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The effects of lactation on impulsive behavior in vasopressin-deficient Brattleboro rats

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ABSTRACT

Vasopressin (AVP)-deficient Brattleboro rats develop a specific behavioral profile, which—among other things—include altered cognitive performance. This profile is markedly affected by alterations in neuroendocrine state of the animal such as during lactation. Given the links between AVP and cognition we hypothesized that AVP deficiency may lead to changes in impulsivity that is under cognitive control and the changes might be altered by lactation. Comparing virgin and lactating AVP-deficient female Brattleboro rats to their respective controls, we assessed the putative lactation-dependent effects of AVP deficiency on impulsivity in the delay discounting paradigm. Furthermore, to investigate the basis of such effects, we assessed possible interactions of AVP deficiency with GABAergic and serotonergic signaling and stress axis activity, systems playing important roles in impulse control. Our results showed that impulsivity was unaltered by AVP deficiency in virgin rats. In contrast a lactation-induced increase in impulsivity was abolished by AVP deficiency in lactating females. We also found that chlordiazepoxide-induced facilitation of GABAergic and imipramine-induced enhancement of serotonergic activity in virgins led to increased and decreased impulsivity, respectively. In contrast, during lactation these effects were visible only in AVP-deficient rats. These rats also exhibited increased stress axis activity compared to virgin animals, an effect that was abolished by AVP deficiency. Taken together, AVP appears to play a role in the regulation of impulsivity exclusively during lactation: it has an impulsivity increasing effect which is potentially mediated *via* stress axis-dependent mechanisms and fine-tuning of GABAergic and serotonergic function.

Introduction

Arginine-vasopressin (AVP) is a peptide hormone produced in the supraoptic and paraventricular nuclei of the hypothalamus (Rhodes et al., 1981; Sokol et al., 1976). Its primary physiological function is to stimulate water retention by increasing the water permeability of the distal tubules of the kidneys (Flamion and Spring, 1990; Wade et al., 1981). However, AVP also acts at vasopressin receptors at several brain areas (Buijs et al., 1978) to regulate a number of neuroendocrine and behavioral processes (Antoni, 1993).

The Brattleboro homozygous recessive rat does not synthesize AVP (Bohus and de Wied, 1998) and is thus a useful model for studying the role of AVP in behavioral processes. Brattleboro rats develop a unique physiological and behavioral profile as a result of lacking a functioning AVP system. Among other things, these rats show normal baseline hypothalamus–pituitary–adrenal (HPA) axis activity and decreased HPA axis reactivity to a variety of stressors (Zelena et al., 2009), slightly reduced anxiety (Fodor et al., 2012) and depression-like behavior (Fodor et al., 2012; Mlynarik et al., 2007). Additionally, they display social deficits (Engelmann and Landgraf, 1994; Feifel et al., 2009; Schank, 2009) and impairments in cognitive performance (Aarde and Jentsch, 2006; Colombo et al., 1992; Varga et al., 2013). The behavioral effects of AVP deficiency are thought to depend on the neuroendocrine state of the individual, e.g. in several cases on the specific physiological conditions during lactation. For example, AVP deficiency does not alter baseline HPA axis activity in virgin females, while it dampens chronic hyperactivity of the HPA axis in lactating female rats (Fodor et al., 2013), an effect that contributes to maternal neglect and mild anxiolysis (Fodor et al., 2012).

Prior work has shown that cognitive performance can be altered by changes in impulsivity (Bizot and Thiebot, 1996). Impulsivity is generally characterized by a failure to resist a drive to respond to environmental stimuli (motor impulsivity) and by responses without consideration of alternatives and/or future consequences (choice impulsivity) (Evenden and Ryan, 1996; Kim and Lee, 2011; Solanto et al., 2001). While it is possible that impulsivity impacts cognitive performance, it is also probable that cognitive and various physiological processes affect impulsivity (Aron, 2007). Thus, as cognitive functions are altered in AVP-deficient rats, one might assume that impulsive behavior is also affected.

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In the present study, firstly we aimed to identify the effects of AVP deficiency on impulsive behavior. As AVP deficiency can alter behavioral processes in a lactation-dependent manner, we also studied possible interactions between AVP activity and lactation in the regulation of impulsivity using virgin and lactating female AVP-deficient and control Brattleboro rats. Specifically, we used the delay discounting paradigm to study impulsive behavior. In this paradigm preference of a delayed, large reward over a smaller, immediate reward is tested with the employment of an operant conditioning procedure. Typically, impulsive individuals tend to choose the latter type of reinforcer in similar paradigms (Adriani and Laviola, 2003; Adriani et al., 2003b; Bizot et al., 1999; Evenden and Ryan, 1996, 1999; Thiebot et al., 1985). Prior to investigations of impulsive behavior, we also assessed cognitive performance of AVP-deficient rats during the training phase of the delay discounting paradigm.

