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# Multimodal early-life stress induces biological changes associated to psychopathologies

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ABSTRACT

Evidences suggest the contributive role of early-life stress (ELS) to affective and anxiety disorders. Chronic exposure to the same stressor may generate habituation, while the exposure to different and repeated stressors gradually promotes maladaptive plasticity. Therefore, to further understand the effects of heterotypic stressors during early life period, male Wistar rat pups (P1–P21) were exposed to Multimodal ELS paradigm. Results indicate pups did not habituate to multimodal ELS and neonates respond to both physical and psychogenic stressors. Adult rats that underwent ELS protocol showed significant lower sucrose intake, decreased latency to immobility in the forced swim test and increased latency to light compartment in the light-dark test when compared to control group. Although it has been shown that ELS-induced changes in hippocampus can be used as biomarkers, multimodal ELS did not significantly alter BDNF, Tyrosine Kinase B (TrkB) receptor expression or neurogenesis in the hippocampus. Taken together, these findings indicate that multimodal ELS protocol can be an interesting experimental model for understanding long-term psychiatric disorders associated with stress. Indeed, our data with neurogenesis, BDNF and TrkB, and conflicting data from the literature, suggest that additional studies on synaptic plasticity/intracellular cascades would help to detect the underlying mechanisms.

## 1. Introduction

Adversities in early life are associated with vulnerability to psychopathologies later in life, resulting in long-term impact in emotional function (Chrousos, 2009; Gold et al., 1988; Heim and Nemeroff, 2002; Juruena, 2013; Lippmann et al., 2007; Lupien et al., 2009).

Patients suffering with stress-related psychiatric disorders appear to be especially sensitive to the effects of early life stress (ELS), which is supported by epidemiological data (Baes et al., 2014; Juruena, 2013). For instance, traumatic childhood experiences such as abuse, neglect and parental losses increase the incidence of Major Depression (MD) (Anda et al., 2006) which could reach in adult life between 59% and 75% (Widom et al., 2007). Depression affects millions of people and causes significant impairments on patient's quality of life (Ustün et al., 2004). Epidemiological data ranks depression in the top fourth causes of global burden of disease (Mathers and Loncar, 2006; Murray and Lopez, 1997), thus representing a serious problem of public health worldwide. Projections estimate that in 2020 nearly one in ten people on the planet (676 million) will present a depressive episode (World Health Organization, 2017).

Anxiety disorders are often observed as comorbid with depression, indicating common neurobiological mechanisms between these mental illnesses (Craske et al., 2009; Craske and Stein, 2016). People that experience early emotional traumas are 1.9 to 3.6 fold more likely to develop anxiety disorders (Fernandes and Osório, 2015). Depression-anxiety comorbidity is strongly associated with impairment in health, as well as in emotional functions (Kroenke et al., 2007).

A better understanding of how stress in early life impacts brain and behavior in adulthood is, therefore, of fundamental importance to the study of the neurobiology of psychiatric disorders. Although plenty of evidences indicate that ELS exposure leads to behavioral changes in adulthood (Bale et al., 2011; Gee and Casey, 2015; Lai and Huang, 2011; Loi et al., 2014; Lupien et al., 2009; Walker et al., 2017), there are some reports proposing that there is a period during early life in which the response to stress is reduced or absent, also known as the stress hyporesponsive period (SHRP). Interestingly, ELS experiences can

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disrupt the Stress SHRP, turning the HPA-axis responsive to stressors (Daskalakis et al., 2015) and there is growing evidence that brain functioning during development is affected by ELS (Cowan et al., 2016). In light of these data the use of animal models can provide a better understanding of the pathological mechanisms that increases vulnerability to depression and other stress-related psychiatry disorders, since the relationship between environmental factors, as well as the underlying brain mechanisms involved in behavioral changes, may be better examined (Abelaira et al., 2013).

A number of animal models have been developed to investigate the underlying changes caused by ELS on the developing brain. Since the 1970's, studies that started with Levine and colleagues have demonstrated that early developmental manipulations related to maternal care, commonly, by maternal separation (MS) paradigm, result in intermittent stress and provoke a profound and lasting impact on emotionality and stress response (Levine, 1967; Levine, 2005; Nishi et al., 2014). Since then, most ELS long-lasting effects were obtained by employing protocols that require long periods of separation (with daily separation for 3 h, or maternal deprivation for 24 h) or a second hit (Holmes et al., 2005). But only a few studies have shown significant structural changes in limbic brain regions, in the HPA axis and in behavior in adulthood (Loi et al., 2014).

