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Gene-environment interactions in a rat model of depression. Maternal separation affects neurotensin in selected brain regions

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ABSTRACT

Although the etiology of major psychiatric disorders has not been elucidated, accumulating evidence indicates that both genetic and early environmental factors play a role.
We have previously demonstrated behavioral and neurochemical changes both in non-manipulated genetic rat models of depression, such as Flinders Sensitive Line (FSL) and Fawn Hooded (FH), and in normal rats following maternal separation (MS). The aim of the present study was to extend this work by exploring whether neurotensin (NT), a peptide implicated in several psychiatric disorders, is altered in a new animal model based on gene – environment interactions.
More specifically, we used the FSL rats as a genetic model of depression and the Flinders Resistant Line (FRL) as controls and subjected them to MS. Pups randomly assigned to the MS procedure were separated from the dam as a litter for 180 min daily between postnatal day 2 to 14. On postnatal day 90, rats were weighed and sacrificed by a two second high energy focused microwave irradiation and several brain regions were obtained by micropuncture. Neurotensin-like immunoreactivity (NT-LI) was measured by radioimmunoassay (RIA).
The results showed that the FSL rats compared to the FRL rats have higher baseline NT-LI concentrations in the temporal cortex and periaqueductal gray and a markedly different response to maternal separation. The only observed change following maternal separation in the FRL rats was an NT-LI increase in the periaqueductal gray. In contrast, in the FSL significant increases were found in the nucleus accumbens, hippocampus, and entorhinal cortex and a decrease was seen in the temporal cortex after MS.
The present study revealed baseline regional differences in NT-LI concentrations between the FSL and FRL strains and demonstrated that early MD differentially affects the two strains. The relevance of these alterations for depression as well as possible mechanisms underlying this gene-environment interaction are discussed.

1. Introduction

Although the etiology of major psychiatric disorders, such as schizophrenia, depression, generalized anxiety disorders and addiction, has not been fully elucidated, mounting evidence indicates that both genetic and early environmental factors play a role. Thus, investigations in monozygotic twins showed concordance rates of 40–70% for these disorders (Gottesman and Shields, 1982; Kendler et al., 2000; Verhulst et al., 2015), while environmental studies indicate that both prenatal and early postnatal factors are of importance. For instance, offspring born to mothers suffering from famine during pregnancy have higher risk to develop schizophrenia (Susser et al., 1996) and depression (Brown et al., 2000). Moreover, significantly increased frequency of early parental loss has been found in patients suffering from schizophrenia (Ellenbroek and Cools, 1998), depression (Agid et al., 1999), or anxiety disorders (Kendler et al., 1992). Likewise, childhood abuse or neglect has been associated with many different psychiatric disorders (Gregorowski and Seedat, 2013).

Cumulatively these findings lead to the key question of how these two sets of factors: genes and environment interact. Within the field of clinical research, with the development of sophisticated (and cheaper) molecular genetic techniques, gene environment interaction studies have flourished (Cerda et al., 2010; Duncan and Keller, 2011; Modinos et al., 2013). For instance, there is substantial evidence that the effects of early life and late life stressors on the development of depression are moderated by genetic alterations in the serotonergic system or the stress system. Thus individuals with the so-called *s*-allele of the serotonin transporter exposed to life stressors have an increased

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risk of developing major depressive disorders (Sharpley et al., 2014). Likewise, individuals with a variation in the *FKBP5* gene (which influences the ability of cortisol to alter gene-expression) exposed to traumatic life events had a significantly earlier age on onset of depression (Zimmermann et al., 2011).

However, although gene environment interactions can be studied in humans, it is virtually impossible to ascertain a causal relationship with psychopathology. In this respect, animal research offers a distinct advantage as it is more feasible to manipulate a single dependent variable, while controlling other environmental (and genetic) factors. Consequently, in order to study gene-environment interactions we used a genetic animal model of depression and, subjected these animals to early repeated maternal separation.

In the present study we used the Flinders Sensitive Line (FSL) rats as a model of depression and the Flinders Resistant Line (FRL) as controls. The FSL strain developed by Overstreet (Overstreet et al., 1979) exhibits a number of features resembling depression, such as reduced bodyweight and locomotor activity, increased rapid eye movement (REM) sleep and cognitive deficits (Overstreet and Wegener, 2013). In addition, these animals show a state of "anhedonia", i.e. reduced sucrose consumption when exposed to chronic mild stress (Overstreet et al., 1997). Finally, the increased immobility of the FSL rats in the Porsolt swim test, often considered to be a sign of depressive behavior, can be reversed by treatment with antidepressants (Caberlotto and Hurd, 1999; El Khoury et al., 2006; Overstreet, 1993; Yadid et al., 2000). Overall, these data support the assumption that the FSL line represents a useful genetic model of depression with predictive, face and possibly also construct validity (Overstreet and Wegener, 2013).

