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Exploratory, anxiety and spatial memory impairments are dissociated in mice lacking the LPA_1 receptor

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ABSTRACT

Lysophosphatidic acid (LPA) is a new, intercellular signalling molecule in the brain that has an important role in adult hippocampal plasticity. Mice lacking the LPA_1 receptor exhibit motor, emotional and cognitive alterations. However, the potential relationship among these concomitant impairments was unclear. Wild-type and $maLPA_1$-null mice were tested on the hole-board for habituation and spatial learning. $MaLPA_1$-null mice exhibited reduced exploration in a novel context and a defective intersession habituation that also revealed increased anxiety-like behaviour throughout the hole-board testing. In regard to spatial memory, $maLPA_1$ nulls failed to reach the controls' performance at the end of the reference memory task. Moreover, their defective working memory on the first training day suggested a delayed acquisition of the task's working memory rule, which is also a long term memory component. The temporal interval between trials and the task's difficulty may explain some of the deficits found in these mice. Principal components analysis revealed that alterations found in each behavioural dimension were independent. Therefore, exploratory and emotional impairments did not account for the cognitive deficits that may be attributed to $maLPA_1$ nulls' hippocampal malfunction.

1. Introduction

Lysophosphatidic acid (LPA, 1-acyl-2-*sn*-glycerol-3-phosphate), acting through 6 G protein-coupled receptors (LPA_{1-6}), has gained increasing attention over the last few years as an intercellular messenger with several effects on different target tissues (Anliker & Chun, 2004; Birgbauer & Chun, 2006; Choi et al., 2010; Chun 2005, 2007; Fukushima, Ishii, Contos, Weiner, & Chun, 2001; Ishii, Fukushima, Ye, & Chun, 2004; Moolenaar, van Meeteren, & Giepmans, 2004; Noguchi, Herr, Mutoh, & Chun, 2009; Rivera & Chun, 2008). A growing body of evidence indicates that the LPA pathway is involved in normal and abnormal brain development and function (Anliker & Chun 2004; Choi, Lee, & Chun, 2008; Chun, 2005; Estivill-Torrus et al., 2008). The most extensively studied of these receptors is LPA_1 (Chun, 2005; Contos, Fukushima, Weiner, Kaushal, & Chun, 2000; Estivill-Torrus et al., 2008; Fukushima et al., 2002; Herr & Chun, 2007; Kingsbury, Rehen, Contos, Higgins, & Chun, 2003; Matas-Rico et al., 2008).

Recently, Santin et al. (2009) described the behavioural phenotype of the $maLPA_1$-null mouse, a stable variant of the LPA_1-null mutant strain formerly characterised by Contos et al. (2000) and described in Estivill-Torrús et al. (2008). Impaired spatial memory retention, abnormal use of searching strategies, altered exploration in the open field and increased anxiety-like responses in the elevated plus maze have been reported in the absence of retinal and auditory malfunctions. However, concomitant neurological deficits were observed in olfaction and somesthesis, limb reflexes, co-ordinated limb use and neuromuscular strength (Santin et al., 2009). Interestingly, these behavioural alterations are accompanied by impairments in both hippocampus and cerebral cortex that may be partially responsible for the phenotype (Estivill-Torrus et al., 2008; Matas-Rico et al., 2008).

The complexity of the behavioural phenotype exhibited by the $maLPA_1$-null mice with impairments in several behavioural domains is frequently observed when transgenic mice are used in research (e.g. Acevedo, Pfankuch, Ohtsu, & Raber, 2006; Kalueff, Fox, Gallagher, & Murphy, 2007; Santin et al., 2009). However, the

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potential relationship among sensorimotor, emotional and cognitive variables is not generally well-addressed and may lead to inaccurate interpretations. To date, it is known that anxiety-related behaviours, exploration and cognition may reflect dissociated or common processes in animal testing (Matzel, Grossman, Light, Townsend, & Kolata, 2008; Miyagawa et al., 1998; Ohl, Roedel, Binder, & Holsboer, 2003; Ohl, Roedel, Storch, Holsboer, & Landgraf, 2002). In this regard, it has been reported that memory could be influenced by the rodent's inborn anxiety or by its reactivity to a stressor (Herrero, Sandi, & Venero, 2006; Ribeiro et al., 1999; Wright, Lightner, Harman, Meijer, & Conrad, 2006). The relevance of this point is emphasized in reports that suggest that the performance of some mouse strains in certain tasks may reflect the strain's anxiety-related behaviour, rather than cognitive functions (Dockstader & van der Kooy, 2001; Ohl et al., 2002). It is important to note that stressors, such as a novel environment or forced swimming, are usually an unavoidable part of the experimental setting even when studying non-emotional cognitive processes. Furthermore, the degree of aversion varies from one task to another, and that may explain disparate memory results between procedures (Hodges, 1996). On the other hand, anxiety levels could be related to increased or reduced locomotion (Kameda et al., 2007; Ramos & Mormède, 1998), and motor activity could influence anxiety and memory when their assessment involves spatial–temporal parameters (Brody & Holtzman, 2006; Kalueff et al., 2007; Strekalova, Spanagel, Dolgov, & Bartsch, 2005).