Therefore, rodent models findings have been supported upon protocols based on one type of psychological stressor, altering the amount of maternal care, which may not necessarily reflect a translational perspective. Even though MS models have provided a vast amount of data on the effects of reduced maternal input on pup development, this manipulation may differ from the human condition since the mother is typically present but infants and children grow up in chronic rather than intermittent stress (Molet et al., 2014). Moreover, the odds of developing depressive disorders with exposure to physical stressors were greatest in prospective studies (Norman et al., 2012). Chronic variable stress protocol, which applies different modalities of stressors, is commonly employed in adult life. Although this type of paradigm is extensively used, its impacts have not been studied early in life.

Development of brain structures related to stress system seems to be tuned specifically to modalities relevant to the early-life period. Also, the chronic exposure to the same stressor may generate habituation (Gadek-Michalska & Bugajski, 2003) while the exposure to different and repeated stressors gradually changes the electrical, proliferative and morphological characteristics of neurons (Joëls et al., 2007). Therefore, protocols employing chronic variable stress during early life could provide a valuable tool to investigate stress effects on neurodevelopment and behavior in adulthood. Although this type of paradigm is extensively used in adults (Willner, 2017), its impacts have not been studied early in life.

There is mounting evidence of multiple dysregulated mechanisms that can be considered interesting biomarkers but reduced levels of growth factors and altered endocrine/metabolic function stand out (Schmidt et al., 2011). Although neurotrophic factors are known to play an important role in the growth and survival of neurons (Mpofana et al., 2016), the role of brain derived neurotrophic factor (BDNF) or its main receptor, tyrosine kinase B (TrkB) in the pathophysiology of depression is in fact complex and not completely understood (Castrén and Rantamäki, 2010). Nevertheless, a clear direction (increase vs decrease) of the expression of either hippocampal BDNF or TrkB after ELS has not been detected (Daskalakis et al., 2015), neither it has been reported a distinction between dorsal and ventral hippocampal sub-regions. Additionally to BDNF-TrkB signaling, disturbed adult neurogenesis may also contribute to hippocampus malfunctioning and symptom development in depressed patients (Lee et al., 2013).

Therefore, it is conceivable that the controllability and predictability of stimuli are important developmental determinants for the adult capacity to cope with environmental demands (Koolhaas et al., 2011). Considering this, our study investigated the effects of a new variable protocol of early life stress on behavioral and molecular changes related to depression, later in adulthood, with the aim to provide a new framework for understanding how changes in neuroendocrine function and their relationship with depression and anxiety behavior can be better explored.

## 2. Material and methods

### 2.1. Animals

Female rats between 18 and 21 days of pregnancy were acquired from the Central Vivarium of the University of São Paulo, Ribeirão Preto Campus (USP-RP) and monitored twice a day until birth. Animals were housed in transparent acrylic boxes (45x32x17cm), at the Animal Facility of the Physiology Department of the Ribeirão Preto School of Medicine at the University of São Paulo, under controlled ventilation and temperature (25 ± 2 °C) with light/dark cycle of 12 h (lights on 7:00 A.M.) and with free access to water and regular chow. After birth, litters were culled to 6 males and 2 female pups. On PND21, the pups were weaned and males were separated. In all protocols described below, procedures (undisturbed or stress) were applied to the entire litter, which were randomly assigned. Different batches of animals were used in each experiment (Table 1).

All procedures involving animals were conducted accordingly to the Ethical Principles in Animal Experimentation, avoiding unnecessary or intentional pain and discomfort. This study was approved by the Ribeirão Preto Medical School - USP Ethics Committee (CETEA) under the protocol number 80/2014.

### 2.2. Experiment 1—effects of different modalities of acute stressors during early life on plasmatic corticosterone concentration

To test the hypothesis that pups present a different hormonal response to different modalities of stressors, naïve animals at PND12 were acutely exposed to different modalities of stressors (same employed on ELS protocol). Maternal separation for 60 min (n = 8) or 10 min (n = 6); restraint with agitation for 60 min (n = 8) or 10 min (n = 8); cold exposure for 10 min (n = 8); propylenoglycol injection (n = 8) or an undisturbed control group (n = 19). After stressor exposure animals were returned to their homecages and were decapitated 30 min after the end of each stressor. Trunk blood was collected between 8:00 and

**Table 1**
Experimental design.

| | Condition | P3 | P12 | P21 | P22 | P82–92 | P88–94 | P90–94 | n |
|---|---|---|---|---|---|---|---|---|---|
| Experiment 1 | Acute multiple stressors on P12 | | Trunk blood | | | | | | 6–19 |
| Experiment 2 | Multimodal ELS 1-21 | Trunk blood | Trunk blood | Trunk blood | Trunk blood/HPA axis organs | | | | 8–17 |
| Experiment 3 | Multimodal ELS 1-21 | | | | | SCT | FST | Trunk blood/fresh brain collection/HPA axis organs or perfusion | 12–14 |
| Experiment 4 | Multimodal ELS 1-21 | | | | | SCT | LDT | Fresh brain collection | 10–13 |

SCT – sucrose consumption test, FST – forced swim test, LDT – light dark test.