Title: How do hypothalamic nonapeptides shape youth's sociality? A systematic review on oxytocin, vasopressin and human socio-emotional development

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Title: How do hypothalamic nonapeptides shape youth's sociality? A systematic review on oxytocin, vasopressin and human socio-emotional development.

Running title: Nonapeptides and human socio-emotional development

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Highlights

- Little attention has been given to the role of nonapeptides in early human sociability
- Evidence is suggestive of their contribution to different dimensions of normal and pathological socio-affective functioning
- The field may benefit from standardization of procedures and techniques
- Differences between life stages may exist and should be described
- This knowledge may inform drug development in paediatric populations

Abstract

The hypothalamic nonapeptides oxytocin and vasopressin are important modulators of socio-affective behaviours in a wide variety of animal species, including humans. Nevertheless, there is little research addressing their possible roles on socio-affective dimensions of human behaviour across development, during which considerable behavioural and physiological change occurs. Questions still remain about the extent to which findings from adults may directly apply to earlier phases of human development. In this article, we systematically summarize and discuss all existing studies investigating the developmental association of endogenous levels of hypothalamic neuropeptides oxytocin and vasopressin with human social behaviour or on its disruption in paediatric populations. Evidence is sparse insofar as there are still relatively few developmental studies and limited due to correlational research designs and unreliability of methods currently used for neuropeptide measurements in biological fluids. The findings to date generally converge with adult evidence, but also suggest that important differences between age stages may exist. Further studies focusing these differences may prove critical for informing drug development for socio-affective deficits in paediatric populations.

Key-Words: oxytocin; vasopressin; social cognition; paediatric populations; neurodevelopment disorders; autism

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1. INTRODUCTION

Even after decades of research, the two nine-amino-acid hypothalamic neuropeptides, oxytocin (OT) and vasopressin (AVP), continue to capture the attention and fuel scientific curiosity of diverse fields: from neurobiology to the social sciences. Many studies from different areas of knowledge have supported the notion that, along with their well-known functions in labour, breastfeeding and/or water balance, these hypothalamic nonapeptides have evolved as important modulators of socio-affective behaviours in a wide variety of animal species, including humans(1-3). Indeed, a wealth of evidence from animal models' studies has demonstrated their modulation of adult social behaviour, including social recognition(4), maternal behaviour(5), social bonding(6, 7), communication(8) and aggression(9). Joining this evidence, a flood of studies in humans has implicated these neuropeptides in human adult socio-affective behaviour, including, but not limited to, generosity, cooperation, visual attention to social stimuli, regulation of negative affect processing, social learning (for an extensive review see(10, 11)). However, despite popular assumptions that these neuropeptides are critical elements of human social behaviour in youth, including social bonding during infancy, their role in childhood and adolescence - when considerable changes in behaviour and physiology occur(12) - has received considerably less attention from researchers. Most studies reported to date, both in human and non-human animals, have used adult samples.

Social competence in adulthood stems from social learning during development(13, 14). Indeed, sociality is a highly dynamic dimension of human behaviour, characterized by intense changes during psycho-emotional ontogenesis(15): e.g. an increase in social network complexity, the emergence of a social-self, the development of perspective-taking, an increase in the regulation of emotional behaviour, among others(15, 16). These behavioural changes are accompanied by pronounced and prolonged structural modifications in several brain regions implicated in social cognition, namely parts of prefrontal, parietal and superior temporal cortices(16). Although already present in the prenatal developing brain, preclinical evidence suggests that the expression of the receptors of AVP (arginine vasopressin receptor 1A [V1aR]) and OT (oxytocin receptor [OTR]) change quantitatively and topologically during pre- and post-natal development(17-20). Their distribution patterns in the brain depend on age and species, with quantitative levels being modulated by gonadal hormones and experience. Changes in OTR and V1aR expression during pre- and post-natal human brain development seem to exist as well. Data from the Human Brain Transcriptome project (http://hbatlas.org) indicate that, for instance, V1aR mRNA expression peaks in the human neocortex during the second trimester of prenatal development, then slowly decays through the rest of the pre- and post-natal life. Human OTR expression seems to be high at birth and for the first 3 years in the neocortex, with prolonged expression in the medial prefrontal cortex. Less is known about the developmental trajectories of expression for CD38 and Leucyl And Cystinyl Aminopeptidase

(LNPEP), a molecular player involved in OT release(21) and one of the enzymes responsible for both OT and AVP degradation(22). Considering that, at least in the brain, several elements of these nonapeptides' machinery seem to dynamically evolve during the post-natal period, one may speculate these changes are functionally relevant and underpin significant differences between OT/AVP impacts on behaviour during different developmental periods. Unravelling the intricacies behind OT and AVP biology during early-ages and their implications for the development of several socio-affective dimensions of behaviour may represent a critical step toward a better understanding of the role of these peptides play in the regulation of social behaviour and/or its disruption during illness. Moreover, both OT and AVP activity in early-life have a long-term impact on the brain and behaviour(21). A full understanding of the neurobiological mechanisms underpinning social behaviour must, therefore, include a complete and detailed characterization of the biological and neural circuits underlying socio-emotional changes during development.

Studies in young non-human animals have been critical for identifying the causal implications of OT and AVP in social behaviour during infancy and childhood(19). Direct manipulation of OT/AVP and/or their receptors (pharmacological and genetic editing) has an effect on processes involved in early-life sociality, including parental engagement(23), familiarity preferences(24), distress vocalization during separation (25) or social contact between neonates(26). Moreover, in animal models single and chronic manipulations of OT in the perinatal period have affected sex-specific adult sexual preferences and brain neurochemistry (for a comprehensive review of the preclinical evidence in early ages see(19) and (27)). Although few in number, these studies converge to show that exposure to these peptides during critical periods of post-natal development may induce permanent changes in the organization of their signalling machinery in several areas of the brain and in behaviour. Moreover, these changes seem to persist through adult-life(28). Additionally, some studies have shown early experience may itself impact on the development of OT and AVP biology(28).

Although similarities in social behaviour exist between human and non-human animals (including the neonatal, childhood and adolescence periods), human behaviour is naturally intricate and accompanied by some particularities (for instance, while rodent social behaviour strongly relies on olfactory cues, humans mostly rely on visual and auditory cues(29)). Moreover, species-related specificities have been described in terms of hypothalamic nonapeptides physiology(30). As for other animals, a full understanding of the biological mechanisms underpinning human social behaviour must include a complete and detailed characterization of the biological circuits underlying socio-emotional development across the life span during development(31). Building on this assumption, an increasing wave of studies have tried to uncover the role of OT and AVP in socio-emotional behaviour in paediatric ages and examine the extent to which findings in young non-human animals may be applied to

humans (Fig.1). Contributing to the urgency of this field is the fact that one of the most promising proposed applications of these molecules as drug therapies are the neurodevelopmental disorders, such as the autism spectrum disorders, which typically affect paediatric populations(32). A better understanding of the contributions of these peptides to socio-affective processing early in life may be critically important for directing therapeutic advances in these age periods.

Traditionally, human research on hypothalamic nonapeptides relies on 1) acute pharmacological manipulation of OT and AVP through intranasal administration; 2) correlative studies of OT and AVP measurements in peripheral fluids with socio-emotional phenotypes; 3) behavioural and imaging genetic association studies of OT and AVP related polymorphisms with socio-emotional behaviour and its related brain underpinnings. It is not surprising that the intervention profile of the experimentally controlled pharmacological designs positions this approach at the leading edge of research designed to discover the role of hypothalamic nonapeptides in human behaviour. However, the ethical questions associated with pharmacological studies in paediatric ages have precluded its expansion to these ages(33). For this reason, research has mostly relied on attempts to establish associations between measurements of these peptides in peripheral biological fluids and behavioural/brain phenotypes.

In order to facilitate an objective overview of such findings, we systematically summarize and discuss all existing studies that have investigated the role of the hypothalamic neuropeptides OT and AVP in human social behaviour or on its disruption during childhood and adolescence. We understand that there are limitations associated with the peripheral measurements of these peptides used in most of the existing studies (such as the use of peripheral surrogate nonapeptide levels, whose ability to index central hypothalamic nonapeptides has been subject to intense criticism(34) and the uncertainties about how these measures in different biological fluids correlate between each other(35)). We highlight and discuss the potential issues and limitations that may be critical to a comprehensive interpretation of these findings. The reviewed findings are discussed in light of existing animal knowledge and coordinated with the current mechanistic frameworks of OT/AVP roles in human social cognition, affective processing or psychopathology. We hope our review will serve to: 1) summarize suggestive evidence from studies early in life that may inform further intervention studies in paediatric populations; 2) increase awareness of the importance of further research on the role of nonapeptides in emotional-social development during paediatric ages and its importance for translational research; and 3) help researchers identify pertinent questions that may speed advances in the field.

2. METHODS

2.1. Search strategy

We followed the *PRISMA guidelines for systematic reviews* to identify relevant studies for inclusion in the current review (Figure 2). A search in *Medline* was performed to identify all studies examining associations between OT and/or AVP endogenous levels and socio-emotional behaviour and/or psychopathology in infants, children, and/or adolescents using the query: ("oxytocin" OR "vasopressin") AND ("children" OR "infant" OR "adolescents" OR "pre-adolescents" OR "youth") AND ("social" OR "emotional" OR “affective” OR "disorder"). All references in the retrieved articles were also manually checked to detect any previously missed articles. Duplicated studies were removed using ENDNOTE smart group function.

2.2. Selection criteria

Inclusion and exclusion criteria were tailored to obtain all studies, published up to 7th of March of 2017 (irrespective of publication date and subjects’ age, sex or ethnicity), which were original reports on the association between endogenous levels of OT and/or AVP socioemotional behaviour and/or psychopathology. One report published between the end date of our searching and this manuscript revision was included as an additional record. Reports also had to be written in English, available in full-text and published in a peer-reviewed journal. Exclusion criteria were: being a review or meta-analysis, non-English written, non-peer reviewed or a proceedings publication, or animal study, or a registered study’s protocol, or studies with adults, or genetics association studies without assessments of OT/AVP levels or studies not focusing on socio-emotional behaviour or psychopathology.

2.3. Systematic review

For each retrieved study, the following variables were recorded: aim; number, sex and age of participants; methods used for OT/AVP measurements; conditions where biological sampling occurred; instruments/paradigms/stimuli used for evaluating socio-emotional behaviour and/or psychopathology; main findings. For the studies conducted in clinical samples, additional information about the diagnosis and the sample size of cases and controls were also retrieved. For simplicity, core findings are then presented and discussed in 5 separate sections, namely: 1) Studies with infants; 2) Studies with pre-school and school children; 3) Studies with pre-adolescents and adolescents; 4) Studies with children after adverse early-life environment; 4) Studies with clinical populations (Autistic Spectrum Disorders (ASD), and others).

2.4. Quality assessment

Each study was appraised on a 12-quality requirement score list (detailed in Table 1). For this, information was retrieved from the main article or online supplementary material. For

each item, a score of 0–3 was attributed dependent on whether there was strong (3), some (2), little (1) or no evidence (0) that it was adhered to. The sum of the 12 items' scores, divided by the maximum sum applicable to the respective study modality, was then used as an indication of the general quality of the study (low: ≤69%, medium: 70–90%, high: ≥90%). No study was discarded on the basis of a poor general quality score.

3. RESULTS

3.1 Overview

A total of 45 studies (which were published over the past 24 years) were reviewed. From these, 5 studies were conducted with infants; 3 in pre-school and school children; 4 in pre- and adolescents; 3 in post-institutionalized and maltreated children; and 29 in clinical samples, spanning ASD, Attention Deficit Hyperactivity Disorder (ADHD), Obsessive Compulsive Disorder (OCD), depression, anxiety, conduct disorders and Prader-Willi syndrome.

