Investigating the human locus coeruleus-norepinephrine system in vivo : discussions on the anatomy, involvement in cognition and clinical applications

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Chapter 1

General Introduction

The LC-NE system: Functions and malfunctions

The neuromodulator norepinephrine (NE) is involved in multiple cognitive processes including attention, learning, and emotions, and has been shown to be disturbed in psychiatric and neurological disorders such as anxiety disorder, post-traumatic stress disorder (PTSD), Alzheimer's disease, and schizophrenia. Most of the NE released in the brain originates from the locus coeruleus (LC), a brainstem nucleus with noradrenergic projections to multiple brain regions. This neuroanatomical formation of the noradrenergic system makes it well suited to rapidly and globally modulate brain function in response to changes in the environment, when cognitive flexibility and increased attention are required, such as when confronted with an important or life-threatening stimulus. Arousal, vigilance and cognitive flexibility in these situations increase the chances of survival and prepare the organism for immediate action. Nonetheless, if this state of arousal and vigilance is prolonged, as it happens in cases of chronic stress, the same mechanism becomes problematic and can lead to disorders such as anxiety disorder and PTSD, instead of being beneficial for survival. Therefore the LC-NE system can be functional and promote survival or be malfunctional and convert into the driving mechanism behind a disorder.

The LC-NE system: Norepinephrine

Norepinephrine (NE), also called noradrenaline, is a catecholamine that functions as a neurotransmitter, neuromodulator and hormone in the brain and body. In the brain, NE is produced mainly by the brainstem nucleus LC. Outside the brain, NE is used as a neurotransmitter by sympathetic ganglia located near the spinal cord or ganglia located in the chest, abdomen and other visceral organs (Hamill, Shapiro, & Vizzard, 2012) and is released into the bloodstream by the adrenal glands in the kidneys. The later provides the name to NE, given that "norepinephrine" (from Greek) and "noradrenaline" (from Latin) means "alongside the kidneys". Regardless of where it is released, NE binds to and activates adrenergic receptors located on the cell surface. As a "classical neurotransmitter", NE transfers information to the postsynaptic neuron. In addition, NE modulates effects produced by other neurotransmitters such as glutamate and gamma amino butyric acid (GABA), and it is this latter function that makes NE a "neuromodulator".

As a neurotransmitter, neuromodulator and hormone, NE plays a crucial role in the function of cognition and the sympathetic nervous system. Indeed, many sympathomimetic drugs (compounds which mimic the effects of endogenous agonists of the sympathetic nervous system) are used to treat high blood pressure (e.g., beta-blockers) but at the same time have effects on brain and cognition (e.g., beta-blockers can impede the consolidation or retrieval of traumatic memories; (Kroes et al., 2016; Lonergan, Olivera-Figueroa, Pitman, & Brunet, 2013).

Norepinephrine: Structure, biosynthesis and receptor types

NE is synthesized from the amino acid tyrosine in the adrenal medulla and postganglionic neurons of the sympathetic nervous system. Tyrosine converts to dopamine mainly in the cytoplasm while dopamine converts to NE mainly inside the neurotransmitter vesicles (Musacchio, 1975). The metabolic pathway is:

Phenylalanine → Tyrosine → L-DOPA → Dopamine → Norepinephrine

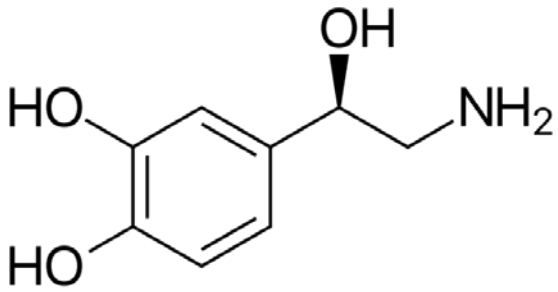

Figure 1. Chemical diagram of the structure of a norepinephrine molecule.

NE effects occur after its binding to noradrenergic receptors. To date, two receptor types have been identified: alpha receptors (divided into subtypes α1 and α2) and beta receptors (divided into subtypes β1, β2, and β3; Rang, 2014). All these receptors are G protein-coupled receptors. After its release, NE is cleared from the synaptic cleft by the NE transporter (NET). In **Chapter 6**, we use the NE transporter blocker atomoxetine in order to manipulate NE levels in healthy human participants.

The central LC-NE system: The LC-NE system in the brain

The LC is located in the brainstem, adjacent to the 4th ventricle. It is a bilateral structure, meaning that there are two LCs in the brain: one at each side of the floor of the 4th ventricle. Although there is a large interindividual variability regarding the exact size and location of the LC, it is estimated that the LC contains approximately 15,000 neurons in each hemisphere of a healthy adult brain (Mather, Clewett, Sakaki, & Harley, 2016). Although this size is as small as a grain of rice (Mouton, Pakkenberg, Gundersen, & Price, 1994), the LC has a substantial influence on brain function due to its wide, ascending, projections to forebrain and midbrain regions such as amygdala, thalamus, hippocampus, basal ganglia, the cortex, and the cerebellum (Aston-Jones, Foote, & Bloom, 1984). At the same time, the LC has connections to the spinal cord (Sara & Bouret, 2012). All these projections make the LC the dominant source of NE in the central nervous system (i.e., the brain and the spinal cord).

