Title: FACILITATION OF CENTRAL NERVOUS SYSTEM SEXUAL RESPONSES
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BACKGROUND OF THE INVENTION

Sexual dysfunction has been a persistent problem, more frequent in an aging population, a problem that has only recently been addressed with frank evaluation, scientific investigation and effective treatment. Male impotence, especially male erectile dysfunction, has received the most attention. Female sexual dysfunction has been considered in the context of male erectile dysfunction, in part because of the anatomical and physiological parallels between the male and female genitalia, and in part, with the hope that effective treatments for male erectile dysfunction could provide some relief for female sexual dysfunction.

The incidence of female sexual dysfunction (FSD) may surpass the incidence of male sexual dysfunction. However, the physiological and pathological mechanisms of FSD are yet to be fully understood. Emotional, relational, situational, experiential, physiological, and pharmacological variables all interact in each woman to produce a particular array of sexual outcomes. The FSDs have been categorized into specific disorders: desire, arousal, orgasmic, and sexual pain. These categories conveniently provide working definitions and an accepted lexicon for researchers and therapists. However, there is potential to incorrectly assume that these disorders are fully independent of each other. Both case studies and epidemiology studies demonstrate that these disorders can overlap and may be interdependent. In some cases, it may be possible to identify the primary disorder that led to the others, but in many cases, this may be impossible. The current definitions are independent of etiology and primacy.

Female sexual arousal disorder (FSAD) is the persistent or recurrent inability to attain, or to maintain, sufficient sexual excitement, which causes personal distress. It may be expressed as lack of subjective excitement, lack of genital response, such as lubrication and swelling, or lack of other somatic responses. Female sexual arousal disorder is one form of female sexual dysfunction, and is associated with the excitement phase. See Basson, R., et al., Report of the international consensus


The role of the central nervous system in male and female sexual function has been studied in several experimental models. Several neuroanatomical approaches have been used, including the c-fos labeling method (Morgan, J. I., et al., Mapping patterns of c-fos expression in the central nervous system after seizure, Science 1987 237:192-196), and transneuronal tracing studies using pseudorabies virus. It has been reported that c-fos immunoreactivity in the brain was induced after sexual behavior in the medial preoptic nucleus, posteromedial part of the bed nucleus of the stria terminalis, posterodorsal part of the medial amygdala, and the paraventricular part of the subparafascicular thalamic nucleus (Coolen, L.M., et al., Fos immunoreactivity in the rat brain following consummatory elements of sexual behavior: a sex comparison. Brain Res. 1996 738: 67-82). Transneuronal tracing studies have demonstrated that consistently labeled neurons in the forebrain included the paraventricular nucleus of the hypothalamus (PVN), the medial preoptic area (MPOA), lateral hypothalamic area, and the central tegmental region (Marson, L., et al., Central nervous system innervation of the penis as revealed by the transneuronal transport of pseudorabies virus. Neuroscience 1993 55: 263-280).

Sexual activity causes an increase in plasma oxytocin in human females that peaks during orgasm (Carmichael, M.S., et al., Relationships among cardiovascular, muscular, and oxytocin responses during human sexual activity, Arch. Sex Behav. 1994 Feb; 23(1):59-79). The subjective intensity of the orgasm is related to plasma oxytocin levels in multiorgasmic women (Carmichael, M.S., et al., Plasma oxytocin

Today, although modern functional imaging techniques have been used to study the neurophysiology of sexual function, the functional anatomy of human sexual responses is still poorly understood. Among these techniques, functional brain imaging techniques bridge the gap between the neural systems and the behavioral neurosciences. Functional magnetic resonance imaging (fMRI) is able to detect discrete areas related to metabolic change resulting from neuronal activity, which is visualized indirectly by detecting changes in signals related to regional cerebral blood flow and local deoxyhemoglobin concentrations in the activated cortex. The significant advantages of fMRI are 1) the noninvasive signal does not require injections of radioactive isotopes or other contrast media, 2) the total scan time required can be very short, i.e., on the order of 1.5 to 2 minutes per run, and 3) the high in-plane resolution of the functional image is generally about 1.5 x 1.5 mm. In a study done to identify the functional neuroanatomy of the female brain associated with visually evoked sexual arousal using fMRI, the following regions of the brain were significantly activated when the women viewed an erotic film: the inferior frontal cortex, insula gyrus, cingulated gyrus, caudate nucleus, corpus callosum, globus pallidus, inferior temporal cortex, and thalamus (Park, K., et al., Blood-oxygenated-level-dependent functional magnetic resonance imaging for evaluating cerebral regions of female sexual arousal response, Urology 2001 57 (6): 1189-1194).

Topical application of alprostadil with a skin penetration enhancer (DDAIP) has been shown by color duplex ultrasonography to produce significant increases in peak systolic velocity (PSV) and end diastolic velocity (EDV) when pre-application and post-application values were compared (Becher, E.F., et al., Clitoral hemodynamic changes after a topical application of alprostadil, J. Sex & Martial Ther., 2001 27:405-410). The value of this study is reduced by the absence of a placebo control. While the authors suggested that topical vasoactive drugs might help in the differential diagnosis of the vascular component of female sexual dysfunction. However, the study cannot support such a statement. The small (18 women) subject population included normal women as well as a majority (14/18) of subjects reporting “some degree of female sexual dysfunction,” but the effects of the
topical application of alprostadil on normal and sexually dysfunctional subjects were not analyzed separately.

Anatomy of the Vagina

The vagina is the canal that connects the uterus with the external genital organs. Its design easily accommodates penetration of a rigid penile erection. At the posterior end the rounded neck of the uterus, the cervix, projects into the space known as the fornix or vaginal vault. Anteriorly, two pleats of sensitive tissue, the labia minora, surround the opening of the vagina and are further protected by larger folds known as the labia majora.

The walls of the vagina consist of three layers - an inner mucosa, an glandular mucous membrane epithelium, an intermediate, highly vascularized muscularis layer, and an outer supportive fibrous mesh. The vaginal mucosa is a mucous type stratified squamous cell epithelium that undergoes hormone-related cyclical changes, such as a slight keratinization of the superficial cells during the menstrual cycle. The muscularis portion comprises smooth muscle and an extensive arborization of blood vessels that may swell during intercourse. The surrounding fibrous layer provides structural support to the vagina; this layer consists of elastin and collagen fibers that allow for expansion of the vaginal vault during sexual arousal or childbirth. Large blood vessels run within the mucosa, and nerve plexuses are present within muscular and adventitial layers. The vagina has many rugae or folds that are necessary for the distensibility of the organ during intercourse and childbirth. Smaller ridges lend to the frictional tension that exists during intercourse.

The arterial supply to the vagina is derived from an extensive network of branching vessels surrounding it from all sides. The anterior branch of the internal iliac artery continually bifurcates as it descends through the pelvis with a series of the newly generated vessels, each supplying the vagina to some degree. After giving off an obturator artery branch, the umbilical, and the middle rectal arteries diverge off to supply a superior and inferior vesical artery, respectively. Between the umbilical and the mid-rectal branches there is a generation of a uterine artery, which further bifurcates to give the vaginal artery. The internal pudendal and accessory pudendal artery also send a branch to the vaginal artery. Finally, the common clitoral artery
sends a branch to the vaginal muscularis.

The neurologic innervation of the vagina originates from two separate plexuses, the superior hypogastric plexus and the sacral plexus. The hypogastric nerve plexus descends on the great vessels spreading into an inferior hypogastric plexus, which systematically branches further into an uterovaginal nerve. The somatic pudendal nerve originates off the pelvic splanchnic branches from the secret plexus. Pudendal branching innervates the vagina towards the opening of the introitus as the perineal and posterior labial nerves.

Immunohistochemistry studies have been utilized to better understand the innervation of the human vaginal mucosa. In a study by Hilliges et al. using protein gene product 9.5, more distal areas of the vagina had significantly more nerve fibers compared to the more proximal parts, and the anterior wall showed a denser innervation than the posterior wall (Hilliges, M. et al., Innervation of the human vaginal mucosa as revealed by PGP 9.5 immunohistochemistry, Acta Anatomica 153: 119 (1995)). Graf et al. studied the distribution patterns and the occurrence of helospectin and pituitary adenylate cyclase activating polypeptide (PACAP) immunoreactivity (Graf, A.H., et al. Helospectin and pituitary adenylate cyclase activating polypeptide in the human vagina, Regul. Pept. 55: 277 (1995)). They confirmed a dense network of vasoactive intestinal peptide (VIP) immunoreactive nerve fibers showing sub-populations of helospectin and LI-type PACAP. Nerve fibers of the vagina had previously been shown to be active in association with specific peptides that include VIP, peptide histidine methionine (PHM), calcitonin gene related peptide (CGPP), and galanin. Genital vasodilation and subsequent increase in vaginal blood flow and lubrication have been observed upon exposure of vessels to VIP. VIP has been implicated as the neurotransmitter for mediating vaginal vasodilation and the formation of lubricating fluid during sexual arousal. Helospectin and PACAP, a potent vasodilator, belong to the same peptide family as VIP and PHM, and recent observations have been made to the effect that distributions and co-localizations of helospectin and VEP as well as PACAP and VIP have been reported in the mammalian gastrointestinal tract.

The vaginal canal is lubricated primarily from a transudate originating from the subepithelial vascular bed passively transported through the interepithelial spaces,
sometimes referred to as intercellular channels. Additional moistening during intercourse comes from secretion of the paired greater vestibular or Bartholin's glands.

