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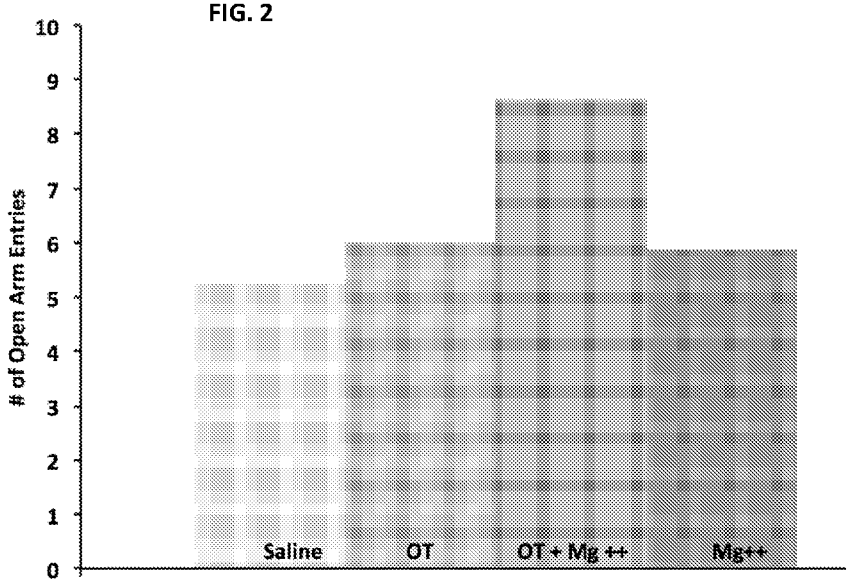
(54) Title: MAGNESIUM-CONTAINING OXYTOCIN FORMULATIONS AND METHODS OF USE

FIG. 2



(57) Abstract: Disclosed are methods and compositions for the treatment of autism spectrum disorder, related disorders and symptoms of such disorders, comprising co-administration of an oxytocin peptide and magnesium ions. Co-administration of an oxytocin peptide and magnesium ions results in a synergistic or enhanced effect on reducing social and communication deficits in a patient suffering from an autism spectrum disorder.

MAGNESIUM-CONTAINING OXYTOCIN FORMULATIONS AND METHODS OF USE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/321,654, filed April 12, 2016, the disclosure of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The invention relates to methods and compositions comprising an oxytocin peptide and magnesium ions for the treatment of autism spectrum disorder, related disorders and symptoms of such disorders.

BACKGROUND OF THE INVENTION

[0003] Oxytocin is a naturally occurring nine-amino acid neuropeptide that is primarily produced in the paraventricular and supraoptic nuclei of the mammalian hypothalamus. It is released in to the central nervous system *via* distributed neural pathways and into peripheral circulation *via* the posterior pituitary. The intramuscular injection or intravenous infusion of synthetic oxytocin (Pitocin®) is currently approved in the United States to produce or improve uterine contractions to facilitate vaginal delivery and to control postpartum hemorrhage. Intranasal oxytocin (Syntocinon®) had been approved in the United States for stimulating milk letdown to facilitate breast-feeding from 1960 until 1997. While the nasal spray of Syntocinon® was withdrawn from the United States market at the request of the manufacturer, intranasal oxytocin is still marketed outside of the United States in countries such as Switzerland, Portugal, or Brazil. Use of oxytocin peptides in treatment of autism spectrum disorder has recently been demonstrated. See WO 2004/030524 A2 and WO 2008/042452 A1, the disclosures of which are incorporated herein by reference.

[0004] Autism spectrum disorder has become increasingly more prevalent in the human population and is typically recognized by certain behaviors and characteristics, such as impairment in communication skills and/or social interaction, lack of eye contact, and/or an inability to form and/or maintain social relationships. Children and adults diagnosed with autism spectrum disorder can exhibit one or more of the behaviors and characteristics mentioned above to varying degrees. Symptoms often observed in individuals with autism

spectrum disorder are persistent deficits in social communication and social interaction, social anxiety, and restricted repetitive behaviors, interests, and activities. Other behaviors and characteristics also observed in persons with autism spectrum disorder include an aversion to physical contact, generalized anxiety, a monotone voice or an inability to modulate volume of voice, failure to develop peer relationships, lack of shared enjoyment and interests and lack of social or emotional reciprocity. Other disorders that display social and communication deficits can include social anxiety disorder, obsessive-compulsive disorder, social (pragmatic) communication disorder, and neurodevelopmental disorders including but not limited to attention deficit hyperactivity disorder, Prader-Willi syndrome, Timothy syndrome, Fragile-X syndrome, Rett syndrome, or Williams syndrome which exhibit symptoms similar to those displayed in autism spectrum disorder. People with autism spectrum disorder tend to have communication deficits, such as responding inappropriately in conversations, misreading nonverbal interactions, or having difficulty building friendships appropriate to their age. In addition, people with autism spectrum disorder may be overly dependent on routines, highly anxious and sensitive to changes in their environment, or intensely focused on inappropriate items (e.g., inanimate objects and/or narrow interests in specific topics). Again, the symptoms of people with autism spectrum disorder vary widely and fall on a continuum, with some individuals showing mild symptoms and others having very severe symptoms. There are no drug therapies available for the core deficits in social communication and social interaction, or restricted repetitive behaviors, interests, and activities in persons with autism spectrum disorder and related disorders, and there remains an urgent need for such treatment.

[0005] Oxytocin has been shown to improve the core symptoms of autism, in particular social and communication deficits and associated anxiety symptoms. Human clinical trials have demonstrated efficacy of intranasal oxytocin in treating autism spectrum disorder, related disorders and symptoms of such disorders. *See, e.g.*, Yatawara et al., *Mol. Psychiatry* **2015**, 1-9; Gorka et al., *Neuropsychopharmacology* **2015**, 40(2):278-286; Anagnostou et al., *Mol. Autism* **2012**, 3(1):16; Guastella et al., *Psychoneuroendocrinology* **2009**, 34(6):917-923. However, these trials have shown wide variability in the response that people with autism spectrum disorder and related disorders have to treatment with oxytocin. Thus, there exists a need for an oxytocin peptide formulation capable of providing a more pronounced effect in the treatment of autism spectrum disorder and related disorders.

BRIEF SUMMARY OF THE INVENTION

[0006] Provided are methods and compositions comprising an oxytocin peptide and magnesium ions for the treatment of an autism spectrum disorder, related disorders and symptoms of such disorders, comprising co-administration of an oxytocin peptide and magnesium ions via craniofacial mucosal administration (e.g., intranasal administration). The methods and magnesium-containing oxytocin peptide formulations described herein provide enhanced efficacy in treating autism spectrum disorder compared to oxytocin alone.

[0007] In one aspect, the invention provides a method for treating an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or anxiety, comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect. The oxytocin peptide and the magnesium ions may be co-administered concurrently or sequentially. In some embodiments, the oxytocin peptide is administered concurrently with the magnesium ions either in the same unit dose or in separate unit doses or formulations. In some embodiments, the oxytocin peptide and the magnesium ions are administered sequentially. For example, the oxytocin peptide is administered at a time period after administration of the magnesium ions. In some embodiments, the subject is a human.

[0008] The oxytocin peptide and the magnesium ions may be administered via the same route or different routes to a subject in need thereof. In some embodiments, the oxytocin peptide is administered via craniofacial mucosal administration (e.g., nasal, buccal, sublingual or ocular administration). In one embodiment, the oxytocin peptide and the magnesium ions are both administered intranasally in the same formulation.

[0009] In some aspects, interleukin-6 (IL-6) is used as a biomarker of potential efficacy of administration of the oxytocin peptide in a subject according to a method described herein for the treatment of an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or anxiety; and to select a subject for application of the methods. In some embodiments, the method comprises measuring the level of IL-6 in a subject and administering to a subject having an elevated IL-6 level an effective dose of an oxytocin peptide and magnesium ions.

[0010] In one aspect, the method for treating an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or anxiety, comprising administering to a subject in need thereof an

effective dose of an oxytocin peptide and magnesium ions, wherein co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect, further comprises administering to the subject an effective dose of interleukin-6 (IL-6), wherein administration of IL-6 induces the elevation of oxytocin receptor expression.

[0011] In some embodiments, the oxytocin peptide is human oxytocin consisting of Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly (SEQ. ID NO:1). In some embodiments, the effective dose of the oxytocin peptide is about 0.5 μg to about 2000 μg, preferably about 8 μg to about 1000 μg, more preferably about 15 μg to about 120 μg. In some embodiments, the effective dose of the magnesium ions administered is about 50 μg to about 68 mg, preferably about 50 μg to about 34 mg, more preferably about 1 mg to about 3 mg. In some embodiments, the method comprises administering a magnesium salt (e.g., magnesium citrate and/or magnesium chloride) in an amount to provide about 50 μg to about 68 mg of magnesium, or about 50 μg to about 34 mg of magnesium, or about 1 mg to about 3 mg of magnesium. In some embodiments, the method comprises administering magnesium citrate or magnesium chloride in an amount to provide about 50 μg to about 68 mg of magnesium, or about 50 μg to about 34 mg of magnesium, or about 1 mg to about 3 mg of magnesium. In some embodiments, the effective dose of the oxytocin peptide and the magnesium ions comprises about 0.5 μg to about 2000 μg, or about 15 μg to about 120 μg (e.g., about 60 μg or about 66 μg) of the oxytocin peptide administered in an aqueous solution containing about 0.11% to about 2.8% (preferably about 1.1% to about 1.6%, e.g. about 1.36 %) (w/v) magnesium.

[0012] In some embodiments, the invention provides a method of reducing one or more symptoms associated with an autism spectrum disorder. The symptoms treatable by the method include any social or communication deficits treatable by an oxytocin peptide, such as deficits in eye contact, social anxiety, generalized anxiety, accuracy in determining complex social cues, empathy, and communication abilities including expressive language functioning.

[0013] In some embodiments, the invention provides a method for the treatment of a disorder manifesting one or more symptoms associated with an autism spectrum disorder. In some embodiments, the disorder is social anxiety disorder, obsessive-compulsive disorder, social (pragmatic) communication disorder, and neurodevelopmental disorders including but not limited to attention deficit hyperactivity disorder, Prader-Willi syndrome, Timothy syndrome, Fragile-X syndrome, Rett syndrome, or Williams syndrome, which exhibit symptoms similar to those displayed in autism spectrum disorder.

[0014] In one embodiment, the invention provides a method for treating an autism spectrum disorder comprising administering (e.g., intranasally) to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect. In one embodiment, the invention provides a method for treating an autism spectrum disorder comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein the effective dose of the oxytocin peptide and the magnesium ions is administered intranasally in a liquid formulation, and the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.

[0015] In one embodiment, the invention provides a method for treating Prader-Willi syndrome comprising administering (e.g., intranasally) to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect. In one embodiment, the invention provides a method for treating Prader-Willi syndrome comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein the effective dose of the oxytocin peptide and the magnesium ions is administered intranasally in a liquid formulation, and the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.

[0016] In one embodiment, provided is a method for treating social and communication deficits comprising administering (e.g., intranasally) to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect. In one embodiment, the invention provides a method for treating social and communication deficits comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein the effective dose of the oxytocin peptide and the magnesium ions is administered intranasally in a liquid formulation, and the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.

[0017] In one embodiment, provided is a method for treating anxiety comprising administering (e.g., intranasally) to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect. In one embodiment, the invention provides a method for treating anxiety comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein the effective dose of the oxytocin peptide and the magnesium ions is administered intranasally in a liquid

formulation, and the volume of the liquid formulation administered is between about 5 µL and about 1000 µL.

[0018] In some of these embodiments, the effective dose of the oxytocin peptide is about 0.5 µg to about 2000 µg. In some of these embodiments, the effective dose of the magnesium ions is about 50 µg to about 68 mg. In some of these embodiments, the effective dose of the oxytocin peptide and the magnesium ions comprises about 15 µg to about 120 µg of the oxytocin peptide administered in an aqueous solution containing about 1.1% to about 1.6% (w/v) of magnesium. In some of these embodiments, the effective dose of the oxytocin peptide and the magnesium ions comprises about 66 µg of the oxytocin peptide administered in an aqueous solution containing about 1.36% magnesium. In some of these embodiments, the weight ratio between the dose of the oxytocin peptide administered and the dose of the magnesium ions administered is between about 1:1 to about 1:1000. In some of these embodiments, the molar ratio between the dose of the oxytocin peptide administered and the dose of the magnesium ions administered is between about 1:40 to about 1:40000. In some of these embodiments, the volume of the liquid formulation administered is between about 50 µL and about 200 µL. In some of these embodiments, the liquid formulation is administered using a metered nasal device in 1 to 4 units of about 50 µL per unit (e.g., spray or puff). In some of these embodiments, the oxytocin peptide is human oxytocin (SEQ. ID NO:1).

[0019] In some of these embodiments, the liquid formulation is contained in a device for intranasal administration. In some of these embodiments, the device for intranasal administration is a nasal pump apparatus. In some of these embodiments, the nasal pump apparatus comprises a reservoir bottle attached to a pump actuator. In some of these embodiments, the pump actuator is metered to deliver a specified volume of about 50 µL. In some of these embodiments, the nasal pump apparatus comprises a reservoir bottle attached to an aerosolizer. In some of these embodiments, the nasal pump apparatus comprises one of more of the following: (i) a filter for preventing back flow, (ii) a metal-free fluid path, and (iii) a plastic material stable to gamma-radiation.

[0020] Further provided is a magnesium-containing oxytocin peptide formulation described herein for use in a method of treating an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or anxiety, in a subject in need thereof. Also provided is a use of a magnesium-containing oxytocin peptide formulation described herein in the manufacture of a medicament for the treatment of an autism spectrum disorder, a disorder manifesting one or

more symptoms associated with an autism spectrum disorder, social and communication deficits, or anxiety.

[0021] Also provided is a kit comprising a magnesium-containing oxytocin peptide formulation described herein contained in a device for intranasal administration such as a nasal pump apparatus and suitable packaging. The kit may further comprise instructions for administering the magnesium-containing oxytocin peptide formulation in a subject in need thereof for the treatment of an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or anxiety.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] FIG. **1** shows the effect of saline, oxytocin, a combination of magnesium citrate and oxytocin, and magnesium citrate in a rat model of social behavior.

[0023] FIG. **2** shows the effect of saline, oxytocin, a combination of magnesium citrate and oxytocin, and magnesium citrate in a rat model of anxiety.

[0024] FIGS. **3**A and **3**B show the effect of magnesium citrate, oxytocin, and combinations of magnesium citrate and oxytocin in an elevated plus maze rat model of anxiety.

DETAILED DESCRIPTION OF THE INVENTION

[0025] The invention provides, *inter alia*, a method for treating an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or anxiety, in a subject in need thereof, by craniofacial administration (e.g., intranasal administration) of an oxytocin peptide and magnesium ions, or a magnesium-containing oxytocin peptide formulation described herein. The oxytocin peptide and the magnesium ions are administered in an effective dose that produces a synergistic or enhanced effect compared to administration of the oxytocin peptide alone.

Definitions

[0026] As used herein, "oxytocin peptide" refers to a substance having biological activity associated with natural oxytocin. Oxytocin peptide can be a naturally occurring endogenous peptide, fragments, analogues or derivatives thereof. Oxytocin peptide can also be a non-endogenous peptide, fragments, analogues or derivatives thereof. In one aspect, the oxytocin

peptide is human oxytocin. In other aspects, the oxytocin peptide may be an analogue or derivative of human oxytocin.

[0027] As used herein, an "analogue" or "derivative" refers to any peptide analogous to naturally occurring oxytocin wherein one or more amino acids within the peptide have been substituted, deleted, or inserted. The term also refers to any peptide wherein one or more amino acids (for example one, two or three amino acids) have been modified, for example by chemical modification. In general, the term covers all peptides which exhibit oxytocin activity but which may, if desired, have a different potency or pharmacological profile.

[0028] As used herein, unless otherwise specified, the term "treatment" or "treating" refers to an approach for obtaining a beneficial or desired result, such as a clinical result. For an autism spectrum disorder and related disorders, beneficial or desired clinical results include, but are not limited to, alleviation of a symptom and/or diminishment of the extent of a symptom, for example, social and/or communication deficits and/or repetitive behaviors and/or anxiety. Social and communication deficits can include but are not limited to impairment in communication skills and/or social interaction, lack of eye contact, and/or an inability to form and/or maintain social relationships.

[0029] "Synergism", "synergy" or "synergistic effect" refers to a joint action of two or more compounds in such a manner that one supplements or enhances the action of the other to produce an effect greater than that which would be predicted or expected by adding the effects of given doses of two or more compounds if given individually. When two or more agents, used in combination, produces an overall effect (e.g., improvement in social and communication deficits and/or a reduction in anxiety) that is greater than individual effects of any of them in equivalent quantities that would be expected or predicted by summing the effects of the individual agents, it is said that a "synergistic effect" is achieved. When use of two or more agents in combination results in faster onset of effect and/or longer lasting effect than would occur following administration of the individual agents used alone in equivalent quantities, a "synergistic effect" is considered achieved also.

[0030] "Craniofacial mucosal administration" refers to delivery to the mucosal surfaces of the nose, nasal passageways, nasal cavity; the mucosal surfaces of the oral cavity including the gingiva (gums), the floor of the oral cavity, the lips, the tongue, the sublingual oral surfaces, including the frenulum of tongue and the floor of the mouth, and the mucosal surfaces of or around the eye including the conjunctiva, the lacrimal gland, the nasolacrimal ducts, and the mucosa of the upper or lower eyelid and the eye.

[0031] "Intranasal administration" or "administered intranasally" refers to delivery to the nose, nasal passageways or nasal cavity by spray, drops, powder, gel, film, inhalant or other means.

[0032] The "inferior region of the nasal cavity" refers generally to the portion of the nasal cavity where the middle and inferior turbinate bones protrude and is a region of the nasal cavity that is significantly innervated by the trigeminal nerve. The "superior region of the nasal cavity" is defined by the upper third and cribriform plate region wherein olfactory innervation is located.

[0033] A "subject" or "patient" as used herein refers to a mammal, including but not limited to a human. Mammals include, but are not limited to, farm animals (such as cows), sport animals, pets (such as guinea pigs, cats, dogs, rabbits and horses), primates, mice and rats. In one embodiment, a subject is a human.

[0034] It should be noted that, as used herein, the singular form "a", "an", and "the" includes plural references unless indicated otherwise. Additionally, as used herein, the term "comprising" and its cognates are used in their inclusive sense; that is, equivalent to the term "including" and its corresponding cognates.

[0035] Where a range of values is provided, it is intended that each intervening value between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the disclosure. For example, if a range of 1 μg to 8 μg is stated, it is intended that 2 μg, 3 μg, 4 μg, 5 μg, 6 μg, and 7 μg are also explicitly disclosed, as well as the range of values greater than or equal to 1 μg and the range of values less than or equal to 8 μg. If a range of 10-14% is stated, it is intended that 10%, 11%, 12%, 13%, and 14% are also explicitly disclosed. Furthermore, each smaller range in a stated range between any stated value or intervening value and any other stated or intervening value in that stated range is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither, or both limits are included in the smaller ranges is also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

Oxytocin Peptide

[0036] Oxytocin was one of the first peptide hormones to be isolated and sequenced. Natural oxytocin is a nine amino acid cyclic peptide hormone with two cysteine residues that

form a disulfide bridge between positions 1 and 6. The amino acid sequence for human oxytocin is Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly (SEQ ID NO:1).

