Massage-like stroking of rats

Distress or “antistress”?

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Abstract

Massage is an ancient treatment that still is commonly used in humans. Massage is reported to have several beneficial effects including activation of the relaxation and growth response that is proposed to be mediated by oxytocin (OT). In the present thesis the effects of repeated massage-like stroking (stroking) in rats has been studied. The experimenter restraints the rat with one hand and with the other hand the abdomen of the rat was firm but gently stroked at a speed of 30-40 strokings/min for 5 minutes. In order to analyze the individual behavioral treatment response the stroking sessions were video recorded (paper III and IV).

In paper I, the effects of stroking on plasma levels of gastrin, insulin, CCK, somatostatin and glucose were investigated. Plasma levels of gastrin and insulin decreased after 14 stroking sessions, whereas plasma levels of glucose and body weight increased.

In paper II, rats were treated postnatally with either stroking early in life or with OT injections. Both postnatal stroking and OT-treatment decreased the diastolic blood pressure measured with the tail-cuff method in adulthood in these rats.

In paper III, female rats surgically prepared with telemetric blood pressure equipment were used. The rats were their own controls and the experiment started with a 5 days control period followed by 10 days with stroking. Each stroking session was video recorded and blood pressure measured with telemetry. Blood pressure increased during stroking and was maintained high during the 10 days stroking period compared to the control period. Latency time to relaxation decreased during the stroking period.

In paper IV, the effects of stroking on social interaction were studied. Stroking in male rats did not alter the social interaction or the plasma levels of OT and corticosterone. However, home-cage dominance as well as interaction between dominance and stroking altered the social behavior.

In conclusion, most of the results indicate that the rat experiences distress rather than anti-stress during stroking. Since rats had to be restrained during stroking, the sympathetic nervous system probably was activated. This might have hidden the suggested stroking induced reflex activation of the parasympathetic nervous system. For future studies another animal model that can be stroked without restraint, i.e. dog is recommended.

Keywords: massage-like stroking, blood pressure, social interaction, corticosterone, glucose, oxytocin, gastrin, insulin, CCK.

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In memory of my mother

and to Olov
Love, Mika and Caspian

Per Aspera Ad Astra
(Genom svårigheterna mot stjärnorna ☺)

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Holst, S., Lund, I., Petersson, M. and Uvnäs-Moberg, K. 2005. Massage-like stroking influences plasma levels of gastrointestinal hormones, including insulin, and increases weight gain in male rats, *Auton Neurosci*, 120(1-2)/2005, pp. 73-79.

II Holst, S., Uvnäs-Moberg, K. and Petersson, M. 2002. Postnatal oxytocin treatment and postnatal stroking of rats reduce blood pressure in adulthood, *Auton Neurosci*, 99(2)/2002, pp. 85-90.

III Holst, S., Sjöquist, M. and Dahlborn, K. Acute responses in behaviour and telemetric blood pressure registration during massage-like stroking in female rats (manuscript).

IV Holst, S., Sjöquist, M. and Dahlborn, K. Home cage dominance-subordination relationships influence social behaviour more than massage-like stroking in male rats (manuscript).

Abbreviations

ACTH	Adrenocorticotropic hormone
ANOVA	Analysis of Variance
AVP	Arginine vasopressin
BNST	Bed of Nucleus of the Stria Terminalis
CCK	Cholecystokinin
CNS	Central Nervous System
CRH	Corticotopin Releasing Hormone
CSF	Cerebrospinal Fluid
DBP	Diastolic blood pressure
DMX	Dorsal Motor Nucleus of the Vagus
GR	Glucocorticoid receptor
HPA-axis	Hypothalamic-Pituitary-Adrenal-axis
5-HT	Serotonin
i.c.v.	Intra cerebroventricular
LC	Locus Coeruleus
MR	Mineralocorticoid receptor
NA	Noradrenalin
NTS	Nucleus of the Solitary Tract
OT	Oxytocin
PAG	Periaqueductal Gray
PVN	Paraventricular Nucleus
RIA	Radioimmunoassay
SBP	Systolic blood pressure
s.c.	subcutaneous
SD	Sprague-Dawley rat, Standard deviation
SEM	Standard Error of the Mean
SON	Supraoptical Nucleus
W	Wistar rat

