Psychoneuroendocrinology (2015) 51, 11—23

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/psyneuen

Restoration of peripheral V2 receptor vasopressin signaling fails to correct behavioral changes in Brattleboro rats

Diána Balázsfi[a,b], Ottó Pintér[a], Barbara Klausz[a], Krisztina B. Kovács[a], Anna Fodor[a,b], Bibiána Török[a], Mario Engelmann[c,d], Dóra Zelena[a,*]

[a] *Hungarian Academy of Sciences, Institute of Experimental Medicine, 1083 Budapest Szigony 43, Budapest, Hungary*
[b] *János Szentágothai School of Neurosciences, Semmelweis University, Budapest, Hungary*
[c] *Institut für Biochemie und Zellbiologie (M.E.), Otto-von-Guericke-Universität, Magdeburg, Germany*
[d] *Center of Behavioral Brain Science, Magdeburg, Germany*

Received 11 July 2014; received in revised form 10 September 2014; accepted 10 September 2014

KEYWORDS
DDAVP;
Anxiety;
Depression;
Recognition memory;
Amygdala;
Lateral septum

Abstract Beside its hormonal function in salt and water homeostasis, vasopressin released into distinct brain areas plays a crucial role in stress-related behavior resulting in the enhancement of an anxious/depressive-like state.

We aimed to investigate whether correction of the peripheral symptoms of congenital absence of AVP also corrects the behavioral alterations in AVP-deficient Brattleboro rats. Wild type (WT) and vasopressin-deficient (KO) male Brattleboro rats were tested.

Half of the KO animals were treated by desmopressin (V2-receptor agonist) via osmotic minipump (subcutaneous) to eliminate the peripheral symptoms of vasopressin-deficiency. Anxiety was studied by elevated plus maze (EPM), defensive withdrawal (DW) and marble burying (MB) tests, while depressive-like changes were monitored in forced swimming (FS) and anhedonia by sucrose preference test. Cell activity was examined in septum and amygdala by c-Fos immunohistochemistry after 10 min FS.

KO rats spent more time in the open arm of the EPM, spent less time at the periphery of DW and showed less burying behavior in MB suggesting a reduced anxiety state. KO animals showed less floating behavior during FS revealing a less depressive phenotype. Desmopressin treatment compensated the peripheral effects of vasopressin-deficiency without a significant influence on

* Corresponding author. Tel.: +36 1 2109400/290; fax: +36 1 2109951.
E-mail address: zelena.dora@koki.mta.hu (D. Zelena).

http://dx.doi.org/10.1016/j.psyneuen.2014.09.011

the behavior. The FS-induced c-Fos immunoreactivity in the medial amygdala was different in WT and KO rats, with almost identical levels in KO and desmopressin treated animals. There were no differences in central and basolateral amygdala as well as in lateral septum.
Our data confirmed the role of vasopressin in the development of affective disorders through central mechanisms. The involvement of the medial amygdala in the behavioral alterations of vasopressin deficient animals deserves further attention.

1. Introduction

Stress is defined as an adaptive reaction to disturbed homeostasis due to internal or external hazards (Holsboer and Ising, 2010). Prolonged and repeated exposures to unavoidable, aversive stimuli may lead to a dysregulation of the hypothalamic–pituitary–adrenocortical (HPA) axis, the major endocrine component of the stress response. A maladaptive stress response is thought to be causally linked to a variety of human disorders including mood disorders (Chrousos and Gold, 1992; Chrousos, 1998; Holsboer, 2000; McEwen, 2000; Engelmann et al., 2004). The physiological foundation of the HPA axis is constituted by parvocellular neurons of the hypothalamic paraventricular nucleus (PVN). These neurons secrete the corticotropin-releasing hormone (CRH) into the portal blood vessels to trigger adrenocorticotropin (ACTH) release from the anterior pituitary (Antoni, 1993). ACTH reaches the adrenal gland through the systemic circulation to stimulate the synthesis and release of glucocorticoids. In addition to CRH, parvocellular cells can also synthesize and release arginine–vasopressin (AVP). AVP is also capable of triggering ACTH release after interaction with V1b receptors in the anterior pituitary, potentiating the effect of CRH (Antoni, 1993; Holmes et al., 2003).

Originally, the endocrine function of AVP was primarily linked to the salt and water homeostasis in mammals. The hypothalamic–neurohypophyseal system (HNS) (Cunningham and Sawchenko, 1991; Johnson et al., 1993; Kato et al., 1995) is constituted by magnocellular neurons located in the PVN and hypothalamic supraoptic nucleus (SON) sending their axons to the posterior pituitary, where AVP is released into the general circulation to control antidiuresis and vasoconstriction/glycogen metabolism. There is growing evidence that the HNS provides as a second neuronal–humoral system that is activated during stress (Hatton, 1990; Engelmann et al., 1998). Hyperactivity of the HNS may lead to a sustained somato-dendritic release of AVP into the extracellular fluid of both the PVN and SON (Engelmann et al., 1998). Under prolonged and repeated stressor exposure, the locally high neuropeptide concentrations may partially 'leak out of the nuclei' and reach remote limbic brain areas in neurophysiologically relevant concentrations (e.g. septum from the PVN or amygdala from the SON) (Engelmann et al., 2004; Landgraf, 2006). To differentiate between the roles of AVP signaling originating in the PVN vs. SON, repeated forced swimming (FS) was chosen as stressful stimulus. Previous studies showed that in response to repeated FS, AVP is increasingly released into the extracellular fluid of PVN, but not SON (Engelmann et al., 1998). In more remote brain areas, AVP acts as neuromodulator and neurotransmitter via local V1 receptors (Johnson et al., 1993; Lolait et al., 1995; Vaccari et al., 1998; Hernando et al., 2001; Stemmelin et al., 2005). Different lines of investigations confirmed that AVP acting in these brain areas regulates behavior including anxiety, social learning and memory processes (Stemmelin et al., 2005; Engelmann, 2008).

The literature is rich in articles describing the role of AVP in anxiety and depression (e.g. (Landgraf and Wigger, 2002; Scott and Dinan, 2002; Griebel et al., 2003; Keck, 2006; Landgraf, 2006; Frank and Landgraf, 2008; Simon et al., 2008; Surget and Belzung, 2008; Ryckmans, 2010). As both psychiatric disorders are considered to be stress related and AVP has a regulatory role in the HPA axis (Engelmann et al., 2004; Makara et al., 2004; Murgatroyd and Spengler, 2011), this connection is reasonable to propose. In particular, the strong connection between chronic stress and depression (see e.g. chronic mild stress as a model of depression (Duman, 2010; Yan et al., 2010; Overstreet, 2012)) and the observed upregulation of AVP synthesis in parvocellular PVN neurons and V1b receptors at the anterior pituitary during chronic stress (Dallman, 1993; Aguilera et al., 1994) suggested the involvement of altered AVP signaling within the HPA axis in pathophysiological processes. Clinical studies found an increased AVP concentration in the cerebrospinal fluid (De Bellis et al., 1993; Heuser et al., 1998) and in the plasma (van Londen et al., 1997, 1998; de Kloet et al., 2008) of depressive subjects, especially in suicide victims (Inder et al., 1997; Brunner et al., 2002). Postmortem studies have found an elevated number of AVP-expressing neurons, increased AVP level in the PVN (Purba et al., 1996; Merali et al., 2006) and an enhanced AVP mRNA in the SON (Meynen et al., 2006). Additionally, a single nucleotide polymorphism of the AVP V1b receptor has been found to protect against major depression (van West et al., 2004). Preclinical studies using the first orally active non-peptide V1b antagonist (SSR 149415) resulted in a reduction of floating in the FS test (Griebel et al., 2002). However, a clinical study using SSR 149415 was unable to proof an effect on anxiety; and there was only a mild, not always reproducible, effect on depressive symptoms (Griebel et al., 2012). Therefore the question arose about the role central V1 receptors may play in anxiety and depression. The fact that genetically AVP-deficient patients with diabetes insipidus show less anxious phenotype together with memory impairment (Bruins et al., 2006), and that diabetes insipidus is often accompanied by memory impairment in other patients as well (Nabe et al., 2007), suggests the possible involvement of

vasopressin signaling via peripheral V2 receptors in behavioral processes.

Rats of the AVP-deficient Brattleboro strain provide an interesting tool to study the role of AVP in neurophysiological processes. This strain was derived naturally from Long Evans rats in the early 1960s (Sokol and Valtin, 1965; Sokol and Zimmerman, 1982). A spontaneous point mutation resulted in an altered AVP precursor which was unable to enter the secretory pathway (Evans et al., 2000; Surget and Belzung, 2008). As a result, homozygous AVP-deficient Brattleboro rats (KO) show diminished HPA axis responses to different acute stressors (Wiley et al., 1974; Conte-Devolx et al., 1982; Fink et al., 1992), accompanied by reduced depressive- and anxiety-like behavioral profile (Mlynarik et al., 2007). More specifically, AVP-deficient rats showed higher preference for a sweet solution, spent considerably shorter time immobile in the FS test and less time self-grooming on the elevated plus maze (EPM) accompanied by a higher level of dexamethasone-induced suppression of corticosterone response to restraint stress. However, the lack of centrally produced AVP results in a variety of peripheral effects including *diabetes insipidus* that also affects motor behavior (Zelena et al., 2009b). All emotional and cognitive tests in laboratory rodents measure motor behavior, and the outcome is interpreted in terms of emotionality and learning and memory. This bears the risk that behavioral changes triggered by the physiological consequences of the absence of peripherally circulating AVP may be mis-interpreted as central actions of the neuropeptide.

