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Research report

Effects of chronic stress in adolescence on learned fear, anxiety, and synaptic transmission in the rat prelimbic cortex

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HIGHLIGHTS

- Chronic stress in adolescence decreases excitatory synaptic transmission in the prelimbic cortex.
- Chronic stress in adolescence slows the extinction of learned fear and enhances anxiety-like behavior.
- Stress-induced alterations of the prelimbic cortex and learned fear were reversed in adulthood.
- The anxiogenic effect of chronic stress in adolescence was still present in adulthood.

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ABSTRACT

The prelimbic cortex and amygdala regulate the extinction of conditioned fear and anxiety, respectively. In adult rats, chronic stress affects the dendritic morphology of these brain areas, slowing extinction of learned fear and enhancing anxiety. The aim of this study was to determine whether rats subjected to chronic stress in adolescence show changes in learned fear, anxiety, and synaptic transmission in the prelimbic cortex during adulthood. Male *Sprague Dawley* rats were subjected to seven days of restraint stress on postnatal day forty-two (PND 42, adolescence). Afterward, the fear-conditioning paradigm was used to study conditioned fear extinction. Anxiety-like behavior was measured one day (PND 50) and twenty-one days (PND 70, adulthood) after stress using the elevated-plus maze and dark-light box tests, respectively. With another set of rats, excitatory synaptic transmission was analyzed with slices of the prelimbic cortex. Rats that had been stressed during adolescence and adulthood had higher anxiety-like behavior levels than did controls, while stress-induced slowing of learned fear extinction in adolescence was reversed during adulthood. As well, the field excitatory postsynaptic potentials of stressed adolescent rats had significantly lower amplitudes than those of controls, although the amplitudes were higher in adulthood. Our results demonstrate that short-term stress in adolescence induces strong effects on excitatory synaptic transmission in the prelimbic cortex and extinction of learned fear, where the effect of stress on anxiety is more persistent than on the extinction of learned fear. These data contribute to the understanding of stress neurobiology.

1. Introduction

The Austro-Hungarian researcher Hans Selye introduced the concept of stress, which he defined as a complex biological response common to all living organisms induced by environmental threats (i.e., stressors) [55]. Stress is oriented to restoring homeostasis and adaption to environment pressures [7,32]. When the threat is too intense and persistent, stress responses may become maladaptive and affect the brain [32,52]. In adulthood, chronic stress impairs limbic structures such as the hippocampus and amygdaloid complex, and the medial prefrontal cortex (mPFC) in both animal models and humans [27,42,62–64]. These brain areas regulate anxiety and fear [14,22,40].

Anxiety is a long-lasting state of apprehension elicited by threats that are not immediately present [12], while fear is an adaptive state activated by real threats, which begins rapidly and dissipates once the threat disappears [12]. The amygdala and *Bed Nucleus of Stria Terminalis* (BNST) are involved in anxiety-like behaviors, while the central nucleus of the amygdala (CeA) and the periaqueductal gray (PAG) nucleus neuronal pathway are associated with fear

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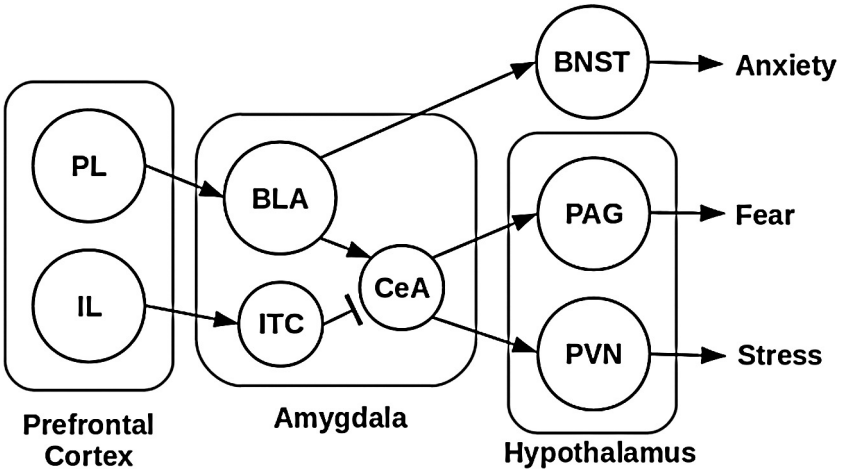