Introduction

Massage/massage-like stroking

Massage is an ancient treatment, probably one of the oldest in the world. Writings, paintings and sculptures describing various methods of massage can be found in virtually all recorded civilizations, such as Babylon, Assyria, China, India, Greece, Rome and Egypt. Laying on of hands on a sick person is the prototype of any treatment which can be recognized in terms used to designate therapeutics, such as the Swedish "behandling" or in the English word "handling". The word massage is thought to have its origin from the Arabic word mass, to touch, or the Greek word massein, to knead (Kamenetz, 1985).

There are various techniques used in massage such as stroking, connective tissue massage, kneading and friction or deep massage. The different techniques have different targets and different effects. Stroking is a light movement of the hands over the skin in a slow, rhythmic fashion. Connective tissue massage is a kind of deeper stroking motion, specifically used to free subcutaneous connective tissue adhesions. Kneading consists of grasping, lifting, squeezing or pushing the massaged tissue. The goal of deep massage is to loosen adhesions between deeper structures, such as ligaments, tendons and muscles. The used method consists of monotone circular movements. The method used in this thesis is stroking (Kamenetz, 1985).

Massage has also been given to animals, e.g. horse massage, dog massage, cow massage and has been reported to be appreciated by the animals (e.g. Schmied et al, 2007).

Background

Effects of massage in humans

Massage given to humans has been reported to stimulate and relaxe tissues and muscles, increase blood circulation, promote nutrition to cells and decrease tension and stress. Massage has been reported to help a number of symptoms such as back pain, cystic fibrosis, fibromyalgia, migraine headache, anxiety and smoking cravings (Cho & Snyder, 1996; Field et al, 1996; Hernandez-Reif et al, 1998, 1999 a&b and 2001; Moyer et al, 2004). Massage has also been shown to have an impact on immunological parameters (Field et al, 2002).

After only one session of massage anxiety, negative mood, pain, plasma levels or urinary levels of cortisol, noradrenalin (NA), adrenalin and blood pressure has been reported to decrease and β-endorphin to increase (Kaada & Torsteinbö, 1989; Kim et al, 2001; Moyer et al, 2004; Hernandez-Reif et al, 2004). A sex-dependent change in plasma levels of the peptide hormone oxytocin (OT) and neuropeptide Y (NPY) has been reported after a single session of massage (Wikström et al, 2003).

Repeated sessions of massage has been reported to decrease depression, hostility and pain and increase urinary dopamine and serotonin values in breast cancer patients (Hernandez-Reif et al, 2004). Repeated sessions of massage decreased pain. The effect is sustained 2 days to 6 weeks after the termination of treatments (Moyer et al, 2004).

Mechanisms of massage/massage-like stroking

The known mechanisms involved in the effects of massage/stroking are reported as promotion of parasympathetic activity. The pressure applied

during massage/stroking could thereby stimulate vagal activity, which leads to reduction of stress hormones and physiological arousal (Uvnäs-Moberg 1998; Field 1998). Tactile sensory stimulation, pain, temperature and touch, are mediated via nerve fibers that project from the skin to the dorsal root ganglia. Depending on the type of tactile stimulation different receptors and nerve fibers are activated. Within the spinal cord two different systems ascend. The anterolateral system transfers pain, temperature and crude touch, via myelinated Aδ-fibers, thin unmyelinated C-fibers and CT-fibers. The CT-afferents project mainly to lamina II and further to the insular cortex (Wiklund Fernström, 2004), see below for further description. The anterolateral system ascends to the cerebral cortex in the white matter of the spinal cord. The axons end in the reticular formation of the medulla and pons, in the tectum of the midbrain and in the ventral posterior lateral nucleus of the thalamus. The neurons in these nuclei in turn send axons to the somatic sensory cortex, to the basal ganglia and a number of areas in the cortex and to the parietal lobe (Martin & Jessell, 1991) (Fig. 1).

