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).

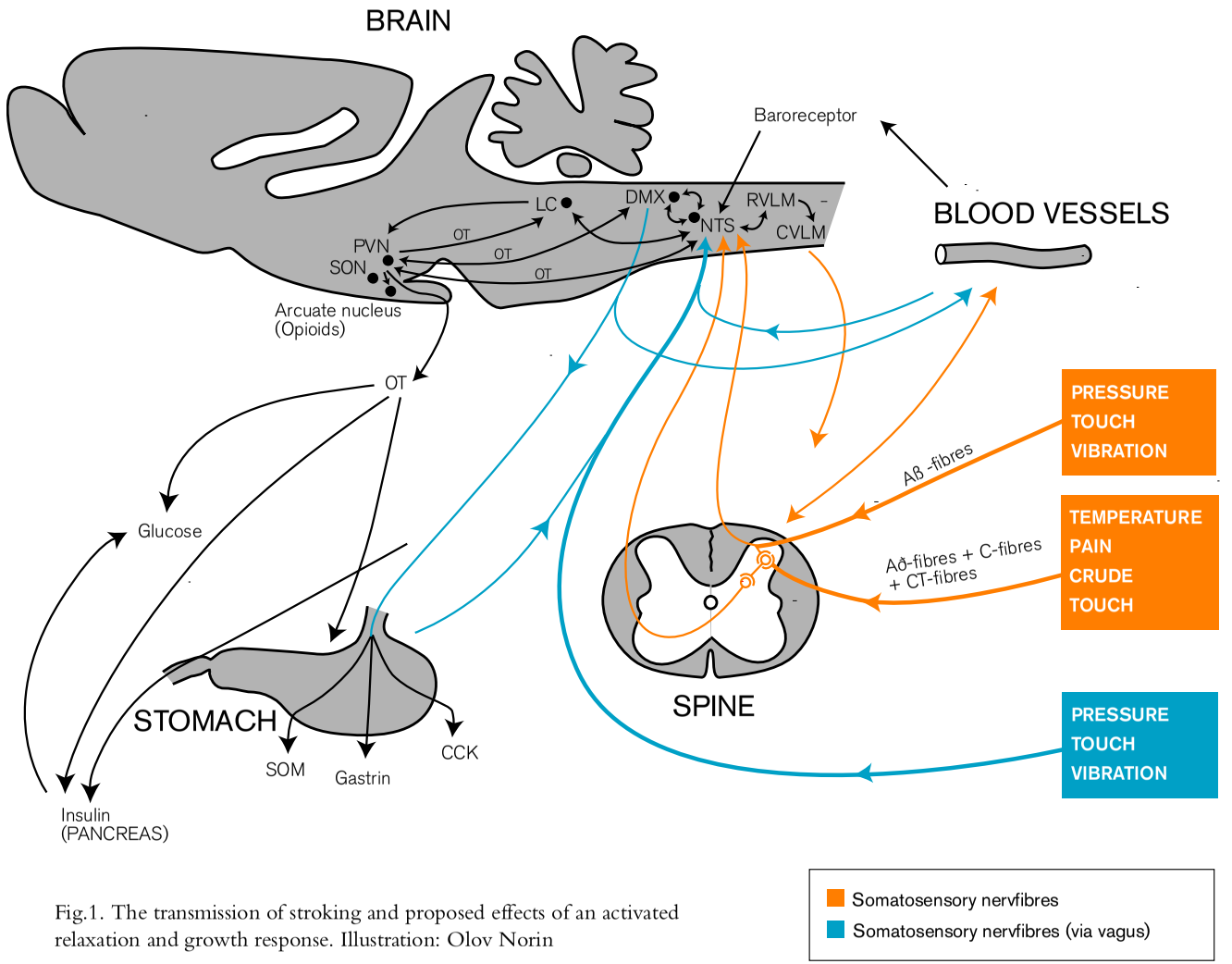

Fig.1. The transmission of stroking and proposed effects of an activated relaxation and growth response. Illustration: Olov Norin

Massage-like stroking in the anaesthetized rat

Previous work on effects of sensory stimulation on the autonomic nervous system has been performed on anaesthetized rats. Sato (et al, 1987; 1997) and coworkers (Kurosawa et al, 1982, Araki et al, 1984) showed that the somatosensory sympathetic reflex was activated by somatosensory stimulation, also from the visceral organs. Both parasympathetic and sympathetic activation have been shown, depending on the site of stimulation. Brushing, electro-acupuncture, vibration and thermal stimulation reduced the release of catacholamines from the adrenal glands (Kurosawa et al, 1982; Araki et al, 1984; Sato 1997) and increased plasma levels of gastrin, CCK and OT (Stock & Uvnäs-Moberg, 1988; Uvnäs-Moberg et al, 1992). Stroking with hands on anaesthetized rats decreased blood pressure (Kurosawa et al, 1995) (Fig. 2).

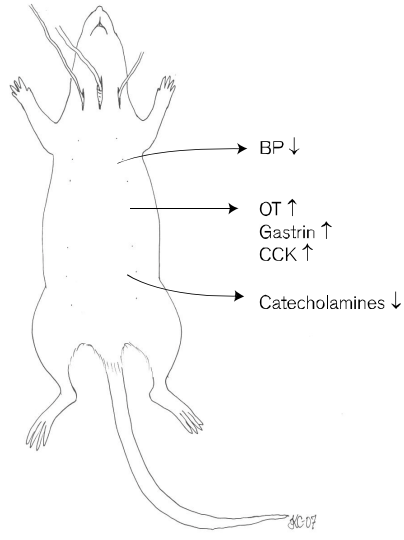

Fig. 2. Schematic picture of results obtained by sensory stimulation in anaestethized rat. Illustration: Katarina Cvek.

Massage-like stroking in the conscious rat

In order to study massage-like stroking (stroking) in conscious rats a model has been devised by Kanetake (1982). The model consists of holding the rat with one hand and stroking it with the other, see materials and methods for further description. Single sessions of stroking with this method in conscious rats has been reported to decrease locomotor activity (Uvnäs-Moberg et al, 1996) and increase blood pressure, measured with the tail-cuff method, immediately after stroking and then drop up to 180 min after the stroking ended (Lund et al, 1999). Repeated strokings of conscious rats has been reported to increase the nociceptive threshold and increase OT in both plasma and in the periaductal gray (PAG) (Lund et al, 2002) (Fig. 3).

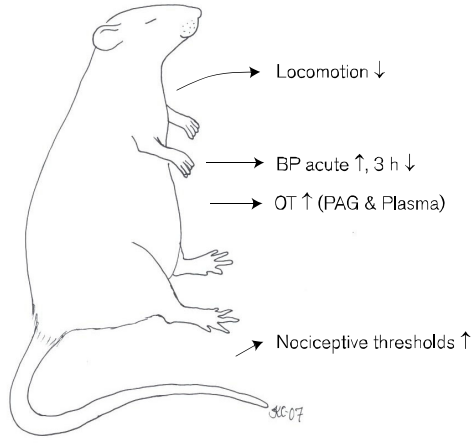

Fig. 3. Schematic picture of results obtained by sensory stimulation in conscious rat. Illustration: Katarina Cvek.

Restraint stress

Restraint, or immobilization, by holding the rat or placing it in a plastic tube is associated with increased blood pressure (Tavares & Correo, 2006) and plasma levels of corticosterone (Calvo & Volosin, 2001; Romeo et al, 2007) and levels of OT both peripherally (Callahan et al, 1992) and within the CNS (Hesketh et al, 2005) (Fig. 4).

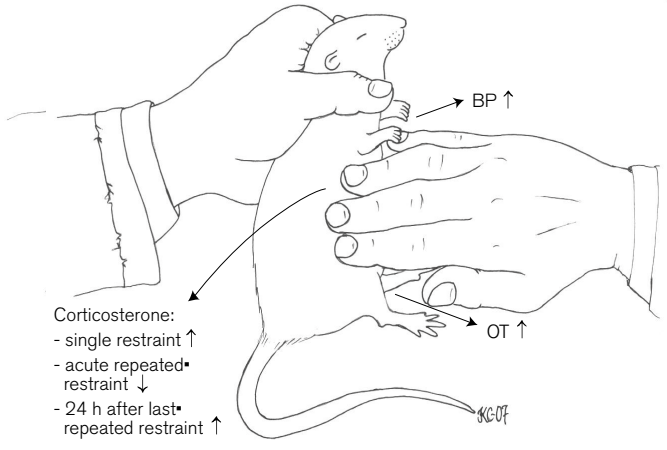

Fig. 4. Schematic picture of results obtained by restrain in conscious rat. Illustration: Katarina Cvek.

If the restraint is not noxious and of short duration (ca 1h), the rat is able to habituate to repeated restraint and both blood pressure (McDougall et al, 2000 & 2004) and plasma levels of corticosterone will then gradually decrease (Pitman et al, 1990). Repeated restraint alters plasma levels of corticosterone measured both directly and 24h after the last restraint. In comparison to basal levels, repeated restraint in male rats decreased plasma levels of corticosterone directly after the last restraint and increased the plasma levels of corticosterone 24h after the last restraint (Zelena et al, 2003; Chadda & Devaud, 2005). In comparison to plasma levels of corticosterone sampled after a single restraint the plasma levels sampled acutely after repeated restraint were lower (Chadda & Devaud, 2005), probably due to habituation to the restraint.

The habituation to the restraint is regulated on a central level including regulation of CRH by the PVN and gamma-aminobutyric acid (GABA) regulating the PVN. Also the affinity and amount of mineralocorticoid- (MR) and glucocorticoid receptor (GR) influences the habituation process (Calvo & Volosin, 2001; Armario et al, 2004).

Restraint stress has not been shown to change social interaction (Chaouloff et al, 1994; Gregus et al, 2005), but has been shown to reduce the time spent in the open arms of an elevated plus-maze (Calvo & Volosin, 2001).

Sex-dependent differences in response to restraint have been found. In female rats the plasma levels of corticosterone were increased acutely after the last repeated restraint compared to the increase after a single restraint (Chadda & Devaud, 2005). However, female rats were less vulnerable to uncontrolled stress since they learned how to escape electrical foot shock, in contrast to male rats who exhibited learned helplessness (Dalla et al, 2007). In addition, the dominance-subordination relationship has been reported to alter the stress response (Zhukov & Vinogradova, 2002; Diaz-Berciano et al, 2007).

Pre- and Postnatal effects

The neonatal period is of great importance for health and wellbeing in adulthood. Both neonatal physiological and psychological factors influence the adult life. For example Barker (2004) and colleagues have coined the concept of fetal origin of adult disease after the discovery that low birth weight is highly correlated with increased rates of coronary heart disease and the related disorders stroke, hypertension and non-insulin dependent diabetes. During the pre- and postnatal development, the organs and systems of the body go through critical periods when they adjust to the environment. The fetal HPA-axis is, for example, affected by the passage of corticosterone from the mother to the fetus (Weinstock, 2001). Developmental plasticity is defined as the phenomenon by which one genotype gives rise to a range of different environmental conditions during development and enables the production of phenotypes that are better matched to their environment (West-Eberhard, 1998).

By subjecting pregnant female rats to environmental stressors such as flashing light, restraint or putting their cage on a shake table prenatal stress impairs the biology and behavioral adaptation to stress in the adult offspring (Kinsley et al, 1988; Weinstock, 2001). Prenatal stress decreases GR and MR levels in the hippocampus (Reul et al, 1994) and according to the

glucocorticoid cascade hypothesis, the stress during pregnancy increases the plasma levels of corticosterone in the mother and the fetus, leading to the decrease of GR and MR in the fetal hippocampus and limbic system. This is followed by an increased activity in the HPA-axis, which results in increased levels of corticosterone and CRH in the developing brain. This may in turn alter the synaptic development and neurotransmitter activity in the brain, thus resulting in alterations in behavior in adulthood (Sapolsky et al, 1986; Weinstock, 2005). Prenatal stress reduces NA in the LC and in the cortex, as well as the α_2-receptor binding (Peters, 1984; Kofman, 2002). Prenatal stress results in the HPA-axis hyperactivation and dysregulation in adult animals, as characterized by stronger and more prolonged responses to stress, i.e. increased plasma levels of corticosterone for a longer period of time after restraint stress than controls (Sapolsky et al, 1986; Dellu et al, 1996; Maccari et al, 1995; Koubovec et al, 2005).

Maternal care in rats is defined by the degree of interaction between the mother and her young and influences the physiology and psychology of the adult animal. Pups that have experienced more maternal care measured as a high frequency of pup licking/grooming and arched-back nursing (referred to as LG-ABN), become less stress-sensitive as adults, measured as decreased startle response, and shorter latency to eat food in a novel environment (Zhang et al, 2004).

The difference in neurochemistry between rats that have experienced different levels of maternal care is suggested to depend on the intensity of the mother-pup interaction as there is no difference in the time spent with the pups, the weight gained or the number of pups raised to weaning, thus implicating that the amount of maternal care is sufficient in order to fulfill the physiological requirements. Studies involving cross fostering confirm that the changes are indeed mediated through the maternal behavior and not through genetic inheritance. If a litter of rat pups is divided so half of the litter stays with the genetic mother whereas half the litter is changed with half a litter from another dam, the pups will develop the same amount of maternal care as the dam that reared them. Thus, as adults the adopted pups developed the same amount of maternal care as their foster mothers and had the same amount of the above mentioned receptors as the genetic offspring to the foster mothers. The genetic offspring were also reared by the same dam, i.e. their biological mother (Weaver et al, 2004; Zhang et al, 2004).

