

SEXUALLY DIMORPHIC LONG-TERM EFFECTS OF AN EARLY LIFE EXPERIENCE ON AMPA RECEPTOR SUBUNIT EXPRESSION IN RAT BRAIN

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Abstract—Neonatal handling, an experimental model of early life experiences, is known to affect hypothalamic–pituitary–adrenal (HPA) axis function, thus increasing adaptability, coping with stress, cognitive abilities and in general brain plasticity-related processes. AMPA receptors (AMPA) mediate fast synaptic transmission at excitatory glutamatergic synapses in the CNS and are crucial during neuronal development, synaptic plasticity and structural remodeling. AMPARs are composed of four types of subunits, designated as AMPA glutamate receptor subunits (GluA1, GluA2, GluA3 and GluA4), which combine to form tetramers. The present study addressed the question of whether neonatal handling (15 min daily maternal separation from postnatal day 1 (PND1) to PND21) might have an effect on GluA1–4 mRNA levels in adult rat male and female brain using *in situ* hybridization. We have identified selective sexually dimorphic effects of neonatal handling on the mRNA expression levels of AMPAR subunits in adult rat hippocampus and nuclei of the amygdaloid complex. In the dorsal hippocampus GluA1 mRNA levels were increased in handled males, while they were decreased in handled female animals. In the ventral hippocampus and the amygdaloid complex GluA2 mRNA was lower in handled females, while no

effect was observed in handled males. Furthermore, we observed that neonatal handling induced in both sexes decreases of GluA2 mRNA in the dorsal hippocampus, as well as in the somatosensory and occipital cortex, of GluA3 mRNA in most hippocampal areas, amygdaloid complex and cortical regions studied, and of GluA4 mRNA in the ventral hippocampus. These results show that glutamatergic transmission is markedly affected by an early experience. The neonatal handling-induced alterations in AMPAR subunit composition are in line with the increased brain plasticity, the more effective HPA axis function, and in general the more adaptive behavioral phenotype known to characterize the handled animals. © 2013 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: neonatal handling, early handling, brief maternal separation, limbic system, brain plasticity, *in situ* hybridization.

INTRODUCTION

Neonatal handling is a firmly established animal model of early life experiences, having well-documented long-lasting effects on the stress response, emotional reactivity and cognitive abilities. In this experimental paradigm the pups are separated from their mother briefly (15 min daily) during the first 3 weeks of life (Levine, 1957). This separation, however, is of short duration and when the pups are returned to their nest the mother shows increased maternal care toward them (Levine, 1994; Liu et al., 1997; Pryce et al., 2001; Fenoglio et al., 2006). Neonatal handling results in increased number of GR receptors in the adult brain (Meaney and Aitken, 1985; Meaney et al., 1996; Wilber et al., 2008) as well as reduced corticotropin-releasing hormone (CRH), adrenocorticotropin (ACTH) and corticosterone release following exposure to stressful stimuli (Plotsky and Meaney, 1993; Bhatnagar and Meaney, 1995; Vallee et al., 1996, 1997; Liu et al., 1997). These neurochemical changes permanently alter the programming of the hypothalamic–pituitary–adrenal (HPA) axis function, resulting in increased ability of the organism to respond to, cope with and adapt to stressful stimuli (Levine, 1957; Meaney and Aitken, 1985; Meaney et al., 1991; Meerlo et al., 1999; Fernandez-Teruel et al., 2002; Fenoglio et al., 2006). As a corollary, handled rats experience less fear in novel environments, tend to explore more their surroundings, and display lower emotionality (Meaney et al., 1991;

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Abbreviations: ACTH, adrenocorticotropin; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AMPARs, AMPA receptors; ANOVA, analysis of variance; Ba, basal amygdaloid nucleus; CeA, central amygdaloid nucleus; CRH, corticotropin-releasing hormone; dCA1, dorsal field 1 of Ammon's horn; dCA3, dorsal field 3 of Ammon's horn; dDG, dorsal dentate gyrus; GluA1–4, AMPA glutamate receptor subunits 1–4; HPA axis, hypothalamic–pituitary–adrenal axis; IL, infralimbic cortex; LTP, long-term potentiation; MeA, medial amygdaloid nucleus; NMDA, *N*-methyl-D-aspartate; Occip, occipital cortex; PBS, phosphate-buffered saline; PND, postnatal day; PrL, prelimbic cortex; RT, room temperature; SEM, standard error of the mean; Sms In, somatosensory inner layers; Sms Out, somatosensory outer layers; Str, Striatum; vCA1, ventral field 1 of Ammon's horn; vCA3, ventral field 3 of Ammon's horn; vDG, ventral dentate gyrus.

Fernandez-Teruel et al., 1997; Vallee et al., 1997; Meerlo et al., 1999).