The goal of the present study was to investigate whether behavioral changes observed in Brattleboro rats are due to the lack of AVP signaling in the central nervous system vs. its peripheral effects. As V2 receptors are expressed predominantly at the periphery e.g. in the renal collecting duct, we used a chronic subcutaneous treatment with a V2-receptor agonist, desmopressin (DDAVP; 1-deamino-8-D-arginine vasopressin) to compensate the peripheral lack of AVP (Robinson, 1976; Bankir, 2001; Makaryus and McFarlane, 2006). To analyze behavioral consequences of this treatment we used a battery of different tests primarily aimed at investigating emotional behavior and learning and memory that have not previously been applied in similar studies using Brattleboro rats and DDAVP treatment (Zelena et al., 2006; Feifel et al., 2007). Anxiety-related behavior was studied by EPM, marble burying test (MB), defensive withdrawal (DW) and anhedonia was detected by sucrose preference (Misslin and Cigrand, 1986). The FS test is routinely used to investigate the efficacy of antidepressant treatment to modulate the behavior in lab rodents (Porsolt et al., 1977, 1978). For examination of recognition memory we used the object discrimination test (OD) (Ennaceur and Delacour, 1988; Varga et al., 2014). We hypothesized that the AVP deficit in KO rats would lead to attenuated anxiety- and depressive-like behavior. If this effect is not causally linked to the symptoms of the *diabetes insipidus* then DDAVP treatment should not significantly alter this behavioral profile. We extended our study by analyzing the impact of FS on c-Fos synthesis in the lateral septum and amygdala as AVP signaling in both brain areas is known to modulate the behavioral response during the test in an opposite manner.

2. Material and methods

2.1. Animals

Adult male Brattleboro rats were obtained from the local breeding colony of the Institute of Experimental Medicine, Budapest, Hungary. The animals were kept under controlled laboratory conditions with food and water ad libitum. KO animals originated from heterozygous mothers and KO fathers (Zelena et al., 2003). The heterozygous and KO offspring were separated based on the measurement of their water consumption at 5 weeks of age, and only the KO animals were used in the present study. A separate wild-type (WT) line was bred out from the Brattleboro line because Long Evans rats could not be used as a proper control, having already been separated for more than 50 years (Bohus and de Wied, 1998). After mating heterozygous males and females and separating the homozygous KO animals from the offspring, the remaining WT or heterozygous animals were bred with KO males and females. For the selection of parent stocks the genotype of their offspring was determined by measuring their hypophyseal AVP content and those having no KO offspring were considered to be WT. The parents used for the WT and heterozygous line are closely related to each other (heterozygous mothers are the daughters of WT mothers), keeping the genetic background of the two lines as close as possible (Zelena et al., 2009a).

In the third examined group (DDAVP) KO rats were implanted subcutaneously with an osmotic minipump (Alzet osmotic minipump, 2002, 0.5 μl/h/14 days) containing desmopressin (DDAVP, 10 ng/h, V2 receptor agonist, Ferring Lieciva, Czech Republic) under short ether anesthesia (~3 min intervention). Concomitant WT and KO animals underwent sham operation. The KO, WT and DDAVP groups were paired according to their age (9–12-week-old at the beginning of experimentation), rather than according to body weight (body weights: WT: 379.9 ± 12.1 g, KO: 265 ± 6.6 g and DDAVP: 287.5 ± 6.5 g).

All manipulations of the animals were approved by the local committee for animal health and care and performed according to the European Communities Council Directive recommendations for the care and use of laboratory animals (2010/63/EU).

2.2. Experiments

2.2.1. Series 1

These experiments were performed with 30 animals (10 in each of the groups WT, KO and DDAVP). Within the first 24–48 h after implantation of the osmotic minipump water intake normalized (Figure 1A). Therefore, on day 4, after recovery, behavioral testing started. Three sessions in the defensive withdrawal (DW) apparatus (on day 4 morning, on day 5 morning and afternoon) were followed by two forced swimming (FS, one on each of day 6 and 7). On the following day (day 8) was another DW session combined with an airpuff as an aversive stimulus. Three days later (day 11), anhedonia was examined by sucrose/alcohol preference test for 24 h. Finally, 2 days later (day 14) animals were tested on the elevated plus-maze (EPM).

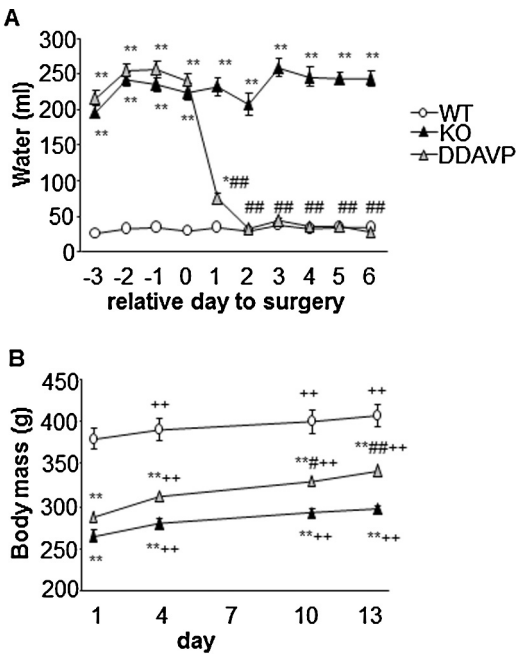

Figure 1 Effects of DDAVP treatment on distinct physiological parameter. (A) After the osmotic minipump implantation KO rats' (day 0) water consumption normalized within 1—2 days, thus the compensation of the peripheral lack of AVP was successful. (B) Body mass increased in all groups, and DDAVP treated group gain significantly more mass than KO rats. *Y* axis reflects the days after minipump implantation. Data from experimental series 1; $n = 10$ for each group; WT: wild type, KO: homozygous vasopressin-deficient rat; DDAVP: desmopressin treated KO rats; $^{*}p < 0.05$, $^{**}p < 0.01$: KO and DDAVP vs. WT; $^{\#\#}p < 0.01$: DDAVP vs. KO; $^{++}p < 0.01$ vs. first measurement

2.2.2. Series 2

These experiments involved a separate group of 15 animals (five in each of the groups WT, KO and DDAVP). First (day 5) an OD test was conducted followed by MB 2 days later (day 7). One week later (day 13), rats were exposed to FS and 2 h later deeply anesthetized and perfused for subsequent immunohistochemical analysis of the brain tissue (see below).

2.3. Behavioral tests

All tests (except MB and anhedonia, for details see below) were videotaped and analyzed later by an experimenter blind to the treatments by means of a computer-based event recorder (H77, Budapest, Hungary).

2.3.1. Defensive withdrawal

The DW test (Takahashi et al., 1989) used a modified setup of the open field activity box. This open field (100 cm × 100 cm with 50 cm side walls) contained a dark gray PVC tube (diameter 10 cm × 21 cm), dividing the total area into two compartments: an illuminated area and a dark area. The open end of the tube was faced one corner at a distance of 10 cm (Engelmann et al., 1996). The box and the tube were cleaned with tap water between the exposures. We analyzed the latency to leave the tube and the percentage of time spent in tube and in the open area (which is divided into center and periphery). A reduced time spent in open area and the elevation of the latency can be interpreted as anxiety-like behavior.

Before the 4th session in the DW apparatus we used an airpuff for a second to the rat's nose and eyes (VMD31 compressed air, Italy) (Engelmann et al., 1996).

2.3.2. Forced swimming

Rats were individually placed in a glass cylindrical tank (45 cm tall and 14 cm in diameter) filled with tap water (at 20 ± 1 °C) at a height of 30 cm. The animals were forced to swim for 10 min and then removed, gently dried with paper towels and returned to their home cages. We measured the percentage of time spent by the animal in a typical immobile posture (*floating*) during the swimming session (rated as depression-like behavior). Floating was defined as immobility of the animal with movements that keep its head above the water only (Porsolt et al., 1977). *Climbing/struggling* was defined as vigorous movements of the four limbs, with the fore paws breaking through the water's surface, along the wall of the tank (Wongwitdecha et al., 2006). *Swimming* differs from climbing that the rats make coordinated and sustained movements with all four limbs, usually traveling around the interior of the cylinder, but did not break the surface of the water (Bravo and Maswood, 2006). During *diving* the rats submerged entire head and body beneath the water surface (Arunrut et al., 2009; Pinter et al., 2011).

2.3.3. Anhedonia

Sucrose preference, alcohol preference, and total fluid consumption were measured using a two-bottle, free-choice test over a single 24 h period in the home cage (Fodor et al., 2012). One bottle contained 2.5% sucrose in water, whereas the other bottle contained 8% ethanol (ETOH) in a 2.5% sucrose—water solution.

2.3.4. Elevated plus maze

The EPM apparatus was made of metal and painted dark grey (elevated 70 cm above the floor, arm length: 50 cm; arm width: 15 cm; central platform: 15 cm × 15 cm; closed arm walls height: 40 cm). Each rat was transferred in the homecage from the housing room to the brightly lit (~50 Lux) test room. Immediately after arrival in the test room, the animal was placed on the central arena of the elevated plus maze, with the head facing a closed arm. EPM exposure lasted 5 min. The percentage of time spent in open arms and open/total (open plus closed) arm entries ratio (an entry was defined as having three paws of animal in a defined compartment) were calculated and used as measures of anxiety-like behavior. The number of closed arm entries was used to estimate the general locomotor activity of the animal.