The dorsal column-medial leminscal system mainly transfers location, pressure, flutter or vibration, via fast myelinated Aβ-fibers and thin unmyelinated C-fibers. The dorsal column-medial leminscal system leads to the cerebral cortex through the middle of the spinal cord. The axons finally project to the ventral posterior lateral nucleus of the thalamus. The neurons in this nucleus in turn send axons to the somatic sensory cortex (Martin & Jessell, 1991) (Fig. 1).

In addition, sensory stimulation is conducted through afferents that project to the medulla without transmission through the spinal cord. The whole ventral side, including the abdomen and the urogenital organs, contains sensory afferents that project via the vagal nerve directly to medulla oblongata. Axons from the nucleus of the solitary tract (NTS) are then able to reach the paraventricular nucleus (PVN) and the dorsal motor nucleus of the vagal nerve for further activation (Norgren & Smith, 1988; Raybould et al, 1988; Komisaruk & Sansone, 2003).

The stimulation of Aβ fibers and CT-fibers by non-noxious sensory stimulation may influence vagal nerve activity (Olson et al, 1992; Eriksson et al, 1996 a&b), which may lead to release of OT from nerve terminals in the dorsal motor nucleus of the vagal nerve (DMX) (Buijs, 1983; Verbalis et al, 1986) and in plasma (Lund et al, 2002).

It has also been suggested that massage inhibits noxious signals to the brain via elevated seretonin (5-HT) levels (Field, 1998). Different types of sensory stimulation result in elevated cerebrospinal fluid (CSF) levels of opioids, i.e. electro acupuncture increases endomorphins, enkephalins,

dynorphins and endorphins dependent on the frequency of stimulation. Opioids are well known inhibitors of pain, and pain inhibition has been reported as one effect of massage (Andersson & Lundeberg, 1995).

The CT-fibers

Recently, a new type of nerve fibers was discovered called the CT-fibers, or CT-afferents. They are thin, unmyelinated fibers, but unlike the common C-afferents they have a separate, less sensitive type of mechanoreceptor and also lack substance P, which leads to the conclusion that their principal function is non-nociceptive. Since they do not respond to fast moving stimuli they have poor discriminative capacity of touch and the CT-afferents consequently are not important for the cognitive and discriminative aspects of tactile stimulation (Wiklund Fernström, 2004). Instead they respond to slowly moving stimuli, like stroking. They have therefore been suggested to be of importance for behavioral, hedonic/emotional and hormonal aspects of tactile stimulation and limbic functions. The CT-afferents project mainly to lamina II and further to the insular cortex via the anterolateral system. Functional magnetic resonance imaging (fMRI) analysis shows that stimulation of the CT-afferents activates the subcortical insular region (Olausson et al, 2002; Hofbauer et al, 2006). The insular region is also activated by maternal and romantic love (Bartels & Zeki, 2000 & 2004).

Central cardiovascular regulation

The regulation of blood pressure is almost entirely controlled by the autonomic nervous system. The far most important part of the autonomic nervous system regulating blood pressure is the sympathetic nervous system, although the parasympathetic nervous system via the vagus nerve is important for regulation of heart function.

The vasoconstrictor system is centrally regulated through the vasomotor center within the reticular substance of the medulla and lower third of the pons. This center transmits impulses through the sympathetic vasoconstrictor fibers to almost all blood vessels in the body. The vasomotor center is divided into three areas: a vasoconstrictor area (C1) that releases NA, which fibers are distributed from C1 through the spinal cord in order to excite vasoconstrictor neurons in the sympathetic nervous system, a vasodilator area (A1) that has fibers projecting to C1 in order to inhibit the vasoconstriction, thereby causing vasodilatation and finally a sensory area (A2) situated in the

NTS receiving signals from the vagus and glossopharyngeal nerves affecting the C1 and A1 areas (Dodd & Role, 1991).