The central LC-NE system: Connecting forebrain, midbrain, brainstem and the periphery

The LC in the brain is activated in parallel with the autonomic system in the body. NE released in the forebrain facilitates attention, processing and cognitive flexibility. Central activation of neuromodulatory neurons in concert with peripheral arousal (related to LC via the spinal cord, the vagus nerve, the nucleus tractus solitarius (NTS), the PGi and

from the paraventricular nucleus of the hypothalamus) prepare the organism for a reorientation or reset of cortical networks and an adaptive response (Nieuwenhuis, De Geus, & Aston-Jones, 2011; Sara & Bouret, 2012; Valentino & Van Bockstaele, 2008).

In case of a confrontation with important stimuli or in situations of stress and fear, the high NE levels manifest both at a higher cognitive level by modulating forebrain regions involved in sensory processing, attention and vigilance, and at a more peripheral level, by preparing the body for the fight-and-flight response and controlling autonomic responses (Valentino & Van Bockstaele, 2008). In **Chapters 4, 5 and 6** we examine the involvement of the LC-NE system in higher-order cognition (cognitive flexibility and tasks that require attention and cognitive control) as well as its relation with peripheral measures (pupil dilation, cortisol, alpha-amylase). In addition, in **Chapter 6**, we use stress in order to manipulate NE levels in healthy human participants.

LC-NE system in the periphery: The peripheral autonomic nervous system (the sympatho-adrenomedullary system)

The peripheral autonomic nervous system (ANS) is activated in parallel to the centrally-acting LC-NE system and these two systems together co-ordinate the reaction of the brain and body, especially in stressful or threatening situations, when rapid reaction may be needed. The ANS is divided into a sympathetic system, which promotes action, and a parasympathetic system, which facilitates relaxation. These two sub-systems interact with each other and function in an antagonistic or (in some cases) in a synergetic fashion. Activation of the sympathetic limb of the sympatho-adrenomodulatory axis leads to increased levels of epinephrine (from the adrenal medula) and NE (from sympathetic nerve ends). Peripheral epinephrine acts on the central LC-NE system through activation of the vagal afferents to the NTS, and from there to the LC. Another pathway that might explain the relatively fast response of the LC is the input that the LC receives from the dorsal horn of the spinal cord (Cedarbaum & Aghajanian, 1978).

Except from the above-mentioned afferent pathways, there are also efferent pathways which send information from the LC towards brain nuclei and the periphery. The LC has an output to sympathetic and parasympathetic preganglionic neurons of the intermediolateral cell column of the spinal cord, and innervates other autonomic nuclei, such as the Edinger-Westphal nucleus, the paraventricular nucleus, the caudal raphe, and the rostroventrolateral medulla. The LC also projects to the dorsal and ventral horns of the spinal cord, respectively (Bouret & Sara, 2004; Hancock & Fougerousse, 1976; Jones & Yang, 1985; Leong, Shieh, & Wong, 1984; Samuels & Szabadi, 2008a). The parallel activation of the centrally acting LC-NE system and the ANS facilitate an organism's fast response to a stimulus (e.g., threat) and prepare the body for the relevant response (flight-or-fight; flight-or-fight; Sara & Bouret, 2012; Valentino & Van Bockstaele, 2008).

LC-NE system in the periphery: The hypothalamic–pituitary–adrenal axis

Aside from its involvement in ANS regulation, the LC is also involved in neuroendocrine function by projections to the neuroendocrine cells of the hypothalamic–pituitary–adrenal

(HPA) axis. The HPA axis is activated in parallel to the ANS but the activation of this system is slower and has longer-lasting effects. As part of this axis, the paraventricular nucleus of the hypothalamus is activated, which secretes corticotropin-releasing hormone and arginine vasopressin. These hormones act on the anterior pituitary to promote the secretion of adrenocorticotropic hormone, which stimulates the adrenal cortex to initiate the synthesis and release of corticosteroids (cortisol in humans; cortisol in humans; Oyola & Handa, 2017; van Bodegom, Homberg, & Henckens, 2017). In **Chapters 5 and 6**, we assess biomarkers and hormones involved in ANS and HPA-axis regulation (i.e., alpha-amylase and cortisol).