2.3.5. Marble burying

The MB test was performed as a modified version (Li et al., 2006) of the test originally described for testing mice (Broekkamp et al., 1986; Njung'e and Handley, 1991; Borsini et al., 2002; Nicolas et al., 2006; Egashira et al., 2007). The rats were placed individually in a plexiglass cage (41.3 cm × 26 cm × 29.8 cm high, Ferplast, Geo Maxi, Italy) with a 5 cm deep layer of bedding holding 15 marbles on its

surface (25 mm diameter; arranged in a 3 × 5 pattern; space between two marbles ~4.5 cm). A session in the MB test lasted 30 min. A trained observer counted and noted every fifth minute the number of buried and half-buried marbles.

2.3.6. Object discrimination

One hour before the test rats were put into a plexiglass box (Ferplast, Geo Maxi, Italy) with food and water ad libitum to freely explore the new environment. Then one of two objects (i.e. glass jar: 6.5 cm diameter, 8.5 cm length and metal jar: 9.5 cm diameter, 4 cm length) was introduced in one of the corners of the box. The rat was allowed to explore this object (subsequently called 'familiar') for 4 min; then the object was removed. After 30 min, the 'familiar' object was placed together with the other (subsequently called 'non-familiar') object into the box for another 4 min. The objects and their location in the box (left—right) were randomized to avoid place preference. The discrimination index (DI) was calculated from the time spent exploring/sniffing the objects: DI = (investigation duration non-familiar − investigation duration familiar)/(investigation duration non-familiar + investigation duration familiar) (Varga et al., 2014).

2.4. Immunohistochemistry

Two hours after a single 10 min FS animals were anesthetized with a ketamin—xylazin—pipolphen cocktail (50—10—5 mg/kg in 2 ml/kg, intraperitoneal) and intracardially perfused with saline followed by 300 ml of 4% paraformaldehyde (0 °C). The brains were removed and post-fixed in the same solution at 4 °C overnight. On the following day brains were washed with phosphate-buffered saline (PBS: 1.56 g NaH_2PO_4, 30.8 g $NaHPO_4{\cdot}12H_2O$, 5 g NaCl and 0.2 g MgCl in 1 l distilled water) and transferred into 20% sucrose in PBS. After 2 days brains were removed from PBS, shock-frozen (−70 °C), packed in aluminium foil, and kept at −80 °C until sectioning. Frozen sections (30 μm) were cut in coronal plane on a sliding microtome and were put into antifreeze liquid at −20 °C.

The brain sections were processed for c-Fos immunoreactivity. Before the immunoreaction brain sections were washed 3 × 10 min with PBS, followed by 0.2% Triton-X-100 and H_2O_2 solutions to inactivate the endogenous peroxidase. After that sections were washed again with PBS for 3 × 5 min, then incubated in 2% normal Horse Serum for 20 min to block non-specific proteins activity, which followed by a new PBS washing for 3 × 10 min. Sections were incubated overnight in polyclonal rabbit primer antibody (Santa Cruz Biotechnology, USA, sc-52; 1:5000 dilution, 4 °C) followed by PBS washing for 3 × 10 min. The sections were then rinsed and exposed to an anti-rabbit secondary antibody (Jackson Immunoresearch Laboratories, 1:1000) for 1 h, then washed with PBS for 2 × 10 min. An avidin-biotin complex (Vectastain ABC kit, 1:1000) peroxidase procedure with nickel-diaminobenzidine as the chromogen was used to visualize the immunoreactivity. The sections were covered with DePeX.

Quantification of the c-Fos immunoreactivity was done in defined septal and amygdaloid nuclei. Section planes were standardized according to the atlas of Paxinos and Watson (Paxinos and Watson, 1998) by an experimenter blind to the treatment groups using sections matching the same coordinates of the brain atlas for each animal. Microscopic images were digitized by an OLYMPUS BX51 camera using a magnification 10×. The average of four sections taken at 80 μm intervals was used for the analysis of each structure, including both hemispheres; that is, eight measures per area and animal. To select c-Fos immunopositive nuclei as targets for quantification, a PC-based software (Image J, http://rsbweb.nih.gov/ij/) was used and targets were subsequently identified in the captured images by gray level thresholding. For each brain area, the threshold for the labeled signal was defined according to the background staining. Size criteria were applied to exclude structures other than c-Fos immunopositive nuclei from measurement, therefore only particles with a minimum area of 20 pixels were considered as a cell nucleus. The number of c-Fos positive nuclei was calculated as the average number obtained of all analyzed images per one rat.

2.5. Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using the ANOVA/MANOVA module of the STATISTICA 11.0 software package (Tulsa, OK, USA). One (factor 'group': WT, KO, DDAVP) or two way ANOVA (factors 'group' and 'time'; with repeated measures on the second factor) were applied. In case the main effect of ANOVA was significant multiple pairwise comparisons were made by the Newman—Keuls method. The difference of the discrimination index from 0 (no discrimination value) was tested with one sample *t* test. Pearson correlations were calculated by multiple linear regression analysis. Data are expressed as mean ± SEM and the level of significance was set at $p < 0.05$.

3. Results

3.1. Physiological parameters

The higher water consumption of KO vs. WT animals remained significant throughout (factor 'group': $p < 0.001$). Within 24—48 h after implantation of the minipumps water intake reached the level of the WT animals in DDAVP treated rats, illustrating the successful treatment of the peripheral symptoms of the diabetes insipidus (Figure 1A; both factor 'time' and 'group' × 'time' interaction: $p < 0.001$).

The body mass increased in the animals of all groups during the course of the experiment (factors 'group', 'time' and 'group' × 'time' interaction: $p < 0.001$). Initially, the body mass of the KO rats was ~30% lower than that of WT animals (first measurement, factor 'group': $p < 0.001$). Although in DDAVP treated animals the body mass increased significantly after onset of the treatment (last measurement, factor 'group': $p < 0.001$), it did not reach the level of the WT group until the end of the study (Figure 1B).

We also measured the mass of the adrenal glands, to obtain a parameter allowing some insight into the glucocorticoid supply. The absolute adrenal mass was significantly lower in KO than in WT animals (factor 'group': $p < 0.001$). However, if referenced to the body mass (calculated as

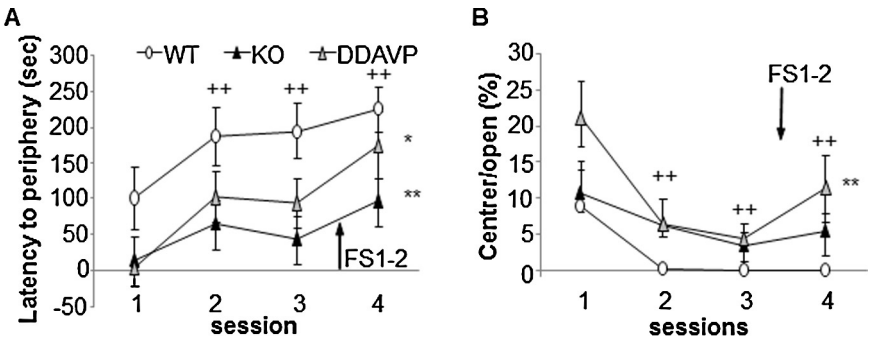

Figure 2 Effects of DDAVP treatment on behavioral parameters measured in the defensive withdrawal test combined with forced swim test and airpuff. (A) During the experiment the latency to enter the periphery increased after the first session then during the second and third test animals were habituated to this environment, and in these sessions latency did not change. After forced swim and airpuff the WT group stayed longer in the safe compartment than the other groups. (B) In the fourth session — after FS and airpuff exposure — WT rats spent significantly less time in the center of the DW apparatus compared to KO and DDAVP animals. Data from experimental series 1; $n = 10$ for each group; WT: wild type, KO: homozygous vasopressin-deficient rat; DDAVP: desmopressin treated KO rats; FS: forced swim test for 10 min; $^{*}p < 0.05$ $^{**}p < 0.01$: KO and DDAVP vs. WT main group effect; $^{++}p < 0.01$ vs. first session

relative organ mass) the difference between the groups disappeared (data not shown).

3.2. Behavioral parameters

3.2.1. DW test

Figure 2 shows the results obtained in the DW test. The first three sessions were used to habituate the animals to the DW apparatus. After habituation the animals swam twice for 10 min. The latter was done to trigger the release of AVP into the extracellular fluid of the PVN (Engelmann et al., 1998). Airpuff startle applied before the fourth DW session was previously reported to increase anxiety (Engelmann et al., 1996) and thought to enhance the differences between the treatment groups. Statistical analysis revealed that during the successive sessions the animals spent an increasing duration in the tube, and the latency to enter the periphery was increased (factor 'time': $p < 0.001$; Figure 2A). WT animals showed significantly increased latency to leave the tube compared to the other groups (factor 'group': $p < 0.01$). Specifically, the latency to leave the tube failed to differ between the DDAVP treated and KO rats during the sessions 1—3. After forced swimming and airpuff-startle, during session 4, the latency of leaving the tube in the DDAVP group did not differ significantly both from KO and WT animals (Figure 2A).