Intense maternal care increases hippocampal GR mRNA expression and glucocorticoid negative feed back sensitivity and decreases CRH mRNA levels and CRH receptor levels in the LC. In response to stress the adult

offspring have reduced plasma levels of ACTH and corticosterone (Zhang et al, 2004).

Dams with high LG-ABN have higher levels of OT-receptors in brain regions known to mediate the expression of maternal care in rats; i.e. the central nucleus of the amygdala, BNST, the medial preoptic area and the lateral septum (Francis et al, 2000). If the pups are given OT-antagonist during the postnatal period, they fail as adults to develop the same amount of licking as their mothers had (Pedersen & Boccia, 2002). Maternal care influences parental behavior also in male rats. In males parental behavior is regulated by the vasopressin receptor V1a, and elevated numbers of this receptor were found in the central nucleus of the amygdala in adult male rats reared by a mother with high LG-ABN (Francis et al, 2002).

Levine et al, (1956) defined handling as a short period of maternal separation, e.g. 15 min a day for the first and/or second week of life. According to a general hypothesis this brief separation in rats increases the maternal care, which mediates the effects of handling (Levine, 2005). The mother is thought to increase her maternal care when she and the pups are reunited after the separation, compared to a mother who is let alone with her pups. The preponderance of the effects of handling is similar to those of high levels of maternal care. Handling decreases NA, which is probably an effect of an increased α_2-receptor density in the LC and in the NTS (Pieretti et al, 1991; Meaney, 1996; Liu et al, 2000) and decreases basal blood pressure in spontaneously hypertensive rats (SHR) (Tucker & Johnson, 1984). In response to restraint stress postnatally handled rats have decreased plasma levels of ACTH and decreased concentration of NA in the PVN (Liu et al, 2000). Handling may also reverse behavioral abnormalities induced by prenatal stress (Wakshlak & Weinstock, 1989).

Postnatal OT-treatment (pharmacological dose: 1mg/kg sc daily during the first 14 days of life) increased the density of α_2-agonist binding sites in the NTS and in the hypothalamus (Diaz-Cabiale et al, 2004) decreased blood pressure measured with tail-cuff (systolic blood pressure from 170 to 150 mm Hg; diastolic blood pressure from 150 to 130 mm Hg) and decreased plasma levels of corticosterone directly after stress (Olausson et al, 2003) in 4 months old rats. Postnatal OT-treatment (3μg/pup) on the first day of life did not change the levels of OT in the pituitary in 60-day old rats. However, postnatal OT-antagonist (0.3 μg/pup)-treatment the first day of life increased the levels of OT in the pituitary in 60-day old male rats and decreased OT in 60-day old female rats (Young et al, 2005).

In summary, increased pup-dam interaction dampens the vulnerability to stress in adulthood (Brake et al, 2004). The pup-dam interaction consists of

different factors, of which one is touch. The hypothesis in paper II was that touch mediates the beneficial effects of the increased interaction, and so the effects of postnatal stroking on blood pressure in adulthood were investigated. As stroking in adult rats has been reported to increase plasma levels of OT, and administration of OT to adult rats may result in similar effect patterns as stroking in adult rats, OT may mediate some of the beneficial effects of touch. Therefore the effects of pharmacological doses of postnatal OT (1mg/kg) on blood pressure in adulthood were also investigated. The pharmacological dose of OT (1mg/kg) was used in order to make sure that a sufficient amount of OT crossed the blood-brain barrier according to Jones & Robinsson (1982). In addition, pups that had been subjected to prenatal stress were treated postnatally with OT in order to investigate if OT would be able to reverse the effects of prenatal stress on blood pressure.

Aims of the thesis

The overall aim of the present thesis was to investigate the effect of repeated stroking on plasma levels of corticosterone, glucose, OT and gastrointestinal hormones including insulin, blood pressure and social interaction. In addition, the model of stroking described by Kanetake (1982) was evaluated.

Specific aims:

- To study the individual behavioral treatment response of repeated stroking, i.e. the willingness of the rat to receive stroking.
- To investigate the effect of repeated stroking on plasma levels of corticosterone, glucose and OT.
- To investigate the effects of repeated stroking on plasma levels of gastrin, CCK, somatostatin, insulin and on weight gain.
- To study the effect of repeated stroking on blood pressure measured with telemetry.
- To validate the tail-cuff measurements compared to telemetric measurements.
- To study the effect of postnatal stroking and high doses (1mg/kg s.c.) of postnatal OT-injections on blood pressure measured with tail-cuff in adulthood.
- To study the effect of repeated stroking on social interaction.
- To investigate factors that may interfere with the effects of repeated stroking; the restraint grip and home cage subordination relationships.

Materials och methods

Animals

In paper I, II (parent animals), and IV Sprague-Dawley (SD) rats (300g; Scanbur-BK AB, Sollentuna, Sweden), were used. In paper III Wistar (W) rats were used. A total of 85 SD rats and 11 W rats were used. The rats in paper I and II were housed in the animal facility at the department of Physiology and Pharmacology, Karolinska Institutet, Sweden. The rats in paper III and IV were housed in the animal facility at the domestic animal center, SLU, Uppsala, Sweden.

The animals were allowed to habituate to the animal department for 1 week before the experiments started. The rats were maintained under constant controlled conditions of light-dark cycle (12:12 h, lights on 06.00), temperature 19-21°C and 21-24°C respectively and relative humidity (55-60%). Food (R36: Ewos, Södertälje, Sweden; RM1, SDS, Witnam, Essex, UK) and tap water were freely available in the home cage. The females were normally housed three-four per cage or, during pregnancy, one per cage (Macrolon IV; 595 mm x 380 mm x 200 mm) or in a larger cage (595 mm x 380 mm x 500 mm) with walls of wire netting. The males were housed two to three per cage. The animals were sacrificed by decapitation or euthanized with barbiturates. All experiments were approved by the Ethics Committee for Animal Experiments in Stockholm or Uppsala, Sweden.

Study design for male and female offspring

The females were mated over night. The females included in the experiments with postnatal stroking were left undisturbed the whole pregnancy. The females included in the experiments with postnatal OT-

administration were divided in two groups on day one of pregnancy: the control group, which was left undisturbed during the whole pregnancy and the prenatal stress group.

The prenatal stress group was stressed both by light and by having their cages shaken (Fride & Weinstock, 1989). The cages were put on top of one another on a shake table and attached with tape. A lamp was directed towards the cages with a distance that differed between 10-35 cm. The intensity of the light varied from 30-710 lux depending on the position of the cage, which was regularly changed so that all cages had been in all positions for an equal number of sessions. The shaking and the light were randomly turned on/off by two separate pre-programmed timers. The two treatments were given in their own separate order. Sometimes the treatments overlapped, but they were always applied with the same time duration. These treatment periods, 16 of shaking and 16 of light, were 15 minutes long. The stress treatment was performed three nights a week through all the three weeks of pregnancy.

Since the rats often gave birth during the afternoon, the day after parturition was set as day 1. At day 1 litter size was adjusted to 10 pups per litter. The offspring included in the experiments with postnatal stroking were divided into 5 groups with 2 pups in each group of which stroking was one and control group another. All the other rats were used in other experiments not mentioned in this thesis. The pups in the control group were picked up and put back down and left untreated while the pups in the stroked group received stroking for 5 min on a daily basis, day 1–7 after birth. Offspring included in the experiments with postnatal OT-administration were divided into 2 groups with 5 pups in each group. The pups in the control group were injected (s.c.) once a day with NaCl (0.9%) and the pups in the OT-group were injected (s.c.) once a day with OT (1mg/kg) day 1–14 after birth. The pups were sexed and weaned at day 21.

Body weight

In Papers I and IV all rats were weighed prior to the stroking during the whole experimental period. Weight measurements (Mettler PE200) were accurate to a tenth of a gram.

Postnatal study design

All the experiments were performed in adult rats. The rats considered as adult at the age of 4–7 months, they are middle aged at 13–16 months and old at the age of 20–24 months (Vallée et al, 1999).

Blood pressure and heart rate were measured at the age of 8 months in the postnatally stroked rats.

The blood pressure and heart rate were measured at the age of 8 (males) and 7 (females) months in the postnatally OT-treated rats.

OT-injections

OT (Polypeptides, USA) was dissolved in physiological saline and injected in a volume of 1 mg/ml in rats with a weight over 10 g and 10 mg/ml in pups with a weight under 10 g.

Stroking

Pups

Rats were held with their backside down and stroked on their ventral (~3 cm^2) side of the abdomen with a one-inch thin camel hair brush. The pups were stroked for 5 minutes a day from day 1-7 with a speed of approximately 20 cm/s and with a frequency of 0.67 Hz, i.e. 40 strokes/min. Two pups were stroked at the same time to avoid isolation stress. The untreated pups were picked up at the same time as the stroked group but received no stroking.

Adult animals

The massage-like stroking (stroking) was performed on a daily basis (paper III) or every second day (paper I and IV) on 3 to 14 occasions. The rat was placed with its hind legs on a surface made of a material that prevented the rat from slipping. During the stroking the rat was held across the scapula and neck region as described by Kanetake (1982). The rat was held with one hand. Two fingers were held under the fore limbs and two fingers over the shoulders so the fingers made a harness. The grip was firm but without squeezing the rat. The experimenter used the other hand to gently but firmly stroke the rat for 5 min on the ventral side of the abdomen with a speed of ~20 cm s-1 and frequency of 0.50–0.67 Hz (i.e. stroking every 1.5–2 s or at 30–40 strokes min-1), and with an estimated pressure of 100 mm H_20 according to description by Kurosawa et al, (1995).

This kind of stroking acutely lowers blood pressure in anaesthetized rats more than stroking of the lateral side of the abdomen with the same frequency, or the ventral side with other frequencies or other time-spans (Kurosawa et al, 1995; Uvnäs-Moberg et al, 1996).

Controls to Stroking

Pups

In paper II the untreated pups were picked up at the same time as the stroked group but received no stroking.

Adult rats

In paper I the controls were held in the same grip as the stroked rats but without receiving stroking. Later on this was changed because the grip may be experienced as stressful. In Paper III the rats serve as their own control sitting on a plastic surface without any physical contact with the experimenter. In paper IV the rats were sitting in the lap of one of the two experimenters, with physical contact but without caressing or stroking.

Measurements of blood pressure and heart rate

Tail-cuff

In paper II blood pressure and heart rate were measured on conscious animals by placing a cuff and a microphone (Kent RTBP-002, Somedic Sales, Farsta, Sweden) on the base of the tail. The cuff was connected to a Grass 7P8 sphygmomanometer and a Grass 7P8DC amplifier with a printer. The rats were kept on a heating pad beneath a lamp in order to increase the vasodilatation of the blood vessels in the tail. Measurements were made as soon as the rat lay still and the heart rate could be clearly measured. The rats were habituated to the entire test procedure for 2–3 weeks before

Tail-cuff equipment. Photo: Elin Spangenberg

the actual testing started. The mean value from 4 measurements of blood pressure and heart rate was calculated.

In paper III the blood pressure was measured by tail-cuff on conscious animals by placing a cuff and a microphone (ML125 NIBP (Non-Invasive Blood Pressure), AD Instruments Pty, Australia) on the base of the tail. The cuff was connected to a PowerLab (AD Instruments Pty, Australia) and analyzed with the software Chart5 for Windows. The rat was then placed on the plastic surface, in a towel, so that the telemetric blood pressure could be recorded simultaneously. The experimenter placed one or two hands on the back of the rat to be able to feel movements and prevent the rat from moving.

The rats were habituated to the cuff and the microphone on day one to three. A total of 2–5 consecutive cycles (inflation/deflation) were performed on each rat and day, without awaiting the pulse to be detectable. Each measurement period was 5–10 min long.

Telemetry

Telemetric blood pressure was measured using a biotelemetry system (Data Sciences International, St Paul, MN, USA). The pressure transmitters (model no.TA11PA-C40; Data Sciences) were implanted according to description in paper III and the radio signal generated was detected using a flat receiver board located under the cages (model no. RA 1020; Data Sciences) or under the rat during experimental procedures. The receiver was, in turn, connected to a consolidation matrix, connected to a computer with the Dataquest A.R.T. (TM) Silver Acquisition 4.00 program, to monitor systolic and diastolic blood pressure. The blood pressure was measured every 10 s during experiment and every 5'th min in the home cage. The transmitters were switched off with a magnet between readings. Recording in the home cage started approximately 1month after surgery and the experiment started 3–4 months after surgery.

Social interaction test

Social interaction was measured in a circular open field arena, which was circular and made of stainless steel. The arena had a diameter of 90 cm, surrounded by a 25 cm high black wall. The floor was a grey metal grid. The arena was lit by two lamps, facing outwards, with white light and a light intensity of ca 200 lux. One digital video camera was placed vertically above the arena and recorded the social behavior for later scoring. The experimenter remained outside the room during the social interaction test.

