

Nasal Oxytocin: Facts and Routes

João Paulo Correia Lima*
and Avelino Luiz Rodrigues

Department of Clinical Psychology and
Nucleus of Neuroscience and Behavior,
Institute of Psychology, University of Sao
Paulo, Brazil

Abstract

Research about the oxytocin effects over behaviour is growing up and the main technic used in this research is the nasal oxytocin administration, but there is some controversy if the blood-brain barrier could or not stop the oxytocin entrance in the central nervous system. The authors present some arguments and anatomical structures that can permit the oxytocin entrance in the central nervous system despite de blood-brain barrier, describing direct and indirect paths, justifying the data collected in experiments based on nasal oxytocin administration, in a hypothetical way (not yet demonstrated).

Keywords: Oxytocin; Human behaviour; Blood-brain barrier; Neurotransmitter; Brain

*Corresponding author: João Paulo
Correia Lima

✉ joaoaclima@usp.br

Psychologist for Neuroscience and Behavior,
Institute of Psychology, University of Sao Paulo,
Brazil

Tel: +55-11-32852420
+55-11-983439754

Received: December 03, 2018; **Accepted:** March 18, 2019; **Published:** March 25, 2019

Citation: Lima JPC, Rodrigues AL (2019) Nasal Oxytocin: Facts and Routes. Acta Psychopathol Vol.5 No.1:1

Introduction

The importance of oxytocin in human behaviour and its effects on stress systems is growing and the research on this topic is increasing every day. If we type "oxytocin and behaviour" in Academic Google, a simple one, for instance, by year, we can found this result: 2015 (3 560 occurrences), 2016 (3 920 occurrences), 2017 (4 260) and 2018 (4 490). And it is not only about the slight increasing year by year, but the cumulative effect, revealing a strong interest in the field. Just an example of a classical area of research involving oxytocin (labour induction, for instance): in 2018 we can found 2 680 occurrences. It can give a picture of the present status in the field.

Many researches about that have been conducted recently [1], and a great part of them use the intranasal oxytocin administration methodology. A single view search on sites over the internet, like Academic Google, PubMed or Sibi-USP, with the words "intranasal oxytocin administration" show us hundreds or even thousands of papers using this methodology: 16.100 to be exact (research made in 02.26.2019). Despite widespread use of the technique, there are still many doubts and uncertainties about how oxytocin reaches the brain and other issues [2-4].

At first sight, one can think that there's little space for doubts here because the nasal oxytocin administration will increase the oxytocin blood levels and blood goes everywhere in the body.

To the brain too and, *ergo*, once there, starts its action as a neurotransmitter. And since there's a lot of blood in the brain, a lot of it carries oxytocin and the drug will finish its journey in some oxytocin receptor. Case closed! But, like other things in life, nothing is that simple.

The reason for this is that the brain is an organ that requests a special environment to work and because its function of central processing and coordinating functions, needs special care [5]. And we have to think, also, in the protection against toxic molecules or pathological organisms [5]. For these reasons, our brains have to have a particular control over the movement of particles, particularly in the "in" direction. These barriers and its possible effects over the oxytocin nasally administered is the focus of this paper. We hope our efforts will be useful.

The Three Barriers

The simple line of thinking "diffusion and get the effect" is not that simple because of the three barriers [6,7]. These barriers keep the brain safe and in optimal conditions to work.

These three walls are:

- a) arachnoid blood-cerebrospinal fluid;

- b) Blood-Cerebrospinal Fluid Barrier (Blood-CSF) and
- c) is the blood-brain barrier, (BBB, for short)

First wall – meninges

This is a physical barrier.

There are three layers covering and protecting the central nervous system (CNS) from chemicals, pathogens and mechanical treats: dura mater, arachnoid and pia mater [6]. We can see the anatomical representation of these meningeal layers with its spaces in **Figure 1**.

As we can see in [8], the outermost layer is dura mater, lying directly underneath the bones of the skull and vertebral column. Dura mater is thick and resistant, formed by connective tissue very rich in collagen fibres, containing vases and nerves.