Summarized descriptions of each study core findings are presented in tables 2, 3, 4, 5 and 6.

The methodology and the target population of the identified studies varied considerably (for an overview see Fig 3). The studies' populations included neonates, babies, children, pre- and adolescents but also participant's parents or other caregivers. Moreover, while some studies focused on the application of validated psychological instruments destined to evaluate socioemotional behaviour or psychopathology, others focused on controlled lab-based paradigms or ecological evaluation of child-parents' interactions. There is considerable heterogeneity in methodological approaches across studies: in the time interval spanned by the measurement, in the type of biological fluids chosen for analysing neuropeptides' levels, spanning plasma, urine, saliva and cerebrospinal fluid, in the method used for sample collection and in the assay techniques used for neuropeptide measurements (including the use of both extracted and unextracted samples; ELISA and RIA techniques), which makes absolute comparisons difficult and precludes meta-analytic synthesis of the core findings.

3.2. Quality assessment

There were 13 studies scoring "High", 31 studies scoring "Medium" and 1 study scoring "Low". An overview of the studies' compliance with our criteria is presented in Table 1.

4. DISCUSSION

4.1 General discussion

The majority of studies we review herein were “moderate” quality. The most common factors which resulted in a medium quality rating were as follows: the low effort to include important demographic and neuropsychological variables in data collection and analysis, the poor description of participant’s inclusion and exclusion criteria, or of the study’s a priori hypothesis (including direction of main effects and interactions), the small sample sizes and the poor description of the fluid sampling conditions and neuropeptides’ measurements protocol.

Overall, although increasing over the past years, the studies investigating the role of the hypothalamic nonapeptides OT and AVP in socio-emotional behaviour during childhood and adolescence are still scarce in number. Possible explanations may include the methodological and ethical challenges inherent to the research on paediatric populations(33). A significantly higher focus has been made on OT research, possibly due to well-grounded preclinical evidence supporting its role in motherhood and mother-infant interaction(36), with only a few studies (22% of all reviewed) focusing on AVP. This is surprising given the preclinical research supporting the role of AVP in social cognition during early-life as well. Importantly, such findings suggest that AVP may counteract OT actions in ‘primordial’ social behaviour, such as maternal separation distress vocalizations and early social odour learning(19), e.g. while OT levels correlated positively with increased maternal separation distress, AVP tended to correlate negatively. Some of the current working hypotheses suggest AVP, acting centrally may elevate vigilance and defensiveness, possibly serving in some cases as an antagonist to the effects of OT — and vice-versa. Conversely, OT may have the capacity to reduce fear and calm the sympathetic responses to stressful stimuli, including those associated with social behaviour(37). In adults, the exact relationship between OT and AVP actions in social behaviour is not yet clear (but some studies suggest that both peptides may act on the same direction to promote sociality)(37).

This research has been particularly biased towards ASD (47% of the total studies herein reviewed). This focus may reflect efforts to explore its putative role in the physiopathology of these disorders in order to inform their suggested use as an adjunctive targeted pharmacological treatment (namely in ASD, where the vast majority of preliminary clinical trials of intranasal OT have been performed(38)).

Limitations common to the vast majority of the studies here reviewed derive mainly from: 1) the use of correlational designs in all studies, which limits the possibility to establish causality and reduces the impact of the evidence gathered(39); 2) the use of peripheral surrogate nonapeptide levels, whose ability to index central hypothalamic nonapeptides has been subject to intense criticism(34); and 3) the use of methods of sample processing and

peptide quantifications whose specificity remains under debate (namely concerning the use of extracted vs unextracted samples and/or the use of ELISA vs RIA techniques)(35) – which is discussed below; 3) the small sample sizes recruited for the bulk of the studies, which raises important questions about the actual statistical power achieved (particularly when the reliability of these measures is as yet unknown).

Peripheral concentrations of OT and AVP (in plasma, urine and saliva) have been used as a proxy for central concentrations. However, the validity of this approach has not been demonstrated. A recent systematic search and meta-analysis of correlations between central and peripheral OT concentrations found a modest positive association. However, this association seem to be restricted to measurements after intranasal OT administration or after experimentally induced stress, i.e. not between baseline levels(34). Regarding the inference of central nonapeptide levels, we note that OT and AVP release in deep brain tissue may not accurately be reflected even in the measured levels at the CSF (and the same applies for peripheral measurements such as blood, saliva or urine). In contrast with more primitive species where OT and AVP transmission derives mainly from diffusion through the CSF in the ventricular system, a controlled axonal synaptic release of these neuropeptides has been described in higher species (for instance rodents) and is likely applicable to the human brain(27). In fact, some studies suggest now that, the release of central and peripheral OT may be dissociated and involve different signalling mechanisms(40) and/or sub-anatomical pathways(41). At this point in time, it is not clear whether saliva, plasma and urine levels of OT do really correlate positively with each other(34).

It is also unclear whether the potential biological interpretations of these measures in each of these fluids might be and which potential mechanisms link them to behaviour or brain function. In fact, their use has been grounded on the assumption they may indeed reflect inter-individual differences in OT/AVP physiology, namely their secretion and signalling in the brain. A recent study aiming to biologically validate OT measurements in saliva and urine in primates suggested that 1) urinary OT measurements may be suitable for establishing baseline levels, as they may represent the build-up of the previous day's concentrations, and 2) salivary OT measurements may be suitable for assessing changes following specific events(42). It is possible that similar conclusions might be observed for humans, but current evidence is insufficient to support such conclusions. Additionally, if this is the case, it is also not clear which fluid may better index, for example, OT/AVP secretion or in which fluid inter-individual differences in these measures may be more strongly affected by differences in metabolism. For instance, while the acidic environment of urine may help to preserve these small peptides, the presence of degrading enzymes in blood are likely to promptly metabolize them(43).These aspects pose important limitations to the critical interpretation of bio-behavioural studies such as those discussed in the current review. A comprehensive evaluation of the validity of these measures, including an assessment of their temporal reliability and the ability of single-

measurements to represent individual nonapeptides' basal physiology, is sorely needed. As soon as this information can be gathered, a richer and complete analysis of the findings already achieved will be possible.

The validity of the techniques for quantifying neuropeptides has been one of the most pressing questions in the field, since accurate determination of OT levels is crucial to motivate, support, and inform research (particularly in paediatric ages) and clinical interventions(35). We stress that, for now, no simple answer to this question exists. Some studies suggest the use of unextracted samples lack reliability and likely target other molecules than OT, namely degraded forms of the peptide lacking functional relevance(44) and other recent studies applying a standard proteomic method of peptide identification and quantification, mass spectrometry, suggest that biological fluid extraction may discard a significant proportion of the total fraction of OT, corresponding to the amount of peptide linked to large proteins(45). If so, aspects such as the biological significance of bound OT, as well as the importance of the exchange rate between bound and unbound peptide, should be clarified. One study also suggested that RIA quantification may not be sensitive enough to detect OT concentrations both in extracted and non-extracted plasma samples(44), raising concerns about the common assumption that this method should be used as gold standard. More recently, the liquid-chromatography mass spectrometry approach has been apointed as a potential standard method for peptide measurement in biological fluids (45, 46). Despite this recent recommendation, none of the most recent reviewed studies made use of this technique.

We cannot exclude the possibility that, similar to the results observed for intranasal OT research, significant publication bias may exist. This has been a prominent question in the field and effort has been recently made to minimize reporting bias through an intense incentive to pre-register studies. We note that despite of this current intense discussion, none of the reviewed studies have implemented this procedure.

Bearing these limitations in mind, below we separately discuss the studies here reviewed, contextualizing the findings achieved in terms of the current theories evoked to explain the role of OT and AVP in socio-emotional behaviour and establishing, as much as possible, the parallel with preclinical studies addressing similar questions.

4.2 Infants

Overall, the studies reviewed in this section are generally compatible with the notion that already in the first months of life, the OTergic system is: 1) active in human infants; 2) measurable; and 3) reacts to social contact in a predictable way. Moreover, the findings achieved support current theories suggesting that social engagement during the first months of life may be supported by the OTergic system, which may drive affiliative and attachment

behaviours in early social life(47). Indeed, the infant OT system seems to react to episodes of interaction with parents(48); remarkably, OT variations are correlated between infants and fathers and predict parent-child behavioural synchrony(48). This relates well with behavioural and neuroimaging inter-subject correlation studies in adult males, suggesting OT increases inter-personal social coordination via increasing the synchrony of brain activity(49, 50)). These observations are consistent with animal research showing that OT (versus an OT antagonist) administration increases social contact cohesion among dam and pups(51). At the same time, they also align with recent findings, suggesting OT may participate in interspecific attachment behaviour, such as those observed between domestic dogs and their human owners (52, 53).

Children with higher CSF OT levels appear to more actively seek parental social interaction for soothing and have a greater interest in social interaction as measured at 6 months of age(54). The only exception to these working hypotheses resides in one study developed in hospitalized premature infants which reported a negative correlation between infant OT levels and social engagement(55). While discordant with all other findings of this section, one should not forget that this study targeted a special infant population characterized as clinically concerning and submitted to high physiological demands to maintain organic homeostasis. In fact, social engagement was mainly quantified during breastfeeding and it is a possible scenario that the premature infants have blocked any extraneous stimuli, such as stimuli from maternal social engagement behaviours, in an effort to maintain physiologic equilibrium during feeding(56, 57). Apparent mixed results regarding the association of OT levels with infant crying are presented as well. While in infants submitted to maternal massage, lower OT levels were associated with increased crying(58), in another study, higher levels of OT were associated with higher crying when parents were not present(54). We speculate that the critical contextual aspect justifying these differences is the parents' presence and availability. Previous animal research showed that central administration of OT reduces the frequency of isolation-induced distress vocalizations in pups and chicks(59). It is reasonable to hypothesize that, when parents are in the vicinity and readily available, the release of higher amounts of OT – possibly naturally evoked by the parents' presence - may happen to reduce stress and the amount of crying (assuming that crying represents a distress vocalization), functioning then as a social buffer. This hypothesis is in agreement with current theories evoked to explain the stress attenuating effects of intranasal OT when a conspecific is present, in adults(60). Conversely, when parents are not available, release of OT may occur to promote parental seeking, expressed through crying vocalization, acting, thus, as a motivational system designed to reduce stress through affiliation. This theory is supported by evidence in adults, suggesting that stress may increase OT release and may constitute a physiological fingerprint of the "tend-and-befriend" strategy of coping with stress(61, 62). The mechanisms responsible for parental detection that may be relevant to activate the OT system remain however to clarify. One may speculate during this early stage affective touch and C-tactile stimulation in the skin may be particularly relevant. Indeed, previous research has shown that low intensity, non-noxious, stimulation of cutaneous somatosensory nerves triggers OT release and is

associated with increased social motivation, plus reduced physiological and behavioural reactivity to stressors(63, 64).

The two studies examining correlations between children and parents' OT responses converge to support the hypothesis that OT physiology may be transmitted cross-generation between parents and children, probably via explicit parental behaviour(48). One of these studies also demonstrated that intranasal OT intervention in the father is accompanied by increased OT infant's responses to parent-child interaction(65). OT-driven parental responses may, thus, interact with other contextual factors to shape infants' OTergic responses to social stimuli(48).

We also highlight that the only study comparing male and female infants' levels of baseline salivary OT did not find any significant differences (48). This study aligns with some adult studies aiming to inspect if nonapeptides levels measured in different biological fluids may vary between males and females and from where no convincing evidence could be gathered so far. However, no study to date has thoroughly compared plasma, CSF or urine levels of OT or AVP between sexes during infancy. Sex differences in OTergic tonus, as well as its potential involvement in sexual dimorphic infants' socio-emotional behaviour, remain thus to be tested.

Studies examining relationships between AVP and socio-emotional behaviour at these neurodevelopmental stages were non-existent.