LC-NE system in the periphery: The vagus nerve

LC activity is also linked with that of the tenth cranial nerve (also called "the vagus nerve" – meaning "the nerve that wanders" in Latin). The vagus nerve is the longest nerve in our body and communicates the state of the viscera to the brain and vice versa. The vagus nerve innervates the brain and its auricular branch innervates the external auditory canal and parts of the external ear. In the rest of the body, the main part of the vagus nerve travels down and innervates the viscera (i.e., internal organs: heart, spleen, kidneys, liver, stomach, lungs, small intestines and colon; i.e., internal organs: heart, spleen, kidneys, liver, stomach, lungs, small intestines and colon; Berthoud & Neuhuber, 2000; Ruffoli et al., 2011; Waldman, 2009; Yuan & Silberstein, 2016). Importantly, the vagus nerve projects to the NTS, which in turn projects directly and indirectly to the LC (Berridge & Waterhouse, 2003). Animal studies have found that vagus nerve stimulation (VNS) increased the firing rate of NE neurons in the LC (Dorr & Debonnel, 2006; Raedt et al., 2011; Roosevelt, Smith, Clough, Jensen, & Browning, 2006), and increased NE levels in the prefrontal cortex (Follesa et al., 2007), basolateral amygdala (Hassert, Miyashita, & Williams, 2004), and cerebrospinal fluid (Martlé et al., 2015). Importantly, this increase in NE levels occurred in a dose-dependent manner, and returned to baseline after termination of VNS (Raedt et al., 2011; Roosevelt et al., 2006).

To date, activation of the vagus nerve happens invasively via a surgical procedure using a pacemaker-like device (vagus nerve stimulation, VNS) or non-invasively via the stimulation of the auricular brunch of the vagus nerve on the ear, using an iPod-like device (transcutaneous VNS, tVNS). In **Chapter 5** we use tVNS to monitor the noradrenergic, arousal-related effects of tVNS and validate the impact of this technique on the LC-NE system. In **Chapter 6**, we use tVNS at different levels of stimulation intensity in order to differentially manipulate NE levels in healthy human participants.

LC-NE system in the periphery: Alpha-amylase, cortisol and pupil responses

Adrenaline, noradrenaline and cortisol are the three major hormones that are secreted in situations of high arousal and elevated stress, and that are related to the LC-NE system (Bremner, 2006). The adrenergic and the corticosteroid systems influence each other as they are both part of the HPA axis, and part of systems that influence each other in

situations of high arousal and elevated stress—the sympathetic-adrenomedullary system and the HPA axis. This interaction has been presented in detail above.

As already mentioned, cortisol is a glucocorticoid stress hormone that correlates with HPA-axis activation (Bosch et al., 2009; Hill, Taylor, Harmer, & Cowen, 2003; Oyola & Handa, 2017; van Bodegom et al., 2017). Salivary cortisol is mediated by noradrenergic inputs to the hypothalamus (Bosch et al., 2009; Dunn, Swiergiel, & Palamarchouk, 2004; Hill et al., 2003) and is sensitive to pharmacologically induced changes in central NE activity (Chamberlain, Muller, Cleary, Robbins, & Sahakian, 2007; Warren, Wilson, et al., 2017). Thus, cortisol can index LC-NE activity. Salivary alpha-amylase (SAA) is a digestive enzyme that is released by the saliva glands in response to local sympathetic nervous system activity (Bosch, Veerman, de Geus, & Proctor, 2011). SAA is a proxy marker of sympathetic-adreno-medullary activation (Bosch et al., 2009; Bosch et al., 2011) and, given that this system is directly activated by central NE, SAA has been suggested as a biomarker of central NE activity (Ehlert, Erni, Hebisch, & Nater, 2006; Speirs, Herring, Cooper, Hardy, & Hind, 1974; van Stegeren, Rohleder, Everaerd, & Wolf, 2006; Warren, van den Brink, Nieuwenhuis, & Bosch, 2017). SAA secretion is increased during stress and correlates with blood plasma NE during arousing activities such as exercise (Bosch et al., 1996; Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996).

In **Chapter 5** we assess levels of cortisol and SAA in order to monitor the noradrenergic, arousal-related effects of tVNS and validate the potential of this technique to modulate the LC-NE system. Consecutively, in **Chapter 6**, we asses cortisol and alpha-amylase as a biomarker of LC-NE activity after tVNS, a pharmacological, and a stress manipulation.

Studies of primates and rodents show that LC activity correlates with baseline pupil diameter (Joshi, Li, Kalwani, & Gold, 2016; Reimer et al., 2014) and the magnitude of task-evoked pupil dilations (Aston-Jones & Cohen, 2005; Joshi et al., 2016; Varazzani, San-Galli, Gilardeau, & Bouret, 2015). In line with this, fMRI studies in humans have shown that BOLD activity in the LC covaries with pupil size at rest and during simple decision-making tasks (de Gee et al., 2017; Murphy, Vandekerckhove, & Nieuwenhuis, 2014). The relationship between pupil size and LC may be mediated by activity in the rostral ventrolateral medulla, which projects to the LC and also innervates the peripheral sympathetic ganglia regulating the pupil (Nieuwenhuis, De Geus, et al., 2011). Based on these findings, many studies have used stimulus-evoked pupil dilation as an indirect measure of phasic activity of the human LC-NE system (e.g., Einhäuser, Stout, Koch, & Carter, 2008; Gilzenrat, Nieuwenhuis, Jepma, & Cohen, 2010; Jepma & Nieuwenhuis, 2010). We report pupil dilation as a biomarker of LC-NE activity after administration of a loud noise (accessory stimulus; **Chapter 4**) and after tVNS (**Chapter 5**).