Estrogen effects on the maintenance and function of female genitalia have been well documented in studies. Estrogen receptors have been shown to exist throughout the vaginal epithelium, in stromal cells, and in the smooth muscle fibers in the muscularis. Weaker conformations of estrogen such as estriol appear more effective in stimulating the vagina as opposed to the uterus. Thickness and rugae of the vaginal wall, as well as vaginal lubrication, have been shown to be estrogen dependent. Although this fluid production has been shown to be hormone-dependent both in the resting state and during sexual excitement, quantitative changes apparently do not occur during the menstrual cycle. An insufficient amount of estrogen will result in thin vaginal walls more easily susceptible to trauma with a decreased ability to heal, as well as a drier and less acidic vaginal environment more vulnerable to infection. Vaginal dryness is associated with ovarian failure and is effectively controlled by estrogen replacement therapy. Some women who are not sexually active may not notice the extent of vaginal atrophy but when coitus does resume, pain and discomfort from intercourse can be considerable.

Anatomy of the Clitoris

The clitoris is the homologue of the penis arising from the embryological genital tubercle. The clitoris consists of a cylindrical, erectile organ composed of three parts: the outermost glans or head, the middle corpus or body, and the innermost crura. The glans of the clitoris is visualized as it emerges from the labia minora, which bifurcate to form the upper prepuce anteriorly and the lower frenulum posteriorly. The body of the clitoris consists of two paired corpora cavernosa of about 2.5 cm in length and lacks a corpus spongiosum. The body extends under the skin at the corona to the crura. The two crura of the clitoris, formed from the separation of the most proximal portions of the corpora in the perineum, attach bilaterally to the undersurface of the symphysis pubis at the ischiopubic rami. A fibrous tunica albuginea ensheaths each corporal body made up of lacunar space sinusoids surrounded by trabecula of vascular smooth muscle and collagen
connective tissue. No retractor clitoridis muscle exists in humans as it does in other animals such as cattle and sheep, however a supporting suspensory ligament does hold the clitoris in the introital region.

The main arterial supply to the clitoris is from the illo-hypogastric-pudendal arterial bed. The internal pudendal artery is the last anterior branch off the internal iliac artery. Distally, the internal pudendal artery traverses Alcock's canal, a position of the obturator fascia and lies on the inner side in apposition to the ischio-pubic ramus. In this latter location, the artery is susceptible to blunt perineal trauma. The internal pudendal artery terminates as it supplies the inferior rectal and perineal artery, which supplies the labia. The common clitoral artery continues to the clitoris. This artery bifurcates into a dorsal clitoral artery and a cavernosal clitoral artery.

Autonomic efferent innervation of the clitoris passes from the pelvic and hypogastric nerves to the clitoris through the urogenital diaphragm. Pelvic nerve stimulation results in clitoral smooth muscle relaxation and arterial smooth muscle dilation. There is a rise in clitoral cavernosal artery inflow, an increase in clitoral intracavernous pressure which lead to tumescence and extrusion of the glans clitoris.

Anatomical studies using female rats have indicated that the major neuronal input to the clitoris was seen in spinal segments from L5-S1, and to a lesser extent in T12-L4 as well as S2-S4. When a label that is taken up by nerve terminals and transported retrogradely to the nerve cell bodies (pseudorabies virus) was injected into the clitoris, labeled nerve cell bodies were found in the brain in multiple locations, including the nucleus paragigantocellularis, raphe pallidus, raphe magnus, Barrington's nucleus, ventrolateral central gray, hypothalamus, and the medial preoptic region. This implies a multisynaptic circuit of neurons may be involved in clitoral neurological control rather than just a simple somatic reflex connection.

Morphological studies have been performed using wheat germ agglutinin conjugated with horseradish peroxidase (WGA / HRP) injected into the clitoris of the female cat to compare afferent pathways to the entire population of pudendal nerve afferents. Central projections of the clitoral afferents were identified in the L7-S3 segments with the most prominent labeling in S1-S2. In the same study, electrophysiological analysis of the clitoris performed under constant mechanical pressure stimulation indicated both phasic and tonic discharges in L7-S2, but most
prominently in S1. In contrast electrical stimulation of the clitoris evoked discharges at S1 only. The neurotransmitters mediating clitoral and arterial smooth muscle dilation remain undetermined, however preliminary studies suggest that nitric oxide is involved. Histochemical studies have revealed VIP and neuropeptide Y (NPY) immunoreactive nerves in the clitoral erectile tissues. Somatic sensory pathways originate from the clitoral skin. There exists a dense collection of Pacinian corpuscles innervated by rapidly adapting myelinated afferents, as well as Meissner's corpuscles, Merckel tactile disks, and free nerve endings. These sensory afferents pass from the dorsal clitoral nerve to the pudendal nerve.

The Grafenbergh Spot

The Grafenbergh spot (or G-spot) can also play a role in female sexual arousal. The current information regarding the Grafenbergh zone (also known as Grafenbergh spot, or G-spot) was recently summarized (Goldstein, I., et al., “Female Sexual Dysfunction” pp. 507-557, at 523 in Jardin, A, et al., editors, Erectile Dysfunction, (First International Consultation on Erectile Dysfunction, co-sponsored by the World Health Organization (WHO), International Consultation on Urological Diseases (ICUD) and Societe Internationale d'Urologie (SIU), held July 1-3, 1999, Paris. 2000). Grafenbergh reported that the digital stroking of the anterior vagina along the urethra, especially in the region of the base of the bladder, sexually aroused female subjects greatly (Grafenbergh E. (1950): The role of the urethra in the female orgasm. Int. J. Sexology. 3: 145-148). In a number of women this region swelled up to the size of a kidney bean and projected into the vaginal lumen. Few took any notice of this finding. The area was rediscovered and renamed the G-spot in honor of Grafenberg (Ladad, A.K., et al., (1982): The G spot and other recent discoveries about Human Sexuality. Holt, Rinehart & Winston, New York). Other investigators could not locate a “spot” but found, rather than a punctate locus, a general excitable area along the whole length of the urethra running along the anterior vaginal wall (Hoch Z. (1986): Vaginal erotic sensitivity by sexological examination. Acta Obstet. et Gynecol. Scand. 65: 768-773). When this was stimulated manually, the sexual arousal induced was almost immediate. Alzate & Londono located the erotic sensitive area in closer relation to the bladder base than the urethra (Alzate H. & Londono M.L.
(1984): Vaginal erotic sensitivity. J. Sex & Marital Therapy. 10: 49-56). Lenck, et al. localized by ultrasound in the living subjects the underlying structure in the anterior vaginal wall that gave the erotic sensations on stimulation as the urethral sphincter confirming it by dissection in the cadaver (Lenck L. Ch., et al., (1992): Sphincter uretral (point G) correlations anatomo-cliniques. Revue Français de Gyncologie et Obstrique. 87: 65-69.). Other investigators have implied that the G spot/area represents that part of the urethra that contains the periglandular or paraurethral tissue, corresponding to the female equivalent of the prostate (See Zaviacic M. & Whipple B. (1993): Update on the female prostate and the phenomenon of female ejaculation. J. Sex Research. 30: 148-151, for references). These glands are present to a greater or lesser degree in about 90% of women.

Prostaglandins

The prostaglandins are a series of cyclic derivatives of certain unsaturated fatty acids. They are found in a variety of tissues, including the prostate gland, the seminal vesicles, the lungs and the brain. These naturally occurring prostaglandins are derived by cyclization of 20-carbon unsaturated fatty acids such as arachidonic acid. See Lehninger, Albert L., *Biochemistry*, 2d ed. (1975) (hereinafter “Lehninger”), p. 300.

Prostaglandins as a class of compounds have diverse pharmacologic activity, including stimulation of gastrointestinal and reproductive smooth muscle, relaxation and contraction of respiratory smooth muscle, hypotensive activity, inhibition of fatty acid lipolysis, inhibition of blood platelet aggregation, and inhibition of gastric acid secretion. Therapeutic utility of prostaglandins in general is correspondingly broad. As for prostaglandin E₁ (“PGE₁”) in particular, this compound, salts thereof, and lower alkyl esters thereof are well known and disclosed, e.g., in U.S. Pat. Nos. 3,069,322 (Bergstrom et al.), 5,219,885 (Froelich et al.) and in J. Org. Chem. 1974, 37, 2921. PGE₁ has found utility in the treatment of peripheral occlusive diseases, acute myocardial infarction, angina pectoris, acute ischemic stroke, asthma, gastrointestinal ulcers, ulcers of the skin, and organ rejection. Various routes of administration have been described, including oral, intravenous, buccal, rectal, intra-arterial, subcutaneous, and sublingual. The preferred route of administration of PGE₁,
will of course be dependent on the particular intended therapeutic use.

Prostaglandins are well known to those skilled in the art. This class of drugs includes those derivatives of prostanoic acid (5-octylcyclopentanehexaptoic acid) referred to as A-I series prostaglandins. Prostaglandin nomenclature is well known and disclosed, e.g., in page 409, Remington's Pharmaceutical Sciences, 18th Edition, 1990, A. R. Gennaro, Ed., Mack Publishing Company, Easton, Pa. The term “prostaglandin” as used generically herein refers to the prostaglandin free acid and pharmaceutically acceptable derivatives thereof, including PGE₁, PGA₁, PGB₁, PGF₁α, 19-hydroxy-PGA₁, 19-hydroxy-PGB₁, PGE₂, PGA₂, PGB₂, 19-hydroxy-PGA₂, 19-hydroxy-PGB₂, PGE₃, PGF₃α, carboprost tromethamine, dinoprost tromethamine, dinoprostone, liproprost, gemeprost, metenoprost, sulprostone and tiaprost as well as salts and esters thereof. Preferred prostaglandins for use in the formulations of this invention include those prostaglandins comprising a β-hydroxyketone moiety, including D-series and E-series prostaglandins, preferably E-series prostaglandins such as prostaglandin E₁, including pharmaceutically acceptable salts and lower alkyl esters thereof (the term “lower alkyl” as used herein means straight chain or branched chain alkyl containing one to four carbon atoms). Of the lower alkyl esters, the ethyl ester of prostaglandin E₁ (commercially available from Sigma Chemical Company, St. Louis, Mo., and preparable as disclosed, e.g., in U.S. Pat. No. 5,219,885, incorporated herein by reference) is preferred.

