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Altered Hippocampal Synaptic Physiology in Aged Parkin-Deficient Mice

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Abstract

We examined synaptic function in the hippocampus of aged mice deficient for the Parkinson's disease-linked protein, parkin. Surprisingly, heterozygous but not homozygous parkin-deficient mice exhibited impairments in basal excitatory synaptic strength. Similarly heterozygous mice exhibited broad deficits in paired-pulse facilitation, while homozygous parkin-deficient mice exhibited more restricted deficits. In contrast to the measurements of basal synaptic function, synaptic plasticity was not altered in aged heterozygous parkin-deficient mice, but was enhanced in aged homozygous parkin-deficient mice, due to an absence of age-related decline. These findings of differential synaptic phenotypes in heterozygous vs. homozygous parkin deficiency suggest compensatory responses to genetic abnormalities could play an important role during the development of pathology in response to parkin deficiency.

Keywords

Parkin; PARK2; Hippocampus; LTP; Plasticity; EPSP; Mouse; Compensatory; Aging; CA1

Introduction

Mutations in the PARK2 gene, which encodes the ubiquitin ligase, parkin, cause autosomal recessive juvenile parkinsonism (Kitada et al. 1998). Parkin is found throughout the brain, including the hippocampus (Horowitz et al. 1999; Stichel et al. 2000). Despite a lack of consistent overt motor phenotypes in parkin knockout mice created by several different groups (Goldberg et al. 2003; Itier et al. 2003; Palacino et al. 2004; Perez and Palmiter 2005; Von Coelln et al. 2004), non-motor alterations including behavioral phenotypes indicative of hippocampal dysfunction have been described in some of these mice (Itier et al. 2003; Perez and Palmiter 2005; Zhu et al. 2007). Experiments in dissociated hippocampal neurons have demonstrated disrupted excitatory function in response to acute modulation of parkin levels (Helton et al. 2008). While parkin mutations cause parkinsonism in a recessive manner, the

genetics may be more complex, and the risk and phenotypes conferred by heterozygous mutations is an active area of investigation (Lee et al. 2009; Lohmann et al. 2009; van Nuenen et al. 2009). In addition, compensatory mechanisms, which may be especially pronounced in homozygous knockouts, could mask detection of the primary physiological deficits caused by parkin mutations. Therefore, to best detect any neurophysiological disruptions resulting from parkin deficiency, we examined synaptic physiology in both homozygous (*parkin*^{-/-}) and heterozygous (*parkin*^{+/-}) parkin-deficient mice in comparison to wild-type (*parkin*^{+/+}) controls. In particular we focused on the properties of excitatory neurotransmission in aged mice modeling life-long parkin deficiency, using coisogenic wild-type and knockout mice on a 129 background (Perez and Palmiter 2005).

Materials and Methods

Animals

Parkin-deficient mice generated with a targeted deletion of *Park2* exon 2 were used (Perez and Palmiter 2005). To avoid potential confounds arising from use of non-coisogenic mice, all experiments were performed using wild-type and mutant mice on a coisogenic 129S4 background that, unlike mice back-crossed onto a B6 background, are expected to be identical at all loci except *Park2*. Littermate *parkin*^{+/+}, *parkin*^{+/-}, and *parkin*^{-/-} males were generated from breeding of heterozygous males and heterozygous females. After weaning, experimental animals were group housed with mixed genotype littermates in cages of up to five mice prior to experimentation. Genotyping was performed by PCR analysis, and experimenters were blind to genotype during data acquisition and analysis.

Brain Slices

Four hundred micrometer thick coronal hemi-brain slices containing hippocampus were prepared using a vibrating sectioning system and were recorded from in oxygenated Artificial Cerebrospinal Fluid (ACSF) at room temperature, containing (in mM) 119 NaCl, 2.5 KCl, 1.3 MgSO₄, 2.5 CaCl₂, 1 Na₂ HPO₄, 26.2 NaHCO₃, 11 glucose, perfused at a rate of 2 ml/min. Slices were prepared and stored at room temperature in oxygenated solution identical to the ACSF used for recording, with the exceptions that the MgSO₄ concentration was elevated from 1.3 to 6 mM during slicing and storage and 119 mM NaCl was replaced with 210 mM sucrose during slicing.

