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Functional alpha7 nicotinic receptors are expressed on immature granule cells of the postnatal dentate gyrus

Danielle John[a,*], Irina Shelukhina[b], Yuchio Yanagawa[c,d], Jim Deuchars[a], Zaineb Henderson[a]

[a]School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK
[b]Department of Molecular Basis of Neurosignaling, Laboratory of Molecular Toxinology, Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow V-437, Russia
[c]Department of Genetic and Behavioural Neuroscience, Gunma University Graduate School of Medicine, Maebashi 371-8511, Japan
[d]Japan Science and Technology Agency, CREST, Sanbancho, Chiyoda-ku, Tokyo 102-0075, Japan

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ABSTRACT

Neurogenesis occurs throughout life in the subgranular zone of the dentate gyrus, and postnatal-born granule cells migrate into the granule cell layer and extend axons to their target areas. The α7*nicotinic receptor has been implicated in neuronal maturation during development of the brain and is abundant in interneurons of the hippocampal formation of the adult brain. Signalling through these same receptors is believed also to promote maturation and integration of adult-born granule cells in the hippocampal formation. We therefore aimed to determine whether functional α7*nicotinic receptors are expressed in developing granule cells of the postnatal dentate gyrus. For these experiments we used 2–3 week-old Wistar rats, and 2–9 week old transgenic mice in which GABAergic interneurons were marked by expression of green fluorescent protein. Immunohistochemistry indicated the presence of α7*nicotinic receptor subunits around granule cells close around the subgranular zone which correlated with the distribution of developmental markers for immature granule cells. Whole-cell patch clamp recording showed that a proportion of granule cells responded to puffed ACh in the presence of atropine, and that these cells possessed electrophysiological properties found in immature granule cells. The nicotinic responses were potentiated by an allosteric α7*nicotinic receptor modulator, which were blocked by a specific α7*nicotinic receptor antagonist and were not affected by ionotropic glutamate or GABA receptor antagonists. These results suggest the presence of functional

Abbreviations: α7*nAChR, alpha7 subunit-containing nicotinic receptor; α-btx, alpha bungarotoxin; BSA, bovine serum albumin; D-AP5, D-(−)-2-amino-5-phosphonopentanoic acid; DG, dentate gyrus; DhβE, dihydro-ß-erythrodine; GAD67, glutamate decarboxylase 67; GFP, green fluorescent protein; NBQX, 2,3,-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulphonamide; PNU120596, N-(5-chloro-2,4-dimethoxyphenyl)-N′-(5-methyl-3-isoxazo lyl)-urea; TBA, tris-buffered ACSF

*Corresponding author. Present address: Division of Biological Sciences, University of California, San Diego, La Jolla, CA 92093-0357, USA.
E-mail address: djohn@ucsd.edu (D. John).

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somato-dendritic α7*nicotinic receptors on immature granule cells of the postnatal dentate gyrus, consistent with studies implicating α7*nicotinic receptors in dendritic maturation of dentate gyrus neurons in adult brain.

1. Introduction

The dentate gyrus (DG) of the hippocampal formation, a region important for spatial and episodic memory (Lisman, 1999; Burgess et al., 2002), is a well-established site of continual neurogenesis in the mammalian brain (Altman, 1962; Altman and Das, 1965; Kaplan and Hinds, 1977; Seki and Arai, 1995; Gage, 2000; Cameron and McKay, 2001), where the processes of ontogenetic developmental neurogenesis and adult neurogenesis are considered to overlap (Amrein et al., 2011). The DG is made up of a molecular layer, granule cell layer, subgranular zone and the hilus. The molecular layer consists mainly of the dendrites of the principal neurons of the DG, i.e. the granule cells, and these dendrites receive extensive glutamatergic input from the entorhinal cortex and from mossy cells in the hilus. The granule cells themselves, densely packed into the granule cell layer, target the principal neurons in CA3 of the hippocampus and possess collaterals that synapse onto mossy cells and local GABAergic interneurons (Amaral and Dent, 1981). The GABAergic interneurons of the DG are located in the subgranular zone, hilus and molecular layer and their terminals are concentrated in the granule cell and molecular layer of the DG (Halasy and Somogyi, 1993; Houser, 2007).