4.3 Preschool and school children

Despite heterogeneity in the aspects of the socio-emotional behaviour that were targeted, the reviewed studies with children at pre-school and school ages were very much in agreement to support a positive association of OT with: 1) positive social relationships and engagement both with parents(66); 2) generosity(67); and 3) visual attention to social cues(68). Indeed, salivary OT was found to be associated with child best-friend social reciprocity, defined as give-and-receive interactions, adaptation to each other's needs, communications, requests and engagement in shared activity(66). This result is congruent with research in rhesus monkeys describing an association between central OT levels at 18 months and the expression of affiliative social behaviour with peers including allo-grooming, sitting in contact, clinging, and touching(69). Nevertheless, one study examining the association between salivary OT and generosity at these ages suggested that, similar to observations of adult monkeys(70), some degree of sexual dimorphism may exist regarding, at least, the association between OT and generosity: the association between OT and generosity tends to be stronger for girls than boys(67). Thus separate analyses by sex are needed to more fully characterize the role of OT socio-emotional behaviour and related neurocorrelates during childhood and adolescence. The results (at least for girls) are generically consistent with current theories, suggesting that OT may be involved in human in-group favouritism(71-73), an association that seems to be present already during childhood.

Regarding attention to social cues, a positive and a negative association between salivary OT levels and fixation time in the eyes or in the mouth during exploration of a human face, respectively, was found in one study(68). This observation is much in agreement with pharmacological studies of OT in healthy and ASD adults showing that OT potentiation increases visual fixation, facial element critical for social interaction and communication, for example, mutual gaze(74). These findings support one of the most cited theories to explain OT's actions in social cognition, the social salience hypothesis, which suggests that OT may have evolved to amplify the salience of social cues(75).

We identified one study that reported a decrease in salivary(68) OT levels with age, in childhood. We were not able to find any studies examining relationships between AVP and socio-emotional behaviour during infancy of childhood.

4.4 Pre-adolescents and adolescents

The four studies reviewed in this section were mostly developed to examine associations between endogenous OT and negative affect regulation(61, 76-79) through social physical comfort. Relevant observations included an increase in urinary OT in children/pre-adolescents who are comforted by their mothers with direct physical contact after a stressful test situation. This result is in accordance with animal research showing skin-to-skin contact between mother and pup are positively correlated with the pups' hypothalamic OT concentrations(80). However, the fact that OT levels did not change overall for children who rested alone or received no maternal comforting following the stressor is in contrast with findings in adults, that suggest physical and psycho-social stress per se may elicit OT release(62). In terms of mode of comforting, OT increase was similar in physical-tactile and voice-only comforting(76), as seen in other mammals, but was not observed when "contact" was assessed as instant messaging(78). This suggests cognitive perception of a comforting message is not enough to elicit OT release. Another study in adolescents replicated the effect on OT when comfort comes from parents but not when from best-friends (particularly for males); contrarily to expectation, these results suggest that friends do not take over the parental social buffering role by age 15-16, at least for males(61). Although a single and not yet replicated finding, this study suggest that parental support is critical to regulate OT's response to stress, even in adolescence, when peers have increasingly salient roles in social life. Specific parental support effects in stress-induced OT release may, therefore, represent a conserved biological mechanism, evident early in life, by which parental attention and contact may help to regulate negative affect years later (at least until adolescence). One study found that CSF and plasma OT correlate negatively with parental reports of anxiety, supporting current theories that suggest OT may reduce anxiety and thereby influence social behaviour. Interestingly, this study reported that although CSF OT's concentrations are significantly higher than in plasma, they are positively correlated at a medium effect size(79). While further replication studies are needed to confirm the utility of plasma measurements as surrogates of CSF OT concentrations, for now there is no reason to believe that, at least at these age

periods, the same inconsistencies found in adults regarding the associations between peripheral and CSF measurements may apply. Further studies examining this question will be welcomed.

Physiology-wise, one study reported a decrease in urinary(61) OT levels with age, in adolescents when compared to pre-adolescents. Moreover, the same study reported lower levels of urinary OT in males than in females. This observation supports the idea that there may be significant sex differences in OT baseline at multiple points in development. OT has been associated with more prosocial, passive coping strategies in the face of stress, which some authors suggest may be directly related to sex differences in coping strategies between men and women (e.g., tend and befriend vs. fight or flight)(81). This notion is partially supported in this study by the finding that while urinary OT levels decreased in face of a stressful challenge in boys when they were paired with friends, this effect was not observed in females. This observation suggests that OT may indeed have a major role in female regulation of negative affect through affiliative bonds. However, we should not lose sight of the fact that consistent evidence of significant differences on OT production and signalling in adults have not been reported to date(82).

Studies examining a relationship between AVP and socio-emotional behaviour at these age stages were not found.

4.5 Adverse early-life environment

The four studies reprised in this section converge to show that early-life environment, in the form of children's institutionalization, maltreatment or parental depression/distress, influences hypothalamic nonapeptides's physiology for children. Reports include basal and stimuli-induced atypical responses of both OT and AVP systems. One study showed that, even for similar baseline levels, adopted children that had been neglected in orphanages had lower urinary OT levels after interaction with their adoptive mothers than family-reared children. In contrast, while adopted children presented lower levels of basal AVP, these children did not differ from controls in social interaction-induced variation AVP(83). These results are congruent with animal research showing that naturally occurring variations in quality of maternal care are associated with OT functioning in the youth(80, 84) or that early deprivation of contact with the mother in primates and rodents caused reduction of central OT levels across development(85-87).

Complementing these findings, mother's chronic depression and father's distress were concomitantly associated with a decrease in salivary OT both for children and parents(88). These findings are consistent with the hypothesis OT physiology can be transmitted across generations and may reflect a decrease in explicit parental affiliative behaviours during depression. Overall, this study highlights the importance of early-intervention during parental depression in order to prevent long-term effects on children's nonapeptides physiology. Potential therapeutic strategies may include intranasal OT augmentation for the parents.

Additionally, preclinical studies suggested that OT augmentation during adulthood in animals previously subjected to neglect in early life may remediate some of the socio-affective deficits present in these animals(89). Similar studies in humans are lacking, data from such studies may help pave the way for targeted therapies aiming at improving socio-affective functioning after early adverse experience. Interestingly, and contrary findings came from two studies showing that increased salivary OT was found both in: 1) children reared in settled environments after an early-period of maltreatment (interestingly, not observed in those reared in unsettled environments) (90); and 2) girls who have experienced early childhood maltreatment, including an atypically high OT response to a social stressor(77). These findings are compatible with a series of studies showing increased OT responses in female prairie voles after an acute stressor(91). In humans, salivary OT also increased in response to physical or psychosocial stressors, for both females and males(62). Current theories suggest that mammals deal with stressful experiences by seeking social support, which seems to be especially adaptive for females(92, 93). Nevertheless, we note that the collective analysis of the studies published until now does not permit drawing clear inferences about the effects of early care neglect on responsiveness of the OT system during adulthood. In fact, while sexual dimorphic response to early life stress may underpin the apparent disparate findings presented in the studies included in this section, it is not yet clear whether early life stress results in an attenuation or in an over-activation of OT secretion - both chronic or acute after a stressful event - and if these effects are significantly different between males and females.

While attenuation as an outcome would be easily reconciled with the increased incidence of social deficits observed in these populations, over-activation could reflect a compensatory mechanism circumventing an epigenetic attenuation of signalling at the receptors level. Indeed, recent work has demonstrated that low maternal care is associated with increased OT receptor promoter methylation levels, a process which typically represses gene expression(94). Consistent with these findings, another recent study reported a decrease in the expression of OT receptor in peripheral blood cells of mothers maltreated during childhood, although no differences could be identified in baseline plasmatic OT between maltreated and non-maltreated individuals(95). For maltreated mothers, expression of OT receptors correlated negatively with Childhood Trauma scores(95). Dynamic changes in OT receptor methylation in response to psychosocial stress have been reported as well(96, 97). The mechanisms behind these associations between early maternal care and increased OT receptor methylation/decreased receptor expression in adulthood remain to be determined. It is possible that persistent elevation of stress-related molecules associated with maternal care deprivation induce persistent epigenetic phenomena regulating nonapeptides' genes that may act to shape OT responses later in life. Further studies will be critical to illuminate these questions and prompt new targeted early-interventions to prevent/minimize deleterious effects of early care neglect on children's functioning.

4.6 Clinical samples

4.6.1 Autism spectrum disorders

There is mixed evidence regarding the hypothesis of nonapeptide deficiencies in ASD, even though it tends to be supportive. While 8 studies support the hypothesis that ASD patients present lower levels of OT than typically developing controls, no difference was seen in 5 studies, and 2 others found the opposite association. Studies diverge considerably in the types of biological sample used to assess nonapeptide levels, conditions of assessment (namely in respect to the time of the day when sample was collected, the presence of fasting), the approach used for sample collection (that is, the use of RIA vs ELISA), and extracted vs unextracted samples. All of these factors have been shown to affect OT and AVP measurements and may, at least partially, explain the disparate results reported (35). Also, in at least one study reporting no difference, the sample size is quite small, which may have limited power to detect small but meanignful differences. Nevertheless, the findings in humans seem to reproduce those that have been reported regarding the OT system in animal models of ASD (genetic, pharmacological or environmental), insofar as studies using different models have diverged between increases, decreases or absence of alterations in OT or its receptor expression in several regions of the rodent brain (such as the amygdala, hippocampus, lateral septum or the cortex)(98). For an extensive exploration of these studies, readers are referred to a recent review on this preclinical evidence(98).

OT is typically synthesized as a prohormone that is sequentially processed to peptides in a chain of enzymatic hydrolysis reactions, whose final step is catalysed by the peptidylglycine alpha-amidating monooxygenase (PAM)(99). These peptides are the bioactive amidated form (OT) and the C-terminal extended peptides, OT-Gly, OT-Gly-Lys and OT-Gly-Lys-Arg, collectively designated as OT-X(99). Interestingly, one study reporting decreased levels of OT in ASD patients extended its approach to quantify OT's C-terminal peptides and found them to be increased(100). Specifically, OT-X/OT was found to be elevated significantly in ASD patients. This study suggests that disruption of OT's peptide processing may be involved in its dysregulation during ASD. To date, alterations in PAM expression and/or activity during ASD remain to be described. Moreover, no study to date has inspected the contribution of PAM genetic variability to ASD liability. Further studies pursuing these questions may help to more fully characterize the role of peptide processing disruption in ASD and bring new insights on the molecular mechanisms underlying these disorders.

Regarding AVP, 4 studies failed to find significant associations between its levels and ASD diagnosis, with only 1 study reporting lower plasmatic and salivary AVP levels in ASD. Although limited by methodological constraints related to peptide measurements, a joint analysis of the available data suggests this picture may be more complex than previously thought, due to the fact that ASD represents a heterogeneous group of disorders, which makes diagnosis and treatment a substantial challenge(101). We suggest that inconsistencies in the ASD nonapeptides literature may reflect some of this diagnostic heterogeneity and that further studies evaluating the extent to which nonapeptide dysregulation may represent an

endophenotype associated with specific ASD subtypes will be particularly useful. These studies should be very valuable in this early-phase of clinical trials of OT augmentation therapy in ASD, because it is possible that only a subset of ASD patients with OT's deficits may really benefit from this intervention(102). We note that in one of the studies reporting higher OT plasma levels in ASD cases, alterations in the OT signalling cascade could be observed as well. It remains unclear whether this elevation in OT levels is truly associated with an effective increase in OT tonus or to some other step in the signalling cascade. It is possible also that elevations of OT levels may correspond to a compensatory response to alterations in receptor signalling observed in some ASD patients, namely its repression. In fact, several DNA methylation sites (CpG dinucleotides) of the OT receptor gene show significant increases in their degree of methylation in peripheral blood cells and temporal cortex samples of ASD patients, when compared to controls(103). Evidence supporting similar alterations for OT, AVP or AVP receptors is not yet available. Further studies combining concomitant assessments of nonapeptides' baseline and evoked responses, as well as epigenetic analysis of the genes involved in their signalling cascade in the same experimental setup may help to address this question and provide a more complete picture regarding nonapeptides signalling for individuals with ASD. Importantly, even in the studies where no appreciable differences in OT levels were observed between ASD and control groups, OT and AVP levels were found to correlate with autistic symptomology. Moreover, several measures of social function, such as theory of mind or social communication were significantly correlated for both ASD and healthy participants (although these associations were not replicated in all studies). We speculate that even if peripheral OT/AVP levels do not have diagnostic or physiopathological relevance, they may represent clinically useful prognostic biomarkers of social functioning in ASD patients.