Showing that AVP deficiency decreases impulsivity in lactating rats, our second aim was to assess the basis of such effects. GABAergic and serotonergic signaling play important roles in the regulation of impulsive behavior; pharmacological manipulation of these systems leads to changes in impulsivity (Bizot et al., 1999; Evenden and Ko, 2005). As AVP activity was shown to alter both GABAergic and serotonergic function (Auerbach and Lipton, 1982; Hermes et al., 2000; Ramanathan et al., 2012; Schwarzberg et al., 1981; Wang et al., 2002), we studied whether AVP deficiency exerts its impulsivity altering effects *via* possible GABAergic and serotonergic interactions. To assess such interactions, we investigated impulsive behavior in AVP-deficient virgin and lactating female Brattleboro rats following treatment with a benzodiazepine, which has been reported to increase impulsivity (Evenden and Ko, 2005; Thiebot et al., 1985; Wolff and Leander, 2002), or a selective serotonin reuptake inhibitor, which has been reported to decrease impulsivity in several studies (Bizot et al., 1988; Miyazaki et al., 2011). In addition to measurements of impulsive behavior, HPA axis activity (i.e. corticosterone levels) was also assessed, as the HPA axis has been reported to be altered by AVP deficiency (Fodor et al., 2013; Makara et al., 2012) and to play a role in impulsivity (Torregrossa et al., 2012).

Material and methods

Subjects

We compared AVP-deficient homozygous female rats with homozygous control (+/+) rats. AVP-deficient and control Brattleboro rats came from a colony maintained in our Institute. The breeding stock was started from breeder rats provided by Harlan Laboratories (Indianapolis, USA). The parents of control rats were homozygous for the non-mutated gene, while AVP-deficient subjects originated from breeding pairs composed of AVP-deficient fathers and heterozygous mothers. Heterozygous mothers always derived from control and AVP-deficient parents, to keep the genetic background of the two lines close. Animals were kept on a light/dark cycle of 12 h with the lights on at 0700 h. The temperature and humidity were kept at 23 ± 2 °C and 60 ± 10%, respectively. Virgin female rats were isolated one week before the start of experimentation and housed individually until the end of all experiments. Female rats that were studied during lactation were mated at the age of 75–115 days and were isolated approximately one week before delivery. Female subjects mated with males of different homozygous genotype, i.e. AVP-deficient females mated with control males, while control females mated with AVP-deficient males. With this design the genotype of all pups was heterozygous; therefore, litter genotype did not differ between subjects and it could not alter maternal behavior. Virgin and lactating females were the same age at the time of experimentation. One day after delivery litters were culled to three males and three females to control for the behavioral effects of quantity and quality of pups. Pups were housed with their dam throughout the experiments (except for during experimentation in the delay discounting boxes). Tap water was available ad libitum. Rat chow was limited to 6 pellets a day (approximately 20 g total) to increase exploration during the delay discounting experiments. Food was provided immediately after the daily training/testing sessions. The weight of each rat was measured daily. Food restriction was adjusted where necessary to maintain the rats at a minimum of 80% of their starting weight. Pups were also evaluated daily to monitor their development. All animals survived experimentation and showed no sign of pain or discomfort throughout our studies. All experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were reviewed and approved by the Animal Welfare Committee of the Institute of Experimental Medicine, Budapest, Hungary.

Drugs and doses

The benzodiazepine, chlordiazepoxide (CDP), and the tryciclic antidepressant, imipramine (IMI), were dissolved in saline. These drugs were administered intraperitoneally 15 min (CDP) or 60 min (IMI) before the start of the experiment at a dose of 0 (vehicle) or 10 mg/kg in a volume of 1 ml/kg. The doses, volume, injection routes and pretreatment time were determined based on previous studies (Evenden and Ko, 2005; Evenden, 1998).

Delay discounting apparatus and procedure

Experiments assessing impulsive behavior were conducted using automated operant chambers equipped with two nose-poke holes with infrared sensors and LED lights, a chamber light and a feeder device with a magazine into which food pellets were dropped (Med Associates, St. Albans, VT, USA). Chambers were placed inside sound-attenuated wooden cubicles and were controlled via computers running Med-PC IV software (Med Associates, St. Albans, VT, USA).

