula as a source of negative reward signals in dopamine neurons. *Nature*, **447**, 1111-1115.

Matsumoto, M. & Hikosaka, O. (2009a) Representation of negative motivational value in the primate lateral habenula. *Nat. Neurosci.*, **12**, 77-84.

Matsumoto, M. & Hikosaka, O. (2009b) Two types of dopamine neuron distinctly convey positive and negative motivational signals. *Nature*, **459**, 837-841.

Matsumoto, N., Yahata, F., Kawarada, K., Kamata, K. & Suzuki, T.A. (1994) Tooth pulp stimulation induces c-fos expression in the lateral habenular nucleus of the cat. *NeuroReport*, **5**, 2397-2400.

McAllen, R.M., Farrell, M., Johnson, J.M., Trevaks, D., Cole, L., McKinley, M.J., Jackson, G., Denton, D.A. & Egan, G.F. (2006) Human medullary responses to cooling and rewarming the skin: a functional MRI study. *Proc. Natl Acad. Sci. U S A*, **103**, 809-813

McGaugh, J.L., McIntyre, C.K. & Power, A.E. (2002) Amygdala modulation of memory consolidation: interaction with other brain systems. *Neurobiol. Learn. Mem.*, **78**, 539-552.

Melzack, R. & Wall, P.D. (1982) *The Challenge of Pain*. Penguin Books, London.

Mesulam, M.M. (2004) The cholinergic innervation of the human cerebral cortex. *Prog. Brain Res.*, **145**, 67-78.

Mesulam, M.M., Mufson, E.J., Levey, A.I. & Wainer, B.H. (1983) Cholinergic innervation of cortex by the basal forebrain: cytochemistry and cortical connections of the septal area, diagonal band nuclei, nucleus basalis (substantia innominata), and hypothalamus in the rhesus monkey. *J. Comp. Neurol.*, **214**, 170-197.

Middleton, F.A. & Strick, P.L. (2001) Cerebellar projections to the prefrontal cortex of the primate. *J. Neurosci.*, **21**, 700-712.

Mishra, S.K. & Hoon, M.A. (2013) The cells and circuitry for itch responses in mice. *Science*, **340**, 968-971.

Mogil, J.S. (2009) Animal models of pain: progress and challenges. *Nat. Rev. Neurosci.*, **10**, 283-294.

Molliver, D.C., Wright, D.E., Leitner, M.L., Parsadanian, A.S., Doster, K., Wen,

D., Yan, Q. & Snider, W.D. (1997) IB4-binding DRG neurons switch from NGF to GDNF dependence in early postnatal life. *Neuron*, **19**, 849-861.

Morrison, S.F. & Nakamura, K. (2011) Central neural pathways for thermoregulation. *Front. Biosci. (Landmark Ed)*, **16**, 74-104.

Mufson, E.J., Ginsberg, S.D., Ikonomovic, M.D. & DeKosky, S.T. (2003) Human cholinergic basal forebrain: chemoanatomy and neurologic dysfunction. *J. Chem. Neuroanat.*, **26**, 233-242.

Mufson, E.J., Counts, S.E., Perez, S.E. & Ginsberg, S.D. (2008) Cholinergic system during the progression of Alzheimer's disease: therapeutic implications. *Expert. Rev. Neurother.*, **8**, 1703-1718.

Muller, M., Triaca, V., Besusso, D., Costanzi, M., Horn, J.M., Koudelka, J., Geibel, M., Cestari, V. & Minichiello, L. (2012) Loss of NGF–TrkA signaling from the CNS is not sufficient to induce cognitive impairments in young adult or intermediate-aged mice. *J. Neurosci.*, **32**, 14885-14898.

Nagashima, K., Nakai, S., Tanaka, M. & Kanosue, K. (2000) Neuronal circuitries involved in thermoregulation. *Auton. Neurosci.-Bascic.*, **85**, 18-25.

Naidich, T.P., Duvernoy, H.M., Delman, B.N., Sorensen, A.G., Kollias, S.S. & Haacke, E.M. (2009) *Duvernoy's Atlas of the Human Brain Stem and Cerebellum*. Springer, Vienna.

Nakamura, K. & Morrison, S.F. (2010) A thermosensory pathway mediating heat-defense responses. *Proc. Natl Acad. Sci. U S A*, **107**, 8848-8853.

Palm, N.W., Rosenstein, R.K. & Medzhitov, R. (2012) Allergic host defences. *Nature*, **484**, 465-472.