The biosynthesis of prostaglandins has been well characterized. See, e.g., Lehninger at p. 687. In a typical biosynthetic pathway, exemplified by production of PGE₂, the essential fatty acid linoleic acid is converted into the 20-carbon arachidonic acid, which is then acted upon by prostaglandin synthase, a dioxygenase enzyme.

Oxygen atoms are added at carbon atoms 9 and 15, and the product is cyclized by formation of a bond between carbon atoms 8 and 12. In the presence of reduced glutathione, this cyclized product undergoes conversion into prostaglandin PGE₂. Other types of naturally occurring prostaglandins are derived from different polyunsaturated fatty acids.

In about the 1960s, prostaglandins were isolated from a particular species of Caribbean coral, which made them more widely available for research. Catanzarite,
Valerian A. and Gary Aisenbrey, *Contemporary OB/GYN* (October 1987), p. 22. A large number of natural and synthetic analogues of the prostaglandins are now known. Lehninger at 687.

The prostaglandins are known to produce various effects over a very wide range of biological activities of a hormonal or regulatory nature. Prostaglandins have been reported to both lower and raise blood pressure, to inhibit gastric secretion, dilate bronchi, inhibit lipolysis, antagonize vasopressin-induced anti-diarrhesis, constrict the pupil, increase and decrease the intraocular pressure and produce contraction of the uterus. See, e.g., Ganong, William F., *Review of Medical Physiology*, 7th ed. (1975), p. 226 (hereinafter “Ganong”). The naturally occurring prostaglandins all appear to be capable of affecting the control of vascular and other smooth muscle contractions. In the central nervous system, prostaglandins are known to modify responses to certain synaptic transmitters. They have been reported to mimic the actions of some hormones and to inhibit the actions of certain others.

Topical and transdermal administration of PGE₁ and PGE₂ derivatives have been described, e.g., in U.S. Pat. Nos. 4,889,845 (Ritter et al.), 4,515,810 (Chow et al.), and 5,219,885 (Froelich et al.) and in Japanese Kokai 2-264725 (Morimoto et al.) and 63-135333 (Nakano et al.). In order for a transdermal formulation of PGE₁ or a derivative thereof to be effective and suitable it is desirable that the formulation have a high transdermal flux rate, allowing a therapeutically effective blood level of the drug to be achieved or maintained when the formulation is applied to a relatively small area of the skin. Furthermore PGE₁ readily undergoes certain reactions and rearrangements (see e.g., Lee et al. J. Chromatography, 1991, 555: 73). This instability of the prostaglandin can be problematic in providing a suitable transdermal formulation.

**SUMMARY OF THE INVENTION**

The present invention provides objective methods of assessing sexual function in a female subject and methods of determining central nervous system responses characteristic of specific sexual dysfunctions. In other preferred embodiments, the
present invention provides methods of screening topical compositions that are effective for modulating central nervous system sexual responses. In preferred embodiments, the present invention provides objective methods that can supplement subjective reporting and questionnaires in the study, diagnosis and treatment of female sexual dysfunctions, such as female sexual arousal disorder.

In certain preferred embodiments, the present invention provides a method for assessing sexual function in a female subject, including the steps of recording baseline neuronal activity in at least one central nervous system region of the female subject that is associated with sexual responses; applying a test topical composition to a region of the female subject’s genitalia; recording neuronal activity in the central nervous system region after the application of the test topical composition; recording neuronal activity in the central nervous system region during a presentation of a neutral stimulus after the application of the test topical composition; recording neuronal activity in the central nervous system region during a presentation of an erotic stimulus after the application of the test topical composition; applying a placebo topical composition to a region of the female subject’s genitalia; recording neuronal activity in the central nervous system region after the application of the placebo topical composition; recording neuronal activity in the central nervous system region during a presentation of a neutral stimulus after the application of the placebo topical composition; recording neuronal activity in the central nervous system region during a presentation of an erotic stimulus after the application of the placebo topical composition; and comparing the female subject’s baseline neuronal activity in the central nervous system region to the neuronal activity after the application of the test or placebo topical compositions in the absence of stimuli and during the presentation of neutral or erotic stimuli to the neuronal activity recorded in the same central nervous system region under the same conditions in a population of healthy subjects.

Suitable stimuli are those that elicit a change in central nervous system neuronal activity via a sensory modality including, but not limited to, sight, touch, hearing, smell, taste and proprioception. In certain preferred embodiments, the neutral and erotic stimuli are mechanical stimuli. In other preferred embodiments, the neutral and erotic stimuli are visual stimuli. In embodiments in which the subject is a
woman, the neutral stimulus is a documentary video recording and the erotic stimulus is an erotic video recording. Typically the neutral and erotic stimuli are directed to the same sensory modality. However, the neutral and erotic stimuli can be directed to different sensory modalities.

In further preferred embodiments, the method of the present invention can be used to compare the female subject’s baseline neuronal activity in the central nervous system region to the neuronal activity after the application of the test or placebo topical compositions in the absence of stimuli and during the presentation of neutral or erotic stimuli to the neuronal activity recorded in the same central nervous system region under the same conditions in a population of female subjects diagnosed as suffering from a sexual dysfunction such as a desire disorder, an arousal disorder, an orgasmic disorder and a sexual pain disorder.

In particularly preferred embodiments, the method of the present invention is applied to healthy women or women suffering from a sexual dysfunction. Typically, the central nervous system region is at least one of the hypothalamic periventricular nucleus, the basomedial amygdala nucleus, parietal cortex, Broca’s area, insula, gyri fusiformis, superior frontal gyrus or anterior frontal lobe.

In certain preferred embodiments, the neuronal activity that is recorded is metabolic activity, and the metabolic activity is preferably recorded using functional magnetic resonance imaging. Functional magnetic resonance imaging provides the ability to correlate anatomical and functional information, provide an average result for a group of female subjects and calculate statistical measures. Results from studies on different groups of female subjects under the same or different conditions and be stored and compared. In other embodiments, other recording methods such as positron emission tomography (PET), single photon emission computed tomography (SPECT) and hybrid CT (computed tomography)/PET. In other preferred embodiments, the neuronal activity that is recorded is electrical activity and is recorded by standard methods known in the art, such as electrophysiological methods or optical methods using voltage-sensitive dyes.

Generally the test topical composition comprises an active agent, preferably a vasoactive agent and a pharmaceutically acceptable carrier. A preferred vasoactive agent is prostaglandin E1. The choice of the pharmaceutically acceptable carrier is
governed, in part, by the active agent. When the active agent is prostaglandin E1, a preferred pharmaceutically acceptable carrier comprises ethanol, ethyl laurate, modified guar gum, dodecyl N,N-dimethylamino isopropionate HCl, modified guar gum, buffer, sodium hydroxide and water. Typically the placebo compound consists essentially of the pharmaceutically acceptable carrier alone. Preferably the test and placebo compositions are administered to the female subject in a double-blind protocol with sufficient time allowed between administrations to ensure wash-out of the composition administered previously. Typically the test or placebo compositions are applied to the clitoris, the anterior vaginal wall or both the clitoris and the anterior vaginal wall. Typically, when the female subject is a woman, the neutral stimulus is a documentary video recording and the erotic stimulus is an erotic video recording.

In other embodiments, the method of the present invention is used for monitoring the progress of the treatment of a female subject suffering from a sexual dysfunction by applying the method on more than one occasion during the course of treatment and comparing the results obtained on each occasion. In further embodiments, the method of the present invention is used for determining the patterns of central nervous system activity characteristic of specific clinically determined sexual dysfunctions: desire disorders, arousal disorders, such as female sexual arousal disorder, orgasmic disorders or sexual pain disorders.

In other aspects, the present invention provides a method for screening topical compositions effective for modulating central nervous system sexual responses including the steps of recording the baseline neuronal activity in at least one central nervous system region of a female subject that is associated with sexual responses; applying a test topical composition to a region of the female subject’s genitalia; recording neuronal activity in the central nervous system region after the application of the test topical composition; applying a placebo topical composition to a region of the female subject’s genitalia; recording neuronal activity in the central nervous system region after the application of the placebo topical composition; and comparing the neuronal activity after the application of the test topical composition to the neuronal activity after the application of the placebo topical composition and baseline neuronal activity to determine if the test topical composition was effective for
modulating central nervous system sexual responses, thereby screening a topical composition.

Generally the test topical composition comprises an active agent, preferably a vasoactive agent and a pharmaceutically acceptable carrier. The choice of the pharmaceutically acceptable carrier is governed, in part, by the active agent. Typically the placebo compound consists essentially of the pharmaceutically acceptable carrier alone. Preferably the test and placebo compositions are administered to the female subject in a double-blind protocol with sufficient time allowed between administrations to ensure wash-out of the composition administered previously.

In preferred embodiments, the female subject is a woman or a non-human mammal. Typically the test or placebo compositions are applied to the clitoris, the anterior vaginal wall or both the clitoris and the anterior vaginal wall. Typically, the central nervous system region that is associated with sexual responses is at least one of the hypothalamic periventricular nucleus, the basomedial amygdala nucleus, parietal cortex, Broca's area, insula, gyri fusiformis, superior frontal gyrus or anterior frontal lobe.

Typically, the neuronal activity that is recorded is electrical activity that is recorded by standard methods, such as electrophysiological methods or optical methods using voltage-sensitive dyes or optical intrinsic signals. In other preferred embodiments, the neuronal activity that is recorded is metabolic activity, and a preferred recording method is functional magnetic resonance imaging.