In between two tests the arena was cleaned with soapy water and dried with tissue paper to avoid olfactory cues.

The social interaction was measured by letting two rats meet in the arena for 20 min. The rats were allowed to explore in pairs with their cage mate for 10 min in the open field on one occasion two days before the social interaction test started.

The video recording was analyzed with EthoLog® 2.25 (Ottoni, 2000). The latency time, frequency and duration of each behavior were registered. The rat initiating each behavior was registered in order to calculate the individual frequency of behaviors.

Individual behavioral treatment response

In order to investigate the willingness of the rat to receive stroking, the stroking sessions were video recorded. The individual behavioural treatment response was then calculated according to:

- Frequency of activity: in paper IV The total number of movements of head and/or legs. In paper III the activity was divided into subgroups: Frequency of low activity; the total number of movements of legs. Frequency of high activity; the total number of times the rat moved so the experimenter lost the grip during stroking, or that the rat put all four feet on the plastic surface and the experimenter had to stop stroking.
- Duration of relaxation: Each relaxation period was added together to get total duration time during the stroking session. The relaxation was identified as lowering in abdominal tonus, identified by that the skin folds and the body moves backwards-downwards.
- Frequency of relaxation (FR): the number of relaxation periods during the stroking session (Only in paper III).
- Duration of each period of relaxation (D/FR): the average duration of a relaxation period (Only in paper III).
- Latency to first relaxation: The time from the start of the stroking to the first period of relaxation.

Collection and treatment of plasma samples

In paper I rats were sacrificed by decapitation. Immediately following decapitation, trunk blood was collected in ice-chilled tubes containing heparin (Lövens läkemedel, Malmö, Sweden) (10 IU/ml) and Trasylol®

(Bayer, Germany) (500 IU/ml). The blood samples were centrifuged and plasma was removed and frozen (-20 °C).

In papers III and IV a blood sample was taken from all rats from the lateral saphenous vein. The blood was collected in ice-chilled tubes containing 10 IU ml-1 of EDTA (Lövens Läkemedel, Malmö, Sweden) and 500 IUml-1 of Trasylol® (Bayer, Germany). Approximately 400 µl blood was sampled.

In paper IV the rats were euthanized with barbiturate (Pentothal® Natrium, Electra-Box Pharma; 150mg/kg, ip) and terminal cardiac puncture was performed with a 19–21G needle and 5 ml syringe. A total of 2-4 ml blood was draw and collected in ice-chilled tubes containing 10 IU ml-1 of EDTA (Lövens Läkemedel, Malmö, Sweden) and 500 IUml-1 of Trasylol® (Bayer, Germany). Blood samples were centrifuged at +4 °C and thereafter plasma was separated, frozen and stored at -20 °C until analysis.

Radioimmunoassays (RIA)

Gastrin and insulin were determined with Radioimmunoassay (RIA) directly in plasma as previously described (Petersson et al, 1999b).

Somatostatin and CCK were determined by RIA after SEP-PAK® C18 extraction (Waters Corporation, Milford, Mass., USA), as previously described (Petersson et al, 1999b).

Corticosterone was radioimmunoassayed by the commercially available kit; Coat-A Count Rat Corticosterone® (Diagnostic Products Corporation, Los Angeles, CA, USA). Limit of detection for corticosterone was 13.58 ng/ml.

Enzyme linked immunosorbent assay (ELISA)

OT was determined by a commercially available ELISA kit (Electra-Box Diagnostica AB, Sweden) modified by Norrby et al (personal communication) after extraction with acetone (GR, Merck, Darmstadt, Germany) and petrolium benzene (GR, boiling range 40–60 °C, Merck, Darmstadt, Germany) with a recovery of 99.1%. Limit of detection for OT was 12.87 pg/ml.

Spectrophotometry

Glucose was measured with GOD-PAP spectrophotometric method (Cat. No. 14365, Diagnostica Merck, Darmstadt, Germany).

Statistical analysis

P-values of 0.05 or less were regarded as statistically significant. All data was analysed with the computer software program Statistica® version 6.0 or 7.0. For data that followed criteria for parametric tests student's t-test or analysis of variance (ANOVA) were used. The ANOVA was followed by Fisher's LSD-test or Bonferroni test. For non-parametric analysis the Mann-Whitney U-test was used. Correlations were calculated with Spearmans Rank. In paper I and II the results are presented as means ± standard deviation (SD). In paper III and IV the results are presented as means ± standard error of the mean (SEM).

Results and Comments

In the present thesis the following results were obtained (Table 1):

Table 1. *Results of the present thesis in comparison with antistress – the relaxation and growth response and with the general stress response.*

Parameter	"Antistress"	Paper I	Paper II	Paper III	Paper IV	"Stress"
Blood pressure	↓	–	↓	↑	–	↑
Glucocorticoids	↓	–	–	↔	↔	↑
Plasma glucose	↔	↑	–	↔	–	↑
Gastrointestinal hormones	↑	↓	–	–	–	↓
Oxytocin	↑	–	–	↔	↔	↑↓
Body weight gain	↑	↑	–	–	↔	↓
Social interactions	↑	–	–	–	↔	↓

Individual behavioral treatment response (Papers III and IV)

The individual behavioral treatment response was recorded to measure the willingness of the rat to receive stroking.

The latency time to relaxation of the abdomen gradually decreased.The activity during stroking measured as movement of head or foot (LA and Activity) and to move so much that the experimenter had to stop stroking (HA) depended more on the individual traits of the rat than of the day of stroking.

Comment: The rats appear to gradually accept and relax the abdomen in response to stroking. However, some individuals never accepted the stroking, which may be due to individual coping strategies.

Plasma levels of corticosterone, OT and glucose (Papers I, III, IV and unpublished)

The plasma levels of corticosterone varied in response to repeated stroking (fig. 5). The results of the plasma levels of glucose were also varied. In two experiments the plasma levels of glucose were unchanged whereas they increased directly after the last repeated stroking in one experiment and decreased 24h after the last repeated stroking in another experiment (fig. 6). The plasma levels of OT were unchanged with repeated stroking (fig. 7). Plasma levels of OT from the rats stroked with 3 or 14 sessions are published elsewhere (Lund et al, 2002). The plasma levels of OT did not differ between the groups after 3 sessions of stroking, ranging in the interval of 20 to 70 pmol/l. After 14 sessions of stroking plasma levels of OT increased in stroked rats ranging in the interval of 90 to 150 pmol/l whereas control rats of 60 to 70 pmol/l (Lund et al, 2002). The rats used in paper IV and for the unpublished data were euthanized with pentobarbiturate before their blood was drawn by cardiac puncture.

Comment: In paper I the increase of plasma levels of glucose could be due to the decreased plasma levels of insulin.

The plasma levels of glucose are high in most of the experiments and this may be a stress response, this despite the rats in paper I were decapitated in a clean room with change of clothes in between each rat. In addition, in the unpublished data the rats were euthanized with pentobarbiturates, which are known to increase plasma levels of glucose.

In the unpublished data the plasma levels of OT were high, which may be due to the terminal cardiac puncture. During the cardiac puncture the

blood pressure decreases rapidly, which provokes a release of AVP with a simultaneous release of OT.

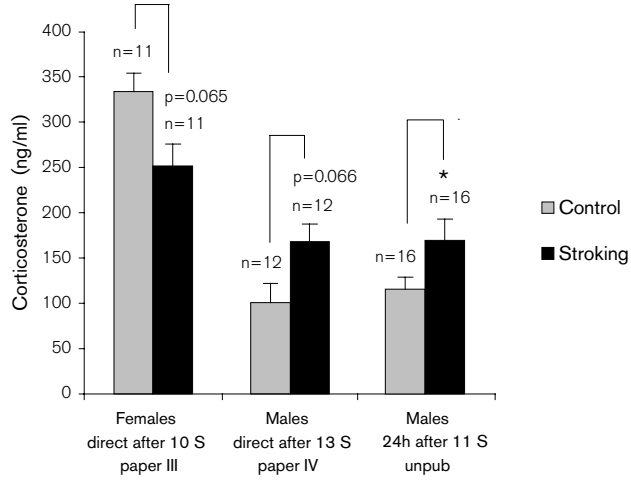

Fig. 5. Plasma levels of corticosterone direct after the last stroking in females and in males as well as 24h after the last stroking. Data is presented as means ±SEM. *p<0.05. In paper IV stroking was performed every second day, in the other papers the strokings was performed on a daily basis. The rats in paper IV were SD, the others W.

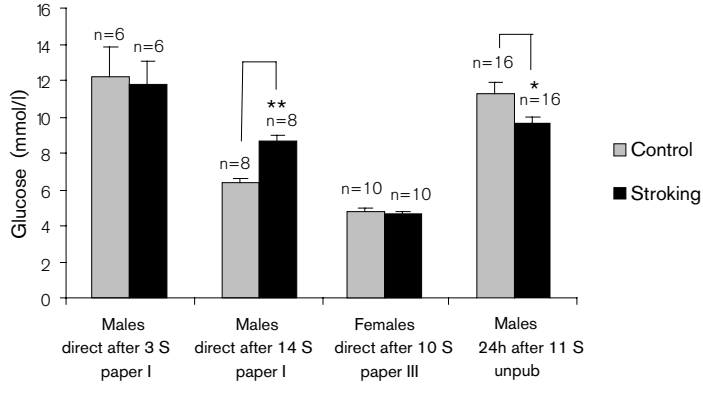

Fig. 6. Plasma levels of glucose direct and 24h after a different number of stroking. Data is presented as means ±SEM. *p<0.05, **p<0.01. In paper I stroking was performed every second day, in the other papers the strokings was performed on a daily basis. The rats in paper I were SD, the others W.

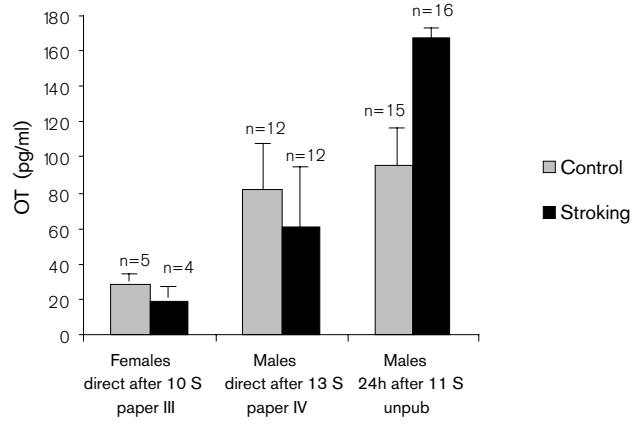

Fig. 7. Plasma levels of OT direct and 24h after stroking on a daily basis in male rats. Data is presented as means ±SEM. In paper IV stroking was performed every second day, in the other papers the strokings was performed on a daily basis. The rats in paper IV were SD, the others W.

Blood pressure (Papers II and III)

Adult female rats

Stroking in female adult rats acutely increased both their systolic and diastolic blood pressure, measured with telemetry, compared to their control period (fig. 8). The increase was sustained through out the 10 day long period of stroking. In addition, telemetric blood pressure was increased just by letting the rat sit on the plastic surface on the experimental area in comparison with the home cage. The blood pressure was further increased in response to validation of the tail-cuff method compared to the control period (fig. 9). The measurements with tail-cuff did not correlate to measurements with telemetry.

Comment: Both during stroking and validation of the tail-cuff method the rats were restrained. It is possible that the restraint caused the increased blood pressure. The variation in the measurements with tail-cuff was not as great as in the telemetric measurements, which may lead to an incorrect assumption of a low and stable blood pressure. The telemetric measurements show that the blood pressure differs a lot both due to the individual and to the situation. In addition, in order to get a blood pressure measurement with tail-cuff the vessels in the tail of the rat have to dilate, which may be difficult if the rat is cold or a bit stressed.

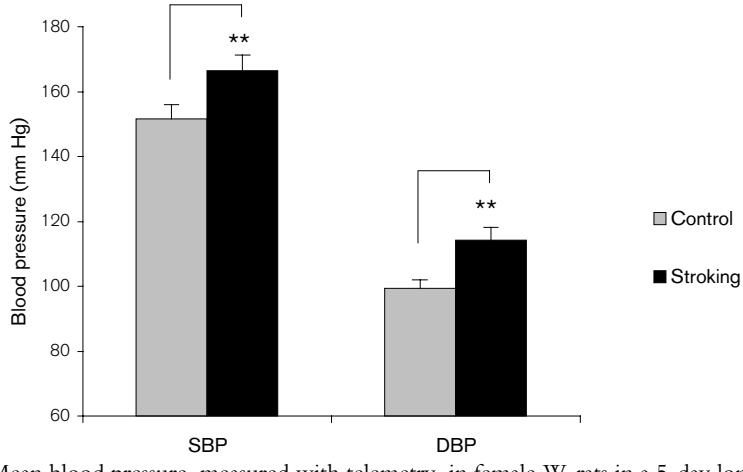

Fig. 8. Mean blood pressure, measured with telemetry, in female W-rats in a 5-day long control period and during 10 sessions of stroking on a daily basis (paper III). Data is presented as means ±SEM. **$p<0.01$.