The following layer, underneath the dura mater, is named arachnoid. Very delicate membrane, juxtaposed to the dura mater, which is separated by a virtual space, the *subdural space*, containing a small amount of liquid necessary to lubricate the contact surfaces of the two membranes. Arachnoid consists of layers of connective tissue; is avascular and does not receive any innervation. The arachnoid is separated from the pia mater by the subarachnoid space, which contains cerebrospinal fluid [8].

It is also considered as belonging to the arachnoid layer the delicate structures that cross the subarachnoid space to connect to the pia mater (the third layer), and are called *arachnoid trabeculae*. These *trabeculae* resemble, in appearance, like a spider web, hence the name “arachnoid”, due to its similarity to the spider web. Between these *trabeculae*, we found the liquor or cerebrospinal fluid. Liquor (cerebrospinal fluid – CSF, *liquor cerebrospinalis*) is a clear, colourless fluid found in CNS either intracerebrally in the ventricular system of the brain or extracerebrally in the subarachnoid space [8].

In a very interesting revision [9], quoted that the Dyes' work, when injected into the blood, tend to stain dura mater but not the arachnoid, suggesting that the arachnoid, rather than dura mater, prevents entry of blood-borne substances into the subarachnoid space. In the same way, these authors describe the important work of Nabeshima et al., when ultrastructural electron microscopical and freeze-fracture studies described the arachnoid as a multi-layered epithelium with tight junctions between cells of the outer continuous 1-3 cellular layers-the arachnoid barrier cell layer-that forms an effective seal covering the inner dural surface [9].

Once more, the same authors: “the pia and the inner layer of arachnoid comprise one cell type-the leptomeningeal cell-covering the outer-most layer of nervous tissue of the brain, the glia limitans, which is composed of a dense multilayered meshwork of astrocytic processes covered by an outer basement membrane” [9]. So, there is a very effective barrier between the CSF and the nervous tissue itself.

In summary, we have, within dura mater, the vascularization. Underneath this, there is a very tiny space (sub-dural), and after this, we found the arachnoid and the cerebrospinal fluid. After

this “sea” of CSF, there's the pia mater, and only then the nervous tissue. But whatever comes in the blood stops in the arachnoid and will not reach this fluid and the pia mater itself. Our nasal introduced oxytocin have to found another way to get to the brain.

Second wall – blood-cerebrospinal fluid barrier (blood-CSF)

“Liquor”, or cerebrospinal fluid, is produced by the choroid plexus and ventricular ependyma in two steps: It begins when a filtered form of plasma moves from fenestrated capillaries in the choroid plexus into an interstitial space - this fluid then needs to pass through the epithelium cells lining the choroid plexus into the ventricles, an active process requiring the transport of sodium, potassium and chloride that draws water into CSF by creating osmotic pressure [10]. Unlike blood passing from the capillaries into the choroid plexus, the epithelial cells lining the choroid plexus contain tight junctions between cells, which act to prevent most substances flowing freely into CSF [11].

The liquor performs the following functions [8]:

- 1 – Mechanical and supportive: the brain is essentially fully immersed in the cerebrospinal liquid causing a reduction of its real weight (around 1500g) to an equivalent of about 25 g. This mechanism protects the brain against the damage caused by its own weight.
- 2 – Protective: CSF acts as a shock absorber, protects the brain from a sudden pressure or temperature changes and the components of the immune system present in the fluid (leukocytes, immunoglobulins etc.) provide protection against various pathogens as well.
- 3 – Metabolic: liquor helps to maintain the correct composition of the environment surrounding nervous tissue cells (homeostasis). It also partially provides the supply of nutrients and disposal of the metabolic waste products and forms an environment through which diffusion of various signal molecules (like neurotransmitters) takes place.

The blood-cerebrospinal fluid barrier is a fluid-brain barrier that is composed of a pair of membranes that separate blood from CSF and CSF from brain tissue: the blood-CSF boundary at the choroid plexus is a membrane composed of epithelial cells and tight junctions that link them and the brain-CSF boundary is the arachnoid membrane, which envelops the surface of the brain [12].