During the training phase, animals were placed inside a chamber for 30 min daily for 5 days. A response on one of the nose-poke holes was rewarded with one 45 mg food pellet (small reward), while a response on the other hole resulted in five 45 mg food pellets (large reward). Both types of reward were presented immediately after the response and were followed by a 25 s timeout with the chamber light switched on. Chamber light was used as a cue which could be associated with the reward after responding on one of the nose-poke holes. It is a common practice to associate visual or auditory cues with the feedback to accelerate learning in operant conditioning procedures (Panlilio et al., 2012). During the timeout period, responses were not rewarded but were registered. To avoid side preference, the nose-poke hole on which responding was rewarded with five food pellets was randomly assigned to either the left or the right side between animals. Animals were placed in the same chamber with the same nose-poke hole side assignment throughout the experiment. After each session ended, the chambers were cleaned with 70% ethanol and were dried with paper towels. All experiments were conducted in the early hours of the light phase. At the end of the training phase, the animals were expected to respond on the nose-poke hole that was paired with the large reward in approximately 90% of all trials (Adriani et al., 2003a).

After two days of rest, the animals underwent the test phase. During this phase, each animal was placed in a chamber for 30 min daily for 8 days. The procedure was similar to that described for the training phase, but a delay was inserted before the large reward. The delay was fixed for each daily session and was increased progressively over subsequent days (10, 20, 30, 45, 60, 80, 100 and 120 s). Responses during these delays were not rewarded, but they were recorded by the software. Sessions of the test phase were conducted at the same time as sessions of the training phase. During the test phase, subjects were expected to shift their preference from the nose-poke hole rewarded by the delayed large reward to the nose-poke hole rewarded by the immediate small reward (Adriani and Laviola, 2003; Adriani et al., 2003b).

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During the training sessions, we recorded the preference of the nose-poke hole paired with the large reward (large reward preference) to assess learning capabilities. Increases of greater magnitude in large reward preference indicated quicker learning. During the test phase, large reward preferences were indicative of non-impulsive choices. This variable is negatively associated with choice impulsivity, which refers to an inability to prefer a larger, delayed reward over an immediate smaller one (Kim and Lee, 2011). Because a slight difference in large reward preference was observed between the treatment groups on the last day of the training phase (see Results), large reward preference during the test phase was calculated as a percentage of the large reward preference on the last day of training (%). With this method, we were able to assess the changes in large reward preference throughout the testing phase. The number of inadequate responses (the sum of responses during timeouts and delays), which reflects the number of premature, impulsive responses, was also evaluated. With this measure, we were able to assess motor impulsivity, which is defined as the inability to inhibit inappropriate actions (Kim and Lee, 2011).

Blood sampling and hormone measurements

For corticosterone measurements, trunk blood was collected in ice-cold plastic tubes following decapitation in the early hours of the light phase. After sampling, blood was centrifuged at 4 °C, and the serum was separated and stored at −20 °C until analysis. Serum corticosterone was measured in 10 μl unextracted serum by a radioimmunoassay (RIA) using a specific antibody developed in our institute as described earlier (Zelena et al., 2003). The corticosterone antibody was raised in rabbits against corticosterone-carboxymethyloxime bovine serum albumine. ^{125}I-labeled corticosterone-carboxymethyloxime-tyrosine methyl ester was used as tracer. The interference from plasma transcortin was eliminated by inactivating transcortin at a low pH. Assay sensitivity was 1 pmol. The intraassay coefficient of variation was 7.5%. All the samples from a particular experiment were measured in one RIA.

Experimental design

Each experiment was performed on a separate set of animals and was analyzed separately. All experiments were carried out in the early hours of the light phase.

Assessment of cognitive performance and impulsivity in virgin females

In *Experiment 1*, we examined learning and impulsive behavior in virgin AVP-deficient ($N = 10$) and control female Brattleboro rats ($N = 10$). Subjects underwent 4 days of partial food restriction then underwent the training phase of the delay discounting paradigm (assessment of cognitive performance), throughout which they were food restricted. The last day of the training phase was followed by two days of rest then subjects underwent the test phase of the delay discounting procedure. Animals underwent three additional daily sessions after the last day of the test phase with 120 s delay. Before these sessions, each animal received either an injection of vehicle, 10 mg/kg CDP or 10 mg/kg IMI in a random order, with one treatment each day. The effects of CDP and IMI on impulsivity were compared to the effects of control (vehicle) treatment received each day.