In certain preferred embodiments, the method also includes the steps of recording neuronal activity in the central nervous system region during a presentation of a neutral stimulus after the application of the test topical composition; recording neuronal activity in the central nervous system region during a presentation of an erotic stimulus after the application of the test topical composition; recording neuronal activity in the central nervous system region during a presentation of a neutral stimulus after the application of the placebo topical composition; and recording neuronal activity in the central nervous system region during a presentation of an erotic stimulus after the application of the placebo topical composition.
Typically, when the female subject is a woman, the neutral stimulus is a documentary video recording and the erotic stimulus is an erotic video recording.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Figures 1A-1F are graphical representations of the results of electrophysiological experiments showing the extracellularly recorded action potential (spike) activity of neurons in the hypothalamic paraventricular nucleus of a female rat, where Figure 1A is a graphical representation of the spike rate of a neuron as a function of time during the experiment, showing the effect of the application of a placebo, (arrow, 12) or a topical prostaglandin E₁ composition, (arrow, 14) via cannulae positioned at the clitoris and anterior vaginal wall (corresponding to the human G-spot), where a comparison of Figure 1B, Figure 1C and Figure 1D shows little increase in spike activity after application 12 of the placebo, while comparison of Figure 1D, Figure 1E and Figure 1F shows an increase in spike activity after application 14 of the topical prostaglandin E₁ composition.

Figures 2A-2F are graphical representations of the results of electrophysiological experiments showing the extracellularly recorded action potential (spike) activity of neurons in the hypothalamic paraventricular nucleus of a female rat, where Figure 2A is a graphical representation of the spike rate of a neuron as a function of time during the experiment, showing the effect of the application of a placebo, (arrow, 22) or a topical prostaglandin E₁ composition, (arrow, 24) via cannulae positioned at the clitoris and anterior vaginal wall (corresponding to the human G-spot), where a comparison of Figure 2B, Figure 2C and Figure 2D shows little increase in spike activity after application 22 of the placebo, while comparison of Figure 2D, Figure 2E and Figure 2F shows an increase in spike activity after application 24 of the topical prostaglandin E₁ composition.

Figure 3 is a graphical representation summarizing the results of experiments on seven neurons in the PVN similar to shown in detail in Figures 1A-F and 2 A-F, shown that the application of the placebo did not, on average, significantly change the neuron firing, while the application of the topical prostaglandin E₁ composition significantly (p< 0.05) changed the neuron firing.
Figures 4A-4E are graphical representations of the results of electrophysiological experiments showing the extracellularly recorded action potential (spike) activity of neurons in the basomedial amygdala nucleus of a female rat, where Figure 4A is a graphical representation of the spike rate of a neuron as a function of time during the experiment, showing the effect of the application of a placebo, (arrow, 42) or a topical prostaglandin E₁ composition, (arrow, 44) via cannulae positioned at the clitoris and anterior vaginal wall (corresponding to the human G-spot), where a comparison of Figure 4B, Figure 4C and Figure 4D shows little increase in spike activity after application 42 of the placebo, while comparison of Figure 4D and Figure 4E shows an increase in spike activity after application 44 of the topical prostaglandin E₁ composition.

Figures 5A and 5B are graphical representations of the results of electrophysiological experiments in which extracellularly recorded action potential (spike) activity of neurons in the hypothalamic paraventricular nucleus of a female rat showed convergence of excitatory effects of electrical stimulation 52 (Figure 5A) and application 53 of the topical prostaglandin E₁ composition (Figure 5B).

Figures 6A and 6B are graphical representations of the results of electrophysiological experiments in which extracellularly recorded action potential (spike) activity of neurons in the basomedial amygdala nucleus of a female rat showed convergence of excitatory effects of intravaginal distension 62 (Figure 6A) and application 63 of the topical prostaglandin E₁ composition (Figure 6B).

Figures 7A and 7B are graphical representations of the results of electrophysiological experiments in which extracellularly recorded action potential (spike) activity of neurons in the basomedial amygdala nucleus of a female rat showed convergence of excitatory effects of genital stretching 72 (Figure 7A) and application 73 of the topical prostaglandin E₁ composition (Figure 7B).

Figures 8A-8F are grayscale reproductions of color photomicrographs of immunocytochemical sections of the brains of female rats that had been sacrificed after a 30 minute treatment with the placebo cream (Figures 8A, 8B and 8C) or after a 30 minute treatment with the topical prostaglandin E₁ composition (Figures 8D, 8E and 8F) showing an increase in oxytocin-like immunoreactivity in the PVN of animals treated with the topical prostaglandin E₁ composition. The groups of
photographs increase in magnification by a factor of two in two steps so that the magnification in Figures 8C and 8F is twice that of Figures 8B and 8E and four times that of Figures 8A and 8D. The third ventricle 112 can be seen; treatment with the topical prostaglandin E₁ composition but not the placebo cream revealed bilateral groups 120 and 122, of neurons having oxytocin-like immunoreactivity.

Figures 9A-9F are grayscale reproductions of color photomicrographs of immunocytochemical sections of the brains of female rats that had been sacrificed after a 30 minute treatment with the placebo cream (Figures 9A, 9B and 9C) or after a 30 minute treatment with the topical prostaglandin E₁ composition (Figures 9D, 9E and 9F) showing an increase in oxytocin-like immunoreactivity in the supraoptic nucleus (SON) of animals treated with the topical prostaglandin E₁ composition. The groups of photographs increase in magnification by a factor of two in two steps so that the magnification in Figures 9C and 9F is twice that of Figures 9B and 9E and four times that of Figures 9A and 9D. Treatment with the topical prostaglandin E₁ composition but not the placebo cream revealed a group 202 of neurons having oxytocin-like immunoreactivity.

Figures 10A-10D are grayscale reproductions of color photomicrographs of immunocytochemical sections of the brains of female rats that had been sacrificed after a 30 minute treatment with the placebo cream (Figure 10A) or after a 30 minute treatment with the topical prostaglandin E₁ composition (Figures 10B, 10C and 10D) showing an increase in oxytocin-like immunoreactivity and co-localization with increase c-fos expression in the supraoptic nucleus 302 of animals treated with the topical prostaglandin E₁ composition. The groups of photographs increase in magnification by a factor of two in two steps so that the magnification in Figure 10D is twice that of Figure 10C and four times that of Figures 10A and 10B. Single labeled 310 and double-labeled 312 neurons were observed in the SON 302.

Figure 11 is a schematic representation 400 in which activated sensory neurons 410 connect with neurons in the spinal cord 420 and hypothalamus 430. Neurons in the paraventricular and supraoptic nuclei of hypothalamus 430 that end in the posterior lobe of the pituitary 440 release oxytocin 452 which produces both uterine responses 462 and breast responses 464. Prolactin 454 produced by cells in the anterior lobe of the pituitary 440 also affects the breast tissue 464.
Figure 12 is a schematic diagram of the brain regions interacting in the limbic system.

Figures 13A-C are averaged functional magnetic resonance images (fMRI) in horizontal brain sections of normal subjects (N=5) showing brain regions in which visual erotic stimulation after application of a topical prostaglandin E₁ composition to the clitoris and G-spot caused a significant increase in brain activity compared to application of the placebo cream. In Figure 13A and Figure 13B, anterior 610 and posterior 612 regions of significantly increased activity were identified on the left side of the brain. In Figure 13C, bilateral regions of increased activity 622, 624, 626 and 628 and decreased activity 630 are shown. Figure 13D is a reference diagram of a lateral view of the left side of a human brain showing the location of cerebral lobes, major sulci and the cerebellum.

Figure 14 is an averaged functional magnetic resonance image (fMRI) in horizontal brain sections of female sexual arousal disorder patients (N=5) showing regions 710, 712 in the right parietal lobe in which visual erotic stimulation after application of a topical prostaglandin E₁ composition to the clitoris and G-spot caused a significant decrease in brain activity compared to application of the placebo cream.

Figures 15A-B show averaged functional magnetic resonance images (fMRI) in horizontal brain sections of normal subjects (N=5) showing in Figure 15A increased activity in the left Broca’s area 812, the right insula 810 and in Figure 15B the right parietal lobe 820, 822. Figure 15C is a reference diagram of a lateral view of the left side of a human brain showing the location of functional areas such as Broca’s area.

Figures 16A-D show averaged functional magnetic resonance images (fMRI) in horizontal brain sections showing areas of increased activity 910, 912 (Figure 16A); 920, 922 (Figure 16B); 932, 933, 934, 938 (Figure 16C) and 952, 955 (Figure 16D).

Figure 17 is a schematic diagram illustrating an embodiment 1000 of the present invention comprising the steps of imaging the subject’s central nervous system in the baseline condition 1010, providing a first test composition 1020, providing a neutral stimulation 1030, imaging the subject’s central nervous system
during the neutral stimulation 1040, providing an erotic stimulation 1050, imaging
the subject's central nervous system during the erotic stimulation 1060 and
comparing the images of the subject's central nervous system 1070.

DETAILED DESCRIPTION OF THE INVENTION

Application of a topical prostaglandin composition including a skin
penetration enhancer to clitoris and the anterior vaginal wall (Graefenberg spot)
produced increased activity in central nervous system areas associated with sexual
behavior. In electrophysiological studies in rat, a topical administration of alprostadil
cream to the female genitalia evoked increased neural activity in the paraventricular
nucleus (PVN) of the hypothalamus and the basomedial nucleus of the amygdala
(BMA), two areas of the central nervous system that have been demonstrated to be
involved in the regulation of female sexual activity. A parallel set of studies
demonstrated that such topical administration of alprostadil cream to the female
genitalia evoked an increase in oxytocin-like immunoreactivity in the PVN and the
supraoptic nucleus (SON) of the female rat.

The results suggest that the left superior frontal gyrus and right anterior frontal
lobe are the activation areas associated with the alprostadil cream administration in
sexual arousal response. The arousal effect of the alprostadil cream was erotic
stimulation dependent. Different activation patterns in healthy and FSAD women
may be associated with etiological factors.