[0037] There are processes described for the production of oxytocin, see for example U.S. Pat. No. 2,938,891 and U.S. Pat. No. 3,076,797; in addition, oxytocin is commercially available. A variety of peptide analogues and derivatives are available and others can be contemplated for use within the invention and can be produced and tested for biological activity according to known methods. Oxytocin analogues may include, but are not limited to, 4-threonine-1-hydroxy-deaminooxytocin, 4-serine-8-isoleucine-oxytocin, 9-deamidooxytocin, 7-D-proline-oxytocin and its deamino analogue, (2,4-diisoleucine)-oxytocin, deamino oxytocin analogue, 1-deamino-1-monocarba-E12-Tyr(OMe)]-OT(dCOMOT), 4-threonine-7-glycine-oxytocin (TG-OT), oxypressin, deamino-6-carba-oxytoxin (dC60), L-371,257 and the related series of compounds containing an ortho-trigluoro-ethoxyphenylacetyl core such as L-374,943. Other exemplary oxytocin analogues include 4-threonine-1-hydroxy-deaminooxytocin, 9-deamidooxytocin, an analogue of oxytocin containing a glycine residue in place of the glycinamide residue, (2,4-diisoleucine)-oxytocin, an analogue of oxytocin with natriuretic and diuretic activities, deamino oxytocin analogue; a long-acting oxytocin analogue, 1-deamino-1-monocarba-E12-[Tyr(OMe)]-OT(dCOMOT), carbetocin, (1-butanoic acid-2-(O-methyl-L-tyrosine)-1-carbaoxytocin, deamino-1 monocarba-(2-O-methyltyrosine)-oxytocin [d(COMOT)]), [Thr4-Gly7]-oxytocin (TG-OT), oxypressin, Ile-conopressin, deamino-6-carba-oxytoxin (dC60), d[Lys(8)(5/6C-Fluorescein)]VT, d[Thr(4), Lys(8)(5/6C-Fluorescein)]VT, [HO(1)][Lys(8)(5/6C-Fluorescein)]VT, [HO(1)][Thr(4), Lys(8)(5/6CFluorescein)]VT, d[Om(8)(5/6C-Fluorescein)]VT, d[Thr(4), Om(8)(5/6C-Fluorescein)]VT, [HO(1)][Om(8)(5/6C-Fluorescein)]VT, [HO(1)][Thr(4), Om(8)(5/6C-Fluorescein)]VT, and 1-deamino-oxytocin in which the disulfide bridge between residues 1 or 6 is replaced by a thioether, and desamino-oxytocin analogues in which the disulfide bond is replaced by a diselenide bond, a ditelluride bond, a telluroseleno bond, a tellurosulfide bond or a selenosulfide bond (e.g., the peptide analogues of oxytocin described in PCT patent application WO 2011/120,071, incorporated herein by reference). Peptides for use within the invention can be peptides that are obtainable by partial substitution, addition, or deletion of amino acids within a naturally occurring or native peptide sequence. Peptides can be chemically modified, for example, by amidation of the carboxyl terminus (-NH_2), the use of D amino acids in the peptide, incorporation of small non-peptidyl moieties, as well as the modification of the amino acids themselves (e.g. alkylation or esterification of side chain R-

groups). Such analogues, derivatives and fragments should substantially retain the desired biological activity of the native oxytocin peptide. In some embodiments, the oxytocin analogue is 4-serine-8-isoleucine-oxytocin or 9-deamidooxytocin. In some embodiments, the oxytocin analogue is carbetocin. The present disclosure also embrace other known oxytocin analogs, for example, the peptidic oxytocin receptor agonists described in PCT patent application WO 2012/042371 and Wiśniewski, et al. *J. Med. Chem.* **2014**, 57:5306-5317, the entire content of which is incorporated herein by reference. In some embodiments, the oxytocin analogue is a compound selected from Compound Nos. 1-65 described in Tables 1-3 in Wiśniewski, et al. *J. Med. Chem.* **2014**, 57:5306-5317. In some embodiments, the oxytocin analogue is a selected from the group consisting of Compound No. 31 ([2-ThiMeGly7]dOT), Compound No. 47 (carba-6-[Phe2,BuGly7]dOT), Compound No. 55 (carba-6-[3-MeBzlGly7]dOT) and Compound No. 57 (carba-1-[4-FBzlGly7]dOT, also referred to as merotocin).

[0038] In some embodiments, oxytocin or an oxytocin analogue is isotopically labeled by having one or more atoms replaced by an isotope having a different atomic mass. Examples of isotopes that may be incorporated into the disclosed compounds include isotopes of hydrogen (e.g., ^{2}H and ^{3}H), carbon (e.g., ^{13}C and ^{14}C), nitrogen (e.g., ^{15}N), oxygen (e.g., ^{18}O and ^{17}O), phosphorus (e.g., ^{31}P and ^{32}P), fluorine (e.g., ^{18}F), chlorine (e.g., ^{36}Cl) and sulfur (e.g. ^{35}S). The isotopically labeled compound may be administered to a subject or other subject and subsequently detected, yielding useful diagnostic and/or therapeutic management data, according to conventional techniques. Further, the isotopically labeled compound may be administered to a subject or other subject in need thereof, yielding therapeutically advantageous absorption, distribution, metabolism and/or elimination profiles. All isotopic variations of the oxytocin peptide, e.g. human oxytocin or an analogue or derivative thereof, whether radioactive or not, are contemplated.

[0039] In some embodiments, the oxytocin peptide is human oxytocin consisting of Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly (SEQ. ID NO:1).

[0040] An "international unit" (IU, UI or IE) is an internationally accepted unit of activity used to quantify vitamins, hormones and vaccines. It defines the amount of a substance that gives a unit of activity as determined using a defined biological assay in order to standardize preparations from multiple source materials. Similarly, a USP unit is a defined dosage unit established by the United States Pharmacopeia in cooperation with the Food and Drug Administration in order to ensure the identity, strength, quality, purity and consistency of a drug product. In general, USP units are equal to International Units, due to harmonization

efforts. By convention, for oxytocin, 1 unit of activity is generally defined as equal to approximately 2 micrograms of synthetic oxytocin peptide; or 1 mg is equal to 500 units (Stedman's Medical Dictionary). Therefore, as used herein, one "IU" or "International Unit" of an oxytocin peptide is the amount of the oxytocin peptide that has the same biological activity or produces the same level of a biological effect (e.g. contractile response of rat uterine strips) as approximately 2 micrograms of the synthetic peptide. An analogue with weaker activity would require more material to achieve the same level of biological effect. Determinations of drug potency are well known to those skilled in the art and may include either in vitro or in vivo assays using synthetic oxytocin as a reference. Atke and Vilhardt *Acta Endocrinol.* **1987**: 115(1):155-60; Engstrom et al. *Eur. J. Pharmacol.* **1998**: 355(2-3):203-10.

Magnesium-Containing Oxytocin Peptide Formulations

[0041] In the method of the present invention for the treatment an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or anxiety, comprising administering to a subject in need thereof in an effective dose of an oxytocin peptide and magnesium ions, the oxytocin peptide and the magnesium ions may be administered in a magnesium-containing oxytocin peptide formulation or composition. In one aspect, the magnesium-containing oxytocin peptide formulation or composition comprises the oxytocin peptide and the magnesium ions in an amount that produces a synergistic or enhanced effect when used in the treatment of an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or anxiety.

[0042] The relative proportion of the oxytocin peptide and the magnesium ions in the magnesium-containing oxytocin peptide formulation is important in achieving optimal synergistic or enhanced effect. The optimal amounts of the oxytocin peptide and the magnesium ions may depend on the specific disorder or symptoms, the type of synergistic or enhanced effect desired, and other factors such as the route of administration. For example, the amount of magnesium may be important to achieve a faster onset of effect; the amount of oxytocin may be important to achieve a longer-lasting effect and the relative ratio between oxytocin and magnesium may be important to achieve maximum improvement in social functioning, reduction of social and communication deficits, and/or decrease in anxiety.

[0043] In some embodiments, the magnesium-containing oxytocin peptide formulation or composition is a liquid formulation comprising between about 0.01 mg/mL and about 16

mg/mL of the oxytocin peptide. In some embodiments, the amount of the oxytocin peptide in the liquid formulation is greater than about (lower limit) 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1 or 2 mg/mL. In some embodiments, the amount of the oxytocin peptide in the liquid formulation is less than about (upper limit) 16, 12, 10, 8, 6, 4, 2, 1.6, 1.2, 1, 0.8, 0.6, 0.4, 0.3, 0.2 or 0.1 mg/mL. That is, the amount of the oxytocin peptide in the liquid formulation is anywhere in the range of from about 0.01 to 16 mg/mL in which the lower limit is less than the upper limit. In some embodiments, the magnesium-containing oxytocin peptide formulation or composition comprises between about 0.01 mg/mL and about 12 mg/mL, between about 0.05 mg/mL and about 16 mg/mL, between about 0.1 mg/mL and about 12 mg/mL, between about 0.1 mg/mL and about 8 mg/mL, between about 0.1 mg/mL and about 4 mg/mL, between about 0.1 mg/mL and about 2 mg/mL, between about 0.1 mg/mL and about 1.6 mg/mL, between about 0.1 mg/mL and about 1.2 mg/mL, between about 0.1 mg/mL and about 1 mg/mL, between about 0.1 mg/mL and about 0.8 mg/mL, between about 0.1 mg/mL and about 0.4 mg/mL, between about 0.1 mg/mL and about 0.3 mg/mL, between about 0.2 mg/mL and about 16 mg/mL, between about 0.2 mg/mL and about 12 mg/mL, between about 0.2 mg/mL and about 10 mg/mL, between about 0.2 mg/mL and about 8 mg/mL, between about 0.2 mg/mL and about 6 mg/mL, between about 0.2 mg/mL and about 4 mg/mL, between about 0.2 mg/mL and about 2 mg/mL, between about 0.2 mg/mL and about 1.6 mg/mL, between about 0.2 mg/mL and about 1.2 mg/mL, between about 0.2 mg/mL and about 1 mg/mL, between about 0.2 mg/mL and about 0.8 mg/mL, between about 0.2 mg/mL and about 0.6 mg/mL, between about 0.2 mg/mL and about 0.4 mg/mL, between about 0.2 mg/mL and about 0.3 mg/mL, between about 0.3 mg/mL and about 16 mg/mL, between about 0.3 mg/mL and about 12 mg/mL, between about 0.3 mg/mL and about 10 mg/mL, between about 0.3 mg/mL and about 8 mg/mL, between about 0.3 mg/mL and about 4 mg/mL, between about 0.3 mg/mL and about 3 mg/mL, between about 0.3 mg/mL and about 1 mg/mL, between about 0.3 mg/mL and about 0.5 mg/mL, between about 0.5 mg/mL and about 16 mg/mL, between about 0.5 mg/mL and about 10 mg/mL, between about 0.5 mg/mL and about 5 mg/mL, between about 0.5 mg/mL and about 1 mg/mL, between about 1 mg/mL and about 16 mg/mL, between about 1 mg/mL and about 10 mg/mL, or between about 1 mg/mL and about 5 mg/mL of the oxytocin peptide. In a preferred embodiment, the magnesium-containing oxytocin peptide formulation or composition comprises between about 0.1 mg/mL and about 2 mg/mL, between about 0.15 mg/mL and about 1.5 mg/mL, or between about 0.2 mg/mL and about 1.2 mg/mL of the oxytocin peptide. In one embodiment,

the oxytocin peptide is human oxytocin consisting of Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly (SEQ. ID NO:1).

[0044] In some embodiments, the magnesium-containing oxytocin peptide formulation or composition is a liquid formulation comprising between about 5 IU/mL and about 8000 IU/mL of the oxytocin peptide. In some embodiments, the amount of the oxytocin peptide in the liquid formulation is greater than about (lower limit) 5, 25, 50, 75, 100, 150, 200, 250, 500, 750 or 1000 IU/mL. In some embodiments, the amount of the oxytocin peptide in the liquid formulation is less than about (upper limit) 8000, 6000, 5000, 4000, 3000, 2000, 1000, 800, 600, 500, 400, 300, 200, 150, 100 or 50 IU/mL. That is, the amount of the oxytocin peptide in the liquid formulation is anywhere in the range of from about 5 to 8000 IU/mL in which the lower limit is less than the upper limit. In some embodiments, the magnesium-containing oxytocin peptide formulation or composition comprises between about 500 IU/mL and about 6000 IU/mL, between about 25 IU/mL and about 8000 IU/mL, between about 50 IU/mL and about 6000 IU/mL, between about 50 IU/mL and about 4000 IU/mL, between about 50 IU/mL and about 2000 IU/mL, between about 50 IU/mL and about 1000 IU/mL, between about 50 IU/mL and about 800 IU/mL, between about 50 IU/mL and about 600 IU/mL, between about 50 IU/mL and about 500 IU/mL, between about 50 IU/mL and about 400 IU/mL, between about 50 IU/mL and about 200 IU/mL, between about 50 IU/mL and about 150 IU/mL, between about 100 IU/mL and about 8000 IU/mL, between about 100 IU/mL and about 6000 IU/mL, between about 100 IU/mL and about 5000 IU/mL, between about 100 IU/mL and about 4000 IU/mL, between about 100 IU/mL and about 3000 IU/mL, between about 100 IU/mL and about 2000 IU/mL, between about 100 IU/mL and about 1000 IU/mL, between about 100 IU/mL and about 800 IU/mL, between about 100 IU/mL and about 600 IU/mL, between about 100 IU/mL and about 500 IU/mL, between about 100 IU/mL and about 400 IU/mL, between about 100 IU/mL and about 300 IU/mL, between about 100 IU/mL and about 200 IU/mL, between about 100 IU/mL and about 150 IU/mL, between about 150 IU/mL and about 8000 IU/mL, between about 150 IU/mL and about 6000 IU/mL, between about 150 IU/mL and about 5000 IU/mL, between about 150 IU/mL and about 4000 IU/mL, between about 150 IU/mL and about 2000 IU/mL, between about 150 IU/mL and about 1500 IU/mL, between about 150 IU/mL and about 500 IU/mL, between about 150 IU/mL and about 250 IU/mL, between about 250 IU/mL and about 8000 IU/mL, between about 250 IU/mL and about 5000 IU/mL, between about 250 IU/mL and about 2500 IU/mL, between about 250 IU/mL and about 500 IU/mL, between about 500 IU/mL and about 8000 IU/mL, between about 500 IU/mL and about 5000 IU/mL, or between about 500

IU/mL and about 2500 IU/mL of the oxytocin peptide. In a preferred embodiment, the magnesium-containing oxytocin peptide formulation or composition comprises between about 50 IU/mL and about 1000 IU/mL, between about 75 IU/mL and about 750 IU/mL, or between about 100 IU/mL and about 600 IU/mL of the oxytocin peptide. In one embodiment, the oxytocin peptide is human oxytocin consisting of Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly (SEQ. ID NO:1).

[0045] The amount of magnesium present in the formulation may also be expressed in percentage by weight (w/v) (grams of magnesium or Mg^{2+} per 100 mL of solution), in mg/mL (milligrams of magnesium or Mg^{2+} per milliliter of solution), or in molarity ("M" – defined as moles of magnesium or Mg^{2+} per liter of the solution; or "mM" – defined as millimoles of magnesium or Mg^{2+} per liter of the solution).

[0046] In some embodiments, the magnesium-containing oxytocin peptide formulation or composition is a liquid formulation comprising between about 1 mg/mL and about 30 mg/mL of magnesium or magnesium ions (Mg^{2+}). In some embodiments, the composition comprises between about 11 mg/mL and about 15 mg/mL of magnesium or magnesium ions. In some embodiments, the amount of the magnesium or magnesium ions in the liquid formulation is greater than about (lower limit) 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 mg/mL. In some embodiments, the amount of the magnesium or magnesium ions in the liquid formulation is less than about (upper limit) 30, 25, 20, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6 or 5 mg/mL. That is, the amount of magnesium or magnesium ions in the liquid formulation is anywhere in the range of from about 1 to 30 mg/mL in which the lower limit is less than the upper limit. In some embodiments, the magnesium-containing oxytocin peptide formulation or composition is a liquid formulation comprising between about 0.01 mg/mL and about 16 mg/mL (preferably between about 0.1 mg/mL and about 2 mg/mL, more preferably between about 0.15 mg/mL and about 1.5 mg/mL, or about 0.33 mg/mL) of the oxytocin peptide and between about 1 mg/mL and about 30 mg/mL (or between about 3 mg/mL and about 30 mg/mL, between about 4 mg/mL and about 30 mg/mL, between about 5 mg/mL and about 30 mg/mL, between about 8 mg/mL and about 30 mg/mL, between about 10 mg/mL and about 30 mg/mL, preferably between about 11 mg/mL and about 15 mg/mL, or about 13 mg/mL, or about 12 mg/mL) of magnesium or Mg^{2+}. In some embodiments, the magnesium-containing oxytocin peptide formulation or composition is a liquid formulation comprising between about 50 mM and about 1500 mM of magnesium or magnesium ions (Mg^{2+}). In some embodiments, the amount of the magnesium or magnesium ions in the liquid formulation is greater than about (lower limit) 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550 or 600

mM. In some embodiments, the amount of the magnesium or magnesium ions in the liquid formulation is less than about (upper limit) 1500, 1200, 1000, 750, 700, 650, 600, 550, 500, 450, 400, 350, 300 or 250 mM. That is, the amount of magnesium or magnesium ions in the liquid formulation is anywhere in the range of from about 50 to 1500 mM in which the lower limit is less than the upper limit. In some embodiments, the magnesium-containing oxytocin peptide formulation or composition is a liquid formulation comprising between about 5 IU/mL and about 8000 IU/mL (preferably between about 50 IU/mL and about 1000 IU/mL, more preferably between about 75 IU/mL and about 750 IU/mL, or about 150 IU/mL) of the oxytocin peptide and between about 1 mg/mL and about 30 mg/mL (preferably between about 11 mg/mL and about 15 mg/mL, or about 13 mg/mL, or about 12 mg/mL) of magnesium or Mg^{2+}. In some embodiments, the magnesium-containing oxytocin peptide formulation or composition is a liquid formulation comprising between about 5 IU/mL and about 8000 IU/mL (preferably between about 50 IU/mL and about 1000 IU/mL, more preferably between about 75 IU/mL and about 750 IU/mL, or about 150 IU/mL) of the oxytocin peptide and between about 50 mM and about 1200 mM (or between about 100 mM and about 1200 mM, between about 150 mM and about 1200 mM, between about 200 mM and about 1200 mM, between about 300 mM and about 1200 mM, between about 400 mM and about 1200 mM, preferably between about 400 mM and about 600 mM, or about 500 mM) of magnesium or Mg^{2+}.

[0047] Any magnesium salt (such as a water-soluble magnesium salt) may be used to provide the magnesium ions in the magnesium-containing oxytocin peptide formulation. The magnesium salt used in the magnesium-containing oxytocin peptide formulation may be selected based on a number of factors such as the amount of free magnesium ions that can be delivered when the formulation is administered, the solubility of the magnesium salt in the media for a liquid formulation, the acidity/basicity of the counter ion, and/or the dissociation constant of the salt. For example, in a liquid formulation, the magnesium salt needs to be sufficiently soluble in the liquid media to deliver the magnesium ions in concentration required for producing synergistic or enhanced effect with the oxytocin peptide. Other factors may also be considered when selecting the magnesium salt, such as compatibility with other substances in the formulation and ability of the counter ion to perform other functions in the formulation. For example, magnesium citrate is sufficiently soluble in an aqueous solution to provide the desirable amount of magnesium or desirable magnesium ion concentration; citrate salts are pharmaceutically acceptable; the citrate can be part of the buffering agents; and magnesium citrate may add a pleasant flavor for the formulation. The magnesium ions in

the magnesium-containing oxytocin peptide formulation may be provided by using one or more magnesium salts. A magnesium salt in the magnesium-containing oxytocin peptide formulation may be a magnesium salt used initially in preparing of the magnesium-containing oxytocin peptide formulation, or formed in situ during preparation of the magnesium-containing oxytocin peptide formulation. For example, magnesium chloride may be used initially in preparing the formulation; and upon addition of citric acid to the formulation, magnesium citrate may be formed in situ. In such instance, the magnesium ions in the magnesium-containing oxytocin peptide formulation are provided by both magnesium chloride and magnesium citrate.