Assessment of cognitive performance and impulsivity in lactating females

Experiment 2 was conducted in a similar manner as described for *Experiment 1*, except that the subjects were control ($N = 10$) and AVP-deficient ($N = 10$) lactating female rats that had delivered 1–5 days before the first day of the test phase. Food restriction was started immediately after delivery.

Assessment of HPA axis activity in virgin and lactating females

In *Experiment 3*, we assessed HPA axis activity in two separate sets of virgin and lactating AVP-deficient and control female Brattleboro rats. The first set of animals consisted of control ($N = 12$) and AVP-deficient ($N = 12$) lactating rats from which blood was sampled on the 10th day after delivery and control ($N = 6$) and AVP-deficient ($N = 8$) virgin rats which were in the same age as lactating rats at the time of blood sampling. This time point had been selected to coincide with the lactation day at the end of the training phase of the delay discounting test (see *Experiments 1* and *2*). For the second set of animals blood sampling was carried out in a similar manner (experimental groups were lactating control ($N = 17$), lactating AVP-deficient ($N = 17$), virgin control ($N = 11$) and virgin AVP-deficient ($N = 16$)), except blood was sampled from lactating rats on the 20th days following delivery. Blood was sampled from virgin rats which were at the same age as lactating rats at the time of blood sampling.

Statistical analyses

Data were presented as the mean ± standard error of the mean. Behavioral variables of the delay discounting test (large reward preference and inadequate responses) were analyzed using repeated measures analysis of variance (ANOVA) (training and test phases of *Experiment 1* and *2*: factor 1: genotype; repeated factor: days or delay; during pharmacological treatments in *Experiment 1* and *2*: factor 1: genotype; repeated factor: treatment). Corticosterone levels were analyzed using factorial ANOVA (*Experiment 3*: factor 1: genotype; factor 2: lactation). ANOVA assumptions were evaluated by Levene's test. Duncan tests were performed for post-hoc analyses when a main effect was significant, and Bonferroni corrections were applied for multiple comparisons. *P* values less than 0.05 were considered statistically significant.

Results

Cognitive performance and impulsivity in AVP-deficient and control virgin females

Large reward preference was significantly increased throughout the training phase in virgin females ($F_{days}(4,72) = 16.1$; $p < 0.01$). Rats lacking AVP exhibited a reduced general preference for the large reward compared to control animals ($F_{genotype}(1,18) = 7.37$; $p = 0.01$) (Fig. 1). No significant interaction between days of the test phase and genotype was observed ($F_{genotype \times delay}(4,72) = 1.66$; $p = 0.17$).

Delay significantly decreased large reward preference in virgin female rats across days ($F_{delay}(7,126) = 13.25$; $p < 0.01$), but genotype failed to alter this variable ($F_{genotype}(1,18) = 1.35$; $p = 0.26$; $F_{genotype \times delay}(7,126) = 1.69$; $p = 0.12$) (Fig. 2a). The number of inadequate responses was also affected by delay, as they increased throughout the test phase ($F_{delay}(7,126) = 5.55$; $p < 0.01$), but it was not altered by genotype ($F_{genotype}(1,18) = 1.09$; $p = 0.31$; $F_{genotype \times delay}(7,126) = 1.03$; $p = 0.41$) (Fig. 2b).

In virgin females, large reward preference was unaltered by genotype, treatment or the interaction between these two factors ($F_{genotype}(1,18) = 1.06$; $p = 0.32$; $F_{treatment}(2,36) = 0.52$; $p = 0.6$; $F_{genotype \times treatment}(2,36) = 0.89$; $p = 0.42$) during the three-day period of pharmacological treatments (Fig. 2c). Inadequate responses were unchanged by genotype ($F_{genotype}(1,18) = 0.3$; $p = 0.59$), but they were significantly altered by treatment ($F_{treatment}(2,36) = 19.67$; $p < 0.01$) (Fig. 2d). Post-hoc comparisons revealed that CDP increased the number of inadequate responses, while IMI decreased inadequate responding compared to vehicle treatment. The effects of these treatments were independent of genotype ($F_{genotype \times treatment}(2,36) = 1.37$; $p = 0.26$).

