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Research report

Learning, memory and long-term potentiation are altered in *Nedd4* heterozygous mice

Daria Camera[a], Harold A. Coleman[b], Helena C. Parkington[b], Trisha A. Jenkins[a], David V. Pow[a], Natasha Boase[c], Sharad Kumar[c], Philip Poronnik[a,d,*]

[a] Health Innovations Research Institute, School of Medical Science, RMIT University, P.O. Box 71, Bundoora, VIC 3083, Australia
[b] Department of Physiology, Monash University, Melbourne, VIC 3800, Australia
[c] Centre for Cancer Biology, University of South Australia, Adelaide, SA 5001, Australia
[d] Department of Physiology, School of Medical Sciences, The University of Sydney, Camperdown, NSW 2006, Australia

HIGHLIGHTS

- Nedd4 expression is abundant in the hippocampus.
- *Nedd4* heterozygous mice show significant decrease in long-term spatial learning and memory.
- A significant decrease in long-term potentiation (LTP) was observed in the *Nedd4* heterozygous mice.

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ABSTRACT

The consolidation of short-term memory into long-term memory involves changing protein level and activity for the synaptic plasticity required for long-term potentiation (LTP). AMPA receptor trafficking is a key determinant of LTP and recently ubiquitination by Nedd4 has been shown to play an important role via direct action on the GluA1 subunit, although the physiological relevance of these findings are yet to be determined. We therefore investigated learning and memory in *Nedd4*$^{+/-}$ mice that have a 50% reduction in levels of Nedd4. These mice showed decreased long-term spatial memory as evidenced by significant increases in the time taken to learn the location of and subsequently find a platform in the Morris water maze. In contrast, there were no significant differences between *Nedd4*$^{+/+}$ and *Nedd4*$^{+/-}$ mice in terms of short-term spatial memory in a Y-maze test. *Nedd4*$^{+/-}$ mice also displayed a significant reduction in post-synaptic LTP measured in hippocampal brain slices. Immunofluorescence of Nedd4 in the hippocampus confirmed its expression in hippocampal neurons of the CA1 region. These findings indicate that reducing Nedd4 protein by 50% significantly impairs LTP and long-term memory thereby demonstrating an important role for Nedd4 in these processes.

1. Introduction

Cognitive functions such as learning and memory result from activity dependent changes in the strength of excitatory synapses resulting in synaptic plasticity. Long-term potentiation (LTP) is widely accepted to be the molecular and cellular basis underpinning these cognitive processes. Since majority of fast excitatory synaptic transmission is mediated by alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR), they must be tightly regulated in the post-synaptic membrane. AMPARs are tetrameric and comprised of four highly homologus subunits (A1–4), with subunit compositions showing development and region specific expression. In the hippocampus, which is an important area for learning and memory, the majority of AMPARs are composed of a GluA1/2 combination with GluA2/3 heteromers being less prominent [1]. GluA1 containing AMPAR are integral to LTP and previous studies in GluA1 knockout mice have shown that LTP is reduced in both the hippocampus and cortex [2,3]. AMPARs are continuously trafficked (endocytosed, recycled and re-inserted) out of and into the membrane [4], with this regulated insertion and removal of AMPAR integral to the dynamic process of synaptic plasticity.

Post-translation modification of AMPARs, such as phosphorylation and ubiquitination has gained considerable attention since

Abbreviation: Nedd4, neural precursor cell expressed developmentally down-regulated 4.

* Corresponding author. Fax: +61 29063316.
E-mail address: philip.poronnik@sydney.edu.au (P. Poronnik).

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AMPAR undergo activity dependent translocation to and from the membrane. Activity-dependent synaptic plasticity is vital for memory and learning processes and relies on adequate trafficking of AMPAR at the synapse [5–7]. For example, phosphorylation of AMPARs is crucial in determining their function and membrane trafficking. There are a number of studies that have also shown that ubiquitination of AMPAR subunits (GluA1/2) is also important for determining membrane trafficking [8–12].

Nedd4 (neural precursor cell expressed developmentally down-regulated 4) is a HECT (homologous to the E6-AP carboxyl terminus) type ubiquitin ligase (E3) that has been shown to ubiquitinate the GluA1 subunit of AMPAR [9,12]. More specifically, Nedd4 over-expression was shown to produce AMPAR ubiquitination, a decrease in AMPAR surface expression and decreased excitatory transmission [9]. In addition, Nedd4 has been recently shown to rapidly redistribute to dendritic spines in rat cultured neurons upon AMPAR activation. These findings highlight that Nedd4 is important for homeostatic downscaling of synaptic strength [13]. Currently there is a growing list of targets for Nedd4 suggesting this ubiquitin ligase may play an important role in neuronal function [14,15], however, little is known regarding the physiological role of Nedd4 in vivo.

We have recently demonstrated that the 50% reduction in Nedd4 levels seen in heterozygous mice produced subtle but significant alterations in gait. This may be due in part to a re-distribution of GluA1 [16]. The present study investigated the consequences of a reduced level of Nedd4 in spatial learning and memory and assessed whether LTP is affected based on the known interaction between Nedd4 and AMPAR. Hippocampal based LTP is well understood and extensively studied in rodents and the use of in vivo behavioral characterisation, including spatial memory and learning paradigms using a Morris water maze or Y maze, is an important approach to start to determine the contribution Nedd4 to these important cognitive processes.

