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

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Chapter 7

Lay summary in English:

A scientific journey in simple words

This PhD dissertation: an explorer's journey

In the 21st century humans have created very detailed maps of planet Earth, have mapped other planets, and are even in search of extraterrestrial life. However, surprisingly enough, they have not yet succeeded at mapping the structures that are inside their own brain, or understanding their behavior and cognition. Surprising that we don't know what is in our brain, isn't it? This PhD dissertation is a journey in understanding parts of our brain, cognition, physiological functions and behavior and it does so by investigating the locus coeruleus-norepinephrine (LC-NE) system in humans *in vivo* (in living humans, not brains of dead donors as it has been usually done until now).

To make the content of this PhD dissertation more clear for the layman reader, the research of this thesis is likened to the journey of 13^{th} and 15^{th} century explorers. Explorers like Marco Polo and Christopher Columbus tried to map and understand planet "Earth", while we try to map and understand planet "Brain and Cognition" by concentrating on the LC-NE system.

What is the locus coeruleus-noradrenergic system?

The locus coeruleus, a small brainstem nucleus, is the main source of the chemical norepineprine in the brain and is involved in a number of cognitive functions as well as several neurological and psychiatric disorders. In this dissertation we study the human LC-NE system, the anatomy of this tiny brainstem nucleus and the involvement of the LC-NE system in stress, arousal, cognitive flexibility and physiology (hormones & pupil responses).

The 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 autonomic nervous system and the slower activated hypothalamic- pituitary- adrenergic axis (HPA-axis).

The LC-NE system is put into action to face environmental challenges, in parallel with the recruitment of the autonomic nervous system, 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 an 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 (Sara & Bouret 2012; Nieuwenhuis et al., 2011; Pfaff et al., 2012). Thus, the LC mobilizes the brain for action while the (sympathetic) autonomic nervous system mobilizes the body; therefore, the LC-NE system as a whole is involved in mobilization of both the brain and the periphery.

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 aimed to bridge the gap between animal studies and theoretical work by acquiring data in living human subjects.

This PhD dissertation: Holistic approach

When studying planet "Earth", explorers of the 15^{th} century were interested in learning about geography and mapping methods in order to reach new lands (e.g., new paths to the Indies), the treasures that they expected to find there, the flora, and the civilizations. One can imagine that depending on their knowledge, different members of the crew would concentrate on different tasks in order to realize the exploration. One member would concentrate on creating a map, another on navigating the boat, another on examining the trade winds, another on exploring the expected treasures. However, after this segregation of tasks, they needed to come together and bridge their knowledge in order to make the trip possible and to survive in the new land. Thus, for making the exploration a success, it was important to always return to the holistic level and have a good overview of the broad picture.

In a similar way, when studying the planet "Brain and Cognition", researchers tend to segregate the different parts in order to be able to deeply 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 is based on different levels of brain and body functions that act together in harmony: 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 were 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. **Chapters 2 and 3** deal mainly with the anatomy of the LC, while **Chapters 4, 5 and 6** concentrate on cognition and human physiology. Additionally, **Chapters 5 and 6** take a clinical approach and collectively deal with clinical applications of tVNS (medical device), the hormones alpha-amylase and cortisol, bodily physiological responses, stress, and pharmacology.

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

Anatomical Chapters (Chapters 2 & 3)- Creating and assessing robustness of a brain atlas

Chapter 2 and 3 of this PhD dissertation describe anatomical studies that provided methodological developments in the visualization of the LC in living humans.

What prevents us from looking inside our brainstem?

The challenges are of two kinds: technical and anatomical. Regarding the technical challenges, there is a need for specific technological advancements, and these have only recently taken place. This means that no matter how hard the researcher-explorers of the past tried to map these structures in living humans, such an enterprise was simply impossible because the timing was not right: one is dependent on technological advances that take quite some time to become feasible and be implemented in brain research. Regarding the anatomical challenges, some structures are difficult to map because they are small, their location and size varies a lot among different individuals, and also in some cases are located in parts of the brain that cannot be easily visualized in living humans within the MRI scanner due to motion artifacts (e.g., they are located in brain tissue close to the vessels or the fourth ventricle and pulsate when blood or cerebrospinal fluid is rushing). The individual differences in the location and size of these structures signify that in different people these brain structures can be a bit higher or lower, and the size and shape can also differ. Under these circumstances, the explorer-researcher, like a modern Christopher Columbus, starts a journey to explore West Indies (i.e., the LC in her case) but runs the risk of accidentally ending up in America (i.e., another, nearby brain nucleus).

What might be the solution?

