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ORIGINAL INVESTIGATION

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Neuropeptide expression in rats exposed to chronic mild stresses

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Abstract To investigate a possible link between some neuropeptides and depression, we analyzed their mRNA levels in brains of rats exposed to chronic mild stresses (CMS; a stress-induced anhedonia model), a commonly used model of depression. Rats exposed for 3 weeks to repeated, unpredictable, mild stressors exhibited an increased self-stimulation threshold, reflecting the development of an anhedonic state, which is regarded as an animal model of major depression. In situ hybridization was employed to monitor mRNA levels of neuropeptide Y (NPY), substance P and galanin in several brain regions. In the CMS rats, NPY mRNA expression levels were significantly decreased in the hippocampal dentate gyrus but increased in the arcuate nucleus. The substance P mRNA levels were increased in the anterodorsal part of the medial amygdaloid nucleus, in the ventromedial and dorsomedial hypothalamic nuclei and the lateral hypothalamic area, whereas galanin mRNA levels were decreased in the latter two regions. These findings suggest a possible involvement of these three peptides in mechanisms underlying depressive disorders and show that similar peptide changes previously demonstrated in genetic rat models also occur in the present stress-induced anhedonia model.

Keywords Depression · Galanin · Mood · Neuropeptide · Neuropeptide Y · Substance P

Abbreviations CMS: Chronic mild stress · FRL: Flinders resistant line · FSL: Flinders sensitive line · NPY: Neuropeptide tyrosine (Y) · REM: Rapid eye movement

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Introduction

Depressive disorders are among the most prevalent psychiatric disorders, with a lifetime incidence of 15–25% according to different reports. They are believed to be induced and modified by a wide variety of genetic and environmental factors. Several behavioral and genetic animal models have been developed with the aim to (1) investigate neurochemical alterations in the brain that may underlie depressive behavior and (2) provide a test system for development of new antidepressant agents. These animal models include the chronic mild stress (CMS) model (Willner et al. 1987), the Flinders sensitive line (FSL) of rats (Overstreet 1993), the "depressed" fawn-hooded rat strain (Mathé et al. 1998) and early life trauma (Kalin and Carnes 1984; Plotsky and Meaney 1993; Ladd et al. 1996).

The CMS model (Willner et al. 1987; Moreau et al. 1998) is a behavioral method for simulating a core symptom of depression, anhedonia (the decreased capacity to experience pleasure). Rats exposed to a series of CMSs, such as overnight illumination, deprivation of food and/or water, cage tilt and change of cage-mate exhibit a decreased intake/preference for sweet solutions, a decreased sensitivity to rewarding electrical brain stimulation in the ventral tegmental area (Moreau et al. 1998) and a variety of other changes consistent with a depressive-like state, such as decrease in sexual and aggressive behaviors, decrease in locomotor activity (Willner et al. 1987;

D'Aquila et al. 1994; Gorka et al. 1996) and disrupted sleep patterns (Moreau et al. 1995; Cheeta et al. 1997). In CMS rats, abnormalities have also been detected in the immune system and in the hypothalamus–pituitary–adrenal (HPA) axis, including adrenal hypertrophy (Muscat and Willner 1992) and corticosterone hypersecretion (Ayensu et al. 1995). Clinically effective antidepressant drugs such as tricyclics and selective serotonin reuptake inhibitors reverse the behavioural phenotype of these CMS-type rodent models of depression (Overstreet 1993; Moreau et al. 1998).

The FSL rats, an example of a genetic animal model of depression, and their controls—Flinders resistant line (FRL) rats—were derived from Sprague–Dawley animals and were selected for their high FSL or low FRL sensitivity to an irreversible cholinesterase inhibitor, di-isopropyl fluorophosphate (Overstreet 1993). The FSL rats display behaviors that are similar to some seen in depressed humans, such as increased immobility after exposure to stressors, reduced body weight, increased REM sleep, disturbance in learning, submissiveness, and decreased response to rewards (Overstreet 1993). The FSL model is well established and expresses alterations in neurotransmitter functions that have been implicated in depressive disorders (Overstreet 1993; Janowsky et al. 1994).

Neuropeptides represent a class of messenger molecules that have a wide distribution both in the central and peripheral nervous system (Hökfelt et al. 2000). There is evidence that some of the neuroactive peptides, in particular neuropeptide Y (NPY), play a role in the pathophysiology of depression and in the effects of antidepressant drugs. For example, clinical studies have demonstrated changes in NPY-like immunoreactivity (LI) in the cerebrospinal fluid (CSF) and plasma of depressed subjects (Widerlöv et al. 1988; Hashimoto et al. 1996; Nilsson et al. 1996). In bipolar depression, NPY mRNA levels are decreased in the prefrontal cortex (Caberlotto and Hurd 1999). Moreover, chronic antidepressant treatment has been shown to affect peptidergic systems. Thus, different antidepressant drug treatments or electroconvulsive therapy increase brain NPY-LI (Stenfors et al. 1989; Mathé et al. 1990, 1997; Wahlestedt et al. 1990; Mathé 1999; Husum et al. 2000, 2001) and NPY mRNA levels (Weiner et al. 1992; Zachrisson et al. 1995a,b) in discrete brain regions. Marked changes in neuropeptide levels have also been seen in the above-mentioned genetic animal models. Indeed, levels of NPY, neurokinin A and neurotensin were changed in fawn-hooded rats when compared with Wistar control rats, and in FSL compared with FRL rats (Mathé et al. 1998; Jiménez Vasquez et al. 2000a,b). In addition, expression of NPY and NPY Y1 receptors was altered in FSL rats (Caberlotto et al. 1998). Recently, Bellido et al. (2002) have shown an increased density of galanin binding sites in the dorsal raphe of FSL rats. Finally, it was recently reported that a neurokinin 1 (NK1) receptor antagonist has clinical efficacy in the treatment of major depression, in agreement with evidence for increased release of substance P in amygdala in a guinea-pig depression model (Kramer et al. 1998).

The CMS model of depression has been studied much less with regard to altered neuropeptide expression. Therefore, in the present in situ hybridization study on brains from chronically stressed anhedonic animals and controls, we analyzed peptide expression in brain areas related to emotion, stress and defense reactions—that is the amygdala, hippocampus and hypothalamus—with focus on NPY, substance P and galanin transcripts.

Materials and methods

Behavioral experiments

All procedures described here are in compliance with ethical principles and guidelines for scientific experiments on animals (Ethical Committee Swiss Academies of Sciences and Medical Sciences, 1995, and Karolinska Institutet's and Stockholms norra djurförsöksetiska nämnd).

The CMS model

Male albino Wistar rats (Ibm: RoRo; Biological Research Institute, Fullinsdorf, Switzerland) weighing approximately 350 g at the start of the experiment were used. Rats were maintained individually in macrolon type-III containers under standard laboratory conditions. Animals (14 stressed and 14 controls) were housed in the same quarters, except when otherwise indicated. Animals were anesthetized with an intraperitoneal (i.p.) injection of 90 mg/kg ketamine hydrochloride and 10 mg/kg Xylazin 2% in solution in physiological saline and administered atropine sulfate (0.125 mg i.p.) to prevent excessive bronchopulmonary secretion. Properly insulated stainless-steel bipolar electrodes (MS 303/1, Plastic Products Co., Roanoke, VA, USA) were stereotaxically implanted unilaterally in the ventral tegmental area of the midbrain (2 mm anterior lambda, 0.3 mm lateral from the midline suture, and 8.5 mm ventral from the skull surface). The electrode assembly was secured to the skull by three stainless-steel screws and autopolymerizing resin. A histophilic and antiseptic plastic film (Nobecutan) was sprayed to close the wound. Animals were maintained post-operatively in a warm environment until fully awake and were given post-operative analgesic treatment (0.05 mg/kg buprenorphine). They were allowed at least 5 days recovery before starting training.