In short, given that this dissertation aimed to investigate the LC-NE system in a holistic manner and from different perspectives, we assessed biomarkers in the body (periphery) that have been linked with the activation of the LC-NE system and its involvement in arousal, stress and cognition.

LC-NE system in the brain & the periphery: Functions

The LC-NE system exerts its action in the brain and body via neuronal (electrical) but also neurochemical and hormonal pathways. It influences the brain via the connections it has with multiple brain regions, and it influences the periphery via the connections it has with other brainstem nuclei, the spinal cord, and the vagus nerve; but also due to the involvement of the LC-NE system in two systems that are well studied in the stress literature: the fast and rapidly activated peripheral ANS and the slower activated HPA axis. It therefore comes as no surprise that there is a great similarity between conditions that activate the LC in the brain and conditions that activate the sympathetic nervous system in the periphery: the LC mobilizes the brain for action while the sympathetic system mobilizes the body.

The LC-NE system is put into action to face environmental challenges, in parallel with the recruitment of the ANS, which responds to homeostatic challenges, stressors, and other stimuli that are important for the organism, and in turn determines general arousal level. The autonomic activation promotes the physiological response, whereas the LC promotes an efficient and appropriate cognitive response through its action in the forebrain. In this way, the LC-NE system plays and important role in cognition and in the orienting reflex, which includes physiological responses such as changes in pupil dilation and heart rate, activated by arousing or motivationally significant stimuli or unexpected changes in the environment (Nieuwenhuis, De Geus, et al., 2011; Pfaff, Martin, & Faber, 2012; Sara & Bouret, 2012).

A significant number of studies have aimed to illuminate these functions of the LC-NE system, but due to technical and anatomical challenges, a large part of this research has been limited to animal subjects or computational models. Research conducted in the context of this PhD dissertation aims to bridge the gap between animal studies and theoretical/computational frameworks by acquiring data in human subjects.

Physiology: The LC exhibits two modes of activity

Studies in non-human primates have suggested that the LC has two distinct modes of discharge: a phasic and a tonic mode (Aston-Jones & Cohen, 2005). During bursts of phasic discharge, LC neurons fire in a highly synchronized manner as a consequence of direct electrical coupling between individual neurons of the LC (Ishimatsu & Williams, 1996). During the tonic discharge, the LC shows a constant background activity characterized by non-synchronicity, uncoupling, and random bursts (Usher, Cohen, Servan-Schreiber, Rajkowski, & Aston-Jones, 1999).

The two types of discharge influence each other in a way that the magnitude of phasic discharge is related to the level of ongoing tonic discharge. Phasic discharge is optimal at moderate levels of tonic discharge and is diminished in situations where tonic discharge is low (e.g., in slow-wave sleep) or when tonic discharge surpasses a moderate level (e.g., in stress; e.g. in stress; Aston-Jones, Rajkowski, & Cohen, 2000; Usher et al., 1999; Valentino & Van Bockstaele, 2008).

The two modes of discharge are related to different types of contexts and behavior. High phasic discharge in the LC is observed when the animal is shown a stimulus that is salient or motivationally significant. Strong tonic discharge occurs in situations of distractible behavior or stress. It has been suggested that there is an inverted-U relation between LC activity and task performance, similar to the classical Yerkes-Dodson relationship between arousal and performance (Aston-Jones, Rajkowski, & Cohen, 1999). Performance is poor at very low levels of LC tonic discharge when the organisms is non-alert. It becomes optimal at moderate LC tonic activity (phasic LC mode; when the phasic discharge is high but tonic is moderate). Finally, performance becomes poor at high levels of tonic LC activity (tonic mode, when tonic activity is high but phasic activity is diminished; Aston-Jones & Cohen, 2005; Aston-Jones et al., 1999).

The fact that the LC exhibits a dual mode of activity has inspired influential theories about how the LC-NE system affects cognition and behavior. These theories are briefly presented below.

Theories regarding effects of the LC-NE system on cognition and behavior

The adaptive gain theory

The adaptive gain theory suggests that the role of the LC is to regulate global neural gain in order to maximize utility in a given context. It does so by balancing the trade-off between tonic and phasic activity. For instance, by gradually shifting between tonic and phasic modes of activity, the LC regulates the trade-off between exploration and exploitation. The LC phasic mode promotes exploitative behavior by facilitating processing of task-relevant information (through the phasic response) but at the same time filtering out irrelevant stimuli (through relatively low tonic activity). In this mode, the organism is highly concentrated on the ongoing task and harvests rewards that this action offers. In contrast, the LC tonic mode promotes distractibility and disengagement from the task. This facilitates exploration and search for alternative, perhaps more productive, behaviors.

