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Research report

Locomotor response to novelty correlates with differences in number and morphology of hypothalamic tyrosine hydroxylase positive cells in rats

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ABSTRACT

Individual differences in the intensity of locomotor response to a new environment (exploratory reaction) are generally used as a model to study individual vulnerability to stress and drug addiction. In the present work we studied the number, distribution and morphology of the hypothalamic cells expressing tyrosine hydroxylase (TH+ cells) (immunohistochemical and immunofluorescent staining) in male Wistar rats divided based on high (HR), midline (MR) or low (LR) locomotor activity in response to novelty. Morphology and total number of TH+ cells were analyzed for A11–A15 dopaminergic groups. We found correlation between the total number of hypothalamic TH+ cells in the whole A11–A15 area and the locomotor activity. The differences were most pronounced in some of the hypothalamic nuclei, i.e. in the rostro-caudal extension of the A11, A12 and A14 structures, where the HR rats had a significantly higher number of TH+ cells in comparison to the MR and LR rats.

Morphology analysis of TH+ cells showed HR/MR/LR differences in single cell area and perimeter and, to a lesser extent, in the other morphometric parameters such as length of the major and minor axes, or circularity factor.

The results suggest that the behavioral traits which characterize the HR animals and are correlated with increased susceptibility to stress and propensity to develop drug addictions can be determined by the number, distribution, activity and perhaps the morphology of the cells in the dopaminergic systems.

1. Introduction

Both among animals and humans, particular individuals differ in their behavioral and physiological reactivity to environmental challenges. This variability can be determined by genetic factors and/or by previous life experiences (Cabib et al., 2000; Miserendino et al., 2003; Wahlsten et al., 2003). Enhanced reactivity to life stressors has been found to be correlated with an increased propensity to develop drug addiction (Deminiere et al., 1989; Piazza et al., 1989, 1990, 1991; Deroche et al., 1992; Ambrosio et al., 1995; Elmer et al., 1995) and with increased sensation seeking motivation (Dellu et al., 1996; Piazza et al., 1993; Kabbaj et al., 2000; Kabbaj and Akil, 2001). Moreover, self-reported measures of sensation-seeking have been associated with a variety of psychiatric disorders such as alcoholism and drug addiction (Zuckerman and Neeb, 1979), which makes it important to understand the biological basis of this personality trait (Kabbaj and Akil, 2001).

Individual behavioral variability is frequently modeled using locomotor reactivity to novelty (Deminiere et al., 1989; Piazza et al., 1989; Piazza and Le Moal, 1996). When placed in a new environment, some individuals (as tested in rats and mice) show vigorous and long-lasting exploratory activity (high responders, HR), whereas others become quiescent after a short period of exploration (low responders, LR). HR rats react with higher corticosterone release to stressful stimuli (Piazza et al., 1990, 1991; Dellu et al., 1996; Kabbaj et al., 2000) and they are more sensitive to reinforcing properties (Piazza et al., 1989, 1990, 1991; Deminiere et al., 1992; Deroche et al., 1993; Ambrosio et al., 1995; Elmer et al., 1995) and behavioral effects of various drugs of abuse (Hooks et al., 1991, 1992a,b,c; Piazza and LeMoal, 1996; Miserendino et al., 2003). Locomotor activity, response to stress and drug self-administration are all related to the central dopaminergic transmission (Wise and Rompre, 1989; Le Moal and Simon, 1991; Piazza and Le Moal, 1996; Smith et al., 1997), therefore the dopaminergic brain systems were the first to be analyzed as a potential neurobiological substrate of HR/LR behavioral differences. The HR animals were found to be in a

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hyperdopaminergic state both in resting conditions and after pharmacological and environmental stimulation. This behavioral type was also showed to have higher basal levels of dopamine in the nucleus accumbens (Hooks et al., 1992a).

Dopaminergic neurons contain tyrosine hydroxylase (TH), the enzyme that limits the rate of catecholamine synthesis. Histochemical detection of this enzyme or its mRNA is the standard method of tracing dopaminergic neurons and evaluating brain dopaminergic activity. This method is well documented for mesencephalic dopaminergic cells in rats that are undifferentiated (e.g. Tohyama and Takatsuji, 1998; Spiga et al., 2003) and differentiated in terms of locomotor activity (e.g. Miserendino et al., 1993, 2003; Lucas et al., 1998; Jerzemowska et al., 2012). The largest concentration of dopaminergic neurons is found in midbrain structures such as the retrorubral field (A8 dopaminergic group), substantia nigra (A9 dopaminergic group), and the ventral tegmental area (A10 dopaminergic group). These cells have several functions, including modulation of motor control (A9 dopaminergic cells), and are involved in reward mechanisms (A10 dopaminergic cells) (e.g. Turiault et al., 2007). In our previous study (Jerzemowska et al., 2012) we found a correlation between the number of midbrain cells containing the tyrosine hydroxylase enzyme (TH+ cells) and locomotor activity in response to novelty. In addition, the HR/LR differences had a specific regional distribution: the HR rats had a higher total number of TH+ cells in the A9 and in the anterior part of the A10 dopaminergic group. In contrast, the LR rats had a higher number of TH+ cells in the parabrachial pigmented nucleus (A10 dopaminergic group) and in the posterior part of the substantia nigra (A9 dopaminergic group).