Paus, T. (2001) Primate anterior cingulate cortex: where motor control, drive and cognition interface. *Nat. Rev. Neurosci.*, **2**, 417-424.

Pezet, S. & McMahon, S.B. (2006) Neurotrophins: mediators and modulators of pain. *Annu. Rev. Neurosci.*, **29**, 507-538.

Pinsky, L. & DiGeorge, A.M. (1966) Congenital familial sensory neuropathy with anhidrosis. *J. Pediatr.*, **68**, 1-13.

Ploghaus, A., Tracey, I., Clare, S., Gati, J.S., Rawlins, J.N. & Matthews, P.M. (2000) Learning about pain: the neural substrate of the prediction error for aversive events. *Proc. Natl Acad. Sci. U S A*, **97**, 9281-9286.

Ramnani, N. (2006) The primate cortico-cerebellar system: anatomy and

function. *Nat. Rev. Neurosci.*, **7**, 511-522.

Redgrave, P., Prescott, T.J. & Gurney, K. (1999) The basal ganglia: a vertebrate solution to the selection problem? *Neuroscience*, **89**, 1009-1023.

Reichardt, L.F. (2006) Neurotrophin-regulated signalling pathways. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, **361**, 1545-1564.

Roosterman, D., Goerge, T., Schneider, S.W., Bunnett, N.W. & Steinhoff, M. (2006) Neuronal control of skin function: the skin as a neuroimmunoendocrine organ. *Physiol. Rev.*, **86**, 1309-1379.

Sanchez-Ortiz, E., Yui, D., Song, D., Li, Y., Rubenstein, J.L., Reichardt, L.F. & Parada, L.F. (2012) TrkA gene ablation in basal forebrain results in dysfunction of the cholinergic circuitry. *J. Neurosci.*, **32**, 4065-4079.

Saper, C.B. (2002) The central autonomic nervous system: conscious visceral perception and autonomic pattern generation. *Annu. Rev. Neurosci.*, **25**, 433-469.

Sarter, M., Hasselmo, M.E., Bruno, J.P. & Givens, B. (2005) Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. *Brain Res. Brain Res. Rev.*, **48**, 98-111.

Sarter, M., Gehring, W.J. & Kozak, R. (2006) More attention must be paid: the neurobiology of attentional effort. *Brain Res. Rev.*, **51**, 145-160.

Schweighofer, N., Doya, K. & Kuroda, S. (2004) Cerebellar aminergic neuromodulation: towards a functional understanding. *Brain Res. Brain Res. Rev.*, **44**, 103-116.

Shen, E.H., Overly, C.C. & Jones, A.R. (2012) The Allen Human Brain Atlas: comprehensive gene expression mapping of the human brain. *Trends Neurosci.*, **35**, 711-714.

Shibata, M., Goto, N., Goto, J. & Nonaka, N. (2012) Nuclei of the human raphe. *Okajimas Folia Anat. Jpn.*, **89**, 15-22.

Silos-Santiago, I., Molliver, D.C., Ozaki, S., Smeyne, R.J., Fagan, A.M., Barbacid, M. & Snider, W.D. (1995) Non-TrkA-expressing small DRG neurons are lost in TrkA deficient mice. *J. Neurosci.*, **15**, 5929-5942.

Simon, C., Hayar, A. & Garcia-Rill, E. (2012) Developmental changes in glutamatergic fast synaptic neurotransmission in the dorsal subcoeruleus

nucleus. *Sleep*, **35**, 407-417.

Singer, T. (2006) The neuronal basis and ontogeny of empathy and mind reading: review of literature and implications for future research. *Neurosci. Biobehav. R.*, **30**, 855-863.

Singer, T., Seymour, B., O'Doherty, J., Kaube, H., Dolan, R.J. & Frith, C.D. (2004) Empathy for pain involves the affective but not sensory components of pain. *Science*, **303**, 1157-1162.

Smeyne, R.J., Klein, R., Schnapp, A., Long, L.K., Bryant, S., Lewin, A., Lira, S.A. & Barbacid, M. (1994) Severe sensory and sympathetic neuropathies in mice carrying a disrupted Trk/NGF receptor gene. *Nature*, **368**, 246-249.

Snider, W.D. & McMahon, S.B. (1998) Tackling pain at the source: new ideas about nociceptors. *Neuron*, **20**, 629-632.

