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# Altered glutamate receptor and corticoliberin gene expression in brain regions related to hedonic behavior in rats

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**Abstract**

Present experiments in rats were aimed to verify the hypothesis that glutamatergic neurotransmission and stress hormones play a role in impairment of hedonic behavior, a sign of depression-like state. On the basis of individual variability in sucrose preference, test rats were divided into anhedonic and hedonic groups. Anhedonic animals showed higher basal concentrations of adrenocorticotropin and corticosterone but reduced hormonal responses during novelty stress compared to hedonic animals. Acute administration of citalopram (10 mg/kg ip) induced similar effects in both groups. Corticotropin-releasing hormone (CRH) mRNA levels in hypothalamic paraventricular nucleus (PVN) were higher in anhedonic rats. Oxytocin (OT) and vasopressin gene expression in the PVN and proopiomelanocortin (POMC) expression in the anterior pituitary failed to show any significant differences. Gene expression of NR1 receptor subunit of *N*-methyl-D-aspartate (NMDA) glutamate receptor in the ventral tegmental area (VTA) was found to be lower in anhedonic rats. In the nucleus accumbens (NAc) and the hippocampus of anhedonic animals, higher mRNA levels of NR2A subunit compared to those of hedonic rats were detected. Thus, low sucrose preference is associated with altered HPA axis activity, NMDA receptor subunits and CRH gene expression in selected brain regions. These mechanisms may operate in the disposition to develop hedonic deficit in some mental disorders.


*Keywords:* Anhedonia; Stress response; Reward; CRH; NMDA; Serotonin

## 1. Introduction

Mechanisms and pathways underlying reward processes have been intensively investigated, and various paradigms describing animal hedonic behavior have been developed. Frequently employed approaches for detecting impairment of hedonic behavior in models of depression (Willner, 1997) or withdrawal from drug abuse (Lieblich et al., 1991) are based on the response to a natural reinforcer, namely, intake of palatable food in rodents.

The intake of sweet substances such as sucrose or saccharine shows high individual variability in rats (Sills and Vaccarino, 1994; Gosnell et al., 1995). Individual differences in sucrose intake correlate with differences in response to drug reinforcers such as amphetamine (DeSousa et al., 2000), morphine (Sills and Vaccarino, 1998) or cocaine (Gosnell, 2000). Presynaptic dopamine function in the nucleus accumbens (NAc) was found to be altered in low sucrose feeders (Sills and Crawley, 1996).

Impaired intake of or preference for sweet substances has been observed after exposure to stress stimuli. This has been described for chronic mild stress model of depression and for exposure to stimuli such as social defeat or inescapable shocks (Katz, 1982; Willner, 1997; Duncko et al., 2001; Von Frijtag et al., 2000; Griffiths et al., 1992). Stress-induced anhedonia was found to be associated with increased corticotropin-releasing hormone (CRH) gene expression in the hypothalamus (Duncko et al., 2001) and increased CRH immunoreactivity in the bed nucleus of the stria terminalis (Stout et al., 2000), indicating that this neuropeptide might participate in the mechanisms involved.

Glutamate is another regulatory substance suggested to play a role in the stress response as well as reward processes (Jezova et al., 1995; Wolf, 1998; Tzschentke, 2001). Hippocampal neurons have a high density of glutamate receptors (Blackstone et al., 1992) and gluco- and mineralocorticoid receptors, which are important in the regulatory feedback of the hypothalamus–pituitary–adrenocortical axis (Jacobson and Sapolsky, 1991). Glutamate receptor

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antagonists can interfere with stress-induced neuroendocrine (Jezova et al., 1995; Zelena et al., 1999) as well as behavioral alterations (Papp and Moryl, 1994). With regard to brain areas related to reward, a direct interaction between glutamatergic and dopaminergic transmission was observed in medial prefrontal cortex (mPFC) and NAc (Pirot et al., 1996; Smith-Roe and Kelley, 2000). Special attention is given to dopamine–glutamate interaction in the ventral tegmental area (VTA), where dopaminergic neurons projecting to the mPFC and NAc are localized (Smith et al., 1996).