We also stress that although some evidence suggests OT baseline levels are lower in ASD patients, their OT system seems to respond to social interaction similarly to control subjects(104), which argues against the possibility of the complete loss of OT responsiveness in ASD. Evidence supporting this conclusion is scarce and replications are needed, nevertheless it opens interesting windows of opportunity for therapeutic strategies. For instance, once sustained, social-based psychotherapy training may retune the OT system to normal baseline functioning without the need for a chronic drug therapy. Further studies and clinical trials exploring this possibility seem timely and warranted. From the studies reviewed here, only one apparent exception emerged: OT secretion in response to a hydrocortisone (a typical stress hormone) was not observed for the ASD sample, although present in controls(105). Although this is a single study and it requires replication, it suggests the possibility that specific impairments in stress-mediated OT release may be present in ASD.

Evidence suggesting that, even if production and secretion of OT is not altered in at least some ASD patients, significant impairment of signalling can occur, came from a single study reporting that, while in controls salivary OT correlated with visual attention to social stimuli, this correlation was not observed in ASD patients (even when OT levels were similar

between groups) (59). This hypothesis is further supported by the observation that OT receptor expression may be decreased in the brain of ASD patients(106).

Another point deserving mention refers to the putative sexual dimorphism present in the nonapeptide involvement in ASD. Indeed, one study reported that OT deficits may be specific of ASD males, although another study comparing both sexes has failed to find differences. The latter study also reported that while higher OT levels were associated with higher anxiety in ASD girls, the same association was not found in ASD boys. Moreover, AVP, which was positively associated with restricted and repetitive behaviours in girls with ASD, was negatively (but non-significantly) associated with these behaviours in boys with ASD(107). We speculate that, similarly to that observed for healthy adults, OTergic modulation of socio-emotional behaviour in youth with ASD may present sexual dimorphism and, thus, findings obtained from male samples should not be presumed to be extrapolatable for females. Both-sex studies are necessary in order to fully clarify the role of OT in ASD physiopathology and its potential as a drug therapy, even acknowledging the higher prevalence of ASD in males(108). Alterations in the age-changes of OT secretion were reported in ASD as well, raising new hypothesis about a possible OT system maturation disruption in this disorder(109).

4.6.2 Others (ADHD, OCD, Depression, Anxiety, Conduct problems and Prader-Willi syndrome)

Expanding the focus from ASD to other clinical samples, 8 studies were conducted to examine the association between CSF, plasma or salivary OT/AVP concentrations and ADHD, OCD, Depression, Anxiety, Conduct problems or Prader-Willi syndrome's diagnosis and symptom-related dimensions. Remarkable findings include: 1) an association between AVP and OT and symptomatic dimensions for individuals diagnosed with OCD, including concomitant depressive and anxiety symptoms(110); 2) a decrease in OT levels in ADHD patients and its association with symptom-related dimensions, such as impulsivity, and general social cognition, including empathic abilities(111, 112); 3) an exacerbation of OT responses to child-mother interactions for children diagnosed with separation anxiety disorder(113); 4) elevations of OT during adolescent treatment-resistant depression, although no significant association with depressive symptoms were identified(114); 5) a negative association between salivary OT and callous-unemotional traits for cases diagnosed with conduct disorder(115); 6) an elevation of plasma levels of OT for PWS cases(116), although in apparent contradiction with recent studies where positive effects of OT augmentation on anxiety, social function and appetite drive were reported for PWS children(117).

While significant associations between levels of hypothalamic nonapeptides and domains of pathological social cognition, such as social anxiety or callous-unemotional traits, are consistent with prior research findings, their association with domains such as impulsivity or obsessive-compulsive phenomena are surprising and may reflect roles of these neuropeptides not previously considered by researchers. Indeed, impulsivity and obsessive-

compulsive symptoms have been associated mainly with imbalances of dopamine and serotonin(118, 119). However, we note that several interactions between the OT and the dopamine or the serotonin systems have been demonstrated in preclinical studies using animal models and are likely to exist in the human brain as well(120, 121). We cannot, thus, exclude the possibility that effects of OT/AVP on other neurochemical systems might mediate the proposed actions of these neuropeptides on cognitive processes not involving direct social processing. The links between OT and feeding behaviour constitutes an emergent field of research that receives support from preclinical studies using animal models that suggest OT may present anorexinogenic properties - limiting the intake of palatable food(122, 123). These findings are not easily reconciled with the apparent increase of OT observed in PWS patients, where hyperphagia has been a pivotal clinical marker of the disease(124) . However, previous studies reported a decrease in OTR gene expression and density in the hypothalamic paraventricular nucleus of PWS males. It may be that this increase in the release of OT to the periphery may thus represent a feedback response to decreased OTR signalling attempting to maintain tonic signalling in homeostatic levels.

Overall, one should note the evidence obtained for neuropsychiatric clinical populations does not permit drawing definitive conclusions about the extent to which observations of altered levels of these peptides in biological fluids may be directly implicated in the genesis of aberrant behaviour or if they might represent a general defensive signal of the body to disease. Indeed, the observation of several deficits relevant to human neurodevelopmental disorders in OT genes using animal knockouts support the hypothesis of links between the OT-system and the pathogenesis of social deficits(125, 126), characteristic of disorders such as ASD. However, cumulative evidence over the last years has been supporting the notion that OT may be an important higher regulator of the immune function, both in the CNS (through microglia activity regulation(127)) and in the body's periphery (promoting thymus and bone marrow development, that strengthens immune defence and maintains immune homeostasis)(128, 129). Thus, we should not exclude that general physiological responses of the OT system to disease may cause variations of its levels in bodily fluids that may not necessarily be related to socio-affective impairment (but instead reflect other aspects of OT's physiology).

5. CONCLUSION

An increasing number of studies exploring the association between endogenous OT/AVP and socio-emotional behaviour or its disruption during neuropsychiatric /neurodevelopmental disorder in youth have been conducted over the last 24 years. Studies are limited by their correlational designs, constraints relating to the reliability of the methodologies currently used for quantifying nonapeptides in biological fluids, and uncertainties relating to the validity of peripheral measurements as proxies of central OT release. Each of these issues limit the possibility of drawing definitive conclusions based on

the evidence presented. Despite this, the accumulating evidence is generally suggestive of a of nonapeptide(s) involvement in socio-emotional functioning and its disruption during disease. Similarly to findings observed for adults, disparities relating to unreplicated or opposite findings in independent studies have been observed in paediatric populations. Moreover, context-dependent and sexually dimorphic effects have been reported as well (see BOX1). These studies have also provided preliminary hints concerning peculiarities of the hypothalamic nonapeptide(s) physiology during the different age stages (which may be particularly informative since in post-natal developmental periods prior to complete sexual maturation, adult studies may indeed be less representative). Although the core findings tend to converge with evidence coming from adults, they also suggest that important differences between age stages may exist: for instance, while in adult males, OT has been associated with trust and generosity to in-group members(130), the only study conducted to date with a similar design in a paediatric sample found the opposite association(67). Interestingly, opposite findings to those observed in adult samples have been reported in young and adolescent animal studies with direct OT pharmacological manipulation as well – for instance, an impairment in the classical pair-bonding observed in male voles when treated with low-dose OT between weaning and sexual maturity(27). While preliminary, these findings support the idea that dynamic changes in the modulation of social and affective processing during development by hypothalamic nonapeptides may exist, which challenges the generalizability of findings from adult samples. However, considering the absence of neurophysiological measures in the vast majority of the studies we included here, this review is limited in the insights it may provide regarding the neurobiological circuits underpinning the contribution of nonapeptides to behaviour at these ages.

Paediatric neurodevelopmental disorders constitute one of the most promising translational applications of intranasal OT. This field will benefit from standardizing the methods used for measurement and reporting on hypothalamic nonapeptides to facilitate quantitative synthesis. Additionally, the field would benefit from a careful examination of the extent to which OT measurements in peripheral fluids may serve as surrogates for OT brain physiology. From that point of view, the advent of studies exploring and validating mass-spectrometry quantification of neuropeptides in biological fluids, a non-biased approach not depending on antibody specificity, will be particularly useful. Also, even though there are ethical and logistic constraints, ideally, future studies using both animal and human participants in paediatric ages should increase their reliance on controlled and standardized direct manipulations of nonapeptide levels in pharmacological designs, overcoming some of the criticisms surrounding OT/AVP probing in peripheral fluids.

Several questions concerning the role of nonaneptides in social cognition and affective processing in paediatric neurodevelopmental/neuropsychiatric health and pathology remain to be explored (we propose some in BOX2). As methodological and ethical constraints are dealt with, inquisitive neuroscientists, children and adolescent psychiatric care providers and, specially, young patients will stand to gain.

Conflict of interest

The authors declare no conflict of interest.

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BOX1: Highlighted suggestions from current evidence

1) OT is putatively implicated in **infant-parental attachment and reciprocal behaviour;**

2) OT is putatively implicated in **prosocial behaviour and social engagement** with both **peers and parents** during childhood;

3) There is suggestive **cross-generation transmission** of OT physiological pathways;

4) OT is putatively implicated in parental, more than peer, support for **social buffering of stress responses** (highlighting the importance of **physical cues** – touch or vocal), during childhood and adolescence;

5) OT is putatively involved in the regulation of **visual attention to social relevant cues**, such as the eye's region of a human face;

6) **Early life adversity** putatively affects youth's nonapeptidergic responses (basal and evoked), although the direction and the adaptive vs pathological value of these effects remain to clarify;

7) **OT and AVP secretion and/or processing is putatively impaired** in **ASD**;

8) ASD patients, in which lower levels of OT were observed, seem to **conserve functional OT secretion systems during social interaction**;

9) **Non-invasive measurements of nonapeptides in peripheral biological fluids may index some aspects of social cognition**, such as theory of mind or social communication, even in patients where dysregulation of these peptides was not observed;

10) **Dysregulation of both OT and AVP systems may exist in a bundle of disorders other than ASD**, involving primarily social symptoms but also other psychopathological dimensions such as impulsivity or obsessive-compulsive symptoms.

BOX 2: Questions for future research

1. What are the **biological mechanisms** involved in OT's and AVP's regulation of socio-emotional behaviour early in human life? What **brain areas** or **neurochemical systems** are involved in their modulatory role?

2. Are there significant **differences between adult and paediatric populations** regarding the modulatory role of hypothalamic nonapeptides in socio-emotional processing? What aspects are different? Can the results from adults be directly extrapolated to children and adolescents?

3. Are there significant **sex differences** between males and females in respect to OT/AVP modulation of socio-emotional behaviour, even in pre-puberty developmental stages? Which differences are these? Can the results from male studies be directly extrapolated to females during these phases of human development?

4. What are the **biological mechanisms** involved in **cross-generation transmission** of OT pathways? Are they presenting in AVP's physiology as well?

5. Can **animal models** be reliably used to foster research on the roles of hypothalamic nonapeptides in socio-emotional processing during development? Which **species** may more adequately model human socioemotional ontogenesis?