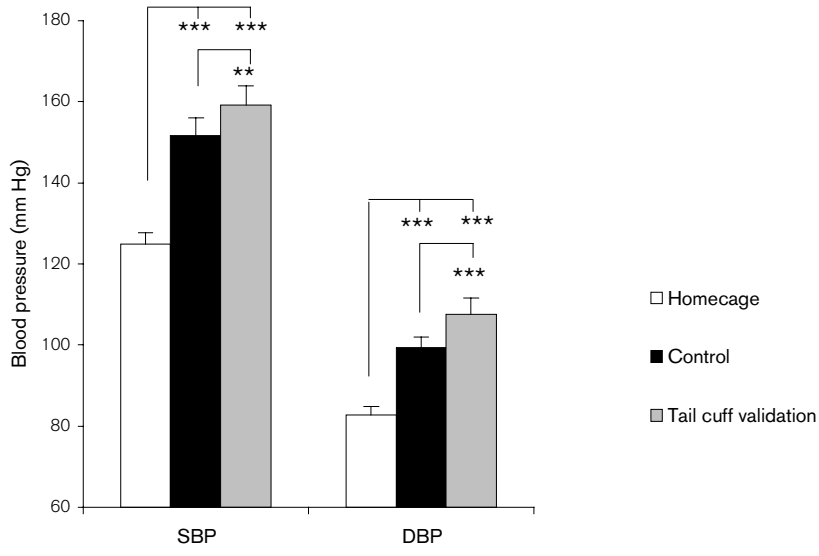

Fig. 9. Mean blood pressure in female W-rats, measured with telemetry, in the home cage (1h), during the 5-day long control period and during the 5-day long tail-cuff validation (paper III). Data is presented as means ±SEM. **p<0.01, ***p<0.001.

Postnatally OT-treated Male rats

Prenatally stressed rats had a significantly higher systolic blood pressure, measured with tail-cuff, than the unstressed rats (fig.10).

Postnatal OT-treatment decreased diastolic blood pressure, measured with tail-cuff (fig. 10).

Comments: Besides the desired effect on the CNS, pharmacological doses of OT may influence the blood pressure also in the periphery by acting on receptors for AVP, for example V1a.

Postnatally OT-treated Female rats

There was no effect of prenatal stress on the blood pressure.

Postnatal OT-treatment decreased systolic blood pressure measured with tail-cuff, in the prenatally stressed rats. Diastolic blood pressure measured with tail-cuff, was decreased by postnatal OT-treatment.

Comments: Postnatal OT-treatment decreased both systolic and diastolic blood pressure in contrast to the male rats. OT–injections (1mg/kg s.c.) to adult rats has been reported to decrease blood pressure more and for a longer period in female rats during oestrus, since the female steroid oestrogen

potentiates the effect of OT (Petersson et al, 1999c). However, there was no difference in the state of oestrus in the different treatment groups.

Postnatally stroked Male rats

Postnatally stroked male rats had a decreased diastolic blood pressure, measured with tail-cuff, compared to the postnatally untreated male rats (Fig. 10).

Comments: The lower diastolic blood pressure also in the controls may be an effect of handling since the mother of the pups often compensate with maternal care after separation. If the separation is brief, the extra maternal care may reduce stress sensitivty in adulthood (Brake et al, 2004; Levine, 2005).

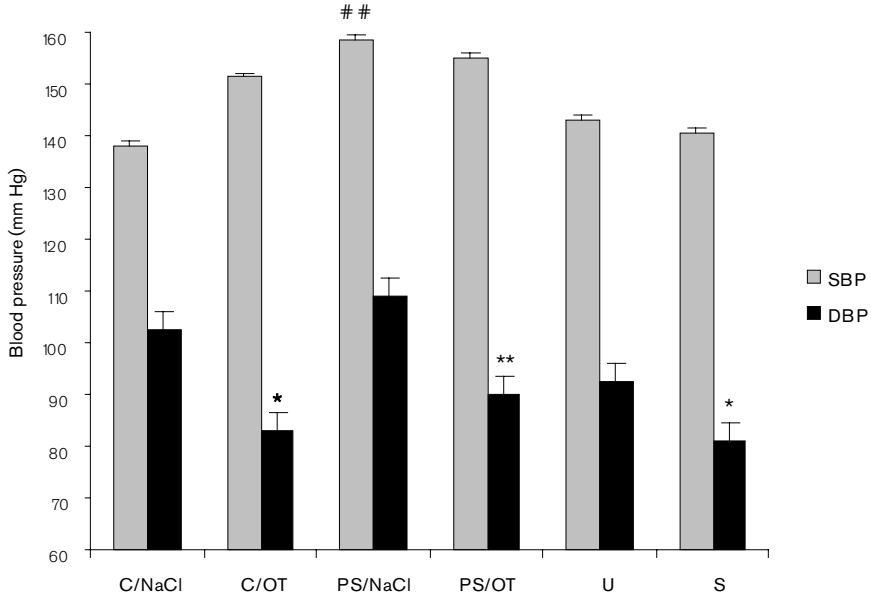

Fig. 10. Blood pressure measured with tail-cuff in adulthood after pre- and postnatal treatments. Systolic blood pressure=SBP, Diastolic blood pressure=DBP, C=control; prenatally unstressed (n=18). PS=Prenatal stress (n=23), U=Untreated (n=5), S=postnatal stroking (n=9), NaCl=postnatal NaCl-treatment, OT= postnatal OT-treatment. a=significant effect of prenatal stress ($F=7.10$; $p=0.011$). b=significant effect of postnatal OT-treatment ($F=14.21$; $p<0.001$). *=$p<0.05$, **=$p<0.01$ compared to control (postnatal NaCl-treated control, prenatally stressed and postnatally NaCl-treated control, and untreated control). ##= $p<0.01$ compared to postnatal NaCl-treated control.

Gastrointestinal hormones (Paper I)

In paper I plasma levels of insulin (Fig. 12) and somatostatin (Fig. 13) decreased after 3 sessions of stroking. After 14 sessions of stroking plasma levels of gastrin (Fig. 11) and insulin (Fig. 12) decreased and plasma levels of somatostatin (Fig. 13) and CCK (Fig. 14) were unchanged.

Comment: The gastrointestinal hormones often decrease or increase simultaneously, therefore also CCK would have been expected to decrease when gastrin and insulin did. If the effects of repeated stroking on CCK were calculated with 3 treatments and 14 treatments together, repeated stroking decreased CCK significantly (p=0.042), probably indicating that the plasma levels of CCK were influenced in the same way as gastrin and insulin.

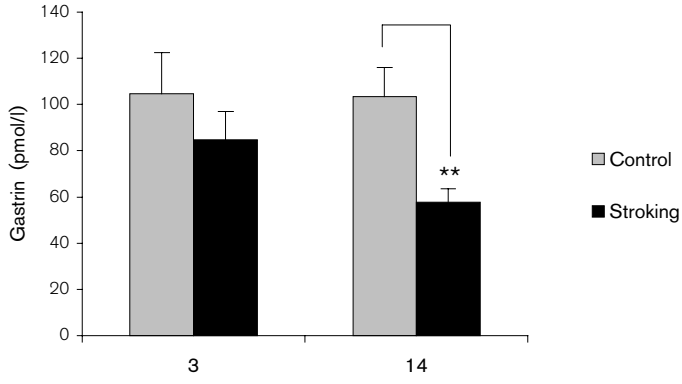

Fig. 11. Plasma levels of gastrin directly after 3 and 14 strokings every second day. Data is presented as means ±SEM. **p<0.001.

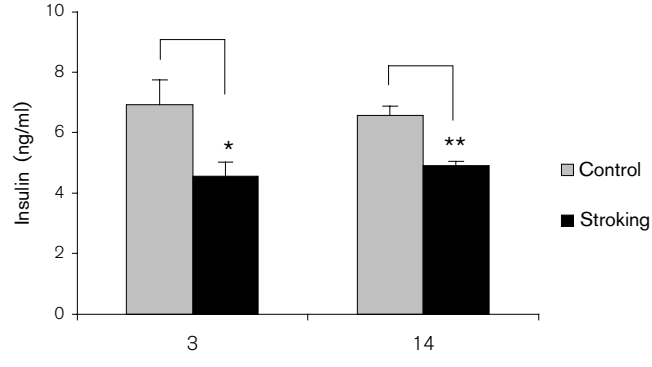

Fig. 12. Plasma levels of insulin directly after 3 and 14 strokings every second day. Data is presented as means ±SEM. *p<0.05 **p<0.001.

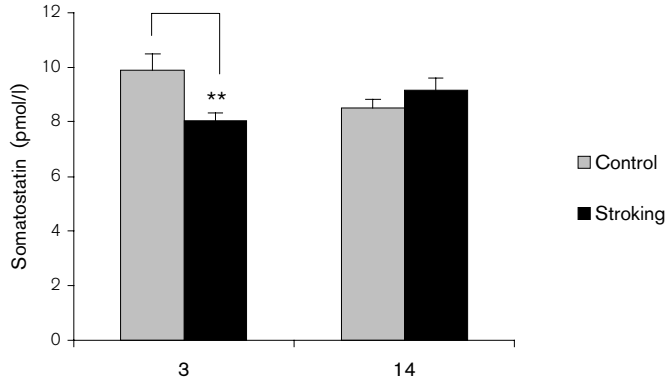

Fig. 13. Plasma levels of somatostatin directly after 3 and 14 strokings every second day. Data is presented as means ±SEM. **p<0.001.

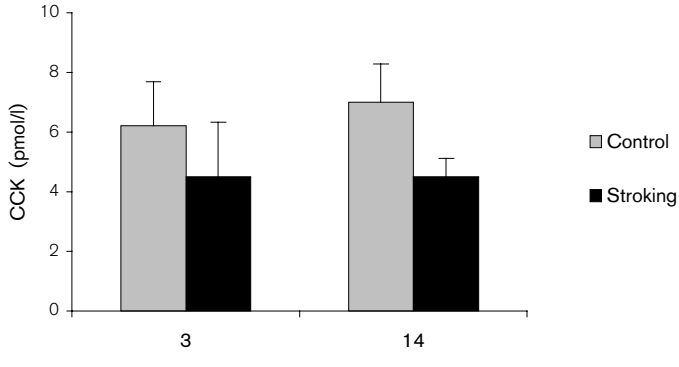

Fig. 14. Plasma levels of CCK directly after 3 and 14 strokings every second day. Data is presented as means ±SEM.

Weight gain (Papers I and IV)

The rats in paper I that received 14 sessions of stroking had a significantly higher weight gain compared to the controls (fig. 15). However, the rats receiving 13 sessions (paper IV) of stroking did not change their weight gain in comparison to controls.

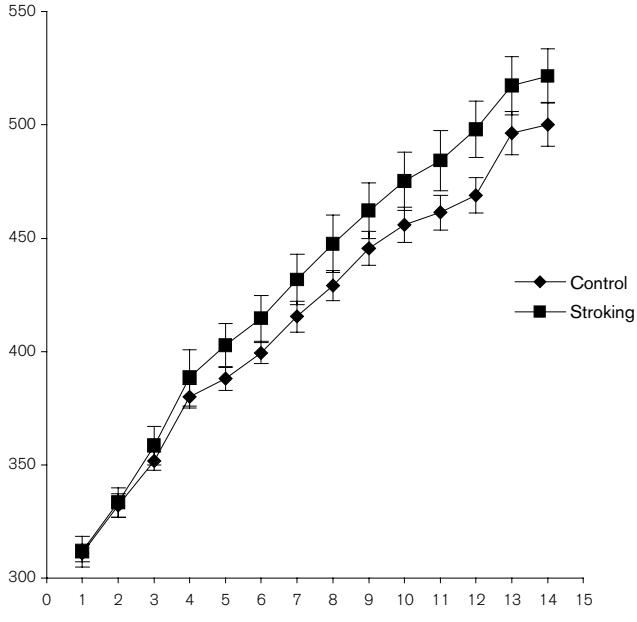

Fig. 15. Weight gain of rats exposed to 14 sessions of stroking (paper I).

Comment: As stroking has been proposed to activate the parasympathetic system, which is more energy saving, the rats would have been expected to weigh more without changing their food intake. However, an increased weight gain could only be seen in one of the experiments.

Social interaction (Paper IV)

The social interaction was not changed due to stroking, but due to the home cage dominance-subordination-relationship. Two dominant individuals interacted longer and more frequently than two subordinate individuals.

Comment: The result of increased social interaction in a pair of dominant rats is in line with previous studies of social interaction. Dominant rats are more often active, they can afford to "act out". In addition, dominant rats more often adopt active coping styles than subordinate rats (Ebner et al, 2005).

The behaviour of each individual in the first social interaction test correlated with the second social interaction test, indicating that the individual behaviour remained constant. The somewhat different patterns of social interaction in the first and second test might be explained by the fact that the rats did not meet the same rat or a rat with the same treatment in the two social interaction tests (fig. 16 & 17).