Among intact rats, a subgroup of animals demonstrates spontaneously decreased hedonic behavior, resembling hedonic impairment observed in stress-induced experimental depression. The present series of experiments was aimed to verify the hypotheses that (1) spontaneous anhedonia is associated with alterations in neuroendocrine response during stress; (2) neuroendocrine response to an antidepressant treatment is altered in spontaneously anhedonic rats; and (3) glutamatergic transmission plays a role in hedonic impairment.

## 2. Methods

### 2.1. Animals

Male Sprague–Dawley rats, each weighing 310–470 g, were used in the present series of experiments. Two weeks before and during the experiment, the animals were housed singly in wire cages at a temperature of 23 °C and on a 12:12-h light–dark cycle (0600–1800 h light). Rats had ad libitum access to water and standard laboratory chow pellets except the water deprivation before the sucrose preference test when only chow pellets were available. All animal experiments were approved by the Animal Care Committee of the Institute of Experimental Endocrinology, Slovak Academy of Sciences, and are in compliance with the European Communities Council Directive 86/609/EEC.

### 2.2. Assessment of hedonic behavior (sucrose preference test)

Sucrose preference was measured in four preference tests to separate the animals into two groups. After overnight water deprivation, animals were exposed to two bottles containing tap water and 1% sucrose solution for 3 h. The test was performed between the 7th and 10th hour of the light phase on four consecutive days. After 3 h, the volume of consumed water and sucrose was measured and the percentage of sucrose solution from the total liquid ingested was calculated. According to the preference of sucrose solution during 4 days, the rats were divided into two groups. Animals with low sucrose preference (below 60%) observed on at least 3 days as well as in average formed the anhedonic (nonpreferring) group, whereas the other rats were assigned to the hedonic (preferring) group.

### 2.3. Cannulations

On the second day after the last preference test, the rats were anaesthetized with pentobarbital sodium, and polyethylene cannulas (Intramedic PE 50; Clay Adams, Parsipanny, NJ, USA) were placed into the tail artery for blood sampling and into the peritoneal cavity for drug administration (Experiment 2), as described previously (Jezova et al., 1995). On the next day, the animals were exposed to a stress stimulus or pharmacological treatment.

### 2.4. Experimental procedures

#### 2.4.1. Stress exposure (Experiment 1)

The animals were exposed to a novel environment (novelty stress). Before the procedure, blood samples were taken for measurement of basal hormone levels. Afterwards, the animals were transported into another room. During the whole procedure, the rats remained in their home cages. Blood samples were obtained from indwelling tail artery catheters at 0, 5 and 15 min after changing the room.

#### 2.4.2. Citalopram challenge (Experiment 2)

Rats were acutely treated with the selective serotonin reuptake inhibitor citalopram. During the whole procedure, the animals remained undisturbed in their home cages. After the control blood samples were taken from a tail artery catheter, the rats were injected with citalopram (Seropram, Lundbeck, 10 mg/kg) via the intraperitoneal canulla. Blood samples were taken at 0, 7, 15, 30 and 60 min after the treatment. Rats were decapitated 24 h after pharmacological treatment and organs were removed for further analysis.

### 2.5. Blood and tissue collection

Blood samples were collected from indwelling tail artery catheters. Blood was sampled into tubes containing EDTA as anticoagulant, centrifuged at 4 °C, and after separation, the plasma was stored at −20 °C until assayed. The brain regions for in situ hybridization (hypothalamus) were quickly removed, frozen in isopentane at −30 °C and stored at −70 °C. The brain regions for PCR (hippocampus, VTA, NAc) were removed, frozen in liquid nitrogen and stored at −70 °C until assayed.