2. Methods

2.1. Animals

A Nedd4 knockout line (Nedd4Gt(IRESBetageo) 249Lex) was obtained from the Mutant Mouse Regional Resource Center. This line is derived by gene trap with a retroviral insertion disrupting the Nedd4 gene between exons 17 and 18 (www.mmrrc.org/strains/11742/011742.html). Primer genoN4 WT5′ (5′ GGA GTC TTT GGA TAT TGT AAG AGC 3′) and genoN4 WT and KO 3′ (5′ GAG CGT GCG CCT CAC AAG TAT GA 3′) were used to amplify a 226 bp fragment from the wild-type allele; genoN4 KO5′ (5′ AAA TGG CGT TAC TTA AGC TAG CTT GC 3′) and genoN4 WT and KO 3′ to amplify a 137 bp fragment from the Nedd4 disruption allele (Kumar et al., 1997). Mice were bred and maintained at SA Pathology initially and transported to the Florey Neuroscience Institute for testing. Nedd4 heterozygotes ($Nedd4^{+/-}$) and wild-type controls ($Nedd4^{+/+}$) were singly housed a week prior to testing and maintained on a 12:12 h light/dark cycle. Ethics approval was obtained from the Howard Florey Neuroscience animal ethics committee, and all studies were conducted in accordance with the guidelines of the National Health and Medical Research Council of Australia Code of Practice for the care and use of animals for experimental purposes.

2.2. Behavioral experiments

Two month old mice, 8–13 for each genotype, were habituated in the testing room overnight with low lighting (10 lx) prior to each test. The Morris water maze test was carried out first. The animals were then rested for 1 week prior to testing in the Y-maze.

2.2.1. Morris water maze

In the Morris water maze test, a pool (1.5 m diameter) was placed in the center of a designated behavioral room (5 m length × 3.8 m wide), with a camera mounted on the ceiling and connected to a video recorder and tracking software (Ethovision XT, Noldus Information Technology, The Netherlands). The pool was filled with water rendered opaque using non-toxic water based white paint, and maintained at a temperature of 24 ± 1 °C as previously described [17]. A clear platform (15 cm diameter) was placed 1 cm below the water. Four extra-maze cues were used (3 × 2 posters and 1 × 3 dimensional object) for spatial navigation. Mice were randomly assigned a platform location, termed the 'home quadrant' and this remained consistent throughout the learning phase of the test. The learning trial was conducted over 6 consecutive days, with each day consisting of four trials. Each day the mouse was introduced into a random entry point facing the wall of the pool in each of the four quadrants (assigned NE, NW, SE and SW). The mouse was then allowed to explore the maze and the trial terminated either when the mouse located the hidden platform or after 120 s had lapsed. Mice that did not locate the platform were guided to the platform. In both instances mice were required to sit for 30 s before being removed from the pool. Mice were placed in a warm box with a heat lamp to prevent hypothermia for a minimum of 2 min before the next trial. The latency to find the hidden platform was recorded for each trial. After completion of the learning trials, the probe trial was conducted on day 7. The platform was removed from the pool, and the mouse was introduced to the quadrant opposite its home quadrant and allowed to swim for 60 s. The latency to enter the home quadrant, number of entries into the home quadrant and time spent in the home quadrant was recorded. Ethovision XT was used to video, track and record the parameters for the Morris water maze.

2.2.2. Y-maze

The Y-maze apparatus consisted of three identical arms (30 cm length × 14 cm wide × 30 cm high) as previously described [18]. In the trial phase, a partition was placed so that only two arms were accessible. A handful of bedding from the home cage of the mouse was placed in the Y-maze and the mouse allowed 10 min to explore the two accessible arms. After a two hour inter-trial interval, during which time the mouse was returned to its home cage, the partition was removed, and the mouse placed back into the Y-maze for 5 min with access to all three arms. Between each pairing the sawdust was replaced, and the arena cleaned with 70% alcohol. Ethovision XT software (Noldus Information Technology, The Netherlands) was used to video track and collect data. Duration of time spent in the novel versus familiar arms, latency to enter the novel arm and the number of entries into the novel versus familiar arms was assessed.

2.3. Immunohistochemistry

Mice ($n = 3$ for each genotype) were euthenized with sodium pentobarbital (100 mg/kg i.p) and perfused with 0.1 M phosphate buffered saline (PBS), pH 7.2 followed by cold 4% paraformaldehyde (PFA) in 0.1 M PBS. Brains were removed, and placed in 4% PFA for 2 h, after which they were cryoprotected in 30% sucrose solution overnight. Frozen 50 μm thick free floating sagittal sections, were prepared using a freezing cryostat (Leica, Germany). Sections were collected in a 1 in 6 series and stored at −20 °C in cryoprotectant (30% sucrose, 1% polyvinylpyrrolididone, and 30% ethylene glycol) solution. Sections were washed in PBS, before being incubated for 1 h at room temperature in blocking buffer (5% normal goat serum and 0.03% Triton-X 100). Sections were washed twice more with PBS, then primary antibody was applied in blocking buffer for 72 h at 4 °C mouse anti-Nedd4 (1:2000, BD Transduction Laboratories). Sections were washed 4 times in PBS and then incubated with