[0048] In some embodiments, the magnesium-containing oxytocin peptide formulation or composition comprises one or more magnesium salts selected from the group consisting of magnesium citrate, magnesium chloride, magnesium sulfate, magnesium acetate, magnesium lactate, magnesium stearate, magnesium oxide, magnesium carbonate, magnesium glycinate, magnesium maltate, magnesium taurate, magnesium gluconate, magnesium succinate, and magnesium pyrophosphate. In some embodiments, the magnesium-containing oxytocin peptide formulation or composition is a liquid formulation comprising a magnesium salt (e.g., magnesium citrate or magnesium chloride) in an amount to provide between about 1 mg/mL and about 30 mg/mL of magnesium. In some embodiments, the composition comprises a magnesium salt in an amount to provide between about 1 mg/mL and about 30 mg/mL of magnesium ions (Mg^{2+}). In some embodiments, the magnesium-containing oxytocin peptide formulation or composition is a liquid formulation comprising one or more magnesium salts (e.g., magnesium citrate and/or magnesium chloride) in an amount to provide between about 1 mg/mL and about 30 mg/mL of magnesium or magnesium ions (Mg^{2+}). In some embodiments, the composition comprises one or more magnesium salts in an amount to provide between about 11 mg/mL and about 15 mg/mL of magnesium or magnesium ions. In some embodiments, the magnesium-containing oxytocin peptide formulation or composition is a liquid formulation comprising between about 0.01 mg/mL and about 16 mg/mL (preferably between about 0.1 mg/mL and about 2 mg/mL, more preferably between about 0.15 mg/mL and about 1.5 mg/mL, or about 0.33 mg/mL) of the oxytocin peptide and a magnesium salt (e.g., magnesium citrate or magnesium chloride) in an amount to provide between about 1 mg/mL and about 30 mg/mL (or between about 3 mg/mL and about 30 mg/mL, between about 4 mg/mL and about 30 mg/mL, between about 5 mg/mL and about 30 mg/mL, between about 8 mg/mL and about 30 mg/mL, between about 10 mg/mL and about 30 mg/mL, preferably between about 11 mg/mL and about 15 mg/mL, or about 13 mg/mL, or

about 12 mg/mL) of magnesium or Mg^{2+}. In some embodiments, the magnesium-containing oxytocin peptide formulation or composition is a liquid formulation comprising between about 5 IU/mL and about 8000 IU/mL (preferably between about 50 IU/mL and about 1000 IU/mL, more preferably between about 75 IU/mL and about 750 IU/mL, or about 150 IU/mL) of the oxytocin peptide and one or more magnesium salts (e.g., magnesium citrate and/or magnesium chloride) in an amount to provide between about 1 mg/mL and about 30 mg/mL (preferably between about 11 mg/mL and about 15 mg/mL, or about 13 mg/mL, or about 12 mg/mL) of magnesium or Mg^{2+}. In some embodiments, the magnesium-containing oxytocin peptide formulation or composition is a liquid formulation comprising between about 5 IU/mL and about 8000 IU/mL (preferably between about 50 IU/mL and about 1000 IU/mL, more preferably between about 75 IU/mL and about 750 IU/mL, or about 150 IU/mL) of the oxytocin peptide and one or more magnesium salts (e.g., magnesium citrate and/or magnesium chloride) in an amount to provide between about 50 mM and about 1200 mM (or between about 100 mM and about 1200 mM, between about 150 mM and about 1200 mM, between about 200 mM and about 1200 mM, between about 300 mM and about 1200 mM, between about 400 mM and about 1200 mM, preferably between about 400 mM and about 600 mM, or about 500 mM) of magnesium or Mg^{2+}.

[0049] The relative amount of the oxytocin peptide and the magnesium ions in the magnesium-containing oxytocin peptide formulation or composition detailed herein may be defined by a weight ratio or a molar ratio. The weight ratio between the amount of the oxytocin peptide and the amount of magnesium or magnesium ions in the formulation or composition is referred to as the "OT/Mg (w) ratio". For example, in a magnesium-containing oxytocin peptide formulation or composition having an OT/Mg (w) ratio of about 1:40, for each 1 mg of the oxytocin peptide present in the formulation or composition, the magnesium or magnesium ions present in the formulation or composition is about 40 mg. The molar ratio between the amount of the oxytocin peptide and the amount of magnesium or magnesium ions in the formulation or composition is referred to as the "OT/Mg (m) ratio". For example, in a magnesium-containing oxytocin peptide formulation or composition having an OT/Mg (m) ratio of about 1:1600, for each 1 μmol of the oxytocin peptide present in the formulation or composition, the magnesium or magnesium ions present in the formulation or composition is about 1600 μmol.

[0050] In some embodiments, the magnesium-containing oxytocin peptide formulation or composition has an OT/Mg (w) ratio between about 1:1 and about 1:1000. In some embodiments, the OT/Mg (w) ratio in the formulation or composition is less than about

(upper limit) 1:1, 1:2, 1:5, 1:10, 1:20, 1:30, 1:40, 1:45, 1:50, 1:60, 1:80, 1:100 or 1:200. In some embodiments, the OT/Mg (w) ratio in the formulation or composition is greater than about (lower limit) 1:1000, 1:800, 1:500, 1:250, 1:200, 1:150, 1:100, 1:80, 1:60, 1:50, 1:40, 1:30, 1:20, 1:10 or 1:5. That is, the OT/Mg (w) ratio in the formulation or composition is anywhere in the range of from about 1:1 to 1:1000 in which the upper limit is more than the lower limit. In some embodiments, the formulation or composition has an OT/Mg (w) ratio between about 1:2 and about 1:200. In some preferred embodiments, the formulation or composition has an OT/Mg (w) ratio of about 1:30, about 1:35, about 1:40, about 1:45, or about 1:50. In some embodiments, the formulation or composition has an OT/Mg (w) ratio between about 1:2 and about 1:1000, between about 1:2 and about 1:800, between about 1:2 and about 1:500, between about 1:2 and about 1:250, between about 1:2 and about 1:150, between about 1:2 and about 1:100, between about 1:2 and about 1:80, between about 1:2 and about 1:60, between about 1:2 and about 1:50, between about 1:2 and about 1:40, between about 1:2 and about 1:30, between about 1:2 and about 1:20, between about 1:2 and about 1:10, between about 1:2 and about 1:5, between about 1:5 and about 1:1000, between about 1:5 and about 1:800, between about 1:5 and about 1:500, between about 1:5 and about 1:200, between about 1:5 and about 1:100, between about 1:5 and about 1:80, between about 1:5 and about 1:60, between about 1:5 and about 1:50, between about 1:5 and about 1:40, between about 1:5 and about 1:30, between about 1:5 and about 1:20, between about 1:5 and about 1:10, between about 1:10 and about 1:1000, between about 1:10 and about 1:800, between about 1:10 and about 1:500, between about 1:10 and about 1:200, between about 1:10 and about 1:100, between about 1:10 and about 1:80, between about 1:10 and about 1:60, between about 1:10 and about 1:50, between about 1:10 and about 1:40, between about 1:10 and about 1:30, between about 1:10 and about 1:20, between about 1:20 and about 1:1000, between about 1:20 and about 1:800, between about 1:20 and about 1:500, between about 1:20 and about 1:200, between about 1:20 and about 1:100, between about 1:20 and about 1:80, between about 1:20 and about 1:70, between about 1:20 and about 1:60, between about 1:20 and about 1:50, between about 1:20 and about 1:40, between about 1:20 and about 1:30, between about 1:30 and about 1:1000, between about 1:30 and about 1:800, between about 1:30 and about 1:500, between about 1:30 and about 1:200, between about 1:30 and about 1:100, between about 1:30 and about 1:80, between about 1:30 and about 1:70, between about 1:30 and about 1:60, between about 1:30 and about 1:50, between about 1:30 and about 1:40, between about 1:35 and about 1:45, between about 1:40 and about 1:1000, between about 1:40 and about 1:800, between about 1:40 and about 1:500, between about 1:40 and

about 1:200, between about 1:40 and about 1:100, between about 1:40 and about 1:80, between about 1:40 and about 1:70, between about 1:40 and about 1:60, between about 1:40 and about 1:50, between about 1:50 and about 1:1000, between about 1:50 and about 1:800, between about 1:50 and about 1:500, between about 1:50 and about 1:200, between about 1:50 and about 1:100, between about 1:50 and about 1:90, between about 1:50 and about 1:80, between about 1:50 and about 1:70, between about 1:50 and about 1:60, between about 1:60 and about 1:1000, between about 1:60 and about 1:800, between about 1:60 and about 1:500, between about 1:60 and about 1:200, between about 1:60 and about 1:100, between about 1:60 and about 1:90, between about 1:60 and about 1:80, between about 1:60 and about 1:70, between about 1:80 and about 1:1000, between about 1:80 and about 1:800, between about 1:80 and about 1:500, between about 1:80 and about 1:200, between about 1:80 and about 1:100, between about 1:100 and about 1:1000, between about 1:100 and about 1:800, between about 1:100 and about 1:500, between about 1:100 and about 1:200, between about 1:200 and about 1:1000, between about 1:200 and about 1:800, between about 1:200 and about 1:500, or between about 1:500 and about 1:1000. In one embodiment, the oxytocin peptide is human oxytocin consisting of Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly (SEQ. ID NO:1).

[0051] In some embodiments, the magnesium-containing oxytocin peptide formulation or composition has an OT/Mg (m) ratio between about 1:40 and about 1:40,000. In some embodiments, the OT/Mg molar ratio in the formulation or composition is less than about (upper limit) 1:40, 1:80, 1:100, 1:150, 1:175, 1:200, 1:250, 1:280, 1:300, 1:400, 1:500, 1:560, 1:800, 1:1000, 1:1100, 1:1200, 1:1600, 1:1700, 1:1800, 1:2000, 1:2400, 1:3200, 1:4000 or 1:8000. In some embodiments, the OT/Mg molar ratio in the formulation or composition is greater than about (lower limit) 1:40000, 1:30000, 1:20000, 1:10000, 1:7500, 1:5000, 1:4000, 1:3000, 1:2500, 1:2000, 1:1600, 1:1200, 1:1100, 1:1000, 1:800, 1:600, 1:400 or 1:200. That is, the OT/Mg (w) ratio in the formulation or composition is anywhere in the range of from about 1:40 to 1:40000 in which the upper limit is more than the lower limit. In some embodiments, the formulation or composition has an OT/Mg (m) ratio between about 1:80 and about 1:8000. In some preferred embodiments, the formulation or composition has an OT/Mg (m) ratio of about 1:175, about 1:280, about 1:560, about 1:1100, about 1:1200, about 1:1400, about 1:1600, about 1:1700, about 1:1800, or about 1:2000. In some embodiments, the formulation or composition has an OT/Mg (m) ratio between about 1:80 and about 1:40000, between about 1:80 and about 1:30000, between about 1:80 and about 1:20000, between about 1:80 and about 1:10000, between about 1:80 and about 1:7500, between about

1:80 and about 1:5000, between about 1:80 and about 1:3000, between about 1:80 and about 1:2000, between about 1:80 and about 1:1600, between about 1:80 and about 1:1200, between about 1:80 and about 1:800, between about 1:80 and about 1:400, between about 1:80 and about 1:200, between about 1:175 and about 1:40000, between about 1:175 and about 1:30000, between about 1:175 and about 1:20000, between about 1:175 and about 1:10000, between about 1:175 and about 1:5000, between about 1:175 and about 1:3000, between about 1:175 and about 1:2400, between about 1:175 and about 1:2000, between about 1:175 and about 1:1700, between about 1:175 and about 1:1600, between about 1:175 and about 1:1200, between about 1:175 and about 1:1100, between about 1:175 and about 1:800, between about 1:175 and about 1:560, between about 1:175 and about 1:400, between about 1:175 and about 1:280, between about 1:200 and about 1:40000, between about 1:200 and about 1:30000, between about 1:200 and about 1:20000, between about 1:200 and about 1:10000, between about 1:200 and about 1:5000, between about 1:200 and about 1:3000, between about 1:200 and about 1:2400, between about 1:200 and about 1:2000, between about 1:200 and about 1:1600, between about 1:200 and about 1:1200, between about 1:200 and about 1:800, between about 1:200 and about 1:400, between about 1:280 and about 1:40000, between about 1:280 and about 1:30000, between about 1:280 and about 1:20000, between about 1:280 and about 1:10000, between about 1:280 and about 1:5000, between about 1:280 and about 1:3000, between about 1:280 and about 1:2400, between about 1:280 and about 1:2000, between about 1:280 and about 1:1700, between about 1:280 and about 1:1600, between about 1:280 and about 1:1200, between about 1:280 and about 1:1100, between about 1:280 and about 1:800, between about 1:280 and about 1:560, between about 1:280 and about 1:400, between about 1:400 and about 1:40000, between about 1:400 and about 1:30000, between about 1:400 and about 1:20000, between about 1:400 and about 1:8000, between about 1:400 and about 1:4000, between about 1:400 and about 1:3000, between about 1:400 and about 1:2400, between about 1:400 and about 1:2000, between about 1:400 and about 1:1600, between about 1:400 and about 1:1200, between about 1:400 and about 1:800, between about 1:560 and about 1:40000, between about 1:560 and about 1:30000, between about 1:560 and about 1:20000, between about 1:560 and about 1:8000, between about 1:560 and about 1:4000, between about 1:560 and about 1:3000, between about 1:560 and about 1:2400, between about 1:560 and about 1:2000, between about 1:560 and about 1:1700, between about 1:560 and about 1:1600, between about 1:560 and about 1:1200, between about 1:560 and about 1:1100, between about 1:560 and about 1:800, between about 1:800 and about 1:40000, between about 1:800 and about 1:30000, between

about 1:800 and about 1:20000, between about 1:800 and about 1:10000, between about 1:800 and about 1:5000, between about 1:800 and about 1:3000, between about 1:800 and about 1:2400, between about 1:800 and about 1:2000, between about 1:800 and about 1:1600, between about 1:800 and about 1:1200, between about 1:1100 and about 1:40000, between about 1:1100 and about 1:30000, between about 1:1100 and about 1:20000, between about 1:1100 and about 1:10000, between about 1:1100 and about 1:5000, between about 1:1100 and about 1:4000, between about 1:1100 and about 1:3000, between about 1:1100 and about 1:2400, between about 1:1100 and about 1:2000, between about 1:1100 and about 1:1700, between about 1:1100 and about 1:1600, between about 1:1200 and about 1:40000, between about 1:1200 and about 1:30000, between about 1:1200 and about 1:20000, between about 1:1200 and about 1:10000, between about 1:1200 and about 1:5000, between about 1:1200 and about 1:4000, between about 1:1200 and about 1:3000, between about 1:1200 and about 1:2400, between about 1:1200 and about 1:2000, between about 1:1200 and about 1:1600, between about 1:1400 and about 1:1800, between about 1:1600 and about 1:40000, between about 1:1600 and about 1:30000, between about 1:1600 and about 1:20000, between about 1:1600 and about 1:10000, between about 1:1600 and about 1:5000, between about 1:1600 and about 1:3000, between about 1:1600 and about 1:2400, between about 1:1600 and about 1:2000, between about 1:1700 and about 1:40000, between about 1:1700 and about 1:30000, between about 1:1700 and about 1:20000, between about 1:1700 and about 1:10000, between about 1:1700 and about 1:5000, between about 1:1700 and about 1:3000, between about 1:1700 and about 1:2400, between about 1:1700 and about 1:2000, between about 1:2000 and about 1:40000, between about 1:2000 and about 1:30000, between about 1:2000 and about 1:20000, between about 1:2000 and about 1:10000, between about 1:2000 and about 1:5000, between about 1:2000 and about 1:4000, between about 1:2000 and about 1:3000, between about 1:2000 and about 1:2400, between about 1:2400 and about 1:40000, between about 1:2400 and about 1:30000, between about 1:2400 and about 1:20000, between about 1:2400 and about 1:10000, between about 1:2400 and about 1:5000, between about 1:2400 and about 1:4000, between about 1:2400 and about 1:3000, between about 1:3000 and about 1:40000, between about 1:3000 and about 1:30000, between about 1:3000 and about 1:20000, between about 1:3000 and about 1:10000, between about 1:3000 and about 1:4000, between about 1:4000 and about 1:40000, between about 1:4000 and about 1:30000, between about 1:4000 and about 1:20000, between about 1:4000 and about 1:10000, between about 1:8000 and about 1:40000, between about 1:8000 and about 1:30000, between about 1:8000 and about 1:20000, or between about 1:10000 and about 1:40000. In one embodiment, the oxytocin

peptide is human oxytocin consisting of Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly (SEQ. ID NO:1).

[0052] In some embodiments, the magnesium-containing oxytocin peptide formulation or composition comprising an oxytocin peptide and magnesium ions further comprises one or more pharmaceutically acceptable carriers (thus constituting a pharmaceutical composition) and optionally other ingredients, such as excipients, vehicles, emulsifiers, stabilizers, preservatives, buffers, and/or other additives that may enhance stability, delivery, absorption, half-life, efficacy, pharmacokinetics, and/or pharmacodynamics, reduce adverse side effects, or provide other advantages for pharmaceutical use. Exemplary excipients include solubilizers, surfactants and chelators. For example, formulations may include, methyl-β-cyclodextrin (Me-β-CD), edetate disodium, arginine, sorbitol, NaCl, methylparaben sodium (MP), propylparaben sodium (PP), chlorobutanol (CB), benzyl alcohol, zinc chloride, ethyl alcohol, didecanoyl L-α-phosphatidylcholine (DDPC), polysorbate, lactose, citrate, tartrate, acetate, and/or phosphate.

[0053] Liquid carriers include, but are not limited to, water, saline, aqueous dextrose, and glycols particularly (when isotonic) for solutions. The carrier can also be selected from various oils, including those of petroleum, animal, vegetable or synthetic origin (e.g. peanut oil, olive oil, soybean oil, mineral oil, sesame oil, and the like). Suitable pharmaceutical excipients include, but are not limited to, starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the like. The compositions can be subjected to conventional pharmaceutical processes, such as sterilization, and can contain conventional pharmaceutical additives, such as preservatives, stabilizing agents, reducing agents, anti-oxidants, chelating agents, wetting agents, emulsifying agents, dispersing agents, jelling agents, salts for adjusting osmotic pressure, buffers, and the like. A liquid carrier may be hypotonic or isotonic with body fluids and may have a pH within the range of 3.5-8.5. The use of additives in the preparation of peptide and/or protein-based compositions, particularly pharmaceutical compositions, is well-known in the art. In some embodiments, the composition has a pH of about 2 to about 7. In some embodiments, the composition has a pH of about 4 to about 7. In a preferred embodiment, the pH of the formulation/composition is about 4.5.

[0054] In some embodiments, the magnesium-containing oxytocin peptide formulation or composition may further comprise one or more mucosal delivery-enhancing agents selected from (A)-(K): (A) solubilization agents; (B) charge modifying agents; (C) pH control agents;

(D) degradative enzyme inhibitors; (E) mucolytic or mucus clearing agents; (F) ciliostatic agents; (G) membrane penetration-enhancing agents; (H) modulatory agents of epithelial junction physiology, such as nitric oxide (NO) stimulators, chitosan, and chitosan derivatives; (I) vasodilator agents; (J) selective transport-enhancing agents; and (K) stabilizing delivery vehicles, carriers, supports or complex-forming species with which the oxytocin peptide is effectively combined, associated, contained, encapsulated or bound to stabilize the active agent for enhanced mucosal delivery. Membrane penetration-enhancing agents in Group (G) may be (i) a surfactant, (ii) a bile salt, (iii) a phospholipid or fatty acid additive, mixed micelle, liposome, or carrier, (iv) an alcohol, (v) an enamine, (iv) an NO donor compound, (vii) a long-chain amphipathic molecule, (viii) a small hydrophobic penetration enhancer; (ix) sodium or a salicylic acid derivative; (x) a glycerol ester of acetoacetic acid, (xi) a cyclodextrin or beta-cyclodextrin derivative, (xii) a medium-chain fatty acid, (xiii) a chelating agent, (xiv) an amino acid or salt thereof, (xv) an N-acetylamino acid or salt thereof, (xvi) an enzyme degradative to a selected membrane component, (xvii) an inhibitor of fatty acid synthesis, (xviii) an inhibitor of cholesterol synthesis; or (xiv) any combination of the membrane penetration enhancing agents of (i)-(xviii). In various embodiments of the invention, an oxytocin peptide may be combined with one, two, three, four or more of the mucosal delivery-enhancing agents recited in (A)-(K). These mucosal delivery-enhancing agents may be admixed, alone or together, with the oxytocin peptide, or otherwise combined therewith in a pharmaceutically acceptable formulation or delivery vehicle. The magnesium-containing oxytocin peptide formulation or composition described herein may provide increased bioavailability of the oxytocin peptide following delivery thereof to a mucosal surface (e.g., in the nasal cavities) of a mammalian subject.

[0055] The lists of carriers and additives discussed herein are by no means complete and a worker skilled in the art can choose carriers and excipients from the GRAS (generally regarded as safe) list of chemicals allowed in pharmaceutical preparations and those that are currently allowed by the U.S. Food and Drug Administration in topical and parenteral formulations, and those that become allowed in the future. (See also Wang et al., (1980) *J. Parent. Drug Assn.*, 34:452-462; Wang et al., (1988) *J. Parent. Sci. and Tech.*, 42:S4-S26.)