Thus, according to the adaptive gain theory, changes in the mode of LC activity orchestrate shifts in behaviors in line with environmental requirements, in order to maximize utility in a specific context (Aston-Jones & Cohen, 2005; Usher et al., 1999).

The network reset theory

As mentioned above, the LC-NE system is activated in response to salient stimuli. According to the network reset theory, when these stimuli are detected, the LC sends a phasic “reset” signal that reorganizes neural networks to facilitate behavioral and cognitive shifts in a way that promotes optimal behavioral performance (Hermans et al., 2011; Sara, 2009; Sara & Bouret, 2012). In **Chapter 5,** we test whether the LC-NE system is involved in cognitive shifts by assessing task switching, a pure form of cognitive flexibility.

The unexpected uncertainty theory

Yu and Dayan (2005) suggest that NE signals unforeseen changes of task context, signaled by strongly unexpected observations. This 'unexpected uncertainty' originates from changes in environmental parameters that require an appropriate update of predictions about the environment, and thus a change in behavior. In contrast, according to this theory, the neuromodulator acetylcholine signals known ('expected') uncertainty in a given task context.

The glutamate amplifies noradrenergic effects (GANE) theory

Based on the 'glutamate amplifies noradrenergic effects' (GANE) theory, the LC-NE system promotes neural representations of goal-relevant information through the 'ignition' of local hotspots that contain the neurotransmitter glutamate. High-priority perceptual representations are favored over low-priority representations due to the collaborating action and timely release of glutamate and phasic NE (Mather, Clewett, Sakaki, & Harley, 2015). Local glutamate–NE effects occur in parallel to more broad-scale actions, resulting in a "winner-takes-more / loser-takes-less" dynamic: high-priority items are even more likely to be remembered, whereas low-priority items are even more likely to be forgotten.

The LC-NE system and stress theory

Although not falling under the category of a "typical" theory, work performed by Valentino and Van Bockstaele regarding the LC-NE system under stress, is very relevant for the research presented in this dissertation, and thus merits acknowledgement in this section. Valentino and Van Bockstaele (2008) draw a link between the dual modes of LC activity (tonic or phasic) and the stress context. Interactions between stress-related neurotransmitters that act on LC neurons regulate shifts between these modes of discharge in response to a stressor and make the LC-NE system a key player in behavioral and cognitive aspects of stress response.

During periods of elevated tonic LC activity, such as in life-threatening situations, phasic responses are diminished in order to facilitate a shift towards an exploratory mode. This response is optimal in a challenging environment and enhances chances for survival, so the ability of LC neurons to switch between phasic and tonic activity would be advantageous for rapidly modifying behavior in response to a stressor or after stress cessation.

The mode of LC activity is modulated by afferent, stress-released neurotransmitters, especially excitatory amino acids, corticotropin-releasing hormone and endogenous opioids secreted onto LC neurons. By biasing the activity of LC neurons towards a particular mode of discharge, these chemicals can favor advantageous behaviors in specific situations. On the other hand, LC neurons, through changes in their discharge rate, facilitate the cognitive and behavioral branches of the stress response.

The theories mentioned above, are to a large extent complementary to each other and extend the adaptive gain theory to different contexts and enriched perspectives. The theories agree that the LC-NE system promotes behavioral adaptation to the demands of the environment. However, each of them focuses on different mechanistic aspects of the way in which NE coordinates such behavioral adaptations.

This dissertation: Holistic approach

When studying brain and cognition, researchers tend to segregate the different parts in order to be able to study the system of interest, but it is important to always return to the holistic level in the end. The beauty of human cognition is that it functions by bringing different levels together in harmony and in a holistic approach: from cell, to synapse, from neuron to neuromodulatory networks, from central neuromodulators to hormones that are secreted in the body, from anatomy to physiology and cognition.

Therefore, this dissertation approaches human cognition and the study of the LC-NE system in a holistic manner. To this end, all chapters are written by taking into consideration theoretical knowledge about the LC-NE system with regard to brain anatomy, cognitive functions, neuromodulation (mainly NE), physiological responses, and clinical applications. Each chapter concentrates on one of these factors to a higher degree but all the other factors are also involved or assessed in some way. The first two chapters deal mainly with the anatomy of the LC, yet there is always a link with the other factors and especially the clinical application of MRI scans and LC integrity as a biomarker for neurological and psychiatric diseases. The last three chapters concentrate on cognition and physiology (pupil responses, P300 component of ERP), but always taking into consideration the structural connections of the LC-NE system. Finally, the last two chapters have a clinical approach and collectively deal with clinical applications of tVNS (medical device), alpha-amylase, cortisol, physiological responses, stress, and pharmacology.

