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Neonatal tactile stimulation decreases depression-like and anxiety-like behaviors and potentiates sertraline action in young rats

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ABSTRACT

It is well known that events which occur in early life exert a significant influence on brain development, what can be reflected throughout adulthood. This study was carried out in order to assess the influence of neonatal tactile stimulation (TS) on behavioral and morphological responses related to depression-like and anxiety-like behaviors, assessed following the administration of sertraline (SERT), a selective serotonin re-uptake inhibitor (SSRI). Male pups were submitted to daily TS, from postnatal day 8 (PND8) to postnatal day 14 (PND14), for 10 min every day. On PND50, adult animals were submitted to forced swimming training (15 min). On PND51, half of each experimental group (UH and TS) received a single sub-therapeutic dose of sertraline (SER, 0.3 mg/kg body weight, i.p.) or its vehicle (C, control group). Thirty minutes after injection, depression-like behaviors were quantified in forced swimming test (FST, for 5 min). On the following day, anxiety-like behaviors were assessed in elevated plus maze (EPM), followed by biochemical assessments. TS per se increased swimming time, decreasing immobility time in FST. Besides, TS per se was able to increase frequency of head dipping and time spent in the open arms of EPM, resulting in decreased anxiety index. In addition, groups exposed to TS showed decreased plasma levels of corticosterone per se. Interestingly, while TS exposure significantly potentiated the antidepressant activity of a subtherapeutic dose of SERT, this drug was able to exacerbate TS-induced anxiolytic activity, as observed in FST and EPM, respectively. Decreased plasma levels of both corticosterone and cortisol in animals exposed to TS and treated with SERT are able to confirm the interesting interaction between this neonatal handling and the antidepressant drug. From our results, we conclude that neonatal TS is able to exert beneficial influence on the ability to cope with stressful situations in adulthood, preventing depression and favorably modulating the action of antidepressant drugs.

1. Introduction

Depression is a serious and expensive psychiatric disease (Mula, 2013) characterized by feelings of sadness, guilt, worthlessness and hopelessness (Jia et al., 2010; Petersen et al., 2001), affecting about 10–30% of women and 7–15% of men worldwide (Kessler et al., 2005). According to the World Health Organization (WHO), depression is the fourth leading cause of morbidity in the world with perspectives to become the second by 2020 (Kessler et al., 2011). Although the etiology of depression is considered multifactorial and is not well elucidated yet, its connection with deficiency of brain monoamines activity has been the mainstay for its treatment (Elhwuegi, 2004). Unfortunately, about 50% of patients who start treatment for depression do not respond to antidepressant medication (Thase, 2004), leading us to think about the existence of interferences so far unknown.

Tactile stimulation (TS) is a form of neonatal handling similar to the maternal behavior of licking and affection (Schanberg and Field, 1987), which exerts solid influence on normal brain development (Chiba et al., 2012). Some studies show that TS is able

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to affect the hypothalamic–pituitary–adrenal (HPA) axis function (Katsouliet al., 2014), improving the ability of the adult organism to adapt to stressful stimuli (Stamatakis et al., 2009) and helping in the recovery from neonatal brain injuries (Richardset al., 2012; Rodrigues et al., 2004). In addition, studies by our group have shown beneficial influence of TS to prevent preference for psychostimulant drugs (Antoniazzi et al., 2014 a,b), being also able to change responsiveness to benzodiazepine drugs (Boufleur et al., 2012).

Sertraline (SERT) is a usual antidepressant drug that belongs to the selective serotonin reuptake inhibitors (SSRIs) (Khan et al., 2005; Young et al., 2009), whose primary mechanism of action is inhibition of the serotonin (5-HT) transporter located in the presynaptic membrane of mesolimbic brain areas (Belmaker and Galila Agam, 2008). Indeed, 5-HT is a neurotransmitter responsible for mood regulation (Peirson and Heuchert, 2000) and for numerous functions in the central and peripheral nervous system (McGeer et al., 1988). In this sense, 5-HT is important in pathogenesis and treatment of different neuropsychiatric disorders including depression (Lucki et al., 1988). In line with this, forced swimming test (FST) is an animal model used to assess depression-like behavior (Cryan and Lucki, 2000), which was firstly described by Porsolt et al. (1978). FST involves two sequential sessions of exposure to deep water, from where animals cannot escape. The first session is in fact a stressor, assumed to induce a state of behavioral despair (Porsolt et al., 1978; Borsini and Meli, 1988; Castagne et al., 2009) interpreted as depression-like symptoms.

Considering the promising benefits of TS in early life, the current study was developed to assess the influence of this neonatal handling on the antidepressant activity of SERT in rats. Behaviors involving depression and anxiety-like symptoms were assessed, as well as the activity of HPA axis.