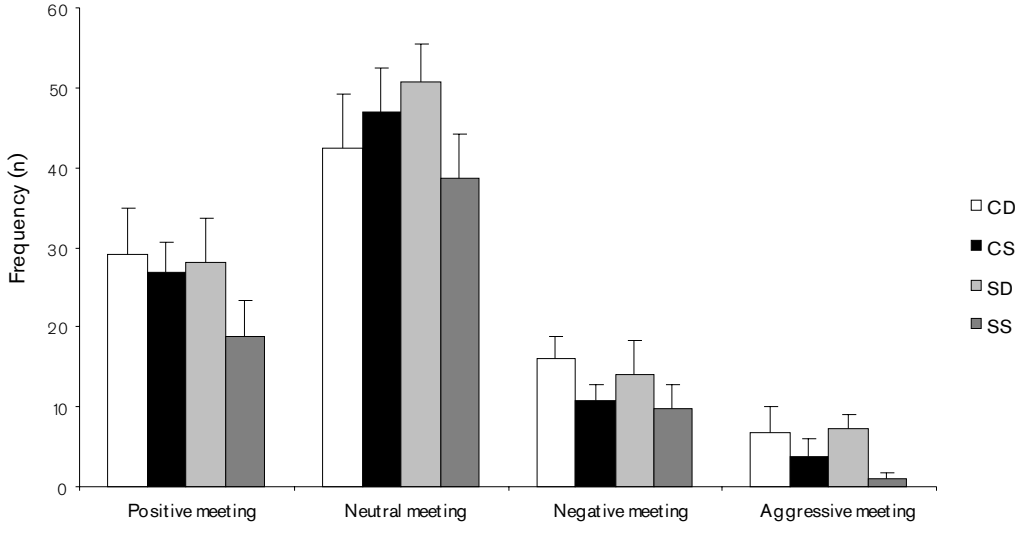

Fig. 16. Frequency of social meeting based on individual, first social interaction test.

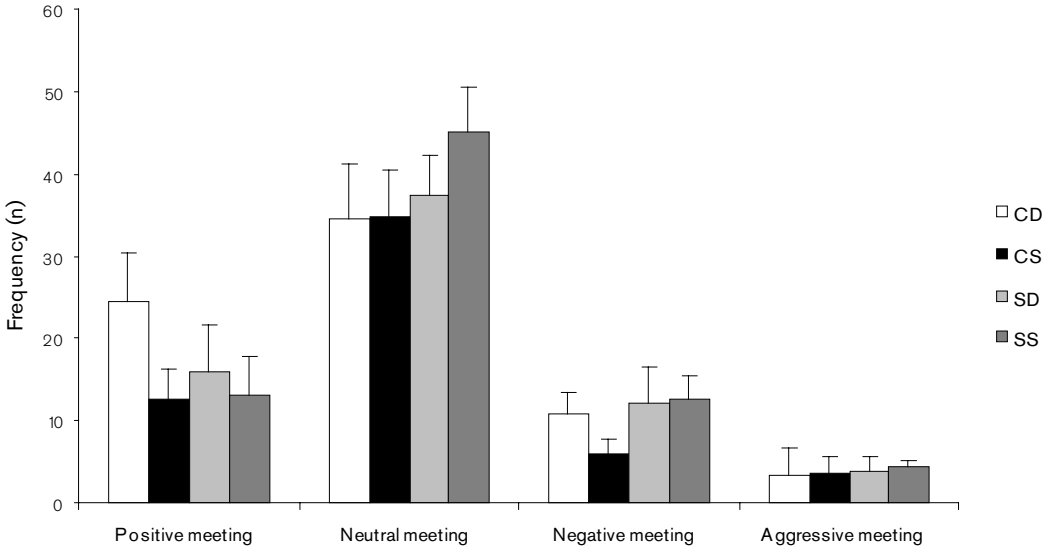

Fig.17. Frequency of social meeting based on individual, second social interaction test.

General discussion

In the title of this thesis the question if rats that receive massage-like stroking experiences distress or antistress was asked.

According to the data reported in the table, in the summary of the thesis, giving an outline over the main results from the different studies most of the results indicate that the rat rather experiences distress than antistress during stroking. Since the adult rats had to be restrained during stroking this response might primarily be due to an activation of the sympathetic nervous system. However, in the pups that received the massage-like stimulation by brushing the abdomen with a soft brush and without any restrain a decrease in diastolic blood pressure was registered in adulthood.

Three different experimenters performed the massage-like stroking in the adult rats in this thesis and they all reported that they could subjectively feel when the rats got relaxed during the stroking session. When analyzing the video tapes this could also be recorded as a motion of the rat body from front to hind legs backwards and downwards and with the abdominal skin making large folds. Occasionally, the rats were "vibrating" and moving the jaws like they were sham-chewing. If the explanation by Sato et al, (1997) is right that stimulation of the ventral abdomen of the rat induces a reflex response. This might have its origin in parasympathetic activation or sympathetic activation or a combined response from both systems. A similar contemporaneous response has for instance been reported in the horse when using a twitch. In the horse heart rate falls and opioids are released simultaneously as plasma levels of cortisol and vasopressin increase (Hydbring et al, 1996).

Every day people all over the world pays money to get different kinds of massage treatments since they experience that massage has a lot of positive effects including pain relief (Hernandez-Reif et al, 2001), stress reduction but also relief of many diseases (see for example Field, 1998). Theories

behind what massage might do include blocking pain signals to the brain (gate control theory; desription in Stanton-Hicks & Salamon, 1997), activating the parasympathetic nervous system, stimulating the release of endorphins and serotonin (Field et al, 2005), preventing fibrosis (Hernandez-Reif, 1999a) or anxiety (Kim et al, 2001).

Are all these people cheated by some commercial interest or is there a true beneficial effect by this ancient treatment?

The results from this thesis will not be able to give the answer neither of this question nor the question put in the title, however, some more aspects concerning the findings will be discussed below.

The general impact of stroking on the plasma levels of glucose and corticosterone as well as the increased and sustained, increase of telemetric blood pressure resembles the effects of repeated restraint (Pitman et al, 1990; Zelena et al, 2003; McDougall et al, 2000 & 2004), so, as mentioned above, the prodominant answer to the title in this thesis is distress. The rats in paper III who served as their own control had higher plasma levels of corticosterone during the tail-cuff validation than during stroking. However, the blood pressure was higher during stroking than during tail-cuff validation. The increased blood pressure during stroking may also be influenced by the reflex that is activated by touching the skin so the sensory afferents are stimulated as propsed by Sato et al, (1997).

The response to the stroking also appears to depend on the individual coping strategy and of personality traits. In paper III high plasma levels of corticosterone correlated with decreased systolic blood pressure. Rats with longer periods of relaxations often had increased plasma levels of corticosterone. Further studies are needed for verification but according to coping strategies (Ebner et al, 2005) a suggestion would be that if rats experience stroking as stressful they either "acts out" or have high plasma levels of corticosterone. Coping strategies are often accompanied with home cage dominance-subordination relationships, where a rat that "acts out" has a coping strategy that is more often connected to dominant ranking whereas a passive coping strategy more often is connected to a subordinate ranking (Ebner et al, 2005). In addition, different personality traits may interfere with the activity of the rat and females are often more active (Ray & Hansen, 2004).

The difficulty of interpreting the results is rendered by the different control groups. In the first paper the rats were held in the same grip as the stroked rats but without being stroked; restraint of the rat. In the second paper the rat pups were handled, the handling may have increased the maternal care and in that way improved the status of the rat in the same way

as the brushing was meant to. In the third paper the rats served as their own control, sitting on the plastic surface of the experimental area. In the forth paper the rats were sitting on the lap of one of the two experimenters and received physical contact but without being stroked. The rats from the unpublished results were sitting on a plastic surface in the experimental area next to a rat that was being stroked. The different control groups mirrors the diverging effects that come from stroking a restrained rat.

The rats in the control group in the first paper are likely to have experienced restraint stress. The plasma levels of insulin and gastrin were in that sense, decreased by stroking during restraint, in comparison to only restraint. If restraint is able to decrease gastrin and insulin the plasma levels of insulin and gastrin would have been the same in both groups. The stress response to restraint may not have been big enough to decrease the plasma levels of gastrin and insulin or the stroking was experienced as more stressful by the rats than just restrained. However, activation of the vagus nerve is involved in the release of gastrointestinal hormones (Uvnäs-Moberg et al, 1992) so the difference of the plasma levels of gastrin and insulin are probably due to stroking and involvement of the vagus nerve rather than of stress.

In comparison with 3 strokings the plasma levels of gastrin decreased after 14 strokings. Uvnäs-Moberg (1994, 1997a) has suggested that the effects of repeated stroking are opposite to effects after a single strokings because the long-term effects may be a response to the effects after a single stroking. The decrease in plasma levels of gastrin and insulin may in that sense be a response to previously increased plasma levels of the same hormones after vagal stimulation by a single stroking.

Uvnäs-Moberg (1994, 1997a) has also suggested that the effects are due to a release of OT in response to somatosensory stimulation. The increased plasma levels of OT would in turn affect gastrin and insulin. Repeated injections with pharmacological doses of OT (1mg/kg s.c.) also decreased gastrointestinal hormones (Petersson et al, 1999c) and the rats in paper I had increased plasma levels of OT (Lund et al, 2002). However, generally the increase of OT in response to stroking appears to be too occasional for OT to be an important regulator of the decrease of gastrin and insulin. In addition, the physiological gain of a decrease is not known and Uvnäs-Moberg (1997a&b) rather call attention to the effects of increased gastrointestinal hormones when presenting the relaxation and growth response.

The results of this thesis could not verify an activation of the relaxation and growth response in connection with stroking, in particular the plasma

levels of OT remained unchanged after stroking. Another candidate other than OT to affect the outcome of massage in humans could be opioids since stroking or massage have been shown to decrease pain and to increase plasma levels of β-endorphin (Kaada & Torsteinbo, 1989), which are worth investigating.

A suggestion for optimal effects of massage/stroking is to activate the CT-fibers and the vagal somatosensory afferents by rhythmic stroking, given to a receiver who is able to receive the stroking and is presumed to lead to a relaxing effect without interfering stress stimuli such as restraint or noise, but in a calm and nice surrounding. According to Uvnäs-Moberg (1997a) each session of stroking is suggested to be short but regularly recurrent rather than occasional and long.

In conclusion, stroking of rats could not be concluded to either be just distress or just antistress, since the grip of the rat during stroking may provoke a stress response. To create an optimal surrounding and gently stroke an unrestrained dog could be a scope for future research.

Conclusions

This thesis stresses that the model for stroking by Kanetake (1982) may provoke responses to restraint stress.

This thesis presents a new method of studying stroking: the individual behavioral treatment response, which measures the behavior of the rat during stroking.

This thesis encourages further studies of postnatal stroking in rat pups, since the model decreases blood pressure in adulthood and corresponds with the natural correlate to stroking the parental care of the pup.

Specific conclusions:

- The individual behavioral treatment response showed that rats react differently to stroking. However, in general the rats appeared to accept stroking as shown from gradually decreased latency time to relaxation and that the mean duration of relaxation was more than half the time of the stroking session.
- The plasma levels of corticosterone and glucose differed in response to repeated stroking. The plasma levels of OT did not change due to repeated stroking.
- The plasma levels of gastrin and insulin decreased and plasma levels of CCK and somatostatin were unchanged after 14 sessions of stroking whereas weight gain increased in paper I and was unchanged in paper IV.
- Repeated stroking of female adult rats increased their blood pressure measured with telemetry.
- The blood pressure measurements with tail-cuff differed from the blood pressure measurements with telemetry. The measurements of systolic blood pressure with tail-cuff were lower than the measurements with telemetry. The measurements of diastolic blood

pressure with tail-cuff were higher than the measurements with telemetry.

- Postnatal stroking decreased diastolic blood pressure measured with tail-cuff in adulthood. Postnatal OT-injection decreased diastolic blood pressure measured with tail-cuff in adulthood. Future studies are suggested to measure the blood pressure with telemetry instead of tail-cuff.
- Stroking did not change social interaction but may interfere with the home cage dominance-subordination relationship.
- The home cage dominance-subordination relationship influenced the social interaction. A meeting with two dominant individuals had higher levels of social interaction than a meeting with two subordinate individuals.
- The grip of the rat during stroking may provoke responses that are similar to responses to repeated restraint.

Future studies

Since the stroking of Kanetake (1982) may provoke stress reactions in the rat and the results are contradictory, further studies of stroking in animals are suggested to be performed in animals that don't need to be restrained during stroking, e.g. pet and companion animals.

Future studies are encouraged since many other studies have reported beneficial health effects of massage or stroking in humans and animals as well as in the interaction between humans and animals. Since massage has been shown to decrease pain, one topic of interest would be the effect of stroking on opioids in the brain.

The willingness of the animal to receive strokings is suggested to be measured with the individual behavioural treatment response. The difference in acceptance to receive stroking may be hard to detect otherwise.

Populärvetenskaplig sammanfattning

Massage är en gammal behandlingsmetod som innebär att hud och muskulatur dras i, knådas, stryks eller pressas. Massage har rapporterats hjälpa mot många symptom, exempelvis ryggont, premenstruella symptom (PMS), brännskador, cystisk fibros, fibromyalgi, migrän, huvudvärk, ångest och återfall till rökning.