Another parameter extracted from the behavioral profile of the animals in the DW apparatus (quotient of the time rats spent in center/open field) reached nearly zero in WT animals during sessions 2—4 (factor 'session': $p < 0.001$; Figure 2B). In contrast, throughout these sessions DDAVP treated rats spend significantly more time in the center (factor 'group': $p = 0.01$). Also, KO animals tended to spent more time in the center ($p = 0.09$).

3.2.2. FS test

Statistical analysis revealed a significant difference between WT and KO rats during both the two FS sessions in Experiment 1 and the session in Experiment 2 (Figure 3; data are

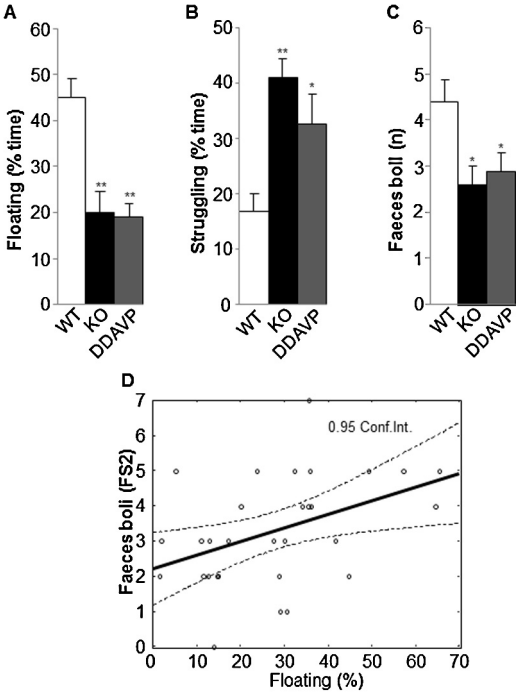

Figure 3 Effects of DDAVP treatment on the behavior in the forced swim test. This figure shows exemplarily the behavioral profile obtained during the second FS session. (A) WT rats showed significantly more floating *(depressive-like behavior)* than DDAVP treated and untreated KO rats. (B) Struggling revealed also a significant difference between groups. (C) The number of boli in the water differed significantly between WT and KO/DDAVP-treated rats. (D) Regression analysis of the floating time and the number of feces boli during the second session (raw data are on part A and C). Data from experimental series 1; $n = 10$ for each group; WT: wild type, KO: homozygous vasopressin-deficient rat; DDAVP: desmopressin treated KO rats; FS: forced swim test for 10 min; $^{*}p < 0.05$ $^{**}p < 0.01$: KO and DDAVP vs. WT

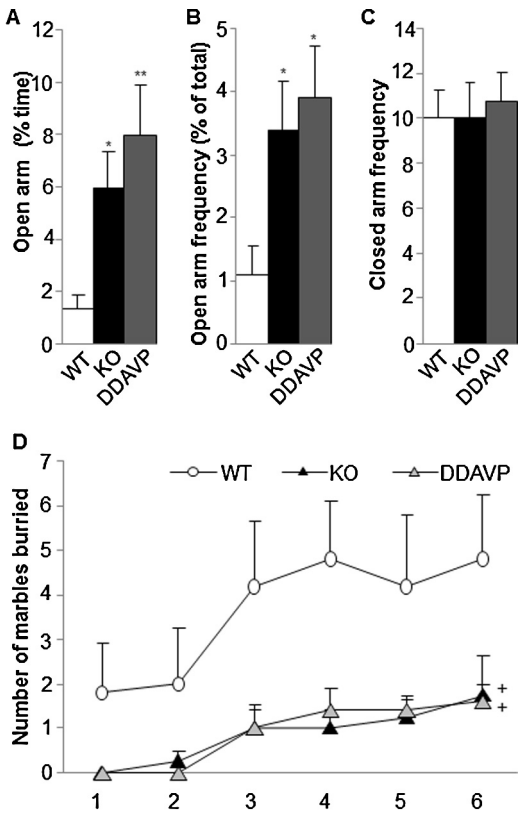

Figure 4 Effects of DDAVP treatment on anxiety-like behavioral responses in elevated plus maze (A—C) and marble burying test (D). (A) Percentage spent on open arm, (B) percentage of entries into the open arms and (C) absolute number of entries into the closed arms of the EPM. (D) Number of marbles buried. A—C: data from experimental series 1; $n = 10$ for each group; D: Data from experimental series 2; $n = 5$ for each group; WT: wild type, KO: homozygous vasopressin-deficient rat; DDAVP: desmopressin treated KO rats; $^{*}p < 0.05$; $^{**}p < 0.01$ KO and DDAVP vs. WT; $^{+}p < 0.05$ KO and DDAVP vs. WT main group effect

shown from session 2 of Exp. 1 and Figure 6C; Exp. 2) in the percentage of time spent floating (factor 'group': $p < 0.05$; Figures 3A and 6C). Subsequent analysis revealed that both DDAVP treated and KO rats spend significantly less time floating than WT animals. The time spend in active behavior was also significantly different between the groups, however, during Experiment 1 only struggling (factor 'group': first: $p = 0.002$; second: $p = 0.001$; Figure 3B), whereas during Experiment 2 only swimming (factor 'group': $p < 0.05$; Figure 6C) were detected to differ statistically significantly.

Interestingly, the number of faeces boli found at the end of the 10 min swimming session paralleled the floating behavior (Figure 3C and D). Namely this parameter was significantly lower in KO vs. WT animals (factor 'group': $p < 0.05$); but DDAVP failed to significantly alter the number of faeces boli when compared to KO animals. There was a positive correlation between the time spent floating and the number of faeces boli both during the first ($p = 0.02$; $r = 0.417$) and also during the second session ($p = 0.018$; $r = 0.427$).

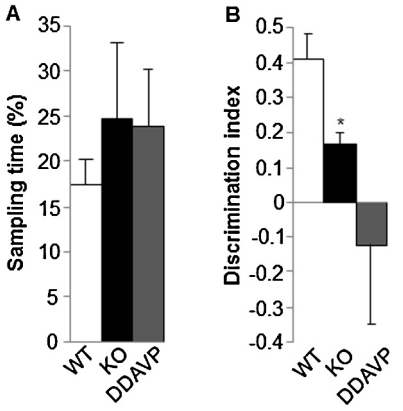

Figure 5 Effects of DDAVP treatment on short-term object discrimination. (A) All groups spent a similar duration investigating the to-be-recognized object during sampling. (B) Based on discrimination index (see Material and methods section) WT animals showed significantly better discrimination abilities than AVP deficient rats during choice. Data from experimental series 2; $n = 5$ for each group; WT: wild type, KO: homozygous vasopressin-deficient rat; DDAVP: desmopressin treated KO rats; $^{*}p < 0.05$ KO vs. WT

3.2.3. Anhedonia test

In contrast to previous reports (Mlynarik et al., 2007) we were unable to find an enhanced sucrose preference in KO animals (Supplementary Figure 1).

3.2.4. EPM test

Statistical analysis revealed that the percentage of time spent on the open arms differed significantly between the groups tested (factor 'group': $p < 0.01$; Figure 4A). Post hoc analysis showed that WT rats spent significantly less time on the open arm than KO or DDAVP treated groups; the two later groups did not differ in their reduced anxiety-like behavior. The other main parameter, the open arm frequency (percentage of open and close arm frequency as a locomotion independent measure) represented also a significant difference between groups (factor 'group': $p = 0.02$; Figure 4B). During the post hoc comparison the WT animals entered less to the open arm (lower frequency) than untreated or DDAVP treated KO rats. Locomotion, estimated upon the closed arm frequency, did not differ between the groups (Figure 4C).

3.2.5. Marble burying test

There was a significant difference between the three groups (factor 'group': $p = 0.012$) during the marble burying test, and the number of buried marbles depended upon the time (factor 'time': $p < 0.01$; Figure 4D). During the test the WT animals buried an increasing number of marbles, whereas KO and DDAVP rats buried 1—2 marbles only.