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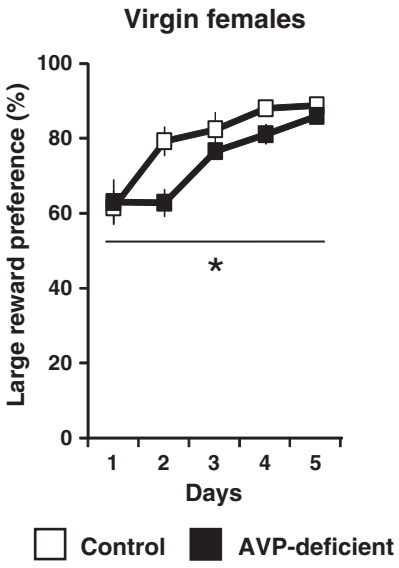

Fig. 1. Effects of vasopressin (AVP) deficiency on learning in virgin female Brattleboro rats in the training phase of the delay discounting paradigm (*Experiment 1*). AVP-deficient rats showed an overall decreased learning ability. * under the line denotes an overall significant difference between the two genotypes ($p < 0.05$).

Cognitive performance and impulsivity in AVP-deficient and control lactating females

Large reward preference was significantly increased during the training phase in all lactating rats regardless of their genotype ($F_{days}(4,72) = 61.68$; $p < 0.01$; $F_{genotype}(1,18) = 1.17$; $p = 0.29$; $F_{genotype \times delay}(4,72) = 2.33$; $p = 0.06$) (Fig. 3).

In lactating females, large reward preference decreased across days ($F_{delay}(7,126) = 22.09$; $p < 0.01$). Overall, the preference for the large reward was higher in AVP-deficient animals compared to control animals ($F_{genotype}(1,18) = 5.13$; $p = 0.03$), but no significant interactions between genotype and delay were observed ($F_{genotype \times delay}(7,126) = 1.02$; $p = 0.42$) (Fig. 4a). The number of inadequate responses was significantly increased throughout the test phase ($F_{delay}(7,126) = 11.67$; $p < 0.01$), but it was unaltered by genotype ($F_{genotype}(1,18) = 3.7$; $p = 0.07$). A significant interaction between genotype and delay was observed ($F_{genotype \times delay}(7,126) = 6.2$; $p < 0.01$), as post-hoc comparisons revealed, the increase in the number of inadequate responses was greater in control animals than in AVP-deficient rats (Fig. 4b).

Large reward preference was unchanged by genotype or treatment in lactating females during the three-day pharmacological treatment period ($F_{genotype}(1,18) = 1.68$; $p = 0.21$; $F_{treatment}(2,36) = 1.16$; $p = 0.33$) (Fig. 4c). A significant interaction between genotype and treatment was observed ($F_{genotype \times treatment}(2,36) = 5.29$; $p < 0.01$). Post-hoc analyses revealed that both CDP and IMI significantly decreased large reward preference only in AVP-deficient rats compared to vehicle treatment. The number of inadequate responses was decreased in AVP-deficient rats compared to control animals and increased by CDP treatment compared to vehicle treated subjects, while IMI caused no significant changes in this variable ($F_{genotype}(1,18) = 7.4$; $p = 0.01$; $F_{treatment}(2,36) = 3.7$; $p < 0.03$) (Fig. 4d). No significant interaction was observed between genotype and treatment ($F_{genotype \times treatment}(2,36) = 2.76$; $p = 0.08$).

HPA axis activity in AVP-deficient and control virgin and lactating female rats

AVP deficiency did not alter corticosterone levels 10 days after delivery in lactating or virgin rats ($F_{genotype}(1,34) = 0.15$; $p = 0.7$;

