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c-Fos expression in the hypothalamic paraventricular nucleus after a single treatment with a typical haloperidol and nine atypical antipsychotics: a pilot study

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Objective. The aim of the present study was to find out whether acute effect of different doses of selected antipsychotics including aripiprazole (ARI), amisulpride (AMI), asenapine (ASE), haloperidol (HAL), clozapine (CLO), risperidone (RIS), quetiapine (QUE), olanzapine (OLA), ziprasidone (ZIP), and paliperidone (PAL) may have a stimulatory impact on the c-Fos expression in the hypothalamic paraventricular nucleus (PVN) neurons.

Methods. Adult male Wistar rats weighing 280–300 g were used. They were injected intraperitoneally with vehicle or antipsychotics in the following doses (mg/kg of b.w.): ARI (1, 10, 30), AMI (10, 30), ASE (0.3), HAL (1.0, 2.0), CLO (10, 20), RIS (0.5, 2.0), QUE (10, 20), OLA (5, 10), ZIP (10, 30), and PAL (1.0). Ninety min later, the animals were anesthetized with Zoletil and Xylariem and sacrificed by a transcardial perfusion with 60 ml of saline containing 450 µl of heparin (5000 IU/l) followed by 250 ml of fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The brains were postfixed in a fresh fixative overnight, washed two times in 0.1 M PB, infiltrated with 30% sucrose for 2 days at 4 °C, frozen at –80 °C for 120 min, and cut into 30 µm thick serial coronal sections at –16 °C. c-Fos profiles were visualized by nickel intensified DAB immunohistochemistry and examined under Axio-Imager A1 (Zeiss) light microscope.

Results. From ten sorts of antipsychotics tested, only six (ARI-10, CLO-10 and CLO-20, HAL-2, AMI-30, OLA-10, RIS-2 mg/kg b.w.) induced distinct c-Fos expression in the PVN. The antipsychotics predominantly targeted the medial parvocellular subdivision of the PVN.

Conclusions. The present pilot study revealed c-Fos expression increase predominantly in the PVN medial parvocellular subdivision neurons by action of only several sorts of antipsychotics tested indicating that this structure of the brain does not represent a common extra-striatal target area for all antipsychotics.

Key words: aripiprazole, amisulpride, asenapine, haloperidol, clozapine, risperidone, quetiapine, olanzapine, ziprasidone, paliperidone, c-Fos immunohistochemistry, PVN, rat

The PVN has been shown to be one of the most important control centers in the brain (Swanson and Sawchenko 1980, 1983; Herman et al. 2003, 2008). It has been described as a complex nucleus, comprising of neurons, which potentially play a significant neuroendocrine role in controlling the hypothalamic-pituitary-adrenal (HPA) axis [corticotropin-releasing hormone (CRH) neurons projecting to the median eminence] (Sawchenko and Swanson 1985; Silverman et al. 1989; Lennard et

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al. 1993), the thyroid axis [thyrotropin-releasing hormone (TRH) neurons projecting to the median eminence] (Segerson et al. 1987; Blake et al. 1992; Merchenthaler and Liposits 1994; Alexander et al. 2005), the reproductive axis [dopamine and oxytocin (OXY) neurons projecting to the median eminence or posterior pituitary] (Hou-Yu et al. 1986), the growth and development (somatostatin neurons projecting to the median eminence) (Ishikawa et al. 1987; Merchenthaler et al. 1989), and the regulation of body fluid balance [vasopressin (AVP) and OXY neurosecretory cells projecting to the posterior pituitary] (Hill 2012). The PVN is also the principal source of descending autonomic pathways sending direct projections to the parasympathetic preganglionic neurons (the salivatory nuclei, the dorsal motor nucleus of the vagus, the nucleus ambiguus) and sympathetic preganglionic neurons in the intermediolateral cell column of the spinal cord (Ranson et al. 1998; Hallbeck 2000; Hill 2012). These PVN descending fibers are peptidergic, using OXY and AVP as major transmitters, but the fibers are positive also for CRH, enkephalin, dynorphins, TRH, somatostatin, vasoactive intestinal polypeptide, and angiotensin II (Sawchenko and Swanson 1985; Ceccatelli et al. 1991; Hallbeck et al. 2001). Anatomical studies have demonstrated the basic cell architecture of the PVN in association with their recognized functional clarification, which allows to perform approximate functional classification also in experimentally activated neurons without phenotype identification of the c-Fos-bearing cells (Swanson et al. 1986). Based on the cytoarchitectonic analysis, the PVN has been divided into eight distinct subdivisions, three magnocellular and five parvocellular (Swanson and Kuypers 1980) distributed within three rostral-caudal (anterior, middle, posterior) levels, which generally indicate also the distribution and spatial organization of CRH, OXY, and AVP subset of neuros (Hou-Yu et al. 1986).