However, there are five small dopaminergic clusters in the hypothalamic regions (A11–A15 dopaminergic groups) (Dahlströem and Fuxe, 1964; Nelson et al., 1996; Turiault et al., 2007) that have not been well described yet in rats differing in locomotor activity in resting conditions. It has been demonstrated that the A11 neurons from the caudal hypothalamic periventricular nucleus project into the spinal cord; the A13 neurons from the zona incerta project locally into the hypothalamus and are engaged in gonadotropin-releasing hormone control; and that most of the dopamine cells in the A12 group (the arcuate nucleus) and A14 and A15 groups (the preoptic area/anterior hypothalamus) are endocrine neurons. These neurons control prolactin secretion and growth hormone secretion from the anterior pituitary gland and melanocyte-stimulating hormone secretion from the intermediate lobe (Turiault et al., 2007). Moreover, it is known that the hypothalamus plays a role in specific and nonspecific behavioral activation (responses directed to food and water intake and undirected locomotory drive) (e.g. Valenstein et al., 1969; Valenstein, 1975; Jerzemowska et al., 2013), and also influences susceptibility to drug abuse (Jama et al., 2008). Hida et al. (1999) examined the A8–A16 dopaminergic cells by using a double-labeling procedure combining immunohistochemistry for TH enzyme and histochemistry for the type of monoamine oxidase (MAO) that shows affinity for dopamine. However, we have not found reports about these hypothalamic dopaminergic cells in rats differing in reactivity to novel stimuli.

The aim of the present study was to investigate whether there is a difference in the hypothalamic dopaminergic cells (A11–A15 groups) between rats belonging to peripheral behavioral groups (low; LR and high; HR) and in rats with the midline response to a new environment (midline, MR) in resting conditions. We examined the number of cells containing the TH (TH+ cells) (immunohistochemical method for TH-expression) in the dorsal (DA) and posterior (PH) hypothalamic areas, subparafascicular thalamic nucleus (SPF) (A11 dopaminergic cells), arcuate nucleus (ARC) (A12 dopaminergic cells), dorsomedial hypothalamic nucleus (DM) with zona incerta (ZI) (A13 dopaminergic cells), anterior hypothalamic area (AH), periventricular (Pe) and ventro-medial (VMH) hypothalamic nuclei (A14 dopaminergic cells), and supraoptic nucleus (SO) (A15 dopaminergic cells) in both left and right hemispheres in rats differing in the level of spontaneous behavioral response to novelty. We investigated the total number of TH+ cells in each of the examined dopaminergic groups separately and also in the whole hypothalamic dopaminergic region (A11–A15). Possible differences in morphology of the hypothalamic dopaminergic cells between the HR/MR/LR rats were also investigated using immunofluorescent staining, by assessing area, perimeter, length of the major and minor axes, and the circularity factor of these cells.

2. Method

2.1. Animals

All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The principles of the care and use of laboratory animals in research, in accordance with the rules of the Local Ethical Committee of the Medical University of Gdansk, were strictly followed and all the protocols were reviewed and approved by the Committee. All efforts were made to minimize both animals' discomfort and the number of animals used.

The study was performed in male Wistar rats (The Institute of Work Medicine, Łódź, Poland, $n = 40$) weighing approximately 250–300 g at the time of arrival at the laboratory. They were housed individually in light (12 h on/12 h off) and temperature (22 °C) controlled environment with food and water available ad libitum. In order to minimize stress caused by the experimental procedures, the animals were handled daily for about two weeks before the experiment and throughout the experiment cycle, i.e. during the novelty test as well as after the test and until their sacrifice. All the animals were kept in the same room but in separate cages (one animal per cage) and they had visual, auditory and olfactory contact with one another. The rats were handled in the animal room and also in the room where they were sacrificed individually at the end of the experiment. Minimal handling was carried out for about 1–3 min for each animal, every day.

2.2. Novelty test

The level of spontaneous locomotor activity in response to novelty was determined according to the method of Piazza et al. (1989) with some modifications described later (e.g. Lucas et al., 1998; Wrona et al., 2005; Jerzemowska et al., 2012). The novel environment was a clear Plexiglas cage (430 mm × 430 mm × 200 mm) equipped with 15 photoelectric cells placed on both axes of the cage (an actometer Opto Varimex Minor – Columbus, USA).

The rats were placed in the actometer for 2 h (4.00–6.00 p.m.) and their horizontal activity was automatically recorded. The number of photocell counts cumulated over 2 h for each animal was used as an index of individual responsiveness to the new environment. Only the upper and lower five rats (regarding the distribution of the index) were used for the neurochemical study (according to Lucas et al., 1998). Thus, high responders (HR = 5) were those animals whose activity score was above the value of 4500, and rats with novelty response score below 2500 were low responders (LR = 5). The remaining rats, with a median activity score (±SE), i.e. between 2500 and 4500, were labeled as the midline group (MR = 30). The rats from the MR group were randomly selected ($n = 5$) for immunohistochemical and immunofluorescent staining procedures and statistical analysis.