In conclusion, all the studies support that alprostadil genital application could
act on the genitai area nerve terminal to enhance/facilitate neurotransmission or
reflex. The consequences of the effects will further improve/enhance the visual erotic
stimulation triggered central sexual responses (from desire to orgasm). The topical
effect of alprostadil was essential to improve the neuropathological conditions to
further enhance the normal sexual reflex arc. This effect was evidenced by the above
electrophysiology, immunocytochemistry and fMRI studies. The novel nerve reflex
facilitation effects can be useful for the treatment of FSAD as well as some
neurodegenerative conditions, such as diabetes, and other neuropsychological sexual
dysfunction, including dysfunctions of desire and orgasm.
Before describing the present invention in detail, it is to be understood that this invention is not limited to particular drugs or drug delivery systems, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

It must be noted that, as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a vasoactive agent" includes a mixture of two or more such drugs; reference to "a penetration enhancer" includes mixtures of two or more enhancers, and the like.

In describing and claiming the present invention, the following terminology will be used in accordance with the definitions set out below.

"Subject" means mammals and non-mammals. "Mammals" means any member of the class Mammalia including, but not limited to, humans, non-human primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, and swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice, and guinea pigs; and the like. Examples of non-mammals include, but are not limited to, birds, and the like. The term "subject" does not denote a particular age or sex.

A "therapeutically effective amount" means an amount of a compound that, when administered to a subject for treating a disease, is sufficient to effect such treatment for the disease. The "therapeutically effective amount" will vary depending on the compound, the disease state being treated, the severity or the disease treated, the age and relative health of the subject, the route and form of administration, the judgment of the attending medical or veterinary practitioner, and other factors.

The term "drug" or "pharmacologically active agent" as used herein is intended to mean a compound or composition of matter which, when administered to an organism (human or animal) induces a desired pharmacologic and/or physiologic effect by local and/or systemic action. As noted above, the pharmacologically active agents used in conjunction with the present invention are vasoactive agents.

By "transdermal" drug delivery, applicant is using the term in its conventional sense, i.e., to indicate delivery of a drug by passage into and through the skin and the underlying tissues and into the blood stream. By "transmucosal" drug delivery,
applicant intends delivery of a drug by passage of a drug through the mucosal and underlying tissue into the blood stream. The compositions, systems, and methods of the invention, unless explicitly stated otherwise, should be presumed to be equally applicable to either transdermal or transmucosal modes of drug delivery.

“Penetration enhancement” or “permeation enhancement” as used herein relates to an increase in the permeability of the skin or mucosal tissue to a selected pharmacologically active agent, i.e., so that the rate at which the drug permeates through the skin or mucosal tissue is increased. “Carriers” or “vehicles” as used herein refer to carrier materials suitable for transdermal or transmucosal drug administration, and include any such materials known in the art, e.g., any liquid, gel, solvent, liquid diluent, solubilizer, or the like, which is nontoxic and which does not interact with other components of the composition in a deleterious manner.

By an “effective” amount of a drug or pharmacologically active agent is meant a nontoxic but sufficient amount of the drug or agent to provide the desired effect.

In order to carry out the method of the invention, a composition suitable for topical application comprising a selected vasoactive agent is administered about fifteen minutes to about one hour prior to the time of desired effect. Preferably, the topical composition is applied once, twice or three times within a twenty-four hour period.

A dose of a prostaglandin E₁ in an amount sufficient to enhance engorgement or vaginal secretion is topically administered to a woman. The appropriate doses of the particular vasodilating agent may be readily determined using known methods. The female response may be measured using methods described in Masters, W. H. and Johnson, V. E., *Human Sexual Response*, Little, Brown, and Co., Boston (1966) which is incorporated herein by reference. Engorgement and redness of the external genitalia can be assessed by visual inspection. Methods for measuring blood flow, including Doppler ultrasonic velocimetry, thermography using for example an isothermal blood flow transducer, radioscintigraphic methods and vaginal photoplethysmography may be used as well as other methods well known in the art.

In addition, measuring the contraction of the distal 1/3 as is characteristic of the plateau phase of female sexual response of the vagina may be measured using methods and equipment well known in the art including but not limited to strain
gauges or other devices for measuring muscular contraction or muscle tension.

A preferred embodiment of the present invention involves the topical administration of at least 0.1 mg to about 6 mg of prostaglandin E₁ to a female subject. In a preferred embodiment of the present invention, about 0.1 mg to about 2 mg of prostaglandin E₁ is administered topically. In another preferred embodiment of the present invention, about 1.4 mg to about 6 mg of prostaglandin E₁ is administered topically to a female. In another preferred embodiment of the present invention, about 1 mg to about 3 mg of prostaglandin E₁ is administered topically to a female.

The composition is suitable for topical application, and comprises a vasoactive prostaglandin, more preferably prostaglandin E₁, a penetration enhancer, a polymer thickener, a lipophilic component, and an acidic buffer system. In some embodiments, the polymer thickener is a polyacrylic acid polymer. In other preferred embodiments, the polymer thickener is a polysaccharide gum or a modified polysaccharide gum. The lipophilic component is selected from the group consisting of the C₁ to C₈, aliphatic alcohols, the C₂ to C₃₀ aliphatic esters and mixtures thereof. The acidic buffer system is chosen to provide a suitable pH to minimize irritation of skin and mucous membranes. The composition is typically in the form of a cream, lotion, gel or other form suitable for topical application to skin and mucous membranes.

In a preferred embodiment, the topical composition comprises an effective amount of a vasoactive prostaglandin; a penetration enhancer selected from the group consisting of an alkyl-(N-substituted amino) alkanoate, an alkyl-2-(N,N-disubstituted amino) alkanoate, an (N-substituted amino) alkanoil alkanoate, an (N,N-disubstituted amino) alkanoil alkanoate, pharmaceutically acceptable salts thereof and mixtures thereof; a polymer thickener selected from the group consisting of a polyacrylic acid polymer, a polysaccharide gum, a modified polysaccharide gum and mixtures thereof; a lipophilic component; and a buffer system wherein the pH of the composition is 3.0 to 7.4. Preferably the vasoactive prostaglandin is selected from the group consisting of prostaglandin E₁, prostaglandin E₁ alkyl esters, pharmaceutically acceptable salts thereof and mixtures thereof. In preferred embodiments, the composition further comprises polyethylene glycol.
In preferred embodiments, the topical composition suitable for topical application comprises 0.001 weight percent to 1 weight percent of a vasoactive prostaglandin selected from the group consisting of PGE₁, pharmaceutically acceptable salts thereof, lower alkyl esters thereof and mixtures thereof; 0.01 weight percent to 5 weight percent modified polysaccharide gum; 0.5 weight percent to 10 percent weight dodecyl N,N-dimethylamino isopropionate or pharmaceutically acceptable salts thereof; 0.5 weight percent to 10 weight percent of a lower alcohol selected from the group consisting of ethanol, propanol, isopropanol and mixtures thereof; 0.5 weight percent to 10 weight percent of an ester selected from the group consisting of ethyl laurate, isopropyl myristate, isopropyl laurate and mixtures thereof, based on the total weight of the composition, and a buffer system wherein the pH of the composition is 3.0 to 7.4. In certain embodiments, the composition further comprises 1 weight percent to 25 weight percent polyethylene glycol 400, based on the total weight of the composition.

A preferred topical composition comprises about 0.07 weight percent to about 0.4 weight percent of prostaglandin E₁ and a pharmaceutically acceptable excipient.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Alprostadil cream (%w/w)</th>
<th>Placebo cream (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl Alcohol, dehydrated, USP</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>dodecyl N,N-dimethylamino isopropionate (DDAIP) HCl</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>prostaglandin E₁</td>
<td>0.4</td>
<td>0</td>
</tr>
<tr>
<td>Ethyl Laurate, FCC</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Guar Gum, modified</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Sodium Hydroxide Solution, 1M</td>
<td><strong>Qs pH 5.5</strong> (adjust pH of cream)</td>
<td><strong>Qs pH 5.5</strong> (adjust pH of cream)</td>
</tr>
<tr>
<td>Sterile Water for Injection, USP, buffered to pH 5.5 with 0.1M Potassium Phosphate Monobasic, NF</td>
<td><strong>qs 100</strong></td>
<td><strong>qs 100</strong></td>
</tr>
</tbody>
</table>

Exemplary placebo and active compositions are detailed in Table 1.

In other embodiments, compositions can be formulated as follows.

Composition I (Table 2, below) was prepared from two parts, A & B. Part A was formed by dissolving about 0.4 parts prostaglandin E₁ (Alprostadil USP) in about 5
parts ethyl alcohol. Next, about 5 parts ethyl laurate were mixed into the alcohol-
prostaglandin E₁ solution. Part B was prepared starting from a pH 5.5 water-buffer
solution. The water-buffer solution was prepared by adding sufficient potassium
phosphate monobasic to purified water to create a 0.1 M solution. The water-buffer
solution diluted to a final concentration of about 0.05M and about pH 5.5, adjusted
with a strong base solution (1 N sodium hydroxide) and a strong acid (1 N phosphoric
acid). Suitable buffer concentrations range from about 0.005M to about 1.0M.
Preferred buffer concentrations range from about 0.05M to about 0.2M. In several
preferred embodiments the buffer concentration is 0.1M. Propylene glycol (about 5
parts) was added to the water / buffer solution, and then the polyacrylic polymer
(about 1 part) was dispersed in the propylene glycol / water / buffer solution. All
parts specified herein are parts by weight.