Although antipsychotic studies regarding the c-Fos activation have been oriented to many different extra-striatal brain structures, little attention has been given to the PVN. Differences in c-Fos expression after acute treatment with clozapine, chlorpromazine, and fluphenazine have been evaluated in appetite-related centers of the rat brain including the arcuate, paraventricular hypothalamic, and paraventricular thalamic nuclei and the nucleus incertus (Rajkumar et al. 2013). Increased c-Fos immunoreactivity after acute olanzapine treatment, which dose-dependently disrupted various components of the maternal behavior, has been demonstrated, among others, in the central amygdala and the ventral tegmental area (Zhao and Li 2012). Effect of aripiprazole and haloperidol has been investigated on c-Fos-like immunoreactivity presence in the prefrontal cortex and amygdala (Park et al. 2011a). In situ hybridization of c-fos mRNA expression has been reported in rat prefrontal cortex, striatum, nucleus accumbens, and lateral septum after treatment with typical (haloperidol, fluphenazine) and atypical (sulpiride, clozapine, OPC-14597) antipsychotics indicating that from the striatal structures only the nucleus accumbens shell portion responded by increased c-fos mRNA expression (Semba et al. 1996). c-Fos immunohistochemistry has also been used to identify brain regions associated with inverse incentive learning after haloperidol sensitization on the bar test in rats (Pezarro Schimmel et al. 2015). Divergent effect of mosapramine and other antipsychotic drugs including clozapine, haloperidol, and risperidone on the c-Fos protein expression has been demonstrated in the medial prefrontal cortex, nucleus accumbens and dorsolateral striatum of the rat (Fujimura et al. 2000). Distinctive patterns of Fos expression has been observed after acute administration of typical antipsychotic chlorpromazine and atypical antipsychotics (clozapine, thioridazine, and raclopride) in the rat forebrain (Wan et al. 1995). The functional neuroanatomical profile of the newly introduced atypical agent ziprasidone has been studied and demonstrated that acute administration of ZIP induced a time-dependent increase in the density of c-Fos-positive nuclei in the shell and core of the nucleus accumbens, lateral and medial caudate putamen, and lateral septum (Jennings et al. 2006).

Previously, we have shown a substantial diversity in the stimulatory effect of the selected antipsychotics including haloperidol, clozapine, olanzapine, and risperidone on the quantity of c-Fos immunostained profiles in the PVN (Kiss et al. 2010). The aim of the present study was to test the effect of different doses of antipsychotics including aripiprazole (ARI), amisulpride (AMI), asenapine (ASE), haloperidol (HAL), clozapine (CLO), risperidone (RIS), quetiapine (QUE), olanzapine (OLA), ziprasidone (ZIP), and paliperidone (PAL) on the c-Fos expression in the PVN neurons.

Materials and Methods

Animals. Adult male Wistar rats weighing 280–300 g were used. They were housed in an animal facility with controlled temperature (22±1 °C), light (12-hour light/dark cycle with lights on at 06:00 h),

and humidity (55%). Animals were provided with dry pellets representing a regular rat chow and tap water ad libitum. Principles of the laboratory animal care and the experimental procedures used were approved by the State Veterinary and Food Administration of the Slovak Republic Committee and the investigation conditions were in accordance with the guidelines for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health.