Solution Nr 1: assess if your map is trustworthy and create a better map

To avoid mixing the Indies with America in the brain, you need to know if your map is trustworthy. For this, you need to practically assess if the map that was created does indeed lead to Indies and not somewhere else. This can be done by letting different explorers use the same map: will all of them end up in the same region with 100% accuracy? It can also be done by using the same map at different time points: will one explorer end up in the same region with 100% accuracy if she makes the voyage several times? This is exactly what we did in **Chapter 2** but we did so for the brain**.**

Additionally, to avoid mixing the Indies with America in the brain, one could create a better map, this can be a probabilistic map. This is exactly what we did in **Chapter 2.** Contrary to the common practice where human brain maps are created only from donors after their death, our probabilistic brain map is based on MRI images of living humans (called "*in vivo*" in scientific jargon).

Our probabilistic map is also more accurate compared to simple brain maps because it aligns all the different images of the LC, one on top of the other, and quantifies the similarities and the differences. In this way the final output is a map that shows a representative image of the core of the LC but also some extra borders around it in order to quantify the certainty of the structure localization (e.g., how certain are you that you are in the LC?).

To compare this with an explorer's trip, our probabilistic map is also more accurate compared to simple maps because it aligns and takes into consideration not only one single map, but all the different maps depicting Indies (e.g., the one created by cartographer Toscanelli, another one by Henricus Martellus etc). In this way, the final output is a map that shows a more representative, accurate image of the Indies: it provides the core of the land but also some extra borders around it in order to quantify the certainty of the structure localization—that is, how certain are you that you are indeed in the Indies?

Finally, we measured the reliability of imaging the LC in humans: is the trip to Indies and the related activities reliable? Does it even make sense to try to make a map and embark to this trip? We also made the LC brain atlas publicly available, so it can be used for free (http://www.nitrc.org/projects/prob_lc_3t). Give it a try!

Solution Nr 2: compare the available maps and start using higher-resolution camera

A second solution would be to compare the available maps and start using technological improvements such as a higher-resolution camera (7 Tesla MRI scanner). This is what we did in **Chapter 3**. We used special MRI sequences that prior literature indicated as promising for imaging structures like the LC. We assessed the effectiveness of these MRI sequences in visualizing the LC by comparing them with each other and by comparing them with scans made with a "low-resolution camera" (3 Tesla MRI scanner).

In sum, in **Chapter 2** we assessed if the map is trustworthy and created a better map to increase the chances that travelers end up in the Indies (LC) and not in America (another brainstem nucleus). In **Chapter 3** we compared the available maps (special scans for LC visualization) and started using a higher-resolution camera (7T MRI scanner), which should in theory improve the visualization of the desired structure.

Explore an alternative route to reach the Indies

Psychophysiological chapter (Chapter 5) - Exploring an alternative, less invasive, inexpensive route to reach the desired destination (LC-NE system)

Apart from designing a map, assessing the map's robustness, and using state-of-the-art technology to further improve a map, a good explorer is also interested in finding alternative, cheaper, easier, less dangerous, and more accessible routes to reach their destination. Indeed, based on the theories of Greek scholars like Pythagoras, Aristotle and Ptolemy that the planet is actually a sphere, Christopher Columbus trusted astronomer Toscanelli's map and theory that sailing west across the Atlantic would be a quicker way to reach the Indies (Spice Islands). Columbus hypothesized that by traveling west, he'd open up new trade routes and he would be able to reach the Indies via an alternative route. He started his journey to test this hypothesis. We did the same in **Chapter 5**. Given the anatomical connections between the LC-NE system and the vagus nerve (the longest nerve of the human body, connecting the brain with the gut), and findings that transcutaneous vagus nerve stimulation can be a new, non-invasive, cheaper, and easier to use method to stimulate the vagus nerve, we hypothesized that we could use this method to modulate the LC-NE system. So we started a journey to test this hypothesis.

You have reached your destination. What treasures can this land offer?

LC-NE system, cognition, physiology, & hormones (Chapter 4 and 6) - What kind of cognitive functions is the LC-NE system involved in?

Suppose you assessed the risks and reliability of the trip, you created the map and you tried to improve it **(Chapter 2 & 3),** you found some signs to support an alternative, not dangerous route to your destination (**Chapter 5)** and now you are ready to reach this destination. What do you expect to find there? Based on prior knowledge, Columbus was expecting his destination (Indies) to be a land of spices. In the same way, we expected the LC-NE system to be involved in specific cognitive phenomena: a) the accessory stimulus effect (improved performance caused by a loud noise irrelevant to the task at hand), and b) switching between tasks (cognitive flexibility). In Columbus' case, those who reached India, confirmed that India has spices. However, Columbus reached America, a land full of gold and cotton instead of spices. Similarly, we found evidence that the LC-NE is involved in the accessory stimulus affect (spices, bingo!), but is not involved in task switching (cotton, oops!).

Results of this PhD project - What did we find?