Sobreviela, T., Clary, D.O., Reichardt, L.F., Brandabur, M.M., Kordower, J.H. & Mufson, E.J. (1994) TrkA-immunoreactive profiles in the central nervous system: colocalization with neurons containing p75 nerve growth factor receptor, choline acetyltransferase, and serotonin. *J. Comp. Neurol.*, **350**, 587-611.

Sparks, D.L. (2002) The brainstem control of saccadic eye movements. *Nat. Rev. Neurosci.*, **3**, 952-964.

Sukhov, R.R., Cayouette, M.H., Radeke, M.J., Feinstein, S.C., Blumberg, D., Rosenthal, A., Price, D.L. & Koliatsos, V.E. (1997) Evidence that perihypoglossal neurons involved in vestibular-auditory and gaze control functions respond to nerve growth factor. *J. Comp. Neurol.*, **383**, 123-134.

Swanson, A.G. (1963) Congenital insensitivity to pain with anhydrosis. A unique syndrome in two male siblings. *Arch. Neurol.-Chicago*, **8**, 299-306.

Swanson, A.G., Buchan, G.C. & Alvord, E.C. (1965) Anatomic changes in congenital insensitivity to pain. *Arch. Neurol. -Chicago*, **12**, 12-18.

Tuszynski, M.H., Thal, L., Pay, M., Salmon, D.P., U, H.S., Bakay, R., Patel, P., Blesch, A., Vahlsing, H.L., Ho, G., Tong, G., Potkin, S.G., Fallon, J., Hansen, L., Mufson, E.J., Kordower, J.H., Gall, C. & Conner, J. (2005) A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. *Nat. Med.*, **11**, 551-555.

Van Bockstaele, E.J., Akaoka, H. & Aston-Jones, G. (1993) Brainstem afferents

to the rostral (juxtafacial) nucleus paragigantocellularis: integration of exteroceptive and interoceptive sensory inputs in the ventral tegmentum. *Brain Res.*, **603**, 1-18.

Warden, M.R., Selimbeyoglu, A., Mirzabekov, J.J., Lo, M., Thompson, K.R., Kim, S.Y., Adhikari, A., Tye, K.M., Frank, L.M. & Deisseroth, K. (2012) A prefrontal cortex-brainstem neuronal projection that controls response to behavioural challenge. *Nature*, **492**, 428-432.

Yamuy, J., Sampogna, S. & Chase, M.H. (2000) Neurotrophin-receptor immunoreactive neurons in mesopontine regions involved in the control of behavioral states. *Brain Res.*, **866**, 1-14.

Zhang, F.X., Lai, C.H., Tse, Y.C., Shum, D.K. & Chan, Y.S. (2005) Expression of Trk receptors in otolith-related neurons in the vestibular nucleus of rats. *Brain Res.*, **1062**, 92-100.