Treatments. After acclimatization for several days in the animal facilities, the experimental animals received antipsychotics in the following doses: aripiprazole 1, 10, 30 mg/kg (ARI/1, ARI/10, ARI/30), amisulpride 10 and 30 mg/kg (AMI/10, AMI/30), asenapine 0.3 mg/kg (ASE), haloperidol 1 and 2 mg/kg (HAL/1, HAL/2), clozapine 10 and 20 mg/kg (CLO/10, CLO/20), risperidone 0.5 and 2 mg/kg (RIS/0.5, RIS/2), quetiapine 10 and 20 mg/kg (QUE/10, QUE/20), olanzapine 5 and 10 mg/kg (OLA/5, OLA/10), ziprasidone 10 and 30 mg/kg (ZIP/10, ZIP/30), and paliperidone 1 mg/kg (PAL)/body weight (b.w.). The individual antipsychotics were dissolved in 40 µl of 20% acetic acid diluted to 1 ml distilled water and adjusted to pH 6 using 10 N NaOH. They were administered intraperitoneally. Controls received antipsychotic dissolvent. Ninety min after the treatments, the rats were anesthetized by a combined treatment of Zoletil (30 mg/kg, Virbac, Carros, France) and Xylariem (15 mg/kg, Riemser, Germany) in the volumes 0.1 ml and 0.24 ml/300 g b.w., respectively and sacrificed by a transcardial perfusion with 60 ml of saline containing 450 µl of heparin (5000 IU/l, Zentiva, Slovakia) followed by 250 ml of fixative containing 4% paraformaldehyde (Sigma-Aldrich, Germany) in 0.1 M phosphate buffer (PB, pH 7.4). The brains were postfixed in a fresh fixative overnight, washed two times in 0.1 M PB, infiltrated with 30% sucrose for 2 days at 4 °C, cut into 30 µm thick coronal sections using Reichert-Jung, Cryo-cut E (Austria), and collected in a cryoprotectant solution at –20 °C until used. All the drugs used have been purchased in Sigma-Aldrich (Germany).

Brain areas investigated. The selection of coordinates for the hypothalamic PVN brain location was performed based on the rat brain atlas Paxinos and Watson (2007). The PVN-containing photomicrographs (4–6 sections/brain) were captured in AxioImager A1 light microscope (Carl Zeiss) coupled to a video camera and monitor. The photomicrographs with the presence c-Fos particles in the PVN were in more detail evaluated in the Adobe Photoshop 7.0.

Fos immunohistochemistry. Free-floating sections were repeatedly washed in cold 0.1 M phosphate buffer (PB) and preincubated in a blocking solution containing 0.3% H_2O_2 in 0.1 M PB (Fisher Scientific, Fair Lawn, NJ, USA) for 20 min at room temperature (RT). Then the sections were rinsed 3 × 10 min in 0.1 M PB and incubated with a polyclonal anti-c-Fos rabbit serum (12-7) diluted 1:1500 in 0.1 M PB containing 4% normal goat serum (Gibco, Grand Island, NY, USA), 0.5% Triton X-100 (Koch-Light Lab. Ltd., Colnbrook Berks, England), and 0.1% sodium azide (Sigma-Aldrich, Germany) for 48 h at 4 °C. After several rinsing in PB, the sections were incubated with biotinylated goat anti-rabbit IgG (1:500, VectorStain Elite ABC Kit, Vector Lab., Burlingame, CA, USA) in PB for 90 min at RT. Next PB rinsing was followed by incubation with the avidin-biotin peroxidase complex (1:250) for 90 min at RT. After several washings in 0.05 M sodium acetate buffer (SAB, pH 6.0), Fos antigenic sites were visualized by nickel-enhanced 3,3′-diaminobenzidine tetrahydrochloride (0.0625% DAB, 2.5% nickel chloride, Sigma-Aldrich, No. 7718-54-9), in SAB containing 0.0006% hydrogen peroxide. The heavy metal-intensification of DAB yielded black staining in the c-Fos labeled nuclei. Developing of the color reaction was monitored under microscope, which lasted 6–10 min. After several washings in SAB, the tissue sections were mounted on positive charged adhesive slides, kept at RT for 120 min, coverslipped with Pertex (Stockholm, Sweden), and stored in histological boxes.

