

IS COGNITION ALTERED IN THE EKER RAT MODEL OF TUBEROUS SCLEROSIS COMPLEX?

Enhanced Episodic-Like Memory and Kindling Epilepsy in a Rat Model of Tuberous Sclerosis

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J Neurochem 2006;96:407–413

Tuberous sclerosis complex (TSC) is a common neurological autosomal-dominant syndrome caused by mutations in the TSC1 or TSC2 genes. TSC starts in early childhood and is characterized by cerebral hamartomas (benign tumours), severe epilepsy and cognitive deficits such as mental retardation and autism. The hamartomas are characterized by loss of the remaining wild-type TSC allele, and clinical data implicate cerebral hamartomas in the generation of epileptic seizures, which may play a significant role in the development of mental retardation. The TSC2 mutation predicts alterations in mitogen-associated protein kinase (MAPK) and, together with the TSC1 mutation, in mammalian target of rapamycin (mTOR) signalling pathways. Both pathways are involved in neuronal plasticity.

We therefore hypothesized that the heterozygous mutation itself, besides cerebral hamartomas, contributes to the pathogenesis of cognitive deficits and possibly also epilepsy. Here, we show that young adult TSC2^{+/-} rats, which are virtually free of cerebral hamartomas, exhibit enhanced episodic-like memory and enhanced responses to chemically induced kindling. The activation of cyclic adenosine monophosphate (cAMP) in the hippocampus results in stronger induction of phospho-p42-MAPK in TSC2^{+/-} rats than in wild-type animals. Thus, the cognitive phenotype and, possibly, epilepsy in TSC patients may result not only from the focal hamartomatous lesions but also, from altered neuronal plasticity in the heterozygous tissue.

Impaired Synaptic Plasticity in a Rat Model of Tuberous Sclerosis

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Eur J Neurosci 2006;23:686–692

Tuberous sclerosis complex (TSC) is a common hereditary disorder caused by mutations in either the TSC1 or TSC2 genes, and characterized by severe epilepsy, cerebral hamartomas and mental retardation. We have used rats that are heterozygous for an autosomal-dominant germline mutation in the TSC2 gene (TSC2^{+/-} rats) to examine the consequences of TSC2 mutations for hippocampal synaptic plasticity. While basal synaptic transmission in the Schaffer collateral–CA1 synapse was not altered, paired-pulse plasticity was significantly enhanced in TSC2^{+/-} rats (interpulse intervals 20–200 ms). Moreover, TSC2^{+/-} rats exhibited a marked reduction of different forms of synaptic plasticity. Long-term potentiation (LTP) elicited following high-frequency tetanization of Schaffer collaterals was sig-

nificantly decreased from 1.45 ± 0.05-fold potentiation to 1.15 ± 0.04 (measured after 60 min). This difference in LTP levels between TSC2^{+/-} and wild-type rats also persisted in the presence of the γ -aminobutyric acid (GABA)_A receptor antagonist bicuculline. In addition to changed LTP, the level of long-term depression (LTD) elicited by different forms of low-frequency stimulation was significantly less in TSC2^{+/-} rats. These results suggest that TSC2 mutations may cause hippocampal synapses to lose much of their potential for activity-dependent synaptic modification. An understanding of the underlying molecular pathways may suggest new therapeutic approaches aimed at inhibiting the development of the profound mental retardation in TSC.

COMMENTARY

The major neurological manifestations of tuberous sclerosis complex (TSC) are epilepsy, mental retardation, and autism. Knowledge of the molecular aspects of TSC is expanding rapidly (1). The responsible genes, *TSC1* and *TSC2*, are

implicated in complex cellular signaling pathways governing cell growth and differentiation. Mutation of *TSC1* or *TSC2* causes the TSC phenotype, with disordered cell growth leading to benign tumor growth in several organs, including brain. In contrast, very little is known about the mechanisms of epilepsy and cognitive impairment in this common genetic disorder. The level of cognitive ability in TSC, which ranges from normal to severe mental retardation, is related in part to the severity of the epilepsy (2). Understanding of the correlation between genotype and phenotype in many aspects of TSC is hindered by the limited availability of relevant animal models; existing animal models lack the structural brain abnormalities and epilepsy that are features of human TSC (3).

Cognition has not been studied extensively in animal models of TSC. The present reports by Waltereit, von der Brélie, and colleagues begin to dissect some aspects of cognition in the Eker model of TSC. The Eker rat is a spontaneous germline mutation of *TSC2*, the gene that encodes tuberin, a GTPase activating protein. Eker rats develop renal tumors but only rarely exhibit neuropathological changes. However, recent reports describe subtle neuropathological abnormalities in Eker rats, especially when the remaining *TSC2* allele is inactivated by a “second hit,” causing loss of heterozygosity. A second hit (somatic mutation) consisting of either early postnatal irradiation (4) or aging (5) led to the appearance of neuropathological changes, including cytomegalic cells and large, dysmorphic neurons. In addition, irradiated Eker rats had a shorter latency to flurothyl-induced seizures (4). In the present report by Waltereit et al., Eker rats displayed enhanced chemical kindling to pentylenetetrazole compared with wild type animals. These studies suggest that Eker rats might express hyperexcitable network properties that could underlie an enhanced predisposition to seizures and that this model might be used to study epilepsy mechanisms in TSC (6).