Investigations of experimental early maternal separation and deprivation in primates and rodents, have convincingly demonstrated that stress in infant animals results in altered adult behavior and neurochemistry. A variety of protocols exists, which are often subdivided into two different classes: maternal deprivation (MD) generally refers to a single 24 h period of separating pups from their mothers, and maternal separation (MS) generally referring to repeated shorter (3–6 h) periods of mother-pup separation. MD has been found to lead to schizophrenia-like abnormalities in adulthood (Ellenbroek and Riva, 2003; Husum et al., 2002; Marco et al., 2015). On the other hand, MS induces abnormalities that phenotypically resemble depression, such as altered hypothalamus-pituitary-adrenal (HPA) axis activity (Ladd et al., 1996; Plotsky and Meaney, 1993), reductions in locomotor activity (Matthews et al., 1996), alterations in the Porsolt swim test (MacQueen et al., 2003) and signs of anhedonia (Matthews and Robbins, 2003). Moreover, alterations in the swim test induced by MS can be reversed by prior treatment with antidepressant drugs (El Khoury et al., 2006; MacQueen et al., 2003). These data indicate that similar to the genetic FSL model, MS results in a number of depressive like characteristics.

In addition, we have previously demonstrated comparable neurochemical changes in non-manipulated genetic rat models of depression, such as FSL and Fawn Hooded (FH), and in normal rats following maternal separation (Caberlotto and Hurd, 1999; Husum et al., 2001; Husum and Mathe, 2002; Husum et al., 2002; Jimenez-Vasquez et al., 2001; Jimenez-Vasquez et al., 2000; Mathe et al., 1998). In particular, alterations in neuropeptide Y (NPY), tachykinins (neurokinin A (NKA) and substance P (SP)) and calcitonin gene-related peptide (CGRP) were shown. The aim of the present study was to extend this work by investigating changes in neurotensin, a peptide implicated in both schizophrenia and depression. Moreover, rather than looking at either a genetic or an environmental model, we investigated the interactive effects of these two factors.

Neurotensin is a tridecapeptide originally isolated from the bovine hypothalamus with a wide range of behavioral and physiological effects, including hypotension, analgesia, sedation, hypothermia and reward (Caceda et al., 2006; Ferraro et al., 2016; Nemeroff, 1980). While there is extensive literature on neurotensin in schizophrenia, psychosis and addiction (Binder et al., 2001; Kinkead and Nemeroff, 2002; Liu and Borgland, 2015; Richelson et al., 2003; Sarhan et al., 1997), there is a paucity of papers dealing with affective disorders and no publications regarding neurotensin in animal models of depression. However, neurotensin interacts with the dopaminergic system which is intricately related to reward and, by extension, anhedonia. Although the neurobiology of reward is complex, there is convincing evidence that the dopaminergic cells within the ventral tegmental area (VTA) are involved in reward and a reduction in cell firing has been associated with aversion and anhedonia (Chaudhury et al., 2015). Given that neurotensin was found to inhibit the dopaminergic cells within the VTA (Stuhrman and Roseberry, 2015) and that local injections of neurotensin agonists within the nucleus accumbens block the effects of dopamine agonists (Vadnie et al., 2014), this leads to the suggestion that increases in neurotensin levels in specific brain regions may contribute to anhedonia, a crucial aspect of depression.

In order to investigate this, we exposed FSL and FRL rats to early MS and in adulthood compared neurotensin concentrations in various brain regions associated with depression.

2. Material and methods

2.1. Animals

Adult FSL and FRL females from the rat colonies maintained at the animal facility at the Karolinska Institutet were mated with sexually experienced FSL and FRL males, respectively. Two weeks later the males were removed and the dams were checked for delivery twice daily (08:00 and 16:00). The day of delivery was denoted as postnatal day (PND) 0.

The housing conditions were the same for all animals throughout the study. The animals were given free access to water and food (Lactamin R36, Stockholm, Sweden) and were housed 3–4 per cage under standard conditions of humidity in temperature controlled rooms (23 $\pm$ 1 °C) and 12-h light/dark cycle (lights on at 7:00 a.m.). The experimental procedures were carried out during the light phase. All animal procedures were approved by the Ethical Committee on Animal Experiments and were conducted in conformity with the Karolinska Institutet's Animal Care Guidelines.

2.2. Maternal separation procedure

Pups randomly assigned to the separation procedure (MS180 group) were briefly handled and separated from the dam as a litter for 180 min. Handling and separation were performed daily from PND2 to PND14, beginning at 09:00 a.m. Dams were removed from the maternity cage and placed into individual cages for the duration of the separation. The litters were removed from the nest cages and placed in clean plastic chambers (15 × 15 cm) with shredded paper bedding placed in an incubator set to maintain an ambient temperature at 32 °C (PND 2–5) and 30 °C (PND 6-14). At the conclusion of the separation period, pups were returned to the maternity cage after which the dam was also returned. Control rats (no Handling, NH) were left undisturbed until weaning, except for the routine cleaning of the cages twice weekly. All pups were weaned on PND22 and from then onwards kept 4–5 rats/cage (single gender) under standard conditions with free access to rat chow and tap water ad libitum in a 12-h day/night cycle (lights on at 7:00 a.m.).

2.3. Experimental groups

The day of delivery was designated as PND0. The litters were randomly assigned to either (1) no handling (NH), or (2) maternal separation for 180 min/day (MS180) group. Thus there were four groups of male rats in the study: FRL: NH (n = 9), MS180 (n = 12); FSL: NH (n = 6), MS180 (n = 9).

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