Postnatal betamethasone administration produces delayed changes in the immunohistochemical expression of brain-derived neurotrophic factor and the tyrosine kinase B receptor in the rat cerebellar cortex together with anxiety-like behavior.

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ICNDS Stroke Congress Sep 20-21, 2018 Rome, Italy

ABSTRACT

Postnatal synthetic glucocorticoids (GCs) are widely used in the prevention of chronic lung disease in premature infants and their pharmacologic use is associated with neurodevelopmental delay and various behavioral and brain changes. Previous studies demonstrated that animals that experience stress, either via chronic maternal stress or exogenous GCs administration, exhibited a significant alteration in the neurotrophic factors regulation and behavioral changes associated with anxious behaviors. The brain-derived neurotrophic factor (BDNF) and the tyrosine kinase B receptor (Trk-B) are involved in cerebellar Purkinje cells (PC) dendritic development and maintenance and are located predominantly in the cerebellar molecular layer where PC dendritogenesis occurs. We **hypothesized** that previously demonstrated reductions in protracted lower PC dendritic arborization could be due, at least in part, to possible changes in dendritic expression of BDNF and Trk-B. **Objectives:** We evaluated whether postnatal administration of betamethasone (BET) alters the immunohistochemical (IHC) expression of BDNF and Trk-B in the cerebellar vermis along with anxiety behaviours. **Results:** We found that rats postnatally treated with BET at P1 and P2 showed a significant reduction in the IHC expression of BDNF together with increases in the IHC expression of the TrkB receptor in adolescent (P52 days of age) BET treated animals. Moreover, these changes were accompanied by an increase of anxiety-like behaviors in the elevated plus maze and marble burying test in infant (P22 days of age) and in adolescent rats.

MATERIALS AND METHODS

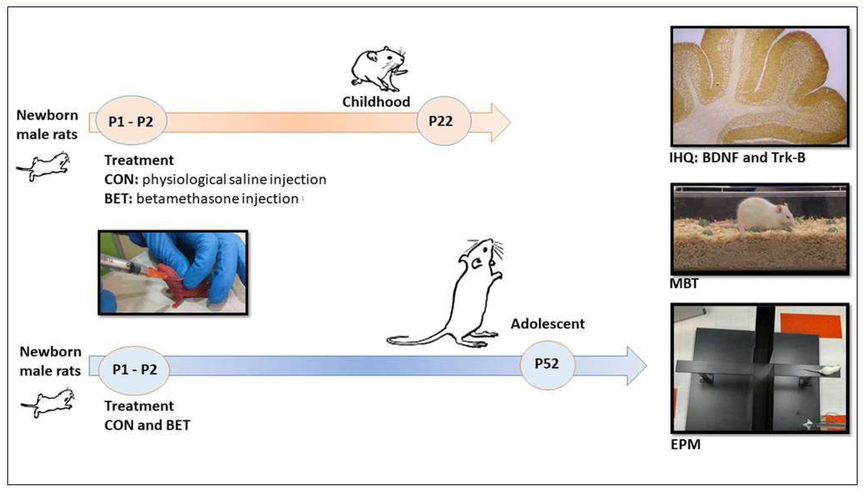

Figure 1. Experimental design. In order to achieve postnatal stress, male pups rats were given betamethasone injection, a daily dose of betamethasone (1µg.g-1 subcutaneously, in the dorsal region of the neck) from the first day after giving birth till postnatal day 2 (P1 and P2). The control group given physiological saline injection of de same form [1]. Pups were then weaned at P21, and they were subjected to behavioral testing in P22 and a other group were evaluated in P52. Behavioral testing included elevated plus maze (EPM) test and marble burying (MBT) test. Behavioral analysis was performed with ANY MAZE software. On day P22 and P52 the tissues were collected to evaluate BDNF BDNF (N-20)sc-546 (1/500), and Trk-B Trk-B (794) SC-12 (1/500) expression in the molecular layer by immunohistochemistry.

RESULTS

1. BEHAVIORAL TESTS

1. 1 BET prenatal exposure increase anxiety –like behaviours in adolescents rats in the elevated plus maze perform.

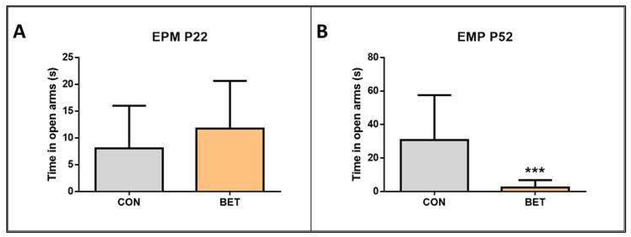

Figure 2: Time in open arms The bar graphs show the time spent in open arms from (5-minutes session). The data as presented as the mean ± standard error of the mean from: (CON) control animals; (BET) betamethasone treated animals. P22 (age in days): N= CON(12);BET(13) and P52 (age in days): N= CON(12);BET(14). The data were analyzed using student test (*** p <00,1).

