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Research report

Altered behaviour and cognitive function following combined deletion of Toll-like receptors 2 and 4 in mice

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HIGHLIGHTS

- TLR2/4 double mutants are neophobic to the novel environment in IntelliCage.
- TLR2/4 double mutants become hyperactive when access to water reward is restricted.
- TLR2/4 double mutants do not show spatial memory impairment.
- TLR2/4 double mutants show impaired performance in visual discrimination task.

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ABSTRACT

Activation of the immune system due to infection or aging is increasingly linked to impaired neuropsychological function. Toll-like receptors 2 and 4 (TLR2, TLR4) are well-characterised for their role in inflammatory events, and their combined activation has been implicated in neurological diseases. We therefore determined whether TLR2 and TLR4 double gene knockout (GKO) mice showed modified behaviour and cognitive function during a 16-day test sequence that employed the automated IntelliCage test system. The IntelliCage features a home cage environment in which groups of mice live and where water reward is gained through performing various tasks centred on drinking stations in each corner of the apparatus. All mice were tested twice, one month apart (the first sequence termed "R1"and the second "R2"). There were fewer corner visits and nosepokes in TLR2/4 GKO compared to wild-type mice during early exploration in R1, suggesting elevated neophobia in GKO mice. Reduced exploration persisted over subsequent test modules during the dark phase. TLR2/4 GKO mice also displayed increased corner visits during drinking sessions compared to non-drinking sessions, but this was not associated with increased drinking. In subsequent, more complex test modules, TLR2/4 GKO mice had unimpaired spatial learning, but showed markedly poorer performance in a visual discrimination reversal task compared to wild-type mice. These results indicated subtle impairments in behaviour and cognitive functions due to double deficiency in TLR2 and TLR4. These finding are highly relevant to understanding the combined actions of TLR2 and TLR4 on neurological status in a range of different disease conditions.

1. Introduction

Toll-like receptors (TLRs) play a vital role in the innate immune system as primary sensors of structurally conserved molecular patterns that are possessed by microbes [1,2]. TLRs belong to a family of type-1 transmembrane pattern-recognition proteins containing leucine-rich repeat (LRR) motifs extracellularly and a Toll/interleukin-1 receptor (TIR) domain intracellularly. They act as regulators of the host innate immune response via a series of signalling pathways initiated through nuclear factor-kappaB (NF-κB) [3] and facilitate the acquisition of adaptive immunity [4]. To date, a total of 10 TLR members have been identified in humans, 12 in mice and at least 6 in other vertebrates ranging from primates to jawed fish [3]. Each recognises different families of extracellular ligands and together they contribute to innate immune processing [5].

Abbreviations: CNS, central nervous system; DSA, drinking session adaptation; FA, free adaptation; GKO, gene knockout; LRR, leucine-rich repeat; NA, nosepoke adaptation; PT, patrolling task; RT, reversal task; R1/2, run 1/2; TIR, Toll/interleukin-1 receptor; TLR, Toll-like receptor; WT, wild-type.

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As well as being key players in immunity, TLRs increasingly are recognised as participating in neurodevelopmental processes [6], neurogenesis [7,8] and neuroplasticity [4] as well as playing metabolic [9] and behaviour-modulating roles [2]. While commonly expressed in a wide range of peripheral immune cells, such as B cells [10], natural killer cells [11], macrophages, monocytes [12] and neutrophils [13], TLRs are also detected in microglia [14], astrocytes [15] and even neuronal progenitor cells in the central nervous system [16].

TLRs 1–9 are highly conserved across humans and rodents during evolution [17] and deletion of various TLRs in mice is associated with a wide range of neurocognitive and/or physiological sequelae. For example, mice deficient in TLR5 display a range of metabolic abnormalities including hypertension, insulin resistance, hyperlipidemia and increased adiposity, which are correlated with an alteration in gut microbiota [9]. Mice lacking functional TLR2 exhibit schizophrenia-like neuropsychiatric disorders, including hyperactivity, reduced anxiety, social withdrawal, prepulse inhibition deficit and cognitive dysfunction [2]. TLR4 was shown to play a developmental role in regulating hippocampus-dependent spatial and contextual cognition [6], while enhanced hippocampal neurogenesis and improved hippocampus-dependent cognition was found in TLR3-deficient mice [7]. A lack of MYD88, an important component of downstream signal transduction in TLR-mediated immunity (except TLR3), caused impaired cognition and motor coordination in mice [18].