Ventral tegmentum self-stimulation procedure

The test chambers consisted of Plexiglas boxes (30×25×25 cm) with a hole (2.5 cm in diameter) located in a side-wall 5 cm above the floor. The rat could interrupt a convergent light beam with a nose poke to trigger

electrical brain stimulation. Bipolar stimulation (0.5-s trains of monophasic square pulses of 0.1 ms duration) was delivered from a constant-current stimulator controlled by a computer which also recorded the number of nose-poke responses per test session. In the training phase, each rat was placed into a test chamber and trained to make a nose-poke response for rewarding intracranial electrical stimulation. The frequency was kept at 70 Hz, and the current intensity was made available individually for each rat so that the highest response rate without observable motor impairment (range 115–400 μA) was maintained. Training continued until stable responding was achieved. Subsequently, the threshold frequency for ventral tegmentum self-stimulation (VTSS) behavior was determined with stimulation intensity maintained constant for each rat. Briefly, the frequency of stimulation varied between 20 Hz and 100 Hz in a stepwise descending and ascending fashion, in steps of 10 Hz, with 2-min testing periods at each level. The VTSS threshold was therefore defined as the mean of ascending and descending frequencies eliciting 15 nose pokes per minute. In the absence of brain stimulation, response rate was usually lower than ten nose pokes per minute, and never exceeded 15. This procedure has been described in greater detail elsewhere (Moreau et al. 1992).

Stress procedure (CMS model)

The CMS procedure was applied for 25 consecutive days. The stress regimen consisted each week of a variety of unpredictable, mild stressors such as repeated periods of confinement to small cages (21×10×9 cm), one period of continuous overnight illumination, one overnight period of food and water deprivation immediately followed by 2 h of access to restricted food, one overnight period of water deprivation immediately followed by 1 h exposure to an empty bottle and one overnight period of group housing in a soiled cage. The various stressors were randomized during each of the weeks, and the procedure was repeated from 1 week to the other. Animals were confined to a smaller cage once-a-day for 1 h. Animals were also maintained on a reversed light/dark cycle from Friday evening to Monday morning (for more details on the procedure used here, see Moreau et al. 1994).

Test procedure

The experiment was started when the self-stimulation threshold of individual rats varied by less than 10% over three consecutive daily test sessions. For a total of 25 days, in two sets of experiments, two groups of four and five rats, respectively, were subjected to the CMS regimen, whereas the control groups (n=5 and 5 rats, respectively) were maintained under standard laboratory conditions. In all groups, the VTSS threshold was determined twice weekly, and the threshold value for each test day was compared with average pretest baseline threshold values. All VTSS measurements were done in the morning prior to any acute stressors. Results are expressed as percentage change in VTSS threshold (anhedonia index). After completion of testing, rats were decapitated. Brains were quickly removed from the skull and frozen on dry ice. Brain samples were then transferred into a −20°C freezer until assayed. Coronal sections (14 μm thick) were cut in a cryostat (Microm, Heidelberg, Germany) and thawed onto "Probe On" slides (Fisher Scientific).

In situ hybridization

Oligonucleotide probes were synthesized by Scandinavian Gene Synthesis AB. The oligonucleotide sequences were complementary to the nucleotides 1671–1714 of NPY mRNA (Larhammar et al. 1987), 145–192 of substance P mRNA (Krause et al. 1987) and 70–189 of galanin mRNA (Vrontakis et al. 1987). The probes were labeled at the 3′ end, as previously described (Dagerlind et al. 1992; also for the rest of the procedure), using terminal deoxynucleotidyl transferase (Amersham) in a cobalt-containing buffer with [^{35}S] dATP (New England Nuclear) and purified with Quiaquick Nucleotide removal Kit (QIAGEN, Hilden, Germany). Tissue sections were dried and incubated for 16–18 h at 42°C in humidified boxes with 10^6 cpm labeled probe per 100 μl of hybridization cocktail containing 50% deionized formamid (J. T. Baker Chemicals BW), 4× standard saline citrate (SSC; 1× SSC = 0.15 M NaCl and 0.01 M sodium citrate), 1× Denhardt's solution [0.02% each of bovine serum albumin, Ficoll (Pharmacia) and polyvinyl pyrrolidone], 0.2 M $NaPO_4$ (pH 7.0), 1% N-lauroylsarcosine, 10% dextran sulphate (Pharmacia), 500 mg/ml denatured salmon testis DNA (Sigma) and 200 mM dithiothreitol (LKB). An excess (100×) of the appropriate cold probe was added to the incubation cocktail of control sections to determine specificity of labeling. After hybridization, the sections were rinsed in 1× SSC, four times for 15 min at 55°C, followed by 30 min at room temperature, immersed in distilled water and air dried. In the first set of experiments (n=4 stressed and 5 control rats), the slides were dipped in NTB2 nuclear track emulsion (Kodak, Rochester, NY, USA) diluted 1:1 with distilled water, exposed in the dark at −20°C for 10–28 days depending on the probe, developed in D19 (Kodak) and analyzed in a Nikon Microphot-FX microscope. Photomicrographs were taken with Kodak T-max 100 film.

In the second set of experiments (n=5 stressed and 5 control rats), locus coeruleus and dorsal raphe were analyzed, and air-dried sections and autoradiographic [^{14}C] Micro-Scale standards (Amersham) were exposed in the same X-ray film cassette to BioMax MR X-ray film (Kodak) for 3–8 days at −20°C. Films were developed in LX 24 and fixed in AL 4 (Kodak).

Quantification and data analysis

The behavioral results were analyzed by a weighted analysis of variance with the fixed effects day and treatment nested in day and with the random effect animal nested in treatment. This analysis was supplemented, where appropriate, by comparisons on individual days carried out with Dunnett's *t* test, using a closed testing procedure as an adjustment for multiple comparisons.

In the first experiment, the number of grains overlying labeled cells in slides from in situ hybridization experiments was counted on cresyl violet-stained sections in a Nikon Microphot microscope. The hippocampus, amygdala and hypothalamus from level Bregma −2.0 mm to Bregma −1.5 mm were examined. Every fifth section of a series was selected (in total 7 sections from each brain). For each neurochemically defined cell population, all neurons labeled with a certain neuropeptide mRNA marker were counted. Cells were considered labeled when the number of silver grains overlying the cytoplasm exceeded five times the background levels. The results were statistically evaluated using the unpaired *t* test.

In the second experiment using X-ray film, autoradiographs and autoradiographic standards were scanned with resolution 800 dpi using UMAX Power Look 3000 Scanner (Umax Systems GmbH, Willich, Germany). The intensity of mRNA labeling was measured from the scanned images using Scion Image 4.0 (Gaithersburg, MD 21701, USA), based on NIH Image by Wayne Rasband, of National Institute of Health, USA. Each image was calibrated to nCi/g values of the autoradiographic standards using a third-degree polynomial function, and signals were measured within the selections of the locus coeruleus or dorsal raphe nuclei. Several sections that were not in focus or had artifacts were excluded from the analysis. Background level was measured from every slide from an outside section area and was ultimately subtracted. The results were statistically evaluated using the two-tailed *t* test.