Parts A and B were mixed and homogenized using a homogenizer. Table 2,
below, contains a list of ingredients and proportions. The resulting composition was
a spreadable, semi-solid suitable for application to the skin and mucous membranes
without the need for supporting devices such as patches and adhesive strips. The
composition was both homogenous in appearance and resistant to separation.
Compositions II-VII were prepared following the same procedure.
Table 2

<table>
<thead>
<tr>
<th>Component</th>
<th>Composition I (weight %)</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noveon AA-1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Ethanol</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Ethyl laurate</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>70% Sorbitol</td>
<td></td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Glycerol</td>
<td></td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>2.5</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>DDAIP</td>
<td></td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>2.5</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>DDAIP HCI</td>
<td></td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>2.5</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Sesame oil</td>
<td></td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>2.5</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Squalene</td>
<td></td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>2.5</td>
<td>2.5</td>
<td></td>
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<tr>
<td>Prehydrated Locust bean gum</td>
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<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Modified Guar Gum</td>
<td></td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Sucrose stearate</td>
<td></td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>0.05M pH 5.5 buffer</td>
<td>78.85</td>
<td>73.85</td>
<td>73.85</td>
<td>73.85</td>
<td>73.85</td>
<td>73.85</td>
<td>73.85</td>
</tr>
<tr>
<td>0.1M pH 5.5 buffer</td>
<td></td>
<td>81</td>
<td>81</td>
<td>81</td>
<td>81</td>
<td>81</td>
<td>81</td>
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<tr>
<td>1 M NaOH</td>
<td>4.75</td>
<td>4.75</td>
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<td>4.75</td>
<td>4.75</td>
<td>4.75</td>
<td>4.75</td>
</tr>
<tr>
<td>Prostaglandin E1</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

In some embodiments, such as Compositions VI and VII, the composition may include a modified polysaccharide gum, suitably a modified galactomannan gum, such as a guar gum. Alternatively, a polyacrylic polymer may be used instead of the polysaccharide gum.

In preferred embodiments, the present invention provides a method for diagnosing sexual dysfunction in a female subject. The method comprises administering an effective amount of a topical prostaglandin composition by applying the topical composition to the genital area of a human female, preferably the clitoris and G-spot.

While this invention has been described by way of preferred embodiments, the examples set out herein are not intended to limit the scope of the invention. Numerous other advantages of the present invention will be apparent from the following detailed description of the invention including the accompanying examples and the appended claims.
EXAMPLE 1

Electrophysiological and Immunocytochemical Studies

The electrophysiological and immunocytochemical study was performed as a placebo-controlled, parallel design, study in female Wistar rats. Placebo cream or 0.4% alprostadil cream as described above in Table 1, as well as electric or mechanical stimuli were used. Neuronal activity in PVN (paraventricular nucleus) and BMA (basomedial amygdala) nucleus of the brain involved in the sexual activity was studied.

Material and Methods

A total of 16 female Wistar rats were anesthetized with urethane and a 3-silastic cannula was inserted intravaginally for application of saline, placebo, and alprostadil cream. The animal was positioned in a stereotaxic frame for recording unit neuronal activity. The placebo and alprostadil cream (0.4%, 75 mg) were randomly applied in succession. Mechanical and electrical stimuli to the external genitalia were used to study neuronal convergency of PVN or BMA. Differences in neuronal unit-firing changes elicited by the alprostadil cream versus placebo were calculated using a paired t-test. Neuronal activity recording sites were confirmed after each experiment.

Immunocytochemical Experiments: Female Wistar rats (n=5 per group) were anesthetized with ethyl ether. An alprostadil (0.4% alprostadil, 75 mg) or a placebo cream was administered intravaginally and to the clitoris for 30 min. Brains were perfused with an ice-cold modified Krebs solution including 5% sucrose for 30 minutes. The brains were quickly removed, treated with fixative, cryoprotected and stored at -80°C for further processing. Coronal sections (20 μm) were stained for detection of oxytocin-like immunoreactivity (OT-LI) and c-Fos-like immunoreactivity (c-Fos-LI) by avidin-biotin-peroxidase complex (ABC) technique using Pierce ImmunoPure Metal Enhanced DAB Substrate Kit. Fluorescent and dual fluorescent immunocytochemical methods were also used to evaluate the expression of OT-LI and c-Fos-LI. Fluorescent sections were viewed and photographed under an Olympus confocal microscope. The c-Fos-IL or oxytocin-IL neurons in PVN, SO, BMA and hippocampus were examined. The OT-LI positive and c-Fos-LI positive
cells in the area of PVN and SON were calculated separately in 10 sections per
animal and analysed by paired t-test.

In general, eight out of 10 (80%) neurons that were recorded in the PVN had
an elevated firing rate following application of the alprostadil cream, with an average
onset time of 40 ± 10.2 sec. Two neurons were unaffected. The mean unit-firing rate
(± SE) increased 75.7 ± 29.2/min after the alprostadil cream application versus -1.7 ±
1.0/min after placebo (P=0.016). Eight out of 11 (73%) neurons that were recorded in
the BMA had an elevated firing rate following application of the alprostadil cream
with an average onset time of 28 ± 4.6 sec, while 3 neurons were unaffected. The
mean unit-firing rate (± SE) increased 12.0 ± 5.6/min after alprostadil cream
application versus 0.3 ± 1.2/min after placebo cream application (P=0.037). In
addition, 3 out of 5 PVN neurons showed responses to electric stimuli while all of the
5 (100%) neurons were excited by the alprostadil cream. The same excitatory
convergency of BMA neurons by mechanical stimuli was observed. Topical
administration of alprostadil cream evoked elevated firing in PVN and BMA in the
CNS that are involved in the regulation of female sexual activity. A convergent effect
of PVN and BMA neurons for the female genital mechanoreceptor- and
chemoreceptor-sensory inputs was observed.

Figure 1 is a graphical representation of the results of electrophysiological
experiments showing the extracellularly recorded action potential (spike) activity of
neurons in the hypothalamic paraventricular nucleus of a female rat, where Figure 1A
is a graphical representation of the spike rate of a neuron as a function of time during
the experiment, showing the effect of the application of a placebo, (arrow, 12) or a
topical prostaglandin E₁ composition, (arrow, 14) via cannulae positioned at the
clitoris and anterior vaginal wall (corresponding to the human G-spot), where a
comparison of Figure 1B, Figure 1C and Figure 1D shows little increase in spike
activity after application 12 of the placebo, while comparison of Figure 1D, Figure 1E
and Figure 1F shows an increase in spike activity after application 14 of the topical
prostaglandin E₁ composition.

Figure 2 is a graphical representation of the results of electrophysiological
experiments showing the extracellularly recorded action potential (spike) activity of
neurons in the hypothalamic paraventricular nucleus of a female rat, where Figure 2A
is a graphical representation of the spike rate of a neuron as a function of time during the experiment, showing the effect of the application of a placebo, (arrow, 22) or a topical prostaglandin E₁ composition, (arrow, 24) via cannulae positioned at the clitoris and anterior vaginal wall (corresponding to the human G-spot), where a comparison of Figure 2B, Figure 2C and Figure 2D shows little increase in spike activity after application 22 of the placebo, while comparison of Figure 2D, Figure 2E and Figure 2F shows an increase in spike activity after application 24 of the topical prostaglandin E₁ composition.

Figure 3 is a graphical representation summarizing the results of experiments on seven neurons in the PVN similar to shown in detail in Figures 1A-F and 2 A-F, shown that the application of the placebo did not, on average, significantly change the neuron firing, while the application of the topical prostaglandin E₁ composition significantly (p< 0.05) changed the neuron firing.

Figure 4 is a graphical representation of the results of electrophysiological experiments showing the extracellularly recorded action potential (spike) activity of neurons in the basomedial amygdala nucleus of a female rat, where Figure 4A is a graphical representation of the spike rate of a neuron as a function of time during the experiment, showing the effect of the application of a placebo, (arrow, 42) or a topical prostaglandin E₁ composition, (arrow, 44) via cannulae positioned at the clitoris and anterior vaginal wall (corresponding to the human G-spot), where a comparison of Figure 4B, Figure 4C and Figure 4D shows little increase in spike activity after application 42 of the placebo, while comparison of Figure 4D and Figure 4E shows an increase in spike activity after application 44 of the topical prostaglandin E₁ composition.

Figures 5A and 5B are graphical representations of the results of electrophysiological experiments in which extracellularly recorded action potential (spike) activity of neurons in the hypothalamic paraventricular nucleus of a female rat showed convergence of excitatory effects of electrical stimulation 52 (Figure 5A) and application 53 of the topical prostaglandin E₁ composition (Figure 5B).

Figures 6A and 6B are graphical representations of the results of electrophysiological experiments in which extracellularly recorded action potential (spike) activity of neurons in the basomedial amygdala nucleus of a female rat
showed convergence of excitatory effects of intravaginal distension 62 (Figure 6A) and application 63 of the topical prostaglandin E₁ composition (Figure 6B).

Figures 7A and 7B are graphical representations of the results of electrophysiological experiments in which extracellularly recorded action potential (spike) activity of neurons in the basomedial amygdala nucleus of a female rat showed convergence of excitatory effects of genital stretching 72 (Figure 7A) and application 73 of the topical prostaglandin E₁ composition (Figure 7B).

Figures 8A-8F are grayscale reproductions of color photomicrographs of immunocytochemical sections of the brains of female rats that had been sacrificed after a 30 minute treatment with the placebo cream (Figures 8A, 8B and 8C) or after a 30 minute treatment with the topical prostaglandin E₁ composition (Figures 8D, 8E and 8F) showing an increase in oxytocin-like immunoreactivity in the PVN of animals treated with the topical prostaglandin E₁ composition. The groups of photographs increase in magnification by a factor of two in two steps so that the magnification in Figures 8C and 8F is twice that of Figures 8B and 8E and four times that of Figures 8A and 8D. The third ventricle 112 can be seen; treatment with the topical prostaglandin E₁ composition but not the placebo cream revealed bilateral groups 120 and 122, of neurons having oxytocin-like immunoreactivity.

Figures 9A-9F are grayscale reproductions of color photomicrographs of immunocytochemical sections of the brains of female rats that had been sacrificed after a 30 minute treatment with the placebo cream (Figures 9A, 9B and 9C) or after a 30 minute treatment with the topical prostaglandin E₁ composition (Figures 9D, 9E and 9F) showing an increase in oxytocin-like immunoreactivity in the supraoptic nucleus (SON) of animals treated with the topical prostaglandin E₁ composition. The groups of photographs increase in magnification by a factor of two in two steps so that the magnification in Figures 9C and 9F is twice that of Figures 9B and 9E and four times that of Figures 9A and 9D. Treatment with the topical prostaglandin E₁ composition but not the placebo cream revealed a group 202 of neurons having oxytocin-like immunoreactivity.