### 2.6. Plasma hormone measurements

Plasma ACTH was measured by a radioimmunoassay using a double antibody technique to separate free and bound fractions as described previously (Jezova et al., 1987). Plasma corticosterone levels were analyzed by radioimmunoassay after dichloromethane extraction of steroids from 10 μl aliquots of plasma (Moncek et al., 2001). Antibodies for ACTH and corticosterone were kindly pro-

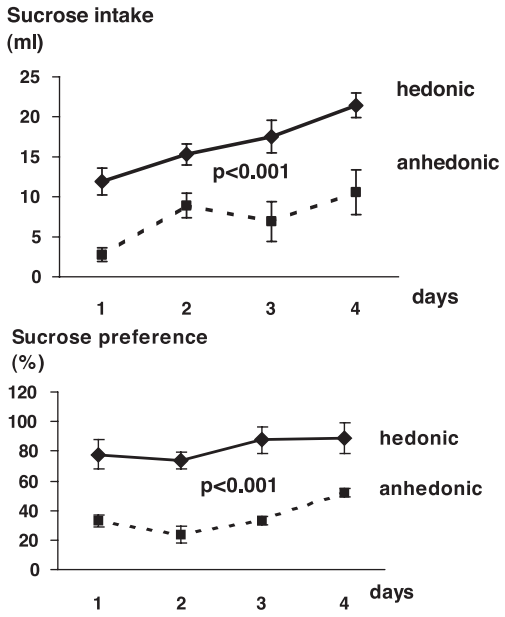


Fig. 1. Sucrose intake and sucrose preference of hedonic and anhedonic group of rats during four days of preference test. Data are expressed as means of 12 (anhedonic group) and 24 (hedonic group) values ± S.E.M.

vided by Prof. G. B. Makara (Budapest) and Prof. C. Oliver (Marseille), respectively.

### 2.7. In situ hybridization

Coronal sections of the brain and anterior pituitary (12 μm) were cut in a cryostat, mounted onto polylysine-coated slides and hybridized as described previously (Skultetyova et al., 1998). Sequentially matched sections of the hypothalamus at the level of the paraventricular nucleus (PVN) were hybridized for CRH, arginine–vasopresine (AVP) and oxytocin (OT) mRNA, whereas sections of the anterior pituitary were hybridized for proopiomelanocortin (POMC) mRNA. The probes were kindly provided by Dr. G. Aguilera, USA. Sections from all animals were processed in the same hybridization and exposed together to Kodak, IBI film (New Haven, CT, USA). The autoradiographic hybridization signal was quantitated using a computerized image analysis system (Scion Image for Windows 4.0.2), and the values for each rat were calculated from average of measurements in two or three matched sections after subtracting the background.