In other words, the nervous system is sheathed in a “leather jacket” of meninges. Between them, there is a “liquid bumper”, the CSF. This cerebrospinal fluid provides not only protection but also nutrition and signal molecules. Whatever is in the CSF can reach the brain. So our nasal administered oxytocin needs to get to the CSF to reach the brain. But for this to happen, it needs to pass through the third wall. Somehow oxytocin nasally administered has to find a way to pass through these epithelial tight junctions of which the third wall is made.

Third wall – the blood-brain barrier (BBB)

In other common endothelium tissues, there are a lot of fenestrations that allow free traffic between the tissues and blood (Figure 2). It is important because this “all area access” system allows the nutrients to reach the tissues, the immune cells to go all around keeping everything under control and for all debris to be collected. But we know that, in the nervous system, it isn't possible. The neurons have the same necessities of nutrients and disposals collection that other tissues, but that can't be done by an “all area access” system because toxins and pathological agents can use the same fenestrations and reach the noble central system Abbot et al. [13].

Despite the danger, this exchange needs to be done, anyway. And it is at the brain endothelial microvessels that blood-CNS changes occur [14]. As its well-known, capillary walls, in general, allow the passage of liposoluble substances, by fenestrations and by pinocytosis. The walls of the capillaries in the CNS do not have such fenestrations, their tight junctions being without intercellular spaces that allow the passage of molecules: there is no pinocytosis, only transcellular transport by liposoluble (non-hydrophilic) processes, by transmembrane transport and by vesicles, also involving glial astrocytes [14]. It is important to stress that oxytocin is hydrosoluble [15]. Some endocrinologists believe oxytocin has little penetration in BBB: small lipophilic molecules have great penetration into this barrier to cerebrospinal fluid,

whereas oxytocin and vasopressin (whose molecular structure is very similar), are large and hydrophilic, and their transport involves a saturable carrier, which would be a limiting factor, since the half-life of the molecule is 19.1 min [16].

However, there's another possibility: The first to demonstrate that peptides and regulatory proteins can cross the BBB was Abba J. Kastin [17], as well as the mechanisms for such transport. In his work he demonstrated that this passage has not only metabolic function but also informational and functional: peptides and regulatory proteins act as information molecules, signalling peripheral events to the CNS in active and selective transport, enabling signalling and communication, respecting the parameters of protection [17].

But besides that, our nasally administered oxytocin has other possible ways to get in the brain.

Windows in the Blood-Brain Barrier

In 1985 Ermisch, Landgraf and Hess have shown that the encephalon is the source and receptor of signal peptides [18]. And they worked over the question if the peptides loaded by the blood would influence the cerebral functions (the BBB would allow a passage so restricted, in the end, that little or no physiological effect would be noticeable). These same authors [18] respond affirmatively to this question: there are encephalic regions where the capillary barriers would be less efficient, especially in the

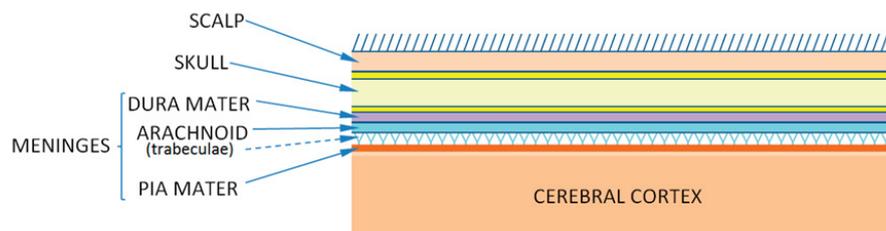


Figure 1 Meningeal layers (based on Machado [8]): after the scalp, we have skull and beneath it the first meningeal layer, dura mater. After a so called “virtual space” we found arachnoid and between this second layer and pia mater, the thinnest layer attached over the cerebral cortex, we have “trabeculae”, a peculiar formation that keep distance between these two layers and is a real space filled with cerebrospinal fluid.