Results

Light microscopic inspection of the serial coronal sections covering the entire PVN extent carried out 90 min after an acute administration of various antipsychotics administered in different doses revealed distinct variabilities in the c-Fos expression response. From ten antipsychotics tested only six ARI/10, CLO/10 and CLO/20, HAL/2.0, AMI/30, OLA/10, and RIS/2.0 mg/kg b.w. (Figures 1C, 1E, 1F, 1H, 1J, 1L, 1S) noticeable enhanced the number of Fos protein positive neurons in the PVN. Most apparent activation in c-Fos-labeled population of cells was observed after ARI/10, CLO/20, and OLA/10 mg/kg b.w. (Figures 1C, 1F, 1L) treatments. Still strong c-Fos signal was observable in the PVN after administration of CLO/10, HAL/2.0, and RIS/2.0 mg/kg b.w. (Figures 1E, 1H, 1S). Only a mild increase in the number of c-Fos immunoreactive cell bodies was noticed after treatment with ARI/1, ARI/30, HAL/1.0,

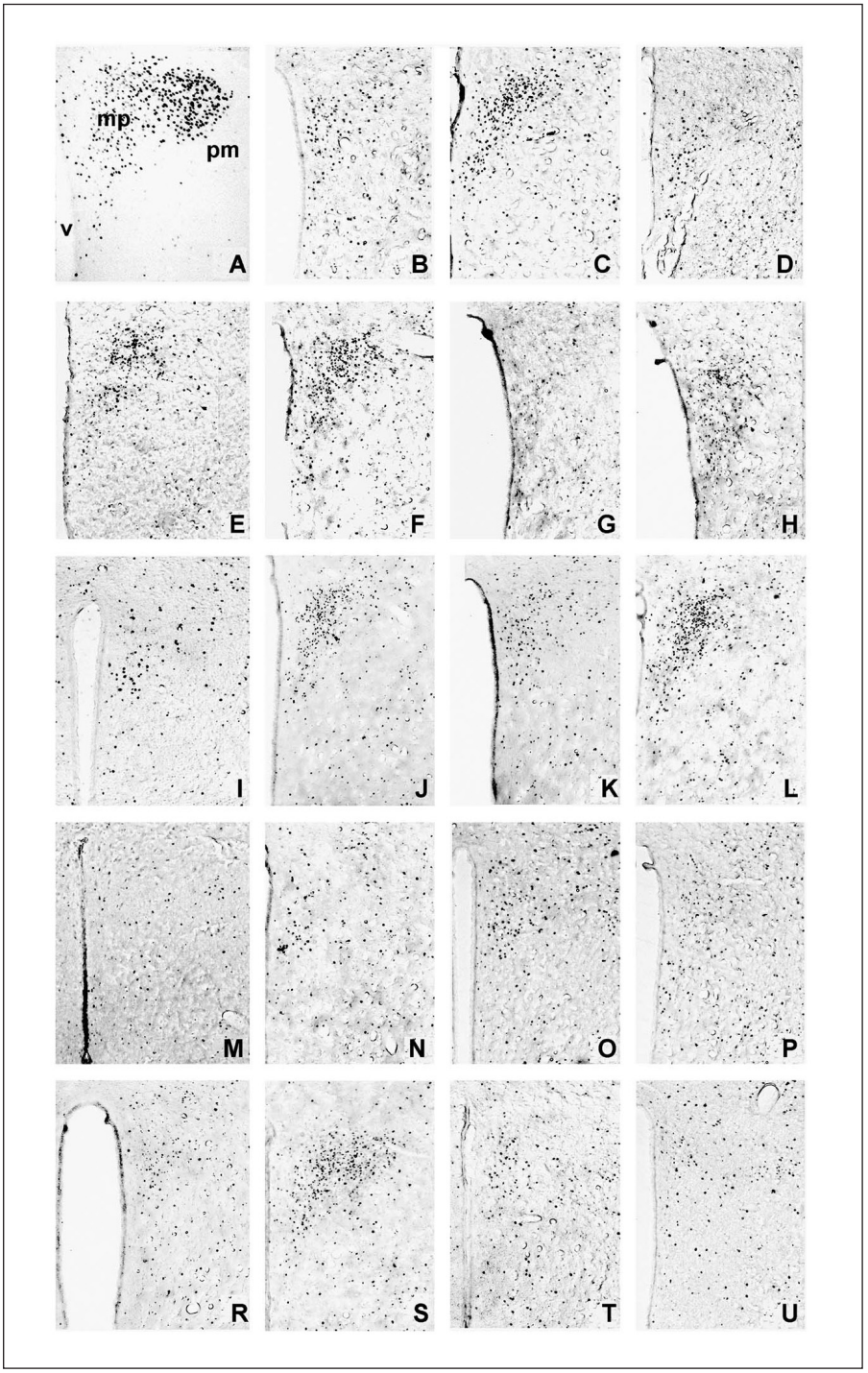

Figure 1. Photomicrographs demonstrating the acute effect of the individual antipsychotics on the c-Fos expression level in the PVN 90 min after the treatment. Most distinct c-Fos expression is seen in the cases of ARI/10 (C), CLO/10 (E) and 20/(F), HAL/2 (H), AMI/30 (J), OLA/10 (L), and RIS/2 (S) mg/kg b.w. treatments. The stimulatory effect of all of them is most marked in the PVN medial posterior subdivision, when compared with the illustrative photomicrograph A clearly depicting both the PVN medial posterior (mp) and posterior magnocellular subdivisions (mp). Lower responses in PVN c-Fos expression are recognizable in ARI/1 (B) and ARI/30 (D), AMI/10 (I), and OLA/5 (K). No evident marks in c-Fos expression stimulation is seen after ZIP/10 (M) and ZI/30 (N), QUE/10 (O), RIS/0.5 (S), PAL (T), and ASE (not shown) treatments, which are comparable with the effect of the vehicle (U) administration.

AMI/10, OLA/5, and RIS/0.5 mg/kg b.w. (Figures 1B, 1D, 1G, 1I, 1K, 1R) in the PVN. Substantially lower c-Fos response, comparable to controls (VEH), was seen after ASE/0.3 (not shown), ZIP/10 and ZIP/30, RIS/0.5, PAL, and QUE/10 and QUE/20 mg/kg b.w. treatments (Figure 1). In contrast, neither of the RIS doses (0.5 or 1 mg/kg) acutely administered to animals elevated the number of c-Fos protein positive neurons in the PVN (Figures 1R and 1S).