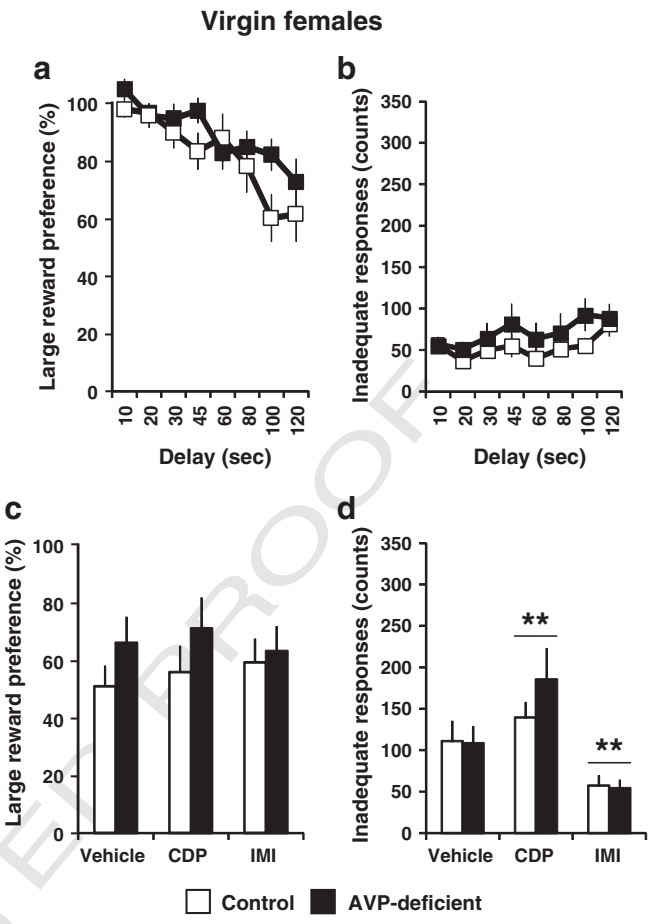

Fig. 2. Effects of vasopressin (AVP) deficiency on impulsivity in virgin female Brattleboro rats in the test phase of the delay discounting paradigm (*Experiment 1*). Control and AVP-deficient rats showed no differences in impulsivity (a–b). Chlordiazepoxide (CDP)-induced enhancement of GABAergic and imipramine (IMI)-induced enhancement of serotonergic activity resulted in no changes in large reward preference, an indicator of choice-impulsivity (c). In both control and AVP-deficient virgins, CDP-treatment caused an increase, while IMI-treatment caused a decrease in the number of inadequate responses, an indicator of motor impulsivity (d). ** above the line denotes a significant difference from control treatment group in post-hoc comparison ($p < 0.01$).

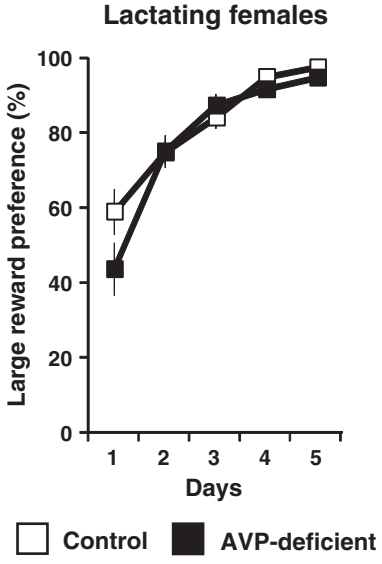

Fig. 3. Effects of vasopressin (AVP) deficiency on learning in lactating female Brattleboro rats in the training phase of the delay discounting paradigm (*Experiment 2*). AVP deficiency had no significant effects on learning ability.

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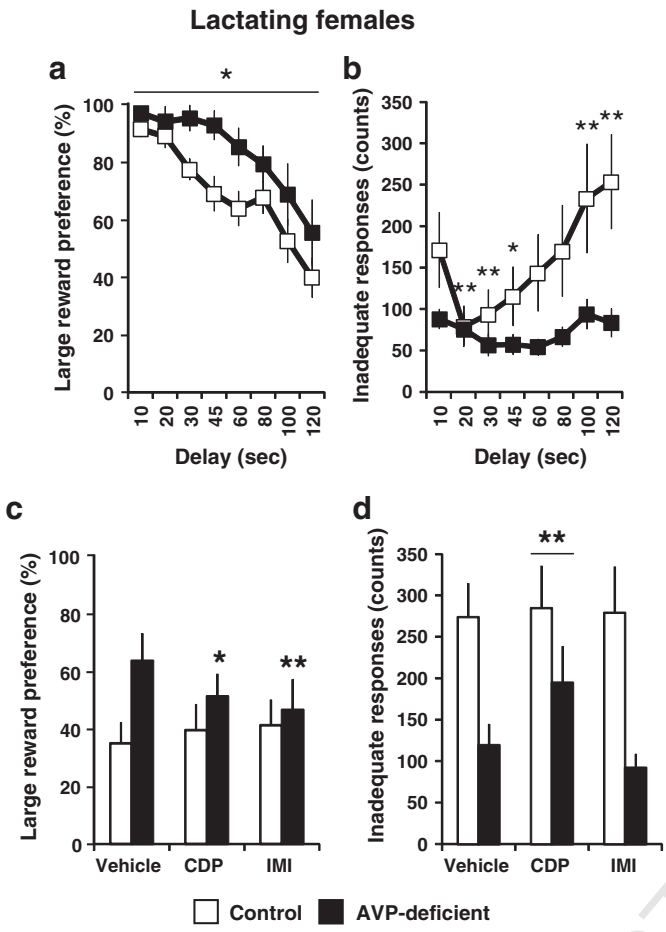

