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# Increased sociability and gene expression of oxytocin and its receptor in the brains of rats affected prenatally by valproic acid

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ABSTRACT

Autism is a neurodevelopmental disorder characterised by the disruption of social interactions. Autistic animal models play a crucial role in neurophysiologic research on this disorder. One of these models is based on rats that have been prenatally treated with valproic acid — VPA rats. The aim of our study performed with this model was to investigate changes in sociability and gene expression of neuropeptides and receptors involved in regulating social behaviour. We focused on gene expression in the hypothalamus, where the neuropeptides oxytocin (OT) and arginine-vasopressin (AVP) are produced, as well as oxytocin receptors (OTR) in certain neuronal structures involved in the creation of social abilities. Our research showed that VPA rats spent more time in the part with an unknown animal and less time in the central part of a three chamber sociability test apparatus than control animals. The latency period of VPA rats before initiating social contact was decreased. In addition, during weaning, VPA female rats spent more time in direct interaction with an unknown rat. We also found that adult VPA rats had an increased expression of OT in the hypothalamic supraoptic and paraventricular nuclei and of OTR in the medial prefrontal cortex, piriform cortex, cortex–amygdala transition zone and the region of the basolateral and basomedial amygdaloid nuclei compared with controls.

To sum up, we observed that a single prenatal injection of VPA increased social behaviour and gene expression of OT and OTR in neurological structures connected with the social behaviour of rats. One unanticipated finding was the absence of one of the core symptoms of autism in VPA rats, suggesting a decreased ability to understand intraspecific communication signals.



## 1. Introduction

Autism is a neurodevelopmental disorder manifested by three core deficits, including abnormal social behaviour and communication and the occurrence of stereotypic activities (American Psychiatric Association, 2000). In particular, social deficit is the most noticeable behavioural deficit observed in autistic populations from childhood to adulthood (Lewis, 1996; Wiener, 1997; Volkmar et al., 2005).

A number of animal models have been created in autism research that imitates changes observed in the autistic population, especially social deficit (Olexová et al., 2012). One animal model of autism is rats that have been treated prenatally with valproic acid — VPA rats (Rodier et al., 1996, 1997; Schneider and Przewlocki, 2005). Valproic acid, a common anticonvulsant, has a teratogenic effect when administered during pregnancy. Its application between gravidity days 11.5 and 12.5 affects the development of the central nervous system, leading to various neuroanatomical changes (Rodier et al., 1996, 1997; Ingram et al., 2000; Markram et al., 2008; Rinaldi et al., 2008; Sui and Chen, 2012) as well as changes in behaviour related to autistic symptoms (Schneider and Przewlocki, 2005; Dufour-Rainfray et al., 2010; Bambini-Junior et al., 2011; Foley et al., 2012; Sandhya et al., 2012). Studies on VPA rats have revealed alterations in various aspects of complex social behaviour. Pubertal and adult VPA rats showed decreased numbers of social interactions (Schneider and Przewlocki, 2005; Sandhya et al., 2012) as well as a decreased frequency of pinning in combination with increased latency to social behaviour in the social interaction test (Schneider and Przewlocki, 2005). In the three chamber sociability test, VPA rats explored unknown animals less frequently (Bambini-Junior et al., 2011; Foley et al., 2012; Kerr et al., 2013) and spent more time in a part without an unknown animal than controls (Kim et al., 2011; Foley et al., 2012; Ali and Elgoly, 2013; Kerr et al., 2013; Bambini-Junior et al., 2014). In the social preference test, VPA rats initiated fewer social contacts with both familiar and unknown animals (Dufour-Rainfray et al., 2010) and spent more time with the familiar animals (Bambini-Junior et al., 2011). The observed changes in social behaviour in VPA rats were more visible in males than in females (Schneider et al., 2008; Kim et al., 2013). In contrast to these studies, Cohen et al. (2013) reported more social exploration and play-fighting in VPA rats as compared to control animals.

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Social behaviour is regulated by various brain structures and neurotransmitters including the neuropeptides oxytocin (OT) and arginine-vasopressin (AVP). The "social brain" in terms of human neuroanatomy includes the structural encoding of faces and rapid face recognition in the fusiform face area (FFA), the dynamic expression of emotions and the interpretation of the actions and intentions of others in the superior temporal sulcus area (STS), the determination of the emotional states of others *via* analysis of facial expressions in the amygdala and interconnected frontal–limbic regions, the visual perception of human bodies in the extra striate body area (EBA), the observation of motor actions of other people in the "mirror system", portions of the parietal cortex, the processing of reward and social reinforcement in the orbitofrontal cortex (OFC), and judgements about other people's intentions and mental states as well as the attribution of emotions to the self and others in the medial prefrontal cortex (mPFC). Therefore, the impairment of social behaviour seen in autistic individuals could be the result of atypical development in these regions (Pelphrey and Carter, 2008). In addition to the areas comprising the "social brain", alteration in other brain structures (Olexová et al., 2012) including the hypothalamus — the structure responsible for the synthesis and secretion of the OT and AVP neuropeptides have also been recognized as being involved in the social deficit observed in autistic populations (Kurth et al., 2011).

The structures involved in social recognition in rats are mainly components of the olfactory system, such as the anterior olfactory nucleus (AON) and the olfactory tubercles as well as the "so called" extended amygdala including the bed nucleus of the stria terminalis (BNST), central and peripheral amygdalar nuclei and the cortex–amygdala transition zones (C xA; Veinante and Freund-Mercier, 1997). The piriform cortex (PirC) also represents a structure that may control olfactory perception and the association among smell, social recognition and emotionality since it is a part of the olfactory circuit and contains an abundance of oxytocin and vasopressin receptors (Ferris et al., 2008).