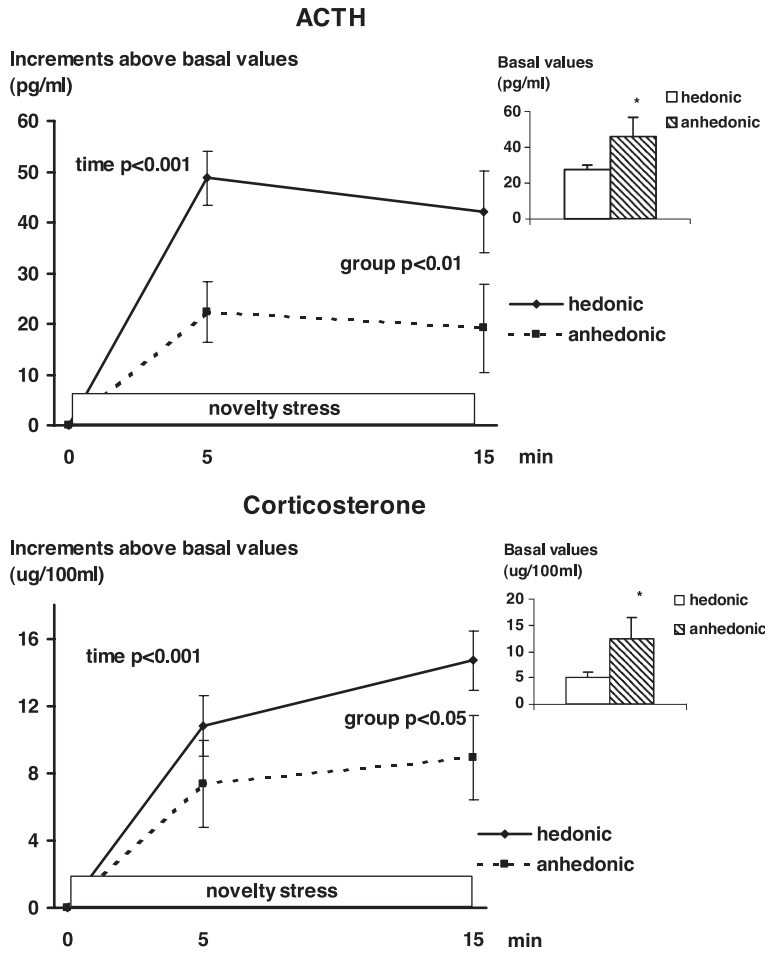


Fig. 2. Plasma ACTH and corticosterone concentrations under basal conditions and during exposure to novel environment. Data are expressed as means of 5 (anhedonic group) and 15 (hedonic group) values ± S.E.M. Statistical significance as compared to values in the hedonic group: $*P < .05$.

### 2.8. Reverse transcription–polymerase chain reaction (RT–PCR)

Total RNA from selected brain regions was extracted by a guanidium thiocyanate-phenol/chloroform method (Chomczynski and Sacchi, 1987). Concentration and purity of RNA preparations were measured by absorption spectroscopy. The quality of RNA was judged from the pattern of ribosomal RNA after gel electrophoresis.

Total cellular RNA (1–2 μg) was reverse-transcribed to cDNA and subjected to PCR amplification as described previously (Schwendt and Jezova, 2001). PCR reactions were carried out in the presence of two sets of primers, one for NR1/NR2A/NR2B and other for β-actin. The number of PCR cycles as well as ratio of NR/β-actin primers was optimized. PCR products were separated on 2% agarose gel stained with ethidium bromide and photographed under UV illumination. Optical density of PCR products was measured with an EDAS densitometric software (Kodak, USA). In each sample, the values for the gene of interest were normalized to housekeeping gene β-actin values to express relative mRNA levels as arbitrary units.

### 2.9. Measurements of ion concentrations and plasma osmolality

Concentrations of natrium, calcium and potassium in plasma were measured by atomic absorption spectrophotometry (Unicam SP 192). Plasma osmolality was determined by cryoscopic osmometry (Osmomat 030, Gonatec).

### 2.10. Open-field test

Open-field behavior was measured in Experiment 2. The test was performed 1 day before the first sucrose preference test during the light phase of the light–dark cycle. The apparatus consisted of a rectangular area of 72 × 48 cm surrounded by a 25-cm high wall. The area was divided into 24 squares of 12 × 12 cm. The field was lighted with a 60-W bulb fixed 50 cm over the field. The rat was placed in one corner of the open field and the activity during the subsequent 5 min was recorded by a video camera. Horizontal and vertical locomotor activity was assessed by measuring the number of squares entered and the number of rears, respectively.

### 2.11. Statistical analysis

The data are expressed as means ± S.E.M. Multiple regression analysis was used to compare sucrose preference between consecutive sessions. One-way analysis of variance (ANOVA) was performed for comparisons of basal (prestress) values and gene expression. The effect of stress exposure or pharmacological treatment was analyzed by means of two-way ANOVA. This was followed by Tukey test for pairwise multiple comparisons when appropriate.