The main purpose of this work is to study exploration, anxiety and spatial memory in maLPA$_1$-null mice, with a focus on the interrelationship among these characteristics, to determine whether motor activity or anxiety impairments might account for cognitive performance. To address this issue, we used the hole-board test and the principal components analysis (PCA) multivariate approach. The hole-board is a frequently used hippocampal-dependent task for measuring spatial learning that is similar to the water maze in that extra-maze cues are used to solve the task (Oades, 1981). Moreover, the hole-board, as well as its modified version, allows the simultaneous evaluation of various potentially interrelated emotional, exploratory and spatial memory measures (Ohl et al., 2002, 2003; Takeda, Tsuji, & Matsumiya, 1998). PCA is useful to resolve variables into the independent dimensions (factors) that underlie behaviour (Ohl et al., 2002, 2003; Ramos & Mormede, 1998). Although PCA has successfully been applied to assess behavioural paradigms and inbred strains, it has been less frequently used in studies using transgenic animals (Carola, D'Olimpio, Brunamonti, Mangia, & Renzi, 2002; Fernandes, Gonzalez, Wilson, & File, 1999; Gross, Santarelli, Brunner, Zhuang, & Hen, 2000; Ohl et al., 2003). In this study, we further show the utility of PCA in analysing behavioural research using mutant mice.

2. Materials and methods

2.1. Animals

The generation and characterization of maLPA$_1$-null mice have been previously described (Estivill-Torrus et al., 2008; Matas-Rico et al., 2008). The original-null mice were obtained by targeted gene disruption using homologous recombination and Cre-mediated deletion in a 129X1/SvJ background. These animals were then backcrossed with C57BL/6J mice. Intercrosses of these mice, as well as with mice generated from one additional backcross (Contos et al., 2000), were begun immediately. An LPA$_1$-null mouse colony, termed maLPA$_1$ from the *Málaga* variant of LPA$_1$ knockout, was spontaneously derived during the original colony expansion by crossing heterozygous foundation parents (maintained in the original hybrid C57BL/6J × 129X1/SvJ background). Intercrosses were performed with these mice and subsequently backcrossed for 20 generations with mice generated within this mixed background. MaLPA$_1$-null mice carrying the *lpa*$_1$ deletion were born at the expected Mendelian ratio, and they survived to adulthood. Targeted disruption of the *lpa*$_1$ gene was confirmed by genotyping (according to Contos et al., 2000), and immunochemistry confirmed the absence of LPA$_1$ protein expression.

Fourteen maLPA$_1$-null male mice and 23 analogous wild-type littermates were used in this study. All mice were approximately 4 months old at the onset of the behavioural testing and were housed singly in standard cages with a 12 h light/dark cycle (lights on at 7:00 a.m.). For 4 days before the experiment, mice were handled daily by the experimenter in the testing room, to get adapted to the experimental conditions, and they were fed a restricted diet so their body weights were reduced to 80–85% of their free-feeding weight. Food restriction processes remained throughout the experiment. Experiments were conducted between 9:00 a.m. and 3:00 p.m. in a testing room illuminated at 300 lux. All procedures were in accordance with the European animal research laws (European Communities Council Directive 86/609/EEC, 98/81/CEE and 2003/65/CE and Commission Recommendation 2007/526/EC) and Spanish National Guidelines for Animal Experimentation and use of genetically modified organisms (Real Decreto 1205/2005 and 178/2004 and Ley 32/2007 and 9/2003).

2.2. Habituation in the hole-board

The hole-board (40 × 40 cm) contained 16 equidistant holes (5.5 cm apart, 2.5 cm diameter, 3 cm depth) placed in the central zone of the apparatus, which was surrounded by an arena 6.5 cm in from the clear Plexiglas walls (20 cm high) of the maze. Several spatial cues (black cards in different geometric shapes) were located on the walls of the testing room to allow mice to orient in space. For habituation, mice spent 1 session of 3 min on 2 consecutive days in the hole-board. All 16 holes were baited with a small food pellet (0.03 gr) in order to habituate mice to visit holes to eat food. After each session, the number of faecal boli laid in the arena was counted, and the apparatus was cleaned with a solution containing neutral soap.

Sessions were videotaped, and locomotion (mm travelled) and thigmotaxis (percent of time spent by the animal in the periphery, defined as the 6.5 cm of arena in from the walls) were registered using a video tracking system (Ethovision XT, Noldus Information Technology, Wageningen, The Netherlands). Frequency of head dipping (the mouse introduced its nose in a hole), rearing (the mouse stood on its hind paws, with forelegs supported or unsupported on the walls), risk assessment (the mouse stretched its head and shoulders, before return to its initial posture) and grooming (the mouse licked/scratched its fur, washed its head and/or licked its tail or genitals) were assessed with an observational software (Smart, 2.5, Panlab, Barcelona. Spain). To control for the fact that animals may not eat the same amount of rewards during the habituation phase, all measures with the exception of thigmotaxis and defecation were expressed as a rate (per minute or per second), and the time each mouse spent eating was observationally recorded and subtracted from the total time employed to calculate the rates. Importantly, the locomotion rate in our study, although expressed in units per second, is not the same as velocity. When velocity is assessed, all the time the animal is resting (not just when eating) should be excluded from analysis (Bothe, Bolivar, Vedder, & Geistfeld 2004).

2.3. Spatial learning in the hole-board

The day after habituation, only a fixed set of 4 holes was baited (0.03 gr of food pellet), in a pattern that remained constant