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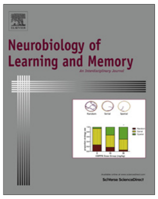

Long-term social isolation in the adulthood results in CA1 shrinkage and cognitive impairment

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ABSTRACT

Social isolation in adulthood is a psychosocial stressor that can result in endocrinological and behavioral alterations in different species. In rodents, controversial results have been obtained in fear conditioning after social isolation at adulthood, while neural substrates underlying these differences are largely unknown. Neural cell adhesion molecule (NCAM) and its polysialylated form (PSA-NCAM) are prominent modulators of synaptic plasticity underlying memory processes in many tasks, including fear conditioning. In this study, we used adult female *Octodon degus* to investigate the effects of long-term social isolation on contextual and cued fear conditioning, and the possible modulation of the synaptic levels of NCAM and PSA-NCAM in the hippocampus. After 6½ months of social isolation, adult female degus showed a normal auditory-cued fear memory, but a deficit in contextual fear memory, a hippocampal dependent task. Subsequently, we observed reduced hippocampal synaptic levels of PSA-NCAM in isolated compared to grouped-housed female degus. No significant differences were found between experimental groups in hippocampal levels of the three main isoforms of NCAM (NCAM180, NCAM140 and NCAM120). Interestingly, social isolation reduced the volume of the hippocampal CA1 subfield, without affecting the volume of the CA3 subregion or the total hippocampus. Moreover, attenuated body weight gain and reduced number of granulocytes were detected in isolated animals. Our findings indicate for the first time, that long-term social isolation of adult female animals induces a specific shrinkage of CA1 and a decrease in synaptic levels of PSA-NCAM in the hippocampus. These effects may be related to the deficit in contextual fear memory observed in isolated female degus.

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1. Introduction

Social isolation in rodents and primates is considered a stressful situation that results in changes in brain structure and function, including increased physiological reactivity to novel stimuli and altered behavior (Baenninger, 1967; Krech, Rosenzweig, & Bennett, 1966; Suomi, Harlow, & McKinney, 1972; Wiberg & Grice, 1963). Usually, animals reared in isolation display several long-lasting changes in behavior recapitulated as “isolation syndrome” (for rev see Fone & Porkess, 2008; Serra, Sanna, Mostallino, & Biggio, 2007), that differ from the behavioral consequences observed when isolation housing takes place in the adulthood (Hall, 1998; Martin & Brown, 2010). Surprisingly, only a few studies have investigated whether cognitive function is altered in socially isolated animals at adulthood. In this regard, controversial results have been found, not only in spatial learning (Arranz, Giménez-Llort, De Castro, Baeza, & De la Fuente, 2009; Moragrega, Carrasco, Vicens, & Redolat, 2003), and working memory (Garrido et al., 2013; Huang, Liang, Ke, Chang, & Hsieh-Li, 2011), but also in fear conditioning (Hsiao, Chen, Chen, & Gean, 2011; Martin & Brown, 2010; Pibiri, Nelson, Guidotti, Costa, & Pinna, 2008), depending on the strain and species evaluated.

The neural cell adhesion molecule (NCAM) is one of the possible candidate molecules that may be involved in cognitive alterations induced by social isolation. NCAM is a glycoprotein of the immunoglobulin superfamily. Alternative splicing of the NCAM gene results in several isoforms of NCAM with different molecular weights. There are three main isoforms of NCAM that present distinctive structural distribution; NCAM120, expressed only in glia; NCAM140, expressed in both neurons and glia, and; NCAM180, expressed only in neurons (Noble et al., 1985). NCAM is involved in different functions in the nervous system, where it mediates Ca^{2+}-independent homophilic and heterophilic cell–cell interactions between neurons and neurons and glia (Dityatev, Dityateva, & Schachner, 2000; Korte, 2001). In addition to the key role that

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NCAM plays during development, in adulthood NCAM promotes synaptic plasticity and synaptogenesis (Bukalo et al., 2004; Dityatev et al., 2000; Kiss, Troncoso, Djebbara, Vutskits, & Muller, 2001; Maness & Schachner, 2007; Sandi, 2004) (for rev see Dityatev, Bukalo, & Schachner, 2008). Moreover, a growing body of data has shown an important role of NCAM in learning and memory processes: (i) its brain expression is modulated after contextual fear conditioning and Morris water maze, two hippocampal dependent tasks (Bisaz, Conboy, & Sandi, 2009; López-Fernández et al., 2007; Sandi, Merino, Cordero, Touyarot, & Venero, 2001; Venero et al., 2006); (ii) administration of antisense oligonucleotides, antibodies, or peptides, that interfere with NCAM function can impair synaptic plasticity and learning and memory abilities of the animals (Arami, Jucker, Schachner, & Welzl, 1996; Cambon, Venero, Berezin, Bock, & Sandi, 2003; Doyle, Nolan, Bell, & Regan, 1992; Lüthl, Laurent, Figurov, Muller, & Schachner, 1994; Venero et al., 2006) and; (iii) peptides that imitate NCAM function can enhance LTP in the hippocampus and improve hippocampal dependent memory tasks (Cambon et al., 2004; Knafo et al., 2002). Essentially, NCAM exerts its functions through activation of intracellular signaling cascades (Hinsby, Berezin, & Bock, 2004; Povlsen, Ditlevsen, Berezin, & Bock, 2003) and/or post-translational modifications, mainly polysialylation (PSA-NCAM) occurring through attachment of long homopolymers of sialic acid, which provides NCAM with anti-adhesion properties (Dityatev et al., 2004; Rutishauser & Landmesser, 1996).