3.2.6. Object discrimination test

During the sampling session all rats spent enough time investigating the to-be-recognized object to become familiar with it (Figure 5A), showing no significant difference in the investigation duration between the different groups. As indicated by the significantly longer investigation duration of the novel object during the discrimination session WT animals showed intact discrimination abilities, while the DDAVP

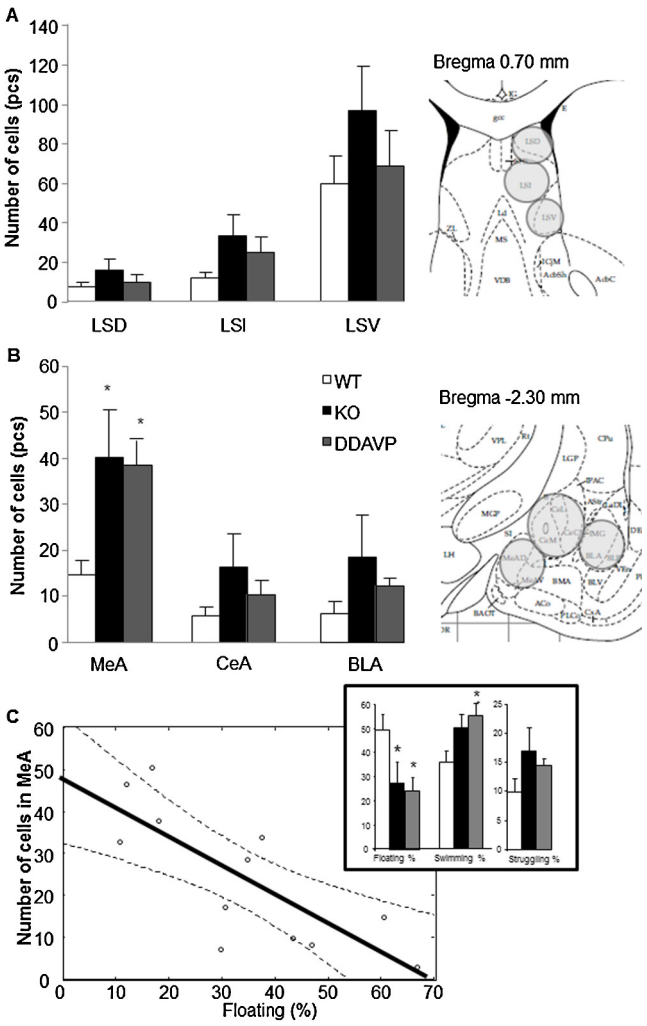

Figure 6 Effects of DDAVP treatment on the stressor exposure induced c-FOS synthesis in septum and amygdala. Number of positively c-Fos-stained cells in distinct nuclei of (A) the septum and (B) amygdala. (C) Regression analysis of the number of c-Fos labeled cells in the MeA and floating duration during FS (see inset for the raw behavioral data). Data from experimental series 2; $n = 5$ for each group; WT: wild type, KO: homozygous vasopressin-deficient rat; DDAVP: desmopressin treated KO rats; LSD: lateral septum dorsal part, LSI: lateral septum intermediate part, LSV: lateral septum ventral part, MeA: medial amygdala, CeA: central amygdala, BLA: basolateral amygdala; $^{*}p < 0.05$ KO and DDAVP vs. WT

group failed to do so (single sample *t* test; Figure 5B). There was a tendency for higher 'discrimination index' of WT animals compared to KO or DDAVP treated rats that just failed to reach statistical significance (factor 'group': $p = 0.05$).

3.2.7. c-Fos immunohistochemistry

After forced swim test we measured c-Fos immunohistochemistry in three distinct parts of both the lateral septum (dorsal: LSD, intermediate: LSI, ventral part: LSV) (Figure 6A) and the amygdala (medial: MeA, central: CeA, basolateral amygdala: BLA) (Figure 6B). The number of labeled cells was similar in all three groups in the different parts of the lateral septum (the highest number of labeled cells found in LSV). However, the number of labeled cells was found to be significantly different in MeA between WT and both KO and DDAVP groups (factor 'group': $p = 0.015$). Further, we found a significant correlation between the number of c-Fos labeled cells in MeA and both the time spent floating ($p = 0.003$; $r = -0.77$) and the time spend swimming ($p = 0.0074$; $r = -0.75$; Figure 6C). The correlation analysis of the other two parts of the amygdala showed a similar tendency, but it failed to reach the level of statistical significance.

4. Discussion

Our data confirm that DDAVP treatment compensated for the peripheral effects of AVP-deficiency and thereby eliminated the symptoms of the *diabetes insipidus*, indicated by the normalized water consumption and the increased body weight gain in DDAVP animals. As subsequently KO and DDAVP treated animals showed a different body weight but failed to differ in their behavioral profile it is unlikely that original differences seen between WT and KO animals can be simply related to body weight differences. Thus, our present results confirm previous findings suggesting an anxiolytic-like and a reduced depressive-like behavioral profile of KO rats (Mlynarik et al., 2007).

One of the most widely used tests for anxiety in laboratory rodents is the EPM. It was used to selectively bread high (HAB) and low anxiety (LAB) rat and mouse lines which were reported to show alterations in the AVP signaling that is probably responsible for high anxiety phenotype in HAB animals (Landgraf and Wigger, 2003; Murgatroyd et al., 2004, Kessler et al., 2007; Landgraf et al., 2007). Our results are in accordance with these studies as we found that the congenital absence of AVP in the brain is accompanied by lower anxiety-like behavioral profile, similarly to LAB animals. In a previous study on Brattleboro animals (Mlynarik et al., 2007) we did not find this difference, but in those experiments heterozygous animals were used as controls, while in the present study WT-animals were used. Nevertheless, the DW, which also tests innate fear from open spaces (Engelmann et al., 1996), showed also similar results, thereby confirming the results on the EPM in the present study. Another facet of innate fear, aimed at testing the aversive impact of novel objects, was studied by the MB test. Again we observed a significant difference between KO and DDAVP vs. WT Brattleboro rats with the latter burying significantly more marbles. Taken together, our data suggest that the behavioral changes observed in three different tests investigating distinct facets of innate fear cannot be corrected by treating the peripheral symptoms of *diabetes insipidus* and are, thus, primarily linked to the action of central AVP signaling. However, we cannot entirely exclude that the developmental effects of AVP-deficiency (Hammock, 2014) contribute to behavioral alterations which cannot be restored by acute/subacute treatment in adulthood. In this context it seems worth noting that different other systemic treatments (e.g. with antipsychotic drugs) were able to 'normalize' distinct behavioral parameters in Brattleboro rats (Feifel et al., 2009; Cilia et al., 2010).

A widely used test for antidepressant development is FS. Here we could repeatedly confirm more active behavioral

response in AVP-deficient Brattleboro rats. This is in line with the assumption that central AVP signaling may play an important role in the development of depressive pathology (Landgraf and Wigger, 2002; Scott and Dinan, 2002; Griebel et al., 2003; Keck, 2006; Landgraf, 2006; Frank and Landgraf, 2008; Simon et al., 2008; Surget and Belzung, 2008; Ryckmans, 2010). Infusion of V1a and V1b antagonists not only into systemic circulation (Overstreet and Griebel, 2005) but also into the PVN (Wigger et al., 2004), septum (Stemmelin et al., 2005) and the amygdala (Ebner et al., 2002) increased the active behavior during FS. Previous studies showed that in response to repeated FS AVP is released not only from the axon terminals into the blood stream, but also from the somata and dendrites of vasopressinergic neurons into the extracellular fluid of the PVN (Engelmann et al., 1998, 2004; Ebner et al., 2002; Wigger et al., 2004). As antisense mRNA of AVP into the septum decreased the anxiety-like behavior in rats, this brain region seemed to be the most important target of somatic-dendritic AVP release (Landgraf et al., 1995).

A particularly interesting finding of the present study concerns the observation of a significant correlation between the percentage of time spend floating and the increased number of c-Fos positive cells in the MeA (Figure 6). Previously it was shown that FS triggers AVP release within the amygdala complex and V1 receptor antagonist treatment significantly reduces floating and increased struggling behavior in male rats (Ebner et al., 2002). Due to technical limitations of the microdialysis technique, a more specific analysis which of the parts of the amygdala complex might have played a particular role in the observed effects was impossible. The present study confirms not only that blocked/absent AVP-signaling within this brain area causes a more active behavioral response during forced swimming, they also extend the previous findings by suggesting that the MeA and, perhaps to a lesser extent the CeA and BLA, plays an important role in the behavioral regulation during defined stressor exposure. Different studies suggested an involvement of amygdaloid V1b receptor in FS behavior (Salome et al., 2006) and V1a receptors in the amygdala, but not in the lateral septum, modulated the behavior in a paradigm for female anxiety (Poirier et al., 2013). In this context it is of note that V1a (Bielsky et al., 2004) and V1b (Egashira et al., 2005) receptor deficient mice failed to show a behavioral difference to WT animals in the FS test. These data suggest a species-specific effect of local AVP signaling.

Interestingly, the number of c-Fos positive cells was not significantly altered in the areas constituting the lateral septum of KO and DDAVP treated rats when compared with WT animals (Figure 6). This sheds new light on data of two other studies reporting a significant impact of V1 receptor antagonist treatment in the lateral septum (Ebner et al., 1999) vs. the MeA (Ebner et al., 2002) on FS behavior of adult male rats. Interestingly, in the septum the treatment effects were exactly the opposite than those observed in the MeA. Therefore, it was concluded that AVP controls the behavioral response in both brain areas that results in a balanced response during FS (Ebner et al., 2002). Upon first glance, the present findings could not only suggest that the MeA plays an important role in the control of FS behavior, but also that the congenital absence of AVP signaling at brain level does not result in a balanced behavioral response. However, a closer look reveals that the behavioral data obtained during FS do not show without doubt that the lack of AVP signaling in the amygdala 'overrules' the absence in the lateral septum. As a consequence, further studies have to analyze whether the behavioral profile is indeed primarily controlled by the 'septum vs. MeA balance' or the result of a downstream manifestation of altered, behaviorally relevant AVP signaling elsewhere in the brain. Therefore, it would be worth testing whether during acute stressor exposure somatic-dendritic release of AVP from the SON vs. PVN might influence the behavioral response in addition to local signaling (Ebner et al., 2002; Ludwig and Leng, 2006).

Anhedonia was suggested to be one of the core symptoms of depression (Di Giannantonio and Martinotti, 2012; Harden et al., 2012; Hill et al., 2012; Warnock et al., 2012). Our experiment investigating anhedonia in KO rats failed to reveal significant differences between the groups. This observation, however, may be hampered by the physiologically disturbed fluid consumption in KO animals and, therefore, does not provide an unbiased outcome.

