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Modeling psychotic and cognitive symptoms of affective disorders: Disrupted latent inhibition and reversal learning deficits in highly stress reactive mice

A. Knapman *, J.-M. Heinzmann, F. Holsboer, R. Landgraf, C. Touma

Research Group of Psychoneuroendocrinology, Max Planck Institute of Psychiatry, Kraepelinstrasse 2-10, 80804 Munich, Germany

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ABSTRACT

Increased stress reactivity has repeatedly been reported in patients suffering from psychiatric diseases including schizophrenia and major depression. These disorders also have other symptoms in common, such as cognitive deficits and psychotic-like behavior. We have therefore investigated if increased stress reactivity is associated with these phenotypic endpoints in an animal model of affective disorders. The stress reactivity mouse model used in this study consists of three CD-1-derived mouse lines, that have been selectively bred for high (HR), intermediate (IR) or low (LR) stress reactivity. Male mice from these three breeding lines were subjected to a reversal learning task and latent inhibition (Li) was assessed using a conditioned taste aversion paradigm. Furthermore, as the dopaminergic system is involved in both Li and reversal learning, the dopamine 1 receptor (D1R), dopamine 2 receptor (D2R) and dopamine transporter (DAT) mRNA expression levels were assessed in relevant brain areas of these animals. The results demonstrate that HR mice show perseveration in the reversal learning task and have disrupted Li. Furthermore, compared to LR mice, HR mice have decreased D2R mRNA levels in the ventral tegmental area, as well as decreased D1R mRNA levels in the cingulate cortex, and an increased expression of D2R mRNA in the nucleus accumbens. Taken together, these results demonstrate that the HR mice display cognitive deficits associated with psychotic-like behavior, similar to those observed in patients suffering from schizophrenia and major depression and could be utilized in the search for better treatment strategies for these symptoms of psychiatric disorders.

1. Introduction

Although many treatment strategies are available, major depression (MD) is not a fully treatable disorder (Holsboer & Ising, 2010; Wong & Licinio, 2001). This particularly applies to the symptoms in the cognitive realm that often subside when the affective symptoms of the disease have successfully been treated (Reppermund, Ising, Lucae, & Zihl, 2009; Reppermund et al., 2007). Typical antidepressants also lack the capacity to treat the psychotic symptoms of major MD (Holtzheimer & Nemeroff, 2006). It is therefore of great importance to better understand the mechanisms underlying these cognitive deficits and psychotic symptoms and to explore new potential targets for their treatment.

Drugs focusing on the dopaminergic system, such as atypical antipsychotics, have shown great promise in treating depressed patients who do not respond to typical antidepressants (Quintin & Thomas, 2004). Atypical antipsychotics are believed to exert their effect by increasing dopaminergic activity in the prefrontal cortex (PFC) implicating the dopaminergic system in these symptoms (Ichikawa, Li, Dai, & Meltzer, 2002; Kuroki, Meltzer, & Ichikawa, 1999; Rollema, Lu, Schmidt, & Zorn, 1997). The dopaminergic system subserves an optimal neuronal function in the PFC (Cropley, Fujita, Innis, & Nathan, 2006), which plays a key role in executive functioning tasks (Carpenter, Just, & Reichle, 2000; Petrides, 1994; Robbins & Arnsten, 2009). Fittingly, executive functioning tasks are the cognitive tasks that depressed patients and schizophrenic patients display the most deficits in (Austin, Mitchell, & Goodwin, 2001; Porter, Gallagher, Thompson, & Young, 2003; Rabin, Sacco, & George, 2009; Reppermund et al., 2007, 2009; Shirayama et al., 2009). Thus, it appears that psychotic MD and schizophrenia (SZ) share certain symptoms and possibly have similar underlying biological underpinnings causing these symptoms. It was therefore our aim to study these symptoms as phenotypic endpoint relevant to both disorders. The focus of this study was the dopaminergic system and the behaviors likely to be subserved by the dopaminergic system in the context of these symptoms.

Dopamine plays a key role in the regulation of latent inhibition (Li). Li is the phenomenon whereby pre-exposure to the to-be conditioned stimulus retards the learning of subsequent pairing of the unconditioned stimulus (US) and the conditioned stimulus (CS) (Lubow, 1973). Disrupted Li is strongly associated with an increased dopaminergic activity in the mesolimbic area (Lubow, 2005;

* Corresponding author. Fax: +49 (0) 89 30622 569.
E-mail address: alanaknapman@hotmail.com (A. Knapman).

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Solomon & Staton, 1982; Weiner, 1990; Young, Joseph, & Gray, 1993) and is considered to be a model of the inability to ignore irrelevant stimuli associated with schizotypy (Baruch, Hemsley, & Gray, 1988; Guterman, Josiassen, Bashore, Johnson, & Lubow, 1996; Rascle et al., 2001; Schmidt-Hansen, Killcross, & Honey, 2009).