Below there is a brief description of the next chapters and an introduction to the studies performed for this PhD dissertation, where a holistic approach in cognitive and clinical neuroscience is applied.

Chapter 2: In vivo visualization of the locus coeruleus in humans: Quantifying the test-retest reliability

Visualization of the LC has been based mainly on ex vivo material (i.e., material of donors after their death) because in vivo localization (in living humans) has been a very difficult enterprise. The reasons for this difficulty are technical and anatomical in nature.

Regarding the technical challenges, there is a need for specific technological advancements, and these have only recently taken place. Until recently the available MRI scans were not able to visualize small brainstem nuclei. This means that no matter how hard the researcher of the past would have tried to visualize and map the LC and other small brainstem nuclei in living humans, such an enterprise was simply impossible

because the timing was not right: one is dependent on technological advances and special MRI scans that require a long time to develop and implement in brain research.

Regarding the anatomical challenges, the LC is difficult to map due to its small size and big inter-individual variability in location and size. Additionally, it is located in parts of the brain that are very challenging to visualize in living humans with MRI scanners (i.e., located close to the vessels and the 4th ventricle that pulsate when blood or cerebrospinal fluid is rushing). This motion creates noise in the visualization in anatomical scans and is particularly problematic in the case of functional MRI (fMRI), which is more sensitive to such motion, rendering co-registration of functional and structural scans almost impossible. Additionally the cerebrospinal fluid running through the 4th ventricle might create noisy signal that can be wrongly perceived as genuine brain activity (i.e., the researcher is detecting noise originating from cerebrospinal fluid but thinks that it is BOLD activity). Finally, MRI research has mainly focused on the cortex and largely ignored the brainstem. Only recently there have been more advances towards this direction (Forstmann, de Hollander, van Maanen, Alkemade, & Keuken, 2017).

Recent developments in neuroimaging methods and scanning protocols have made possible the visualization of the LC by the adaptation of a T1-weighted turbo spin echo (TSE) scan sequence for 3T MRI, which is thought to be sensitive to neuromelanin (Keren et al., 2015; Sasaki et al., 2006), a pigment that is produced in catecholaminergic neurons and exists in large quantities in the LC (Fedorow et al., 2005).

Since the initial publication, numerous studies have used this scanning protocol for visualizing the LC in a variety of applications (Astafiev, Snyder, Shulman, & Corbetta, 2010; Clewett et al., 2016; Keren, Lozar, Harris, Morgan, & Eckert, 2009; Murphy, Vandekerckhove, et al., 2014; Sasaki et al., 2008; Takahashi et al., 2015). Importantly, given that LC dysfunction plays an important role in cognitive and neurodegenerative disorders, such as Parkinson's and Alzheimer's disease (Grudzien et al., 2007; Mravec, Lejavova, & Cubinkova, 2014) and monoamine-related psychiatric disorders such as anxiety, depression (Ressler & Nemeroff, 1999; Schramm, McDonald, & Limbird, 2001) and schizophrenia (van Kammen & Kelley, 1991), it has been suggested that TSE scans may be used as a diagnostic tool for tracking the progression of these disorders or as a biomarker for differential diagnosis. Importantly, this requires a reliable and robust scan protocol that allows delineation of the LC in a reproducible manner across different time points and by different raters/clinicians. Otherwise, there is risk of wrong diagnosis or fallacious treatment plan decisions, with possible deleterious effects for the patient. Aside from its use as a tool for monitoring pathological changes in LC structure, the TSE sequence is also used to identify the LC for region-of-interest analyses in fMRI studies. Both applications require that the contrast generation process is robust and reproducible, and that the scans allow accurate delineation of the LC. Despite its frequent use, to date no study has investigated the reproducibility and inter-observer variability of the LC masks identified using the TSE scan sequence.

In Chapter 2 we aimed to quantify the test-retest reliability of LC imaging by assessing stability of the TSE contrast of the LC across two independent scan sessions and by quantifying its intra- and inter-rater reliability. Additionally, we combined all TSE scans of our study and created a probabilistic LC atlas that quantifies the variability of this structure and can facilitate the spatial localization of the LC in standardized (MNI) space.

We found moderate reproducibility and scan-rescan stability, indicating that the localization and segmentation of the LC in vivo is a challenging, but reliable enterprise. However, clinical or longitudinal studies should be carried out carefully. Our probabilistic atlas results show substantial variability in the spatial location of the LC. In the current atlas (freely available from http://www.nitrc.org/projects/prob_lc_3t) we adopted a quantification approach, resulting in probabilistic information on where the LC is located. This information can, for instance, be used to weigh the measured fMRI signal with the probability of it originating from the LC. It is the first probabilistic atlas for the LC and one of the few attempts to map the brainstem, a field that deserves more attention and is promising to turn the brainstem from a "terra incognita" into a fully mapped and understood region in the future (Forstmann et al., 2017).