Taking into consideration the high prevalence of cognitive disturbances in depression (Murrough et al., 2011) we also studied this aspect of our rats. The short term memory and learning processes were examined by object discrimination, as we did not wanted to confound our results with potential social alterations (Neumann and Landgraf, 2012). We confirmed the positive effect of centrally released AVP in short-term recognition memory. Previous studies used social discrimination tests in Brattleboro rats and showed that AVP injection to the septum improved their social discrimination skills (Dantzer and Bluthe, 1993; Engelmann and Landgraf, 1994; Landgraf et al., 1995). Thus, our data extend the findings using conspecifics as stimuli and show that the absence of central AVP signaling results in an impaired short-term recognition memory also towards objects.

5. Conclusions

In summary, male AVP-deficient Brattleboro rats showed in several tests (DW, FS, EPM, MB) a behavioral profile that can be interpreted as reduced anxiety and/or depressive-like which was not 'normalized' after peripheral replacement of AVP by chronic DDAVP treatment acting via V2 receptors. In addition, KO animals showed—similar to previously reports—-short-term recognition memory deficits. As the DDAVP treated animals behaved similarly to untreated KO rats in all studied tests, it is quite probable that the effect of AVP on affective and memory symptoms is due to its central (neuromodulator/neurotransmitter via V1a and V1b receptors) rather than peripheral role (antidiuretic hormone via V2 receptors). Further studies have to reveal whether indeed the MeA, rather than the lateral septum contributes primarily to the behavioral effects of intracerebrally signaling AVP, e.g. after its release from the somata-dendrites during defined stressor exposure.

Role of the funding source

Supported by DFG grants EN 366/6-1 and EN 366/8-1, Germany and OTKA grants F 48783, IN 67249 and NN 71629,

Hungary. The agencies had no further role in study design; in the collection, analysis or interpretation of the data.

Conflict of interest

We disclose any possible conflict of interest in the conduct and reporting of research.

Acknowledgments

The authors wish to thank Rita Murau for expert technical assistance. All listed authors have contributed significantly to the manuscript and consent to their names on the manuscript. We thank Dr John Menzies (Edinburgh) for polishing the English, and we disclose any possible conflict of interest in the conduct and reporting of research.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2014.09.011.

References

Aguilera, G., Pham, Q., Rabadan-Diehl, C., 1994. Regulation of pituitary vasopressin receptors during chronic stress: relationship to corticotroph responsiveness. J. Neuroendocrinol. 6, 299—304.

Antoni, F.A., 1993. Vasopressinergic control of pituitary adrenocorticotropin secretion comes of age. Front. Neuroendocrinol. 14, 76—122.

Arunrut, T., Alejandre, H., Chen, M., Cha, J., Russo-Neustadt, A., 2009. Differential behavioral and neurochemical effects of exercise, reboxetine and citalopram with the forced swim test. Life Sci. 84, 584—589.

Bankir, L., 2001. Antidiuretic action of vasopressin: quantitative aspects and interaction between V1a and V2 receptor-mediated effects. Cardiovasc. Res. 51, 372—390.

Bielsky, I.F., Hu, S.B., Szegda, K.L., Westphal, H., Young, L.J., 2004. Profound impairment in social recognition and reduction in anxiety-like behavior in vasopressin V1a receptor knockout mice. Neuropsychopharmacology 29, 483—493.

Bohus, B., de Wied, D., 1998. The vasopressin deficient Brattleboro rats: a natural knockout model used in the search for CNS effects of vasopressin. Prog. Brain Res. 119, 555—573.

Borsini, F., Podhorna, J., Marazziti, D., 2002. Do animal models of anxiety predict anxiolytic-like effects of antidepressants? Psychopharmacology (Berl). 163, 121—141.

Bravo, G., Maswood, S., 2006. Acute treatment with 5-HT3 receptor antagonist, tropisetron, reduces immobility in intact female rats exposed to the forced swim test. Pharmacol. Biochem. Behav. 85, 362—368.

Broekkamp, C.L., Rijk, H.W., Joly-Gelouin, D., Lloyd, K.L., 1986. Major tranquillizers can be distinguished from minor tranquillizers on the basis of effects on marble burying and swim-induced grooming in mice. Eur. J. Pharmacol. 126, 223—229.

Bruins, J., Kovacs, G.L., Abbes, A.P., Burbach, J.P., van den Akker, E.L., Engel, H., Franken, A.A., de Wied, D., 2006. Minor disturbances in central nervous system function in familial neurohypophysial diabetes insipidus. Psychoneuroendocrinology 31, 80—91.

Brunner, J., Keck, M.E., Landgraf, R., Uhr, M., Namendorf, C., Bronisch, T., 2002. Vasopressin in CSF and plasma in depressed suicide attempters: preliminary results. Eur. Neuropsychopharmacol. 12, 489—494.

Chrousos, G.P., 1998. Stressors, stress, and neuroendocrine integration of the adaptive response. The 1997 Hans Selye Memorial Lecture. Ann. N. Y. Acad. Sci. 851, 311—335.

Chrousos, G.P., Gold, P.W., 1992. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. J. Am. Med. Assoc. 267, 1244—1252.

Cilia, J., Gartlon, J.E., Shilliam, C., Dawson, L.A., Moore, S.H., Jones, D.N., 2010. Further neurochemical and behavioural investigation of Brattleboro rats as a putative model of schizophrenia. J. Psychopharmacol. 24, 407—419.

Conte-Devolx, B., Oliver, C., Giraud, P., Castanas, E., Boudouresque, F., Gillioz, P., Millet, Y., 1982. Adrenocorticotropin, and corticosterone secretion in Brattleboro rats. Endocrinology 110, 2097—2100.

Cunningham Jr., E.T., Sawchenko, P.E., 1991. Reflex control of magnocellular vasopressin and oxytocin secretion. Trends Neurosci. 14, 406—411.

Dallman, M.F., 1993. Stress update adaptation of the hypothalamic—pituitary—adrenal axis to chronic stress. Trends Endocrinol. Metab. 4, 62—69.

Dantzer, R., Bluthe, R.M., 1993. Vasopressin and behavior: from memory to olfaction. Regul. Pept. 45, 121—125.

De Bellis, M.D., Gold, P.W., Geracioti Jr., T.D., Listwak, S.J., Kling, M.A., 1993. Association of fluoxetine treatment with reductions in CSF concentrations of corticotropin-releasing hormone and arginine vasopressin in patients with major depression. Am. J. Psychiatry 150, 656—657.

de Kloet, C.S., Vermetten, E., Geuze, E., Wiegant, V.M., Westenberg, H.G., 2008. Elevated plasma arginine vasopressin levels in veterans with posttraumatic stress disorder. J. Psychiatr. Res. 42, 192—198.

Di Giannantonio, M., Martinotti, G., 2012. Anhedonia and major depression: the role of agomelatine. Eur. Neuropsychopharmacol. 22 (Suppl 3), S505L 10.

Duman, C.H., 2010. Models of depression. Vitam. Horm. 82, 1—21.

Ebner, K., Wotjak, C.T., Holsboer, F., Landgraf, R., Engelmann, M., 1999. Vasopressin released within the septal brain area during swim stress modulates the behavioural stress response in rats. Eur. J. Neurosci. 11, 997—1002.

Ebner, K., Wotjak, C.T., Landgraf, R., Engelmann, M., 2002. Forced swimming triggers vasopressin release within the amygdala to modulate stress-coping strategies in rats. Eur. J. Neurosci. 15, 384—388.

Egashira, N., Tanoue, A., Higashihara, F., Fuchigami, H., Sano, K., Mishima, K., Fukue, Y., Nagai, H., Takano, Y., Tsujimoto, G., Stemmelin, J., Griebel, G., Iwasaki, K., Ikeda, T., Nishimura, R., Fujiwara, M., 2005. Disruption of the prepulse inhibition of the startle reflex in vasopressin V1b receptor knockout mice: reversal by antipsychotic drugs. Neuropsychopharmacology 30, 1996—2005.

Egashira, N., Tanoue, A., Matsuda, T., Koushi, E., Harada, S., Takano, Y., Tsujimoto, G., Mishima, K., Iwasaki, K., Fujiwara, M., 2007. Impaired social interaction and reduced anxiety-related behavior in vasopressin V1a receptor knockout mice. Behav. Brain Res. 178, 123—127.

Engelmann, M., 2008. Vasopressin in the septum: not important versus causally involved in learning and memory—two faces of the same coin? Prog. Brain Res. 170, 389—395.

Engelmann, M., Ebner, K., Wotjak, C.T., Landgraf, R., 1998. Endogenous oxytocin is involved in short-term olfactory memory in female rats. Behav. Brain Res. 90, 89—94.

Engelmann, M., Landgraf, R., 1994. Microdialysis administration of vasopressin into the septum improves social recognition in Brattleboro rats. Physiol. Behav. 55, 145—149.