During the normal development of the DG the granule cells are born in the ventricular germinal layer and in the subgranular zone, and in the adult brain these cells occupy the outer two thirds of the granule cell layer (Dayer et al., 2003). Neurogenesis continues to occur throughout life in the subgranular zone, and in the postnatal brain the newly formed neurons accumulate in the inner third of the granular layer where they differentiate and become fully integrated into the adult circuitry (Gould et al., 1999; Hastings and Gould, 1999; Wang et al., 2000; van Praag et al., 2002; Schmidt-Hieber et al., 2004; Doetsch and Hen, 2005).

Whilst much is understood about the factors that influence neurogenesis in the postnatal DG (Hagg, 2005; Zhao et al., 2008b), less is known about how the newly-generated granule cells mature and integrate into the adult circuitry of the brain. The α7 subunit-containing nicotinic receptor (α7*nAChR) is known to support neuroplasticity (Broide and Leslie, 1999; Mansvelder and McGehee, 2000; Ji et al., 2001; Kang and Vaucher, 2009) and neurite outgrowth during development (Lipton and Kater, 1989; Role and Berg, 1996; Lauder and Schambra, 1999). The receptor also plays an important role in learning, memory and attention (Dani and Bertrand, 2007) and has been shown to be required for the maturation and synaptic integration of adult-born neurons in the DG (Campbell et al., 2010).

The most common nAChR subtypes expressed in the hippocampal formation are those based on α7 and α4β2 subunits (Deneris et al., 1988; Wada et al., 1989; 1990; Seguela et al., 1993; Dominguez del et al., 1994). They are located postsynaptically on GABAergic interneurons (Alkondon et al., 1998; Frazier et al., 1998a,b, 2003) and presynaptically on GABAergic and glutamatergic axonal terminals (Colquhoun and Patrick, 1997). Localization of α7 nAChR and β2 nAChR subunits has been observed in granule cells in the DG using receptor binding and immunofluorescence respectively (Kaneko et al., 2006), but direct electrophysiological evidence for functional α7*nAChRs has been missing (Frazier et al., 2003). We therefore aimed to provide evidence for functional α7*nAChRs on granule cells in the postnatal DG and to ascertain if these receptors are expressed

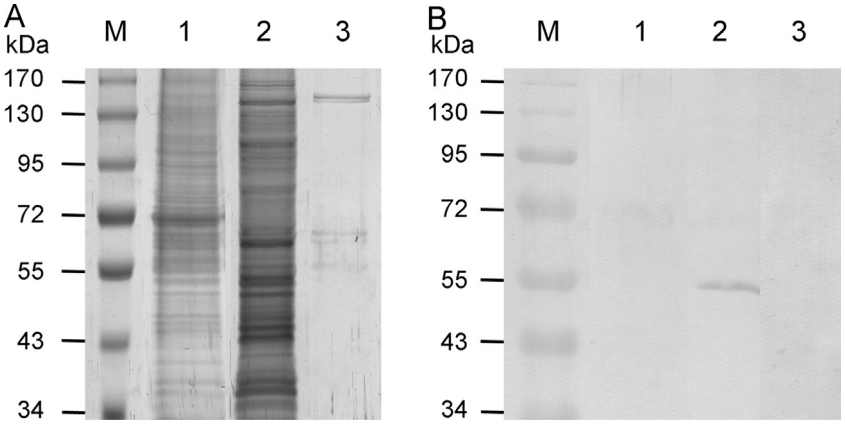

Fig. 1 – Polyclonal α7(8-25) nAChR antibody specificity characterisation by immunodetection of recombinant α7 nAChR affinity purified with α-cobratoxin-sepharose. (A) SDS-PAGE of fractions of GH4C1 cells stably expressed human α7 nAChR (silver stain). (A1) cell lysate, (A2) α-CTX affinity purified proteins; (A3) proteins non-specifically bound by CH Sepharose 4B. (B) Western blot analysis of α7 nAChR immunodetection capability for (B1) GH4C1 cell lysate, (B2) α-CTX affinity purified proteins and (B3) proteins non-specifically bound by CH Sepharose 4B. Abbrevs. M; prestained protein ladder.