Figures 10A-10D are grayscale reproductions of color photomicrographs of immunocytochemical sections of the brains of female rats that had been sacrificed after a 30 minute treatment with the placebo cream (Figure 10A) or after a 30 minute
treatment with the topical prostaglandin E₁ composition (Figures 10B, 10C and 10D) showing an increase in oxytocin-like immunoreactivity and co-localization with increase c-fos expression in the supraoptic nucleus 302 of animals treated with the topical prostaglandin E₁ composition. The groups of photographs increase in magnification by a factor of two in two steps so that the magnification in Figure 10D is twice that of Figure 10C and four times that of Figures 10A and 10B. Single labeled 310 and double-labeled 312 neurons were observed in the SON 302.

Figure 11 is a schematic representation 400 in which activated sensory neurons 410 connect with neurons in the spinal cord 420 and hypothalamus 430. Neurons in the paraventricular and supraoptic nuclei of hypothalamus 430 that end in the posterior lobe of the pituitary 440 release oxytocin 452 which produces both uterine responses 462 and breast responses 464. Prolactin 454 produced by cells in the anterior lobe of the pituitary 440 also affects the breast tissue 464. Figure 12 is a schematic diagram of the brain regions interacting in the limbic system.

In general, treatment with the alprostadil cream resulted in noticeable increases of oxytocin-immunoreactive material in neurons of the PVN and SON compared with the placebo cream administration. Treatment with the alprostadil produced noticeable increases of c-fos-immunoreactive material, a marker of cellular activation, in neurons of PVN and SON of the hypothalamus. Co-localization of oxytocin-immunoreactive neurons and c-fos-immunoreactive neurons in PVN and SON was observed.

The present study using neurophysiological and immunocytochemical approaches provided evidence for the first time that application of topical medication to the female genital area could evoke neuronal excitation in central nervous nuclei that are involved with regulation of sexual behavior. Treatment with the alprostadil cream could evoke excitatory responses in neurons of the central nuclei that are involved in the neurophysiological regulation of female sexual activity. The present study provided evidence that the female sexual mechanoreceptor- and chemoreceptor sensory inputs along the spino-hypothalamus-amygdala pathways produced convergence of neuronal activation of PVN and BMA neurons.

Other measures of neuronal activity can be used in mammals such as rats. Optical measures of electrical activity can be preformed using voltage-sensitive dyes
or intrinsic optical signals. Metabolic measures of neuronal activity can be recorded using functional magnetic resonance imaging, PET, SPECT, hybrid CT/PET or diffuse optical tomography. See, e.g., Siegel, et al., Temporal comparison of functional brain imaging with diffuse optical tomography and fMRI during rat forepaw stimulation, Phys. Med. Biol. 48 (2003) 1391-1403. Preferably the neuronal activity is correlated with the anatomical location of the activated neurons.

EXAMPLE 2

Functional MRI Studies of Healthy Subjects and FSAD Subjects

Cerebral activation patterns were studied using functional magnetic resonance imaging (fMRI) and subjective arousal responses after application of alprostadil cream (900 mcg, 0.4% prostaglandin E1) or placebo cream to the clitoris and G-spot during visual stimulation with neutral or erotic videos.

Briefly, the study group consisted of five healthy subjects and five patients diagnosed with FSAD. The design was a double-blind, placebo-controlled study using fMRI and 5-point subjective sexual arousal response scale. Each subject randomly applied the alprostadil cream or the placebo cream with a minimum 5-day wash off period.

The subjects were examined on a Signa 1.5T TwinSpeed MRI system to obtain the BOLD-fMRI data at baseline, and during the real-time visual stimulation (documentary and erotic video-images). The statistical parametric data were normalized with head motion correction, spatial normalization, spatial filtering, and further analyzed using student t-test with a threshold of \( P<0.01 \) to obtain the statistical parametric maps. All maps were further analyzed with AFNI's ANOVA2 program to detect areas of significant brain activation (\( P<0.01 \)).

Genital application of alprostadil cream elicited activation or inhibition in certain brain areas during visual erotic stimulation in healthy subjects and FSAD patients. The results showed that the arousal effects of the topical alprostadil cream were dependent on visual erotic stimulation. The pattern of activated brain regions seen in healthy subjects was different from that seen in FSAD patients. In healthy women, the activation effects of the alprostadil cream were stronger than placebo in the left superior frontal gyrus and the right anterior frontal lobe, but weaker in the
bilateral parietal lobe. The activation effect of the alprostadil cream in the FSAD patients was weaker in the right parietal lobe activation compared to the placebo. Alprostadil cream administration caused stronger activation in the left Broca’s area, right insula and bilateral parietal lobe in the healthy women as compared to the FSAD subjects. Alprostadil cream administration caused stronger activation in the right prefrontal lobe, left gyri fusiformis and left superior frontal gyrus in the FSAD patients as compared to the healthy subjects. Erotic visual stimulation after application of the placebo cream produced the same proportion (3/5) of reported successful sexual arousal responses in the healthy subjects and the FSAD patients.

All (5/5) of the FSAD patients reported successful sexual arousal responses to erotic visual stimulation after application of the alprostadil cream. The majority (3/5) of the healthy subjects reported successful sexual arousal responses to erotic visual stimulation after application of the alprostadil cream. The results indicate that the left superior frontal gyrus and right anterior frontal lobe are the activation areas associated with the alprostadil cream topical administration. Different activation patterns in healthy and FSAD women may be associated with etiological factors.

The differing patterns of regional brain activation or inhibition facilitated by genital alprostadil application provide evidence of utility of the present method for the diagnosis and treatment of FSAD as well as female sexual dysfunctions having a neuropsychological component, including dysfunctions of desire and orgasm.

The clinical study required approximately 60 minutes at Visit 1 and Visit 2 in addition to a screening visit 14 days before the study period. Subjects were randomly assigned to either use placebo or alprostadil cream first with a wash out time of a minimum of five days between Visit 1 and Visit 2. During the measurement sessions the subjects first underwent a 5-minute baseline fMRI scan before the subject applied either placebo or alprostadil cream, followed by additional fMRI scans while viewing of a 15-minute documentary film and a 15-minute erotic film.

The inclusion criteria for the study were that the female subjects were 21 years old without pregnancy, without planning to be pregnant, have taken the contraception procedures for at least 1 month and subject and partners had provided written, informed consent. The FSAD patients had a score = 40 in the FSDS at Visit 1, and met the diagnostic criteria for Sexual Arousal Disorder according to the AFUD
Consensus Panel definition for at least the past six months. The FSAD patients had been previously “sexually functional”; that is, had experienced sexual arousal and/or orgasm during vaginal intercourse at some point in the past for a period of at least 5 years (acquired versus lifelong disease). The patients provided proof of a normal Thinprep Cytologic Test (TCT) or Pap smear test within the past 6 months; any TCT test or Pap test result with inflammation or inflammatory changes in the absence of clinically significant vaginitis were admitted. The FSAD patients scored = 8 and = 7 in the Covi anxiety scale and the Raskin depression scale, respectively.

Subjects with any of the following conditions or meeting any of the following criteria were excluded from the study: evidence of unresolved sexual trauma or abuse, primary anorgasmia, vaginismus, sexual pain disorder, sexual aversion disorder, or sexually transmitted disease, female sexual dysfunction caused by untreated endocrine disease (eg, hypopituitarism, hypothyroidism, and diabetes mellitus) or surgical procedure (eg, adrenalectomy, hypophysectomy); a history of chronic or complicated urinary tract or vaginal infections, pelvic inflammatory disease, or orthostatic hypotension within the previous 6 months; a history of chronic dyspareunia not attributable to vaginal dryness within previous 12 months; severe vaginitis on pelvic examination; psychoses and/or bipolar disorder, depression, or alcohol/substance abuse (within the past 6 months); used within the previous three months: neuroleptics (eg, risperidone), lithium (eg, lithium carbonate), antidepressants (eg. amitriptyline, fluoxetine, bupropion), mood-stabilizers (eg. benzodiazepines), cognitive-enhancers (eg, donepezil), food supplements, or centrally acting antihypertensives (eg, clonidine) known to affect sexual desire; is currently receiving psychotherapy for the treatment of FSAD; clinically significant hepatic disease as evidenced by AST or ALT >3 times the upper limit of normal, or clinically significant renal disease as evidenced by a serum creatinine > 220 umol/L; a history of myocardial infarction within previous 6 months; significant neurological diseases within the last 6 months i.e., stroke, spinal cord injury, etc.; a known sensitivity to prostaglandin E1; participated in another study with an investigational drug or device during the 30 days prior to study entry, or during the study; a condition which would interfere with the patient’s ability to provide informed consent, to comply with study
instructions, or which might confound the interpretation of the study results, or a condition that would endanger the patient if she participated in this trial.

Figures 13A-C are averaged functional magnetic resonance images (fMRI) in horizontal brain sections of normal subjects (N=5) showing brain regions in which visual erotic stimulation after application of a topical prostaglandin E₁ composition to the clitoris and G-spot caused a significant increase in brain activity compared to application of the placebo cream. In Figure 13A and Figure 13B, anterior 610 and posterior 612 regions of significantly increased activity were identified on the left side of the brain. In Figure 13C, bilateral regions of increased activity 622, 624, 626 and 628 and decreased activity 630 are shown. Figure 13D is a reference diagram of a lateral view of the left side of a human brain showing the location of cerebral lobes, major sulci and the cerebellum.

Figure 14 is an averaged functional magnetic resonance image (fMRI) in horizontal brain sections of female sexual arousal disorder patients (N=5) showing regions 710, 712 in the right parietal lobe in which visual erotic stimulation after application of a topical prostaglandin E₁ composition to the clitoris and G-spot caused a significant decrease in brain activity compared to application of the placebo cream.