Results

Behavioral evaluation of stress-induced anhedonia

The effects of the chronic unpredictable mild stress regimen on VTSS behavior for the first groups of rats (n=4+5) is shown in Fig. 1. Before stress, VTSS frequency threshold values (mean±SEM) for control and stressed groups were 43.9±1.0 Hz and 46.7±0.3 Hz, respectively. Comparisons of data obtained in stressed and non-stressed animals revealed a significant stress effect ($F_{7\,56}$=42.36, P<0.0001) from day 11 to day 25 of the experiment. In stressed animals, VTSS threshold progressively increased (i.e., reward sensitivity progressively decreased) over a period of about 2 weeks of mild stress and then remained consistently high through the rest of the stress period. At day 25 of the stress regimen, VTSS frequency threshold values for control and stressed animals were 44.2±0.5 Hz and 63.7±1.3 Hz, respectively. In non-stressed animals, VTSS threshold did not appreciably vary throughout the entire experiment ($F_{7\,56}$=0.37, P>0.05). Individual anhedonia scores were assigned to each rat.

Neuropeptide mRNA expression

Quantitative evaluation of neuropeptide levels in brain structures from anhedonia rats compared with control animals of the first experiment is shown in Fig 2. Some of the changes in neuropeptide mRNA levels observed via in situ hybridization are illustrated in Figs. 3, 4. The pattern of NPY mRNA expression in control rats was consistent with previous descriptions of the NPY mRNA distribution in the rat brain with scattered, strong hybridization signals found mainly in the cerebral cortex, hippocampus, striatum, and hypothalamus (Gehlert et al. 1987; Morris and Gibbins 1989). A highly significant difference was found between CMS and control animals in the arcuate nucleus (Arc), with higher NPY mRNA levels in CMS rats than in control animals (+38%; P<0.01; Fig. 3a,b). Significant differences were also observed in the dentate gyrus of the hippocampal formation, but here with lower levels of NPY mRNA in CMS (−22%; P<0.05; Fig. 3c) than in control (Fig. 3d) rats. In the second set of experiments, a trend toward an increase in NPY mRNA levels was found in the locus coeruleus of CMS when compared with control rats, but statistical significance was not reached (mean nCi/g ±SE; 169.6±15 versus 146.9±5, respectively; P=0.1).

The expression pattern of substance P mRNA in the control rat brains was in line with previous in situ hybridization results (Warden and Young 1988; Harlan et al. 1989) with high levels in the medial amygdaloid nucleus (MeAD) and several hypothalamic nuclei, such as the ventromedial (VMH), dorsomedial (DMH) and lateral

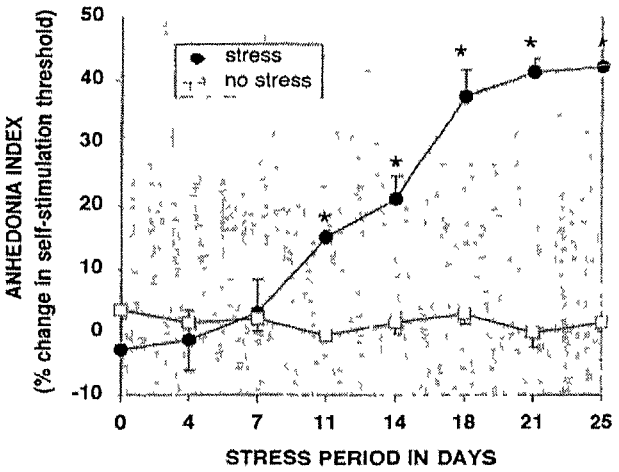

Fig. 1 Stress-induced anhedonia in chronic mild stress (CMS) rats. Variations in self-stimulation threshold in stressed (*black squares*) and non-stressed (*open circles*) animals are shown as a function of stress exposure time (days). *Asterisk* Statistically significant difference (Dunnett's *t* test, P<0.05) with baseline value measured before the stress period (day 0)

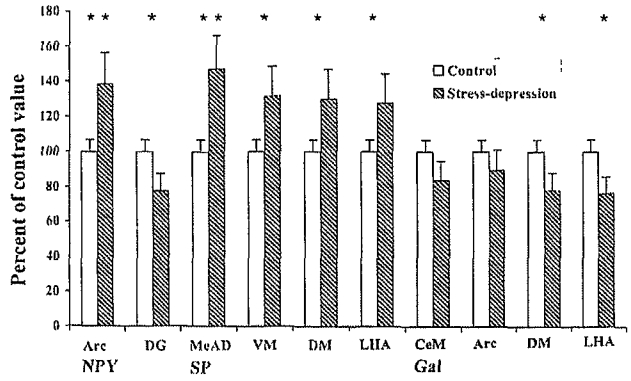

Fig. 2 Neuropeptide mRNA levels (as percentage of control values) in a variety of brain structures from stressed anhedonic (*hatched bars*) and control (*open bars*) animals. *Arc* arcuate nucleus, *DG* dentate gyrus, *MeAD* medial amygdaloid nucleus, *VMH* ventromedial hypothalamus, *DMH* dorsomedial hypothalamus, *LHA* lateral hypothalamic area, *CeM* central nucleus of amygdaloid complex, *NPY* neuropeptide Y, *SP* substance P, *Gal* galanin

hypothalamic area (LHA). Statistically significant increases in substance P mRNA levels were observed in the MeAD of CMS when compared with control rats (+48%; $P<0.01$; Fig. 4a,b). Increased substance P mRNA levels were also observed in the VMH (+32%; $P<0.05$), DMH (+30%; $P<0.05$) and LHA (+28%; $P<0.05$).

Galanin mRNA was expressed in several areas of the rat brain in agreement with published in situ hybridization findings (Jacobowitz and Skofitsch 1991). The most intense signals were observed in the bed nucleus of stria terminalis, the central nucleus of amygdaloid complex (CeM), DMH, Arc and LHA. Compared with controls, galanin mRNA levels were decreased in the LHA (−24%; $P<0.05$) and DMH (−22%; $P<0.05$) (Fig. 4c,d). No significant changes were observed in the Arc and CeN. In the second set of experiments, no significant differences were found in galanin mRNA levels (mean nCi/g±SE) in the locus coeruleus (298.7±22 versus 267±18; $P=0.15$) or in the dorsal raphe nucleus (41.6±1.8 versus 43±5.6; $P=0.3$) between stressed and normal rats, respectively. None of the above-mentioned peptide mRNA signals was