Engelmann, M., Landgraf, R., Wotjak, C.T., 2004. The hypothalamic-neurohypophysial system regulates the

hypothalamic—pituitary—adrenal axis under stress: an old concept revisited. Front. Neuroendocrinol. 25, 132—149.
Engelmann, M., Thrivikraman, K.V., Su, Y., Nemeroff, C.B., Montkowski, A., Landgraf, R., Holsboer, F., Plotsky, P.M., 1996. Endocrine and behavioral effects of airpuff-startle in rats. Psychoneuroendocrinology 21, 391—400.
Ennaceur, A., Delacour, J., 1988. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behav. Brain Res. 31, 47—59.
Evans, D.A., De Bree, F.M., Nijenhuis, M., Van Der Kleij, A.A., Zalm, R., Korteweg, N., Van Leeuwen, F.W., Burbach, J.P., 2000. Processing of frameshifted vasopressin precursors. J. Neuroendocrinol. 12, 685—693.
Feifel, D., Melendez, G., Priebe, K., Shilling, P.D., 2007. The effects of chronic administration of established and putative antipsychotics on natural prepulse inhibition deficits in Brattleboro rats. Behav. Brain Res. 181, 278—286.
Feifel, D., Mexal, S., Melendez, G., Liu, P.Y., Goldenberg, J.R., Shilling, P.D., 2009. The brattleboro rat displays a natural deficit in social discrimination that is restored by clozapine and a neurotensin analog. Neuropsychopharmacology 34, 2011—2018.
Fink, G., Dow, R.C., Casley, D., Johnston, C.I., Bennie, J., Carroll, S., Dick, H., 1992. Atrial natriuretic peptide is involved in the ACTH response to stress and glucocorticoid negative feedback in the rat. J. Endocrinol. 135, 37—43.
Fodor, A., Klausz, B., Pinter, O., Daviu, N., Rabasa, C., Rotllant, D., Balazsfi, D., Kovacs, K.B., Nadal, R., Zelena, D., 2012. Maternal neglect with reduced depressive-like behavior and blunted c-fos activation in Brattleboro mothers, the role of central vasopressin. Horm. Behav. 62, 539—551.
Frank, E., Landgraf, R., 2008. The vasopressin system—from antidiuresis to psychopathology. Eur. J. Pharmacol. 583, 226—242.
Griebel, G., Beeske, S., Stahl, S.M., 2012. The vasopressin V(1b) receptor antagonist SSR149415 in the treatment of major depressive and generalized anxiety disorders: results from 4 randomized, double-blind, placebo-controlled studies. J. Clin. Psychiatry 73, 1403—1411.
Griebel, G., Simiand, J., Serradeil-Le Gal, C., Wagnon, J., Pascal, M., Scatton, B., Maffrand, J.P., Soubrie, P., 2002. Anxiolytic- and antidepressant-like effects of the non-peptide vasopressin V1b receptor antagonist, SSR149415, suggest an innovative approach for the treatment of stress-related disorders. Proc. Natl. Acad. Sci. U S A 99, 6370—6375.
Griebel, G., Simiand, J., Stemmelin, J., Gal, C.S., Steinberg, R., 2003. The vasopressin V1b receptor as a therapeutic target in stress-related disorders. Curr. Drug Targets CNS Neurol. Disord. 2, 191—200.
Hammock, E.A., 2014. Developmental perspectives on oxytocin and vasopressin. Neuropsychopharmacology, http://dx.doi.org/10.1038/npp.2014.120.
Harden, M.T., Smith, S.E., Niehoff, J.A., McCurdy, C.R., Taylor, G.T., 2012. Antidepressive effects of the kappa-opioid receptor agonist salvinorin A in a rat model of anhedonia. Behav. Pharmacol. 23, 710—715.
Hatton, G.I., 1990. Emerging concepts of structure-function dynamics in adult brain: the hypothalamo-neurohypophysial system. Prog. Neurobiol. 34, 437—504.
Hernando, F., Schoots, O., Lolait, S.J., Burbach, J.P., 2001. Immunohistochemical localization of the vasopressin V1b receptor in the rat brain and pituitary gland: anatomical support for its involvement in the central effects of vasopressin. Endocrinology 142, 1659—1668.
Heuser, I., Bissette, G., Dettling, M., Schweiger, U., Gotthardt, U., Schmider, J., Lammers, C.H., Nemeroff, C.B., Holsboer, F., 1998. Cerebrospinal fluid concentrations of corticotropin-releasing hormone, vasopressin, and somatostatin in depressed patients and healthy controls: response to amitriptyline treatment. Depress. Anxiety 8, 71—79.
Hill, M.N., Hellemans, K.G., Verma, P., Gorzalka, B.B., Weinberg, J., 2012. Neurobiology of chronic mild stress: parallels to major depression. Neurosci. Biobehav. Rev. 36, 2085—2117.
Holmes, A., Heilig, M., Rupniak, N.M., Steckler, T., Griebel, G., 2003. Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. Trends Pharmacol. Sci. 24, 580—588.
Holsboer, F., 2000. The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology 23, 477—501.
Holsboer, F., Ising, M., 2010. Stress hormone regulation: biological role and translation into therapy. Annu. Rev. Psychol. 61, 81—109 (C1—11).
Inder, W.J., Donald, R.A., Prickett, T.C., Frampton, C.M., Sullivan, P.F., Mulder, R.T., Joyce, P.R., 1997. Arginine vasopressin is associated with hypercortisolemia and suicide attempts in depression. Biol. Psychiatry 42, 744—747.
Johnson, A.E., Audigier, S., Rossi, F., Jard, S., Tribollet, E., Barberis, C., 1993. Localization and characterization of vasopressin binding sites in the rat brain using an iodinated linear AVP antagonist. Brain Res. 622, 9—16.
Kato, Y., Igarashi, N., Hirasawa, A., Tsujimoto, G., Kobayashi, M., 1995. Distribution and developmental changes in vasopressin V2 receptor mRNA in rat brain. Differentiation 59, 163—169.
Keck, M.E., 2006. Corticotropin-releasing factor, vasopressin and receptor systems in depression and anxiety. Amino Acids 31, 241—250.
Kessler, M.S., Murgatroyd, C., Bunck, M., Czibere, L., Frank, E., Jacob, W., Horvath, C., Muigg, P., Holsboer, F., Singewald, N., Spengler, D., Landgraf, R., 2007. Diabetes insipidus and, partially, low anxiety-related behaviour are linked to a SNP-associated vasopressin deficit in LAB mice. Eur. J. Neurosci. 26, 2857—2864.
Landgraf, R., 2006. The involvement of the vasopressin system in stress-related disorders. CNS Neurol. Disord. Drug Targets 5, 167—179.
Landgraf, R., Gerstberger, R., Montkowski, A., Probst, J.C., Wotjak, C.T., Holsboer, F., Engelmann, M., 1995. V1 vasopressin receptor antisense oligodeoxynucleotide into septum reduces vasopressin binding, social discrimination abilities, and anxiety-related behavior in rats. J. Neurosci. 15, 4250—4258.
Landgraf, R., Kessler, M.S., Bunck, M., Murgatroyd, C., Spengler, D., Zimbelmann, M., Nussbaumer, M., Czibere, L., Turck, C.W., Singewald, N., Rujescu, D., Frank, E., 2007. Candidate genes of anxiety-related behavior in HAB/LAB rats and mice: focus on vasopressin and glyoxalase-I. Neurosci. Biobehav. Rev. 31, 89—102.
Landgraf, R., Wigger, A., 2003. Born to be anxious: neuroendocrine and genetic correlates of trait anxiety in HAB rats. Stress 6, 111—119.
Landgraf, R., Wigger, A., 2002. High vs low anxiety-related behavior rats: an animal model of extremes in trait anxiety. Behav. Genet. 32, 301—314.
Li, X., Morrow, D., Witkin, J.M., 2006. Decreases in nestlet shredding of mice by serotonin uptake inhibitors: comparison with marble burying. Life Sci. 78, 1933—1939.
Lolait, S.J., O'Carroll, A.M., Mahan, L.C., Felder, C.C., Button, D.C., Young 3rd, W.S., Mezey, E., Brownstein, M.J., 1995. Extrapituitary expression of the rat V1b vasopressin receptor gene. Proc. Natl. Acad. Sci. U S A 92, 6783—6787.
Ludwig, M., Leng, G., 2006. Dendritic peptide release and peptide-dependent behaviours. Nat. Rev. Neurosci. 7, 126—136.
Makara, G.B., Mergl, Z., Zelena, D., 2004. The role of vasopressin in hypothalamo—pituitary—adrenal axis activation during stress: an assessment of the evidence. Ann. N. Y. Acad. Sci. 1018, 151—161.
Makaryus, A.N., McFarlane, S.I., 2006. Diabetes insipidus: diagnosis and treatment of a complex disease. Clevel. Clin. J. Med. 73, 65—71.

McEwen, B.S., 2000. The neurobiology of stress: from serendipity to clinical relevance. Brain Res. 886, 172—189.

Merali, Z., Kent, P., Du, L., Hrdina, P., Palkovits, M., Faludi, G., Poulter, M.O., Bedard, T., Anisman, H., 2006. Corticotropin-releasing hormone, arginine vasopressin, gastrin-releasing peptide, and neuromedin B alterations in stress-relevant brain regions of suicides and control subjects. Biol. Psychiatry 59, 594—602.

Meynen, G., Unmehopa, U.A., van Heerikhuize, J.J., Hofman, M.A., Swaab, D.F., Hoogendijk, W.J., 2006. Increased arginine vasopressin mRNA expression in the human hypothalamus in depression: a preliminary report. Biol. Psychiatry 60, 892—895.