[0056] In some embodiments, the magnesium-containing oxytocin peptide formulation or composition, comprising an oxytocin peptide and magnesium ions, wherein the oxytocin peptide and the magnesium ions are in an amount that produces a synergistic or enhanced effect when used in the treatment of an autism spectrum disorder, further comprises one or more solvent or excipient selected from the group consisting of chlorobutanol, benzalkonium,

methyl 4-hydroxybenzoate, propyl 4-hydroxybenzoate, acetic acid, citric acid, glycerol, sodium chloride, sodium monohydrogen phosphate, sorbitol and water. In some embodiments, the magnesium-containing oxytocin peptide formulation or composition further comprises chlorobutanol, acetic acid and water.

[0057] In some embodiments, the magnesium-containing oxytocin peptide formulation or composition, comprising an oxytocin peptide and magnesium ions, further comprises a chitosan-containing excipient (e.g., ChiSys®, http://www.archimedespharma.com/productArchiDevChiSys.html). In some embodiments, the magnesium-containing oxytocin peptide formulation or composition further comprises about 1% of the chitosan-containing excipient. In some embodiments, a chitosan glutamate salt may be preferred for nasal delivery for its superior absorption enhancing ability. In some embodiments, chitosan co-polymer nanoparticles may be used, such as nanoparticles containing chitosan glutamate and a negatively charged polymer (e.g., tripolyphosphate pentasodium). Thiolated chitosans (e.g. chitosan covalently modified with 2-iminothiolane), which have been used in microparticles containing insulin and reduced glutathione, may also be useful as an excipient in the magnesium-containing oxytocin peptide formulation or composition described herein.

[0058] In some embodiments, the magnesium-containing oxytocin peptide formulation or composition, comprising an oxytocin peptide and magnesium ions, further comprises one or more gelling agents, such that the oxytocin peptide formulation forms a gel in the nasal cavity, thus enhancing nasal absorption of the oxytocin peptide. Gelling systems useful in the formulations and methods described herein may include any known gelling system, such as a chemically reactive pectin-based gelling system (e.g., PecSys™, Archimedes Pharma) and a thermoreactive polymer gelling system (e.g., Pluronic® F127, BASF). PecSys™ is a low viscosity aqueous pectin based solution, delivered as a fine mist in which each droplet gels on contact with calcium ions in the nasal mucosa. Other low methoxy pectin could also be employed, e.g., at about 1% concentration. Pluronic® F127 contains ethylene oxide/propylene oxide block copolymers. The gelling temperatures vary depending on the ratios of components and the amount of co-polymer employed in the final formulation. Gelling in the human nasal cavity has been demonstrated for Pluronic® F127 at approximately 18-20% wt/vol, for examples, as used in a vitamin B12 gel supplement (EnerB, Nature's Bounty, NY) and in a gelling sumatriptan, which contains 18% wt/vol Pluronic® F127 and 0.3% wt/vol Carbopol (anionic bioadhesive polymer C934P). The

monomer ratios and concentrations may be adjusted for the intended oxytocin formulations to ensure gelling at 25-37 °C, around the typical temperature of 34 °C in nasal cavity. If the gelation temperature is lower than 25 °C, the formulation could gel at room temperature; if the gelation temperature is above 37 °C, the formulation would not fully gel on contact with the nasal mucosa. In some embodiments, the magnesium-containing oxytocin peptide formulation or composition may further comprise a mucoadhesive agent such as Carbopol. Addition of a mucoadhesive, e.g., addition of up to 0.5% Carbopol, may further lower the gelation temperature.

[0059] In some embodiments, the magnesium-containing oxytocin peptide formulation or composition, comprising an oxytocin peptide and magnesium ions, further comprises a surface active agent, such as a nonionic surfactant (e.g., polysorbate-80), and one or more buffers, stabilizers, or tonicifiers. In some embodiments, the magnesium-containing oxytocin peptide formulation or composition further comprises a propellant. The pH of the nasal spray solution is optionally between about pH 3.0 and 8.5, but when desired the pH is adjusted to optimize delivery of a charged macromolecular species (e.g., a therapeutic protein or peptide) in a substantially unionized state. The pharmaceutical solvents employed can also be a slightly acidic aqueous buffer (pH 3-6). Suitable buffers for use within these compositions are as described above or as otherwise known in the art. Other components may be added to enhance or maintain chemical stability, including preservatives, surfactants, dispersants, or gases. Suitable preservatives include, but are not limited to, phenol, methyl paraben, paraben, *m*-cresol, thiomersal, benzalkonium chloride, and the like. Suitable surfactants include, but are not limited to, oleic acid, sorbitan trioleate, polysorbates, lecithin, phosphotidyl cholines, and various long chain diglycerides and phospholipids. Suitable dispersants include, but are not limited to, ethylenediaminetetraacetic acid (EDTA), and the like. Suitable gases include, but are not limited to, nitrogen, helium, chlorofluorocarbons (CFCs), hydrofluorocarbons (HFCs), carbon dioxide, air, and the like. Suitable stabilizers and tonicifying agents include sugars and other polyols, amino acids, and organic and inorganic salts. In some embodiments, the magnesium-containing oxytocin peptide formulation or composition further comprises a citrate salt, a succinate salt or a pyrophosphate salt.

[0060] In some embodiments, the magnesium-containing oxytocin peptide formulation or composition, comprising an oxytocin peptide and magnesium ions, further comprises an agent capable of upregulating oxytocin receptor expression, such as IL-6.

[0061] To further enhance the mucosal delivery of the oxytocin peptide, an enzyme inhibitor, particularly proteases inhibitors, can be included further in the formulation. Protease inhibitors may include, but are not limited to, antipain, arphamenine A and B, benzamidine HCl, AEBSF, CA-074, calpain inhibitor I and II, calpeptin, pepstatin A, actinonin, amastatin, bestatin, boroleucine, captopril, chloroacetyl-HOLeu-Ala-Gly-NH_2, DAPT, diprotin A and B, ebelactone A and B, foroxymithine, leupeptin, phosphoramidon, aprotinin, puromycin, BBI, soybean trypsin inhibitor, phenylmethylsulfonyl fluoride, E-64, chymostatin, 1,10-phenanthroline, EDTA and EGTA. Other enzyme inhibitors such as bacitracin may also be included in the formulation.

[0062] To enhance delivery into or across a mucosal surface and/or absorption of the oxytocin peptide and the magnesium ions, an absorption-enhancing agent can be included in the formulation. These enhancing agents may enhance the release or solubility (e.g., from a formulation delivery vehicle), diffusion rate, penetration capacity and timing, uptake, residence time, stability, effective half-life, peak or sustained concentration levels, clearance and other desired mucosal delivery characteristics (e.g., as measured at the site of delivery) of the composition. Enhancement of mucosal delivery can thus occur by any of a variety of mechanisms, for example by increasing the diffusion, transport, persistence or stability of the oxytocin peptide, increasing membrane fluidity, modulating the availability or action of calcium and other ions that regulate intracellular or paracellular permeation, solubilizing mucosal membrane components (e.g., lipids), changing non-protein and protein sulfhydryl levels in mucosal tissues, increasing water flux across the mucosal surface, modulating epithelial junctional physiology, reducing the viscosity of mucus overlying the mucosal epithelium, reducing mucociliary clearance rates, and other mechanisms.

[0063] Mucosal absorption enhancing compounds may include, but are not limited to, surfactants, bile salts, dihydrofusidates, bioadhesive/mucoadhesive agents, phospholipid additives, mixed micelles, liposomes, or carriers, alcohols, enamines, cationic polymers, NO donor compounds, long-chain amphipathic molecules, small hydrophobic penetration enhancers; sodium or a salicylic acid derivatives, glycerol esters of acetoacetic acid, cyclodextrin or beta-cyclodextrin derivatives, medium-chain fatty acids, chelating agents, amino acids or salts thereof, *N*-acetylamino acids or salts thereof, mucolytic agents, enzymes specifically targeted to a selected membrane component, inhibitors of fatty acid synthesis and inhibitors of cholesterol synthesis.

[0064] All peptides described and/or contemplated herein can be prepared by chemical synthesis using either automated or manual solid phase synthetic technologies, generally

known in the art. The peptides can also be prepared using molecular recombinant techniques known in the art.

Delivery Systems

[0065] The magnesium-containing oxytocin peptide formulation or composition may be adapted for craniofacial mucosal administration (e.g., nasal, buccal, sublingual or ocular administration). In some embodiments, the composition may further comprise a device for mucosal delivery. In some embodiments, the composition is adapted for buccal and/or sublingual mucosal delivery, which may further comprise a device for buccal and/or sublingual mucosal administration, such as unit dose containers, pump sprays, droppers, squeeze bottles, airless and preservative-free sprays, nebulizers, dose inhalers and pressurized dose inhalers. In some embodiments, the composition is adapted for ocular delivery, which may further comprise a device for conjunctival administration, such as a dropper or a squeeze bottle. In some embodiments, the composition is adapted for intranasal administration, which may further comprise a device for intranasal administration, such as a dropper, pump spray, squeeze bottle, airless and preservative-free sprays, or a nasal pump apparatus, e.g., a nasal pump apparatus comprising a reservoir bottle attached to an aerosolizer.

[0066] Intranasal drug delivery has been a topic of research and development for many years, although it has been only within the past decade that carrier systems have been devised which make delivery of substances effective. (Sayani and Chien, *Critical Reviews in Therapeutic Drug Carrier Systems* **1996**, 13:85-184.) Intranasal delivery has a number of advantageous features including comparatively high bioavailability, rapid kinetics of absorption and avoidance of a first-pass effect in the liver. In some aspects, intranasal administration can allow for delivery of an oxytocin peptide to the nasal cavity and in other aspects, intranasal administration can allow for targeted delivery to the cranial nerves of the nose and/or the brain. Without wishing to be bound by any particular theories, intranasal administration of an oxytocin peptide can target either the olfactory nerve systems or the trigeminal nerve systems or both. The oxytocin peptide may be delivered intranasally in any applicable forms, including but is not limited to a liquid formulation, a solid formulation (e.g., a dry powder formulation), a gel formulation or an emulsion formulation.

[0067] In embodiments where the combination of oxytocin and magnesium ions are administered intranasally, the composition can be prepared as a liquid aerosol formulation combined with a dispersing agent and/or a physiologically acceptable diluent. Alternatively, dry powder aerosol formulations are contemplated, and may contain a finely divided solid

form of the subject compound and a dispersing agent allowing for the ready dispersal of the dry powder particles. With either liquid or dry powder aerosol formulations, the formulation is aerosolized into small, liquid or solid particles in order to ensure that the aerosolized dose reaches the mucous membranes of the nasal passages or the lung. The term "aerosol particle" is used herein to describe a liquid or solid particle suitable of a sufficiently small particle diameter for nasal (in a range of from about 10 microns) or pulmonary (in a range of from about 2-5 microns) distribution to targeted mucous or alveolar membranes. Other considerations include the construction of the delivery device, additional components in the formulation, and particle characteristics. These aspects of nasal or pulmonary administration of drugs are well known in the art, and manipulation of formulations, aerosolization means, and construction of delivery devices, is within the level of ordinary skill in the art.

[0068] In some embodiments, the magnesium-containing oxytocin peptide formulation or composition useful in the methods described herein, wherein the oxytocin peptide and the magnesium ions are in an amount that produces a synergistic or enhanced effect when used in the treatment of autism spectrum disorder, are administered using a device for intranasal delivery. The device may be any device suitable for intranasal administration of the magnesium-containing oxytocin peptide formulation. In some embodiments, the device is suitable for delivery of the oxytocin peptide and the magnesium ions to specific region within the nasal cavity. In some embodiments, the device is suitable for delivery of the oxytocin peptide and the magnesium ions to the inferior two-thirds of the nasal cavity. In some embodiments, the device is suitable for delivery of the oxytocin peptide and the magnesium ions to the upper third of the nasal cavity. In some embodiments, the device is suitable for delivery of the oxytocin peptide to the entire nasal passage.

[0069] In some embodiments, the device for intranasal delivery is a nasal pump apparatus. In some embodiments, the nasal pump apparatus comprises a reservoir bottle attached to a pump actuator. In some embodiments, the pump actuator is metered to deliver a specified volume (e.g. about 5 to about 1000 μL, preferably about 50 to about 150 μL, more preferably about 50 μL or about 100 μL) in a specified distribution of droplet sizes. In some embodiments, the nasal pump apparatus comprises a reservoir bottle attached to an aerosolizer, e.g. an Equadel pump marketed by Aptar Pharma. In some embodiments, the device for nasal administration functions irrespective of the pressure applied to the pump once a threshold value is reached. In some embodiments, the device for nasal administration is a mucosal atomization device (*e.g.*, LMA® MAD NASAL™) that can be added to a syringe. For administration in large mammals, the nasal pump apparatus may comprise a

reservoir bottle attached to a pump actuator that is metered to deliver larger volumes (e.g., about 100 µL to about 600 µL, or higher).

[0070] In some embodiments, the device for intranasal delivery is designed for delivery of multiple doses of the drug formulations. For example, a nasal pump apparatus may comprise a reservoir bottle attached to a pump actuator where the reservoir bottle holds multiple dose of the liquid formulation and the pump actuator is metered to deliver a specified volume that is a fraction of the liquid formulation held in the reservoir bottle. In some embodiments, the pump actuator is metered to deliver about 50 µL of the liquid formulation per spray. The nasal pump apparatus may comprise a filter for preventing back flow in order to reduce contaminant (e.g., bacterial) ingress into the reservoir bottle. In some embodiments, the nasal pump apparatus comprises a metal-free path for delivery of the liquid formulation (e.g., a plastic path). In some embodiments, the pump apparatus uses plastic material that is stable to gamma radiation (used for sterilizing the nasal apparatus). In some embodiments, the device for intranasal delivery is equipped with a multi-dose pump comprising a microbial filter and an auto-blocking mechanism in the pump actuator, for example, a spray device described in US Patent No. 5,988,449.

[0071] In some embodiments, the device for intranasal delivery is a breath-actuated nasal delivery device, such as the devices described in US Patents No. 7,784,460 and 7,854,227. Such devices may improve delivery to a target site deep into the nasal cavity. In some embodiments, a standard metered dose spray device is incorporated into a housing that allows the patient to blow into a mouthpiece to actuate the device. In some embodiments, the device is comprised of a conical sealing nosepiece and a mouthpiece that incorporate a traditional mechanical spray pump (e.g. an Equadel pump marketed by Aptar Pharma), a chargeable spring and a breath actuation mechanism. The system can be used for single or multi-dose delivery. One example of such a liquid delivery device is the OptiMist™ device marketed by OptiNose. When in use, the nasal piece of the device is inserted into the nostril and the mouth piece is blown into. This closes the soft palate, transfers pressure to the nostril, opens passages providing airflow behind the nasal septum and allows air to exit the other nostril (bidirectional flow). Since the device is breath actuated, small particles cannot enter the lungs. Modifications to flow rate and particle size allows for targeting of specific nasal regions.

[0072] In some embodiments, the device for intranasal delivery is a unit-dose metering spray device suited for single administration of the magnesium-containing oxytocin peptide formulation or composition. In some embodiments, the device for intranasal delivery is a

multi-dose metering spray pump apparatus suited for repeated administrations of an oxytocin peptide.

[0073] Drop size, plume volume and flow rate can be modified to target specific nasal regions. The liquid spray may provide droplet size between 5 and 50 microns in order to target olfactory and/or respiratory epithelium. Larger droplets primarily travel down the nasopharynx and are swallowed, while smaller droplets are targeted to the pulmonary tissue. The Mass Median Equivalent Aerodynamic Diameter (MMAD) is used to specify the drop size. The pH of the nasal spray is optimized to deliver charged peptide in mostly an unionized state. The nose will generally tolerate solutions having a pH of about 3-8. The nasal mucosa can generally absorb volumes of approximately 100 µL before saturation occurs and liquid begins to drip out of the nose. Therefore, plume volume may be up to (and including) 100 µL. For use in large mammals, plume volume may be up to (and including) 150 µL or higher (e.g., 600 µL or higher). For infant and pediatric use, or for veterinary use in smaller animals (e.g., rodents, cats), smaller plume volumes (5-50 µL) could be used.

[0074] In some embodiments, the device for intranasal delivery is an ergonomically designed to facilitate patient compliance, such as a pump apparatus with a side-actuation triggering mechanism. In some embodiments, the device for intranasal delivery comprises a metering spray pump working as a closed system, which does not allow air to enter into the pump apparatus thus preventing contamination from airborne germs. In some embodiment, the device for intranasal delivery comprises a metering spray pump working with a filter. The venting air is sucked through a filter assembled inside the pump, keeping airborne germs out of the pump apparatus. In some embodiments, the intranasal delivery device comprising a nasal pump apparatus may further comprise micro-electronic devices that may facilitate data transmission and treatment monitoring.

[0075] In some embodiments, the magnesium-containing oxytocin peptide formulation or composition comprises an oxytocin peptide and magnesium ions wherein the oxytocin peptide and the magnesium ions are contained in any one of the devices for intranasal delivery described herein, and wherein the concentrations of the oxytocin peptide and the magnesium ions are within any of the concentration ranges described herein, as if each and every combination of device and concentration is described individually.

Methods

[0076] The terms "autism spectrum disorder (ASD)" or "autism" refer to a group of complex disorders of brain development. These disorders are characterized, in varying

degrees, by difficulties in social interaction, verbal and nonverbal communication and repetitive behaviors. With the May 2013 publication of the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), all autism disorders were merged into one umbrella diagnosis of ASD. Previously, they were recognized as distinct subtypes, including autistic disorder, childhood disintegrative disorder, pervasive developmental disorder-not otherwise specified (PDD-NOS) and Asperger syndrome. *See* http://www.autismspeaks.org/what-autism. Those skilled in the art will recognize that there is considerable overlap of the symptoms of autism spectrum disorder with many other psychiatric disorders. Examples of disorders which exhibit symptoms similar to those displayed in autism spectrum disorder include, but are not limited to, social anxiety disorder, obsessive-compulsive disorder, social (pragmatic) communication disorder, and neurodevelopmental disorders including but not limited to attention deficit hyperactivity disorder, Prader-Willi syndrome, Timothy syndrome, Fragile-X syndrome, Rett syndrome, and Williams syndrome.

[0077] The DSM-5 provides diagnostic criteria for ASD including: (A) Persistent deficits in social communication and social interaction across multiple contexts, as manifested, currently or by history, by the following illustrative examples: (1) Deficits in social-emotional reciprocity, ranging, for example, from abnormal social approach and failure of normal back-and-forth conversation; to reduced sharing of interests, emotions, or affect; to failure to initiate or respond to social interactions; (2) Deficits in nonverbal communicative behaviors used for social interaction, ranging, for example, from poorly integrated verbal and nonverbal communication; to abnormalities in eye contact and body language or deficits in understanding and use of gestures; to a total lack of facial expressions and nonverbal communication; and (3) Deficits in developing, maintaining, and understanding relationships, ranging, for example, from difficulties adjusting behavior to suit various social contexts; to difficulties in sharing imaginative play or in making friends; to absence of interest in peers; and (B) Restricted, repetitive patterns of behavior, interests, or activities, as manifested, currently or by history, by at least two of the following illustrative examples: (1) Stereotyped or repetitive motor movements, use of objects, or speech (e.g., simple motor stereotypies, lining up toys or flipping objects, echolalia, idiosyncratic phrases); (2) Insistence on sameness, inflexible adherence to routines, or ritualized patterns or verbal nonverbal behavior (e.g., extreme distress at small changes, difficulties with transitions, rigid thinking patterns, greeting rituals, need to take same route or eat food every day); (3) Highly restricted, fixated interests that are abnormal in intensity or focus (e.g., strong attachment to or preoccupation

with unusual objects, excessively circumscribed or perseverative interest); and (4) Hyper- or hyporeactivity to sensory input or unusual interests in sensory aspects of the environment (e.g., apparent indifference to pain/temperature, adverse response to specific sounds or textures, excessive smelling or touching of objects, visual fascination with lights or movement). *See* http://www.autismspeaks.org/what-autism/diagnosis/dsm-5-diagnostic-criteria.

[0078] Autism spectrum disorders (ASD) are characterized by social-interaction difficulties, communication challenges and a tendency to engage in repetitive behaviors. However, symptoms and their severity vary widely across these three core areas. ASD can be associated with intellectual disability, difficulties in motor coordination and attention and physical health issues such as sleep and gastrointestinal disturbances. ASD can be associated with psychiatric symptoms including anxiety and depression. *See, e.g.,* Kim et al., *Autism* **2000**, 4(2):117-132.