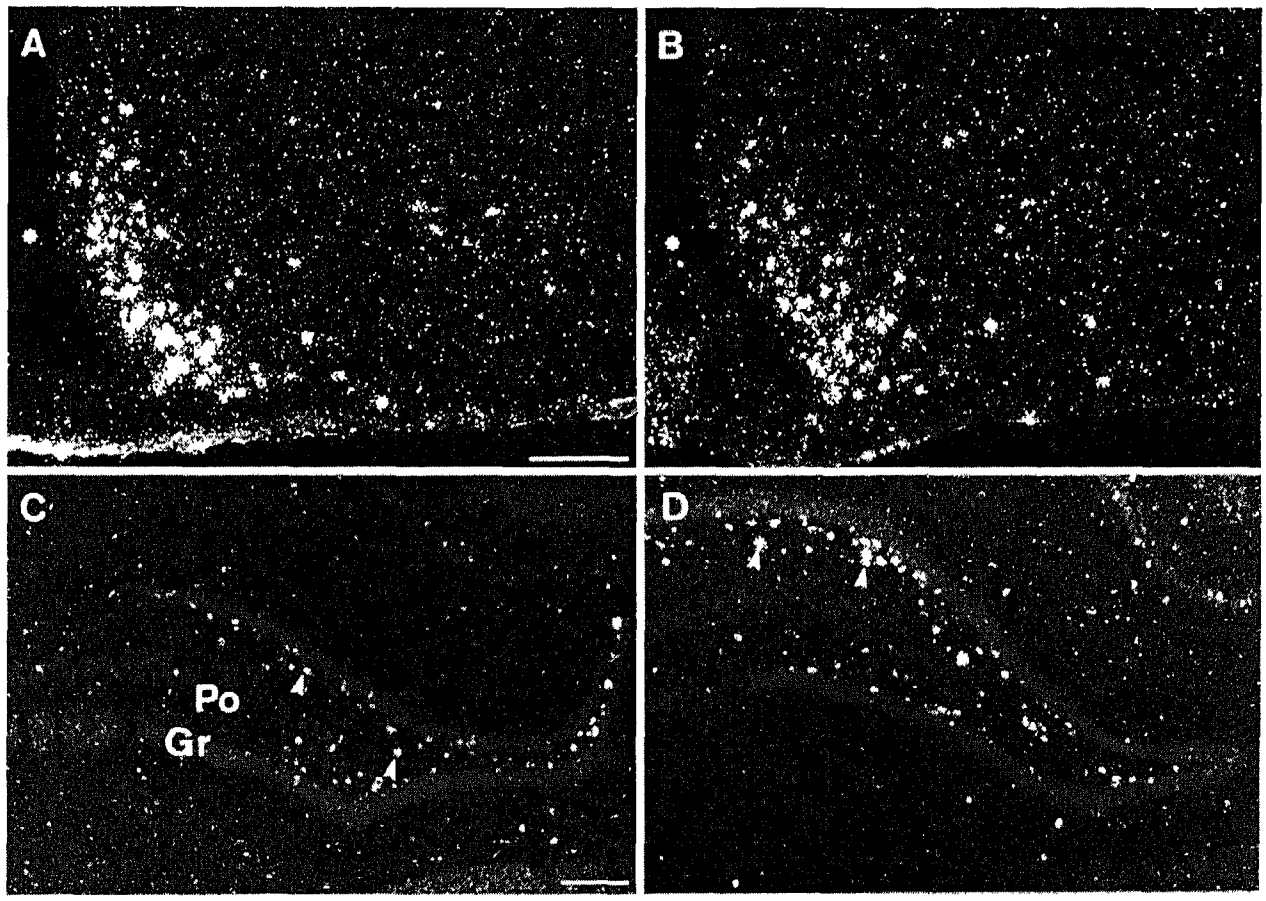

Fig. 3 Darkfield micrographs of the arcuate nucleus (**a,b**) and dentate gyrus (**c,d**) of chronic mild stress (CMS; **a,c**) and control (**b, d**) rats after hybridization with probe complementary to neuropeptide tyrosine (NPY) mRNA. In the CSM rats, there is an increase in NPY mRNA in the arcuate nucleus (**a**) when compared with the control rats (**b**); whereas the neurons in the polymorph layer of the dentate gyrus are more weakly labeled in the CSM (**c**) than in the control (**d**) rats. *Gr* Granular cell layer of the dentate gyrus, *Asterisks* Third ventricle, *Arrowheads* Cell bodies. *Scale bars* 300 μm (**a=b; c=d**)

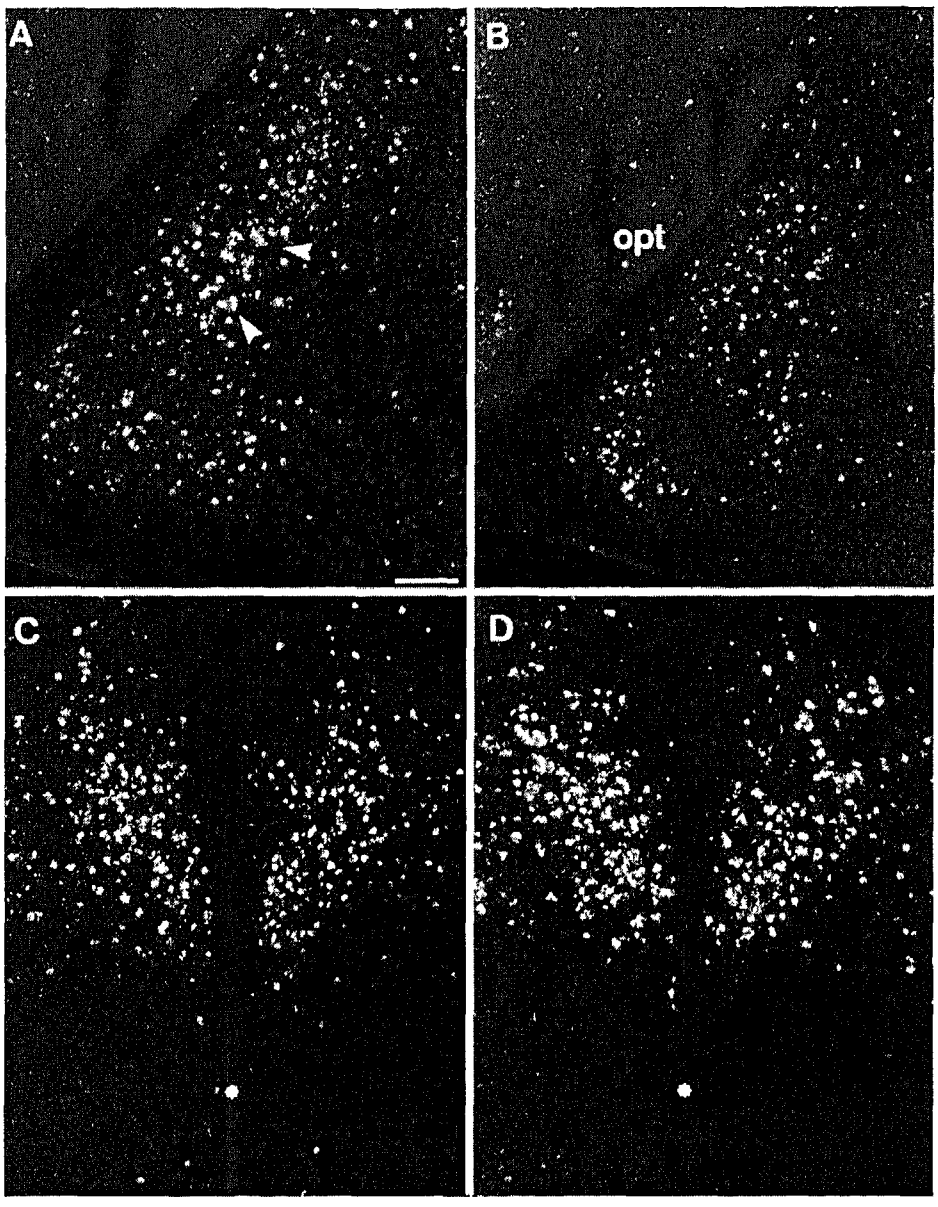

Fig. 4 Darkfield micrographs of the central amygdaloid nucleus (**a**,**b**) and the dorsal medial hypothalamic nucleus (**c**,**d**) of chronic mild stress (CMS; **a**,**c**) and control (**b**,**d**) rats after hybridization with probes complementary to preprotachykinin mRNA (**a**,**b**) and preprogalanin mRNA (**c**,**d**), respectively. Preprotachykinin mRNA signal is increased in the CMS (**a**) when compared with control (**b**) rats; whereas the reversed situation is found for galanin mRNA—that is decreased levels in the CMS (**c**) relative to control (**d**). *rat. opt* optic tract, *Asterisks* Third ventricle. *Scale bar* 300 μm (**a**=**b**; **c**=**d**)