Fig. 4. Effects of vasopressin (AVP) deficiency on impulsivity in lactating female Brattleboro rats in the test phase of the delay discounting paradigm (*Experiment 2*). AVP-deficient rats showed decreased impulsivity (a–b). Both chlordiazepoxide (CDP)-induced enhancement of GABAergic activity and imipramine (IMI)-induced enhancement of serotonergic activity caused a decrease in large reward preference, an indicator of choice-impulsivity, but only in AVP-deficient rats (c). CDP-treatment caused an increase in the number of inadequate responses, an indicator of motor impulsivity in all rats regardless of their genotypes, while IMI-treatment had no effect on the same variable (d). In Fig. 4a * over the line denotes an overall significant difference between the two genotypes in post-hoc comparison ($p < 0.05$); in Fig. 4b * denotes a significant difference from value at 10 second delay ($p < 0.05$); ** denotes a significant difference from value at 10 second delay ($p < 0.01$); in Fig. 4c * denotes a significant difference from control treatment group within the same genotype in post-hoc comparison ($p < 0.05$), ** denotes a significant difference from control treatment group within the same genotype in post-hoc comparison ($p < 0.01$); in Fig. 4d ** over the line denotes a significant difference from control treatment group in post-hoc comparison ($p < 0.01$).

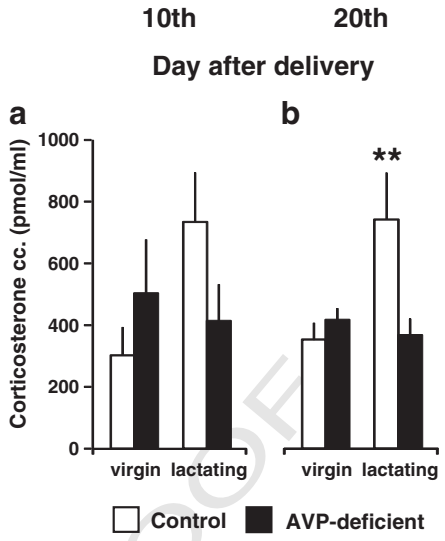

Fig. 5. Effects of vasopressin (AVP) deficiency on corticosterone levels in control and AVP-deficient virgin and lactating females 10 days (a) and 20 days (b) after delivery. AVP-deficiency abolished lactation-induced increases in corticosterone levels on the 20th day after delivery. ** denotes a significant difference from the virgin group within the same genotype in post-hoc comparison ($p < 0.01$).

$F_{lactation}(1,34) = 1.23$; $p = 0.27$; $F_{genotype \times lactation}(1,34) = 2.82$; $p = 0.1$) (Fig. 5a).

Corticosterone was unchanged by genotype or lactation ($F_{genotype}(1,58) = 2.95$; $p = 0.09$; $F_{lactation}(1,58) = 3.52$; $p = 0.06$) when measured 20 days after delivery. A significant interaction was observed between AVP deficiency and lactation, as corticosterone levels were higher in control lactating than control virgin rats, but they did not differ in AVP-deficient lactating and virgin rats ($F_{genotype \times lactation}(1,58) = 5.86$; $p = 0.01$) (Fig. 5b).

Discussion

AVP-deficient virgin females exhibited decreased learning abilities when compared to control animals. However, AVP deficiency did not appear to affect impulsive behavior. CDP increased impulsivity and IMI decreased impulsivity in both genotypes of virgin females. Corticosterone levels were unaltered by AVP deficiency. In lactating females, AVP deficiency did not alter learning, but led to decreased impulsivity. Both CDP and IMI decreased choice impulsivity, but only in AVP-deficient rats. CDP increased motor impulsivity in both genotypes of lactating rats. Lactating rats exhibited elevated corticosterone levels at the end of the test phase of the delayed discounting test, but AVP deficiency dampened this elevation.

Our results demonstrating that reduced learning capabilities in AVP-deficient rats are in line with earlier reports in which Brattleboro rats were shown to exhibit impaired performance in cognitive tasks (Aarde and Jentsch, 2006; Colombo et al., 1992; Varga et al., 2013). Interestingly, lactation seems to alter these effects, as these effects of AVP deficiency did not occur in lactating rats. Taken together, these findings suggest that AVP plays a role in the modulation of cognitive processes and that the specific physiological state during lactation decreases the sensitivity of animals to the effects of AVP deficiency.