Chapter 3: Paving the path for better visualization for the LC: Quantifying the contrast of the human locus coeruleus in vivo at 7 Tesla MRI.

As discussed above, the important role that the LC plays in cognition and its use as a biomarker for assessment of neurodegenerative disorders necessitate accurate visualization of the LC. To date the most frequently used scan at 3T scanners is a T1-weighted TSE scan sequence (e.g., Betts, Cardenas-Blanco, Kanowski, Jessen, & Düzel, 2017; Clewett et al., 2016; de Gee et al., 2017; Keren et al., 2009; Liu et al., 2017). In Chapter 2, we made an attempt to assess the robustness of visualization of the LC at a 3T scanner using this TSE sequence. In Chapter 3, we made a step to further improve LC visualization by using an ultra-high field (7T) MRI scanner. We hypothesized that imaging at higher magnetic field strength might provide a solution to the challenges involved in LC imaging. Higher magnetic field strength increases signal-to-noise ratio and allows imaging at a higher spatial resolution (Cho et al., 2014; Sclocco, Beissner, Bianciardi, Polimeni, & Napadow, 2017; van der Zwaag, Schafer, Marques, Turner, & Trampel, 2016). This, in turn, results in smaller partial volume effects, which in itself can help to improve contrast and thereby detectability (de Hollander, Keuken, & Forstmann, 2015; Kneeland, Shimakawa, & Wehrli, 1986).

Additionally, we examined a number of sequences whose utility for LC imaging has been implied by prior literature. We measured the LC contrast in vivo for these 7T sequences and compared the obtained contrast measures to a frequently used sequence at 3T MRI.

The results indicate that several of the 7T sequences provide detectable contrast between the LC and surrounding tissue. Of the tested sequences, a T1-weighted sequence with spectral presaturation inversion recovery (SPIR) seems the most promising method for visualizing the LC at ultra-high-field MRI. This sequence provides similar contrast of the

LC as the 3T sequence commonly used, but at a higher spatial resolution and with isotropic voxels. The isotropic voxels at 7T are an important advantage given the small size of the LC. Finally, although there is no clear benefit in contrast, a potential advantage of using SPIR is the relatively short acquisition time, which may be desirable in clinical settings to minimize subject motion.

To conclude, in Chapter 3 we made a first step towards the improvement of visualization of the LC in a 7 Tesla MRI scanner. We are the first ones to compare these scan sequences in 7T and develop a TSE scan sequence version for the 7T. Future work can utilize this work and proceed to further development and improvement of MRI scans in order to achieve better visualization of the LC at 3 T, 7T and maybe higher magnetic field scanners.

Chapter 4: The accessory stimulus effect is mediated by phasic arousal: a pupillometry study

As highlighted above, the LC-NE system plays an important role in arousal. Different levels of induced arousal can have beneficial or detrimental effects on cognitive functioning and performance (usually according to an inverted U-shaped function; Yerkes & Dodson, 1908). A phenomenon that has been linked with arousal and positive cognitive performance is the accessory stimulus (AS) effect. It has been shown that people respond faster and more accurately in reaction time (RT) tasks when a visual imperative stimulus is immediately preceded by a task-irrelevant accessory stimulus (AS) presented in a different (e.g., auditory) perceptual modality, compared to when the imperative stimulus is presented alone. Although the information processing stage(s) that benefit from the AS remain debated, there seems to be a consensus that the AS effect is caused by a brief surge of arousal. Indeed, both pioneering and more recent studies have used the terms immediate arousal effect (Hackley & Valle-Inclán, 1999; Kiesel & Miller, 2007; Sanders, 1975) and automatic alertness/arousal (Posner, Klein, Summers, & Buggie, 1973) to refer to the AS effect. Despite the common inference that the AS effect is mediated by a phasic arousal response, there is only some indirect evidence to support this idea.

In Chapter 4, we exploited pupil dilation as a common index of phasic arousal (Beatty & Lucero-Wagoner, 2000) and LC activity (Joshi et al., 2016; Murphy, Vandekerckhove, et al., 2014; Varazzani et al., 2015) and examined its relationship with the AS effect. Participants carried out a demanding choice reaction time task with accessory stimuli occurring on 25% of the trials.

Results showed that participants exhibited the typical AS effect, and the accessory stimuli evoked a reliable early pupil dilation on top of the more protracted dilation associated with the imperative stimulus. Variation in reaction times on AS trials was selectively associated with pupil dilation during the early time window within which the AS had an effect, such that particularly large AS-evoked dilations were associated with especially fast responses. These results provide the first evidence for the long-standing assumption that the AS effect is mediated by AS-evoked phasic arousal.