The baroreflex is activated if the stretch receptors within the walls of large systemic arteries are stretched due to large blood volume. The baroreflex then causes the autonomic nervous system to reduce the arterial blood pressure. The baroreflex is transmitted via Hering's nerve (carotid sinus) and the vagus nerve (arch of the aorta) to the NTS within the medullary area in the brain stem. The NTS integrates the peripherally initiated sensory information of blood pressure, heart rate and respiratory function. Furthermore, it innervates the parasympathetic motor centers, the dorsomedial motor nucleus of the vagus nerve (DMV) and the nucleus ambigus (AMB) and is interconnected with the rostral ventrolateral medulla (RVLM) as well as the caudal ventrolateral medulla (CVLM). The RVLM is a major tonic pressor region, which innervates the sympathetic preganglionic neurons located in the intermediolateral cell column (IML) of the spinal cord. The CVLM receives baroreceptor input from the NTS and modulates RVLM via an inhibitory pathway (Fig. 1) (Dodd & Role, 1991).

The NTS innervates either directly or indirectly other areas that are involved in cardiovascular regulation, such as the insular region and prefrontal cortex, the amygdala, the bed nucleus of the stria terminalis (BNST), the hypothalamic nuclei like the PVN and the raphe cells (Dodd & Role, 1991).

In addition to receiving fibers from the NTS, the hypothalamus also receives fibers from the RVLM, CVLM, and the locus coeruleus (LC). The hypothalamus innervates the same medullary centers as NTS, except for LC. Through these pathways the hypothalamus receives cardiovascular information and modulate sympathetic, parasympathetic and hormonal outflow to the periphery (Dodd & Role, 1991).

The LC is the most prominent cluster of noradrenergic neurons within the brain and is situated bilaterally and posteriorly at the juncture between the pons and the mesencephalon. The dorsal noradrenergic bundle projects to the cortex, hippocampus and cerebellum. The ventral noradrenergic bundle projects to the hypothalamus, hippocampus as well as other parts of the forebrain. The LC thereby influences the noradrenergic transmission and both activates and inhibits the activity of the brain (Rang et al, 1995).

Except for regulation of the circulation, the noradrenergic neurons also participate in the reward system, mood, state of arousal and neuroendocrine regulation. The noradrenergic effects are transferred via the α-adrenoceptors and β-adrenoceptors. The β-adrenoceptors are mostly involved in the

regulation of heart rate and mostly stimulating it when activated (Rang et al, 1995).

There are two groups of α-adrenoceptors: α_1-adrenoceptors that are located postsynaptically and mostly excite the neurons and α_2-adrenoceptors that are located presynaptically and may act as an autoreceptor in order to inhibit noradrenergic transmission (Rang et al, 1995).

Oxytocin

Oxytocin (OT) is a nonapeptide produced in the PVN and the supraoptical nuclei (SON) within the hypothalamus. The magnocellular neurons in the PVN and SON project to the neurohypophysis whence OT is released into the circulation. Classical effects of OT are for example contractions of smooth muscles involved in parturition and milk ejection. OT is also released in response to hyperosmolarity. As early as 1938, OT release was described in response to electrical stimulation of the vagal nerve (Chang et al, 1938).

In addition, a widespread network of oxytocinergic fibers project from parvocellular neurons in the PVN to many different areas within the central nervous system (CNS) (Swanson & Sawchenko, 1980 & 1983; Gimpl & Fahrenholz, 2001). PVN also receives projections from many brain areas including the dorsal vagal complex (DVC), LC, nucleus parabrachialis, BNST, amygdala, hippocampus, and the raphe nuclei and from cell groups within the hypothalamus. Many of these pathways are bi-directional (Swanson & Sawchenko, 1980 & 1983).