Electrophysiological Recordings

In all experiments, synaptic inhibition was blocked using the GABA_A receptor ionophore blocker picrotoxin (50 μM) in the ACSF in order to isolate the properties of excitatory synapses. Recording electrodes consisting of ACSF-filled glass micropipettes were placed in the stratum radiatum of area CA1 and the evoked field excitatory postsynaptic potentials (EPSPs) were measured using a MultiClamp 700A amplifier in current clamp mode with 1000X gain. Presynaptic stimulation was delivered with biphasic 100 μs stimuli via a concentric bipolar stimulating electrode placed in the stratum radiatum of CA1 between the recording electrode and area CA3. The initial slope of the field EPSP responses was used as a measure of synaptic response magnitude in order to avoid artifacts of amplitude measurements caused by reflected population spike contributions. LTP was induced with a

high frequency stimulation protocol consisting of three bursts of 10 stimuli delivered 100 Hz with 15 s intervals between the bursts. Test stimuli were delivered at 0.5 Hz, and mean responses were calculated for each minute of recording during LTP experiments, and group data are presented as the mean $\pm$ SEM for all recordings from each genotype. Input–output curves and paired-pulse ratios for each recording were determined from the mean of 2–3 repetitions of each stimulus protocol, and group data are presented as mean $\pm$ SEM for all recordings from each genotype. Statistical significance was assessed using a Student's *t*-test with a threshold of $P < 0.05$.

Results

Basal Excitation Alterations in Heterozygous vs. Homozygous Parkin Deficiency

Basal excitatory synaptic transmission was examined by measuring the relationship between stimulus intensity and EPSP response magnitude in area CA1 of the hippocampus in parkin-deficient mice and wild-type controls. To evaluate aging-related phenotypes, all measurements were made in mice aged 16–18 months. While basal excitatory transmission in mice with a homozygous deficiency (parkin $^{-/-}$) was indistinguishable from wild-type mice (parkin $^{+/+}$), mice with a heterozygous deficiency (parkin $+/-$) had weaker response amplitudes with significantly reduced maximal responses (Fig. 1). Because reduced excitatory efficacy could be contributed to by either presynaptic or postsynaptic abnormalities, we made measurement of the paired-pulse ratio (PPR), which is the relative strength of a second EPSP evoked shortly after a prior EPSP, and is generally regarded as a property of presynaptic function. PPR measurements were made over a range of inter-stimulus intervals (ISI) in parkin-deficient mice and controls. Parkin $^{-/-}$ mice exhibited a significantly reduced PPR at the shortest interval tested (25 ms), but otherwise did not significantly differ from controls at intervals between 50 and 800 ms, despite a trend toward enhanced PPRs at the longer intervals (Fig. 2a, c). In contrast, parkin $+/-$ mice exhibited significantly reduced PPRs relative to controls at intervals of 25, 50, and 100 ms (Fig. 2b, c). Thus, compared to homozygous parkin deficiency, heterozygous parkin deficiency resulted in a unique deficit in basal transmission, and results in more severe deficits in PPR measurements, indicative of altered presynaptic function.

Synaptic Plasticity Alterations in Heterozygous vs. Homozygous Parkin Deficiency

Because synaptic plasticity, especially long-term potentiation (LTP) of excitatory transmission, is thought to underlie learning and memory, which are observed to be impaired in PD patients (Bruck et al. 2004; Camicioli et al. 2003; Ibarretxe-Bilbao et al. 2008; Riekkinen et al. 1998) and altered in parkin-deficient mice (Itier et al. 2003; Perez and Palmiter 2005; Zhu et al. 2007), we measured LTP in aged (16- to 18-month-old) parkin-deficient mice and controls. Since the strength of LTP declines with age in wild-type mice (Watson et al. 2002, 2006), we also made recordings from young adult (2–3 months) mice for comparison. These recordings revealed either young parkin $+/-$ or parkin $^{-/-}$ mice were significantly different from young wild-type mice (Fig. 3a, c, e). However, aged parkin $^{-/-}$ but not parkin $+/-$ exhibited significantly stronger LTP than old wild-type controls (Fig. 3b, d, f). These results demonstrate that homozygous, but not heterozygous, parkin deficiency uniquely leads to a preservation of LTP strength in old mice, resulting in excessively strong

LTP relative to controls. This result is in contrast with our basal transmission observations, where heterozygous but not homozygous parkin deficiency caused abnormalities in old mice.