Chapter 5: The neuromodulatory and hormonal effects of transcutaneous vagus nerve stimulation as evidenced by salivary alpha-amylase, salivary cortisol, pupil diameter, and the P3 event-related potential

As mentioned above, activity of the LC-NE system is linked to the activity of the vagus nerve, and the vagus nerve can be stimulated in an invasive and non-invasive manner. Invasive VNS is a promising treatment for depression (George & Aston-Jones, 2010; Nemeroff et al., 2006; Vonck et al., 2014) and epilepsy (Ellrich, 2011; Kraus et al., 2013) that likely exerts part of its therapeutic effect by increasing NE release from the LC. tVNS can be achieved by delivering electrical impulses to the auricular branches of the vagus nerve, which are situated close to the surface of the skin of the outer ear (Ellrich, 2011). fMRI studies in healthy humans demonstrate that tVNS elicits widespread changes in cortical and brainstem activity (Frangos, Ellrich, & Komisaruk, 2015; Kraus et al., 2007; Kraus et al., 2013; Yakunina, Kim, & Nam, 2017). In light of the clinical potential of tVNS, it would be valuable to establish if tVNS, like invasive VNS, affects NE, using relatively inexpensive and easy-to-use biomarkers of NE. In Chapter 5, we evaluate the effect of tVNS on NE levels using three accepted biomarkers and one putative biomarker of central NE activity: salivary alpha-amylase (SAA), salivary cortisol, pupil size, and the P3 component of the event-related brain potential (ERP), respectively.

The connection between LC-NE activity and SAA, salivary cortisol, and pupil size has been described above. Regarding the P3 component of the event-related brain potential, it has been suggested that the phasic changes in cortical NE levels are associated with the scalp-recorded P3 component (Chmielewski, Muckschel, Ziemssen, & Beste, 2017; De Taeye et al., 2014; Murphy, Robertson, Balsters, & O'Connell R, 2011; Neuhaus et al., 2007; Nieuwenhuis, Aston-Jones, & Cohen, 2005; Warren & Holroyd, 2012; Warren, Tanaka, & Holroyd, 2011; Wolff, Mückschel, Ziemssen, & Beste, 2018). Events that lead to increased phasic firing of the LC also lead to increased P3 amplitude (Nieuwenhuis et al., 2005). Noradrenergic drugs influence P3 amplitude in both animals (Swick, Pineda, & Foote, 1994) and humans (Brown et al., 2016; Brown, van der Wee, van Noorden, Giltay, & Nieuwenhuis, 2015; de Rover et al., 2015), and lesion of the LC eliminates the P3 in monkeys (Pineda, Foote, & Neville, 1987). Of interest here, the amplitude of the P3 is increased by invasive VNS (De Taeye et al., 2014; Neuhaus et al., 2007; Schevernels et al., 2016).

Despite the common inference that the tVNS effect, similarly to the invasive VNS effect, is mediated by increasing central NE activity, there is only some indirect, limited evidence to support this idea. To explore the claim that tVNS increases central NE, in Chapter 5 we assess SAA, salivary cortisol, pupil size and P3 amplitude across three experiments.

Results show that tVNS significantly increased SAA and salivary cortisol, but did not affect P3 amplitude nor pupil size. These findings suggest that SAA and cortisol, but not pupil size and P3 amplitude, can be used to monitor the arousal-related effects of tVNS.

Chapter 6: Noradrenergic regulation of cognitive flexibility: No effects of stress, transcutaneous vagus nerve stimulation and atomoxetine on task-switching in humans

Cognitive flexibility allows us to adaptively switch between different responsibilities in important domains of our daily life. Previous work has suggested an important role for the LC-NE system in modulating several forms of cognitive flexibility, possibly by global modulation of gain and corresponding levels of decision noise (Aston-Jones & Cohen, 2005; Kane et al., 2017; Warren, Wilson, et al., 2017). However, it is still unknown whether NE levels are also critical for task switching (Kehagia, Cools, Barker, & Robbins, 2009; Kehagia, Murray, & Robbins, 2010), which requires the dynamic transformation of task-set representations from trial to trial.

In Chapter 6, we addressed this question by examining cued task-switching performance after manipulating activity of the LC-NE system using stress induction, tVNS at moderate and high intensity, and administration of the selective NE blocker atomoxetine.

None of the manipulations affected cognitive flexibility, leaving the size of the switch costs and the preparation effect unaffected. The findings were highly consistent, suggesting that NE is not involved in the cognitive flexibility required to switch between relatively abstract rules and sets of stimulus-response mappings. Task-switching performance reflects a complex mix of cognitive control and bottom-up dynamics of task-set representations. Our findings suggest that NE does not affect either of these aspects of cognitive flexibility.

Although stress induction, tVNS and atomoxetine also affect other neuromodulator systems, and the three manipulations do not affect the LC-NE system in a similar way, if task switching is crucially dependent on activity of the LC-NE system, one would expect effects of (some of) these manipulations on task switching performance – which we did not find.