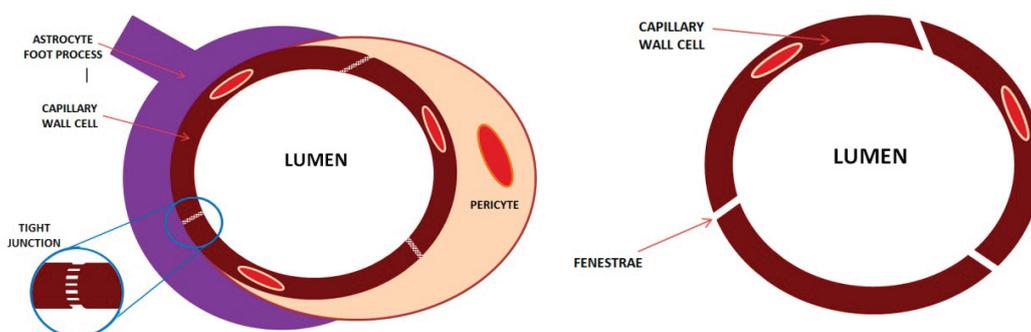


Figure 2 Right CNS capillary, left, peripheral capillary. In the CNS capillary we have a much more selective barrier, with tight junctions between the capillary wall cell (), pericytes and astrocytes that surround them. None of these structures (physical barriers) we can see in the peripheral capillary (base on John Martin [13]).

pineal gland, in the choroid plexus and in the neurohypophysis, where the peptides could reach cellular elements of the tissues by acting on them or by altering the permeability of cells to specific elements (by controlling the physiology of nerve tissue or by providing necessary elements). This “hows” (the mechanisms of transport of peptides in BBB) are very important because of, at least, two reasons: first, to understand how circulating peptides can affect the brain and; second, the great difficulty that some medications have to get access to nervous tissues, which excluded a range of treatments from the list of possible, although feasible, since they are generally water-soluble and therefore excluded from entering the brain by BBB [19].

Adding to this two ways (the BBB allows some informational and regulatory proteins to get to the brain and, in some places, the permeability of this barrier can be changed), we have proper “brain windows” described in several places (next section).

Places the blood-brain barrier does have fenestrations

On the other hand, as we can see in Felten and Józefowicz [19], in the description of the known circumventricular organs, we can find true “brain windows”. In these organs there are no “tight” glued joints of cells of the walls of the vascularization, in the author words: “Devoid of the usual occlusive junctions, in the endothelial appositions, having, instead, fenestrated vascularization. Thus, the circumventricular organs lack a blood-brain barrier”. Below the list of these circumventricular organs (Figure 3):

- a) Vascular organ of lamina terminalis (VOLT)
- b) Median eminence
- c) Neurohypophysis
- d) Pineal gland
- e) subfornical organ

- f) Area postrema
- g) Subcommissural organ

It is important to note that the VOLT, the subfornical organ and the postrema area send fibres to the hypothalamus and other visceral structures and that the fibres of the neurohypophysis itself have wide diffusion in the nervous tissue, besides the median eminence has important control on the hypothalamic releasing factors [20].

It began to be not so strange to us that nasally administered oxytocin can have the results that the literature points out, even influencing behaviours as complex as the social one since there are good possible ways (hypothetically) reach the central nervous system. But, strangely enough, we found no reference to these remarkable circumventricular structures in the discussion of oxytocin and brain barriers in our review.

Peripheral and Central Oxytocin – Some Questions

As said above, nothing is that simple.

Oxytocin is produced in the brain but there is also a peripheral oxytocin production. Not only its effects can be central or peripheral, but also the synthesis can be central or peripheral. Oxytocin can be synthesized in the gastrointestinal tract, heart, testicles, uterus, corpus luteum, placenta, kidneys, pancreas, thymus and adipocytes [21]. There is not much - almost unidentified - information about the significance of oxytocin from these sources on behaviour. And this is very important since the presence of peripheral oxytocin is essential in many experiments, especially those that measure the amount of plasma oxytocin after psychological stimulation, how authors like Barraza and Zak use to do, for instance [22].