OT participates in the regulation of maternal behaviour, sexual behaviour, social recognition and social contact, and can facilitate pair-bonding (Neumann, 2008). AVP, possibly in conjunction with OT, may be of particular relevance to male parental behaviour and male pair bond formation (Carter, 2007). In addition, reduction in the expression of genes for OT in transgenic mice has been associated with altered social behaviours (reviewed in Carter, 2007).

Studies in autistic populations have revealed decreased levels of OT in the blood of autistic children (Al-Ayadhi, 2005; Green et al., 2001; Modahl et al., 1998), but OT-precursor in the blood of autistic children was increased compared to levels seen in the control groups (Green et al., 2001). In contrast, levels of OT in the blood of adult autistic patients were increased (Jansen et al., 2006). AVP was also found to be decreased in the blood of autistic individuals (Green et al., 2001). Knowledge of the relevant gene expression of neuropeptides and their receptors in the autistic population is low. For obvious reasons, it reflects the state of the brain in post-mortem samples of the adult autistic population (Gregory et al., 2009).

As mentioned above, animal models including mice with alterations in the OT and oxytocin receptor (OTR) systems showed a social deficit (Carter, 2007; Olexová et al., 2012); however, the study of Crawley et al. (2007) did not observe any social deficit in OT null mutants or heterozygous mice. These findings support the interpretation that oxytocin plays a highly specific role in social memory, but is not essential for general spontaneous social approaches in mice (Crawley et al., 2007).

Despite the fact that changes in various aspects of social behaviour of VPA rats have been well-studied, information about the oxytocinergic and vasopressinergic system in these animals is missing. Therefore, the aim of our study was to investigate changes in the social behaviour of VPA rats in combination with measurements of OT, AVP and OTR expression in various brain structures involved in the control of social behaviour.

## 2. Methods

### 2.1. Animals

The parental generation — male and female Wistar rats (the Institute of Experimental Pharmacology and Toxicology, Dobra Voda, SR) were housed in standard light conditions (12:12) (lights on 6 a.m.), with food (Dos — 2b OVO, Dobra Voda, SR) and water provided *ad libitum*. After an acclimatisation period (7 days) rats were mated overnight and the presence of spermatozoa in vaginal smear was considered as the first day of gestation (GD 1). On the 12.5th day of gestation, females received a single intraperitoneal injection of sodium valproate (Sigma, USA) dissolved in saline (pH = 7.3; c = 250 mg/ml) at a dose of 600 mg/kg. Control females received saline at the same time (Schneider and Przewlocki, 2005). Females were housed in groups of two or three per cage until GD 20.

After delivery, valproate-treated (VPA) and control (Ctrl) females were housed individually and allowed to raise their offspring until weaning at postnatal day (PND) 21. On PND 1 the litters were culled to 8 animals per litter (4 males, 4 females). After weaning, rats of either sex were housed separately in groups of 4 animals per cage. Animals were anaesthetised with $CO_2$ and sacrificed at the age of PND 79–83; their brains were immediately removed and placed into frozen medium (Cryomount, Histolab AB, Sweden), frozen in dry ice and stored at −80 °C.

Behavioural analyses were conducted on 36 animals, comprising the litters from 3 Ctrl and 3 VPA females (Ctrl: n = 18, males n = 9, females n = 9; VPA: n = 18, males n = 9, females n = 9). For *in situ* hybridisation, the brains of the litter from 5 Ctrl and 4 VPA females (Ctrl: n = 38; males n = 19, females n = 19; VPA: n = 27, males n = 16, females n = 11) were used. The brains of all animals that were tested in sociability tests were also evaluated by *in situ* hybridisation.

### 2.2. Sociability test

Animals were tested during the light phase between 12:00 and 5:00 p.m. in a separate testing room. All animals were transported into the testing room in their home cages and were allowed to acclimatise. Immediately after testing, the animals were returned to their home cages. All animals were tested during three developmental periods: weaning (PND 25), puberty (PND 46) and adulthood (PND 76).

The sociability test was adapted from Crawley (2004). The testing chamber was a Plexiglas box (70 × 70 × 50 cm) divided into three identical communicating compartments. First, the tested animal was placed in the empty central compartment of the testing box and allowed to habituate for 10 min. The doors between compartments were closed during this stage of the test. Next, an unknown rat (male from the parental generation) in a wire cage was placed into the left compartment of the testing box, while the right compartment contained an empty wire cage. The doors were opened and during the 10-minute testing period the number of crosses from one compartment to another as well as the time spent in each compartment was measured. We also measured the latency to initiate social interaction with an unknown rat and the direct social interaction — the time spent by the subject sniffing the stranger rat. We also measured the time spent sniffing the empty wire cage.

### 2.3. In situ hybridisation

The *in situ* hybridisation protocol has been previously described elsewhere (Dzirbíková et al., 2011). Briefly, consecutive frozen cryostat sections of the rat brain (14 μm) were mounted on adhesion slides (SuperFrost® Plus, MenzelGläser, Thermo Fisher Scientific Inc.) for subsequent hybridisation of chosen mRNAs. A synthetic 41-base oligonucleotide probe was used: 5′-GGG CTC AGC GCT CGG AGA AGG CAG