Scheme 1. Simplified schematic diagram of the neural circuitry involved in fear and anxiety-like behavior responses. The amygdala complex, mPFC and hypothalamus are the main structures involved in fear and anxiety. During fear conditioning, sensory information about the US and CS converges on the BLA, leading to the storage of the CS-US association within this nucleus. The BLA is also activated by integrated sensory information from PL. In consequence, the presentation of the CS alone, which after conditioning is considered a predictor of explicit threat, activates a set of BLA neurons, which in turn activates the CeA, but not the BNST. The CeA projects to PAG and PVN, triggering the physiological and behavioral responses of freezing and stress, respectively. During extinction of conditioned fear, CS sensory information enters the mPFC and BLA. It activates IL in the mPFC, which along with the BLA, projects and activates ITC cells. The ITC are GABAergic neurons that project to the CeA, inhibiting this nucleus, leading to the reduction of freezing and stress responses. Sensory information, which is a less explicit predictor of threat, enters a set of neurons in the BLA, which now instead of activating the CeA, activate the BNST. This nucleus activates the PAG and PVN, triggering the physiological and behavioral responses of freezing and stress, respectively.

regulation [12,13,22] (Scheme 1). Anxiety levels are higher in rats subjected to chronic stress during adulthood [9,62], which is associated with dendritic hypertrophy in both the basolateral amygdala (BLA) and BNST [61,63,64]. In addition, chronic stress induces dendritic atrophy in the pyramidal neurons of layers II/III and V of the mPFC in adult rats [5,31,42,49], a brain area that regulates the recall of learned fear [22,38,47,56].

1.1. Effects of stress in adolescence and adulthood

Adolescence is the only transitional period between childhood and adulthood that is characterized in all mammals by several behavioral, hormonal, and neural changes [57,58]. Adolescence is an extended period that includes puberty and culminates in reproductive maturation. Adolescence in male rats is considered to last from post-natal day (PND) 35 to 55 [39], with physical markers of sexual maturation observed from PND 45 to 48 in males [26].

The brain is particularly sensitive to stress in the adolescence [11]. It has been shown that adolescent rats display higher levels of stress responses than do adult animals [31,45]. For example, adolescent rats subjected to chronic stress have higher plasma corticosterone levels than do adults under same condition. As well, the corticosterone levels of adolescent can take twice as long as those adults to return to the baseline after acute stress [16,19]. The rat mPFC and amygdala, and the neuronal connectivity between them begin developing during adolescence and continue into adulthood [8,24]. Chronic stress affects the dendritic morphology of these brain areas in adolescence and adulthood [10,15,63]. As well, it has been shown that after 21 days of stress-free recovery, adult rats continue showing enhanced anxiety-like behavior and dendritic hypertrophy in the amygdala, while mPFC neurons completely restore their dendritic structure and functions [18,48,64]. A comparable study with adolescent mice treated with corticosterone, shows that this hormone decreases spinal density in the infralimbic and orbitofrontal cortices of the mPFC, while in the amygdala this treatment increases spinal density [21]. After a corticosterone-free recovery period, dendritic changes in the prelimbic cortex and amygdala were reversed, while the dendritic changes in the orbitofrontal cortex remained unchanged after the recovery period [21]. It is not known whether the effects of stress on the mPFC and amygdala during adolescence are reversed in adulthood.

1.2. Fear conditioning

Fear conditioning is a three-phase behavioral paradigm used to study the neural circuit of fear [53]. The first phase is conditioning in which the rats are trained in a shuttle box to associate a conditioned stimulus (CS) (a tone) with an unconditioned stimulus (US) (e.g. a foot shock). Once learned, the CS will by itself elicit a conditioned response. For instance, in the fear conditioning paradigm freezing is a behavioral and physiologically conditioned response to fear [25]. It is supposed that fear memories acquired during conditioning are consolidated in the BLA area of the brain [25,43,54] (Scheme 1). The recall of learned fear is associated with increased neuronal activity in the BLA, which in turn activates the CeA. Direct projections are sent from this brain structure to the PAG and paraventricular nuclei (PVN) to elicit defensive fear-behaviors (Scheme 1) [25]. During the fear extinction phase, the CS is presented several times to the rats, which allows them to acquire a new memory that suppresses the retrieval of the previously learned fear. The mPFC regulates the expression of extinction memory via the amygdala [46]. In the recall phase, the CS is presented to the rats and they recall the conditioned fear extinction learned during the extinction phase, which in turn decreases the freezing behavior elicited in the animals [47].

The prelimbic cortex (PL) integrates auditory, contextual, and stress-related signals from several brain areas during fear conditioning to regulate fear expression by the basal amygdala (Scheme 1) [6]. PL activity is key for the expression of fear and memory extinction. For example, in vivo stimulation of the PL increases freezing during the conditioning phase [60]. Chronic stress-induced dendritic atrophy in the PL and dendritic hypertrophy in the lateral amygdala, both in adulthood, have been correlated to failure to express fear extinction [36,48,63,66].

In general, the acquisition of the conditioned fear is regulated by BLA [25,43,54], while the mPFC controls the recall of the extinction of conditioned fear [47,56]. In adulthood, hypertrophy of the rat BLA neurons induced by chronic stress persists after 3 weeks of stress-free recovery, while the morphological alterations induced