What we have is a certain consensus or assumption that the stimulus (whether it is a film, the presence of a loved one or

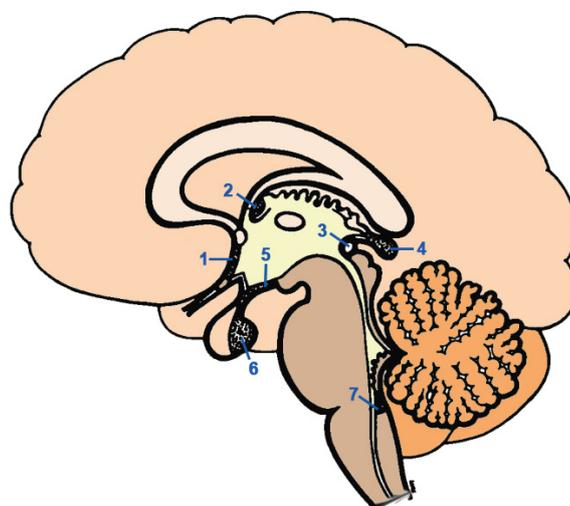


Figure 3 Location of the circumventricular organs: 1 - vascular organ of lamina terminalis; 2 - subfornical organ; 3 - subfornical organ; 4 - pineal gland; 5 - median eminence; 6 – Neurohypophysis; 7 - Area postrema (adapted from Felten DL, Józefowicz RF [19]).

the collaboration in games) causes liberation of oxytocin in the brain, by modifying the activity of the circuits involved in social activities. In addition, it is assumed that plasma oxytocin levels are correlated with their brain levels. It is not so clear to us.

Different Routes? – is it Possible?

This very important question is raised by Churchland and Winkielman [23], that in order to understand the relationship between social stimulus and the plasmatic level of oxytocin, other factors need to be separated: when a human being receives social stimulus it is very likely to cause a reaction in (especially in the heart and intestines), and the heart and intestines are the major contributors to oxytocin plasma levels. In addition, this oxytocin will activate the afferent branches of the vagus nerve and will send signals to the brain [23]. These assertions are confirmed by what we have argued from the polyvagal theory that SNA is influenced by exogenous and endogenous factors [24].

Onaka [25], when studying the central and peripheral neural pathways of oxytocin release during stress, reviewed several studies in which there is a high level of information exchange between blood levels of various substances (such as ghrelin, leptin) that can affect the release of central oxytocin.

It is important to reflect on all this because, as this work and many others use nasal administration of synthetic oxytocin, the access route to the CNS is yet to be clearly determined. Likewise, the way it acts to produce the effects reported in the literature is far from clear and known.

Discussion and More Data

Although the nasal administration is highly convenient because of its non-invasiveness, the question of whether and how this oxytocin supplied exogenously can influence behaviour systems is very relevant and not very clear at the moment. As reported in their review, Churchland and Winkielman [23] found no studies demonstrating how much oxytocin thus introduced into the body affects brain receptors. These authors discuss the work of Born and others, reputed as one of the most cited to support the hypothesis that the oxytocin administered intranasally reaches the brain through the bloodstream via the nasal mucosa [25]. Discussing these paths they point out that it could hardly do so by olfactory bulb neurons, since they would have to actively use the retrograde neuronal pathways and this would take a long time, considering that the observed effect of the intranasal oxytocin is reported in minutes, in addition to running the risk of proteolysis during this retrograde, active path. These authors also point out that the most probable pathway is reaching the ventricular cerebrospinal fluid, in the subarachnoid space and/or via pia mater. As we have seen, endo-encephalically produced oxytocin in the hypothalamus reaches the amygdala and nucleus accumbens. Thus, nasally administered oxytocin would follow the vasopressin pathway described by Born and colleagues [26]:

spray => bloodstream => subarachnoid space => ventricular cerebrospinal fluid => extracellular space => nucleus and neurons

This way could be hypothesized considering, I suppose, the

circumventricular organs. But still, Churchland and Winkielman [23] question about how much of these peptides, such as vasopressin and oxytocin, have to be administered to produce a behavioural effect.

Thus, their findings do not exactly support the protocols used for oxytocin, usually 24 IU. They also comment that often the number of puffs is not mentioned in published works. Besides that, there is also the time between each puff, since an administration followed can cause the excess to flow through the throat instead of being absorbed by the mucosa. For VP, the interval is 30-45 seconds, according to Born and collaborators [26]. It is assumed that the same reasoning is valid for oxytocin, due to the high molecular similarity of the two substances (but it is an assumption, anyway). On the other hand, the exogenous oxytocin may interact with the positive feedback mechanisms that characterize this peptide or, as we have seen, the oxytocin interacts with many other systems, so that one may have an indirect behavioural effect, by action on other systems by summoning different neurotransmitters.