## 3. Results

### 3.1. Hedonic behavior

Analysis of sucrose intake and preference data confirmed the existence of high individual variability in hedonic behavior, and about 30% of rats were claimed to be anhedonic with mean sucrose preference ($F = 123.1$; $P < .001$) and sucrose intake ($F = 18.75$; $P < .001$) significantly lower than that in the hedonic group (Fig. 1). The pattern of variability was stable during the time and data obtained during four consecutive days showed significant positive correlation ($\beta > .43$, $P < .05$).

### 3.2. Stress hormone release and gene expression

Under basal prestress conditions, anhedonic animals showed higher plasma concentrations of ACTH ($F = 5.88$, $P = .028$) and corticosterone ($F = 6.54$, $P = .02$) than hedonic animals. The novelty stress was followed by a rise in plasma ACTH ($F = 8.28$, $P < .001$) and corticosterone ($F = 17.33$, $P < .001$) levels with significant differences between the groups. In anhedonic rats, the stress-induced rise in ACTH

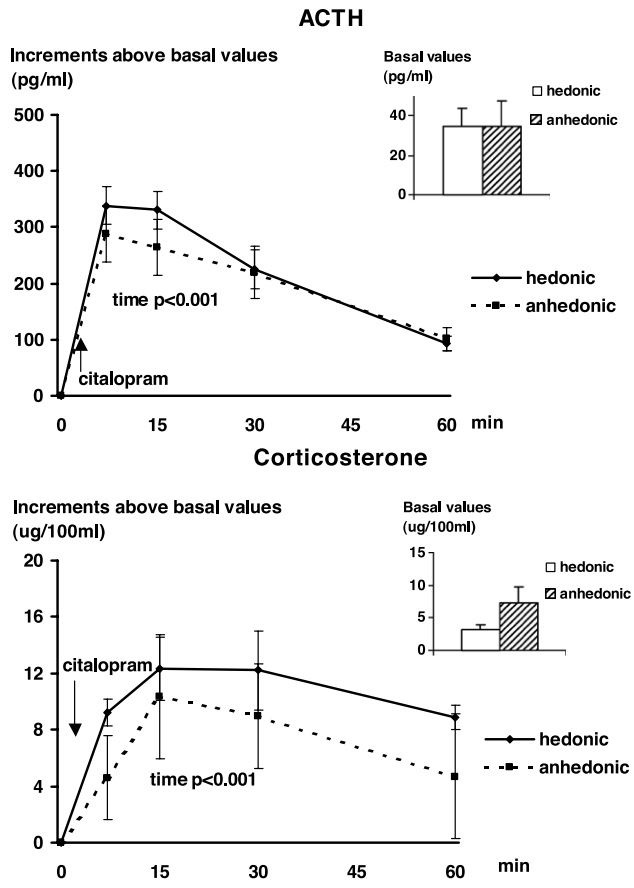


Fig. 3. Plasma ACTH and corticosterone concentrations under basal conditions and after treatment with citalopram (10 mg/kg ip). Data are expressed as means of seven (anhedonic group) and nine (hedonic group) values ± S.E.M.

($F=9.07$, $P<.01$) as well as corticosterone ($F=4.19$, $P=.05$) levels was blunted in comparison to that in hedonic rats (Fig. 2).

Intraperitoneal injection of citalopram resulted in a significant rise in ACTH ($F=32.7$, $P<.001$) and corticosterone ($F=9.99$, $P<.001$) levels in plasma. No differences between the groups were observed (Fig. 3).

In situ hybridization in the hypothalamus showed that levels of mRNA coding for CRH in the PVN were significantly higher ($F=5.94$, $P<.05$) in the anhedonic than in the hedonic group of rats. POMC gene expression in the anterior pituitary failed to show any significant differences. Similarly, OT and AVP gene expression in the PVN did not show any significant differences between hedonic and anhedonic rats (Fig. 4).