The oxytocin receptor (OTR) is a 389 amino acid polypeptide with 7 transmembrane domains and belongs to the class I G protein-coupled receptor family. OTR is often, but not always, localized in the same peripheral and central regions where OT-synthesis is localized. Oestrogen is able to enhance the expression of OTR and the binding of OT to the OTR (Gimpl & Fahrenholz, 2001; Zingg & Laporte, 2003).

The sibling hormone to OT, arginine vasopressin (AVP), is also synthesized within the SON and PVN, but not located in the same neurons as OT. Arginine vasopressin (AVP) has three receptors V1a, V1b and V2. V1a receptors are for example located in smooth muscle cells, the liver, adrenal cortex and the CNS and V1b receptors are for example located in the anterior pituitary, the adrenal medulla and the CNS. V2 receptors are located in the kidney where they mediate the antidiuretic effect of vasopressin. AVP has almost the same affinity for the OTR as for V1a and V1b, but although the OTR is relatively unselective it has about 10-fold

higher affinity for OT than AVP. OT binds to the AVP-receptors but with a lower affinity than AVP (Jard et al, 1987; Kimura, 1995; Ingram et al, 1995; Barberis & Tribollet, 1996; Vaccari et al, 1998; Gallo-Payet & Guillon, 1998; Gimpl & Fahrenholz, 2001; Wersinger et al, 2002; Keverne & Curley, 2004).

There appears to be some controversies concerning the OT regulation of cardiovascular function. OT has both been shown to have no effect and to decrease basal heart rate, as well as to enhance or reduce baroreceptor reflex gain (Michelini, 2001). Pharmacological doses of OT (1mg/kg s.c.) increase blood pressure immediately after injection, whereas a 5-day treatment period decreases blood pressure 10-20 mm Hg measured with the tail-cuff method (Petersson et al, 1999a). The same dose and method of OT-injection has also been reported to increase the activity and binding capacity of α_2-adrenoceptors within the NTS, which may be a mechanism by which OT decreases blood pressure (Petersson, 2002).

OT is able to alter the hypothalamic-pituitary-adrenal-axis (HPA-axis). Under basal conditions OT exerts an inhibitory tone on the HPA-axis, e.g. resulting in decreased plasma levels of corticosterone in rats. Under stressful conditions, such as parturition, OT is able to increase the activity of the HPA-axis. Corticotopin releasing hormone (CRH) is able to induce release of OT. Systemic OT treatment modulates glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) mRNA in the rat hippocampus (Bruhn et al, 1986; Petersson et al, 1999a; Gimpl & Fahrenholz, 2001; Neumann, 2002). Glucocorticoids may increase OTR binding in some areas of the limbic system (Liberzon et al, 1994; Liberzon & Young, 1997; Patchev et al, 1993). OT also potentiates the release of adrenocorticotrophine releasing hormone (ACTH) mediated by CRH, thereby increasing the corticosterone levels. Moreover in humans OT (mean plasma concentration 133.6±2.6 pmol/l after injection) has been shown to act directly on the pituitary gland leading to a decrease in the release of ACTH (Page et al, 1990).

Opioids and OT may interact via nerve fiber projections between the PVN and the arcuate nucleus in the hypothalamus (Bicknell, 1985; Csiffary et al, 1992; Douglas et al, 2002). This interaction is particularly strong during pregnancy and parturition (Russell et al, 1995).

Pharmacological doses of OT (1mg/kg s.c.) increase nociceptive thresholds. This effect can be abolished by the uterus OT-antagonist or the opioid antagonist naloxone. The effect of OT on nociceptive thresholds is sustained for several hours after the termination of OT-treatment and long-term (10 days) after repeated treatments (Petersson et al, 1996a).