Discussion

Parkin Deficiency and Synaptic Transmission

The impaired basal synaptic strength in parkin+/- mice could be mediated by dysregulation of parkin substrates involved in postsynaptic or presynaptic function. Parkin associates with PDZ scaffold proteins in the postsynaptic density (Fallon et al. 2002), and parkin ubiquitinates protein interacting with C-kinase (PICK1), a protein that regulates AMPA receptor trafficking and excitatory synaptic strength (Joch et al. 2007). Furthermore, acute post-synaptic manipulation of parkin has been shown to regulate excitatory synapse number in primary hippocampal neuronal cultures (Helton et al. 2008). At the same time, the altered paired-pulse ratios in parkin+/- and parkin-/- mice suggest presynaptic dysfunction is occurring in aged parkin-deficient mice. Possible mechanism for a presynaptic site of dysfunction is provided by known parkin substrates that could play key roles in presynaptic glutamate release including, Cdcrel-1 (Zhang et al. 2000), glycosylated alpha-synuclein (Shimura et al. 2001), synphilin-1 (Chung et al. 2001), and synaptotagmin XI (Huynh et al. 2003). Thus deregulation of parkin substrates likely leads to the observed phenotypes of abnormal hippocampal neurotransmission, either via direct disruption of synaptic function or through compensatory responses, as discussed below.

Parkin Deficiency and Compensatory Changes in Synaptic Function

Given the impairment of maximal synaptic response magnitude and the broad impairment of paired-pulse facilitation in aged parkin+/- mice, a reasonable expectation would have been that parkin-/- mice, should have more severe impairments on these measures of basal synaptic function. Surprisingly, however, parkin-/- mice exhibited no impairment in maximal response magnitude, and a relatively restricted impairment in paired-pulse facilitation. This raises the possibility that homozygous but not heterozygous parkin deficiency results in recruitment of compensatory mechanisms that overcome the impact of parkin deficiency on basal synaptic function. However, with respect to synaptic plasticity, parkin-/- mice, but not parkin+/- mice exhibited alterations relative to wild-type controls. In particular, aged parkin-/- mice exhibited significantly more robust LTP than wild-type mice, apparently due to an absence of normal age-related decline in the strength of LTP. This is consistent with behavioral measurements reported in mice generated with this mutation, which showed that enhancements in learning and memory developed in parkin-/- mice in comparison to controls as the mice aged (Perez and Palmiter 2005). One explanation for these findings of differential effects of heterozygous and homozygous parkin deficiency is that the compensatory mechanisms that rescue basal synaptic transmission could also result in altered synaptic plasticity in the homozygous parkin-deficient mice. While future experiments will be necessary to determine the underlying mechanisms of altered synaptic functions including potential compensatory mechanisms, various misregulated signaling pathways could be involved. To give just one example, altered oxidative stress and reactive oxygen species levels (ROS) are a key convergent feature of parkinsonism caused by

various factors, including parkin mutations (Greenamyre and Hastings 2004; Rodriguez-Navarro et al. 2007). At the same time, ROS signaling is also necessary for normal hippocampal LTP (Almaguer-Melian et al. 2000; Watson et al. 2006), suggesting a possible role in the altered synaptic physiology.

It is worth noting that other studies of parkin-deficient mice created using different constructs have also examined some aspects of hippocampal synaptic function in the case of homozygous parkin-deficient mice. One study examined LTP in young homozygous animals, and we are in agreement with their finding of normal LTP in young parkin^{-/-} mice compared to parkin^{+/+} controls (Kitada et al. 2009). Another study focused on older homozygous parkin^{-/-} mice and found opposite results from ours, in that basal synaptic strength was impaired in aged parkin^{-/-} mice, but LTP was normal (Itier et al. 2003). Possible explanations for these differences could include technical differences in the gene targeting-constructs (Perez and Palmiter 2005) or differences in the LTP induction protocol. Another interesting possibility is that differential interaction between parkin loss of function and the genetic contexts of the different mouse lines, leads to different phenotypes. Just as humans with different genetic backgrounds show different phenotypes in response to mutations of the same gene, different genetic contexts could result in different interactions between loss of parkin-dependent functions and compensatory responses during aging. In any case, the important concept that emerges is that the effects of disease-linked mutations can be modulated by compensatory mechanisms, resulting in abnormalities in synaptic function that depend on both the dosage of the gene deficiency and likely the genetic context.