Interestingly, PSA-NCAM has been found to be of special relevance in synaptic plasticity as well as learning and memory processes (Becker et al., 1996; Dityatev et al., 2004; Kochlamazashvili et al., 2010; Lüthl et al., 1994; Muller et al., 1996); for rev see (Rutishauser, 2008; Sandi, 2004), including fear conditioning (López-Fernández et al., 2007; Senkov et al., 2006). Previous studies have shown that different types of stressors (i.e. restraint; psychosocial; variable and unpredictable) can modulate brain levels of NCAM and PSA-NCAM (Bisaz, Schachner, & Sandi, 2011; Cordero et al., 2005; Foley, Rønn, Murphy, & Regan, 2003; Murphy, ÓConnell, & Regan, 1996; Murphy& Regan, 1999; Sandi et al., 2003; Touyarot, Venero, & Sandi, 2004; Venero et al., 2002; Wielgat, Walesiuk, & Braszko, 2011). To our knowledge, the effects of long-term social isolation at adulthood on brain PSA-NCAM levels have been studied only in male Wistar rats (Djordjevic, Djordjevic, Adzic, & Radojcic, 2012), but not on adult females of any species. While the effect of social isolation at adulthood on fear conditioning has been studied by different groups on male animals (mice and rats), only one study was done using females (Martin & Brown, 2010). In that work, female mice were evaluated using a randomized testing over oestrous cycle. Here, we decided to use adult female degus (*Octodon degus*) for two reasons: (i) Degus are highly social diurnal caviomorph rodents that, in natural conditions, live in groups(Fulk, 1976), and; (ii) female degus show a large oestrous cycle (lasting 17–21 days) (Mahoney, Rossi, Hagenauer, & Lee, 2011), that facilitates the design and conclusion of the study, and minimizes the problem of cycling fluctuations of hormones every four days that typically occurs over the oestrous cycle of mice and rats.

Recently, PSA-NCAM has also been found to regulate synaptic plasticity and contextual fear memory by interacting on NMDA function (Kochlamazashvili et al., 2010). Specifically, a deficit in PSA-NCAM increased NMDA mediated transmission only when NMDA receptors are composed of NR1/NR2B but not NR1/NR2A. Therefore, we also decided to analyze whether hippocampal synaptic levels of the NMDA subunits NR1 (the constitutive subunit of NMDA) and NR2B were altered by long-term isolation.

In the present study, we investigated the effects of long-term social isolation on cued (hippocampus-independent), and contextual (hippocampus-dependent) fear conditioning, and the possible modulation of NCAM and PSA-NCAM in the hippocampus of adult female degus. We showed that social isolation impaired contextual, but not cued, fear conditioning. In addition, isolated female degus presented reduced synaptic levels of PSA-NCAM in the hippocampus, without affecting synaptic content of total NCAM or NR1 and NR2B subunits of the NMDA receptor in this brain structure. Interestingly, reduced hippocampal CA1 volume was found in isolated, compared to grouped-housed degus, but no differences were found in the volume of CA3 or the whole hippocampus. Long-term social isolation reduced body weight gain, a reliable indicator of chronic stress, without altering plasma cortisol levels and weight of adrenal glands. Given that stress can alter immune function (Avitsur, Padgett, & Sheridan, 2006) and susceptibility to disease and mortality risk especially in aged subjects (Hansel, Hong, Camara, & von Kanel, 2010), we also decided to perform a complete blood count test including white blood cells, that play a key role in the immune system. Interestingly, full blood count revealed a decrease in granulocytes in isolated animals, indicating that social isolation compromise the immune system.

2. Materials and methods

2.1. Animals

In this study we used 12 female *O. degus*, (39–44 months old at the beginning of the experimental procedure), that were obtained from the Animal Service of the University of Murcia. The animals were housed in groups of three per cage in transparent plexiglas cages (48.3 cm length × 26.7 cm width × 20.3 cm height), under a 12:12 light/dark cycle; light on from 08:00 to 20:00 h), with humidity (60%) and temperature (23 ± 1 °C) controlled conditions. The degus were fed *ad libitum* throughout the experiment, using a commercial rat chow (A04 rat–mouse maintenance Panlab). In captivity, degus show a life-span of 6–7 years, although some signs of aging (i.e. falls andsignificant Aβ deposits in blood vessel walls) can already be found in 3-year-old animals (van Groen et al. (2011)).

The fear conditioning was performed during the light period (09:00–15:00 h), that corresponds to their main activity phase since these rodents exhibit, predominantly, diurnal pattern with two crepuscular peaks of activity, around dusk and before lights-on (Otalora, Vivanco, Madariaga, Madrid, & Rol, 2010). Freezing behavior was recorded using a digital camera to enable subsequent evaluation by two researchers (one of them was kept blind concerning the animal groups). To avoid the effect of hormonal fluctuation in 17–21-day regular cycling females, animals were trained in the fear conditioning in a period that corresponds to the diestrous phase of the oestrous cycle.

All procedures related to animal maintenance and experimentation were in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) and the guidelines issued by the Spanish Ministry of Agriculture, Fishing and Feeding (Royal Decree 1201/2005 of October 21, 2005) and were approved by the Institutional Animal Ethics Committee. Efforts were made to minimize the number of animals used, as well as their suffering.

2.2. Experimental groups and design

At the age of 39 months, the animals were divided into two homogenous groups: individual-housed ($N = 6$) and group-housed ($N = 6$). Cardboard panels to avoid visual contact separated the individual cages. After maintenance during six months and a half in these conditions, we observed that two animals (one isolated and one group-housed) showed eyelid drooping. These animals