Chapter 2: Is your map trustworthy and can you create a better map?

Title of the chapter in scientific jargon**:** *In vivo* visualization of the locus coeruleus in humans: Quantifying the test-retest reliability

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. Thus the voyage to the Indies is an enterprise worth trying, but you should be careful and be prepared for the dangers you will face during the trip.

Our probabilistic atlas results show substantial variability in the spatial location of the LC and this map is freely available (http://www.nitrc.org/projects/prob_lc_3t). This atlas 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 promises to turn the brainstem from a "tera incognita" into a fully mapped and understood region in the future (Forstmann et al., 2017). We provide the first probabilistic map. Enjoy the voyage!

Chapter 3: How do the available maps perform and does it make sense to use higher-resolution camera?

Title of the chapter in scientific jargon: Quantifying the contrast of the human locus coeruleus *in vivo* at 7 Tesla MRI

In Chapter 3, we made a first step towards the improvement of visualization of LC in an ultra-high-field (7 Tesla) MRI scanner. We are the first ones to compare these scan sequences at 7T and develop a version of the TSE scan sequence for use at 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 3T, 7T and maybe higher magnetic field scanners.

It should be noted that choosing the correct lenses and resolution is important in enterprise of atlas creation. If you use lenses strong enough to visualize planets, these will not work when you want to visualize the Indies, which is just part of Earth. Some locations are sufficiently visualized with a low- to medium-resolution camera. The LC is visualized decently at low resolution (3T); however, better visualization is needed and high-resolution camera can be promising in improving visualization of the LC. Still some work is needed to achieve perfect visualization. Future work can build on the work done in this chapter.

Chapter 5: Exploring an alternative, less invasive, inexpensive route to reach the desired destination (LC-NE system)

Title of the chapter in scientific jargon: 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

In Chapter 5, we evaluate the effect of tVNS on NE levels using three accepted biomarkers and one putative biomarker of central NE activity: the hormone alpha-amylase, the hormone cortisol, pupil size, and the P3 component of the event-related brain potential (these are brainwaves that are assessed using electroencephalography), respectively. Results show that tVNS significantly increased salivary alpha-amylase and salivary cortisol, but did not affect P3 amplitude or pupil size. These findings suggest that salivary alpha-amylase and cortisol, but not pupil size and P3 amplitude, can be used to monitor the arousal-related effects of tVNS.

Chapter 4: You have reached your destination. Bingo! Your destination is a land full of spices

Title of the chapter in scientific jargon: The accessory stimulus effect is mediated by phasic arousal: a pupillometry study

As highlighted in Chapter 1, the LC-NE system plays an important role in regulating arousal. A phenomenon that has been linked with arousal and enhanced cognitive performance is the accessory stimulus effect (AS effect). The AS effect refers to the phenomenon where people show improved performance when -while they are performing a visual task- they hear an irregular, loud noise from time to time. Importantly, this noise leads to better performance compared to when doing the task alone, despite the fact that it does not provide any information about the task at hand.

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 this chapter we provide the first evidence for this long-standing assumption and we demonstrate that the LC-NE system is involved in the accessory stimulus effect.

Chapter 6: You have reached your destination. Oups! Your destination is not a land of spices- how about some cotton?

Title of the chapter in scientific jargon: 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 and is a multifaceted construct containing learning, updating, cognitive control, decision making and switching between tasks. Previous work has suggested an important role for the LC-NE system in modulating several forms of cognitive flexibility, possibly by modulation of gain and corresponding levels of decision noise. However, it is still unknown whether NE levels are also critical for the task switching, an aspect of cognitive flexibility 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 pharmacological administration of the selective NE blocker atomoxetine. None of the manipulations affected task switching. Our findings suggest that NE does not affect this aspect of cognitive flexibility.

Did they live happily ever after and what comes next?

I don’t know if they lived happily ever after, but I do know what should come next: The exploration of the brain should continue. And of course this journey will have higher impact and will be much more fun if cognitive neuroscientists (explorers) join powers. In the end, a “modern Christopher Columbus” researcher will always benefit from a colleague who – like a “modern Amerigo Vespucci” – would demonstrate that she was wrong to believe that she reached the Indies; she had actually reached an unexplored, wholly new continent previously unknown to Eurasians, a “New World” that would be called “America”. Importantly, modern explorers know that those who do not read history are doomed to repeat it, and can take lessons from 15th century’s explorers in order to avoid mistakes of the past. Therefore, they ought to treat their discoveries in an ethical and respectful manner, promote “fair trade” and open science, embrace a collaborative atmosphere instead of a “rich-get-richer” competitive approach. They should also provide the relevant credits to the relevant people, always remain open to critique and alternative perspectives and, above all, have as their main goal the contribution to knowledge and to a better society instead of self-interest and an accumulation of publications. Amen!