[0079] Oxytocin has been known to treat a number of conditions including anxiety and social and communication deficits in autism spectrum disorders. However, it has been observed that the effect of oxytocin in treating social and communication deficits in autism spectrum disorder varies widely between patients. It is possible that variations in receptor availability and receptor affinity for oxytocin are responsible for the variation in effect. Clinical efforts in using commercial formulations of oxytocin (e.g., Syntocinon®) to treat ASD have been marred by lack of efficacy and poor tolerability. Due to the low potency and high volume of currently available oxytocin formulations, when administered by nasal spray, the amount of drug absorbed is insufficient for efficacy. The present invention provides a method for administering an oxytocin peptide in a more potent formulation and lower volume such that an efficacious amount of the formulation can be delivered using a nasal device for treating an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, or social and communication deficits.

[0080] In one aspect, provided is a method for treating an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or an anxiety, comprising administering to a subject in need thereof an effective amount of an oxytocin peptide and magnesium ions, wherein the effective amount is delivered via intranasal administration in a volume that is readily absorbed in the nasal cavity. In some embodiments, the volume that allows ready absorption of the oxytocin peptide and the magnesium ions in the nasal cavity is between about 5 μL and about 1000 μL. In some embodiments, the molar ratio between the amount of the oxytocin

peptide and the amount of magnesium or magnesium ions is about 1:175, about 1:280, about 1:560, about 1:1100, about 1:1700, or about 1:2000, including any ranges between these ratios.

[0081] In one aspect, provided is a method comprising administering to a subject in need thereof an effective amount of an oxytocin peptide and magnesium ions, wherein the effective amount is delivered via intranasal administration in a volume of between about 5 µL and about 1000 µL. In some embodiments, the molar ratio between the amount of the oxytocin peptide and the amount of magnesium or magnesium ions is about 1:175, about 1:280, about 1:560, about 1:1100, about 1:1700, or about 1:2000, including any ranges between these ratios. In some embodiments, the method is for treating one or more symptoms associated with an autism spectrum disorder. In some embodiments, the method is for treating a disorder manifesting one or more symptoms associated with an autism spectrum disorder. In some embodiments, the method is for reducing social and communication deficits. In some embodiments, the method is for treating or decreasing anxiety.

[0082] Magnesium is involved in many aspects of life and health such as energy production, oxygen uptake, central nervous system function, electrolyte balance, glucose metabolism and muscle activity. Magnesium has also been found clinically effective in decreasing social and communication deficits in children with autism spectrum disorder. *See* Mousain-Bosc et al., *Magnes. Res.* **2006**, 19(1):53-62. Co-administration of oxytocin and magnesium ions of the present invention results in synergistic or enhanced improvement in social behavior and decreases in anxiety relative to administration of oxytocin alone. The mechanisms underlying these effects are unclear, but are likely to involve either non-competitive blockade of the *N*-methyl D-aspartate (NMDA) neurotransmitter receptor, or an increase in affinity of the oxytocin receptor action as an allosteric modulator, or both.

[0083] In some aspects, provided is a method for treating an autism spectrum disorder comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect. In some aspects, provided is a method for alleviating or reducing one or more symptoms associated with an autism spectrum disorder comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect. In some aspects, provided is a method for treating a disorder manifesting one or more symptoms associated with an autism spectrum disorder comprising administering to a subject in need thereof an effective dose of an

oxytocin peptide and magnesium ions, wherein co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect. In some embodiments, the oxytocin peptide and the magnesium ions are administered at a dose that produces an overall effect on alleviating or reducing the symptom that is greater than the sum of the effects of equivalent doses of the oxytocin peptide and the magnesium salt administered individually. In some embodiments, the oxytocin peptide and the magnesium ions are administered at a dose that produces a faster onset of effect and/or a longer lasting effect than would occur following administration of the individual agents used alone in equivalent quantities. Examples of symptoms associated with an autism spectrum disorder include but are not limited to persistent deficits in social communication and social interaction, social anxiety, and restricted repetitive behaviors, interests and activities. Other behaviors and characteristics also observed in persons with autism spectrum disorder include an aversion to physical contact, generalized anxiety, a monotone voice or an inability to modulate volume of voice, failure to develop peer relationships, lack of shared enjoyment and interests and lack of social or emotional reciprocity. Examples of disorders which exhibit symptoms similar to those displayed in autism spectrum disorder include, but are not limited to, social anxiety disorder, obsessive-compulsive disorder, social (pragmatic) communication disorder, and neurodevelopmental disorders including but not limited to attention deficit hyperactivity disorder, Prader-Willi syndrome, Timothy syndrome, Fragile-X syndrome, Rett syndrome, and Williams syndrome.

[0084] Prader-Willi Syndrome is a complex genetic condition that affects many parts of the body and is caused by a loss of function of genes in a particular region of chromosome 15. Individuals with Prader-Willi Syndrome often have mild to moderate intellectual impairment and learning difficulties and many exhibit behavioral problems including temper outbursts, stubbornness, manipulative behavior, and obsessive-compulsive behaviors including skin picking. Other symptoms often observed in individuals with Prader-Willi Syndrome are persistent deficits in social communication and social interaction, anxiety and irritability, and sleep problems.

[0085] In some aspects, provided is a method for treating Prader-Willi syndrome comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions. In some embodiments, co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect. In some embodiments, the oxytocin peptide and the magnesium ions are administered at a dose that produces an overall effect that is greater than the sum of the effects of equivalent doses of the oxytocin peptide

and the magnesium salt administered individually. In some embodiments, the oxytocin peptide and the magnesium ions are administered at a dose that produces a faster onset of effect and/or a longer lasting effect than would occur following administration of the individual agents used alone in equivalent quantities. In one embodiment, the invention provides a method for treating Prader-Willi Syndrome comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein the effective dose of the oxytocin peptide and the magnesium ions is administered intranasally in a liquid formulation, and the volume of the liquid formulation administered is between about 5 μL and about 1000 μL. In some embodiments, the molar ratio between the amount of the oxytocin peptide and the amount of magnesium or magnesium ions is about 1:175, about 1:280, about 1:560, about 1:1100, about 1:1700, or about 1:2000, including any ranges between these ratios.

[0086] In some aspects, provided is a method for alleviating or reducing one or more symptoms associated with Prader-Willi syndrome comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions. Examples of symptoms associated with Prader-Willi syndrome include but are not limited to persistent deficits in social communication and social interaction, anxiety and irritability, and sleep problems. In some embodiments, co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect. In some embodiments, the oxytocin peptide and the magnesium ions are administered at a dose that produces an overall effect on alleviating or reducing the symptom that is greater than the sum of the effects of equivalent doses of the oxytocin peptide and the magnesium salt administered individually. In some embodiments, the oxytocin peptide and the magnesium ions are administered at a dose that produces a faster onset of effect and/or a longer lasting effect than would occur following administration of the individual agents used alone in equivalent quantities. Examples of symptoms associated with Prader-Willi syndrome include but are not limited to persistent deficits in social communication and social interaction, anxiety and irritability, and sleep problems. In one embodiment, the invention provides a method for treating Prader-Willi Syndrome comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein the effective dose of the oxytocin peptide and the magnesium ions is administered intranasally in a liquid formulation, and the volume of the liquid formulation administered is between about 5 μL and about 1000 μL. In some embodiments, the molar ratio between the amount of the oxytocin peptide and the amount of

magnesium or magnesium ions is about 1:175, about 1:280, about 1:560, about 1:1100, about 1:1700, or about 1:2000, including any ranges between these ratios.

[0087] In some aspects, provided is a method for treating anxiety associated with Prader-Willi syndrome comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions. In some embodiments, co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect. In some embodiments, the oxytocin peptide and the magnesium ions are administered at a dose that produces an overall effect on alleviating or reducing anxiety that is greater than the sum of the effects of equivalent doses of the oxytocin peptide and the magnesium salt administered individually. In some embodiments, the oxytocin peptide and the magnesium ions are administered at a dose that produces a faster onset of effect and/or a longer lasting effect than would occur following administration of the individual agents used alone in equivalent quantities. In one embodiment, the invention provides a method for treating anxiety associated with Prader-Willi Syndrome comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein the effective dose of the oxytocin peptide and the magnesium ions is administered intranasally in a liquid formulation, and the volume of the liquid formulation administered is between about 5 μL and about 1000 μL. In some embodiments, the molar ratio between the amount of the oxytocin peptide and the amount of magnesium or magnesium ions is about 1:175, about 1:280, about 1:560, about 1:1100, about 1:1700, or about 1:2000, including any ranges between these ratios.

[0088] In one aspect, the invention provides a method for treating social and communication deficits comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect. In one aspect, the invention provides a method for treating anxiety comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect. In some embodiments, the oxytocin peptide and the magnesium ions are administered at a dose that produces an overall effect on reducing social and communication deficits and/or anxiety that is greater than the sum of the effects of equivalent doses of the oxytocin peptide and the magnesium salt administered individually. In some embodiments, the molar ratio between the amount of the oxytocin peptide and the amount of magnesium or magnesium ions is about 1:175, about 1:280, about 1:560, about 1:1100, about 1:1700, or

about 1:2000, including any ranges between these ratios. In some embodiments, the social and communication deficits is an impairment in communication skills and/or social interaction, a lack of eye contact, and/or an inability to form and/or maintain social relationships.

[0089] In some aspects, provided is a method for treating anxiety associated with autism spectrum disorder comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions. In some embodiments, co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect. In some embodiments, the oxytocin peptide and the magnesium ions are administered at a dose that produces an overall effect on alleviating or reducing anxiety that is greater than the sum of the effects of equivalent doses of the oxytocin peptide and the magnesium salt administered individually. In some embodiments, the oxytocin peptide and the magnesium ions are administered at a dose that produces a faster onset of effect and/or a longer lasting effect than would occur following administration of the individual agents used alone in equivalent quantities. In some embodiments, the molar ratio between the amount of the oxytocin peptide and the amount of magnesium or magnesium ions is about 1:175, about 1:280, about 1:560, about 1:1100, about 1:1700, or about 1:2000, including any ranges between these ratios.

[0090] The oxytocin peptide and the magnesium ions may be administered concurrently or sequentially. In some embodiments, the oxytocin peptide is administered concurrently with the magnesium ions in the same unit dose. In some embodiments, the oxytocin peptide is administered concurrently with the magnesium ions but in separate unit doses or formulations. In some embodiments, oxytocin peptide and the magnesium ions are administered sequentially. In some embodiments, the magnesium ions are administered to the subject in a first administration and then the oxytocin peptide is administered to the subject in a second administration. In some of these embodiments, the oxytocin peptide is administered between about 10 minutes and about 2 hours after administration of the magnesium ions. In some of these embodiments, the oxytocin peptide is administered between about 10 minutes and about 2 hours, between about 10 minutes and about 1 hour, between about 10 minutes and about 30 minutes, between about 20 minutes and about 2 hours, between about 20 minutes and about 1 hour, between about 30 minutes and about 2 hours or between about 30 minutes and about 1 hour after administration of the magnesium ions. In some of these embodiments, the oxytocin peptide is administered about 10 minutes, about 15 minutes, about 20 minutes, about 30 minutes, about 45 minutes, about 60 minutes, about 90 minutes or about 120 minutes after administration of the magnesium ions. In some of these

embodiments, the oxytocin peptide is administered about 10 minutes, about 15 minutes, about 20 minutes, or about 30 minutes after administration of the magnesium ions. In one embodiment, the oxytocin peptide is administered to the subject first and then the magnesium ions are administered to the subject. In some embodiments, the subject is a human.

[0091] Interleukin-6 (IL-6) has been demonstrated to induce the elevation of oxytocin receptor expression in various tissues (e.g., Young et al., *J. Neuroendocrinology*, **1997**; 9:859-65). Thus, serum IL-6 levels may be used as a biomarker of potential efficacy of oxytocin, for example, when nasally administrated with magnesium.

[0092] In some aspects, IL-6 is used as a biomarker of efficacy of administration of the oxytocin peptide in a subject according to a method detailed herein for treating an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or anxiety. and to select a subject for application of the methods. In some embodiments, IL-6 is used to select a subject (e.g., a human) for administration of an oxytocin peptide (e.g., nasal administration of an oxytocin peptide in combination with magnesium ions).

[0093] In some embodiments, the subject is selected for treatment based on the subject having a high level of IL-6. The level of IL-6 may be high as compared to a control or reference. In some embodiments, a level of IL-6 is high compared to a control or reference if it is significantly greater than the control or reference as determined by an appropriate statistical analysis. In some embodiments, a level of IL-6 is high compared to a control or reference if it is at least one standard deviation greater than the control or reference. In some embodiments, the control is a value for the level of IL-6 as determined in age- and gender-matched healthy subjects. In some embodiments, the reference is a reported value for the level of IL-6, such as a value reported for IL-6 in age- and gender-matched healthy subjects. In some embodiments, the level of IL-6 is determined as the level of IL-6 in a sample (such as a tissue or fluid sample) from the subject, including, without limitation, whole blood, serum, plasma, tears, and the like. The level of IL-6 in a sample can be determined by any method known in the art, such as by immunoassay, *e.g.*, ELISA-based assay. *See* for example Yang, C-J., *et al. Neuroscience* 284: 290-296, 2015; Emanuele, E., *et al. Neuroscience letters* 471(3): 162-165, 2010; Ashwood, P., *et al. Brain, behavior, and immunity* 25(1): 40-45, 2011; and Malik, M., *et al. Immunobiology* 216(1): 80-85, 2011.

[0094] In some embodiments, the method for treating an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or anxiety, comprises measuring the level of IL-6 (e.g.,

serum level of IL-6) in a subject and administering to a subject having a high IL-6 level an effective dose of an oxytocin peptide and magnesium ions.

[0095] In one aspect, the method for treating an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or anxiety, comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect, further comprises administering to the subject an effective amount of interleukin-6 (IL-6). In some embodiments, the effective amount of IL-6 results in an increase in expression of the oxytocin receptor (OTR) in the subject.

[0096] In some embodiments, according to any of the methods described herein where IL-6 is administered to the subject, the oxytocin peptide and the IL-6 may be administered concurrently or sequentially. In some embodiments, the oxytocin peptide is administered concurrently with the IL-6 in the same unit dose. In some embodiments, the oxytocin peptide is administered concurrently with the IL-6 but in separate unit doses or formulations. In some embodiments, oxytocin peptide and the IL-6 are administered sequentially. In some embodiments, the IL-6 is administered to the subject in a first administration and then the oxytocin peptide is administered to the subject in a second administration. In some of these embodiments, the oxytocin peptide is administered between about 1 minutes and about 4 hours after administration of the IL-6. In some of these embodiments, the oxytocin peptide is administered between about 1 minutes and about 4 hours, between about 10 minutes and about 4 hours, between about 10 minutes and about 3 hours, between about 10 minutes and about 2 hours, between about 10 minutes and about 1 hour, between about 10 minutes and about 30 minutes, between about 20 minutes and about 4 hours, between about 20 minutes and about 3 hours, between about 20 minutes and about 2 hours, between about 20 minutes and about 1 hour, between about 30 minutes and about 4 hours, between about 30 minutes and about 3 hours, between about 30 minutes and about 2 hours or between about 30 minutes and about 1 hour after administration of the IL-6. In some of these embodiments, the oxytocin peptide is administered about 1 minute, about 10 minutes, about 15 minutes, about 20 minutes, about 30 minutes, about 45 minutes, about 60 minutes, about 90 minutes, about 120 minutes, about 150 minutes, about 180 minutes, about 210 minutes, or about 240 minutes after administration of the IL-6. In some of these embodiments, the oxytocin peptide is administered about 10 minutes, about 15 minutes, about 20 minutes, or about 30 minutes after administration of the IL-6. In one embodiment, the oxytocin peptide is administered to

the subject first and then the IL-6 is administered to the subject. In some embodiments, the subject is a human. In some of these embodiments, magnesium ions are administered concurrently with the oxytocin peptide and/or IL-6, prior to either or both of the oxytocin peptide and IL-6, or after either or both of the oxytocin peptide and IL-6.

[0097] The oxytocin peptide and the magnesium ions may be administered via the same route or different routes to a subject in need thereof. In some embodiments, the oxytocin peptide is administered via craniofacial mucosal administration (e.g., nasal, buccal, sublingual or ocular administration). In one embodiment, the oxytocin peptide and the magnesium ions are both administered intranasally in the same formulation. In one embodiment, the oxytocin peptide is administered via craniofacial mucosa and the magnesium ions are administered systemically, e.g., intravenously, intramuscularly, orally, subcutaneously, or intrathecally.

[0098] In some embodiments, the oxytocin peptide is administered via intranasal administration. In some embodiments, the oxytocin peptide and the magnesium ions are administered via intranasal administration. The oxytocin peptide and/or the magnesium ions can be administered to the mucosa tissue within the nasal cavity using a suitable device for intranasal delivery such as a nasal delivery device described herein. Suitable regions within the nasal cavity include, but are not limited to, the inferior two-thirds of the nasal cavity, or the upper third, or the entire nasal passage. In some embodiments, the oxytocin peptide and/or the magnesium ions are administered to the upper third of the nasal cavity. In some embodiments, the oxytocin peptide and/or the magnesium ions are administered to the lower two thirds of the nasal cavity. In some embodiments, the oxytocin peptide and/or the magnesium ions are administered specifically to reach both the lower two thirds and the upper third of the nasal cavity. In some embodiments, a method is provided for treating an autism spectrum disorder, one or more symptoms associated with an autism spectrum disorder, or a disorder manifesting one or more symptoms associated with an autism spectrum disorder, comprising intranasally administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect. In some embodiments, the method is for treating social and communication deficits or an anxiety.

[0099] In some embodiments, according to any of the methods described herein where IL-6 is administered to the subject, the IL-6 is administered via intranasal administration. The IL-6 can be administered to the mucosa tissue within the nasal cavity using a suitable device for intranasal delivery such as a nasal delivery device described herein. In some embodiments,

the IL-6 is administered systemically, *e.g.*, intravenously, intramuscularly, orally, subcutaneously, or intrathecally.

[0100] In some embodiments, the oxytocin peptide is human oxytocin consisting of Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly (SEQ. ID NO:1). In some embodiments, the effective dose of the oxytocin peptide is about 0.5 μg to about 2000 μg. In some embodiments, the effective dose of the oxytocin peptide is about 0.5 μg to about 1000 μg, about 1 μg to about 1000 μg or about 1 μg to about 2000 μg. In some embodiments, the effective dose of the oxytocin peptide is about 4 μg to about 1000 μg, about 8 μg to about 1000 μg, about 8 μg to about 800 μg, about 8 μg to about 500 μg, about 8 μg to about 400 μg, about 8 μg to about 300 μg, about 8 μg to about 200 μg, about 8 μg to about 100 μg, about 8 μg to about 80 μg, about 8 μg to about 50 μg, about 10 μg to about 1000 μg, about 10 μg to about 500 μg, about 10 μg to about 200 μg, about 10 μg to about 100 μg, about 16 μg to about 1000 μg, about 16 μg to about 800 μg, about 16 μg to about 500 μg, about 16 μg to about 400 μg, about 16 μg to about 200 μg, about 16 μg to about 160 μg, about 16 μg to about 120 μg, about 16 μg to about 80 μg, about 20 μg to about 1000 μg, about 20 μg to about 800 μg, about 20 μg to about 500 μg, about 20 μg to about 200 μg, about 20 μg to about 100 μg, about 30 μg to about 1000 μg, about 30 μg to about 500 μg, about 30 μg to about 300 μg, about 30 μg to about 120 μg, about 30 μg to about 90 μg, about 50 μg to about 1000 μg, about 50 μg to about 500 μg, about 50 μg to about 250 μg, about 50 μg to about 100 μg, or about 50 μg to about 80 μg. In some embodiments, the effective dose of the oxytocin peptide is about 8 μg, about 16 μg, about 32 μg, about 48 μg, about 64 μg, about 80 μg, about 96 μg, about 128 μg, about 256 μg, about 10 μg, about 20 μg, about 30 μg, about 40 μg, about 50 μg, about 60 μg, about 70 μg, about 80 μg, about 90 μg, about 100 μg, about 120 μg, about 150 μg, about 200 μg, about 400 μg, about 600 μg, about 800 μg or about 100 μg. In a preferred embodiment, the effective dose of the oxytocin peptide is about 8 μg to about 120 μg, about 15 μg to about 120 μg, about 30 μg to about 120 μg, or about 66 μg.