6. Can **attachment relationships** be in part modifiable, not only through psycho-education and implementation of behavioural strategies, but also through implementation of **nonapeptides-based pharmacotherapies** for high-risk populations?

7. What are the **biological mechanisms** underlying disruption of OT and AVP signalling after **early-life adversity**? Do they involve **epigenetic reprogramming** of nonapeptides-related genes? Are they **stress-related**? Can the socio-emotional deficits of these subjects be remediated through implementation of **nonapeptides-based augmentation pharmacotherapies**?

8. May **social-based interventions retune the OT system** during ASD and persistently remediate the hypo-oxytocinemia reported in some patients?

9. Can measurements of nonapeptides in **peripheral biological fluids** represent **clinically relevant biomarkers** for paediatric neurodevelopmental and neuropsychiatric disorders (diagnosis, prognosis or treatment response)?

10. Can disparity in plasma and serum OT concentrations represent **ASD-related endophenotypes** associated with different biological sub-types of this heterogeneous

disorder? May they reflect differences in the underlying pathophysiological mechanisms?

11. Can **OT or AVP-based augmentation therapies** be useful in the treatment of asocial symptoms during paediatric neurodevelopmental and neuropsychiatric disorders? Can its application go beyond social dimensions?

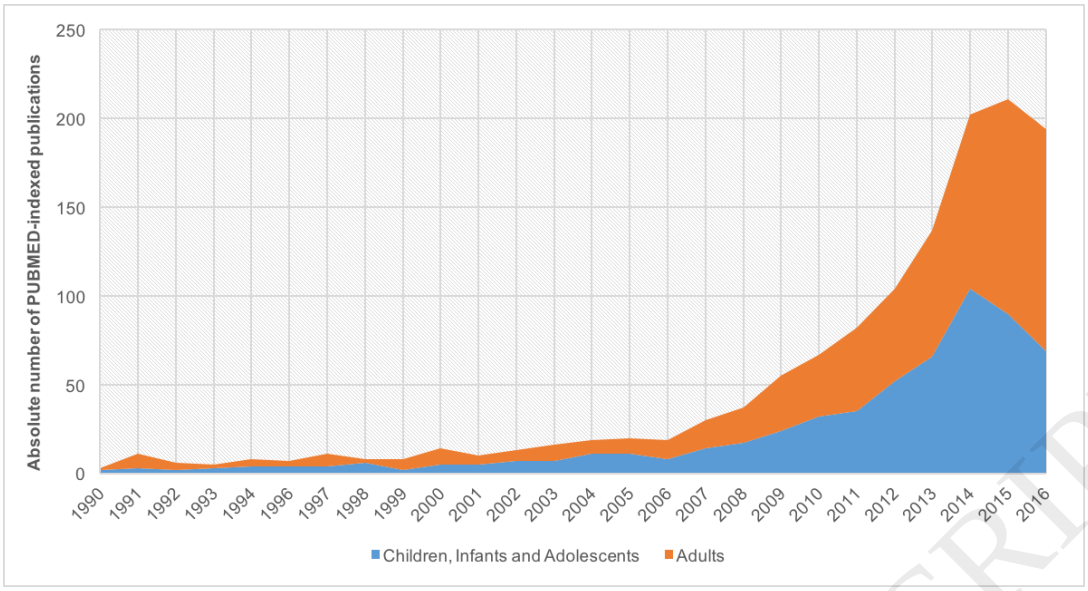
12. Is **chronic** OT or AVP-based augmentation therapies safe during early phases of human psycho-socio-emotional development? Can them entice modifications (positive or negative) persisting into the adult life?

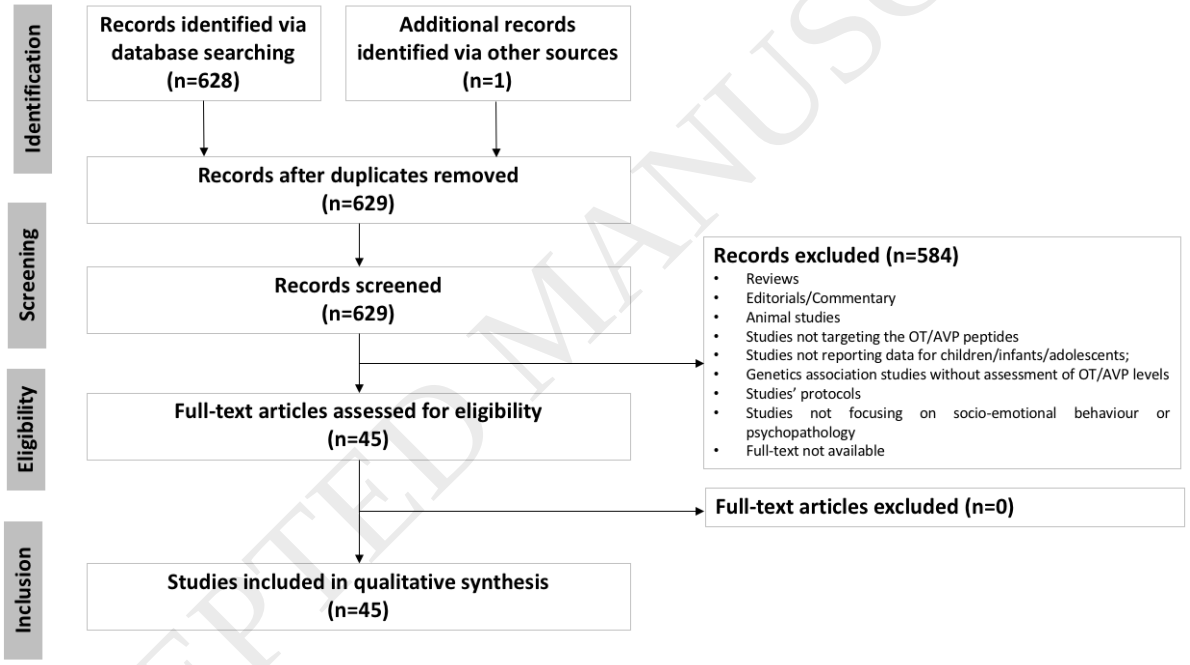
Figures

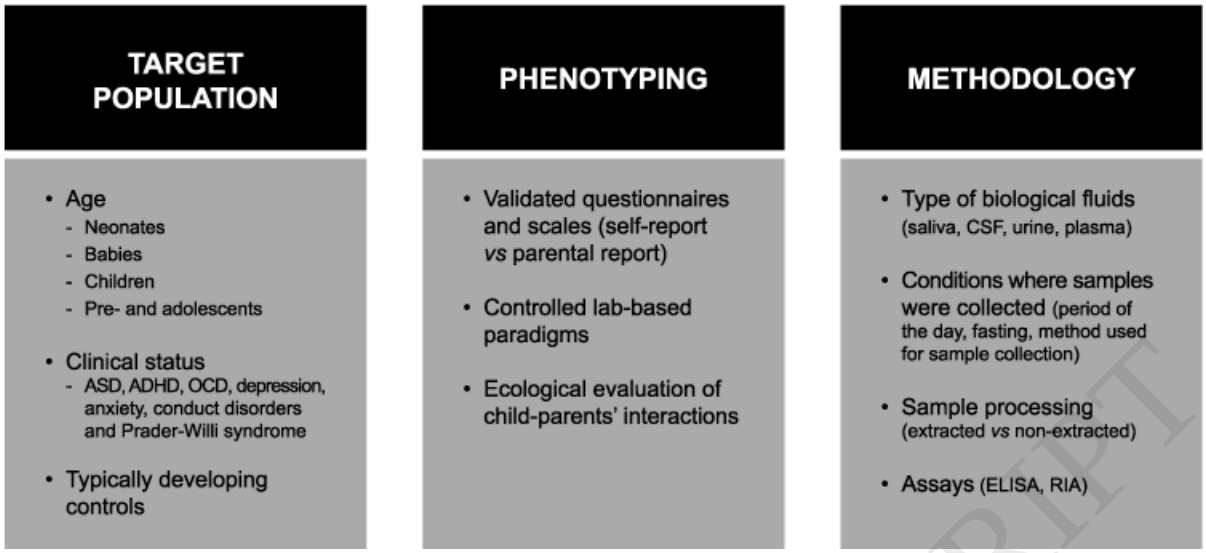
Fig. 1 – Evolution of research interest on hypothalamic peptides oxytocin and vasopressin in adults' and youths' sociability (PUBMED-indexed publications until the end of 2016); source: https://www.ncbi.nlm.nih.gov/pubmed/; queries: "(oxytocin OR vasopressin OR arginine vasopressin) AND (children OR infants OR adolescents) AND social" vs "(oxytocin OR vasopressin OR arginine vasopressin) AND adults AND social"; Accompanying the increasing flood of studies addressing the role of OT/AVP on human social behaviour, an increase in the absolute number of PUBMED-indexed publications exploring the role of these peptides in socio-affective behaviour in paediatric populations could be observed as well – this increase alludes to the crescent interest of the research community on these topics over the last decades;

Fig. 2 – Process of selection of studies for systematic review according to PRISMA guidelines (628 records published up to March 2017 were identified via MEDLINE searching; 1 additional record was identified during the time of the revision of this manuscript and was considered; Duplicates were automatically identified and removed; Titles, asbtracts and key-words were screened to identify potential full-text articles fitting our inclusion and exclusion criteria; 584 records were identified as irrelevant and the remaining 45 assessed for full-text eligibility; no full-text was discarded during this phase, thus all the 45 full-texts were included for qualitative synthesis;)

Fig. 3 – Overview of the characteristics of the reviewed studies (The methodology and the target population of the identified studies varied considerably: The studies' populations included neonates and babies (up to 6 months), children (6 months – 5 years old), pre-adolescents (7-12 years old) and adolescents (12-18 years old) but also participant's parents or other caregivers; while some studies focused on the application of validated psychological instruments destined to evaluate socioemotional behaviour or psychopathology, others focused on controlled lab-based paradigms or ecological evaluation of child-parents' interactions; heterogeneity could be identified in the methodological approaches used, namely in the time interval spanned by the measurement, in the type of biological fluids chosen for analysing neuropeptides' levels, in the method used for sample collection and in the assay techniques used for neuropeptide measurements)







Tables

Table 1. A 12-criteria score list to appraise the quality of the studies reviewed and the percentage of studies complying with the highest (from 0 to 3) within each criterion.