För att undersöka mekanismer och effekter av massage har forskare borstat sövda djur på buken eller ryggen med en pensel, eller stimulerat dem med elektro akupunktur eller värme. När man borstade buken på sövda råttor, minskade frisättningen av katecholaminer (noradrenalin, adrenalin) från binjurarna, vilket bör ses som ett tecken på stressreducering, eftersom stress brukar öka katecholaminerna. När man stimulerade buken med värme eller vibration ökade plasma nivåerna för magtarm-hormonerna gastrin och CCK samt oxytocin, ett hormon som frisätts vid amning och förlossning. När sövda råttor ströks på magen, på ett sätt som liknar massage på människa, sänktes blodtrycket direkt i samband med strykningarna. Vissa forskare menar att resultaten tyder på en reflexmässig stressreducering.

För att undersöka effekter av massage på vakna råttor har en massagemodell för råtta utarbetats, vilken innebär att man håller råttan med ena handen i ett grepp, som är fast och bestämt men utan att klämma den, och med den andra handen stryker råttan över buken ca 40 strykningar per minut i ca 5 minuter. Denna metod har visat sig öka blodtrycket, mätt med ”tail-cuff” (blodtrycksmanschett på svansen), direkt efter avslutad behandling. Om man sedan fortsätter att mäta blodtrycket efter avslutad behandling sjunker det i upp till 3 timmar. Strykningarna har visat sig öka smärttröskeln och sänka råttans aktivitet i en beteende-box. Sammantaget kan det tyda på en stressminskning, även om vissa av mätparametrarna ger samma resultat också efter stress.

Massagemodellen innebär att råttan inte kan röra sig fritt, vilket sannolikt innebär ett obehag som stressar råttan. I försök där man placerat råttan i ett rör eller på annat sätt minskat råttans rörelsefrihet, sk fixering, har man sett stresseffekter i form av ökade nivåer av stresshormonet kortikosteron (råttans kortisol) i blodet, förhöjt blodtryck och ett minskat undersökande beteende. Om råttan under korta stunder sätts i fixeringsrör vid upprepade tillfällen kommer dock stress-svaret inte bli lika stort som första gången eftersom råttan vänjer sig vid och accepterar behandlingen, s.k. habituering.

Frågan denna avhandling försöker besvara är således om råttan upplever strykningarna under fixering som avslappnande eller stressande?

För att studera hur råttorna kan ha upplevt massagemodellen videofilmades de under behandlingen. Tiden de slappnade av i buken, hur lång tid det tog till första gången de slappnade av och hur många gånger de rörde sig registrerades. När råttan slappnade av, slappade muskulaturen i buken och huden veckades. Dessutom studerades effekterna av hur upprepade strykningar påverkade nivåer av mag-tarm hormoner samt kortikosteron, oxytocin och blodsocker. På samma gång som råttan blev struken på magen mättes blodtrycket med telemetri. Telemetri innebär att en blodtrycksmätaren är inopererad, vilket gör att råttan kan röra sig fritt under blodtrycksmätningen och att mätningen i sig inte stressar råttan. Utöver detta så studerades även effekterna av upprepade strykningar avseende sociala interaktioner. Två råttor, som var okända för varandra fick mötas 20 min i en arena.

Forskaren Uvnäs-Moberg har utifrån de effekter, som ses i samspelet mellan det nyfödda barnet och dess ammande mamma, föreslagit att massage aktiverar den s.k. lugn- och ro reaktionen. Denna kännetecknas av att aktiviteten i kroppen förändras från en mer vaksam hållning till avslappning. Lugn- och ro reaktionen har föreslagits leda till läkning, ökad upplagring av näring i kroppen, sänkt blodtryck och till avstressande effekter och ökade sociala interaktioner. Oxytocin föreslås reglera lugn och ro reaktionen på en central nivå, bland annat genom att göra en del av det autonoma nervsystemet mer effektivt. Ökad aktivering leder till ökad matsmältning, dåsighet, läkning, sänkt blodtryck och ökat socialt samspel. Lugn- och ro reaktionens effektivitet föreslås öka med antalet aktiveringar av systemet, t ex via upprepad massage.

Eftersom lugn- och ro reaktionen är baserad på samspelet mellan mor och barn och eftersom ökad omvårdnad av råttungar, sannolikt beroende på den fysiska beröringen, visat sig minska effekter av stress hos råttungarna när de är vuxna, undersöktes hur penselborstningar på magen på råttungar påverkade blodtrycket i vuxen ålder. Den första levnadsveckan fick två

råttungar i taget 5 min pensling med en mjuk brödpensel under tiden de låg på rygg bredvid varandra i en försöksledarens hand. I vuxen ålder mättes blodtrycket med ”tail-cuff”. Som kontroller till de penslade ungarna fick några ungar en hög dos oxytocin den första och andra levnadsveckan. Om massage frisätter oxytocin borde effekterna likna varandra.

Avhandlingens resultat visar att hos de råttor, som fick upprepade strykningar under fixering sjönk mag-tarm hormonerna gastrin och insulin jämfört med hos de råttor som motsvarande gånger bara fixerats. Blodsockernivåerna var höga hos båda grupperna, men högst hos de strukna råttorna i försöket. I ett annat massageförsök satt kontrollråttan på ett bord mellan försöksledarens armar och då var blodsockret lägre hos kontrollråttorna jämfört med de råttor, som fått upprepade strykningar under fixering. Även i det försöket var blodsockernivåerna höga hos alla råttor, vilket kan tyda på stress antingen under försöket eller under avlivningen. Stresshormonet kortikosteron i blodet varierade mellan att vara högre och lägre hos de strukna råttorna i olika försök medan oxytocinnivån i blodet var oförändrad i de försök den mättes.

Blodtrycket mätt med ”tail-cuff” hos vuxna råttor, som blivit penslade på magen med en brödpensel som små var delvis sänkt. Hos de råttor som fått en hög dos av oxytocin som små var det var både förhöjt och sänkt.

Blodtrycket, mätt med telemetri, ökade direkt under strykningarna och fortsatte att vara högre jämfört med när samma råttor tidigare under kontrollperioden på 5 dagar bara satt på ett sittunderlägg. Blodtrycksökningen höll i sig alla de 10 dagar som råttorna blev strukna. Under de efterföljande 5 dagarna utvärderades ”tail-cuff” metoden genom att blodtrycket mättes med ”tail-cuff” och telemetri samtidigt samma gång. Även då var blodtrycket på samma nivå som när råttorna blev strukna.

”Tail-cuff” metoden gav inte samma mätvärden som telemetrimetoden. ”Tail-cuff” tenderade dels att beroende på om det var över eller undertryck som mättes, ge olika mätresultat samt missa de enskilda variationerna inom grupperna. ”Tail-cuff” -metoden påverkas, som nämnts innan, av själva mätningen. Telemetriblodtrycket uppmätt under ”tail-cuff” valideringen var högre jämfört med under kontrollperioden. Antagligen beror det på att råttan då måste sitta stilla när blodtrycket mäts. Råttan hålls då i ett bestämt grepp, något som ju kan upplevas som obehagligt.

De sociala interaktioner, mellan råttor som fått upprepade strykningar under fixering skiljde sig inte från kontrollråttorna, som bara fått sitta i knä på försöksledaren. Sociala interaktioner påverkades istället av råttornas rang i hemburen, där ett möte mellan två dominanta råttor visade ett ökat socialt samspel jämfört med ett möte mellan två undergivna råttor.

I jämförelse med den föreslagna lugn- och ro reaktionens effekter av massage, fanns ingen ökning av oxytocin och mag-tarm hormonerna, ingen entydig sänkning av blodtryck och bara enstaka sänkningar av stresshormonet kortikosteron. Att blodsockernivån ibland höjdes skulle kunna vara en effekt dels av stress, som tidigare nämnts, dels av låga insulinnivåer, eftersom insulin sänker blodsockernivåerna.

I jämförelse med stresseffekter av upprepad fixering överensstämde mönstret delvis genom att kortikosteronet och blodsockret var tydligt påverkade samt att blodtrycket var förhöjt.

Råttorna verkar således påverkas mer av fixeringen än av att bli strukna på magen. Att bli struken på magen verkar dock ha en viss effekt på alla, det är den subjektiva bedömningen av de försöksledare som strukit råttor på magen. Dessutom minskade tiden till första avslappning successivt. Råttans individuella sätt att bearbeta experimentsituationen verkade dock påverka resultatet. Höga nivåer av kortikosteron i plasma under kontrollperioden korrelerade till lägre blodtrycksnivåer under behandling samt ”tail-cuff” utvärdering. Metoden med att registrera råttans beteende under behandling visade sig vara ett bra sätt att detektera varje råttas individuella svar på behandlingen.

Det är möjligt att effekterna av att stryka råttorna på buken skulle bli tydligare om de inte behövde fixeras. Man kan också med fördel välja ut råttor som i högre grad accepterar behandlingen för att få veta vilka effekter strykningar på buken har utan inblandning av stress.

Ett annat förslag är att i framtiden byta djurslag för massagestudier och använda djur som inte behöver fixeras i samband med massage, exempelvis sällskapsdjur.

Sammanfattningsvis visar denna avhandling att fixering under massageliknande strykningar troligtvis ger effekter både av fixeringen i sig och av själva strykningarna. Det kunde inte styrkas om strykningarna aktiverar lugn- och ro reaktionen. I framtiden föreslås att man studerar massage på djurmodeller, som inte kräver fixering.

Referenser

Andersson, S. & Lundeberg, T. 1995. Acupuncture – from empiricism to science: functional background to acupuncture effects in pain and disease. *Med Hypotheses*. 45(3), 271-281.

Altszuler N & Hampshire J. 1981. Oxytocin infusion increases plasma insulin and glucagon levels and glucose production and uptake in the normal dog. *Diabetes* 30(2), 112-4.

Araki, T., Ito, K., Kurosawa, M. & Sato A. 1984. Responses of adrenal sympathetic nerve activity and catecholamine secretion to cutaneous stimulation in anaesthetised rats. *Neuroscience* 12, 289-299.

Armario, A., Valles, A., Dal-Zotto, S., Marquez, C. & Belda, X. 2004. A single exposure to severe stressors causes long-term desensitisation of the physiological response to the homotypic stressor. *Stress* 7(3), 157-172.

Barberis, C. & Tribollet, E. 1996. Vasopressin and oxytocin receptors in the central nervous system. *Crit Rev Neurobiol*. 10(1), 119-154.

Barker, D.J. 2004. The developmental origins of adult disease. *J Am Coll Nutr.* 23(6 Suppl), 588S-595S.

Bartels, A. & Zeki, S. 2000. The neural basis of romantic love. *Neuroreport* 11(17), 3829-3834.

Bartels, A. & Zeki, S. 2004. The neural correlates of maternal and romantic love. *Neuroimage* 21(3), 1155-1166.

Bicknell, R., Chapman, C. & Leng, G. 1985. Neurohypophysial opioids and oxytocin secretion: source of inhibitory opioids. *Exp Brain Res*. 60(1), 192-196.

Björkstrand, E., Eriksson, M. & Uvnäs-Moberg, K. 1996a. Evidence of a peripheral and a central effect of oxytocin on pancreatic hormone release in rats. *Neuroendocrinology* 63(4), 377-383.

Björkstrand, E. & Uvnäs-Moberg, K. 1996b. Central oxytocin increases food intake and daily weight gain in rats. *Physiol Behav*. 59(4-5), 947-952.

Brake, W., Zhang, T., Diorio, J., Meaney, M. & Gratton, A. 2004. Influence of early postnatal rearing conditions on mesocorticolimbic dopamine and behavioural responses to psychostimulants and stressors in adult rats. *Eur J Neurosci*. 19(7), 1863-1874.

Bruhn, T., Sutton, S., Plotsky, P. & Vale, W. 1986. Central administration of corticotropin-releasing factor modulates oxytocin secretion in the rat. *Endocrinology* 119(4), 1558-1563.

Buijs, R., De Vries, G.,Van Leeuwen, F. & Swaab, D. 1983.Vasopressin and oxytocin: distribution and putative functions in the brain. *Prog Brain Res.* 60, 115-122.

Callahan, M., da Rocha, M. & Morris, M. 1992. Sinoaortic denervation does not increase cardiovascular/endocrine responses to stress. *Neuroendocrinology* 56(5), 735-744.

Calvo, N. & Volosin, M. 2001. Glucocorticoid and mineralocorticoid receptors are involved in the facilitation of anxiety-like response induced by restraint. *Neuroendocrinology* 73(4), 261-271.

Chadda, R. & Devaud L. 2005. Differential effects of mild repeated restraint stress on behaviors and GABAA receptors in male and female rats. *Pharmacology, Biochemistry and Behavior* (81), 854-863

Chang, H-C., Lim, R., Lu, Y-m., Wang, C-C., Wang, K-j. 1938. A vago-post-pituitary reflex III. Oxytocic component. Chinese Journal of Physiology. 13, 269-284.

Chaouloff, F. & Baudrie, V. 1994. Coupry I Effects of chlorisondamine and restraint on cortical [3H]ketanserin binding, 5-HT2A receptor-mediated head shakes, and behaviours in models of anxiety. *Neuropharmacology* 33(3-4), 449-456.

Cho, K. & Snyder, M. 1996. Use of hand massages with presence to increase relaxation in Korean-American elderly. *J Nurs Academic Soc.* 26. 623-631.