[0101] In some embodiments, the effective dose of the oxytocin peptide is about 0.25 IU to about 1000 IU. In some embodiments, the effective dose of the oxytocin peptide is about 0.25 IU to about 500 IU, about 0.5 IU to about 500 IU or about 0.5 IU to about 1000 IU. In some embodiments, the effective dose of the oxytocin peptide is about 2 IU to about 500 IU, about 4 IU to about 500 IU, about 4 IU to about 400 IU, about 4 IU to about 250 IU, about 4 IU to about 200 IU, about 4 IU to about 150 IU, about 4 IU to about 100 IU, about 4 IU to about 50 IU, about 4 IU to about 40 IU, about 4 IU to about 25 IU, about 5 IU to about 500 IU, about 5 IU to about 250 IU, about 5 IU to about 100 IU, about 5 IU to about 50 IU, about 8 IU to

about 500 IU, about 8 IU to about 400 IU, about 8 IU to about 250 IU, about 8 IU to about 200 IU, about 8 IU to about 100 IU, about 8 IU to about 80 IU, about 8 IU to about 60 IU, about 8 IU to about 40 IU, about 10 IU to about 500 IU, about 10 IU to about 400 IU, about 10 IU to about 250 IU, about 10 IU to about 100 IU, about 10 IU to about 50 IU, about 15 IU to about 500 IU, about 15 IU to about 250 IU, about 15 IU to about 150 IU, about 15 IU to about 60 IU, about 15 IU to about 45 IU, about 25 IU to about 500 IU, about 25 IU to about 250 IU, about 25 IU to about 125 IU, about 25 IU to about 50 IU, or about 25 IU to about 40 IU. In some embodiments, the effective dose of the oxytocin peptide is about 4 IU, about 8 IU, about 16 IU, about 24 IU, about 32 IU, about 40 IU, about 48 IU, about 64 IU, about 128 IU, about 5 IU, about 10 IU, about 15 IU, about 20 IU, about 25 IU, about 30 IU, about 35 IU, about 40 IU, about 45 IU, about 50 IU, about 60 IU, about 75 IU, about 100 IU, about 200 IU, about 300 IU, about 400 IU or about 50 IU. In a preferred embodiment, the effective dose of the oxytocin peptide is about 4 IU to about 60 IU, about 7.5 IU to about 60 IU, about 15 IU to about 60 IU, or about 30 IU.

[0102] The dose or amount of oxytocin in the combination is, in one embodiment, effective to provide a clinically measurable improvement in a symptom of an autism spectrum disorder or a related disorder. The combination of oxytocin and the magnesium ions provides a synergistic or enhanced effect to improve the autism spectrum disorder or a related disorder. In some embodiments, oxytocin is administered at a sub-therapeutically effective dose relative to a dose of oxytocin administered as a single agent. The dose of oxytocin as a single agent depends, in part, on the route of administration. Accordingly, the dose of oxytocin in the combination therapy described herein will also depend, in part, on the route of administration.

[0103] The optimal dosage of the magnesium ions may depend on the specific disorder or symptom, the type of synergistic or enhanced effect desired, and other factors such as the route of administration. The optimal dose may be measured in the total amount of magnesium ions administered, or the concentration of magnesium ions in the formulation administered. In some embodiments, the effective dose of magnesium ions administered is about 50 µg to about 68 mg. In some embodiments, the effective dose of magnesium ions administered is about 50 µg to about 34 mg, or about 1 mg to about 3 mg. In some embodiments, the effective dose of magnesium ions administered is about 1.3 mg, or about 2.6 mg. In some embodiments, the effective dose of magnesium ions administered is about 1.2 mg, or about 2.4 mg. In some embodiments, the effective dose of magnesium ions administered is about 50 µg to about 17 mg, about 50 µg to about 8 mg, about 50 µg to about 4 mg, about 50 µg to

about 2 mg, about 50 μg to about 1 mg, about 50 μg to about 500 μg, about 100 μg to about 68 mg, about 100 μg to about 34 mg, about 100 μg to about 17 mg, about 100 μg to about 8 mg, about 100 μg to about 4 mg, about 100 μg to about 2 mg, about 100 μg to about 1 mg, about 100 μg to about 500 μg, about 200 μg to about 68 mg, about 200 μg to about 34 mg, about 200 μg to about 17 mg, about 200 μg to about 8 mg, about 200 μg to about 4 mg, about 200 μg to about 2 mg, about 200 μg to about 1 mg, about 200 μg to about 500 μg, about 500 μg to about 68 mg, about 500 μg to about 34 mg, about 500 μg to about 17 mg, about 500 μg to about 8 mg, about 500 μg to about 5 mg, about 500 μg to about 4 mg, about 500 μg to about 3 mg, about 500 μg to about 2 mg, about 500 μg to about 1 mg, about 1 mg to about 68 mg, about 1 mg to about 34 mg, about 1 mg to about 17 mg, about 1 mg to about 8 mg, about 1 mg to about 6 mg, about 1 mg to about 5 mg, about 1 mg to about 4 mg, about 1 mg to about 3 mg, about 1 mg to about 2 mg, about 1.5 mg to about 8 mg, about 1.5 mg to about 6 mg, about 1.5 mg to about 5 mg, about 1.5 mg to about 4 mg, about 1.5 mg to about 3 mg, about 1.5 mg to about 2 mg, about 1.3 mg to about 2.6 mg, or about 1.2 mg to about 2.4 mg. In some embodiments, the magnesium ions are provided using a magnesium salt (e.g., magnesium citrate and/or magnesium chloride).

[0104] In some embodiments, the magnesium salt administered comprises magnesium chloride and the effective dose of the magnesium salt is about 0.48 mg to about 600 mg of magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$, MW 203.3). In some embodiments, the effective dose of magnesium chloride hexahydrate is about 0.48 mg to about 300 mg, about 0.5 mg to about 150 mg, about 0.5 mg to about 75 mg, about 5 mg to about 150 mg, about 5 mg to about 75 mg, about 5 mg to about 50 mg, about 10 mg to about 600 mg, about 10 mg to about 300 mg, about 10 mg to about 150 mg, about 10 mg to about 75 mg, about 10 mg to about 50 mg, about 10 mg to about 30 mg, or about 12 mg to about 24 mg. In some preferred embodiments, the effective dose of magnesium chloride hexahydrate is about 6 mg, about 12 mg, about 18 mg, about 24 mg or about 30 mg.

[0105] In some embodiments, the magnesium salt administered is magnesium citrate and the effective dose of the magnesium salt is about 0.48 mg to about 600 mg of magnesium citrate. In some embodiments, the effective dose of magnesium citrate (e.g., anhydrous magnesium citrate dibasic, MW. 214.4) is about 0.48 mg to about 300 mg, about 0.5 mg to about 150 mg, about 0.5 mg to about 75 mg, about 5 mg to about 150 mg, about 5 mg to about 75 mg, about 5 mg to about 50 mg, about 10 mg to about 600 mg, about 10 mg to about 300 mg, about 10 mg to about 150 mg, about 10 mg to about 75 mg, about 10 mg to about 50 mg, about 10 mg to about 30 mg, or about 12 mg to about 24 mg. In some preferred

embodiments, the effective dose of magnesium citrate (e.g., anhydrous magnesium citrate dibasic, MW. 214.4) is about 6 mg, about 12 mg, about 18 mg, about 24 mg or about 30 mg. In some embodiments, the effective dose of magnesium citrate is about 0.48 mg to about 12 mg, about 0.5 mg to about 10 mg, about 0.5 mg to about 8 mg, about 0.5 mg to about 5 mg, about 0.5 mg to about 2.5 mg, about 0.5 mg to about 1 mg, about 1 mg to about 10 mg, about 1 mg to about 8 mg, about 1 mg to about 5 mg, about 1 mg to about 2 mg, about 2 mg to about 10 mg, about 2 mg to about 8 mg, about 2 mg to about 6 mg, about 2 mg to about 4 mg, about 3 mg to about 10 mg, about 4 mg to about 10 mg, about 4 mg to about 8 mg, about 4 mg to about 6 mg, about 5 mg to about 10 mg, about 5 mg to about 8 mg, about 5 mg to about 7 mg, about 5 mg to about 6 mg, about 6 mg to about 10 mg, about 6 mg to about 8 mg, or about 6 mg to about 7 mg. If other magnesium salts are substituted for magnesium citrate, the effective dose of the magnesium salt provides an amount of magnesium ions equivalent to that provided by magnesium citrate.

[0106] It is intended and understood that each and every dosage of the magnesium ions described herein may be combined with each and every dosage of the oxytocin peptide described herein as if each and every combination is individually stated. For example, in some embodiments, the effective dose of the oxytocin peptide is about 0.5 μg to about 2000 μg and the effective dose of the magnesium ions is about 50 μg to about 68 mg of magnesium. In some embodiments, the effective dose of the oxytocin peptide is about 15 μg to about 120 μg (e.g., about 60 μg or about 66 μg) and the effective dose of the magnesium ions is equivalent to the amount of magnesium ions provided by about 10 mg to about 30 mg (e.g., about 12 mg or about 24 mg) of magnesium citrate.

[0107] In some embodiments, provided is a method for treating an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or an anxiety, comprising administering (for example by intranasal administration) to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein the weight ratio between the dose of the oxytocin peptide administered and the dose of the magnesium ions administered is between about 1:1 to about 1:1000, preferably between about 1:2 to about 1:200, more preferably about 1:20, about 1:30, about 1:35, about 1:40, about 1:45, about 1:50, about 1:60, or any of the OT/Mg (w) ratios described herein for the magnesium-containing oxytocin peptide formulation or composition. In some embodiments, a method is provided for treating an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or an anxiety, comprising

administering (for example by intranasal administration) to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein the molar ratio between the dose of the oxytocin peptide administered and the dose of the magnesium ions administered is between about 1:40 to about 1:40000, preferably between about 1:80 to about 1:8000, more preferably about 1:175, about 1:280, about 1:500, about 1:560, about 1:800, about 1:1000, about 1:1100, about 1:1200, about 1:1400, about 1:1600, about 1:1700, about 1:1800, about 1:2000, about 1:2400, about 1:3000, or any of the OT/Mg (m) ratios described herein for the magnesium-containing oxytocin peptide formulation or composition. In some of these embodiments, the oxytocin peptide is human oxytocin consisting of Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly (SEQ. ID NO:1). In some of this embodiment, the magnesium ions are provided by magnesium citrate and/or magnesium chloride. In some of these embodiments, the social and communication deficits is an impairment in communication skills and/or social interaction, a lack of eye contact, and/or an inability to form and/or maintain social relationships.

[0108] In one embodiment, a method is provided for treating an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or an anxiety, comprises intranasally administering to a subject in need thereof a dose of about 0.5 μg to about 2000 μg (e.g., about 8 μg to about 300 μg, about 15 μg to about 120 μg or about 66 μg) of an oxytocin peptide and a dose of about 50 μg to about 68 mg, about 50 μg to about 34 mg, about 1 mg to about 3 mg, about 1.3 mg, or about 2.6 mg of magnesium or magnesium ions. In one embodiment, the method comprises intranasally administering to a subject in need thereof an effective amount of a magnesium-containing oxytocin peptide formulation or composition described herein. In one embodiment, the method comprises intranasally administering to a subject in need thereof an effective amount of an oxytocin peptide and magnesium ions in a liquid formulation of between about 5 μL and about 1000 μL in volume. In one embodiment, the method comprises intranasally administering to a subject in need thereof an effective amount of a magnesium-containing oxytocin peptide formulation or composition comprising between about 0.01 mg/mL and about 16 mg/mL (e.g., about 0.1 mg/mL and about 16 mg/mL) of oxytocin and between about 1 mg/mL and about 30 mg/mL of magnesium or magnesium ions. In one embodiment, the method comprises intranasally administering to a subject in need thereof an effective amount of a magnesium-containing oxytocin peptide formulation comprising between about 0.01 mg/mL and about 16 mg/mL (e.g., about 0.1 mg/mL and about 16 mg/mL or about 0.15 mg/mL and about 1.5 mg/mL) of oxytocin and between about

1% and about 25% (by weight) (e.g., about 1% to about 15% or about 10% to about 14%) of magnesium citrate. In one embodiment, the method comprises intranasally administering to a subject in need thereof an effective amount of a magnesium-containing oxytocin peptide formulation comprising between about 5 IU/mL and about 8000 IU/mL (e.g., about 50 IU/mL and about 8000 IU/mL or about 75 IU/mL and about 750 IU/mL) of oxytocin and between about 1% and about 25% (by weight) (e.g., about 1% to about 15%, about 10% to about 14%, or about 12%) of magnesium citrate. In one embodiment, the method comprises intranasally administering to a subject in need thereof an effective amount of a magnesium-containing oxytocin peptide formulation comprising between about 0.01 mg/mL and about 16 mg/mL (e.g., about 0.1 mg/mL and about 16 mg/mL or about 0.15 mg/mL and about 1.5 mg/mL) of oxytocin and between about 1% and about 25% (by weight) (e.g., about 1% to about 15%, about 8% to about 12%, or about 10%) of magnesium chloride hexahydrate. In one embodiment, the method comprises intranasally administering to a subject in need thereof an effective amount of a magnesium-containing oxytocin peptide formulation comprising between about 5 IU/mL and about 8000 IU/mL (e.g., about 50 IU/mL and about 8000 IU/mL or about 75 IU/mL and about 750 IU/mL) of oxytocin and between about 1% and about 25% (by weight) (e.g., about 1% to about 15%, about 8% to about 12%, or about 10%) of magnesium chloride hexahydrate.

[0109] In some embodiments, the effective dose of the oxytocin peptide and the magnesium ions comprises about 0.5 µg (or 0.25 IU) to about 2000 µg (or 1000 IU) of the oxytocin peptide administered in an aqueous solution containing about 0.1% to about 2.8% (w/v) of magnesium. In some embodiments, the effective dose of the oxytocin peptide and the magnesium ions comprises about 8 µg (or 4 IU) to about 1000 µg (or 500 IU) of the oxytocin peptide administered in an aqueous solution containing about 0.11% to about 1.65% (w/v) of magnesium. In some embodiments, the effective dose of the oxytocin peptide and the magnesium ions comprises about 15 µg (or 7.5 IU) to about 120 µg (or about 60 IU) (e.g., about 60 µg or 30 IU) of the oxytocin peptide administered in an aqueous solution containing about 1.1% to about 1.6% (e.g., about 1.2% or about 1.35%) magnesium. In one embodiment, the effective dose of the oxytocin peptide and the magnesium ions comprises about 60 µg (or 30 IU) of the oxytocin peptide administered in an aqueous solution containing about 1.2% or about 1.35% of magnesium.

[0110] In some embodiments, the effective dose of the oxytocin peptide and the magnesium ions is administered intranasally in a liquid formulation, and the volume of the liquid formulation administered is between about 5 µL and about 1000 µL. In some

embodiments, the volume administered is between about 5 μL and about 500 μL, between about 5 μL and about 250 μL, between about 5 μL and about 100 μL, between about 5 μL and about 50 μL, between about 10 μL and about 1000 μL, between about 10 μL and about 500 μL, between about 10 μL and about 250 μL, between about 10 μL and about 100 μL, between about 25 μL and about 1000 μL, between about 25 μL and about 500 μL, between about 25 μL and about 250 μL, between about 25 μL and about 100 μL, between about 50 μL and about 1000 μL, between about 50 μL and about 750 μL, between about 50 μL and about 500 μL, between about 50 μL and about 450 μL, between about 50 μL and about 400 μL, between about 50 μL and about 350 μL, between about 50 μL and about 300 μL, between about 50 μL and about 250 μL, between about 50 μL and about 200 μL, between about 50 μL and about 150 μL, between about 100 μL and about 500 μL, between about 100 μL and about 400 μL, between about 100 μL and about 300 μL, or between about 100 μL and about 200 μL. In some embodiments, the volume administered is about 50 μL, about 100 μL, about 150 μL, about 200 μL, about 250 μL, about 300 μL, about 350 μL, about 400 μL, about 450 μL, or about 500 μL. In some embodiments, the effective dose of the oxytocin peptide and the magnesium ions is administered intranasally in a liquid formulation contained in a nasal device described herein.

[0111] The combination of an oxytocin peptide and magnesium ions described herein may be used for the treatment of any social and communication deficits treatable by oxytocin, such as impairment in communication skills and/or social interaction, lack of eye contact, and/or an inability to form and/or maintain social relationships. Thus, provided is a method for treating social and communication deficits comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein the social and communication deficit is impairment in communication skills and/or social interaction, lack of eye contact, and/or an inability to form and/or maintain social relationships. In one embodiment, the method comprises intranasally administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions. In some embodiments, the molar ratio between the amount of the oxytocin peptide and the amount of magnesium or magnesium ions is about 1:175, about 1:280, about 1:560, about 1:1100, about 1:1700, or about 1:2000, including any ranges between these ratios.

[0112] In one embodiment, a method is provided for treating an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or an anxiety, comprising intranasally administering to a subject in need thereof (e.g., a human or veterinary patient) an effective dose of an oxytocin

peptide and magnesium ions, wherein co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect. In some embodiments, the oxytocin peptide is human oxytocin consisting of Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly (SEQ. ID NO:1). In some embodiments, the effective dose of the oxytocin peptide is about 0.5 μg (or 0.25 IU) to about 2000 μg (or 1000 IU), preferably about 8 μg (or 4 IU) to about 1000 μg (or 500 IU), more preferably about 15 μg (or 7.5 IU) to about 120 μg (or 60 IU). In some embodiments, the effective dose of the magnesium ions is about 50 μg to about 68 mg. In some embodiments, the magnesium ions are provided using a magnesium salt (e.g., magnesium chloride and/or magnesium citrate) administered in an amount to provide about 50 μg to about 68 mg of magnesium. In some embodiments, the effective dose of the magnesium ions is provided by using about 0.48 mg to about 600 mg of magnesium citrate. In some embodiments, the effective dose of the magnesium ions is provided by using about 0.42 mg to about 540 mg of magnesium chloride hexahydrate. In some embodiments, the effective dose of the oxytocin peptide and the magnesium ions comprises about 15 μg (or 7.5 IU) to about 120 μg (or 60 IU) (e.g., about 60 μg or 30 IU) of the oxytocin peptide administered in an aqueous solution containing about 1.1% to about 1.54% (e.g., about 1.2% or about 1.35%) (w/v) magnesium. In some embodiments, the effective dose of the oxytocin peptide and the magnesium ions comprises about 10 μg to about 120 μg (e.g., about 66 μg) of the oxytocin peptide administered in an aqueous solution containing about 10% to about 14% (e.g., about 12 %) (w/v) magnesium citrate.

Kits

[0113] Provided herein are kits for carrying out any of the methods described herein. Kits are provided for use in treatment of an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or anxiety. In some embodiments, the kit comprises an oxytocin peptide and magnesium ions, wherein the oxytocin peptide and the magnesium ions are in an amount that produces a synergistic or enhanced effect when used in the treatment of an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or anxiety, and a device for craniofacial mucosal administration (e.g., intranasal administration) in suitable packaging. Kits may further comprise a protease inhibitor and/or at least one absorption enhancer. Kits may further comprise IL-6. Other kits may further comprise instructions providing information to the user and/or health care provider for carrying out any one of the methods described herein. Kits

may further comprise reagents/tools for measuring IL-6 levels in a subject; and optionally instructions for predicting efficacy of nasal oxytocin and magnesium ions.

[0114] Also provided is a kit comprising a magnesium-containing oxytocin peptide formulation described herein contained in a device for craniofacial mucosal administration (e.g., a device for intranasal administration such as a nasal pump apparatus) and suitable packaging. The kit may further comprise instructions for administering the magnesium-containing oxytocin peptide formulation in a subject in need thereof.

[0115] The instructions relating to the use of the kit for carrying out the invention generally describe how the contents of the kit are used to carry out the methods of the invention. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (*e.g.*, a paper sheet included in the kit), but machine-readable instructions (*e.g.*, instructions carried on a magnetic or optical storage disk) are also acceptable.

EXAMPLES

[0116] The invention can be further understood by reference to the following examples, which are provided by way of illustration and are not meant to be limiting.