Quality Assessment Criteria	% of compliance
1. Were the hypothesis and objectives of the study clearly described?	69%
2. Have the authors used methods that can test their hypothesis? Was a valid cognitive task assigned to elicit the desired effects in the participants?	88%
3. Were the study's inclusion criteria for the participants clearly described (e.g. ethnicity, age range, gender, neuropsychiatric and other medical conditions and handedness)?	43%
4. Were there sufficient attempts to account for demographic and neuropsychological variability amongst participants [e.g age, gender, handedness, IQ, level of education]?	38%
5. Were the behavioural/psychological assessments clearly described and the tools used validated?	83%
6. Are aspects related to fluid sampling and neuropeptides measurement clearly described?	45%
7. Did the authors discuss their measurements in face of the ranges of the method's sensitivity?	40%
8. Are missing values or outliers exclusion identified and/or justified?	38%
9. Did the study include an a priori calculation of power to define sample sizes? Did this calculation took into account the reliability of the measures used?	45%
10. Is the statistical methodology clearly explained?	71%
11. Were effect sizes reported for significant results (e.g. Cohen's d, Pearson's correlation, odds ratio or risk ratios)?	47%
12. Was a consensual statistical significance threshold set (i.e. p< 0.05 after correction for multiple comparisons)?	95%

Study	Aims	Sample	Method of OT/AVP measurement	Biological sample collection	Social measures and/or stimulus	Main findings
White-Traut et al. (1998)	To determine whether massage in the newborn would increase urine OT levels;	36 newborn infants born via cesarean section	Urinary OT detected by RIA	NA	Amount of crying during the massage procedure	OT levels significantly ⇓ in infants who cried (surrogate of stress);
Clark et al. (2013)	To understand the role of OT biology in sociability, at term, at 3 months, and at 6 months of age;	18 human neonates (27 – 40 weeks of gestation, 10 vaginal delivery and 8 C-section) (12M, 6F) & their parents	CSF OT levels measured by ELISA	Lumbar puncture within 72h of birth	At term: Parental list of soothing techniques that helped to calm their baby At 3 months: Parent Infant Behavior Questionnaire At 6 months: Parent Infant Behavior Questionnaire and *Relating to Others* subscale from the Socialization section of the Vineland Adaptive Behavior Scales	At term: ⇑ OT levels were associated with needing feeding to be soothed; At 3 months: ⇑ neonatal OT levels were significantly associated with ⇑ crying when parents were not immediately responsive; At 6 months: ⇑ levels of OT were associated with: a ⇑ crying for contact and positively related to the *Relating to Others* subscale of the Vineland instrument;
Feldman et al. (2010)	To examine the cross-generation transmission of OT in humans;	55 infants (4-6 months, 50 with vaginal delivery) and 55 parents (36 mothers and 19 fathers, not couples)	Salivary OT, measured by ELISA	1-4 PM Babies: 1h following the last breastfeeding and 1h prior to the next one Baseline samples collected after 10 minutes without touch between parents and the baby Post-interaction samples collected 15	15-min "play-and-touch" interaction, between parents and their infants, that would include any type of touch they typically use	No differences between the OT levels of male and female infants or between infants interacting with mother or father at the two assessments; Both parental and child display the OT response and ⇑ their OT levels after an episode of joint play; Parent and infant's OT concentrations showed significant correlations at both the pre- and post-interaction assessments;

				minutes after interaction		
Weisman et al (2012)	To study whether intranasal OT intervention to the parent can have parallel effects on the infant;	35 fathers and their 5-month-old infants (17M,18F)	Salivary OT determined by ELISA assay	1 – 5 PM Parents asked to abstain from alcohol or caffeine in the day and avoid food intake 2h before arrival Baseline samples for the father collected in a isolated context without interaction with the child	Face-to-face still-face paradigm Free-play episode coded with measures of the Parent-infant synchrony construct	Infants whose fathers received OT showed an ⇑ in salivary OT between the first (before father-infant interaction) and second assessments; Child OT response correlated with father's touch, ⇑ latencies to father gaze aversion, ⇑ child object manipulation and father-child touch synchrony
Weber et al (2017)	To examine associations among infant plasma OT trajectories and maternal-infant social engagement behaviours during initial hospitalization of premature infants	28 extremely premature infants (average age at birth 27,25 weeks) (14M, 14F)	Plasma OT measured by ELISA (unextracted samples)	11 PM – 2 AM Sample collection started at day 14 of life and performed weekly until baby achieve 34 weeks of post-menstrual age; collection was performed before feeding	Parent-Child Early Relational Assessment	Negative association between infant plasma OT levels and infant social engagement behaviors

Table 2.

Overview of the *studies with infants* (Abbreviations: ELISA – Enzyme Linked Immunosorbent Assay; RIA – Radioimmunoassay; OT – oxytocin; AVP - vasopressin)

Table 3) Studies with Preschool and school Children						
Study	**Aims**	**Sample**	**Method of OT/AVP measurement**	**Biological sample collection**	**Social measures and/or stimulus**	**Main findings**
Feldman et al (2013)	To investigate the cross-generation transfer of OT	50 3-years children 50 child "best-friends"	Salivary OT Measured by ELISA	4 – 8 PM (home) Time between feeding, not including the 30 mins immediately after or before feeding Method: Salivettes	7-min parent-child interaction using age-appropriate toys + 10-min interaction between the child and best friend during structured play – determination of Social Reciprocity composite after behavior coding through the *Coding Interactive Behavior Manual*	Child OT correlated with Maternal and Paternal OT Child OT correlated with ⇑ *Social Reciprocity* with best-friend Maternal CD38 rs3796863 A allele and the interaction of CD38 rs3796863xEarly Maternal Care predicted children OT levels: among children receiving ⇓ maternal care, those with mothers homozygous for the high-risk CC allele had ⇓ OT levels than those whose mothers were A-carriers.
Fujii et al (2016)	To examine the association between salivary OT and generosity in Preschoolers	50 (24M, 26F) (average age 4-5 years)	Salivary OT measured by ELISA after extraction	9 – 11 AM Method: Passive-droll method	Dictator's game	Boys: OT levels were negatively associated with allocations made to both ingroup and outgroup members; Girls: OT levels were positively related to allocations made to ingroup members, and unrelated to allocations made to outgroup members;
Nishizato et al. (2017)	To examine the developmental relationship between salivary OT and social attention during	149 children (76M, 73F) (age range 5-90 months)	Salivary OT measured by ELISA	Method: Salivettes	Eye-tracking during visual exploration of pictures depicting faces, people and objects	Negative association of salivary OT with age; No significant sex difference was observed with regard to salivary OT levels;

	the young childhood					Positive correlations were observed between OT and the fixation time spent on the eye area of the face; negative correlations were observed between OT and the fixation time spent on the mouth area; OT mediates the relationship between age and visual attention to the eye area (Human face); Salivary OT levels in young children are modulated by the *OXTR* rs53576 polymorphism (AA<G-carriers);

Table 3. Overview of studies with prescho ol and school children (Abbreviations: ELISA – Enzyme Linked Immunosorbent Assay; RIA – Radioimmunoassay; CSF – cerebrospinal fluid; OT – oxytocin; AVP - vasopressin)

Table 4) Studies with Pre-adolescents and Adolescents						
Study	**Aims**	**Sample**	**Method of OT/AVP measurement**	**Biological sample collection**	**Social measures and/or stimulus**	**Main findings**
Seltzer et al. (2010)	To study if OT's release is contingent to contact with the mother after stress	61 girls and their mothers (age range 7-12 years)	Urinary OT analyzed by ELISA	Session began at 4 PM Urine samples collected at arrival, baseline (after 30 mins of acclimatization), 30 and 60 minutes post-stressor	1)Trier Social Stress Test for Children 2) Period of 15-min of contact with their mothers (either direct contact or verbal), versus the control group that had no contact.	⇑ in OT following comforting by their mothers involving direct physical contact, and in response to speech with their mothers even in the absence of all other types of somatosensory contact; OT levels did not change for children who rested alone and received no form of maternal comforting following the stressor;
Seltzer et al. (2012)	To examine the OT and cortisol responses of female children after undergoing a stressor	68 girls and their mothers (age range 7-12 years)	Urinary OT analyzed by ELISA	Session began at 4 PM Urine samples collected at arrival, baseline (after 30 mins of acclimatization), 30 and 60 minutes post-stressor	Trier Social Stress Test for Children After stressor: *17* in the "no contact" condition, as a negative control; 17 in the "full contact" condition, as a positive control; 17 in the "verbal contact" condition; 17 in the "instant message" condition;	Children using instant messages to communicate maintained levels of OT that did not differ from OT measures in the negative control group ⇑ levels of OT in children permitted to make either direct contact or contact over the phone; these groups did not differ from one another but they did differ from both the instant message and negative control conditions
Carson et al (2014)	To test (1) whether plasma OT predict CSF OT and (2) test whether OT predicts anxiety;	27 pediatric and adult patients (average age of 16 years) (11M, 16F) Subset (N=10) of child	CSF and plasma OXT concentration were quantified by ELISA (extracted samples)	Within 72h of birth	Parent version of the Spence Children's Anxiety Scale.	CSF OT was significantly ⇑ than plasma OT; Plasma OT significantly and positively predicted CSF OT in all subjects; the same was observed when only children (⩽ 18 years) were included in the analysis; Both plasma and CSF OT negatively predicted anxiety scores in children;

		participants aged 6–18 years old				
Doom et al. (2016)	To examine the effect of social support on oxytocin's response to stress	54 children (28M, 26F), 9-10 years 55 adolescents (25M, 30F), 15-16 years	Urinary OT measured by ELISA	2:30 – 4:30 PM Urine collected 25 mins after arrival and within 10 mins after last saliva sample for cortisol measurements	Social Trier Stress test (subjects prepared for the test with a friend or with a parent)	Adolescents present ⇓ levels of urinary OT than children; Males present ⇓ levels of urinary OT than females; Urinary oxytocin ⇓ across the task when the male participants prepared with a friend but not with a parent - this effect was independent of age; no significant ⇓ was observed for females;

Table 4. Overview of studies with pre- and adolescents (Abbreviations: ELISA – Enzyme Linked Immunosorbent Assay; RIA – Radioimmunoassay; CSF – cerebrospinal fluid; OT – oxytocin; AVP - vasopressin)

Table 5) Studies with children after adverse early-life environment						
Study	**Aims**	**Sample**	**Method of OT/AVP measurement**	**Biological sample collection**	**Social measures and/or stimulus**	**Main findings**
Wiesmer - Fries et al. (2005)	To assess baseline OT/AVP and their responses to social and physical interactions	18 adopted children who had resided in orphanages (6M, 12F) (average age 4,47 years) 21 children reared by their biological parents with age matched and similar high socioeconomic backgrounds (9M, 12F) (average age 4,51 years)	Urinary OT and AVP analyzed by ELISA (after HPLC separation)	Basal levels determined by averaging 12h overninght urine collections from 4 separate mornings Post-interaction samples collected 15 mins after task	30-min interactions between children and their mothers, and between children and an unfamiliar female experimenter, with regularly timed physical contact	OT and AVP were equivalent for boys and girls, in both groups Basal levels of OT did not differ between the adopted and control children; Adopted children present ⇓ baseline levels of AVP than controls; OT levels for biological family-reared children ⇑ after physical contact with their mothers but adopted children did not show this response; AVP levels does not vary as a function of contact neither in the mother nor in the stranger condition in any of the groups;
Seltzer et al. (2014)	To test if (1) physical abuse is associated with OT response to a social stressor and (2) whether OT and cortisol levels are associated after a stressor;	37 "maltreated children" who had experienced verified physical abuse (17M, 21F) 36 "typically developing" children with similar sociodemographic characteristics (18M, 18F) (age range 8 – 11.5 years)	Urinary OT levels analyzed by ELISA	Arrival at 4 PM Baseline urine collected after 30 mins of acclimatization Post-stressor collected 60 mins after stress challenge	Trier Social Stress Test for children to induce a stress response	Girls with histories of physical abuse have ⇑ levels of urinary OT and ⇓ levels of salivary cortisol following a stressor when compared to controls; Abused and control boys do not differ in their hormonal responses; Children's salivary CT responses post-stressor were negatively correlated with urinary OT responses

Mizushima et al. (2015)	To investigate differences in secretion patterns of cortisol (CT) and oxytocin (OT) among children who experienced children maltreatment;	38 maltreated children (18M, 18F): 23 categorized as "Settled" (average age 12.2 years) 15 as "Unsettled" (average age 13.1 years) 26 age- and gender-matched typically developing (TD) children (12M, 14F) (average age 12,6 years)	Salivary OT determined by ELISA	Awakening (within 1h) and bedtime samples Method: Salivettes	ADHD symptoms: Japanese version of the ADHD Rating Scale-IV ASD symptoms: Autism-Spectrum Quotient-children's version (AQ) Strengths and Difficulties Questionnaire (SDQ) Depression Self-Rating Scale for Children (DSRS-C: Japanese version) Traumatic experiences: UCLA PTSD Index for DSM-IV (UPID) the Impact of Event Scale Revised (IES-R) and the trauma symptom checklist for children (TSCC)	Children in "Settled" environments have a marked ⇑ in the bedtime salivary OT level compared with TD children;
Apter-Levy et al. (2013)	To investigate the role of maternal depression in children's salivary OT levels	149 6-years children (46 from depressed mothers, 103 from never-depressed mothers as comparison subjects) (51% M, 49%F)	Salivary OT levels determined by ELISA	Method: Salivettes Sampling in duplicate and the measures averaged	Development and Well-Being Assessment 10-min of mother-child interactions with preselected toys coded with the *Coding Interactive Behavior manual*	⇓ salivary OT levels in the mothers, fathers and children in families with depressed mothers and father's emotional distress when compared with controls