Since cognitive impairment is a usual feature in humans with TSC, the authors of the present reports hypothesized that Eker rats also would be impaired cognitively and that characterizing their in vivo deficits could lead to study of the molecular mechanisms of cognitive dysfunction. However, continuing the baseball analogy (6), we have now been thrown a curve ball, low and inside. Rather than uncovering cognitive *deficits*, Waltereit et al. found that Eker rats had enhanced episodic memory in a specific subtest of spatial learning. The finding that Eker rats performed better than controls on these tests is startling indeed. How can this result be explained? Are Eker rats smarter than controls, at least in the domain of episodic memory? And by analogy, could patients with TSC be more adept at certain cognitive tasks? A detailed look at the underlying taxonomy of memory and the authors' methods and results is warranted.

Memory is traditionally divided into short-term (working) and long-term (reference) types. In humans, long-term

memory is further subdivided into episodic (memory of events occurring at particular places and times) and semantic (memory of facts and general knowledge). Together, reference and working memories are considered subtypes of declarative or explicit memory, which is dependent upon the hippocampus and neocortex. Declarative memory in humans is considered to be roughly equivalent to spatial memory in rodents (7), justifying the widespread use of the water maze and other tests of spatial learning in epilepsy research.

The investigators analyzed spatial learning and memory in *TSC2*^{+/-} heterozygote Eker rats that had no cerebral hamartomas. Therefore, any cognitive difference in mutant rats would not be due to a brain lesion, such as a tuber, or to epilepsy (Eker rats do not have spontaneous seizures). Using the Morris water maze, they tested spatial learning and memory in Eker rats and compared them with wild type controls (8). This paradigm, familiar to most neurobiologists, forces rats to use extra-maze distal visual cues to find and escape onto a hidden platform in a pool of water. Over repeated trials, the time (latency) taken by a rat to swim to the platform and climb onto it decreases progressively. The ability to rapidly find and escape onto the platform is poorer in animals with hippocampal lesions and epilepsy (9). The water maze is considered to test a form of episodic memory, since the rat must remember the location of the platform.

Waltereit et al. found that Eker rats performed no differently than controls in several standard measures of water maze learning—acquisition learning (finding the platform in its fixed position), reversal learning (learning the new location of the platform), or probe trial (after the platform is removed, relative time spent searching for the platform in its previous location). Finding no difference between groups, the investigators upped the ante and subjected the rats to a more challenging spatial memory test, called a “delayed matching-to-place task.” After learning platform position with 15-second intervals between trials, the interval between the first and second trials was increased from 15 seconds to 2 hours. Surprisingly, on this task, Eker rats found the platform significantly *faster* than controls. That is, after this 2-hour “break,” Eker rats did not exhibit the expected delay in finding the platform. This result was replicated using an independent spatial learning task, the radial arm maze. Again, after showing that Eker and control rats learned the radial arm maze task equally well, introducing a longer interval between two trials resulted in fewer arm entry errors among Eker rats. The authors excluded anxiety levels, swimming speed, and running speed as explanations for the performance differences.

Do these results constitute enhanced cognitive function, diminished cognitive function, or neither? The authors argue that despite the apparent improved episodic memory of Eker rats on the delayed matching-to-place task, their performance might also be interpreted as a cognitive disadvantage. That is, Eker rats might find the platform quicker in this task because

their memory is not disturbed by competing memories of previous platform positions. This interpretation is not compelling. Rather than assume that these performance differences represent actual alterations in memory function, more robust behavioral and learning differences would be more reassuring. Only then would it be reasonable to pursue a molecular explanation. Waltereit et al. found alterations in phospho-p42-mitogen-associated protein kinase (phospho-p42-MAPK) induction. That cellular signaling pathway has been implicated in long-term memory and long-term potentiation (LTP) (10), so it would comprise a logical target for TSC-induced plasticity alterations. However, these experiments, despite the intriguing findings, fail to establish a causative link between molecular dysfunction and cognitive impairment.

Using Eker rats, von der Brélie et al. examined synaptic plasticity in hippocampal slices. They found that Eker rats had decreased LTP and long-term depression compared with wild type controls and concluded that the impaired synaptic plasticity in mutant rats could correlate with the cognitive impairments seen in human TSC. The loss of synaptic plasticity was not due to GABA_A-mediated inhibition, since bicuculline had no effect on the decreased LTP and long-term depression. The molecular mechanisms underlying these plastic changes remain to be determined.

The experiments by these two groups represent the first attempts at understanding cognitive dysfunction in an animal model of TSC. As such, the work is pioneering. However, considerable additional evidence must be garnered before attributing the cognition alterations (which are minor and even counterintuitive) with molecular dysfunction in a specific signaling pathway. It is surprising that standard water maze outcomes were not affected in Eker rats; many studies of epilepsy models

using the water maze found deficits in acquisition or retention of spatial memory, which were not observed here. The Eker rat is still at bat—but can it find its way to first base?

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