Example 1: Exemplary preparation of a magnesium-containing oxytocin peptide formulation

Example 1A

[0117] The drug product formulation, which is hypertonic and targeted at pH 4.5, consists of Oxytocin USP (150 IU/mL); Magnesium Chloride USP (as the hexahydrate or anhydrous salt); Citric Acid USP (as the anhydrous or monohydrate form); Sodium Hydroxide NF; and Sterile Water for Injection USP. The quantitative composition is provided in Table 1. The molar ratio of oxytocin to magnesium ions in the formulation is about 1:1679. All ingredients meet the compendial (USP/NF) requirements from the corresponding monographs.

Table 1

Component	Composition		Function
	mg/mL	wt %	
Oxytocin USP[1]	150 IU	Footnote 1	Active ingredient
Magnesium Chloride USP[2]	101.7	10.2	Chelating system
Citric Acid USP[3]	9.6	0.96	
Sodium Hydroxide NF	qs pH 4.5	qs pH 4.5	pH adjustment
Sterile Water for Injection USP	qs	qs	Solvent
Total	ca. 1000	100	

[1] The exact amount of oxytocin to be used is based on its oxytocic activity from the supplier certificate of analysis.

[2] The composition values for magnesium chloride represent those of the hexahydrate; the anhydrous salt may be used as well, with corresponding adjustment of composition.
[3] The composition values for citric acid represent those of the anhydrous form; the monohydrate may be used as well, with corresponding adjustment of composition.

[0118] The drug product is manufactured by dissolving the ingredients in Sterile Water for Injection, sterile filtering and filling into a vial with a snap on preservative free pump, and is tested in general accordance with the July 2002 FDA nasal spray guidance.

[0119] In one example, a 10-L batch of the magnesium-containing oxytocin formulation according to the composition provided in Table 1 was prepared as following: Filled the formulation vessel with water to about 60% of the required batch volume. While stirring at ambient temperature, added in the following order the required quantities of: Sodium Chloride, Citric Acid and Magnesium Chloride hexahydrate. The materials dissolved readily. No heat was required, just gentle stirring. Adjusted the pH of the solution to 4.5 with the addition of 1N NaOH. (If over-titrated, 10% HCl could be used to back-titrate to pH 4.5.) Added the required amount of oxytocin and stirred until dissolved. Added water to bring the batch to the final weight/volume. Stirred until the solution was homogenous.

Example 1B

[0120] The drug product formulation, which is isotonic and targeted at pH 4.5, consists of Oxytocin USP (150 IU/mL); Magnesium Citrate, Sodium Chloride USP; Sodium Acetate Trihydrate USP; Glacial Acetic Acid USP; and Sterile Water for Injection USP. Quantitative compositions are provided in Table 2. The molar ratio of oxytocin to magnesium ions in the formulation is about 1:1992. The target pH of 4.5 is selected based on the optimal formulation stability at or near this pH (Hawe, et al. *Pharmaceut. Res.* **2009**, 26:1679-1688). All ingredients meet the compendial (USP/NF) requirements from the corresponding monographs.

[0121] To prepare a stock oxytocin solution, lyophilized oxytocin (2 mg) is added to 1 mL of water (USP), 0.9% physiological saline or phosphate buffered saline in a 5 mL glass vessel. The solution is stirred until all the oxytocin is dissolved, and the pH is adjusted to between 3.5 and 8.5, producing 1 mL of a 2 mg/mL (about 1000 IU/mL) liquid oxytocin formulation.

[0122] For use as clinical material oxytocin and the excipients are manufactured under current Good Manufacturing Practice and undergo terminal sterilization (aseptic filtration through a 0.2 micron membrane filter) prior to filling within a glass reservoir bottle and sealing with a pump actuator. Various formulation concentrations can be produced from this

example by increasing or decreasing the oxytocin amount. Approximately, 10 doses of oxytocin are obtained from this 1 mL batch volume.

Table 2

Ingredient	Concentration (mg/mL)
Oxytocin USP	0.283
Magnesium Citrate	120
Sodium Chloride USP	4.675
Sodium Acetate Trihydrate USP	6.805
Citric Acid USP	pH 4.5
Sterile Water for Injection USP	qs

Example 2: Rat model of social behavior

[0123] Rats were treated intranasally with 20 µl (10 µl/nostril) of a solution containing saline, 10 µg oxytocin, a combination of 12% magnesium citrate and 10 µg oxytocin (molar ratio of about 1:1127 for oxytocin to magnesium ions), or 12% magnesium citrate. Eight (8) rats were used in each treatment group. Forty minutes after nasal drug administration two animals from the same treatment group were partnered and placed into a testing chamber and their social behavior (sniffing, following, crawling over and under, allogrooming [grooming partner], and play fighting) was recorded for 10 minutes. The time spent on social interaction is shown in FIG. 1. The results show evidence of an enhanced effect of the combination of 12% magnesium citrate and 10 µg oxytocin on improving social behavior.

Example 3: Rat model of anxiety

Example 3A

[0124] Rats were treated intranasally with 20 µl (10 µl/nostril) of a solution containing saline, 10 µg oxytocin, a combination of 12% magnesium citrate and 10 µg oxytocin (molar ratio of about 1:1127 for oxytocin to magnesium ions), or 12% magnesium citrate. Eight (8) rats were used in each treatment group. Fifty minutes after nasal drug administration, the animals were placed into a radial arm maze and their anxiety was assessed by the number of open arm entries that animals made during a 5 minute period. The observed numbers of open arm entries are shown in FIG. 2. Results show evidence of a synergistic effect of the combination of 12% magnesium citrate and 10 µg oxytocin on reducing anxiety.

Example 3B

[0125] Rats were treated intranasally with 20 µl (10 µl/nostril) of a solution containing saline, 3% magnesium citrate, 6% magnesium citrate, 16 µg oxytocin, 10 µg oxytocin, a combination of 3% magnesium citrate and 16 µg oxytocin (molar ratio of about 1:176 for oxytocin to magnesium ions), or a combination of 6% magnesium citrate and 10 µg oxytocin (molar ratio of about 1:563 for oxytocin to magnesium ions). Eight (8) rats were used in each treatment group. Thirty minutes after nasal drug administration, the animals were exposed to 5 minutes of elevated platform stress, immediately followed by placement into an elevated plus maze for 5 minutes. Their anxiety was assessed by the number of open arm entries that animals made during the 5 minute period. The observed numbers of open arm entries are shown in FIGS. 3A and 3B. Animals treated with the combination of 3% magnesium citrate and 16 µg oxytocin (molar ratio of about 1:176 for oxytocin to magnesium ions) had lower anxiety than animals treated with either 3% magnesium citrate alone or 16 µg oxytocin alone, as indicated by the increased number of open arm entries. By contrast, animals treated with the combination of 6% magnesium citrate and 10 µg oxytocin (molar ratio of about 1:563 for oxytocin to magnesium ions) had greater anxiety than animals treated with either 6% magnesium citrate alone or 10 µg oxytocin alone, as indicated by the decreased number of open arm entries.

[0126] To further assess anxiety, latency to open arm entry, time spent in open arms, and number of closed arm entries is determined.

[0127] The experiments are repeated with additional amounts of magnesium citrate and oxytocin, including, for example, 6% magnesium citrate alone, 20 µg oxytocin alone, and a combination of 6% magnesium citrate and 20 µg oxytocin (molar ratio of about 1:281 for oxytocin to magnesium ions).

Example 4: A single subject case study

[0128] A subject (for example a child) with a diagnosis of autism spectrum disorder is administered intranasally a liquid formulation containing between 12 and 24 IU of oxytocin every morning and evening for a period of 3 days. Social functioning and anxiety of the subject are assessed. Following a 4 day washout, the subject is administered intranasally a liquid formulation containing between 3% and 12% magnesium citrate every morning and evening for a period of 3 days, and the social functioning and anxiety of the subject are assessed. Following a 4 day washout, the subject is administered intranasally a liquid

formulation containing a combination of between 12 and 24 IU of oxytocin and between 3% and 12% magnesium citrate every morning and evening for a period of 3 days, and the social functioning and anxiety of the subject are assessed.

Example 5: Human clinical tests

[0129] Using a double-blind, randomized, placebo controlled, parallel design the effects of a 6 week course of twice daily intranasal treatment with a combination of oxytocin and magnesium are tested in male and female subjects, aged 18 to 55 years, with a diagnosis of autism spectrum disorder. The primary efficacy endpoint is the change in scores of social reciprocity measured by the Autism Diagnostic Observation Schedule-II prior to and after the double-blind treatment period. Secondary endpoints consist of one of more of the following:

(1) Changes in scores of communication and restricted and repetitive behavior as measured by Autism Diagnostic Observation Schedule-II prior to and on completion of the double-blind treatment period;

(2) Change in anxiety as measured by the State and Trait Anxiety Inventory assessed prior to and on completion of the double-blind treatment period;

(3) Change in depression as measured by the Center for Epidemiologic Studies Depression Scale assessed prior to and on completion of the double-blind treatment period;

(4) Change in eye gaze to social cues assessed prior to and on completion of the double-blind treatment period;

(5) Changes in facial and voice expressions analyzed from videos recorded in every two weeks during trial period; and

(6) Change in Clinical Global Impression and Global Assessment of Functioning scores assessed in every two weeks during trial period.

[0130] Study inclusion criteria consist of the following:

1) Diagnosed of autism spectrum disorder based on DSM-V;

2) Exceeded cutoff for qualitative abnormalities in reciprocal social interaction (Domain A) in Autism Diagnostic Interview-Revised; and

3) Verbal IQ above 85 and Full IQ above 80 measured with Wechsler Adult Intelligent Scale-III.

[0131] Study exclusion criteria consist of the following:

1) Primary psychiatric diagnosis other than inclusion criteria 1);

2) Current instability due to a comorbid psychiatric diagnosis;

3) History of changes in medications or medication doses of psychotropics within one month of randomization;

4) Under current treatment with psychotropics more than two categories;

5) Under current treatment with atomoxetine or methylphenidate;

6) History of continual treatment of oxytocin;

7) History of sensitivity to oxytocin;

8) History of seizures or traumatic brain injury with loss of consciousness for longer than 5 minutes; and

9) History of alcoholism or substance abuse or addiction.

Example 6: Effects of oxytocin and magnesium in a social anxiety disorder

[0132] Subjects meeting Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition criteria for generalized social phobia are randomized to treatment with nasal placebo (saline) – treatment A, oxytocin alone (30 IU) – treatment B, magnesium alone (10%) – treatment C, or oxytocin (30 IU) plus magnesium (10%) – treatment D.

[0133] After a 1-week, single-blind, placebo, run-in period, patients receive a double-blind, 11-week course of treatment A, B, C, or D. Patients receive treatments twice/day at approximately 12 hour intervals.

[0134] Optionally, serum IL-6 levels are taken at the end of the 1 week run-in period and at the end of the 11 week course.

[0135] Number of responders based on the Clinical Global Impression Global Improvement Item ("much improved" or "very much improved"); mean change from baseline on the Liebowitz Social Anxiety Scale total score are measured. Optinally, the serum level of IL-6 are correlated with the degree of efficacy to determine effectiveness of IL-6 as a predictive biomarker of efficacy.

[0136] The effects of the treatment groups are analyzed.

EXEMPLARY EMBODIMENTS

[0137] The invention is further described by the following embodiments. The features of each of the embodiments are combinable with any of the other embodiments where appropriate and practical.

[0138] Embodiment 1. In one embodiment, there is provided a method for treating an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or an anxiety, comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect.

[0139] Embodiment 2. In a further embodiment of embodiment 1, the oxytocin peptide is administered concurrently with the magnesium ions.

[0140] Embodiment 3. In a further embodiment of embodiment 1, the oxytocin peptide is administered before or after administration of the magnesium ions.

[0141] Embodiment 4. In a further embodiment of any one of embodiments 1 to 3, the oxytocin peptide is administered via craniofacial mucosal administration.

[0142] Embodiment 5. In a further embodiment of embodiment 4, the oxytocin peptide is administered via intranasal administration.

[0143] Embodiment 6. In a further embodiment of embodiment 5, the oxytocin peptide and the magnesium ions are administered via intranasal administration.

[0144] Embodiment 7. In a further embodiment of any one of embodiments 1 to 6, the effective dose of the oxytocin peptide is about 0.5 μg to about 2000 μg.

[0145] Embodiment 8. In a further embodiment of any one of embodiments 1 to 7, the effective dose of the magnesium ions is about 50 μg to about 68 mg.

[0146] Embodiment 9. In a further embodiment of any one of embodiments 1 to 8, the magnesium ions are provided using magnesium chloride and/or magnesium citrate.

[0147] Embodiment 10. In a further embodiment of embodiment 1, the effective dose of the oxytocin peptide and the magnesium ions comprises about 15 μg to about 120 μg of the oxytocin peptide administered in an aqueous solution containing about 1.1% to about 1.6% (w/v) of magnesium.

[0148] Embodiment 11. In a further embodiment of embodiment 1, the effective dose of the oxytocin peptide and the magnesium ions has an oxytocin to magnesium molar ratio between about 1:40 to about 1:40000.

[0149] Embodiment 12. In a further embodiment of any one of embodiments 1 to 11, the method is for treating an autism spectrum disorder.

[0150] Embodiment 13. In a further embodiment of any one of embodiments 1 to 11, the method is for treating a disorder manifesting one or more symptoms associated with an autism spectrum disorder.

[0151] Embodiment 14. In a further embodiment of embodiment 13, the disorder is a social anxiety disorder, an obsessive-compulsive disorder, a social (pragmatic) communication disorder, a neurodevelopmental disorder, an attention deficit hyperactivity disorder, Prader-Willi syndrome, Timothy syndrome, Fragile-X syndrome, Rett syndrome, or Williams syndrome.

[0152] Embodiment 15. In a further embodiment of any one of embodiments 1 to 11, the method is for treating social and communication deficits.

[0153] Embodiment 16. In a further embodiment of any one of embodiments 1 to 11, the method is for treating an anxiety.

[0154] Embodiment 17. In a further embodiment of any one of embodiments 1 to 16, the oxytocin peptide is human oxytocin (SEQ. ID NO:1).

[0155] Embodiment 18. In one embodiment, there is provided a method for treating an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or an anxiety, comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein the effective dose of the oxytocin peptide and the magnesium ions is administered intranasally in a liquid formulation, and the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.

[0156] Embodiment 19. In a further embodiment of embodiment 18, the effective dose of the oxytocin peptide is about 0.5 μg to about 2000 μg.

[0157] Embodiment 20. In a further embodiment of embodiment 18, the effective dose of the magnesium ions is about 50 μg to about 68 mg.

[0158] Embodiment 21. In a further embodiment of embodiment 18, the effective dose of the oxytocin peptide and the magnesium ions comprises about 15 μg to about 120 μg of the oxytocin peptide administered in an aqueous solution containing about 1.1% to about 1.6% (w/v) of magnesium.

[0159] Embodiment 22. In a further embodiment of embodiment 18, the effective dose of the oxytocin peptide and the magnesium ions has an oxytocin to magnesium molar ratio between about 1:40 to about 1:40000.

[0160] Embodiment 23. In a further embodiment of embodiment 21 or 22, the volume of the liquid formulation administered is between about 50 μL and about 200 μL.

[0161] Embodiment 24. In a further embodiment of embodiment 23, the liquid formulation is administered using a metered nasal device in 1 to 4 units of about 50 μL per unit.

[0162] Embodiment 25. In a further embodiment of any one of embodiments 18 to 24, the method is for treating an autism spectrum disorder.

[0163] Embodiment 26. In a further embodiment of any one of embodiments 18 to 24, the method is for treating a disorder manifesting one or more symptoms associated with an autism spectrum disorder.

[0164] Embodiment 27. In a further embodiment of embodiment 26, the disorder is a social anxiety disorder, an obsessive-compulsive disorder, a social (pragmatic) communication disorder, a neurodevelopmental disorder, an attention deficit hyperactivity disorder, Prader-Willi syndrome, Timothy syndrome, Fragile-X syndrome, Rett syndrome, or Williams syndrome.

[0165] Embodiment 28. In a further embodiment of any one of embodiments 18 to 24, the method is for treating social and communication deficits.

[0166] Embodiment 29. In a further embodiment of any one of embodiments 18 to 24, the method is for treating an anxiety.

[0167] Embodiment 30. In a further embodiment of any one of embodiments 18 to 29, the oxytocin peptide is human oxytocin (SEQ. ID NO:1).

[0168] Embodiment 31. In a further embodiment of embodiment 18, the liquid formulation is contained in a device for intranasal administration.

[0169] Embodiment 32. In a further embodiment of embodiment 31, the device for intranasal administration is a nasal pump apparatus.

[0170] Embodiment 33. In a further embodiment of embodiment 32, the nasal pump apparatus comprises a reservoir bottle attached to a pump actuator.

[0171] Embodiment 34. In a further embodiment of embodiment 33, the pump actuator is metered to deliver a specified volume of about 50 µL.

[0172] Embodiment 35. In a further embodiment of embodiment 32, the nasal pump apparatus comprises a reservoir bottle attached to an aerosolizer.

[0173] Embodiment 36. In a further embodiment of any one of embodiments 32 to 35, the nasal pump apparatus comprises one of more of the following:

(i) a filter for preventing back flow,

(ii) a metal-free fluid path, and

(iii) a plastic material stable to gamma-radiation.

[0174] Embodiment 37. In one embodiment, there is provided a composition comprising an oxytocin peptide and magnesium ions, wherein the oxytocin peptide and the magnesium ions

are in an amount that produces a synergistic or enhanced effect when used in the treatment of anxiety.

[0175] Embodiment 38. In a further embodiment of embodiment 37, the oxytocin peptide is human oxytocin (SEQ. ID NO:1).

[0176] Embodiment 39. In a further embodiment of embodiment 37, the composition is a liquid formulation comprising between about 0.01 mg/mL and about 16 mg/mL of the oxytocin peptide.

[0177] Embodiment 40. In a further embodiment of embodiment 37, the composition is a liquid formulation comprising the magnesium salt in an amount to provide between about 3 mg/mL and about 30 mg/mL of magnesium.

[0178] Embodiment 41. In a further embodiment of embodiment 37, the oxytocin peptide and the magnesium ions have a molar ratio between about 1:40 to about 1:40000.

[0179] Embodiment 42. In a further embodiment of embodiment 41, the molar ratio is between about 1:40 to about 1: 800.

[0180] Embodiment 43. In a further embodiment of embodiment 41, the molar ratio is between about 1:800 to about 1: 40000.

[0181] Embodiment 44. In a further embodiment of any one of embodiments 37 to 43, the composition further comprises a device for craniofacial mucosal administration.

[0182] Embodiment 45. In a further embodiment of embodiment 44, the oxytocin peptide and the magnesium ions are contained in the device for craniofacial mucosal administration.

[0183] Embodiment 46. In a further embodiment of embodiment 45, the device is for intranasal administration.

[0184] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be apparent to those skilled in the art that certain changes and modifications may be practiced without departing from the invention. Therefore, the descriptions and examples should not be construed as limiting the scope of the invention.

[0185] All patents, patent applications, documents, and articles cited herein are incorporated by reference in their entireties.

CLAIMS

What is claimed is:

1. A method for treating an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or an anxiety, comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein co-administration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect.

2. The method of claim 1, wherein the oxytocin peptide is administered concurrently with the magnesium ions.

3. The method of claim 1, wherein the oxytocin peptide is administered before or after administration of the magnesium ions.

4. The method of any one of claims 1 to 3, wherein the oxytocin peptide is administered via craniofacial mucosal administration.

5. The method of claim 4, wherein the oxytocin peptide is administered via intranasal administration.

6. The method of claim 5, wherein the oxytocin peptide and the magnesium ions are administered via intranasal administration.

7. The method of any one of claims 1 to 6, wherein the effective dose of the oxytocin peptide is about 0.5 μg to about 2000 μg.

8. The method of any one of claims 1 to 7, wherein the effective dose of the magnesium ions is about 50 μg to about 68 mg.

9. The method of any one of claims 1 to 8, wherein the magnesium ions are provided using magnesium chloride and/or magnesium citrate.

10. The method of claim 1, wherein the effective dose of the oxytocin peptide and the magnesium ions comprises about 15 μg to about 120 μg of the oxytocin peptide administered in an aqueous solution containing about 1.1% to about 1.6% (w/v) of magnesium.

11. The method of claim 1, wherein the effective dose of the oxytocin peptide and the magnesium ions has an oxytocin to magnesium molar ratio between about 1:40 to about 1:40000.