Table 5. Overview of studies with children after early-life adverse environment (Abbreviations: ELISA – Enzyme Linked Immunosorbent Assay; HPLC – High Pressure Liquid Chromatography; ADHD – Attention Deficit and Hyperactivity Disorder; ASD – Autism Spectrum Disorder; OT – oxytocin; AVP - vasopressin)

Table 6. Overview of studies with clinical samples: 6.1) ASD; 6.2) Others (Abbreviations: ELISA – Enzyme Linked Immunosorbent Assay; RIA – Radioimmunoassay; CSF – cerebrospinal fluid; ADHD – Attention Deficit and Hyperactivity Disorder; ASD – Autism Spectrum Disorder; OT – oxytocin; AVP – vasopressin; PWS – Prader-Willi syndrome)

Table 6.1) *Clinical samples* (*ASD)*						
Study	**Aims**	**Sample**	**Method of OT/AVP measurement**	**Biological sample collection**	**Social measures and/or stimulus**	**Main findings**
Modahl et al. (1998)	To determine abnormalities in OT of autistic children	29 autistic boys 30 non-autistic boys used as a control (age 5-12 years)	Plasma OT levels measured by RIA (extracted samples)	Within 2h of noon 2h fasting	*Children measures:* Peabody Picture Vocabulary Test (PPVT-R) *Parent Interviews:* The Vineland Adaptive Behavior Scales (VABS)	Autistic children presented ⇓ OT levels than controls; OT ⇑ with age in the controls but not the autistic children; Elevated OT was associated with ⇑ scores on social and developmental measures for the normal children; For the autistic group, OT was negatively associated with *Daily Living Skills*, *Personal Care*, and *Community Skills* (these relationships were strongest in a subset of autistic children identified as aloof)
Green et al. (2001)	To determine whether there are changes in OT peptide forms in autistic children	28 boys diagnosed with DSM-IV autistic disorder 31 age-matched and nonpsychiatric boys (age range 6-11 years)	Plasma OT levels measured with peptide RIA (extracted samples)	Within 2h of noon 2h fasting	1) Brief questionnaire regarding variables that could affect the measurement of peptide levels; 2) Vineland Adaptive Behavior Scales 3) Stanford Binet fourth edition 4) Copying and Pattern Analysis subtests 5) Peabody Picture Vocabulary Test (PPVT) 6) Autistic Disorders Checklist and an adaptation based on DSM-IV criteria	⇓ in plasma OT, an ⇑ in OT-X and an ⇑ in the ratio of OT-X/OT in the autistic sample. OT-X ⇑ with age only in the autistic group OT-X was not significantly correlated with any of the Vineland domains or intellectual scores in either group.
Al-Ayadhi (2005)	To assess plasma levels of OT and AVP in autistic children	65 children diagnosed with DSM-IV autistic disorder (61M, 4F) 77 healthy age and sex matched controls (71M, 6F) (age range 3,5 – 14 years)	Plasma OT and AVP levels measured by ELISA (unextracted samples)	NA	Diagnosis of autistic disorders	OT and AVP were significantly ⇓ in the autistic group, as compared to the control group No association between age or autism degree and the oxytocin or vasopressin levels in both groups
Corbett et al. (2011)	To evaluate the impact of an intervention to improve socioemotional functioning in autistic children	8 children with autism spectrum disorder (7M, 1F) 8 children, used as peers with typical development (4M, 4F) (age range: 6-17 years)	Plasma OT levels determined by ELISA	NA	*Social Emotional NeuroScience Endocrinology (SENSE) Theatre* (designed to improve socioemotional functioning and reduce stress in autistic children)	No significant impact of intervention on OT levels neither in ASD nor in controls
Zhang et al. (2012)	To assess the efficacy of Transcutaneous electrical acupoint simulation (TEAS)	76 autistic children: 37 receiving TEAS (34M, 3F) 39 control group (34M,5F) (around 4 years)	Plasma OT /AVP levels determined by ELISA (unextracted samples)	9-11 AM Parents present during sampling	Childhood Autism Rating Scale (CARS) (by psychiatry); ABC (by parents); Social Adaptive Development Quotient Scale (ADQ); Parental reported changes in children's behavior, once per weak during the intervention;	OT ⇓ in both the control and TEAS groups, but the decrement was significantly lower in the TEAS group than that of the control; AVP ⇑ significantly in the TEAS group compared with that in the control; OXT and AVP showed a positive correlation in the control group before any intervention
Gordon et al. (2013)	To understand how changes in peripheral OT are	17 children and adolescents with ASD (18M, 3F)	Salivary OT determined by ELISA	Baseline and 30 min post-administration samples	Reading the Mind in the Eyes Test (RMET)	Positive correlation between changes in salivary OT from baseline to 30 min post-administration and activity in the amygdala and BA 25 regions during eyes>vehicles;

	associated with changes in brain activity after intranasal OT	(age range: 8–16.5)		Salivettes	Judgments of social (Eyes) and non-social (Vehicles) meaningful pictures	
Feldman et al. (2014)	To assess 1) baseline OT in pre-schoolers with ASD and 2) test whether OT production may be enhanced by parent–child contact;	40 pre-schoolers with high-functioning ASD (average age of 5,12 years); 40 typically developed controls (matched for mental age)	Salivary OT determined by ELISA	4 samples for each child/parent in different time points in relation to parental interaction Baseline collected after a period of acclimatization; Salivettes	Measurements of OT at baseline and after synchronous parent-child interactions	Children with ASD had ⇓ baseline OT; Following 20 min of parent–child interactions, OT normalised and remained high during social contact; 15 min after contact, OT ⇓ to baseline. OT correlated with parent–child social synchrony in both groups.
Jacobson et al. (2014)	To assess baseline OT and downstream cellular mediators levels in autistic patients	37 autistic patients (25M, 12F) 41 typically developing controls (24M, 17F) (age between 4-6 years)	Plasma OT levels determined by ELISA (extracted samples)	10 – 12 AM 2h fasting	Autism Diagnostic Observation Schedule (ADOS) Autism Diagnostic Interview – Revised (ADI-R) Developmental Quotient; *Kauffman Assessment Battery for Children-Second Edition (KABC-II)* *; Bayley Scales of Infant and Toddler Development – Third Edition (Bayley-III);* Vineland Social-Emotional Early Childhood Scales	Males with autism displayed ⇑ OT levels compared to controls. Children with autism displayed significantly ⇑ mRNA for stimulatory G proteins compared to controls. OT levels correlated strongly positively with c-fos mRNA levels, but only in control participants OT, G-protein, and c-fos mRNA levels correlated inversely with measures of social and emotional behaviors, but only in control participants.
Fujisawa et al. (2014)	To investigate the relationship between visual attention to social stimuli and OT in preschool ASD children;	19 ASD children (16M, 3F) (average age of 4,8 years) 60 typically developed controls (28M, 32F) (average age of 4 years)	Salivary OT determined by ELISA	Salivettes 2 samples averaged	Visual attention to 4 categories of social stimuli (human faces, people and geometry, human motion and finger pointing) assessed by eye-tracking (gaze fixation)	Positive association between OT and fixation duration for an indicated object area in a finger-pointing movie in controls; No significant association in ASD children;
Husarova et al. (2016)	To assess 1) baseline differences in plasmatic OT levels between children with ASD and control healthy typically	19 ASD males 44 healthy age-matched males (age between 2 – 9 years)	Plasma OT determined by ELISA (unextracted samples)	8-9 AM	Autistic symptoms evaluated through Childhood Autism Rating Scale and Autism Diagnostic Interview (ADI), adjusted research version; Parental autistic traits evaluated by Autism Spectrum Quotient (AQ), Systemizing Quotient (SQ) and Empathizing Quotient	ASD children had significantly ⇓ OT than controls; OT positively correlated with ADI Reciprocal Interaction and Communication scores; AQ and SQ of fathers positively correlated with children OT;

	developing children; 2) if plasmatic OT levels in children with autism are associated with the severity of particular autism symptoms and autistic traits of their parents					
Taurines et al. (2014)	To assess baseline differences in plasma concentrations of OT in children and adolescents with ASD and its relationship with ASD symptomology	19 male children and adolescents with ASD 19 male children with ADHD (clinical control group) 17 typically developing male controls (age range 6.0–17.6 years)	Plasma OT determined by RIA (extracted samples)	7:30 – 10 AM Fasting	Autism Diagnostic Interview-Revised (ADI-R) Autism Diagnostic Observation Schedule (ADOS)	No significant differences in OT between ASD and control subjects; ASD patients: autistic symptomalogy correlated with OT levels; ADHD patients present levels of OT significantly ⇓ than ASD patients;
Abdulamir et al. (2016)	To assess baseline differences in plasma concentrations of OT in ASD patients and its relationship with ASD social and cognitive dysfunction	60 male autistic patients 26 age- and gender-matched control subjects (age range 3.0–13.0 years)	Plasma OT determined by ELISA (unextracted samples)	9 AM Overnight fasting	ASD symptomology grading in accordance with DSM - V criteria	ASD patients present ⇓ levels of OT than controls; OT associated with disease severity, especially for the most severe patients;
Parker et al. (2014)	To test the extent to which plasma OT concentrations and OTR SNPs (i) interact to produce ASD phenotypes, (ii) exert	79 children with ASD (62M, 17F) 52 unaffected siblings (29M, 23F) 62 neurotypical control children (40M, 22F) (age range 3.0–	Plasma OT levels were quantified by ELISA (extracted samples) Genotyping was performed in blood DNA for the OXTR SNPs	10 AM – 2 PM	Social phenotyping included the following instruments: Social Responsiveness Scale (SRS) "A Developmental NEuroPSYchological Assessment" (NEPSY-III) Vineland Adaptive Behavior Scales	OT not significantly altered between groups, neither in males nor in females; OT positively predicted theory of mind and social communication performance in all groups; OT concentrations showed significant heritability between ASD-discordant siblings

	differential phenotypic effects in ASD vs. non-ASD children, or (iii) have similar phenotypic effects independent of disease status	12.0 years)	rs2254298 and rs53576		(VABS-2) ADI-R Social Domain Scores	
Miller et al. (2013)	To assess baseline differences in plasma OT in male and female ASD patients and its relationship with ASD social, language and cognitive dysfunction	75 preadolescent and adolescent girls and boys; 40 with high-functioning ASD (21M,19F); 35 typically developing children (19M, 16F) (age range 8.0–18.0 years)	Plasma OT and AVP determined by ELISA (unextracted samples)	Fasting of at least 2h	*Qualification Measures* • *Wechsler Abbreviated Scale of Intelligence* • *Autism Diagnostic Observation Schedule* • *Social Communication Questionnaire* *Autism Symptom Measures* • *Social Responsiveness Scale* • *Children's Communication Checklist-2* • *Repetitive Behavior Scale* *Internalizing Symptom Measures* • *Behavior Assessment System for Children*	Girls showed ⇑ levels of OT than boys; Boys showed ⇑ levels of AVP than girls; There were no significant effects of diagnosis on OT and AVP neither in males nor in females. ⇑ OT values were associated with ⇑ anxiety in all girls, and with ⇑ pragmatic language in all boys and girls. AVP levels were positively associated with restricted and repetitive behaviors in girls with ASD but negatively (non-significantly) associated with these behaviors in boys with ASD;
Zhang et al. (2016)	To assess baseline differences in plasma OT and AVP in ASD patients and its relationship with ASD social and cognitive dysfunction	84 (71M, 13 F) ASD children 85 (71M, 14F), age matched, typically developing children (age range 2.0–7.0 years)	Plasma OT and AVP determined by ELISA (extracted samples)	9-11 AM Fasting overnight and amount of fluids minimized	Autistic symptoms assessed by the Childhood Autism Rating Scale (CARS)	Male ASD children present ⇓ OT than controls, but no significant difference between ASD and control females; No significant differences in AVP levels between diagnostic groups; OT is not correlated between children and their mothers, neither in the ASD nor in the control group; OT was not correlated with total CARS score, but correlated negatively with verbal communication and positively with adaptation dimensions of CARS (TRENDS); Negative correlation between AVP and object use (TREND)
Yang et al. (2017)	To assess baseline differences in OT in ASD patients and its relationship with autistic symptoms	55 ASD children (43M, 12F) 110 (84M, 26F), age matched, typically developing children (age range 2-17 years)	Plasma OT determined by ELISA (extracted samples)	8 – 9:30 AM Fasting in the morning	Autistic symptoms assessed by the Childhood Autism Rating Scale (CARS) and the Autism Behavior Checklist (ABC)	ASD subjects exhibited ⇑ OT than controls; Positive correlations between OT levels and *"adaptation to change score"* and CARS total scores; ASD subjects: OTR SNP rs2254298 genotype was associated with serum OT levels (G-carriers>AA);