The topographic analysis revealed that the majority of c-Fos profiles after the treatments with potential antipsychotics occurred in the PVN medial parvocellular subdivision (mpPVN) at the middle level of the PVN. The distribution of c-Fos profiles is compared to an illustrative c-Fos immunostained PVN section (Figure 1A), which clearly depicts two large PVN subdivisions, i.e. the medial parvocellular (Figure 1A mp) and the posterior magnocellular ones (Figure 1A pm) at the middle level of the PVN. The presence of c-Fos labeling after the treatment with the potential antipsychotics was less distinct in the PVN posterior magnocellular (pmPVN) subdivision as visible when compared to the c-Fos distribution on the illustrative PVN section (Figure 1A).

Discussion

In the present pilot study, a distinct c-Fos expression increase in the PVN was observed 90 min after the treatment with six ARI/10, CLO/10 and 20, HAL/2, AMI/30, OLA/10, and RIS/2 mg/kg b.w. from ten (ARI, AMI, ASE, HAL, CLO, RIS, QUE, OLA, ZIP, PAL) antipsychotics tested. In all the potent antipsychotics, c-Fos-labeled profiles mainly occurred in the mpPVN subdivision and only scattered c-Fos-labeled profiles were detectable in the pmPVN subdivision at the middle level of the PVN.

The present pilot data may speak out for at least three outcomes. First, since only several antipsychotics induced c-Fos expression in the PVN, it is more than probable that the PVN structure will not belong to those extra-striatal structures, which might be considered as a common target area for all the antipsychotic drugs. Second, the antipsychotics used, except the ARI/30, produced a dose-dependent increase in the number of Fos protein positive neurons in the PVN. Third, c-Fos response in the PVN indicates that the effect of the potential antipsychotics is predominantly directed to a subset of neurons located in the mpPVN subdivisions. The last finding is in agreement with our previous study (Kiss et al. 2010), in which antipsychotics OLA/30, CLO/30, RIS/2, HAL/1 after 60 min of the treatment induced c-Fos expression mainly in the mpPVN at the comparable extent as demonstrated in the present pilot study.