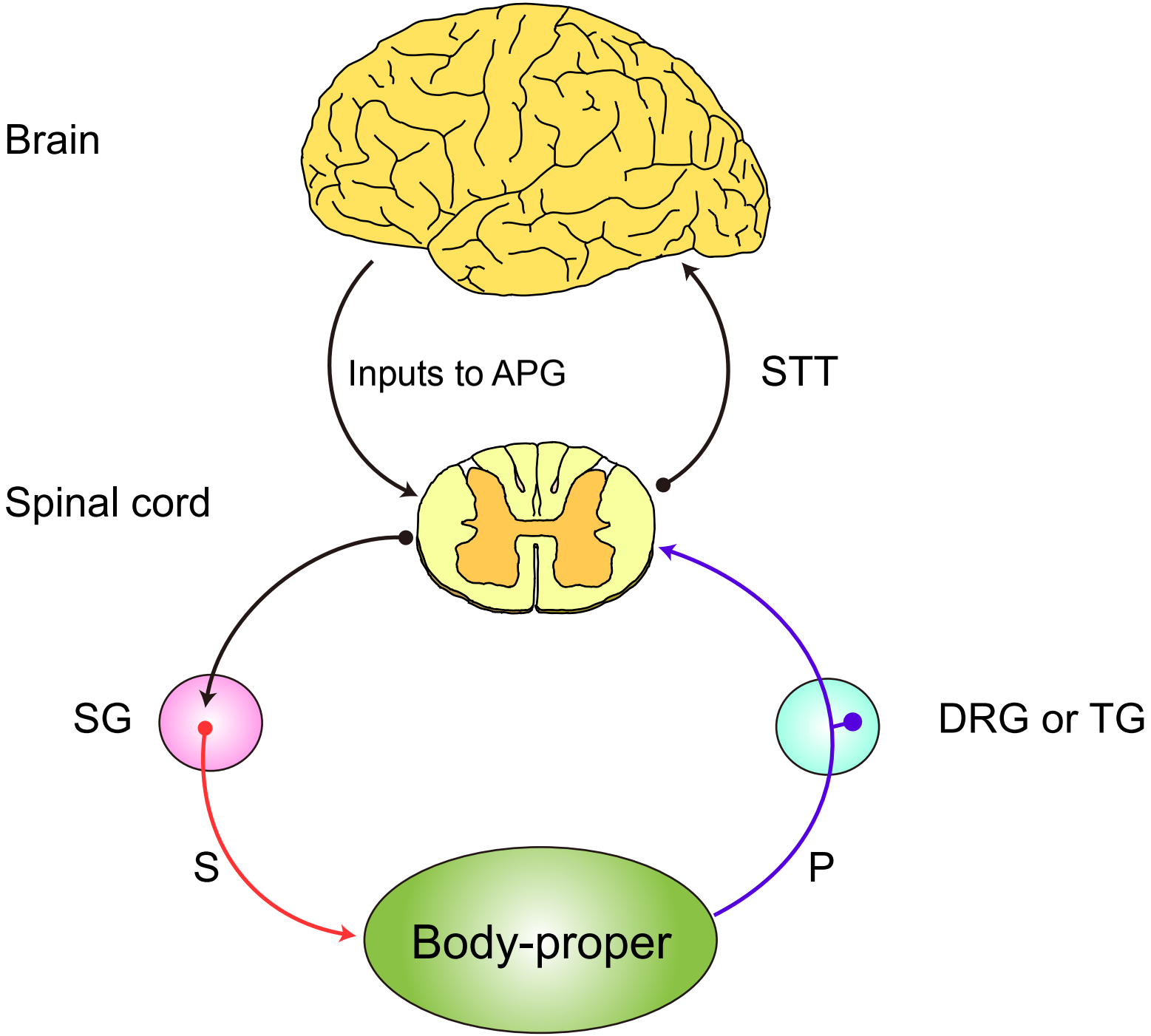
Zhang, Y., Ogata, N., Yozu, A. & Haga, N. (2013) Two-dimensional video gait analyses in patients with congenital insensitivity to pain. *Dev. Neurorehabil.*, **16**, 266-270.

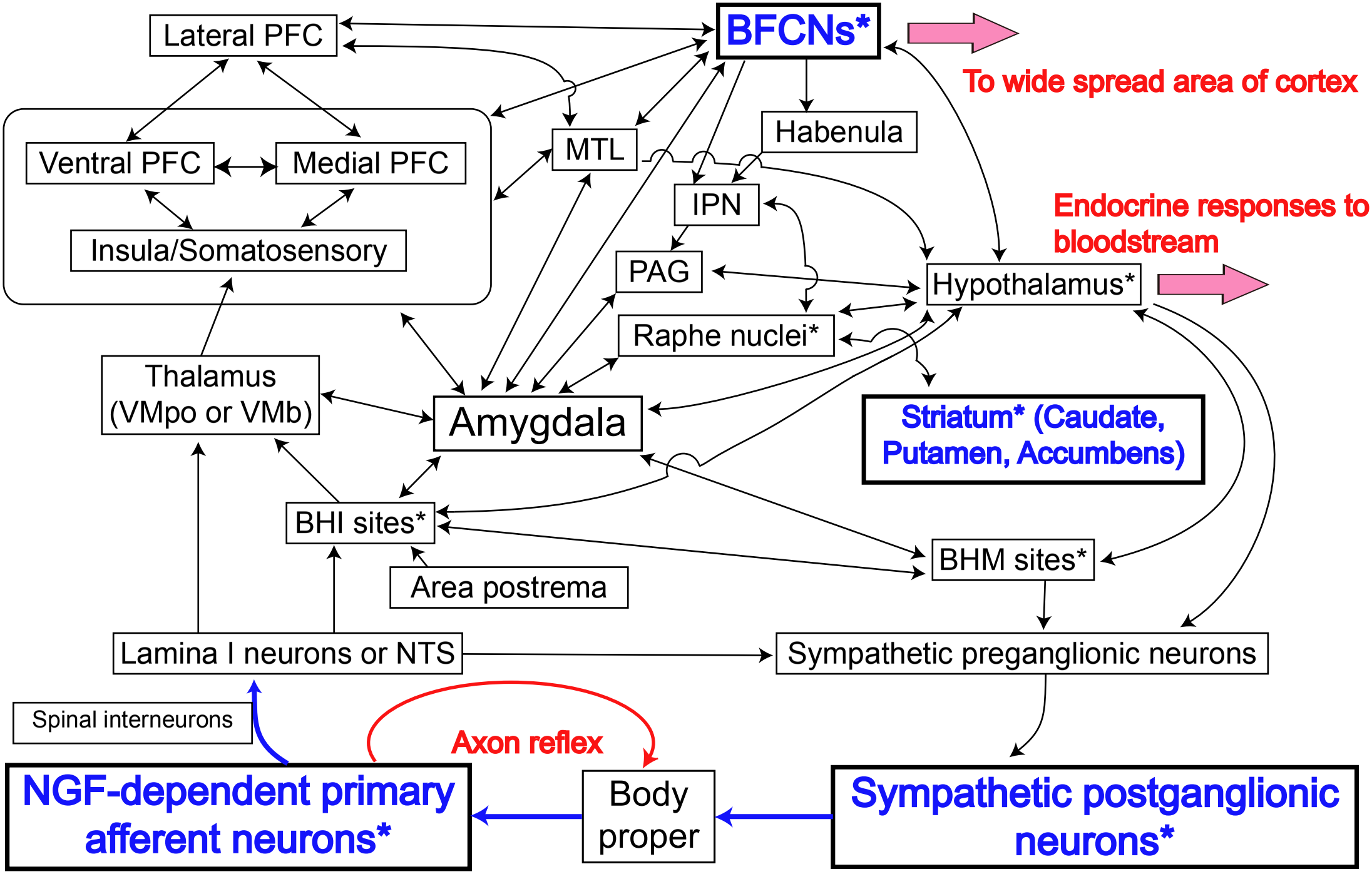
Zhou, F.M., Wilson, C.J. & Dani, J.A. (2002) Cholinergic interneuron characteristics and nicotinic properties in the striatum. *J. Neurobiol.*, **53**, 590-605.