Synaptic Plasticity Dysfunction in Parkinsonism

Combined with the recent discovery of altered synaptic plasticity in the basal ganglia of *Pink1* knockout mice (Kitada et al. 2007), and reports of altered synaptic function in the striatum (Goldberg et al. 2005) and the hippocampus (Wang et al. 2008) of *Dj-1* knockout mice, our discovery of altered hippocampal synaptic plasticity in parkin-deficient mice implicates synaptic plasticity as a potential general feature of genetic models of parkinsonism. Furthermore, abnormal synaptic plasticity has been described in the basal ganglia in neurotoxin models of PD (Kreitzer and Malenka 2007; Picconi et al. 2005) and indications of abnormal synaptic plasticity have also been reported in human Parkinson's disease patients (Prescott et al. 2009). Together these findings suggest that abnormal synaptic plasticity may be a common mediator of pathophysiology in both the basal ganglia and the hippocampus during conditions causing parkinsonism.

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Fig. 1.

Basal synaptic transmission alterations. **a** Excitatory synaptic strength of parkin^{-/-} mice compared to controls is shown with a plot of initial EPSP slope as a function of stimulus amplitude. Example traces from a parkin^{-/-} recording are shown. **b** Synaptic strength of parkin^{+/-} mice compared to parkin^{+/+} controls is plotted. Example traces from a parkin^{+/-} recording are shown. **c** Maximal synaptic strength is shown for all three genotypes using the initial slope at the highest stimulus intensity. Parkin^{+/-} mice have significantly weaker synaptic strength than wild-type controls ($P < 0.05$), while parkin^{-/-} do not differ significantly from controls (parkin^{+/+} $n = 15$ slices from 8 animals, parkin^{+/-} $n = 19$ slices from 10 animals, and parkin^{-/-} $n = 15$ slices from 10 animals)

Fig. 2.

Paired pulse alterations. **a** Paired pulse ratio (PPR) measurements of parkin^{-/-} mice compared to parkin^{+/+} controls is shown with a plot of the ratio of the initial slope of the second pulse divided by the initial slope of the first pulse as a function of inter-stimulus intervals. PPR values >1 indicate paired-pulse facilitation. Example traces from a parkin^{-/-} recording are shown. **b** The PPRs of parkin^{+/-} mice compared to the parkin^{+/+} controls are plotted. Example traces from a parkin^{+/-} recording are shown. **c** PPR of the three shortest ISIs are compared between all three genotypes. Parkin^{-/-} mice have significantly reduced PPR compared to wild-type controls at the 25 ms ISI ($P < 0.05$), while parkin^{+/-} have significantly reduced PPR at 25, 50, and 100 ms ISI ($P < 0.05$). No significant differences were seen in either genotype at the longer ISIs (parkin^{+/+} $n = 10$ slices from 7 animals, parkin^{+/-} $n = 17$ slices from 11 animals, and parkin^{-/-} $n = 14$ slices from 10 animals)

Fig. 3.

Age-related synaptic plasticity alterations. **a** LTP experiments from young adult (2–3 months) parkin^{-/-} mice compared to controls are shown with average initial EPSP slopes plotted during each minute of the experiment. LTP was induced at time 0 and measurements were normalized to baseline levels prior to induction. Example traces from a young parkin^{-/-} and young parkin^{+/+} control recording are shown. **b** LTP experiments and example traces from old (16–18 months) parkin^{-/-} and parkin^{+/+} controls are shown. **c** LTP experiments and example traces from young parkin^{+/+} and parkin^{+/+} controls are shown. **d** LTP experiments and example traces from old parkin^{+/+} and parkin^{+/+} controls are shown. **e** The average magnitudes of LTP measured between 50 and 60 min post-LTP induction are plotted for young and old parkin^{-/-} and parkin^{+/+} controls. The old parkin^{-/-} mice exhibited significantly stronger LTP than the controls ($P < 0.05$), consistent with an absence of normal age-related decline in LTP. **f** The average magnitudes of LTP are plotted for young and old parkin^{+/+} and parkin^{+/+} controls. While there is a trend toward reduced LTP in young parkin^{+/+} mice, no significant differences between parkin^{+/+} mice and parkin^{+/+} controls were observed in either age group (old: parkin^{+/+} $n = 9$ slices from 6 animals, parkin^{+/+} $n = 14$ slices from 11 animals, and parkin^{-/-} $n = 13$ slices from 10 animals,

young: parkin^{+/+} $n = 6$ slices from 6 animals, parkin^{+/-} $n = 14$ slices from 11 animals, and parkin^{-/-} $n = 6$ slices from 5 animals)