In the mpPVN, a wide variety of neuropeptides-synthesizing neurons have been identified (Kiss 1988). Therefore, it has to be taken into the consideration that c-Fos expression increase in the pmPVN subdivision after the potential antipsychotics treatments may notionally target any of the neuronal phenotypes localized in this PVN subdivision including CRH (Swanson et al. 1983; Sawchenko et al. 1984; Lennard et al. 1993), neurotensin (NT) (Ceccatelli et al. 1989), enkephalin (ENK) (Merchenthaler 1992; Beaulieu et al. 1996), somatostatin (Ishikawa et al. 1987; Merchenthaler et al. 1989), vasoactive intestinal polypeptide (VIP) (Toni et al. 1990; Dalcik and Phelps 1993), cholecystokinin (CCK) (Ceccatelli et al. 1989), TRH (Segerson et al. 1987; Merchenthaler and Liposits 1994), galanin (GAL) (Palkovits et al. 1987), AVP (Hou-Yu et al. 1986), and OXY (Hou-Yu et al. 1986). In addition, the coexistence of two or more of these peptides in the mpPVN neurons is a common event, however, their functional significance, except the CRH-AVP co-expression, is not fully understood. Multitude simultaneous occurrence of neuromediators in PVN cellular units might lead to a more divergent functional specification with a possible role in the functional plasticity of fixed anatomical circuit (Kiss 1988). Actually, Ceccatelli et al. (1989) have shown that about 30% of the CRF neurons contain NT and 20% ENK, whereas about 60% of both NT- and ENK-immunoreactive neurons lacked CRF-LI.

From the above-mentioned literature data, it comes out that the CRH neurons form the most extensive and densest cell aggregation (80% from 2000 CRH cells) in the mpPVN subdivision. Park and co-workers (2011b) have shown that haloperidol may stimulate CRF mRNA expression in the PVN under basal conditions, while ZIP administration does not, although in the same experiment, chronic ZIP treatment prevented CRF mRNA expression increase induced by immobilization stress. This clearly head towards the fact that different antipsychotics may play a different role in the regulation of the CRH perikarya in the mpPVN. Actually, in the present study, in agreement with the above-mentioned one, no marked c-Fos induction in the PVN was observed neither after lower nor higher doses of ZIP acute treatment under basal conditions. *In vitro* studies have shown that QUE and OLA are able to acutely reduce the release of CRH from isolated rat hypothalami and hippocampi (Tringali et al. 2009)

and HAL, CLO, and RIS may inhibit the CRH-CAT activity, indicating that some of the antipsychotic drugs by the inhibition of CRH gene promoter activity may negatively affect, i.e. inhibit the activity of the HPA axis (Basta-Kaim et al. 2006). Previously, we have also described the effect of several atypical neuroleptics on the activity of pmPVN magnocellular neurons. Namely, CLO and OLA acute treatments revealed c-Fos colocalizations with both AVP- and OXY-displaying magnocellular perikarya in the pmPVN. This finding clearly indicates that not only parvocellular but also magnocellular perikarya may be a target for some of the antipsychotics (Kiss et al. 2010). However, there is only a limited number of studies in the literature available dealing with the effect of antipsychotics on c-Fos expression in the PVN directed to the analysis of the phenotype character of the activated cells.

In spite of the fact that many antipsychotics act via a number of different receptors, it is still not clear whether the effect of the potential antipsychotics on the population of neurons located in the mpPVN subdivision is direct or indirect. There is also a limited number of studies demonstrating how importantly the individual antipsychotics might be involved in the regulation of the PVN neurons activity and consequently in the regulation of different functional circuits originating or mediating by these PVN neurons. Actually, He and co-workers (2013) have shown that antagonizing of the hypothalamic H_1 receptors by CLO or OLA may time-dependently affect the hypothalamus-brainstem circuits leading to a weight gain.

In summary, the attempt of the present pilot study was to depict an outline of the possible extra-striatal action of different doses of ten antipsychotics selected in the PVN. The c-Fos expression in the mpPVN induced by the potential antipsychotics may speak out for a possibility to affect a wide range of neuronal phenotypes involved in different intrinsic and extrinsic brain circuits.

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