In 2015, Leng and Ludwig [7,27] insist that, in spite of the large number of reports that oxytocin nasal administration has various behavioural effects, very little of what is considered high doses seems to reach the cerebrospinal fluid, recommending great care and attention, since there is a need of appropriate statistical methods and dose-response studies that are not yet conclusive.

We note all the observations, but although the mechanisms of action are not clear, it does not mean *per se* that there is no one or, more serious, that the administration of nasal oxytocin has no behavioural effects, only because we do not know how this procedure produces this effect. Considering the large volume of research with positive results involving oxytocin nasal administration, it seems fairly well established that there are effects, although there are still a number of questions to be answered before these protocols became well established.

We consider, finally, that this gap in our understanding is not an impediment to use the technic. We think that this kind of “methodological maturity” is a thing that comes with time. And this maturation of the field of study is only done with hard work, much research and a large amount of data, which, little by little, are refined and understood. There is extensive literature on the robust support that nasal administration of oxytocin has behavioural effects, which involve stress responses and social situations [3]. The fact that the mechanisms of action are still unclear and the dosage is still not well established does not mean that we cannot or should not proceed with other studies using this technique that, as already indicated, has the facility of being noninvasive, low cost and easy operation. By another side, it should be an excuse to not establishing the protocols.

Supporting this view, we can see the most interesting review of Bethlehem, Honka and Auyeung [28] in which, taking a look at nasal administration studies of oxytocin and fMRI, they found that there is a specific group of social brain regions that are activated with this modality administration of the peptide. And they have also established, based on these studies, that oxytocin has the potential not only to modulate the activity of a group of brain regions, but also the functional connectivity of these

areas. They have also argued that studying the effects of nasal administration on the brain may contribute to our understanding of the neural networks of the social brain.

In spite of these difficulties and the great demand of hard work that is still required in this field, the number of researches with very fruitful results is remarkable, as is also seen in the review of Adam J. Graustella and Colin MacLeod [4]. They begin their presentation bringing the information that the effect of the nasal oxytocin on human social cognition has already been known for 11 years. They find in this study that oxytocin nasal administration improves the detection of social cues and the elaboration and strategic level of processing of this information. Although they also note that there are inconsistencies in several findings in the literature, they attribute such inconsistency to different methods of evaluating social data and ways of understanding different attitudes in the group. We would add to this that such differences in findings could be attributed to the differences between the technics applied because each one activates different behavioural

systems (for example social cues, relationship conflicts, trust games, public speaking and so forth).

Conclusions

In conclusion, the effects of intranasal oxytocin administration are well established. But we have solid barriers on how oxytocin reaches the nervous system simply by diffusion, so the answer to the question “how oxytocin reaches the brain” to do this effect is not an easy one. We have solid data to provide some very strong hypothesis about the ways through oxytocin is carried by blood and can enter the nervous system despite these barriers. These ways can be direct or indirect because the possibility exists that the oxytocin blood levels can activate oxytocin receptors placed in peripheral regions. And, as usual, all hypothesis can be true and have a partial role in the behavioural effect. But, at least at this moment, to our knowledge, this hypothetical waiting for more research.