2. Material and methods

2.1. Animals and experimental procedure

Seven pregnant female *Wistar* rats from the breeding facility at Universidade Federal de Santa Maria (UFSM), RS, Brazil, were housed in Plexiglas cages with free access to food and water. Animals were kept in a room with controlled temperature (22–23 °C) on a 12-h light/dark cycle with lights on at 7:00 a.m. The experimental protocol was approved by the Animal Ethics Committee (Universidade Federal de Santa Maria), affiliated to the National Council for the Control of Animal Experimentations (CONCEA), following international norms of care and animal maintenance. All efforts were made to minimize animal suffering and to reduce the number of animals used in experiments.

The pups date of birth (postnatal day 0-PND0) was monitored, and gender distinguished by larger genital papilla and longer anogenital distance in male vs. female pups (Liu et al., 2008). On the same day, litters were culled to seven pups (four males and three females to ensure adequate nutritional status (Boufleur et al., 2012; Vazquez et al., 2006)), being only male rats designated to the current experimental protocol.

In order to avoid using littermates in the same experimental group, on postnatal day one (PND1) male pups were taken from several litters and randomly assigned to experimental groups ($n = 14$, each group), separated into unhandled (UH) and tactile stimulation (TS). TS groups received stimulus from PND8 to 14, according to the protocol described below. On PND22, litters were weaned and left undisturbed up to 50 days of age, when they were submitted to training of FST, being immersed in water for 15 min. This procedure works as a stressor which is thought to induce a state of behavioral despair (Porsolt et al., 1978; Castagne et al., 2009) related to a "depressive state" (Borsini and Meli, 1988). On PND51, half ($n = 7$) of each experimental group (UH and TS) received a single dose of sertraline (SERT, 0.3 mg/kg body weight i.p.), which corresponds to a sub-therapeutic dose (following pilot studies at our laboratory), or its vehicle (C-control group). SERT (Pharmanostra, India,) was dissolved in NaCl 0.9% plus 0.05 mL Tween 80 (Sigma–Aldrich, Brazil);vehicle (C) consisted of NaCl 0.9% plus 0.05 mL Tween 80.

SERT was the antidepressant drug chosen because it has been frequently reported in studies involving animal models (Ulloa et al., 2010; Mikail et al., 2012; Kaygisiz et al., 2014). Thirty minutes following the injections, animals were submitted to forced swimming test (FST). One hour after FST, anxiety-like symptoms were observed in elevated plus maze (EPM). Following the EPM paradigm, all animals were weighed, anesthetized with sodium pentobarbital (80 mg/kg, i.p.), and euthanized by exsanguination. Blood was collected by cardiac puncture (Fig. 1).

2.2. Neonatal handling

Tactile stimulation (TS) consisted of gently stimulating pups' dorsal surface individually with the index finger from rostral to caudal direction for 10 min (each day) out of the nest by the same experimenter (Antoniazzi et al., 2014a,b; Boufleur et al., 2012; 2013; Rodrigues et al., 2004). TS was applied daily between 12:00 and 14:00 from PND8 until PND14, according to previous studies by our group (data submitted), since most beneficial influence of TS has been observed in pups. After the procedures, pups were returned to their litters, staying the remaining time with their mothers until weaning.

2.3. Behavioral assessments

2.3.1. Forced swimming test (FST)

Behavioral responses related to depression-like symptoms are experimentally assessed in FST, whose method has been described in several studies (Porsoltet al., 1978; Wieland and Lucki1990; Castagne et al., 2009). On the first day (PND50) rats were forced to swim for a 15-min period (pretest session), and carefully dried before returning to their home cages. Twenty-four hours following the test (PND51), all rats were injected with SER or vehicle, as described above, and again submitted to FST for 5 min. Immobility, climbing and swimming times were quantified by trained raters blinded to handling and treatment. In this sense, immobility is no additional activity other than the required to keep the head above water, whereas climbing is defined as upward struggling movements of the forepaws at the side of the cylinder. Movements around the swimming cylinder (Porsolt et al., 1978) are indicative of swimming time.

2.3.2. Elevated plus maze (EPM)

One hour after FST, anxiety-like symptoms were assessed in the EPM paradigm, which is based on the innate fear rodents have for open and elevated spaces (Montgomery, 1955). The apparatus was made of wood and consisted of a plus-shaped platform elevated 50 cm from the floor. Two opposite arms (50 cm × 10 cm) were enclosed by 40 cm high walls whereas the other two arms had no walls. The four arms had a central platform (10 cm × 10 cm) at their intersection, which gave access to any of the four arms. At the beginning of each test, each animal was individually placed in the central platform facing an open arm. Time spent and number of entries in open and closed arms were monitored during a five-minute test. A shorter time spent (expressed as a percentage of total length of the test) and fewer entries in the open arms of the maze indicated anxiety (Hlavacova et al., 2010). Total activity in the maze (total number of arm entries) and number of open and closed arms