Csiffary, A., Ruttner, Z., Toth, Z. & Palkovits, M. 1992. Oxytocin nerve fibers innervate beta-endorphin neurons in the arcuate nucleus of the rat hypothalamus. *Neuroendocrinology*. 56(3), 429-35.

Dalla,C., Edgecomb,C., Whetstone, A. & Shors,T. 2007. Females do not Express Learned Helplessness like Males do. Neuropsychopharmacology. [Epub ahead of print]

Dellu, F., Mayo, W., Vallee, M., Maccari, S., Piazza, P., Le Moal, M. & Simon, H. 1996. Behavioral reactivity to novelty during youth as a predictive factor of stress-induced corticosterone secretion in the elderly – a life-span study in rats. *Psychoneuroendocrinology* 21(5), 441-453.

Díaz-Berciano, C., de Vicente, F. & Fontecha, E. 2007. Modulating effects in learned helplessness of dyadic dominance-submission relations. *Aggress Behav*. 20. [Epub ahead of print].

Diaz-Cabiale, Z., Olausson, H., Sohlström, A., Agnati, L., Narvaez, J., Uvnäs-Moberg, K. & Fuxe, K. 2004. Long-term modulation by postnatal oxytocin of the alpha 2-adrenoceptor agonist binding sites in central autonomic regions and the role of prenatal stress. *J Neuroendocrinol.* 16(3), 183-190.

Dodd, J & Role, L. 1991. Anatomy of Somatic Sensory System. p 770-772. In: Kandle et al (eds). *Principles of Neural Science*. 3'rd edition. Appleton & Lange. Connecticut.

Douglas, A., Bicknell, R., Leng, G., Russell, J. & Meddle, S. 2002. Beta-endorphin cells in the arcuate nucleus: projections to the supraoptic nucleus and changes in expression during pregnancy and parturition. *J Neuroendocrinol.* 14(10), 768-777.

Ebner, K., Wotjak, C., Landgraf, R. & Engelmann, M. 2005. Neuroendocrine and behavioral response to social confrontation: residents versus intruders, active versus passive coping styles. *Horm Behav.* 47(1),14-21.

Eriksson, M., Lindh, B., Uvnäs-Moberg, K. & Hökfelt, T. 1996a. Distribution and origin of peptide-containing nerve fibres in the rat and human mammary gland. *Neuroscience* 70(1), 227-245.

Eriksson, M., Ceccatelli, S., Uvnäs-Moberg K., Iadarola, M. & Hökfelt, T. 1996b. Expression of Fos-related antigens, oxytocin, dynorphin and galanin in the paraventricular and supraoptic nuclei of lactating rats. *Neuroendocrinology* 63(4), 356-367.

Field, T., Ironson, G., Scadfidi, F. Nawrocki, T., Goncalves, A., Burman, I., Pickens, J., Fox, N., Scanberg, S. & Kuhn, C. 1996. Massage therapy reduces anxiety and enhances EEG pattern of alertness and math computations. *Intern J Neuroscience* 86, 197-205.

Field, T. 1998. Massage therapy effects. *Am Psychol.* 53(12), 1270-1281.

Field, T. 2002. Massage therapy. *Med Clin North Am.* 86(1), 163-171.

Field, T., Hernandez-Reif, M., Diego, M., Schanberg, S. & Kuhn, C. 2005. Cortisol decreases and serotonin and dopamine increase following massage therapy. *Int J Neurosci.* 115(10), 1397-1413.

Fisher, B., Baylis, P. & Frier, B. 1987. Plasma oxytocin, arginine vasopressin and atrial natriuretic peptide responses to insulin-induced hypoglycaemia in man. *Clin Endocrinol (Oxf).* 26(2), 179-185.

Francis, D., Champagne F. & Meaney, M. 2000. Variations in maternal behaviour are associated with differences in oxytocin receptor levels in the rat. *Jour of Neuroend.* 12, 1145-1148.

Francis, D., Young, L., Meaney, M. & Insel, T. 2002. Naturally occurring differences in maternal care are associated with the expression of oxytocin and vasopressin (V1a) receptors: gender differences. *J Neuroendocrinol.* 14(5), 349-353.

Fride, E. & Weinstock, M. 1989. Alterations in behavioral and striatal dopamine asymmetries induced by prenatal stress. *Pharmacol Biochem Behav.* 32(2), 425-430.

Gallo-Payet, N. & Guillon, G. 1998. Regulation of adrenocortical function by vasopressin. *Horm Metab Res.* 30(6-7), 360-367.

Gimpl, G. & Fahrenholz, F. 2001. The oxytocin receptor system: structure, function, and regulation. *Physiol Rev.* 81(2), 629-683.

Gregus, A., Wintink, A.J., Davis, A.C. & Kalynchuk L.E. 2005. Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats. *Behav Brain Res.* 156(1), 105-114.

Hernandez-Reif, M., Dieter, J., Field, T., Swerdlow, B. & Diego, M. 1998. Migraine headaches are reduced by mssage therapy. *Intern J Neuroscience.* 96, 1-11.

Hernandez-Reif, M., Field, T., Krasnegor, J., Martinez, E., Schwartzman, M. & Mavunda, K. 1999a. Children with cystic fibrosis benefit from massage therapy. *Journal of pedriatic psychology* 24(2), 175-181.

Hernandez-Reif, M., Field, T. & Hart, S. 1999b. Smoking cravings are reduced by self-massage. *Preventive medicine* 28, 28-32.

Hernandez-Reif, M., Field, T., Krasnegor, J. & Theakston, H. 2001. Lower back pain is reduced and range of motion increased after massage therapy. *Intern J Neuroscience* 106, 131-145.

Hernandez-Reif, M., Ironson, G., Field, T., Hurley, J., Katz, G., Diego, M., Weiss, S., Fletcher, M., Schanberg, S., Kuhn, C. & Burman, I. 2004. Breast cancer patients have improved immune and neuroendocrine functions following massage therapy. *J Psychosom Res*. 57(1),45-52.

Hesketh, S., Jessop, D.S., Hogg, S. & Harbuz, M. 2005. Differential actions of acute and chronic citalopram on the rodent hypothalamic-pituitary-adrenal axis response to acute restraint stress. *J Endocrinol*. 185(3), 373-382.

Hofbauer, R., Olausson, H. & Bushnell, M. 2006. Thermal and tactile sensory deficits and allodynia in a nerve-injured patient: a multimodal psychophysical and functional magnetic resonance imaging study. *Clin J Pain*. 22(1), 104-108.

Hydbring, E., Nyman, S. & Dahlborn, K.1996. Changes in plasma cortisol, plasma β-endorphin, heart rate, haematocrit and plasma protein concentration in horses during restraint and use of a naso-gastric tube. *Pferdeheilkunde*. 12, 423-427.

Ingram, C., Adams, T., Jiang, Q., Terenzi, M., Lambert, R., Wakerley, J. & Moos, F. 1995. Mortyn Jones Memorial Lecture. Limbic regions mediating central actions of oxytocin on the milk-ejection reflex in the rat. *J Neuroendocrinol*. 7(1), 1-13.

Jard, S., Barberis, C., Audigier, S. & Tribollet, E. 1987. Neurohypophyseal hormone receptor systems in brain and periphery. *Prog Brain Res*. 72, 173-187.

Jones, P. & Robinson, I. 1982. Differential clearance of neurophysin and neurohypophysial peptides from the cerebrospinal fluid in conscious guinea pigs. *Neuroendocrinology* 34(4), 297-302.

Kaada, B. & Torsteinbö, O. 1989. Increase of plasma beta-endorphins in connective tissue massage. *Gen Pharmacol*. 20(4), 487-489.

Kamenetz, H. 1985. History of massage. In: Basmajian, J. (ed). *Manipulation, traction and massage*. 3'rd edition. Williams & Wilkins, Baltimore.

Kanetake C. 1982. A method for continuos drawing of blood from the jugular vein and drug injection in mice and rats. *Jpn J Bacteriol*. 37, 943-947.

Keverne, E. & Curley, J. 2004. Vasopressin, oxytocin and social behaviour. *Curr Opin Neurobiol*. 14(6), 777-783.

Kim, M., Cho, K., Woo, H. & Kim, J. 2001. Effects of hand massage on anxiety in cataract surgery using local anesthesia. *J Cataract Refract Surg*. 27(6), 884-890.

Kimura, A., Ohsawa, H., Sato, A. & Sato, Y. 1995 Somatocardiovascular reflexes in anesthetized rats with the central nervous system intact or acutely spinalized at the cervical level. *Neurosci Res*. 22(3), 297-305.

Kinsley, C., Mann, P. & Bridges, R. 1988. Prenatal stress alters morphine- and stress-induced analgesia in male and female rats. *Pharmacol Biochem Behav.* 30(1), 123-128.

Kofman, O. 2002. The role of prenatal stress in the etiology of developmental behavioural disorders. *Neuroscience and Biobehavioral Reviews.* 26(4), 457-470.

Komisaruk, B. & Sansone, G. 2003. Neural pathways mediating vaginal function: the vagus nerves and spinal cord oxytocin. *Scand J Psychol.* 44(3), 241-250.

Koubovec, D., Geerts, L., Odendaal, H., Stein, D. & Vythilingum, B. 2005. Effects of psychologic stress on fetal development and pregnancy outcome. *Curr Psychiatry Rep.* 7(4), 274-280.

Kurosawa, M., Suzuki, A., Utsugi, K. & Araki, T. 1982. Response of adrenal efferent nerve activity to non-noxious mechanical stimulation of the skin in rats. *Neurosci Lett.* 34, 295-300.

Kurosawa, M., Lundeberg, T., Ågren, G., Lund, I. & Uvnäs-Moberg, K. 1995. Massage-like stroking of the abdomen lowers blood pressure in anaesthetised rats: influence of oxytocin. *J Auton Nerv Syst.* 56(1-2), 26-30.

Levine, S., Chevalier, J. & Korchin, S. 1956. The effects ofearly handling and shock on later avoidance behavior. *J Pers.* 24, 475–493.

Levine, S. 2005. Developmental determinants of sensitivity and resistance to stress. *Psychoneuroendocrinology* 30(10), 939-46.

Liberzon, I., Chalmers, D., Mansour, A., Lopez, J., Watson, S. & Young, E. 1994. Glucocorticoid regulation of hippocampal oxytocin receptor binding. *Brain Res.* 650(2), 317-322.

Liberzon, I. & Young, E. 1997. Effects of stress and glucocorticoids on CNS oxytocin receptor binding. *Psychoneuroendocrinology* 22(6), 411-422.

Liu, D., Diorio, J., Day, J., Francis, D. & Meaney, M. 2000. Maternal care, hippocampal synaptogenesis and cognitive development in rats. Nat Neurosci. 3(8), 799-806.

Lund, I., Lundeberg, T., Kurosawa, M. & Uvnäs-Moberg, K. 1999. Sensory stimulation (massage) reduces blood pressure in unanaesthetised rats. *J Auton Nerv Syst.* 78(1), 30-37.

Lund, I., Yu, L-C., Uvnäs-Moberg, K., Rosén, A., Wang, J., Yu, C., Kurosawa, M., Ågren, G., Lekman, M. & Lundeberg, T. 2002. Repeated massage-like stimulation induces long-term effects on nociception – contribution of oxytocinergic mechanisms. *Eur J Neurosci.* Accepted.

Maccari, S., Piazza, P., Kabbaj, M., Barbazanges, A., Simon, H. & Le Moal, M. 1995. Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. *J Neurosci.* 15(1 Pt 1), 110-116.

Martin, J. & Jessell, T. 1991. Anatomy of Somatic Sensory System. p353-366. In: Kandle et al (eds). *Principles of Neural Science.* 3'rd edition. Appleton & Lange. Connecticut.

McDougall, S., Lawrence, A. & Widdop, R. 2004. Differential cardiovascular responses to stressors in hypertensive and normotensive rats. *Exp Physiol.* 90(1), 141-150.

McDougall, S., Paull, J., Widdop, R. & Lawrence, A. 2000. Restraint stress: differential cardiovascular responses in Wistar-Kyoto and spontaneously hypertensive rats. *Hypertension* 35(1 Pt 1), 126-129.

Meaney, M., Diorio, J., Francis, D., Widdowson, J., LaPlante, P., Caldji, C., Sharma, S., Seckl, J. & Plotsky, P. 1996. Early environmental regulation of forebrain gluccocorticoid receptor gene expression: implication for adrenocortical responses to stress. *Dev Neurosci*. 18, 49-72.

Michelini, L. Oxytocin in the NTS. 2001. A new modulator of cardiovascular control during exercise. *Ann N Y Acad Sci*. 940, 206-220.

Mirsky, I.A. 1962. Effect of oxytocin on plasma free fatty acids of non-diabetic and diabetic dogs. *Proc Soc Exp Biol Med*. 110, 42-4.

Moyer, C., Rounds, J. & Hannum, J. 2004. A meta-analysis of massage therapy research. *Psychol Bull*. 130(1), 3-18.

Narayanan, J., Watts, R., Haddad, N., Labar, D., Li, P. & Filippi, C. 2002. Cerebral activation during vagus nerve stimulation: a functional MR study. *Epilepsia* 43(12), 1509-1514.

Neumann, I. 2002. Involvement of the brain oxytocin system in stress coping: interactions with the hypothalamo-pituitary-adrenal axis. *Prog Brain Res*. 139, 147-162.