Tabel 1. Expression profile of *NTRK1* in various human brain regions

Name of top level structure	Name of structure	Numbers of donors*
Basal Forebrain	Septal nuclei	1
	Substantia innominata	6
Striatum	Body of the caudate nucleus	5
	Tail of the caudate nucleus	1
	Nucleus accumbens	3
	Putamen	5
Hypothalamus	Lateral hypothalamic area	1
	Posterior hypothalamic area	1
	Tuberomammillary nucleus	1
Cerebellar Nuclei	Dentate nucleus	5
	Globose nucleus	2
Basal Part of Pons	Pontine nuclei	5
Pontine Tegmentum	Abducens nucleus	4
	Central gray of the pons	1
	Facial motor nucleus	1
	Nucleus subceruleus	1
	Pontine raphe nucleus	4
	Paramedian pontine reticular formation	6
	Trigeminal nuclei	2
Myelencephalon	Arcuate nucleus of medulla	6
	Cochlear nuclei	5
	Cuneate nucleus	2
	Gracile nucleus	1
	Hypoglossal nucleus	1
	Gigantocellular group	3
	Lateral medullary reticular group	6
	Raphe nuclei of medulla	5
	Vestibular nuclei	5

An expression profile of *NTRK1* in various brain regions is derived from a publicly available online resource of gene expression information of the human brain (Allen Human Brain Atlas: http://human.brain-map.org/). In this whole brain microarray data, various brain regions in which the expression z-score of *NTRK1* is higher than 1.0, assessed with two probes (A_23_P34804 and A_24_P265506) in at least one of the brains of six donors, are selected. *Numbers of donors to fulfill the selection criterion.


Brain
Inputs to APG
STT
Spinal cord
SG
DRG or TG
S
P
Body-proper


Lateral PFC
BFCNs*
To wide spread area of cortex
Ventral PFC
Medial PFC
MTL
Habenula
IPN
Endocrine responses to bloodstream
Insula/Somatosensory
PAG
Hypothalamus*
Raphe nuclei*
Thalamus (VMpo or VMb)
Amygdala
Striatum* (Caudate, Putamen, Accumbens)
BHI sites*
BHM sites*
Area postrema
Lamina I neurons or NTS
Sympathetic preganglionic neurons
Spinal interneurons
Axon reflex
NGF-dependent primary afferent neurons*
Body proper
Sympathetic postganglionic neurons*