### 3.3. Glutamate receptors gene expression

Evaluation of *N*-methyl-D-aspartate (NMDA) receptor subunit gene expression in different brain regions revealed selective differences between the groups (Fig. 5). In the VTA, levels of mRNA coding for NR1 subunit of the NMDA receptor were higher ($F=58.02$, $P<.001$) in hedonic than in anhedonic rats. Gene expression of NR2A and NR2B subunits did not show significant difference between the groups. In the NAc, gene expression of NR2A subunit was significantly higher ($F=17.33$, $P<.01$) in anhedonic than that in hedonic rats. Similarly, in the hippocampus, gene expression of NR2A subunit was significantly higher ($F=11.78$, $P<.05$) in anhedonic when compared to those in hedonic rats, while no differences were found for NR1 and NR2B subunits.

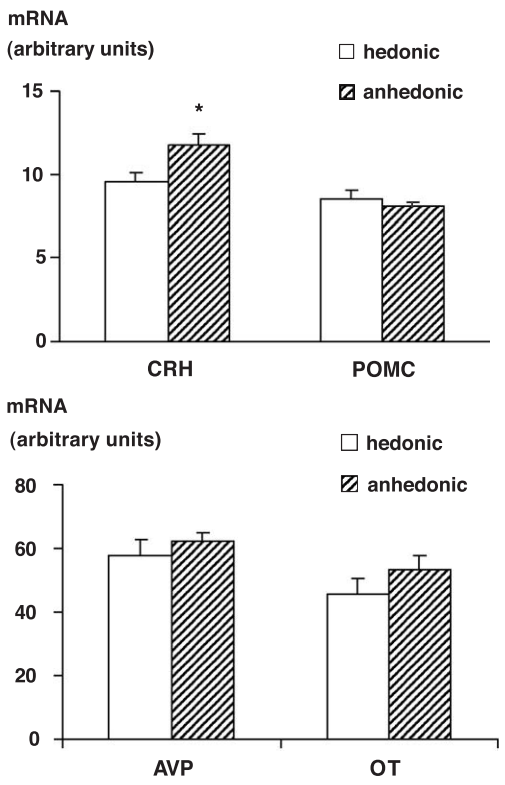


Fig. 4. Corticoliberine (CRH), AVP and OT mRNA levels in the hypothalamic PVN and POMC mRNA levels in the anterior pituitary in hedonic and anhedonic rats. Data are expressed as means of seven (anhedonic group) and nine (hedonic group) values ± S.E.M. Statistical significance as compared to values in the hedonic group: * $P<.05$.

