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# Aging impairs the control of prefrontal cortex on the release of corticosterone in response to stress and on memory consolidation

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**Abstract**

This study investigated the role of the dorsomedial prefrontal cortex (dmPFC) on the activity of the hypothalamus-pituitary-adrenal axis and memory consolidation in young and aged rats. The messenger RNA (mRNA) expression of several gamma-aminobutyric acid (GABA) and glutamate receptor subunits were also evaluated in the prefrontal cortex (PFC) of young and aged rats. Microinjections of picrotoxin ($GABA_A$ antagonist), muscimol ($GABA_A$ agonist), or vehicle were performed into the dmPFC of young adult (3 months) and aged (27 months) male Wistar rats. Plasma corticosterone was measured under acute stress (30-minute restraint) conditions following microinjections. The retention of an inhibitory avoidance response was also evaluated in response of the same treatments. Picrotoxin microinjections into the dmPFC reduced the stress-induced corticosterone concentrations on young but not on aged animals. Aging did not modify the mRNA content of any of the receptor subunits analyzed. Picrotoxin into the dmPFC reduced inhibitory avoidance response in young but not aged animals. Muscimol treatment did not modify any of the parameters evaluated. These results suggest that prefrontal cortex loses its capacity to control hypothalamo-pituitary-adrenal (HPA) axis activity and the consolidation of emotional memory during aging.


*Keywords:* Prefrontal cortex; Corticosterone; Stress; Picrotoxin; Muscimol; Inhibitory avoidance; Microinjection; Rat

## 1. Introduction

Aging is a physiological process associated with a general decline of learning and memory functions (Mora et al., 2007). Some of these changes are thought to be produced, at least in part, by a dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis (Lupien et al., 2009; Sapolsky et al., 1986). In fact, basal corticosterone Levels (Issa et al., 1990; Montaron et al., 2006) and stress-induced levels of corticosterone (Garrido et al., 2010; Issa et al., 1990; Sapolsky et al., 1986) are increased in aged animals. Enhanced levels of corticosterone are thought to produce damage in the prefrontal cortex (PFC) and the hippocampus that would lead in turn to age-related cognitive deficits (Cerqueira et al., 2008; Conrad, 2008; McEwen, 2000; Radley et al., 2006a).

In particular, the PFC is involved in the regulation of the HPA axis activity (Jankord and Herman, 2008). It has been reported that acute stress increases the activity (measured as c-Fos expression) of the PFC (Cullinan et al., 1995; Ostrander et al., 2003; Weinberg et al., 2010) and lesions of the dorsomedial PFC (dmPFC) lead to an enhanced increase of corticosterone in response to stress (Diorio et al., 1993; Radley et al., 2006b, 2009; Sullivan and Gratton, 2002). Furthermore, it has been recently shown that the activation of the medial PFC by local picrotoxin microinjections inhibits the stress-induced increases of corticosterone in young adult rats (Weinberg et al., 2010). These studies suggest that the activation of the dmPFC plays an inhibitory role on the HPA axis activity. In the context of aging, a recent study has shown that acute stress leads to a reduced neuronal activation (measured as *c-fos* expression) in the PFC of aged rats compared with young rats (Nagahara and

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Handa, 1997; Shoji and Mizoguchi, 2010), which suggests the possibility for a blunted activation of the PFC in response to acute stress as the underlying cause of the dysregulation of the HPA axis observed in aged animals.

Previous studies have suggested a close relationship between corticosterone levels and emotional memory. In fact, it has been shown that the stimulation of corticosterone receptors enhances, while their blockade impairs the consolidation of memory related to aversive stressful stimuli in young animals (Roozendaal et al., 2002, 2009a). Moreover, the PFC has been suggested to play a relevant role in the performance of tasks evaluating emotional memory (i.e., inhibitory avoidance task) (Izquierdo et al., 2007; Maaswinkel et al., 1996; Zhang et al., 2011). Because the PFC is thought to modulate corticosterone release in response to stressful events, it can be hypothesized that a deficient regulation of the HPA axis by the PFC during aging can alter memory consolidation. However, few studies have investigated whether changes in the activity of the PFC modulate memory consolidation during aging.

