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Defective synaptic transmission and structure in the dentate gyrus and selective fear memory impairment in the *Rsk2* mutant mouse model of Coffin–Lowry syndrome

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ABSTRACT

The Coffin–Lowry syndrome (CLS) is a syndromic form of intellectual disability caused by loss-of-function of the RSK2 serine/threonine kinase encoded by the *rsk2* gene. *Rsk2* knockout mice, a murine model of CLS, exhibit spatial learning and memory impairments, yet the underlying neural mechanisms are unknown. In the current study, we examined the performance of *Rsk2* knockout mice in cued, trace and contextual fear memory paradigms and identified selective deficits in the consolidation and reconsolidation of hippocampal-dependent fear memories as task difficulty and hippocampal demand increase. Electrophysiological, biochemical and electron microscopy analyses were carried out in the dentate gyrus of the hippocampus to explore potential alterations in neuronal functions and structure. *In vivo* and *in vitro* electrophysiology revealed impaired synaptic transmission, decreased network excitability and reduced AMPA and NMDA conductance in *Rsk2* knockout mice. In the absence of RSK2, standard measures of short-term and long-term potentiation (LTP) were normal, however LTP-induced CREB phosphorylation and expression of the transcription factors EGR1/ZIF268 were reduced and that of the scaffolding protein SHANK3 was blocked, indicating impaired activity-dependent gene regulation. At the structural level, the density of perforated and non-perforated synapses and of multiple spine boutons was not altered, however, a clear enlargement of spine neck width and post-synaptic densities indicates altered synapse ultrastructure. These findings show that RSK2 loss-of-function is associated in the dentate gyrus with multi-level alterations that encompass modifications of glutamate receptor channel properties, synaptic transmission, plasticity-associated gene expression and spine morphology, providing novel insights into the mechanisms contributing to cognitive impairments in CLS.

Introduction

Loss-of-function mutations in the gene encoding the serine/threonine 90-kDa ribosomal S6 kinase 2, RSK2, is the cause of the Coffin–Lowry syndrome (CLS) (Trivier et al., 1996). CLS is a rare syndromic form of X-linked genetic disorder (prevalence 1:50,000 to 1:100,000) characterised in male patients by a triad of symptoms including progressive skeletal abnormalities, growth retardation and severe mental retardation associated with learning and memory disability (Pereira et al., 2010). The *rsk2* gene is subject to strong allelic heterogeneity. Over 140 distinct inactivating mutations distributed throughout the gene have so far been identified in CLS patients, the majority being unique to single families with no apparent correlation with the severity of clinical features (Delaunoy et al., 2001; Jacquot et al., 1998; Touraine et al., 2002). Cognitive deficiencies in CLS patients, though variable in severity, are prominent. The vast majority of male patients are severely affected with a low intelligence quotient ranging from 15 to 60, associated in

Abbreviations: AMPAr, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CLS, Coffin–Lowry syndrome; CREB, cAMP-response element binding protein; CS, Conditioned stimulus; EM, Electron microscopy; EPSC, Excitatory postsynaptic currents; ERK, Extracellular signal-regulated kinase; fEPSP, Field excitatory postsynaptic potential; GluR, Glutamate receptor; IEG, Immediate early gene; ISI, Inter-stimulus interval; ITI, Inter-trial interval; LTP, Long-term potentiation; mGluR, Metabotropic glutamate receptor; MPP, Medial perforant path; MSB, Multiple spine bouton; MSK1/2, Mitogen stress-activated kinase 1 and 2; NMDAr, *N*-methyl-D-aspartate receptor; PPF, Paired-pulse facilitation; PPI, Paired-pulse inhibition; PSD, Postsynaptic density; PTP, Post-tetanic potentiation; RSK, 90-kDa ribosomal S6 kinase; STP, Short-term potentiation; US, Unconditioned stimulus; WT, Wild-type.

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the more severe cases with retarded language skills (Hanauer and Young, 2002).

RSK2 belongs to a family of four highly homologous proteins (RSK1–4) encoded by distinct genes. In both embryonic and adult brains of humans and mice, RSK2 is expressed predominantly in the neocortex, hippocampus and cerebellum, regions essential for cognitive functions (Zeniou et al., 2002). In neurons, RSK2 is directly activated by extracellular signal-regulated kinase-1 and -2 (ERK1/2), thus taking part in one core signalling cascade implicated in synaptic plasticity and memory (Bozon et al., 2003; Davis and Laroche, 2006; Sweatt, 2001, 2004). RSK2 can in turn phosphorylate a number of cytosolic substrates such as BAD, p53, L1CAM, GSK3β, the translation factors eIF4B and eEF2, and nuclear targets, including cAMP-response element binding protein (CREB), CREB-binding protein (CBP), c-FOS, serum response factor (SRF), the oestrogen receptor-α (ERα), nuclear factor-κB (NF-κB), the transcription initiation factor TIF1A and histone H3, indicating a potential role in various cellular processes including cell proliferation, growth and survival, synaptic plasticity, regulation of gene expression programmes and protein synthesis (Anjum and Blenis, 2008; De Cesare et al., 1998; Frodin and Gammeltoft, 1999; Pereira et al., 2010; Poteet-Smith et al., 1999; Romeo et al., 2012; Sassone-Corsi et al., 1999).