Misslin, R., Cigrand, M., 1986. Does neophobia necessarily imply fear or anxiety? Behav. Proc. 42, 45—50.

Mlynarik, M., Zelena, D., Bagdy, G., Makara, G.B., Jezova, D., 2007. Signs of attenuated depression-like behavior in vasopressin deficient Brattleboro rats. Horm. Behav. 51, 395—405.

Murgatroyd, C., Spengler, D., 2011. Epigenetic programming of the HPA axis: early life decides. Stress 14, 581—589.

Murgatroyd, C., Wigger, A., Frank, E., Singewald, N., Bunck, M., Holsboer, F., Landgraf, R., Spengler, D., 2004. Impaired repression at a vasopressin promoter polymorphism underlies overexpression of vasopressin in a rat model of trait anxiety. J. Neurosci. 24, 7762—7770.

Murrough, J.W., Iacoviello, B., Neumeister, A., Charney, D.S., Iosifescu, D.V., 2011. Cognitive dysfunction in depression: neurocircuitry and new therapeutic strategies. Neurobiol. Learn. Mem. 96, 553—563.

Nabe, K., Honjo, S., Ikeda, H., Wada, Y., Nomura, K., Hamamoto, Y., Koh, T., Tatsuoka, Y., Koshiyama, H., 2007. Diabetes insipidus and cognitive function. Med. Hypotheses 69, 764—766.

Neumann, I.D., Landgraf, R., 2012. Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. Trends Neurosci. 35, 649—659.

Nicolas, L.B., Kolb, Y., Prinssen, E.P., 2006. A combined marble burying-locomotor activity test in mice: a practical screening test with sensitivity to different classes of anxiolytics and antidepressants. Eur. J. Pharmacol. 547, 106—115.

Njung'e, K., Handley, S.L., 1991. Evaluation of marble-burying behavior as a model of anxiety. Pharmacol. Biochem. Behav. 38, 63—67.

Overstreet, D.H., 2012. Modeling depression in animal models. Methods Mol. Biol. 829, 125—144.

Overstreet, D.H., Griebel, G., 2005. Antidepressant-like effects of the vasopressin V1b receptor antagonist SSR149415 in the Flinders sensitive line rat. Pharmacol. Biochem. Behav. 82, 223—227.

Paxinos, G., Watson, C., 1998. The Rat Brain in Stereotaxic Coordinates, Fourth Edition. Academic Press, San Diego.

Pinter, O., Domokos, A., Mergl, Z., Mikics, E., Zelena, D., 2011. Do stress hormones connect environmental effects with behavior in the forced swim test? Endocr. J. 58, 395—407.

Poirier, G.L., Cordero, M.I., Sandi, C., 2013. Female vulnerability to the development of depression-like behavior in a rat model of intimate partner violence is related to anxious temperament, coping responses, and amygdala vasopressin receptor 1a expression. Front. Behav. Neurosci. 7, 35.

Porsolt, R.D., Anton, G., Blavet, N., Jalfre, M., 1978. Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur. J. Pharmacol. 47, 379—391.

Porsolt, R.D., Le Pichon, M., Jalfre, M., 1977. Depression: a new animal model sensitive to antidepressant treatments. Nature 266, 730—732.

Purba, J.S., Hoogendijk, W.J., Hofman, M.A., Swaab, D.F., 1996. Increased number of vasopressin- and oxytocin-expressing neurons in the paraventricular nucleus of the hypothalamus in depression. Arch. Gen. Psychiatry 53, 137—143.

Robinson, A.G., 1976. DDAVP in the treatment of central diabetes insipidus. N. Engl. J. Med. 294, 507—511.

Ryckmans, T., 2010. Modulation of the vasopressin system for the treatment of CNS diseases. Curr. Opin. Drug Discov. Dev. 13, 538—547.

Salome, N., Stemmelin, J., Cohen, C., Griebel, G., 2006. Differential roles of amygdaloid nuclei in the anxiolytic- and antidepressant-like effects of the V1b receptor antagonist, SSR149415, in rats. Psychopharmacology (Berl) 187, 237—244.

Scott, L.V., Dinan, T.G., 2002. Vasopressin as a target for antidepressant development: an assessment of the available evidence. J. Affect Disord. 72, 113—124.

Simon, N.G., Guillon, C., Fabio, K., Heindel, N.D., Lu, S.F., Miller, M., Ferris, C.F., Brownstein, M.J., Garripa, C., Koppel, G.A., 2008. Vasopressin antagonists as anxiolytics and antidepressants: recent developments. Recent Pat. CNS Drug Discov. 3, 77—93.

Sokol, H.W., Valtin, H., 1965. Morphology of the neurosecretory system in rats homozygous and heterozygous for hypothalamic diabetes insipidus (Brattleboro strain). Endocrinology 77, 692—700.

Sokol, H.W., Zimmerman, E.A., 1982. The hormonal status of the Brattleboro rat. Ann. N. Y. Acad. Sci. 394, 535—548.

Stemmelin, J., Lukovic, L., Salome, N., Griebel, G., 2005. Evidence that the lateral septum is involved in the antidepressant-like effects of the vasopressin V1b receptor antagonist, SSR149415. Neuropsychopharmacology 30, 35—42.

Surget, A., Belzung, C., 2008. Involvement of vasopressin in affective disorders. Eur. J. Pharmacol. 583, 340—349.

Takahashi, L.K., Kalin, N.H., Vanden Burgt, J.A., Sherman, J.E., 1989. Corticotropin-releasing factor modulates defensive-withdrawal and exploratory behavior in rats. Behav. Neurosci. 103, 648—654.

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

van Londen, L., Goekoop, J.G., van Kempen, G.M., Frankhuijzen-Sierevogel, A.C., Wiegant, V.M., van der Velde, E.A., De Wied, D., 1997. Plasma levels of arginine vasopressin elevated in patients with major depression. Neuropsychopharmacology 17, 284—292.

van Londen, L., Kerkhof, G.A., van den Berg, F., Goekoop, J.G., Zwinderman, K.H., Frankhuijzen-Sierevogel, A.C., Wiegant, V.M., de Wied, D., 1998. Plasma arginine vasopressin and motor activity in major depression. Biol. Psychiatry 43, 196—204.

van West, D., Del-Favero, J., Aulchenko, Y., Oswald, P., Souery, D., Forsgren, T., Sluijs, S., Bel-Kacem, S., Adolfsson, R., Mendlewicz, J., Van Duijn, C., Deboutte, D., Van Broeckhoven, C., Claes, S., 2004. A major SNP haplotype of the arginine vasopressin 1B receptor protects against recurrent major depression. Mol. Psychiatry 9, 287—292.

Varga, J., Klausz, B., Domokos, A., Kalman, S., Pakaski, M., Szucs, S., Garab, D., Zvara, A., Puskas, L., Kalman, J., Timar, J., Bagdy, G., Zelena, D., 2014. Increase in Alzheimer's related markers preceeds memory disturbances: studies in vasopressin-deficient Brattleboro rat. Brain Res. Bull. 100, 6—13.

Warnock, K.T., Yang, A.R., Yi, H.S., June Jr., H.L., Kelly, T., Basile, A.S., Skolnick, P., June, H.L., 2012. Amitifadine, a triple monoamine uptake inhibitor, reduces binge drinking and negative affect in an animal model of co-occurring alcoholism and depression symptomatology. Pharmacol. Biochem. Behav. 103, 111—118.

Wigger, A., Sanchez, M.M., Mathys, K.C., Ebner, K., Frank, E., Liu, D., Kresse, A., Neumann, I.D., Holsboer, F., Plotsky, P.M., Landgraf, R., 2004. Alterations in central neuropeptide expression, release, and receptor binding in rats bred for high anxiety: critical role of vasopressin. Neuropsychopharmacology 29, 1—14.

Wiley, M.K., Pearlmutter, A.F., Miller, R.E., 1974. Decreased adrenal sensitivity to ACTH in the vasopressin-deficient (Brattleboro) rat. Neuroendocrinology 14, 257—270.

Wongwitdecha, N., Kasemsook, C., Plasen, S., 2006. Social isolation alters the effect of desipramine in the rat forced swimming test. Behav. Brain Res. 167, 232—236.

Yan, H.C., Cao, X., Das, M., Zhu, X.H., Gao, T.M., 2010. Behavioral animal models of depression. Neurosci. Bull. 26, 327—337.

Zelena, D., Langnaese, K., Domokos, A., Pinter, O., Landgraf, R., Makara, G.B., Engelmann, M., 2009a. Vasopressin administration into the paraventricular nucleus normalizes plasma oxytocin and corticosterone levels in Brattleboro rats. Endocrinology 150, 2791—2798.

Zelena, D., Mergl, Z., Makara, G.B., 2003. Maternal genotype influences stress reactivity of vasopressin-deficient brattleboro rats. J. Neuroendocrinol. 15, 1105—1110.

Zelena, D., Mergl, Z., Makara, G.B., 2009b. Postnatal development in vasopressin deficient Brattleboro rats with special attention to the hypothalamo—pituitary—adrenal axis function: the role of maternal genotype. Int. J. Dev. Neurosci. 27, 175—183.

Zelena, D., Mergl, Z., Makara, G.B., 2006. The role of vasopressin in diabetes mellitus-induced hypothalamo-pituitary-adrenal axis activation: studies in Brattleboro rats. Brain Res. Bull. 69, 48—56.