Given that an appropriately adjusted stress response is necessary for efficient learning and memory (Joels et al., 2006), it would be reasonable to assume that neonatal handling would lead to improved cognitive abilities. Indeed, it has been shown that in adulthood neonatally handled rats show improved performance in several types of hippocampal-dependent learning paradigms, as in the two-way active avoidance task (Escorihuela et al., 1994; Pryce et al., 2003), in the contextual fear conditioning (Beane et al., 2002) and in spatial tasks, including the Morris water maze (Wong and Jamieson, 1968; Huot et al., 2002; Fenoglio et al., 2005). Furthermore, handled rats are protected from the aging-associated loss of hippocampal neurons and the consequent decline of spatial learning and memory that is observed in non-handled rats (Meaney et al., 1988; Fernandez-Teruel et al., 1997).

AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors (AMPA receptors) have been shown to underlie activity-dependent changes in excitatory synaptic function during different forms of learning (Keifer and Zheng, 2010). AMPARs consist of heterotetrameric complexes made up of at least two of four subunits designated glutamate receptor GluR1–4 (Wenthold et al., 1996) and recently renamed AMPA glutamate receptor subunits (GluA1–A4) (Collingridge et al., 2009). The different subunits confer specific physiological properties to AMPAR channel function, such as kinetics, conductance and permeability. Most notably, GluA2-containing AMPARs are impermeable to divalent cations, whereas those lacking the GluA2 subunit are calcium (Ca^{2+}) permeable (Isaac et al., 2007). Ca^{2+} permeability and subunit composition of AMPARs are dynamically remodeled in a cell- and synapse-specific manner during development and in response to neuronal activity (Liu and Zukin, 2007). The composition of AMPA receptor (AMPA) subunits undergoes dynamic changes following exposure to a variety of *in vivo* experiences, for example, sensory experience (Clem and Barth, 2006; Goel et al., 2006), emotional stress (Liu et al., 2010; Vialou et al., 2010), cocaine use (Conrad et al., 2008; Mameli et al., 2011) and learning (Plant et al., 2006; Clem and Haganir, 2010), as well as social isolation (Schmidt et al., 2010).

Based on the above, we developed the hypothesis that AMPAR subunit levels would be modified as a result of neonatal handling. We further hypothesized that handling-induced effects would probably be sexually dimorphic since it has been previously shown that neonatal handling has a number of sexually dimorphic effects (Smythe et al., 1994; Papaioannou et al., 2002a,b; Park et al., 2003; Stamatakis et al., 2006) and most interestingly influences cognitive abilities differentially in the two sexes (Stamatakis et al., 2008). Therefore, in the present study, we investigated the effect of neonatal handling on the mRNA expression of AMPAR subunits GluA1, GluA2, GluA3 and GluA4 in the following adult rat limbic system structures: hippocampus, amygdala and prefrontal cortex [prelimbic

(PrL) and infralimbic (IL)], as well as in somatosensory and occipital cortex (Occip), brain areas known to be affected by neonatal handling (Meaney and Aitken, 1985; Mitchell et al., 1990; Meaney et al., 1996; Pham et al., 1997; Jaworski et al., 2005; Garoflos et al., 2007, 2008).

EXPERIMENTAL PROCEDURES

Animals

Wistar rats of both sexes were reared in our colony under standard conditions (24 °C; 12-h light/dark cycle, lights on at 8:00 am) and received food and water *ad libitum*. Virgin females were housed with stud males and pregnancy was determined by the presence of sperm in the vaginal smear (day 0 of pregnancy). Prior to birth litters were randomly distributed to either the handled or non-handled groups (six litters in each of the two groups). The litter size and the sex ratio did not differ between the litters employed in the two groups [average litter size [mean \pm SEM (standard error of the mean): non-handled litters 9.1 ± 0.5 (range, 7–11), handled litters 9.2 ± 0.3 (range, 8–10); average sex ratio (males: females, mean \pm SEM): non-handled litters 1.05 ± 0.18 , handled litters 1.08 ± 0.15]. Culling of litters was not performed, since it has been shown that litter size within the range employed does not affect maternal behavior (Deviterne et al., 1990; Champagne et al., 2003). The day of birth was defined as postnatal day 0 (PND0). Following weaning, three to four animals of the same sex, litter and group (handled or non-handled) were placed per cage and were kept under standard housing conditions (see above) in the same room of the animal house. A total of 24 adult animals (PND90–PND100) were used in this study: six male non-handled, six male handled, six female non-handled, and six female handled (one animal of each sex from each litter was employed). Experiments were carried out in agreement with ethical recommendation of the European Communities Council Directives of 24 November 1986 (86/609/EEC) and of 22 September 2010 (2010/63/EU). All efforts were made to minimize the number of animals used and their suffering.

Neonatal handling

We employed a neonatal handling protocol similar to that originally described by Levine (1957) lasting from PND1 until weaning (PND22). More specifically, every day between 9:00–10:00 am the mothers of the pups to be subjected to handling were removed from their home cages and placed separately into cages (always the same cage for each mother throughout the handling period). All offspring of a litter were then removed and placed together in a plastic container lined with paper towels and heated by an infrared lamp (temperature range 32–34 °C). After 15 min the pups, and then their mothers, were returned to their home cages. Non-handled litters were left completely undisturbed in their home cage until weaning.

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