1.2 BET prenatal exposure increase anxiety –like behaviours in infants and adolescents rats in the Marble burying test perform.

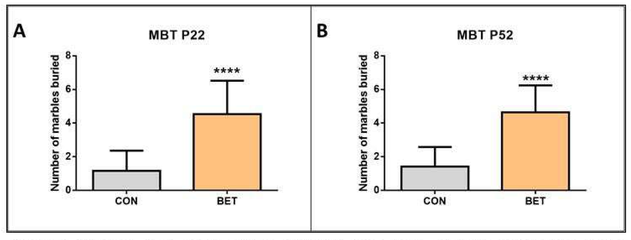

Figure 3: Number of marbles buried. The bar graphs show the total number of buried marbles (10-minute session). The data as presented as the mean ± standard error of the mean from: (CON) control animals; (BET) betamethasone treated animals. P22 (age in days): N= CON(12);BET(13) and P52 (age in days): N= CON(12);BET(14). The data were analyzed using using student test (*** p <00,1).

2. HISTOLOGICAL ANALYSIS

2.1 BET prenatal exposure decrease BDNF and increase Trk-B immunohistochemical expression in the molecular layer of cerebellar vermis of adolescents rats.

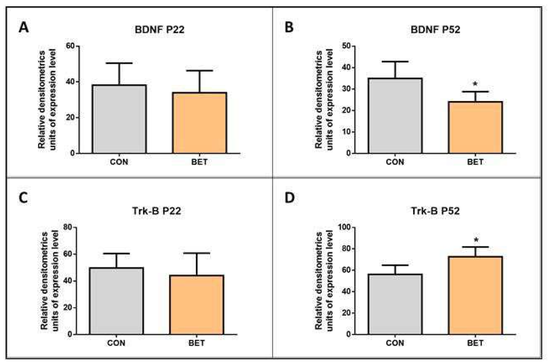

Figure 4: Expression of BDNF and Trk-B in the cerebellar molecular layer in (CON) control animals and (BET) betamethasone treatment. The **BDNF** label intensity of a digital photomicrograph in (**A**) P22 is not different between groups and (**B**) P52 is significant different (p<0,05). The **Trk-B** label intensity of a digital photomicrograph in (**C**) P22 is not different and (**D**) P52 is significant different (p<0,05). The data as presented as the mean + standard error of the mean (N=5, per group). The data were analyzed using student test (* p <0.05).

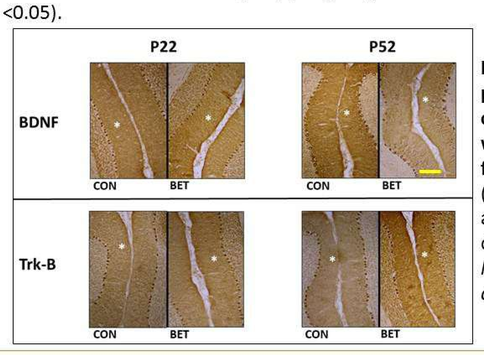

Figure 5: Representative photomicrographs of the cerebellar molecular layer stained with anti-BDNF and anti-Trk-B from: (CON) control animals and (BET) betamethasone treated animals, in P22 and P52 (age in days). *White asterisks: molecular layer. Scale bar (30 um, approximately).*

DISCUSSION

It has been shown that in the cerebellar vermis, BDNF is abundantly expressed in PC and promotes cell maturation[4,5]. Therefore, we hypothesized that BDNF underexpression could be part of a mechanism through which cerebellar PC dendritic deterioration occurs. Similarly, the TrkB receptor is expressed in the cerebellar molecular layer, and the TrkB receptor binds to BDNF to produce powerful neurotrophic effects [6,7]. Because there is a low expression of BDNF (caused by the postnatal administration of BET), an overexpression of its receptor is induced, which corresponds an adaptive neural tissue responses to reductions in neurotransmitters or neuropeptides is the membrane expression of new receptors [8]. On the other hand, our current data support the hypothesis that prenatal GCs lead to anxiety disorders later in life [9], because anxious behaviors in the MBT were detected at P22 suggests that this test may be more sensitive to detecting anxiety-like states in rodents compared with the EPM. Consistent with our findings, early postnatal stressed animals exhibit a significant reduction in BDNF levels and anxiety-like behavior assessed in the EPM. Finally, TrkB receptor expression impairment appeared to be also related with anxiety- like behaviors in the EPM test [10].

CONCLUSIONS

Postnatal betamethasone administration produces delayed changes in the immunohistochemical expression of BDNF and the TrkB receptor in the cerebellar cortex together with anxiety-like behavior in infant and adolescent rats. Furthermore, although in the current study we administered a similar dose of betamethasone used in perinatal medicine [2] and the rat is currently a good mammalian animal model, it is necessary to keep in mind the potential species-specific differences when extrapolates animal data to the human condition [3].

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