In addition to the affective and cognitive symptoms, dysregulation of the hypothalamus–pituitary–adrenal (HPA) axis is commonly observed in patients suffering from MD (de Kloet, Joels, & Holsboer, 2005; Holsboer, 2000; Holsboer & Ising, 2010; Ising et al., 2005), but has also been reported in patients suffering from SZ (Gallagher, Watson, Smith, Young, & Ferrier, 2007; Muck-Seler et al., 2004; Ritsner et al., 2007; Ryan, Sharifi, Condren, & Thakore, 2004). An animal model of affective disorders, the "stress reactivity" mouse model, was therefore established using a selective breeding approach to generate mice with high, intermediate or low stress reactivity (Touma et al., 2008). Briefly, a founder population of CD-1 mice was subjected to a standardized stressor (15-min restraint), and the increase of plasma corticosterone concentrations in response to this stressor was determined. Males and females with very high stress reactivity (HR) were then mated, as were males and females with very low stress reactivity (LR). Their offspring were tested for their stress reactivity in the same manner and so forth for each generation to come (for details see (Touma et al., 2008)). An intermediate reactivity (IR) line was additionally established to serve as a control group with the same inbreeding status as the other two lines. The IR mice present a corticosterone response similar to the mean of the founder population of CD-1 mice (Touma et al., 2008). In this study, these three mouse lines have been utilized to investigate the effect of a hyperactive vs. hypoactive HPA axis reactivity on the dopaminergic system as well as behaviors subserved by the dopaminergic system that are relevant to the psychotic and cognitive deficits observed in MD and SZ. Ultimately, we aim to provide a mouse model for cognitive deficits and psychotic symptoms that could be utilized in the search for better treatment options for these symptoms.

To this end, mice from the stress reactivity model were subjected to a reversal learning task, and Li was assessed in a conditioned taste aversion paradigm. The brains of these animals were subsequently analyzed via *in situ* hybridization to measure dopamine 1 receptor (D1R), dopamine receptor 2 (D2R) and dopamine transporter (DAT) mRNA levels in relevant brain areas.

2. Methods

2.1. Subjects

A total of 48 male mice were used in these experiments. The mice originated from the 11th generation of the stress reactivity mouse model. This mouse model consists of three CD-1-derived mouse lines, selectively bred for high (HR), intermediate (IR) or low (LR) stress reactivity, respectively (Touma et al., 2008). Stress reactivity was determined by the method described below. From each breeding line, HR, IR, and LR, 16 male mice were selected according to their plasma corticosterone increase in the SRT. Within each breeding line, two independent sub-lines, A and B, exist. These sub-lines were never interbred and serve as a replication of the breeding protocol conducted in parallel. The 16 mice of each group comprised of 8 mice from each sub-line. Two weeks prior to the onset of behavioral testing all animals were single housed under standard laboratory conditions in transparent polycarbonate cages (standard macrolon cages type II, $38 \times 22 \times 15$ cm) with food and water available *ad libitum*. Testing and housing rooms were maintained on a 12:12 h light–dark cycle with a constant temperature and humidity of 22 ± 1 °C and $55 \pm 10\%$, respectively. All behavioral tests and hormone measurements were conducted during the first 4 h of the light phase when the animals were still relatively active and corticosterone levels are at their circadian trough. The presented work complies with current regulations covering animal experimentation in Germany and the EU (European Communities Council Directive 86/609/EEC). All experiments were announced to the appropriate local authority and were approved by the 'Animal Welfare Officer' of the Max Planck Institute of Psychiatry.

2.2. Stress reactivity test

All the mice used in these experiments were tested in the "stress reactivity test" (SRT) at the age of approximately eight weeks. The test comprises of an initial blood sample collected from a small incision in the ventral tail vessel, followed by 15 min of restraint stress and finally a reaction blood sample (for details see Touma et al., 2008), collected from a second incision in the ventral tail vessel immediately after the restraint stressor. Corticosterone levels in the blood plasma were analyzed as described below. The test was performed during the first hours of the light phase when corticosterone levels are at their trough level.

2.3. Plasma corticosterone measurements

A radioimmunoassay (RIA) kit (MP Biomedicals, Solon, Ohio, USA) was used with a slight modification to the manufacturer's instructions to determine corticosterone levels in the plasma samples. Only half of the recommended volumes were used for all components to increase the amount of samples that could be analyzed per kit. From the initial sample, 10 μl of plasma was diluted 1:13.5, and for the reaction sample, 10 μl of plasma was diluted 1:100. The difference in dilution ensures that the samples are within the linear part of the standard curve. Inter- and intra-assay coefficients of variation were both below 10%.

2.4. Reversal learning task

Reversal learning is a useful task to test the functional integrity of the PFC in patients and animal models of psychiatric diseases (Clark, Cools, & Robbins, 2004).The mice were tested in the reversal learning task at the age of approximately 16 weeks. A *T*-maze with three 50 cm long arms made out of Plexiglas, mounted on a table with the height of 50 cm was used for this test. The arms could individually be opened or closed via transparent Plexiglas pulley doors. The walls of the start arm were covered in black paper with diagonal white stripes; one of the goal arm's walls was white and the other black. At the end of one of the goal arms an escape tunnel made of chicken wire with a diameter of 5 cm was present leading back to the mouse's home cage which was placed on the floor to ensure that the mouse could neither smell nor see its cage from the center of the maze. At the end of the other arm a 'dummy' tunnel was present that consisted of an identical chicken wire tunnel with a dead end after 20 cm to ensure that the two arms looked identical from the center of the maze where the mouse made its choice of which arm to enter. The mouse was placed in a Plexiglas start box with a trap door. The start box was placed in the beginning of the start arm and the trap door was opened. When the mouse reached the end of the start arm and entered the center zone the pulley door to the start arm was closed so the mouse must choose one of the goal arms. When the mouse had selected one of the goal arms the door to that arm was closed. If the mouse entered the correct arm it was allowed to proceed to its home cage via the escape tunnel. If the incorrect arm was selected the mouse received a mildly aversive air-puff and was left in the arm for 30 s, and subsequently returned to its home cage. The mice were trained to find their home cage by turning left or right and selecting the correct goal arm. They were tested in batches of three (one mouse