observed after addition of an excess of cold probe to the hybridization cocktail.

Discussion

The present study provides evidence that in the rat CMS depression model, transcriptional changes occur in the expression of some neuropeptides in discrete brain regions. We have monitored these changes in mRNA levels by means of in situ hybridization. The transcriptional changes include increases in substance P mRNA levels in amygdaloid and hypothalamic nuclei, an increase in NPY transcript in the Arc and a decrease in the dentate gyrus, and decreased galanin mRNA levels in some hypothalamic nuclei. In agreement with these findings in our CMS paradigm, several types of stress have shown distinct and widespread regulatory effects on many neuropeptides, including corticotropin releasing hormone and somatostatin (Palkovits 2000), and such changes may represent neurochemical mechanisms related to depression.

It could be argued that the gene-expression changes observed here are not the result of CMS exposure only, but would rather reflect the interaction of CMS exposure with the self-stimulation measurements. However, this seems unlikely as such changes were not observed in a group of control unstressed animals exposed to VTSS, therefore ruling out the fact that VTSS alone would have a similar impact on neuropeptide expression as CMS. In addition, similar changes in neuropeptide expression were obtained in genetic rat models of depression, again suggesting that these changes most likely are triggered by stress.

In the present study, we have examined the mRNA levels for SP, NPY and galanin but have not measured the tissue levels of the actual gene products or the releasable pool of these neuropeptides. In general, a good correlation was found between tissue levels of neuropeptides and their mRNA levels, reflecting the fact that tissue levels of neuropeptides are regulated by their release which is indirectly coupled to their biosynthesis and thus to gene transcription and the mRNA level.

Our results on NPY mRNA expression are similar to those obtained in a previous study on FSL/FRL rats by Caberlotto et al. (1998), where an increase of the NPY mRNA transcript levels in Arc and a decrease in hippocampus (as well as changes in other regions not studied by us) were found. NPY peptide levels were also lower in the hippocampus of fawn-hooded "depressed" Wistar rats (Mathé et al. 1998), FSL rats (Jiménez Vasquez et al. 2000a,b) and maternally separated rats (Husum and Mathé 2002) when compared with controls. The decreased expression of NPY in dentate gyrus supports a role for hippocampal NPY in depressive disorders. In fact, studies have shown that administration of antidepressant drugs (Stenfors et al. 1989; Mathé et al. 1990; Weiner et al. 1992; Zachrisson et al. 1995a,b; Husum et al. 2000, 2001) and electroconvulsive treatment (Mikkelsen et al. 1994; Mathé et al. 1997; Husum et al. 2000) increase NPY-LI and NPY mRNA in this region. Since limbic structures are regarded as important anatomic substrates of emotion and mood, and considering the presumed role of NPY in emotional behavior, in particular anxiety and depression (Wahlestedt et al. 1993; Heilig and Widerlöv 1995), low NPY mRNA levels in a hippocampal region may be related to the pathophysiology of stress-induced depression and/or a genetically predisposed state of depression. Furthermore, Redrobe et al. (2002) have shown an antidepressant effect in mouse after intracerebroventricular injection of NPY, mediated via NPY Y1 receptors, using the forced swimming (Porsolt) test. Similarly, an NPY Y1 agonist increased swimming time in the Porsolt test when given intracerebroventricularly to FSL ("depressed") rats but had no effect in FRL rats (Gruber et al. 2004). Finally, there is a link between hippocampal NPY signaling and memory processing (Flood et al. 1987; Morley and Flood 1990), and low NPY levels may thus also relate to the memory deficit reported in depressed humans.

The increased NPY mRNA levels in Arc of CMS rats not only confirms the study on FSL rats by Caberlotto et al. (1998) but also a stress study by Makino et al. (2000). The mechanism of this increased NPY transcription may be through stress activation of the HPA axis, resulting in elevated glucocorticoid and decreased insulin serum levels (McKibbin et al. 1992; Tempel and Leibowitz 1994; Wang et al. 1998), and the subsequent stress-induced reduction of insulin levels that is known to cause a marked increase in NPY gene expression (Makino et al. 2000). The elevation of NPY mRNA in Arc may seem puzzling in view of NPY's known stimulatory effect on feeding (Stanley et al. 1986; Brady et al. 1990), preferentially affecting carbohydrate intake (Stanley and Leibowitz 1985; Morley 1987), whereas "depression" in rats leads to a decrease in sucrose intake and feeding behavior in general (Willner et al. 1987). It may be speculated that the stimulation of NPY-dependent food intake in the animal model studied here is blocked by activation of other stress-induced signaling systems.

An interesting observation in the present study was the mild stress-induced elevation of substance P mRNA levels in the medial amygdaloid nucleus. The role of the amygdala in fear, fear learning and stress responses has been firmly established in experimental animals (Heilig et al. 1993; Davis et al. 1994; Rogan and LeDoux 1996) and humans (Morris et al. 1998; Whalen et al. 1998). Furthermore, the amygdala is considered as a potential site of action for antidepressant drugs. Thus, the tricyclic antidepressant imipramine has similar effects on rat behavior in psychological stress tests after focal injection into amygdala as after systemic administration (Horovitz et al. 1966; Duncan et al. 1986). Increased internalization of substance P via the NK1 receptor occurs in the anterior-basolateral amygdala after maternal separation of guinea-pig pups (Kramer et al. 1998), a psychological stress/depression model. Moreover, repeated administration of antidepressant drugs alters substance P synthesis in some brain regions in rat (Brodin et al. 1987; Riley et al. 1991; Barden et al. 1993; Shirayama et al. 1996). Lithium, which is used in treatment of bipolar disorder, "normalizes" the decreased or increased, respectively, neurokinin A and substance P concentrations in FSL when compared with FRL rats (Husum et al. 2001). All these studies and the present results are in good agreement with a study reporting that an NK1 (substance P) receptor antagonist has clinical efficacy in treatment of major depression (Kramer et al. 1998), indicating that this disorder is associated with enhanced substance P signaling. However, more recent trials have failed to support this initial study.