The main aim of this work was to study the role of the dmPFC in young adult and aged animals, on: (1) the release of corticosterone in response to an acute stress, and (2) memory consolidation, by using an inhibitory avoidance paradigm. These objectives were tested by activating or inhibiting the dmPFC through local microinjections of the gamma-aminbutyric acid (GABA) receptor type A antagonist picrotoxin and agonist muscimol, respectively. Previous studies have shown that picrotoxin and muscimol produce neurochemical and behavioral effects through the reversible activation or inhibition of the PFC (Amat et al., 2005; Canales, 2005; Onge and Floresco, 2010; Weinberg et al., 2010). Also the effects of aging on messenger RNA (mRNA) levels of several glutamate (NR1, GluR2) and $GABA_A$ ($\alpha$1, $\beta$2, $\gamma$2) receptor subunits were evaluated in the PFC.

## 2. Methods

### 2.1. Animals

Young adult (3 months, weight 408 ± 8 g, $n = 29$) and aged (27 months, weight 676 ± 24 g, $n = 28$) male Wistar rats (Universidad de Granada, Spain) were group housed (3–4 animals per cage) and provided with ad libitum food and water and maintained in a temperature-controlled room (22 ± 2 °C) under an inverted light/dark cycle (lights on at 20:00–8:00). The experiments were carried out during the dark phase of the cycle. Aged animals were thoroughly examined for wounds, pain, or tumors. Two aged animals were excluded from the study due to the presence of tumors. All experiments carried out in our laboratory at the Universidad Complutense of Madrid followed the Spanish regulations for the protection of laboratory animals (RD1201/2005).

### 2.2. Microinjections into the dmPFC

Animals were anesthetized with equithesin (2.5 mg/kg intraperitoneally) and stereotaxically implanted in the brain with bilateral guide-cannulae to reach the prelimbic region of the dorsomedial PFC with the following coordinates: + 3.2 mm rostral, 0.8 mm medial, from bregma; and −2 mm from the top of the skull, with the incisive bar set at −2.5 mm for young and −3 mm for aged rats (Paxinos and Watson, 1998). Guide cannulae, 23-gauge stainless-steel (PlasticsOne, Roanoke, VA, USA) were fixed to the skull surface with dental acrylic and 3 stainless-steel anchorage screws. Dummy cannulae, 28-gauge stainless steel, were inserted into the guide to keep it clean and to prevent occlusion. Seven days after surgery, bilateral intra PFC injections were performed by means of injection cannulae, 28-gauge stainless steel, protruding 1 mm below the tip of the guide and attached to a micropump (Harvard Apparatus, Holliston, MA, USA) at a flow rate of 0.25 $\mu$L per minute. A total volume of 0.25 $\mu$L per side was injected (60 seconds) maintaining the injection cannulae in place for 60 seconds to allow the diffusion of the drug and/or vehicle. Flow rate injection and volume per side were set at low levels to try to avoid diffusion of the drug to neighbor brain regions. Each rat received 2 injections (corticosterone measurements and inhibitory avoidance test), with a period of 6–7 days between them, and all animals received a different treatment in the second microinjection. Rats remained in their experimental cages during 1 hour after the corresponding microinjection to minimize effects of the microinjection procedure on basal corticosterone levels.

### 2.3. Drugs

The noncompetitive $GABA_A$ receptor antagonist picrotoxin (Sigma-Aldrich, Spain) (0.20 $\mu$g per side) and agonist muscimol (Tocris Bioscience, UK) (0.25 $\mu$g per side) were freshly dissolved in artificial cerebrospinal fluid (CSF) consisting of (in millimolar: NaCl 137, $CaCl_2$ 1.2, KCl 3, $MgSO_4$ 1, $NaH_2PO_4$ 0.5, $Na_2HPO_4$ 2, glucose 3, pH = 7.3). Artificial cerebrospinal fluid was also used as vehicle treatment. Drug doses were based on previous studies performing microinjections into the PFC, and they are within the range of concentrations probed to be effective in modulating fear conditioning and extinction (Allen et al., 2008; Sierra-Mercado et al., 2011), responses of neurotransmitter systems to stress and local N-methyl D-aspartate (NMDA) receptors blockade (Amat et al., 2005, 2008; Del Arco et al., 2011), or enhancing responses to psychoactive drugs (Canales, 2005). Muscimol and picrotoxin microinjections are in fact well-established methods for transient inhibition and activation, respectively of brain regions (Amat et al., 2005; Berretta et al., 2005; Maeng et al., 2010; Shima and Tanji, 1998; Weinberg et al., 2010).