The interaction between AVP and lactation in the regulation of impulsivity appears to be different than what is observed for cognitive capabilities. The lack of AVP-induced effects on impulsive behavior in virgin rats suggests that AVP does not modulate impulsivity under non-lactating conditions. In contrast, AVP-deficient lactating rats exhibit decreased impulsivity, which might indicate that the neuroendocrine state developing during lactation involves AVP in the regulation of impulsivity. Similar lactation-dependent effects of AVP on impulsivity have not been reported previously, but our findings are consistent with studies showing that the behavioral effects of AVP deficiency (e.g. reduced depressive-like behavior) occur in a lactation-dependent manner in rats (Fodor et al., 2012).

An interesting trend was observed when comparing the number of inadequate responses in the delay discounting test between virgin and lactating females. While the number of inadequate responses showed a small, AVP-independent increase throughout the test phase in virgin rats, the number of inadequate responses increased remarkably with the length of the delays in control, AVP-expressing lactating females. Interestingly, in AVP-deficient lactating subjects, the changes in inadequate responses were similar to those observed in virgin animals (see Figs. 2b and 4b). These differences suggest that AVP does not play a role in the regulation of impulsivity in virgin animals, but that it exerts a robust, impulsivity-increasing effect in lactating rats. In the absence of AVP, lactating female rats exhibit a less impulsive phenotype that is similar to that observed in virgin female rats. Because the AVP system is well known to be over-activated during lactation (Caldwell et al.,

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1987; Landgraf et al., 1991) we can assume that the enhanced AVP activity in lactating rats might be one of the causes of their increased impulsivity. One might hypothesize that moderately increased impulsivity might be beneficial to lactating rats given that rapidly accessible sources would be preferred when energy need is constantly high (Widdowson, 1976).

AVP-deficiency exerted a lactation-dependent impact on the effects of pharmacological manipulations on impulsive behavior. In virgin rats, CDP treatment increased, while IMI treatment decreased impulsivity in a genotype-independent manner, which findings are in line with earlier data (Bizot et al., 1988; Evenden and Ko, 2005; Miyazaki et al., 2011; Thiebot et al., 1985; Wolff and Leander, 2002). In contrast, AVP deficiency altered the effects of pharmacological manipulations of GABAergic and serotonergic signaling on impulsivity during lactation: both treatments increased choice impulsivity in AVP-deficient lactating rats. As this form of impulsivity was unaltered by treatments in virgin AVP-deficient rats, one might assume that AVP only interacts with GABAergic and serotonergic signaling in the regulation of choice impulsivity during lactation. Interestingly, IMI treatment led to an increase in this form of impulsivity of AVP-deficient lactating rats in contrast to its motor impulsivity-decreasing effects in virgin animals; however, similar contrasting effects of serotonergic manipulations on different forms impulsivity had been reported earlier (Harrison et al., 1997; Winstanley et al., 2004). Although the precise effects of CDP and IMI on impulsivity have yet to be fully elucidated, our results demonstrate that AVP interacts with serotonergic and GABAergic signaling similarly in the regulation of choice impulsivity during lactation. Specifically, AVP desensitizes lactating rats to the choice impulsivity-altering effects of GABAergic and serotonergic manipulations, while AVP-deficient lactating rats react to such manipulations. Regarding motor impulsivity, AVP deficiency had no similar impact on the effects of pharmacological treatments, suggesting that interactions between AVP, GABAergic and serotoninergic signaling might be only involved in the regulation of choice impulsivity.

Corticosterone levels were increased in control lactating rats twenty days after delivery, while AVP deficiency dampened this increase, a finding that is consistent with earlier reports (Fodor et al., 2013).These data might suggest that AVP contributes, in part, to maternal increases in impulsivity *via* the enhancement of HPA axis activity, as earlier reports showed that chronic corticosterone treatment leads to increased impulsivity (Torregrossa et al., 2012).

Conclusions

Taken together, our results demonstrate that AVP is crucial for normal cognitive processes in virgin rats, but it does not exert similar effects during lactation. In contrast, AVP alters impulsive behavior in the opposite manner. Namely, it does not play a role in the regulation of impulsive behavior in virgin females, but it has an important impulsivity-increasing effect during lactation, possibly by increasing HPA axis activity. Moreover, AVP apparently desensitizes lactating females to the effects of GABAergic and serotonergic manipulations on choice impulsivity. These results contribute to our knowledge regarding the effects of AVP on cognitive processes and impulsivity, as well as the dependence of these effects on neuroendocrine background. Our findings highlight possible interactions between the AVPergic, GABAergic, serotonergic systems and the HPA axis, which might contribute to the development of maternal impulsive behavior.

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