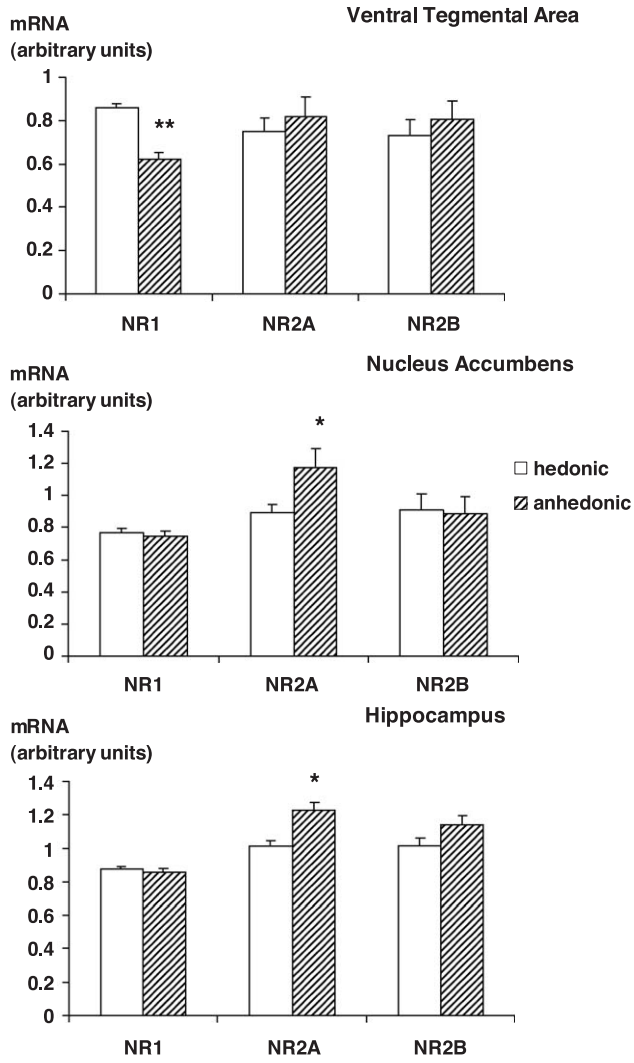


Fig. 5. Gene expression of NMDA receptor subunits in the VTA, NAc and hippocampus in hedonic and anhedonic rats. Data are expressed as means of four (anhedonic group) and five (hedonic group) values ± S.E.M. Statistical significance as compared to values in the hedonic group: * $P<.05$, ** $P<.01$.

### 3.4. Behavioral and physiological parameters

Horizontal and vertical locomotor activity during the open-field test failed to show any significant differences between the hedonic and anhedonic groups (data not shown). Evaluation of physiological parameters such as body weight, hematocrite, plasma osmolality and plasma natrium, potassium and calcium levels revealed no significant differences between the groups (data not shown).

## 4. Discussion

The present series of experiments document that hedonic deficit, defined as low preference for 1% sucrose solution, is associated with altered CRH and glutamate receptor gene expression in brain regions related to stress response and reward. Furthermore, altered HPA axis activity in response to a stress stimulus but not to an antidepressant treatment has been observed in anhedonic rats.

The main alterations of HPA axis activity observed in this study are blunted corticosterone and ACTH responses to novelty stress in anhedonic animals. These data are consistent with behavioral changes described during exposure to elevated plus maze in low sucrose feeders (Desousa et al., 1998). Moreover, our findings are consistent with data from some, though scarce, clinical studies that show blunted stress response in depressed patients (Kathol et al., 1992; Young et al., 2000). Furthermore, higher basal, prestress corticosterone and ACTH levels observed in anhedonic animals resemble those described in experimental models (Ayensu et al., 1995) and patients with depression (Holsboer and Barden, 1996).

The analysis of gene expression of hypothalamic regulatory factors revealed significantly enhanced levels of mRNA coding for CRH in anhedonic animals, which is in agreement with alterations in hypothalamic CRH gene expression reported in experimental depression (Duncko et al., 2001). Thus, in addition to the hedonic impairment, anhedonic rats display neuroendocrine changes similar to those observed in experimental as well as clinical depression. It could be argued that presented changes in neuropeptide gene expression were due to different responses to citalopram treatment made 24 h earlier. However, no changes in parvicellular CRH gene expression were observed 240 min or 24 h following single or repeated citalopram treatment (Jensen et al., 1999; Moncek et al., 2003). Thus, we suggest that the data represent preexisting differences in hedonic and anhedonic rats.

Two other hypothalamic regulatory factors, OT and AVP, have been suggested to play a role in the pathogenesis of affective disorders (Purba et al., 1996; Uvnas-Moberg et al., 1999). The present results do not support the involvement of magnocellular OT and AVP in hedonic behavior; however, the possible role of these factors in symptoms other than anhedonia remains to be elucidated.

Serotonergic transmission is thought to play a crucial role in the development of depressive symptoms (Charney, 1998). In the present study, the serotoninergic regulation of HPA axis function, as examined by a challenge with selective serotonin reuptake inhibitor citalopram, failed to be altered in rats with hedonic deficit. As the surgery associated with tail artery cannulation is known to induce transient activation of stress hormone secretion, it might be expected to interfere with citalopram effects. However, the basal hormone levels measured 24 h after cannulation were in normal range, and we have recently demonstrated that three different chronic stress paradigms do not modify hormonal responses to citalopram treatment (Moncek et al., 2003). Thus, serotonergic transmission in the hypothalamus does not seem to be involved in this behavioral alteration. This finding is in discordance with the suggested role of serotonin in the pathogenesis of depression. However, there are discrepancies within the monoaminergic hypothesis of depression, and other factors were suggested to participate in clinical effects of monoaminergic antidepressants (Bouron and Chatton, 1999; Jezova and Duncko, 2002).