A major projection area of the amygdala is the periventricular zone of hypothalamus, including the dorsomedial and ventromedial nuclei, as well as the lateral hypothalamus/perifornical region. These sites are known to be related to emotional reactions and to play a role in the integration of the autonomic, endocrine and behavioral responses to stress (Smith et al. 1980, 1990; Swanson 1987). Increased expression of substance P mRNA in these three regions may be due to a stimulating influence from amygdala and may be involved in stress-induced responses. Both antidepressants and a substance P antagonist have been shown to affect defensive rage in cats, a behavior related to activation of amygdala and hypothalamus (Dubinsky and Goldberg 1971; Shaikh et al. 1993).

The role of galanin in mood control has been less well explored but this peptide has, dependent on site of administration, been reported to exert both anxiolytic (Bing et al. 1993) and anxiogenic (Moller et al. 1999) effects. Immunohistochemical and in situ hybridization studies on distribution of galanin and galanin mRNA show that the majority of the noradrenergic neurons in the locus coeruleus neurons and the 5-hydroxytryptamine neurons in the raphe nucleus can express galanin (Melander et al.

1986). Galanin hyperpolarizes LC neurons and lowers their firing rate (Seutin et al. 1989; Sevcik et al. 1993; Pieribone et al. 1995; Xu et al. 2001), and this and other findings suggest that galanin may be of importance in depression (Fuxe et al. 1998; Hökfelt et al. 1998; Weiss et al. 1998), which is characterized by lowered extracellular levels of monoamines. In fact, Bellido et al. (2002) found an increased density of galanin binding sites in the dorsal raphe in FSC rats. Palkovits (2000) reported increased galanin mRNA after short-term stress, whereas we here found decreased galanin expression in some nuclei in the CMS model. However, there was a trend towards increased galanin mRNA levels in the locus coeruleus, in agreement with several other studies (Holmes et al. 1995; Sweerts et al. 1999). Such a difference seems also to exist for NPY regulation in the dentate gyrus, where acute stress increases NPY mRNA (Conrad and McEwen 2000), whereas chronic stress, such as that employed in the present and several previous studies on depression models, causes a decrease in NPY mRNA levels (for references, see above). Thus, short- and long-term stress may exert different effects on transcription of neuropeptides.

In conclusion, the present results obtained in the CMS rats suggest that alterations in NPY, substance P and galanin expression in brain areas related to emotion, stress and defense reactions may be involved in the pathophysiology of depression. These findings, together with previously published results, suggest that not only substance P and its receptors but also neuropeptides such as NPY and galanin and their receptors may be interesting targets for development of novel antidepressant therapies.

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References

Aycnsu WK, Pucilowksi O, Mason GA, Overstreet DH, Rezyani AH, Janowsky DS (1995) Effects of chronic mild stress on serum complement activity, saccharin preference and corticosterone levels in Flinders lines of rats. Physiol Behav 57:165–169

Barden N, Daigle M, Picard V, Di Paolo T (1993) Perturbation of rat brain serotonergic systems results in an inverse relation between substance P and serotonin concentrations measured in discrete nuclei. J Neurochem 41:834–840

Bellido I, Diaz-Cabiale Z, Jimenez-Vasquez PA, Andbjer B, Mathé AA, Fuxe K (2002) Increased density of galanin binding sites in the dorsal raphe in a genetic rat model of depression. Neurosci Lett 317:101–105

Bing O, Moller C, Engel JA, Söderpalm B, Heilig M (1993) Anxiolytic-like action of centrally administered galanin. Neurosci Lett 164:17–20

Brady LS, Smith MA, Gold PW, Herkenham M (1990) Altered expression of hypothalamic neuropeptide mRNAs in food-restricted and food-deprived rats. Neuroendocrinology 52:441–447

Brodin E, Ögren S-O, Theodorsson-Norheim E (1987) Effects of subchronic treatment with imipramine, zimelidine and alaproclate on regional tissue levels of substance P-and neurokinin A/neurokinin B-like immunoreactivity in the brain and spinal cord of the rat. Neuropharmacology 26:581–590

Caberlotto L, Hurd YL (1999) Reduced neuropeptide Y mRNA expression in the prefrontal cortex of subjects with bipolar disorders. Neuroreport 10:1747–1750

Caberlotto L, Fuxe K, Overstreet DH, Gerrard P, Hurd YL (1998) Alterations in neuropeptide Y and Y1 receptor mRNA expression in brains from an animal model of depression: region specific adaptation after fluoxetine treatment. Brain Res 59:58–65

Cheeta S, Ruigt G, van Proosdij J, Willner P (1997) Changes in sleep architecture following chronic mild stress. Biol Psychiatry 41:419–427

Conrad CD, McEwen BS (2000) Acute stress increases neuropeptide Y mRNA within the arcuate nucleus and hilus of the dentate gyrus. Mol Brain Res 79:102–109

Dagerlind Å, Friberg K, Bean A, Hökfelt T (1992) Sensitive mRNA detection using unfixed tissue: combined radioactive and non-radioactive in situ hybridization histochemistry. Histochemistry 98:39–49

D'Aquila P, Brain PF, Willner P (1994) Effects of chronic mild stress in behavioral tests relevant to anxiety and depression. Physiol Behav 56:861–867

Davis M, Rainnie D, Cassell M (1994) Neurotransmission in the rat amygdala related to fear and anxiety. Trends Neurosci 17:208–214

Dubinsky B, Goldberg ME (1971) The effect of imipramine and selected drugs on attack elicited by hypothalamic stimulation in the cat. Neuropharmacology 10:537–545

Duncan GE, Breese GR, Criswell H, Stumpf WE, Mueller RA, Covey JB (1986) Effects of antidepressant drugs injected into the amygdala on behavioral responses of rats in the forced swim test. J Pharmacol Exp Ther 238:758–762

Flood JF, Hernandez EN, Morley JE (1987) Modulation of memory processing by neuropeptide Y. Brain Res 421:280–290

Fuxe K, Jansson A, Diaz-Cabiale Z, Andersson A, Tinner B, Finnman U-B, Misane I, Razani H, Wang F-H, Agnati L, Ögren SO (1998) Galanin modulates 5-hydroxytryptamine functions. Focus on galanin fragments/5-hydroxytryptamine 1A receptor interactions in the brain. Ann NY Acad Sci 863:274–290

Gehlert DR, Chronwall BM, Schafer MP, O'Donohue TL (1987) Localization of neuropeptide Y messenger ribonucleic acid in rat and mouse brain by in situ hybridization. Synapse 1:25–31

Gorka Z, Moryl E, Papp M (1996) The effect of chronic mild stress on circadian rhythms in the locomotor activity of rats. Pharmacol Biochem Behav 54:229–234

Gruber SHM, Efendic S, Mathé AA (2004) Neuropeptide Y has antidepressant properties in a rat model of depression. Nord J Psychiatry 58:97

Harlan RE, Garcia MM, Krause JE (1989) Cellular localization of substance P- and neurokinin A-encoding preprotachykinin mRNA in the female rat. J Comp Neurol 287:179–212

Hashimoto H, Onishi H, Koide S, Kai T, Yamagami S (1996) Plasma neuropeptide Y in patients with major depressive disorder. Neurosci Lett 216:57–60

Heilig M, Widerlöv E (1995) Neurobiology and clinical aspects of neuropeptide Y. Crit Rev Neurobiol 9:115–136

Heilig M, McLeod S, Brot M, Heinrich SC, Menzaghi F, Koob GF, Britton KT (1993) Anxiolytic-like action of neuropeptide Y: mediation by Y1 receptors in amygdala and dissociation from food intake effects. Neuropsychopharmacology 8:357–363