Nissen, E., Gustavsson, P., Widström, A. & Uvnäs-Moberg, K. 1998. Oxytocin, prolactin, milk production and their relationship with personality traits in women after vaginal delivery or Cesarean section. *J Psychosom Obstet Gynaecol*. 19(1), 49-58.

Norgren, R. & Smith, G. 1988. Central distribution of subdiaphragmatic vagal branches in the rat. *J Comp Neurol*. 273(2), 207-23.

Norrby, M., Madsen, M., Borg, Alexandersen, C. & Madej, A. Validation of immunoassay kit for determination of plasma oxytocin in different species, with special application for sows during oestrus and service. "In preparation".

Olausson, H., Lamarre, Y., Backlund, H., Morin, C., Wallin, B., Starck, G., Ekholm, S., Strigo, I., Worsley, K., Vallbo, A. & Bushnell, M. 2002. Unmyelinated tactile afferents signal touch and project to insular cortex. *Nat Neurosci*. 5(9), 900-904.

Olausson, H., Uvnäs-Moberg, K. & Sohlström, A. 2003. Postnatal oxytocin alleviates adverse effects in adult rat offspring caused by maternal malnutrition. *Am J Physiol Endocrinol Metab*. 284(3), E475-480.

Olson, B., Hoffman, G., Sved, A., Stricker, E. & Verbalis, J. 1992. Cholecystokinin induces c-fos expression in hypothalamic oxytocinergic neurons projecting to the dorsal vagal complex. *Brain Res*. 569(2), 238-248.

Ottoni EB. 2000. EthoLog 2.2: a tool for the transcription and timing of behavior observation sessions. *Behav Res Methods Instrum Comput*. 32(3), 446-449.

Page, S., Ang, V., Jackson, R., White, A., Nussey, S. & Jenkins, J. 1990. The effect of oxytocin infusion on adenohypophyseal function in man. *Clin Endocrinol (Oxf)*. 32(3), 307-313.

Patchev, V., Schlosser, S., Hassan, A. & Almeida, O. 1993. Oxytocin binding sites in rat limbic and hypothalamic structures: site-specific modulation by adrenal and gonadal steroids. *Neuroscience*. 57(3), 537-43.

Pedersen, C. & Boccia, M. 2002. Oxytocin links mothering received, mothering bestowed and adult stress responses. *Stress* 5(4), 259-267.

Peters, D. 1984. Prenatal stress: effect on development of rat brain adrenergic receptors. *Pharmacol Biochem Behav*. 21(3), 417-422.

Petersson, M., Alster, P., Lundeberg, T. & Uvnäs-Moberg, K. 1996a. Oxytocin increases nociceptive thresholds in a long-term perspective in female and male rats. *Neurosci Lett*. 212, 87-90.

Petersson, M., Alster, P., Lundeberg, T. & Uvnäs-Moberg, K. 1996b. Oxytocin causes a long-term decrease of blood pressure in female and male rats. *Physiol Behav*. 60, 1311-1315.

Petersson, M., Hulting, A-L. & Uvnäs-Moberg, K. 1999a. Oxytocin causes a sustained decrease in plasma levels of corticosterone in rats. *Neurosci Lett*. 264, 41-44.

Petersson, M., Hulting, A-L., Andersson, R. & Uvnäs-Moberg, K. 1999b. Long-term changes in gastrin, cholecystokinin and insulin in response to oxytocin treatment. *Neuroendocrinology* 264, 1-7.

Petersson, M., Lundeberg, T., Uvnäs-Moberg, K. 1999c. Short-term increase and long-term decrease of blood pressure in response to oxytocin-potentiating effect of female steroid hormones. *J Cardiovasc Pharmacol*. 33(1), 102-108.

Petersson, M. 2002. Cardiovascular effects of oxytocin. *Prog Brain Res*. 139, 281-288.

Pieretti, S., d'Amore, A. & Loizzo, A. 1991. Long-term changes induced by developmental handling on pain threshold: Effects of morphine and naloxone. *Behav Neurosci*. 105, 215-218.

Pitman, D., Ottenweller, J. & Natelson, B. 1990. Effect of stressor intensity on habituation and sensitization of glucocorticoid responses in rats. *Behav Neurosci*. 104(1), 28-36.

Rang, H., Dale, M., Ritter, J. 1995. The central nervous system. p492-495. In: Rang et al (eds). *Pharmacology*. 3'rd edition. Churchill Livingstone. Edinburgh.

Ray, J. & Hansen, S. 2004. Temperament in the rat: sex differences and hormonal influences on harm avoidance and novelty seeking. *Behav Neurosci*. 118(3), 488-497.

Raybould, H., Gayton, R. & Dockray, G. 1988. Mechanisms of action of peripherally administered cholecystokinin octapeptide on brain stem neurons in the rat. *J Neurosci*. 8(8), 3018-3024.

Reul, J., Stec, I., Wiegers, G., Labeur, M., Linthorst, A., Arzt, E. & Holsboer, F. 1994. Prenatal immune challenge alters the hypothalamic-pituitary-adrenocortical axis in adult rats. *J Clin Invest*. 93(6), 2600-2607.

Romeo, R., Karatsoreos, I., Jasnow, A. & McEwen, B. 2007. Age- and Stress-Induced Changes in Corticotropin-Releasing Hormone mRNA Expression in the Paraventricular Nucleus of the Hypothalamus. *Neuroendocrinology* 85, 199-206.

Russell, J., Leng, G. & Bicknell, R.1995. Opioid tolerance and dependence in the magnocellular oxytocin system: a physiological mechanism? *Exp Physiol.* 80(3), 307-340.

Sapolsky, R., Krey, L. & McEwen, B. 1986. The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. *Endocr Rev.* 7(3), 284-301.

Sato, A. 1987. Neural mechanisms of somatic sensory regulation of catecholamine secretion from the adrenal gland. *Adv Biophys.* 23, 39-80.

Sato, A., Sato, Y. & Schmidt, R. 1997. The impact of somatosensory input on autonomic functions. p7-69. In: *Physiology Biochemistry and pharmacology* 130. Heidelberg Springer-Verlag. Berlin.

Schmied, C., waiblinger, S., Scharl, T., Leisch, F. & Boivin, X. 2007. Stroking of different body regions by a human: Effects on behaviour and heart rate of dairy cows. *Appl Anim Behav Sci.* Doi: 10.1016/j.applanim.2007.01.013.

Siaud, P., Puech, R., Assenmacher, I. & Alonso, G. 1991. Microinjection of oxytocin into the dorsal vagal complex decreases pancreatic insulin secretion. *Brain. Res.* 546(2), 190-194.

Stanton-Hicks, M. & Salamon, J. 1997. Stimulation of the central and peripheral nervous system for the control of pain. *J Clin Neurophysiol.* 14(1), 46-62.

Stock, S. & Uvnäs-Moberg, K. 1988. Increased plasma levels in response to afferent electrical stimulation of sciatic and vagal nerves and in response to touch and pinch in anaesthetised rats. *Acta Physiol Scand.* 132, 29-34.

Swanson, L. & Sawchenko, P. 1980. Paraventricular nucleus: a site for the integration of neuroendocrine and autonomic mechanisms. *Neuroendocrinology* 31(6), 410-417.

Swanson, L. & Sawchenko, P. 1983. Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. *Annu Rev Neurosci.* 6, 269-324.

Tavares, R. & Corrêa, F. 2006. Role of the medial prefrontal cortex in cardiovascular responses to acute restraint in rats. *Neuroscience* 143(1), 231-240.

Tucker, D. & Johnson, A. 1984. Influence of neonatal handling on blood pressure, locomotor activity, and preweanling heart rate in spontaneously hypertensive and Wistar Kyoto rats. *Dev Psychobiol.* 17(6), 587-600.

Uvnäs-Moberg, K., Lundeberg, T., Bruzelius, G. & Alster, P. 1992. Vagally mediated release of gastrin and cholecystokinin following sensory stimulation. *Acta Physiol Scand.* 146, 349-356.

Uvnäs-Moberg, K. 1994. Role of efferent and afferent vagal nerve activity during reproduction: integrating function of oxytocin on metabolism and behaviour. *Psychoneuroendocrinology* 19(5-7), 687-695.

Uvnäs-Moberg, K., Ahlenius, S., Hillegaart, V. & Alster, P. 1994. High doses of oxytocin cause sedation and low doses cause an anxiolytic-like effect in male rats. *Pharmacol Biochem Behav.* 49(1), 101-106.

Uvnäs-Moberg, K., Alster, P. & Petersson, M. 1996a. Dissociation of oxytocin effects on body weight in two variants of female Sprague-Dawley rats. *Integr. Physiol Behav Sci.* 31, 44-55.

Uvnäs-Moberg, K., Alster, P., Lund, I., Lundeberg, T., Kurosawa, M. & Ahlenius S. 1996. Stroking of the abdomen causes decreased locomotor activity in conscious male rats.. *Physiol Behav.* 60(6), 1409-1411.

Uvnäs-Moberg, K. 1997a. Oxytocin linked antistress effects – the relaxation and growth response. *Acta Physiol Scand Suppl.* 640, 38-42.

Uvnäs-Moberg, K. 1997b. Physiological and endocrine effects of social contact. *Ann N Y Acad Sci.* 807, 146-163.

Uvnäs-Moberg, K. 1998. Oxytocin may mediate the benefits of positive social interaction and emotions. *Psychoneuroendocrinology* 23(8), 819-835

Uvnäs-Moberg, K., Alster, P., Petersson, M., Sohlström, A. & Björkstrand, E. 1998. Postnatal oxytocin injections cause sustained weight gain and increased nociceptive thresholds in male and female rats. *Pediatr Res.* 43(3), 344-348.

Vaccari, C., Lolait, S.J. & Ostrowski, N.L. 1998. Comparative distribution of vasopressin V1b and oxytocin receptor messenger ribonucleic acids in brain. *Endocrinology* 139(12), 5015-5033.

Vallee, M., Maccari, S., Dellu, F., Simon, H., Le Moal, M. & Mayo, W. 1999. Long-term effects of prenatal stress and postnatal handling on age-related glucocorticoid secretion and cognitive performance: a longitudinal study in the rat. *Eur J Neurosci.* 11(8), 2906-2916.

Wakshlak, A. & Weinstock, M. 1989. Neonatal handling reverses behavioural abnormalities induced by prenatal stress. *Physiol Behav.* 48, 289-292.

Weaver, I., Diorio, J., Seckl, J., Szyf, M. & Meaney, M. 2004. Early environmental regulation of hippocampal glucocorticoid receptor gene expression: characterization of intracellular mediators and potential genomic target sites. *Ann N Y Acad Sci.* 1024, 182-212.

Weinstock, M. 2001. Alterations induced by gestational stress in brain morphology and behaviour of the offspring. *Prog Neurobiol.* 65(5), 427-451.

Weinstock, M. 2005. The potential influence of maternal stress hormones on development and mental health of the offspring. *Brain, Behavior, and Immunity* 19(4), 296-308.

Verbalis, J., McCann, M., McHale, C. & Stricker, E. 1986. Oxytocin secretion in response to cholecystokinin and food: differentiation of nausea from satiety. *Science.* 232(4756), 1417-1419.

Wersinger, S.R., Ginns, E.I,, O'Carroll, A.M., Lolait, S.J. & Young, W.S. 3rd. 2002. Vasopressin V1b receptor knockout reduces aggressive behavior in male mice. *Mol Psychiatry* 7(9), 975-984.

West-Eberhard, M. 1998. Evolution in the light of developmental and cell biology, and vice versa. *Proc Natl Acad Sci U S A.* 95(15), 8417-8419.

Wiklund Fernström, K. 2004. Physiological Properties of Unmyelinated Low-Threshold Tactile (CT) Afferents in the Human Hairy Skin. Doctoral thesis. Department of Physiology, Göteborg University, Göteborg.

Wikström, S., Gunnarsson, T. & Nordin, C. 2003. Tactile stimulus and neurohormonal response: a pilot study. *Int J Neurosci.* 113(6), 787-793.

Winslow, J. & Insel, T. 2002. The social deficits of the oxytocin knockout mouse. *Neuropeptides*. 36(2-3), 221-229.

Young, L., Murphy Young, A. & Hammock, E. 2005. Anatomy and neurochemistry of the pair bond. *J Comp Neurol*. 493(1), 51-57.

Zelena, D., Mergl, Z., Foldes, A., Kovacs, K., Toth, Z. & Makara, G. 2003. Role of hypothalamic inputs in maintaining pituitary-adrenal responsiveness in repeated restraint. *Am J Physiol Endocrinol Metab*. 285(5), E1110-1117.

Zhang, T., Parent, C., Weaver, I. & Meaney, M. 2004. Maternal programming of individual differences in defensive responses in the rat. *Ann N Y Acad Sci*. 1032, 85-103.

Zhukov, D. & Vinogradova, K. 2002. Learned helplessness or learned inactivity after inescapable stress? Interpretation depends on coping styles. Integr Physiol Behav Sci. 37(1), 35-43.

Zingg, H. & Laporte, S. 2003. The oxytocin receptor. *Trends Endocrinol Metab*. 14(5), 222-227.

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