Along with other neurotransmitters, glutamate is suggested to play a role in the pathogenesis of depressive symptoms (Papp and Moryl, 1994; Skolnick et al., 2001). Intriguing finding of the present study is that low sucrose preference is associated with altered gene expression of NMDA receptor subunits in the VTA, NAc and hippocampus. Glutamatergic transmission in the VTA is crucial for the regulation of mesolimbic and mesocortical dopaminergic pathways, which represent the neurobiological substrate of reward (Tzschentke, 2001). Accordingly, treatment with addictive drugs, as well as intracranial self-stimulation, was found to be associated with altered gene expression of glutamate receptors in the VTA (Fitzgerald et al., 1996; Carlezon et al., 2001). Thus, down-regulation of NR1 mRNA levels in the VTA could account not only for decreased excitability of VTA dopaminergic neurons but also for hypofunction of reward mechanisms. In the NAc, the increase of NR2A mRNA levels in anhedonic group might result in NR2A-enriched receptors. It has been documented that increased proportion of NR2A to NR1 and NR2B subunits results in restricted $Ca^{2+}$ influx into neurons (Cull-Candy et al., 2001). Altered subunit composition of NMDA receptor complex may contribute to subsensitivity of NAc neurons to glutamate stimulation and to altered function of reward system.

The analysis of glutamate receptor gene expression in the hippocampus revealed increased gene expression of NR2A subunit of NMDA receptor in anhedonic rats. Hippocampal structures are involved in many physiological functions including the regulation of stress response (Jacobson and Sapolsky, 1991; Schwendt and Jezova, 2000). Some studies have described that chronic alterations of corticosterone levels or exposure to stress influenced specifically NR2A and NR2B subunit expression in the hippocampus (Yoneda et al., 1994; Weiland et al., 1997). Furthermore, hippocampus is known to participate in the regulation of appetitive behaviors (Tracy et al., 2001). Alterations in hippocampal glutamatergic transmission are hypothesized to occur in learned helplessness model of depression (Shors et al., 1989) and after treatment with antidepressants (Zahorodna and Bijak, 1999). The presented changes in glutamate receptor gene expression should be considered in relevance to neuroendocrine and behavioral alterations observed in anhedonic rats.

As the sucrose preference testing was associated with water deprivation, it may be argued that the neurochemical

and neuroendocrine differences between hedonic and anhedonic rats could be due to different physiological responses to repeated water deprivation. However, the lack of difference in plasma ion concentrations, plasma osmolality and hematocrit indicates that this is not the case. Moreover, comparable gene expression of paraventricular AVP in both groups of rats doubt the possibility that observed individual differences in sucrose intake might be due to altered water and electrolyte homeostasis.

Decreased locomotor or exploratory activity was reported to occur in animal models of depression. Similar alterations in positively motivated behavior are hypothesized in rats with low sucrose intake (Desousa et al., 1998). Indeed, blunted locomotor response to a psychostimulant treatment in anhedonic animals has been described (Sills and Vaccarino, 1994), but no such differences were reported in spontaneous locomotor or exploratory activity. In the present study, we found no changes in locomotor activity during the open-field test.

In conclusion, we suggest that alterations in stress response and glutamatergic transmission are related to low sucrose preference in rats. Our data indicate that anhedonic rats display behavioral and neurochemical changes similar to those observed in experimental depression and support the hypothesis that the hedonic deficit described is related to a predisposition to develop depressive-like behavior. Neurobiological changes observed may belong to the factors responsible for the predisposition to depressive states in humans.

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