12. The method of any one of claims 1 to 11, wherein the method is for treating an autism spectrum disorder.

13. The method of any one of claims 1 to 11, wherein the method is for treating a disorder manifesting one or more symptoms associated with an autism spectrum disorder.

14. The method of claim 13, wherein the disorder is a social anxiety disorder, an obsessive-compulsive disorder, a social (pragmatic) communication disorder, a neurodevelopmental disorder, an attention deficit hyperactivity disorder, Prader-Willi syndrome, Timothy syndrome, Fragile-X syndrome, Rett syndrome, or Williams syndrome.

15. The method of any one of claims 1 to 11, wherein the method is for treating social and communication deficits.

16. The method of any one of claims 1 to 11, wherein the method is for treating an anxiety.

17. The method of any one of claims 1 to 16, wherein the oxytocin peptide is human oxytocin (SEQ. ID NO:1).

18. A method for treating an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or an anxiety, comprising administering to a subject in need thereof an effective dose of an oxytocin peptide and magnesium ions, wherein the effective dose of the oxytocin peptide and the magnesium ions is administered intranasally in a liquid formulation, and the volume of the liquid formulation administered is between about 5 μL and about 1000 μL.

19. The method of claim 18, wherein the effective dose of the oxytocin peptide is about 0.5 μg to about 2000 μg.

20. The method of claim 18, wherein the effective dose of the magnesium ions is about 50 μg to about 68 mg.

21. The method of claim 18, wherein the effective dose of the oxytocin peptide and the magnesium ions comprises about 15 μg to about 120 μg of the oxytocin peptide administered in an aqueous solution containing about 1.1% to about 1.6% (w/v) of magnesium.

22. The method of claim 18, wherein the effective dose of the oxytocin peptide and the magnesium ions has an oxytocin to magnesium molar ratio between about 1:40 to about 1:40000.

23. The method of claim 21 or 22, wherein the volume of the liquid formulation administered is between about 50 μL and about 200 μL.

24. The method of claim 23, wherein the liquid formulation is administered using a metered nasal device in 1 to 4 units of about 50 μL per unit.

25. The method of any one of claims 18 to 24, wherein the method is for treating an autism spectrum disorder.

26. The method of any one of claims 18 to 24, wherein the method is for treating a disorder manifesting one or more symptoms associated with an autism spectrum disorder.

27. The method of claim 26, wherein the disorder is a social anxiety disorder, an obsessive-compulsive disorder, a social (pragmatic) communication disorder, a neurodevelopmental disorder, an attention deficit hyperactivity disorder, Prader-Willi syndrome, Timothy syndrome, Fragile-X syndrome, Rett syndrome, or Williams syndrome.

28. The method of any one of claims 18 to 24, wherein the method is for treating social and communication deficits.

29. The method of any one of claims 18 to 24, wherein the method is for treating an anxiety.

30. The method of any one of claims 18 to 29, wherein the oxytocin peptide is human oxytocin (SEQ. ID NO:1).

31. The method of claim 18, wherein the liquid formulation is contained in a device for intranasal administration.

32. The method of claim 31, wherein the device for intranasal administration is a nasal pump apparatus.

33. The method of claim 32, wherein the nasal pump apparatus comprises a reservoir bottle attached to a pump actuator.

34. The method of claim 33, wherein the pump actuator is metered to deliver a specified volume of about 50 µL.

35. The method of claim 32, wherein the nasal pump apparatus comprises a reservoir bottle attached to an aerosolizer.

36. The method of any one of claims 32 to 35, wherein the nasal pump apparatus comprises one of more of the following:
(i) a filter for preventing back flow,
(ii) a metal-free fluid path, and
(iii) a plastic material stable to gamma-radiation.

37. A composition comprising an oxytocin peptide and magnesium ions, wherein the oxytocin peptide and the magnesium ions are in an amount that produces a synergistic or enhanced effect when used in the treatment of anxiety.

38. The composition of claim 37, wherein the oxytocin peptide is human oxytocin (SEQ. ID NO:1).

39. The composition of claim 37, wherein the composition is a liquid formulation comprising between about 0.01 mg/mL and about 16 mg/mL of the oxytocin peptide.

40. The composition of claim 37, wherein the composition is a liquid formulation comprising the magnesium salt in an amount to provide between about 3 mg/mL and about 30 mg/mL of magnesium.

41. The composition of claim 37, wherein the oxytocin peptide and the magnesium ions have a molar ratio between about 1:40 to about 1:40000.

42. The composition of claim 41, wherein the molar ratio is between about 1:40 to about 1: 800.

43. The composition of claim 41, wherein the molar ratio is between about 1:800 to about 1: 40000.

44. The composition of any one of claims 37 to 43, further comprising a device for craniofacial mucosal administration.

45. The composition of claim 44, wherein the oxytocin peptide and the magnesium ions are contained in the device for craniofacial mucosal administration.

46. The composition of claim 45, wherein the device is for intranasal administration.

FIG. 1

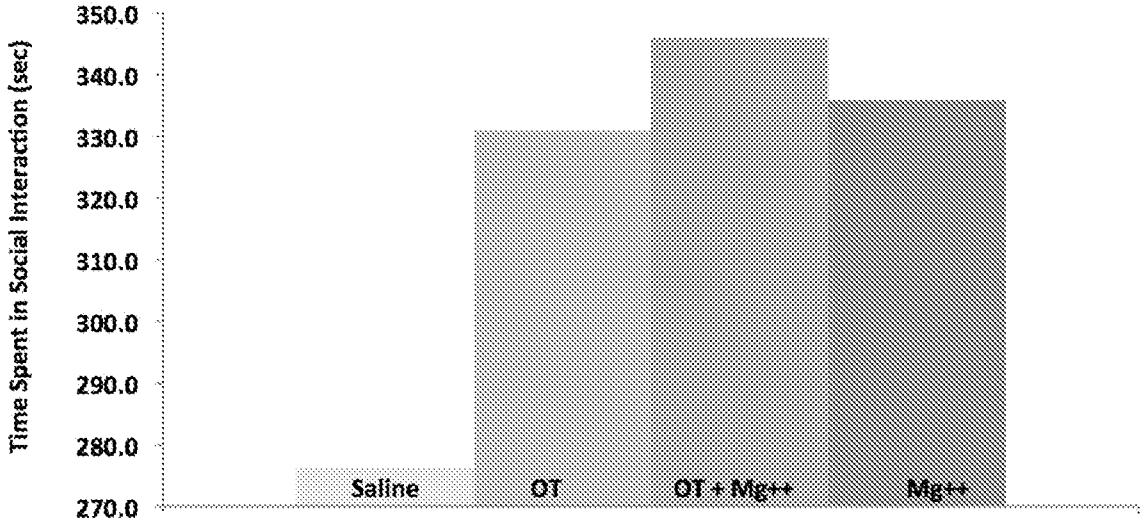

Time Spent in Social Interaction (sec)
350.0
340.0
330.0
320.0
310.0
300.0
290.0
280.0
270.0
Saline
OT
OT + Mg++
Mg++

FIG. 2

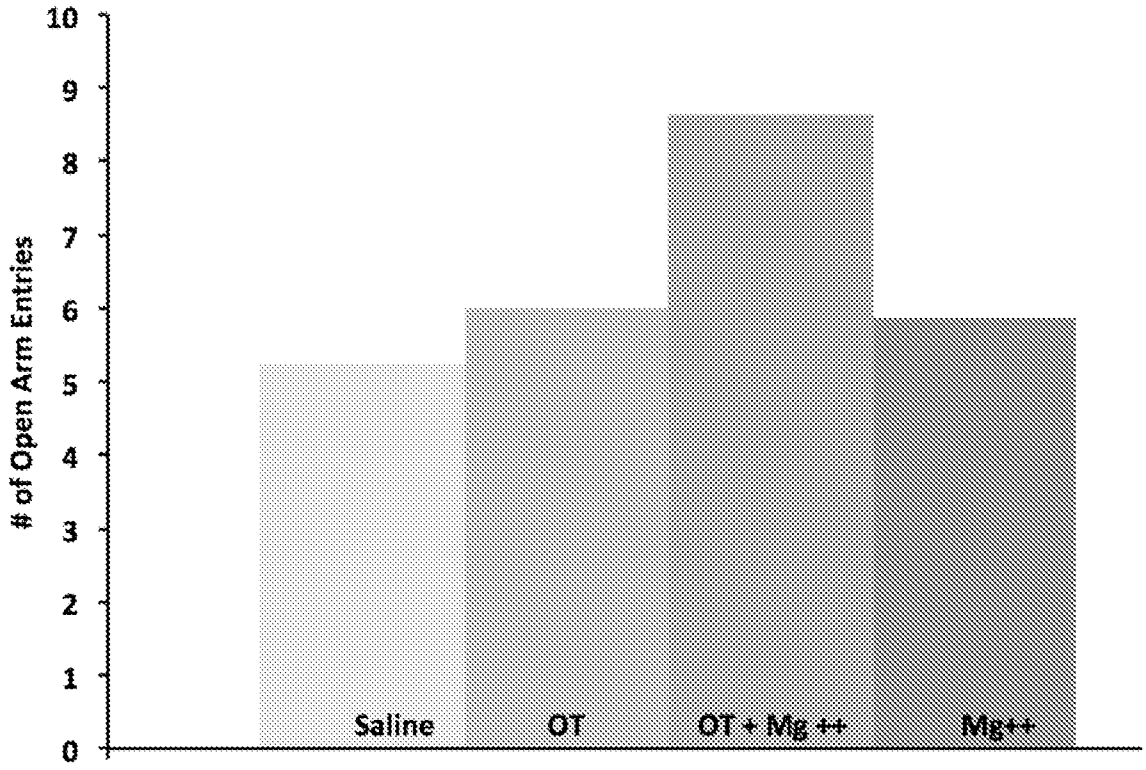

# of Open Arm Entries
10
9
8
7
6
5
4
3
2
1
0
Saline
OT
OT + Mg ++
Mg++

FIG. 3A

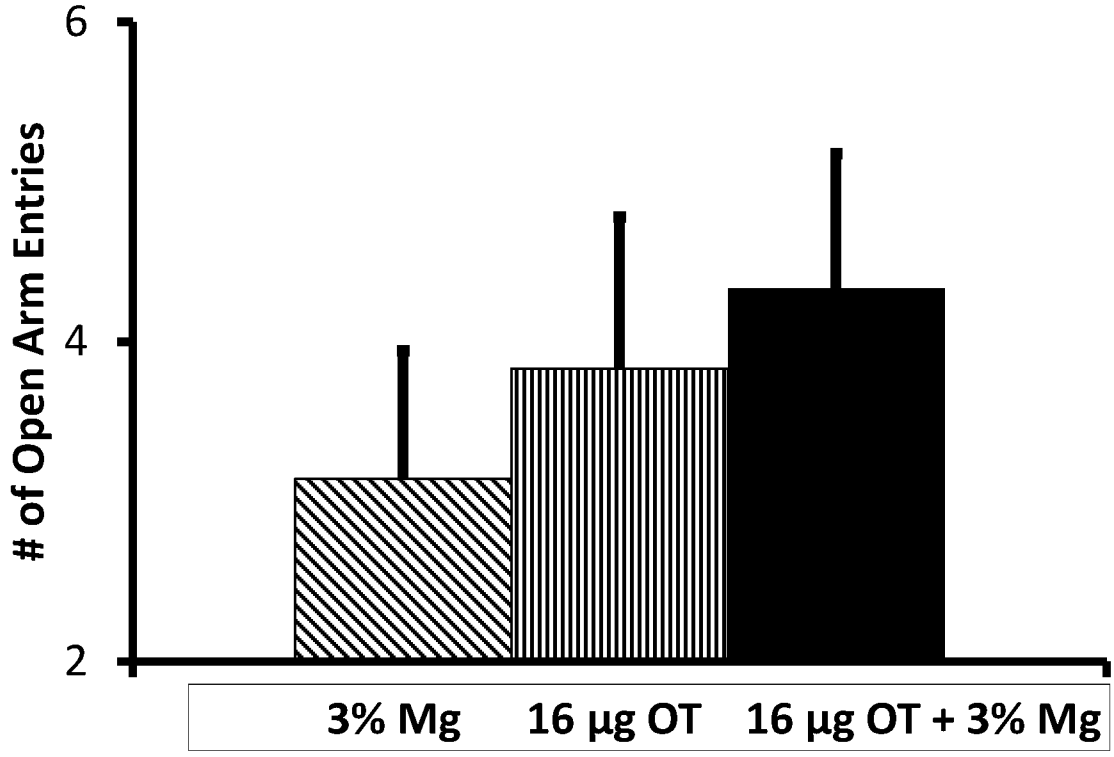
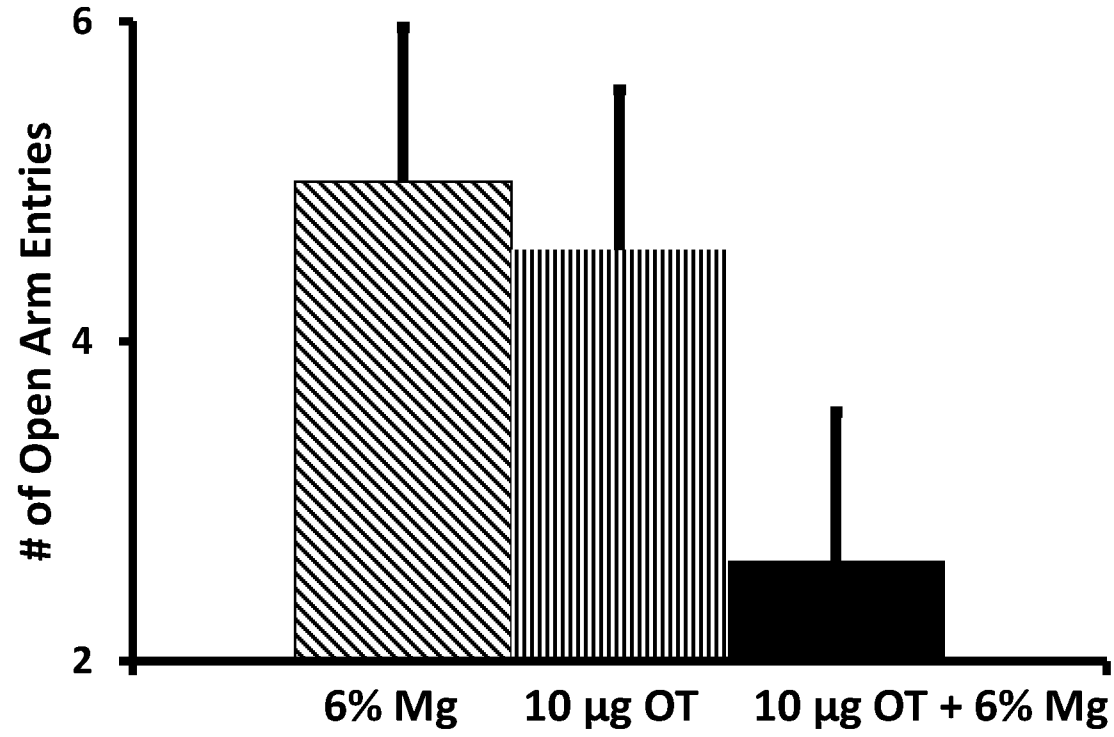

6
4
2
# of Open Arm Entries
3% Mg
16 µg OT
16 µg OT + 3% Mg

FIG. 3B


6
4
2
# of Open Arm Entries
6% Mg
10 µg OT
10 µg OT + 6% Mg

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/27265

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/11, A61P 25/00, A61K 9/12 (2017.01)
CPC - A61K 38/11, A61K 9/12, A61K 9/0043

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2012/0172304 A1 (LEONARD et al.,) 05 July 2012 (05.07.2012) Abstract; para [0019]; para [0057]; para [0072]; para [0079]; para [0084]; para [0089]; para [0109]; para [0136]; para [0159]; para [0221]	1-6, 10, 11, 18-24, 31-36
Y	CHAN et al., Effects of Magnesium ion and oxytocin inhibitors on the uterotonic activity of oxytocin and prostaglandins E2 and F2alpha. Journal of Pharmacology and Experimental Therapeutics. 1 July 1974. Vol 190, No 1, pp 77-87. Especially Abstract; p78, col 1, para 3; p78, col 2, para 3; p80, col 2, para 1	1-6, 10, 11, 18-24, 31-36
Y	US 2014/0342021 A1 (MAGCEUTICS INC.) 20 November 2014 (20.11.2014) Claim 1; para [0010]; para [0023]; para [0097]; para [0133]; para [0138-0139]; para [0141]; para [0143]; para [0155];	2, 10, 11, 20-22

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 July 2017	08 SEP 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/27265

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7-9, 12-17, 25-30
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-6, 10-11, 18-24, 31-36, drawn to methods for treating an autism spectrum disorder.

Group II: Claims 37-46, drawn to a composition comprising an oxytocin peptide and magnesium ions.

-------Please see continuation in first extra sheet----------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-6, 10, 11, 18-24, 31-36

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/27265

Continuation of Box No III Observations where unity of invention is lacking

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I requires method steps for treating an autism spectrum disorder, not required by Group II.

Group II requires a composition of matter, not required by Group I.

Common Technical Features

The feature shared by Groups I and II is an oxytocin peptide and magnesium ions, wherein the oxytocin peptide and the magnesium ions are in an amount that produces a synergistic or enhanced effect when used in the treatment of a symptom of an autism spectrum disorder such as anxiety.

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by US 2012/0172304 A1 to Leonard et al., (hereinafter 'Leonard') in view of the article entitled 'EFFECTS OF MAGNESIUM ION AND OXYTOCIN INHIBITORS ON THE UTEROTONIC ACTIVITY OF OXYTOCIN AND PROSTAGLANDINS E2 AND F2alpha' by Chan et al., (hereinafter 'Chan') [The Journal of Pharmacology and Experimental Therapeutics vol 190, No. 1, 77-87, 1974]

Leonard teaches a method for treating an autism spectrum disorder, a disorder manifesting one or more symptoms associated with an autism spectrum disorder, social and communication deficits, or an anxiety, comprising administering to a subject in need thereof an effective dose of an oxytocin peptide (Abstract - 'Methods and compositions containing oxytocin or an oxytocin analog, such as carbetocin, are provided for the prevention and treatment of autism spectrum disorders, related disorders and symptoms of such disorders. The methods and compositions of this disclosure are effective in the treatment of social withdrawal, eye contact avoidance, repetitive behaviors, anxiety, attention deficit, hyperactivity, depression, loss of speech, verbal communication difficulties, aversion to touch, visual difficulties, comprehension difficulties, and sound and light sensitivity. Additional compositions and methods are provided which employ oxytocin or an oxytocin analog in combination with a secondary or adjunctive therapeutic agent to yield more effective treatment tools against autism spectrum disorders and related disorders.';). Leonard does not expressly state magnesium ions, wherein coadministration of the oxytocin peptide and the magnesium ions produces a synergistic or enhanced effect. Chan teaches the potentiating effect of Magnesium on oxytocin (Abstract - 'The potency of oxytocin was markedly enhanced by 0.5 and 1.0 mM Mg++ in the bathing medium. In 0.5 mM Mg++ both the potency of oxytocin and the maximal response increased... These observations suggest that the potentiating effect of Mg++ on active neurohypophysial peptides cannot be satisfactorily explained on the basis of enhancing hormone-receptor affinity alone. Since Mg++ increases the potency as well as the maximal response to oxytocin, it is probable that Mg++ may in some manner also increase the reactivity or reduce the threshold of excitation-contraction coupling in the contractile system sensitive to the neurohypophysial peptides.'; p78, col 1, para 3 - 'Our studies of the effects of Mg++ on the activities of oxytocin and a series of antioxytocic peptides related to oxytocin revealed that Mg2+ can enhance tile intrinsic activity of neurohypophysial peptides.'; p80, col 2, para 1 - 'Magnesium increased the affinity of oxytocin for the receptor as well as the intrinsic activity of the hormone as indicated by a shift of the dose-response curve to the left and an increase in the maximal response in the presence of 0.5 mM Mg2+'). Since Chan teaches that Magnesium potentiates the neurohypophysial activity of oxytocin, it would have been obvious to one of ordinary skill in the art that magnesium could synergize with the therapeutic administration of oxytocin compositions of Leonard for treatment of autism spectrum disorders.

As the technical feature was known in the art at the time of the invention, it cannot be considered a special technical feature that would otherwise unify the groups.

Groups I and II therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.