Among the different TLRs, TLR2 and TLR4 underlie important inflammatory mechanisms in microbial infection including some that cause CNS complications [17,19,20]. They primarily recognise different cell wall components of Gram-positive and -negative bacteria: for TLR2, signalling is transduced through binding with lipoproteins and peptidoglycans, while the ligands for TLR4 are primarily lipopolysaccharides [21,22]. In addition to exogenous stimuli, TLR 2 and 4 signalling can be activated by host-derived molecules or damage-associated molecular patterns released in the presence of endogenous stress [23]. While they are considered central participants in the pathological brain injury due to ischemia and reperfusion [24], innate TLR ligands also can be protective as TLR2 signalling is neuroprotective, and antagonises the damaging effect, i.e. impaired survival, increased cerebral infarct size and aggravated neurological deficits, caused by TLR4-mediated inflammation [25].

A joint participation of TLR2 and TLR4 in mediating the immunopathology of microbial infection, such as pneumococcal meningitis [26], in the CNS and other neurological injuries, including cerebral ischemia [24], has been widely studied. Although they modulate different immune signalling pathways under pathological conditions [12] and interact with CNS cells differently and independently under normal physiological conditions [2,6], they usually are co-activated under inflammatory conditions [17]. Their physiological roles in the CNS have been much less commonly studied, however. To help address this deficiency, we investigated the interaction between these two TLRs in the CNS in the normal physiological state, by evaluating the effects on mouse behaviour and cognition of a combined deficiency in TLRs 2 and 4.

2. Materials and methods

2.1. Mice

TLR2/4 double knockout mice were generated by crossing TLR2-deficient [22] and TLR4-deficient [27] mice. The resulting F1 progeny were intercrossed, and homozygous double TLR deficient founders were selected to establish the new line. All mice were bred in the animal house of the University of Sydney. Fourteen female gene knockout (GKO) mice aged 7–11 weeks at the beginning of the experiment were used. The control mice carrying wild type TLR-2 and -4 alleles were on a C57BL/6J background. An equal number of age-matched C57BL/6J female mice were purchased from the Animal Resources Centre (Canning Vale, Western Australia). Prior to the experiment, all mice (17–24 g) were allowed to acclimatise to the same standard laboratory animal housing conditions (12 h light/dark cycle with the light phase beginning at 6 am) in a temperature-controlled environment for at least one week. Water and food were available ad libitum to animals housed in groups of 3–6 mice in an individually-vented caging system (Tecniplast, Buguggiate, Italy). Experiments were conducted in compliance with the NSW Animal Research Act (1985—Animal Research Regulation 2010) and the 2004 NHMRC 'Australian code of practice for the care and use of animals for scientific purposes' with approval by the University of Sydney Animal Ethics Committee.

2.2. IntelliCage behavioural and cognitive assessment paradigms

IntelliCage™ (New Behaviour, Zurich, Switzerland; http://www.newbehavior.com), which is integrated with radio-frequency identification technology, is a computer-operated home cage system that has approximately 5 times the floor area (2062.5 cm^2) of a standard mouse housing cage (432 cm^2). Compared to the standard caging system, the IntelliCage is more socially enriched, in that it allows accommodation of up to 16 mice, the behaviours of which are monitored simultaneously and individually. The hardware and testing paradigms of the IntelliCage setup that were employed have been described in detail previously [28].

A total of 14 WT female mice were randomly allocated to one of two IntelliCages to obviate any potential effects resulted from cage variation. An equal number of GKOs were mixed and co-housed with the WT animals in the two IntelliCages. Five days prior to IntelliCage assessment, a sterile transponder (T-IS 8010 FDX-B; Datamars SA, Switzerland) was inserted sub-cutaneously in the dorsal-cervical area of each mouse, and the unique ID code of each transponder was checked every day until the beginning of the cognitive and behavioural testing. The transponder allowed the entry of each mouse into a drinking chamber to be recorded individually, as well as its nosepoking and drinking behaviour, which are described below. In addition, the accessibility of water to each mouse in any one of the 4 drinking corners could be regulated individually.

Mice were subjected to a series of 16 day test paradigms on two occasions with a one month interval in between. During the first run of 16-day assessment (denoted as "R1"), water access acted as a positive reinforcer and the condition required to access water was altered in different test paradigms, which are primarily categorised into two major forms, behavioural and cognitive test paradigms. The testing paradigms have been described in detail elsewhere [28] and are briefly summarised below.

(A) Behaviours assessing test paradigms

Mice were allowed to adapt to the IntelliCage environment freely for 2 days, during which time (free adaptation, FA) they learned to visit the corner chambers to gain water access. They were then trained for 2 days (nosepoke adaptation, NA) to make a single nosepoke in order to access water for 5 s throughout the whole light and day cycle, and then trained for another 2 days (drinking session adaptation, DSA) to incorporate this learning into the next paradigm, which restricted water access to two 3 h sessions (1800–2100 and 0300–0600). During this 6-day period, the spontaneous and adaptive exploratory behaviours of the mice were recorded and compared for genotype variation. Subsequent to the adaptation phase, mice were assessed for their response to either