Tsuji et al (2015)	To assess the impact of maternal massage therapy on salivary OT levels of autistic children	7 ASD males (age range 8-12 years)	Salivary OT measured by ELISA	Paper cups Baseline and post-massage samples collected after a period of free playing	Maternal massage	During the period of massage therapy, the children and mothers exhibited ⇑ OT than during the non-massage period; OT before and after a single massage session was not significantly changed neither in children nor in mothers;
Corbet et al (2016)	To evaluate the relationship between cortisol and OT/AVP in children with ASD under baseline and physiological stress (hydrocortisone challenge) conditions	14 ASD (12M, 2F) (average age 9,70 years) 11 Neurotypical controls (10M,1F) (average age 9,37)	Salivary and plasma OT and AVP measured by ELISA (unextracted samples)	1 – 4 PM Baseline collected after acclimatization to the experimental setting	Autism Diagnostic Observation Schedule (ADOS) Wechsler Abbreviated Scale of Intelligence (WASI) Social Communication Questionnaire (SCQ) Social Responsiveness Scale (SRS)	No differences in baseline OT and AVP salivary and plasma levels between ASD and controls; Controls: OT ⇑ during physiological challenge and remained stable or ⇓ during placebo administration; ASD: OT remained stable or ⇓ during both the physiological challenge and the placebo condition; No significant effects of challenge on AVP levels, neither in controls nor ASD; In the TD group, the SRS was positively associated with baseline AVP; In the ASD group, there was a modest negative correlation between SRS and AVP at baseline (TREND);
Shou et al (2017)	To investigate the relationship between autistic behaviours, circulating AVP and the structure and functional connectivity (FC) of specific brain regions in autistic children compared with typically developing children	14 ASD (12M, 2F) 14 neurotypical controls (10M, 4F) (age range 3-5 years)	Plasma AVP measurements by ELISA (extracted samples)	8-10:30 AM No more than 7 days after MRI Overnight fasting + fluid intake minimized	Childhood Autism Rating Scale The Autism Behavior Checklist (ABC) The Autism Spectrum Quotient Children's Version (AQChild)	AVP negatively correlated with the visual and listening response score in CARS AVP positively correlated with the total volume of the hypothalamus In boys with ASD: positive correlation between the functional connectivity of the left amygdala-supramarginal gyrus and AVP;
Carson et al (2015)	To test whether blood AVP concentrations 1) differed between children with ASD, their ASD discordant siblings, and neurotypical	57 ASD (48M, 9F) 47 ASD discordant siblings (27M, 20F) 55 neurotypical controls (36M, 19F) (age range 3-12 years)	Plasma AVP measurements by ELISA (extracted samples)	Study 1: Samples taken at morning or afternoon or night; CSF samples taken from the cisterna magna orleft ventricle or lumbar puncture; local or general anaesthesia applied; Study 2: 10 AM – 2 PM	NEPSY-II Social and Perception Domain: Affect Recognition and Theory of Mind tasks Social Responsiveness Scale (SRS)	AVP did not differ by group or sex; No relationship between AVP and Theory of Mind score in non-ASD children; AVP positively predicted Theory of Mind score in children with ASD; AVP did not predict Affect Recognition score or SRS Total score in either the ASD or non-ASD group;

	controls; and 2) predicted social functioning					
Oznan et al (2018)	To test the ablity of a multidimensional biomarker based on OT and AVP and their receptors quantification to predict diagnosis status	44 ASD (37M, 7F) (age range 6-12)	Plasma OT and AVP measurements by ELISA (extracted samples) OXTR and V1aR expression quantified in huma lymphocytes by qPCR	10 AM – 5 PM Within 2 weeks of behavioural phenotyping	The core behavioral features of ASD (i.e., social impairments and restricted, repetitive behaviors) were assessed using the Social responsiveness scale and the Repetitive Behavioural scale.	ASD patents do not differ from controls in AVP or OT blood levels, but present ⇓ levels of total neuropeptides receptor expression (sum of the OXTR and AVPR1A gene expression. ⇓ levels of total neuropeptide receptor gene expression and plasmatic OT (when included in a model considering receptors expression, plasmatic OT predicted disease status). Levels of AVP do not predict disease status. ⇓ levels of total neuropeptide receptor gene expression predicted ⇑ social impairments as measured by the SRS total score and ⇑ severity of stereotipy

Table 6.2)	Clinical samples (others)					
Study	**Aim**	**Sample**	**Method of OT/AVP measurement**	**Biological sample collection**	**Social measures**	**Results**
Swedo et al. (1992)	To study CSF OT/AVP levels in children and adolescents with OCD;	43 children and adolescents with primary OCD (26M, 17F) (age range 6-19 years)	Lumbar CSF OT/AVP levels determined by RIA (extracted samples)	9 – 10:30 AM Lumbar puncture Medication free for a minimum of 4 weeks and low monoamine diet for 3 days	Hamilton Depression Rating Scale NMH Global Scales for OCD, depression, anxiety, and overall functioning;	AVP was negatively correlated with several ratings of obsessive-compulsive disorder symptom severity; AVP was ⇑ in a subgroup of patients with checking rituals; OT did not differ between subgroups; OT was positively correlated with depressive symptoms; The ratio AVP/OT was negatively correlated with obsessive-compulsive and depressive symptoms; Comorbid affective disorder was associated with ⇓ AVP; Anxiety disorder was associated with ⇑ OT; OT was ⇑ in a subgroup of patients who did not have family history than in patients with a positive family history;
Demirci et al. (2016)a	To determine the relationship between OT levels and impulsivity in both healthy controls and ADHD patients;	40 male ADHD patients 40 male age matched controls (age range 8-15 years)	Plasma OT measured by ELISA (unextracted samples)	8 AM Overnight fasting	Turgay DSM-IV-based Child and Adolescent Behavior Disorders Screening and Rating scale: Barrrat Impulsiveness Scale-11 (BIS-11)	OT was ⇓ in the ADHD group than in controls; OT negatively correlated with impulsivity and attention subscale scores of BIS-11 in both groups;
Demirci et al. (2016)b	To determine the relationship between aggression, empathy and OT levels in children and adolescents with ADHD;	40 male ADHD patients 40 male age matched controls (age range 7-18 years)	Plasma OT measured by ELISA (unextracted samples)	8-9 AM Overnight fasting Rest period of 25 mins	Buss–Perry Aggression Questionnaire Bryant's Empathy Index (BEI) for Children and Adolescents Reading the Mind in the Eyes test (RMET)	ADHD patients presenedt ⇓ OT; Patients with a hyperactive/impulsive subtype of disease presented ⇓ OT than the inattentive subtype; Negative correlation between OT and aggression scores in the ADHD patients, but not in controls; Positive correlation between OT and RMET scores in both patients with ADHD and controls (in children but not adolescents); Positive correlation between the BEI and OT in patients but not in controls;
Lebowitz et al. (2017)	To evaluate OT's response during youth-mother interaction in clinically anxious youth	41 clinically anxious youth (14M, 27F), (age range 7–16 years)	Salivary OT measurements through ELISA (unextracted)	4 – 5 PM 2h fasting	Anxiety disorders interview schedule—Children and parent (ADIS-C/P) Multidimensional anxiety scale for children (MASC2) Dyadic interaction: subjective coding and evaluation through the application of the *Coding Interactive Behaviour* (*CIB*) system	Affective touch, maternal sensitivity, maternal intrusiveness, youth engagement and youth initiative predicted youth's OT response; When affective touch was high, youth had ⇑ OT response; OT response to a positive youth–mother interaction is ⇑ among youth with Separation anxiety disorder (SAD), compared to clinically anxious youth without SAD;
Sasaki et al (2016)	To determine whether serum levels of OT in	10 TRDIA (6M, 4F) (average age 14,40 years)	Serum OT measurements through ELISA	10 AM – 3 PM	Children's Depression Rating Scale-Revised (CDRS-R)	TRDIA patients present ⇑ OT than non-TRDIA and controls;

	treatment-resistant depression in adolescents (TRDIA) differ from non-treatment-resistant depression (non-TRDIA) or controls.	27 non-TRDIA (11M, 16F) (average age 12.89 years) 25 neurotypical controls (12M, 13F) (average age 12,88 years)	(extracted samples)		Depression Self-Rating Scale for Children-Japanese Version (DSRS-C-J)	No significant correlation between OT and the CDRS-R total scores or DSRS-C-J scores in any group; No differences in OT between the medicated group with adolescent depression and non-medicated group with adolescent depression;
Levy et al (2015)	To examine the relationship between OT and conduct problems (specifically callous-unemotional traits)	67 M adolescent undergoing residential treatment (age range 15-19 years)	Saliva OT levels measured by ELISA	Late noon Salivettes Eating, drinking, smoking and oral hygiene resfrained Sample collected before questionnaires	Inventory of callous-unemotional traits Strengths and difficulties questionnaire Brown Goodwin Questionnaire (BGQ)	OT inversely correlated with conduct problems severity on Strength and Difficulties Questionnaire; Recorded history of antisocial acts did not correlate with current OT; ⇑ odds ratio for significant CU traits among subjects with conduct problems in low-OT vs high-OT subjects;
Bilgiç et al (2016)	to investigate whether serum levels of AVP in children with ADHD differ from those of sex- and age-matched healthy controls	24 ADHD (24M, 10F) 36 controls (23M, 13F) (age range 7-15 years)	Plasma AVP levels measured by ELISA (unextracted samples)	8 – 10 AM Overnight fasting	CPRS-RS (Conners' Parent Rating Scale-Revised Short); CTRS-RS (Conners' Teacher Rating Scale-Revised Short)	No significant differences between ADHD patients and controls in AVP, neither in males nor females;
Johnson et al. (2016)	To assess baseline differences in plasma concentrations of OT between PWS patients and controls	23 PWS children with genetic confirmation (13M, 10F) 18 age matched healthy unrelated siblings without PWS (10M, 8F) (age range 5.0–11.0 years)	Plasma levels of OT determined by immunoassay	Morning Overnight fasting PWS patients under weight control program	PWS diagnosis	⇑ OT levels in children with PWS compared with unrelated and unaffected siblings without the diagnosis of PWS; The diagnosis of PWS predicted OT's level in controlled regression analysis;