Figures 15A-B show averaged functional magnetic resonance images (fMRI) in horizontal brain sections of normal subjects (N=5) showing in Figure 15A increased activity in the left Broca’s area 812, the right insula 810 and in Figure 15B the right parietal lobe 820, 822. Figure 15C is a reference diagram of a lateral view of the left side of a human brain showing the location of functional areas such as Broca’s area.

Figures 16A-D show averaged functional magnetic resonance images (fMRI) in horizontal brain sections showing areas of increased activity 910, 912 (Figure 16A); 920, 922 (Figure 16B); 932, 933, 934, 938 (Figure 16C) and 952, 9955 (Figure 16D).

Figure 17 is a schematic diagram illustrating an embodiment 1000 of the testing module of the present invention in which neuronal activity and anatomical location is imaged using a technique such as fMRI, comprising the steps of imaging the subject’s central nervous system in the baseline condition 1010, providing a first
test composition 1020, providing a neutral stimulation 1030, imaging the subject’s central nervous system during the neutral stimulation 1040, providing an erotic stimulation 1050, imaging the subject’s central nervous system during the erotic stimulation 1060 and comparing the images of the subject’s central nervous system 1070. In preferred embodiments, the imaging of the subject’s central nervous system provides an image of central nervous system function superimposed on an image of central nervous system structure. In a particularly preferred embodiment, the imaging includes the use of functional magnetic resonance imaging, fMRI.

Preferably a single test composition is administered during a testing module, preferably in a double-blind protocol. The presentations of testing modules are suitably separated by a time interval selected to provide for sufficient “washout time” before the subsequent test composition is presented, typically about five days in the present studies. The suitable time interval can vary by the characteristics of the active agent (e.g., lipid solubility), the concentration of the active agent, the mode of administration and the presence of other components, such as penetration enhancers in a topical composition. In preferred embodiments, a plurality of test compositions are presented in a protocol consisting of a plurality of testing modules, including at least one testing module in which a placebo is presented.
CLAIMS

What is claimed:

1. A method for assessing sexual function in a female subject comprising the steps of:
   recording baseline neuronal activity in at least one central nervous system region of the female subject that is associated with sexual responses;
   applying a test topical composition to a region of the female subject’s genitalia;
   recording neuronal activity in the central nervous system region after the application of the test topical composition;
   recording neuronal activity in the central nervous system region during a presentation of a neutral stimulus after the application of the test topical composition;
   recording neuronal activity in the central nervous system region during a presentation of an erotic stimulus after the application of the test topical composition;
   applying a placebo topical composition to a region of the female subject’s genitalia;
   recording neuronal activity in the central nervous system region after the application of the placebo topical composition;
   recording neuronal activity in the central nervous system region during a presentation of a neutral stimulus after the application of the placebo topical composition;
   recording neuronal activity in the central nervous system region during a presentation of an erotic stimulus after the application of the placebo topical composition; and
   comparing the female subject’s baseline neuronal activity in the central nervous system region to the neuronal activity after the application of the test or placebo topical compositions in the absence of stimuli and during the presentation of neutral or erotic stimuli to the neuronal activity recorded in the same central nervous system region under the same conditions in a population of healthy subjects.
2. The method of claim 1 further comprising the step of comparing the female subject’s baseline neuronal activity in the central nervous system region to the neuronal activity after the application of the test or placebo topical compositions in the absence of stimuli and during the presentation of neutral or erotic stimuli to the neuronal activity recorded in the same central nervous system region under the same conditions in a population of female subjects suffering from a sexual dysfunction selected from a desire disorder, an arousal disorder, an orgasmic disorder and a sexual pain disorder.

3. The method of claim 1 wherein central nervous system region is at least one of the hypothalamic periventricular nucleus, the basomedial amygdala nucleus, parietal cortex, Broca’s area, insula, gyri fusiformis, superior frontal gyrus or anterior frontal lobe.

4. The method of claim 1 wherein the neuronal activity that is recorded is metabolic activity or electrical activity.

5. The method of claim 1 wherein the stimuli are mechanical stimuli or visual stimuli.

6. The method of claim 1 wherein the neuronal activity is recorded using functional magnetic resonance imaging, PET, SPECT or hybrid CT/PET.

7. The method of claim 1 wherein the region of the subject’s genitalia comprises the clitoris, the anterior vaginal wall or both the clitoris and the anterior vaginal wall.

8. The method of claim 1 wherein the test topical composition comprises a vasoactive agent and a pharmaceutically acceptable carrier.

9. The method of claim 8 wherein the vasoactive agent is prostaglandin E1.

10. The method of claim 8 wherein the pharmaceutically acceptable carrier comprises ethanol, ethyl laurate, modified guar gum, dodecyl N,N-dimethylamino isopropionate HCl, modified guar gum, buffer, sodium hydroxide and water.

11. The method of claim 9 wherein the placebo topical composition consists essentially of ethanol, ethyl laurate, modified guar gum, dodecyl N,N-dimethylamino isopropionate HCl, modified guar gum, buffer, sodium hydroxide and water.
12. The method of claim 1 wherein the neutral stimulus is a documentary video recording.

13. The method of claim 1 wherein the erotic stimulus is an erotic video recording.

14. A method for monitoring the progress of the treatment of a female subject suffering from a sexual dysfunction comprising applying the method of claim 1 or claim 2 on more than one occasion during the course of treatment and comparing the results obtained on each occasion.

15. A method for screening topical compositions effective for modulating central nervous system sexual responses comprising the steps of:

   recording baseline neuronal activity in at least one central nervous system region of a female subject that is associated with sexual responses;

   applying a test topical composition to a region of the female subject’s genitalia;

   recording neuronal activity in the central nervous system region after the application of the test topical composition;

   applying a placebo topical composition to a region of the female subject’s genitalia;

   recording neuronal activity in the central nervous system region after the application of the placebo topical composition; and

   comparing the neuronal activity after the application of the test topical composition to the neuronal activity after the application of the placebo topical composition and baseline neuronal activity to determine if the test topical composition was effective for modulating central nervous system sexual responses, thereby screening a topical composition.

16. The method of claim 15 wherein the female subject is a mammal.

17. The method of claim 16 wherein the female subject is a woman.

18. The method of claim 15 wherein the region of the subject’s genitalia comprises the clitoris, the anterior vaginal wall or both the clitoris and the anterior vaginal wall.

19. The method of claim 15 wherein the test topical composition comprises an active agent and a pharmaceutically acceptable carrier.
20. The method of claim 19 wherein the active agent is prostaglandin E\textsubscript{1}.

21. The method of claim 19 wherein the placebo topical composition consists essentially of the pharmaceutically acceptable carrier.

22. The method of claim 15 wherein central nervous system region is at least one of the hypothalamic periventricular nucleus, the basomedial amygdala nucleus, parietal cortex, Broca's area, insula, gyri fusiformis, superior frontal gyrus or anterior frontal lobe.

23. The method of claim 15 wherein the neuronal activity that is recorded is electrical activity or metabolic activity.

24. The method of claim 15 wherein the neuronal activity is recorded using functional magnetic resonance imaging, PET, SPECT or hybrid CT/PET.

25. The method of claim 15 further comprising the steps of:
   - recording neuronal activity in the central nervous system region during a presentation of a neutral stimulus after the application of the test topical composition;
   - recording neuronal activity in the central nervous system region during a presentation of an erotic stimulus after the application of the test topical composition;
   - recording neuronal activity in the central nervous system region during a presentation of a neutral stimulus after the application of the placebo topical composition; and
   - recording neuronal activity in the central nervous system region during a presentation of an erotic stimulus after the application of the placebo topical composition.

26. The method of claim 25 wherein the stimuli are mechanical stimuli or visual stimuli.

27. The method of claim 25 wherein the neutral stimulus is a documentary video recording.

28. The method of claim 25 wherein the erotic stimulus is an erotic video recording.
Figure 3
Figure 7A

Figure 7B
Figure 12
Figure 14
Imaging baseline condition

Providing test composition

Providing neutral stimulation

Imaging during neutral stimulation

Providing erotic stimulation

Imaging during erotic stimulation

Comparing images

Figure 17
### PATENT COOPERATION TREATY

**PCT**

**DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT**  
(PCT Article 17(2)(a), Rules 13ter.1(c) and Rule 39)

<table>
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<th>IMPORTANT DECLARATION</th>
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**Appliant**  
NEXMED (HOLDINGS), INC.

This International Searching Authority hereby declares, according to Article 17(2)(a), that no international search report will be established on the international application for the reasons indicated below:

1. **X** The subject matter of the international application relates to:
   - [ ] scientific theories
   - [ ] mathematical theories
   - [ ] plant varieties
   - [ ] animal varieties
   - [ ] essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes
   - [ ] schemes, rules or methods of doing business
   - [ ] schemes, rules or methods of performing purely mental acts
   - [X] schemes, rules or methods of playing games
   - [X] methods for treatment of the human body by surgery or therapy
   - [X] methods for treatment of the animal body by surgery or therapy
   - [ ] diagnostic methods practised on the human or animal body
   - [ ] mere presentations of information
   - [ ] computer programs for which this International Searching Authority is not equipped to search prior art

2. **X** The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:
   - [ ] the description
   - [X] the claims
   - [ ] the drawings

3. [ ] A meaningful search could not be carried out without the sequence listing; the applicant did not, within the prescribed time limit:
   - [ ] furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
   - [ ] furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
   - [ ] pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b).

4. [ ] A meaningful search could not be carried out without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-Bis of the Administrative Instructions, and such tables were not available to the International Searching Authority in a form and manner acceptable to it.

5. Further comments:

**Name and mailing address of the International Searching Authority**  
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Fax: (+31-70) 340-3016

**Authorized officer**  
Irene Rbia-Brand

Form PCT/ISA/203 (April 2005)
A meaningful search is not possible on the basis of all claims because all claims are directed to - Method for treatment of the human or animal body by surgery; Method for treatment of the human or animal body by therapy; Diagnostic method practised on the human or animal body - Rule 39.1(iv) PCT

The applicant’s attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.