Hökfelt T, Xu Z-Q D, Shi T-J, Holmberg K, Zhang X (1998) Galanin in ascending systems. Focus on 5-hydroxytryptamine and noradrenaline. Ann NY Acad Sci 863:252–263

Holmes PV, Blanchard DC, Blanchard RJ, Brady LS, Crawley JN (1995) Chronic social stress increases levels of preprogalanin mRNA in the rat locus coeruleus. Pharmacol Biochem Behav 50:655–660

Horovitz ZP, Pialo JJ, High JP, Burke JC, Leaf RC (1966) Effects of drugs on the mouse-killing (muricide) test and its relationship to amygdaloid function. Int J Neuropharmacol 5:405–411

Husum H, Mathé AA (2002) Early life stress changes concentrations of neuropeptide Y and corticotropin-releasing hormone in adult rat brain. Lithium treatment modifies these changes. Neuropsychopharmacology 27:756–764

Husum H, Mikkelsen JD, Hogg S, Mathé AA, Mörk A (2000) Involvement of hippocampal neuropeptide Y in mediating the chronic actions of lithium, electroconvulsive stimulation and citalopram. Neuropharmacology 39:1463–1473

Husum H, Jiménez-Vasquez PA, Mathé AA (2001) Changed concentrations of tachykinins and neuropeptide Y in brain of the rat model of depression: Lithium treatment normalizes tachykinins. Neuropsychopharmacology 24:183–191

Hökfelt T, Broberger C, Xu Z-Q, Sergeyev V, Ubink R, Diez M (2000) Neuropeptides—an overview. Neuropharmacology 39:1337–1356

Jacobowitz DM, Skofitsch G (1991) Localization of galanin cell bodies in the brain by immunocytochemistry and in situ hybridization histochemistry. In: Hökfelt T, Bartfai T, Jacobowitz D, Ottoson D (eds) Galanin. A new multifunctional peptide in the neuro-endocrine system. Wenner-Gren Center Int. Symp. Ser., vol 58. MacMillan, New York, pp 69–92

Janowsky DS, Overstreet DH, Nurberger JI (1994) Is cholinergic sensitivity a genetic marker for the affective disorders? Am J Med Genet 54:335–344

Jiménez Vasquez PA, Overstreet DH, Mathé AA (2000a) Neuropeptide Y in male and female brains of Flinders Sensitive Line, a rat model of depression. Effects of electroconvulsive stimuli. J Psychiatr Res 34:405–412

Jiménez Vasquez PA, Salmi P, Ahlenius S, Mathé AA (2000b) Neuropeptide Y in brains of the Flinders Sensitive Line rat, a model of depression. Effects of electroconvulsive stimuli and d-amphetamine on peptide concentrations and locomotion. Behav Brain Res 111:115–123

Kalin NH, Carnes M (1984) Biological correlates of attachment bond disruption in humans and nonhuman primates. Prog Neuropsychopharmacol Biol Psychiatry 8:459–469

Kramer MS, Cutler N, Feighner J, Shrivastava R, Carman J, Sramek JJ, Reines SA, Liu G, Snavely D, Wyatt-Knowles E, Hyde JJ, Mills SG, MacCoss M, Swain CJ, Harrison T, Hill RG, Hefti F, Scolnick EM, Cascieri MA, Chicchi GG, Sadowski S, Williams AR, Hewson L, Smith D, Carlson EJ, Hargreaves RJ, Rupniak NM (1998) Distinct mechanism for antidepressant activity by blockade of central substance P receptors. Science 281:1640–1645

Krause JE, Chirgwin JM, Carter MS, Xu ZS, Hershey AD (1987) Three rat preprotachykinin mRNAs encode the neuropeptides substance P and neurokinin A. Proc Natl Acad Sci U S A 84:881–885

Ladd CO, Owens MJ, Nemeroff CB (1996) Persistent changes in corticotropin-releasing factor neuronal systems induced by maternal deprivation. Endocrinology 137:1212–1218

Larhammar D, Ericsson A, Persson H (1987) Structure and expression of the rat neuropeptide Y gene. Proc Natl Acad Sci U S A 84:2068–2072

Makino S, Baker RA, Smith MA, Gold PW (2000) Differential regulation of neuropeptide Y mRNA expression in the arcuate nucleus and locus coeruleus by stress and antidepressants. J Neuroendocrinol 12:387–395

Mathé AA (1999) Neuropeptides and electroconvulsive treatment. J ECT 15:60–75

Mathé AA, Jousisto-Hanson J, Stenfors C, Theodorsson E (1990) Effect of lithium on tachykinins, calcitonin gene-related peptide, and neuropeptide Y in rat brain. J Neurosci Res 26:233–237

Mathé AA, Gruber S, Jimenez PA, Theodorsson E, Stenfors C (1997) Effect of electroconvulsive stimuli and MK-801 on neuropepride Y, neurokinin A, and calcitonin gene-related peptide in rat brain. Neurochem Res 22:629–636

Mathé AA, Jimenez PA, Theodorsson E, Stenfors C (1998) Neuropeptide Y, neurokinin A and neurotensin in brain regions of fawn hooded "depressed," Wistar, and Sprague Dawley rats. Effects of electroconvulsive stimuli. Prog NeuroPsychopharmacol. Biol Psychiatry 22:529–546

McKibbin PE, McCarthy HD, Shaw P, Williams G (1992) Insulin deficiency is a specific stimulus to hypothalamic neuropeptide Y: a comparison of the effects of insulin replacement and food restriction in streptozocin-diabetic rats. Peptides 13:721–727

Melander T, Hökfelt T, Rökaeus Å, Cuello AC, Oertel WH, Verhofstad A, Goldstein M (1986) Coexistence of galanin-like immunoreactivity with catecholamines, 5-hydroxytryptamine, GABA and neuropeptides in the rat CNS. J Neurosci 6:3640–3654

Mikkelsen JD, Woldbye D, Kragh J, Larsen P, Bowlig TG (1994) Electroconvulsive shocks increase the expression of neuropeptide Y (NPY) mRNA in the piriform cortex and the dentate gyrus. Mol Brain Res 23:317–322

Moller C, Sommer W, Thorsell A, Heilig M (1999) Anxiogenic-like action of galanin after intra-amygdala administration in the rat. Neuropsychopharmacology 21:507–512

Moreau JL, Jenck F, Martin JR, Mortas P, Haefely WE (1992) Antidepressant prevents chronic unpredictable mild stress-induced anhedonia as assessed by ventral tegmentum self-stimulation behavior in rats. Neuropsychopharmacology 2:43–49

Moreau JL, Jenck F, Martin JR, Mortas P (1994) Curative effects of the atypical antidepressant mianserin in the chronic mild stress-induced anhedonia model of depression. J Psychiatry Neurosci 19:51–56

Moreau JL, Scherschlict R, Jenck F, Martin JR (1995) Chronic mild stress-induced anhedonia model of depression: sleep abnormalities and curative effects of electroshock treatment. Behav Pharmacol 6:682–687

Moreau JL, Jenck F, Martin JR (1998) Simulation of a core symptom of human depression in rats. Curr Top Pharmacol 4:37–50

Morley JE (1987) Neuropeptide regulation of appetite and weight. Endocr Rev 8:265–287