References

- 1 Lima JPC, Rodrigues AL (2018) Oxytocinergic system: a proposal. *Acta Psychopathol* 4: 14.
- 2 LIMA JPC (2017) Effects of an oxytocin nasal spray over autonomic parameters and cortisol level in model of social stress. Tese de Doutorado, Instituto de Psicologia da Universidade de São Paulo, São Paulo.
- 3 Cardoso Ch, Kingdon D, Ellenbogen MA (2014) A meta-analytic review of the impact of intranasal oxytocin administration on cortisol concentrations during laboratory tasks: moderation by method and mental health. *Psychoneuroendocrinology* 49: 161-170.
- 4 Graustella AJ, Macleo C (2012) A critical review of the influence of oxytocin nasal spray on social cognition in humans: evidence and future directions. *Horm Behav* 61: 410-418.
- 5 Abbott NJ, Friedman A (2012) Overview and introduction: the blood-brain barrier in health and disease. *Epilepsia* 53: 1-6.
- 6 Heldelman Walter (2015) *Atlas of Functional Neuroanatomy*. CRC Press, London.
- 7 Habgood MD, Møllgård K, Dziegielewska KM (2016) The biological significance of brain barrier mechanisms: help or hindrance in drug delivery to the central nervous system? *F1000Research* 5: F1000 Faculty Rev- 313.
- 8 Machado ABM, Haertel LM (2006) *Neuroanatomia funcional*. 3rd Edn. São Paulo: Atheneu.
- 9 Brøchner CB, Holst CB, Møllgård K (2015) Outer brain barriers in rat and human development. *Front Neurosci* 9: 75.
- 10 Sakka L, Coll G, Chazal J (2011) Anatomy and physiology of cerebrospinal fluid. *Eur Ann Otorhinolaryngol Head Neck Dis* 128: 309-316.
- 11 Hall John (2011) *Guyton and Hall textbook of medical physiology* (12th Edn). Philadelphia, Pa: Saunders/Elsevier, p: 749.
- 12 Lathera J, Keep R, Betz LA, Goldstein GW (1999) *Blood-cerebrospinal fluid barrier. basis neurochemistry: molecular, cellular and medical aspects*. 6th Edn. Philadelphia, Lippincott-Raven.
- 13 Martin John (2003) *Neuroanatomy: text and atlas*. 4th Edn. New York, McGraw-Hill.
- 14 Abbott NJ, Patabendige AAK, Dolman DEM, Yusof SR, Begley DJ (2010) Structure and function of the blood-brain barrier. *Neurobiol Dis* 37: 13-25.
- 15 Snell CR, Smyth DG (1977) Biologically active macromolecular forms of oxytocin. [8-Lysine]oxytocin as a suitable ligand. *Biochem J* 165: 43-47.
- 16 McEwen BB (2004) Brain-fluid barriers: relevance for theoretical controversies regarding vasopressin and oxytocin memory research. *Adv Pharmacol* 50: 655-708.
- 17 Banks William A (2015) Peptides and the blood-brain barrier. *Peptides* 72: 16-19.
- 18 Ermisch A, Riihle HJ, Landgraf R, Hess J (1985) Blood-brain barrier and peptides. *J Cereb Blood Flow Metab* 5: 350-357.
- 19 Felten DL, Józefowicz RF (2005) *Atlas de neurociência humana de netter*. Porto Alegre: Ed. ArtMed, P: 328.
- 20 Pardridge William M (2013) Receptor-mediated peptide transport through the blood-brain barrier. *Endocr Rev* 7: 314-330.
- 21 Kiss A, Mikkelsen JD (2005) Oxytocin-anatomy and functional assignments: a minireview. *Endocr Regul* 39: 97-10.
- 22 Barraza JA, Zak PJ (2009) Empathy toward strangers triggers oxytocin release and subsequent generosity. *Ann N Y Acad Sci* 1167: 182-189.
- 23 Churchland PS, Winkielman P (2012) Modulating social behavior with oxytocin: how does it work? What does it mean? oxytocin, vasopressin and social behavior. *Horm Behav* 61: 392-399.
- 24 Porges Stephen W (2011) The polyvagal theory: neurophysiological foundations of emotions, attachment, communication and self-regulation. *J Can Acad Child Adolesc Psychiatry* 21: 313-314.

- 25 Onaka T (2014) Neural pathways controlling central and peripheral oxytocin release during stress. *J Neuroendocrinol* 16: 308-312.
- 26 Born J, Lange T, Kern W, McGregor Gerard P, Bickel U, Fehm Horst L (2002) Sniffing neuropeptides: a transnasal approach to the human brain. *Nat Neurosci* 5: 514-516.
- 27 Leng G, Ludwig M (2016) Intranasal oxytocin: myths and delusions. *Biol Psychiatry* 79: 243-250.
- 28 Bethlehem RAI, Van Honka JAB, Baron-Cohen S (2013) Oxytocin, brain physiology, and functional connectivity: a review of intranasal oxytocin fMRI studies. *Psychoneuroendocrinology* 38: 962-974.