OT has dual opposed effects on insulin via the vagal nerve (Siaud et al, 1991; Björkstrand et al, 1996b). OT decreases insulin levels via the NTS, which is blocked by an OT-antagonist and increases insulin levels via the dorsal motor nucleus of the Vagus nerve (DMX), which can be blocked by atropine. Somatostatin, Cholecystekinin (CCK) and gastrin all decrease after one dos OT-injection administered intra cerebroventricular (1-10μg i.c.v.), these decreases are also attenuated by atropine. Plasma levels of insulin, CCK and gastrin remain low for at least 10 days after a five-day OT-treatment period. (Altszuler & Hampshire, 1981; Siaud et al, 1991; Uvnäs-Moberg et al, 1994; Björkstrand et al, 1996 a&b; Uvnäs-Moberg et al, 1996a; Petersson et al, 1999c). OT increases plasma levels of glucose and is released in response to insulin-induced hypoglycemia (Mirsky, 1962; Fisher et al, 1987).

In the rat, OT increases maternal behavior, sexual behavior, affiliative behavior, grooming, social memory and aggression, whereas anxiety, feeding and learning decreased (Gimpl & Fahrenholz, 2001; Winslow & Insel, 2002).

"Antistress" – the relaxation and growth response

As opposed to the stress and fight and flight reaction an "antistress" reaction called the relaxation and growth response has been proposed by Uvnäs-Moberg (1994, 1997 a&b, 1998). The hypothesis of the relaxation and growth response commences in the physiological and psychological interaction between a mother and her baby during breastfeeding, since suckling stimulates vagal afferents that are proposed to activate the relaxation and growth response.

The stimulation starts with ocular, olfactory and social interaction between the baby and the mother. This interaction is positively correlated with plasma levels of OT. If the mother had a vaginal parturition, the interaction with the baby is increased because the elevated plasma levels of OT released during parturition makes the glia cells in the PVN around the OT-producing cells retract and the OT-releasing cells will then be able to junc together and release OT simultaneously in pulses. This strengthens the OT-mediated response of the mother to the tactile, ocular and olfactory stimulation given by the baby. The response is ejected milk, warmth by dilating blood vessels in the chest, protection, care and social interaction. The baby responds by suckling, warmth and social interaction. Uvnäs-Moberg (1998) believes that there is a correlation between the elevated OT and increased calmness as well as social interaction in the mother behavior. In vaginal delivered women plasma levels of OT have been shown to

correlate positive to socialization measured by an inventory, the Karolinska Scales of Personality (Nissen et al, 1998).

The maternal somatosensory afferents transmit the suckling stimulus to the CNS, which shift the autonomic nervous tone from sympathetic to parasympathetic mainly trough the vagal nerve. The vagal efferents regulate gastrointestinal secretory and motor function as well as the endocrine functions of the gut. The relaxation and growth response decreases sympathetic actions in favor of parasympathetic actions in order to optimize milk production and survival of the baby. The blood flow is directed to the visceral organs and energy conservation is increased by increased release of gastrointestinal hormones, gastrin and CCK and also insulin. The activation of the vagal afferents during suckling, by for example CCK, triggers the sensation of satiety and relaxation, i.e. almost sedation, which allows the mother to be still even if she is stressed – so the baby can eat and grow. In addition CCK and OT take part in the filial imprinting. As previously mentioned, under non stressful (basal) conditions OT dampens the activity of the HPA-axis (Neumann, 2002), which also decreases the stress of the mother.

The vagal afferents influence blood pressure via nerve fibers projecting from the PVN containing AVP or OT that mainly influence the NTS regulation of the heart rate and indirectly the blood pressure (Michilini, 2001) (Fig 1).

Starting from the physiological and psychological interaction between a mother and her baby during breastfeeding, Uvnäs-Moberg proposes that the relaxation and growth response is stimulated by all forms of friendly somatosensory stimulation between two individuals leading to relaxation, decreased activity of the HPA-axis, plasma levels of corticosterone, and blood pressure as well as increased plasma levels of gastrointestinal hormones, insulin and OT, weight gain, nociceptive thresholds and social interaction (Uvnäs-Moberg, 1994, 1997 a&b, 1998).

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