Morley JE, Flood JF (1990) Neuropeptide Y and memory processing. Ann NY Acad Sci 611:226–231

Morris JL, Gibbins IL (1989) Co-localization and plasticity of transmitters in peripheral autonomic and sensory neurons. Int J Dev Neurosci 7:521–531

Morris JS, Friston KJ, Buchel C, Frith CD, Young AW, Calder AJ, Dolan RJ (1998) A neuromodulatory role for the human amygdala in processing emotional facial expressions. Brain 121:47–57

Muscat R, Willner P (1992) Suppression of sucrose drinking by chronic mild unpredictable stress: a methodological analysis. Neurosci Biobehav Rev 16:507–517

Nilsson C, Karlsson G, Blennow K, Heilig M, Ekman R (1996) Differences in the neuropeptide Y-like immunoreactivity of the plasma and platelets of human volunteers and depressed patients. Peptides 17:359–362

Overstreet DH (1993) The Flinders sensitive line rats: a genetic animal model of depression. Neurosci Behav Rev 17:51–68

Palkovits M (2000) Stress-induced expression of co-localized neuropeptides in hypothalamic and amygdaloid neurons. Eur J Pharmacol 405:161–166

Pieribone V, Xu Z-Q, Zhang X, Grillner S, Bartfai T, Hökfelt T (1995) Galanin induces a hyperpolarization of norepinephrine-containing locus coeruleus neurons in the brainstem slice. Neuroscience 64:861–874

Plotsky PM, Meaney MJ (1993) Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. Mol Brain Res 18:195–200

Redrobe JP, Dumont Y, Fournier A, Quirion R (2002) The neuropeptide Y (NPY) Y1 receptor subtype mediates NPY-induced antidepressant-like activity in the mouse forced swimming test. Neuropsychopharmacology 26:615–624

Riley LA, Walker PD, Hart RP, Jonakait GM (1991) Alterations of preprotachykinin (PPT) mRNA in medullary raphe occur following manipulation of serotonin. Ann NY Acad Sci 632:455–456

Rogan MT, LeDoux JE (1996) Emotion: systems, cells, synaptic plasticity. Cell 85:469–475

Seutin V, Verbanck P, Massotte L, Dresse A (1989) Galanin decreases the activity of locus coeruleus neurons in vitro. Eur J Pharmacol 164:373

Sevcik J, Finta EP, Illes P (1993) Galanin receptors inhibit the spontaneous firing of locus coeruleus neurones and interact with μ-opioid receptors. Eur J Pharmacol 230:223–230

Shaikh MB, Steinberg A, Siegel A (1993) Evidence that substance P is utilized in medial amygdaloid facilitation of defensive rage behavior in the cat. Brain Res 625:283–294

Shirayama Y, Mitsushio H, Takashima M, Ichikawa H, Takahashi K (1996) Reduction of substance P after chronic antidepressants treatment in the striatum, substantia nigra and amygdala of the rat. Brain Res 739:70–78

Smith OA, Astley CA, DeVito JL, Stein JM, Walsh KE (1980) Functional analysis of hypothalamic control of the cardiovascular responses accompanying emotional behavior. Fed Proc 39:2487–2494

Smith OA, Astley CA, de Vito JL, Stein JM, Walsh KE (1990) Functional analysis of hypothalamic control of cardiovascular responses to emotion are located in lateral hypothalamus-perifornical region. Am J Physiol 259:R943–R954

Stanley BG, Leibowitz SF (1985) Neuropeptide Y injected in the paraventricular hypothalamus: a power stimulant of feeding behavior. Proc Natl Acad Sci U S A 82:3940–3943

Stanley BG, Kyrkouli S, Lampert S, Leibowitz SF (1986) Neuropeptide Y chronically injected into the hypothalamus: a powerful neurochemical inducer of hyperphagia and obesity. Peptides 7:1189–1192

Stenfors C, Theodorsson E, Mathé AA (1989) Effect of repeated electroconvulsive treatment on regional concentrations of tachykinins, neurotensin, vasoactive polypeptide, neuropeptide Y and galanin in rat brain. J Neurosci Res 24:445–450

Swanson LW (1987) The hypothalamus. In: Swanson LW, Björklund A, Hökfelt T (eds) Handbook of chemical neuroanatomy, vol 5. Elsevier, Amsterdam, pp 1–124

Sweerts BW, Jarrott B, Lawrence AJ (1999) Expression of preprogalanin mRNA following acute and chronic restraint stress in brains of normotensive and hypertensive rats. Mol Brain Res 69:113–123

Tempel DL, Leibowitz SF (1994) Adrenal steroid receptors: interactions with brain neuropeptide systems in relation to nutrient intake and metabolism. Neuroendocrinology 6:479–501

Vrontakis ME, Peden LM, Duckworth ML, Friesen HG (1987) Isolation and characterization of a complementary DNA (galanin) clone from estrogen-induced pituitary tumor messenger RNA. J Biol Chem 262:16755–16758

Wahlestedt C, Blendy JA, Kellar KJ, Heilig M, Widerlöv E, Ekman R (1990) Electroconvulsive shocks increase the concentration of neocortical and hippocampal neuropeptide Y (NPY)-like immunoreactivity in the rat. Brain Res 507:65–68

Wahlestedt C, Pich EM, Koob GF, Yee F, Heilig M (1993) Modulation of anxiety and neuropeptide Y-Y1 receptors by antisense oligodeoxynucleotides. Science 259:528–531

Wang J, Akabayashi A, Dourmashkin J, Yu H-J, Alexander T, Chae HJ, Leibowitz SF (1998) Neuropeptide Y in relation to carbohydrate intake, corticosterone and dietary obesity. Brain Res 802:75–88

Warden MK, Young III WS (1988) Distribution of cells containing mRNAs encoding substance P and neurokinin B in the rat central nervous system. J Comp Neurol 272:90–113

Weiner ED, Mallat A, Papolos DF, Lachman HM (1992) Acute lithium treatment enhances neuropeptide Y gene expression in rat hippocampus. Mol Brain Res 12:209–214

Weiss JM, Bonsall RW, Demetrikopoulos MK, Emery MS, West CHK (1998) Galanin: a significant role in depression? Ann NY Acad Sci 863:364–382

Whalen PJ, Rausch SL, Etcoff NL, McInerney SC, Lee MB, Jenike MA (1998) Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. J Neurosci 18:411–418

Widerlöv E, Heilig M, Ekman R, Wahlestedt C. (1988) Possible relationship between neuropeptide Y (NPY) and major depression—evidence from human and animal studies. Nord Psykiatr Tidsskr 42:131–137

Willner P, Owell A, Sampson D, Sophokleous S, Muscat R (1987) Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology 93:358–364

Xu Z-QD, Tong Y-G, Hökfelt T (2001) Galanin enhances noradrenaline-induced outward current on locus coeruleus noradrenergic neurons. NeuroReport 12(8):1779–1782

Zachrisson O, Mathé AA, Stenfors C, Lindefors N (1995a) Limbic effects of repeated electroconvulsive stimulation on neuropeptide Y and somatostatin mRNA expression in the rat brain. Mol Brain Res 31:71–85

Zachrisson O, Mathé AA, Stenfors C, Lindefors N (1995b) Region-specific effects of chronic lithium administration on neuropeptide Y and somatostatin mRNA expression in the rat brain. Neurosci Lett 194:89–92