Despite increasing insights into the cell-signalling functions in which the RSK2 kinase may be involved in, little is known about the role of RSK2 in neuronal functions *in vivo* and on the physiopathological mechanisms underlying cognitive dysfunction in CLS. A morphometric MRI study of individuals with CLS suggests a particularly important impact on cerebellar, temporal lobe and hippocampal volumes (Kesler et al., 2007). Our own behavioural studies of a *Rsk2*-null mutant mouse model of CLS (*Rsk2*-KO) revealed normal motor functions, but profound delay in spatial learning, severe deficits in long-term spatial memory consolidation and a selective reconsolidation deficit in object-place recognition memory, suggesting hippocampal dysfunction (Davis et al., 2010; Poirier et al., 2007). A recent study also reported deficits in a conditioned place-preference task (Darcq et al., 2011). These mice show no gross anatomical and histological brain abnormality, but increased cortical dopamine presumably caused by tyrosine hydroxylase hyperactivity, associated with over-expression of dopamine receptor type 2 and dopamine transporter (Marques Pereira et al., 2008). In primary cortical neurons it was found that transfection of a kinase-dead RSK2 reduces the frequency of AMPA receptor mediated miniature excitatory postsynaptic currents (Thomas et al., 2005). Moreover a recent transcriptomic analysis of hippocampal tissue from *Rsk2*-KO mice identified differential expression of several genes among which an over-expression of *Gria2* that encodes GluR2, an AMPA receptor subunit thought to decrease Ca^{2+} permeability and channel conductance, suggesting a potential alteration of synaptic transmission (Isaac et al., 2007). An *in vitro* analysis in area CA1 of the hippocampus pointed to the same type of decreased transmission (Mehmood et al., 2011). Thus, together with the profile of behavioural deficits identified in *Rsk2*-KO mice (Poirier et al., 2007), we hypothesised that RSK2 loss-of-function may cause functional and/or structural neuronal abnormalities in the hippocampus.

Here, we first characterised performance of *Rsk2*-KO mice in four distinct fear memory paradigms that differentially engage hippocampal circuitry and found a selective deficit in the consolidation of trace fear memory and moderate deficit in the reconsolidation of contextual fear memory. Together with the previously described impairments in spatial learning and memory (Poirier et al., 2007), this highlights the particular sensitivity of hippocampal-dependent memory to RSK2 loss-of-function. Complementary approaches were then used to explore the role of RSK2 in neuronal functions and identify mechanisms by which RSK2 loss may impair neuronal and cognitive functions. We focused on the dentate gyrus, a region of the hippocampus involved in both spatial memory (Sutherland et al., 1983) and trace fear memory (Moore et al., 2010; Shors et al., 2001) and in which optogenetic activation of a sparse but specific ensemble of neurons has been shown to contribute to fear memory engrams and to be sufficient for the recall of fear memory (Liu et al., 2012). Moreover, although the contribution of specific hippocampal subfields to various forms of memory is as yet not well defined, dentate gyrus neurons, in contrast to CA1 neurons, exhibit large and sustained firing to the CS in trace fear conditioning (Gilmartin and McEchron, 2005). We first analysed in wild-type (WT) and *Rsk2*-KO mice basal synaptic transmission, paired-pulse facilitation and inhibition, long-term potentiation (LTP) *in vivo*, and AMPA and NMDA currents *in vitro*, at perforant path to dentate granule cell synapses, the major cortical input to the hippocampus, and examined basal and LTP-regulated expression of selected proteins and transcription factors that are potential downstream targets of ERK–RSK2 signalling. Second, to investigate the structural integrity of the synaptic network, we analysed the density and morphology of distinct dentate gyrus synapse subtypes using quantitative electron microscopy. The consequences of RSK2 loss on synapse structure and function and on cognitive functions provide new insights into the potential role of *Rsk2* mutations in the pathogeny of intellectual disabilities associated with CLS.

Material and methods

Animals

The generation of *Rsk2*-null mice by homologous recombination has been described previously (Yang et al., 2004). The targeting vector was constructed by inserting a neomycin resistance gene, flanked by two loxP sites and followed by three stop codons (in the three forward reading frames) in exon 2 of *Rsk2*. The construct was linearised and electroporated into 129 × 1/SvJ embryonic stem (ES) cells and NeoR clones were selected. The ES cells carrying the correct mutation were injected into C57BL/6J blastocysts. The resulting mixed-background 129 × 1/SvJ × C57BL/6J mice carrying a targeted allele of *Rsk2* were backcrossed six times with C57BL/6J mice, before the NeoR cassette was removed by crossing with a C57BL/6J/CMV-Cre transgenic line. The resulting mutant mice carried a single loxP followed by three stop codons within exon 2. Females heterozygous for the mutation have been backcrossed for at least 20 generations to C57BL/6 males to generate *Rsk2*-KO and WT littermate mice used for the present study. The genotype of the mice was determined as described previously (Yang et al., 2004). Male mouse siblings, 3–7 months old, were housed in a temperature and light-controlled colony room (12 h light/dark cycle) in groups of 4/5 with food and water *ad libitum*. All experiments were conducted during the light phase. All efforts were made to minimize the number of animals and their suffering throughout the experiments. Experiments were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), EU Directive 2010/63/EU and the French National Committee (87/848). All experiments were conducted blind to the genotype.

Behaviour

Training was conducted in a conditioning chamber (19 × 25 × 19 cm) equipped with black methacrylate walls, a transparent front door, a speaker and a grid floor (here referred to as context A), placed inside an outer sound-attenuating chamber (StartFear System, Panlab). A computer programme (Freezing Software, StartFear System, Panlab) controlled the audio generator to deliver the tone CS (15 s, 80 dB, 1 kHz) and a shock generator wired to the grid floor to deliver scrambled foot-shocks as US (2 s, 0.7 mA). The conditioning context was cleaned with 100% ethanol between each mouse.

In delay cued fear conditioning, the learning trial consisted of a 2-min exploration period followed by two CS–US pairings (2 min inter-trial interval, ITI) with the CS and US co-terminating. Mice (WT: $n = 24$; *Rsk2*-KO: $n = 20$) were returned to their home cages after 2 min. Fear memory was tested 24 h after conditioning and