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MAP1B-dependent Rac activation is required for AMPA receptor endocytosis during long-term depression

Marion Benoist, Rocio Palenzuela, Carlos Rozas, Patricio Rojas, Elena Tortosa, Bernardo Morales, Christian Gonzalez-Billault, Jesus Avila and Jose A Esteban

Corresponding author: Jose Esteban, Centro de Biologia Molecular Severo Ochoa

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

Editor: Karin Dumstrei

1st Editorial Decision 16 February 2013

Thank you for submitting your manuscript to the EMBO Journal. Your study has now been seen by three referees and their comments are provided below. I would also like to apologize for the delay in getting back to you with a decision, but in this case it unfortunately took a bit longer than anticipated to receive the reports.

As you can see, the referees appreciate the data set reporting a role of MAP1B in AMPA receptor endocytosis during LTD. However, they also raise a number of issues that I assume that you should be able to address. I would therefore like to invite you to submit a revised manuscript that addresses the concerns raised in full. I should add that it is EMBO Journal policy to allow only a single round of revision and that it is therefore important to address the raised concerns at this stage.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: http://www.nature.com/emboj/about/process.html

We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

Thank you for the opportunity to consider your work for publication. I look forward to your

revision.

REFEREE REPORTS

Referee #1

Review of Benoist et al.

This manuscript reports that the microtubule-associated protein MAP1B is serving an intriguing function in mature hippocampal neurons. Specifically, MAP1B appears to be important for long-term depression (LTD) by disrupting the internalization of AMPA receptors in dendritic spines, through a Rac/TIAM1 specific pathway. The authors present compelling electrophysiological evidence through the judicious use of wild type and heterozygous MAP1B mutants in acute hippocampal slices. They also are very thorough in the controls that they present to show that MAP1B deficiency has specific effects. By live cell AMPAR imaging (with a SEP-GluA2 probe) in organotypic slices, they show that AMPARs are not internalized as efficiently in MAP1B+/- neurons. The LTD deficiency in MAP1B+/- slices can be rescued by increasing Rac protein but not by inhibiting RhoA. Overall, this is a well documented and convincing study showing that a microtubule associated protein may be functioning to regulate activation of GTPases and thus affect actin filaments in dendritic spines after LTD. These results are especially intriguing given the recently described invasion of dynamic microtubules into dendritic spines undergoing synaptic activation by several other labs. However, there are a few technical aspects of the study that deserve attention and it is unclear how much of an advance these studies make, based on the previous literature.

Major Criticisms

1) In Fig. 4A and D, the images of the MAP1B+/+ example spines in the cells transfected with the SEP-GluA2 (Fig. 4A) or Tomato both almost disappear after NMDA-dependent LTD (and are much dimmer than the MAP1B+/- spines). Although the quantification of SEP-GluA2 (Fig. 4C) or Tomato (Fig. 4E) as a ratio of spine to dendrite gives a quantitative number it would seem that computing how the SEP-GluA2/Tomato ratio changes after NMDA addition would be the most appropriate way to determine if the levels of AMPA receptors are significantly different between the MAP1B wild-type and heterozygotes.

2) Technically, most of the figures are very well presented with appropriate controls. However, in Fig. 3G and H the control for the shRNA treatment (and for the Rac and DN-Rho treatments in Fig. 6B, C and Fig. 7A-D) are uninfected neurons. The authors should at least use scrambled shRNA or better yet, addition of a MAP1B construct not knocked down by the shRNA as a rescue (i.e. for Fig. 3). In Fig. 6 and 7 the DN-Rho serves as a type of control but it would be better to have a vector alone control.

3) In the discussion the authors should explain why they think "...the enhanced LTP we observed in MAP1B+/- animals with weak induction protocols is an indirect consequence of impaired LTD expression." (p. 16) And probably more importantly why they make the claim that MAP1B defects are specific for LTD throughout the manuscript if they are seeing an effect with a more physiological LTP protocol (theta burst stimulation) (Fig. 2C).

4) The authors rightly give credit to previous work that has shown MAP1B involvement in LTP (Zervas et al., 2005) and the role of MAP1B in mGluR-mediated endocytosis of AMPA receptors in the hippocampus (Davidkova and Carroll, 2007). They argue that the 2005 study used a different MAP1B hypomorphic mouse and found opposite results to those presented here. They also state that the 2007 study did not observe AMPAR endocytosis directly, as they have done here. The authors should be given credit for not trying to bury previous results, as many others do, but they should also make a stronger case for the novelty of this study.

Minor Concerns

1) On pg. 10, 1st paragraph - (see below, Fig. 5G-H) should be changed to Supp. Fig. 3.

2) Figure 1 could be added to supplemental data since no difference is detected in any of the measures between wild type and heterozygotes.

Referee #2

In this manuscript the authors investigate the role of the microtubule-associated protein MAP1B in the function of the mature synapse. Using MAP1B heterozygous mice and acute protein down-regulation, they demonstrate that reduction of MAP1B results in impairment of LTD, produced by inhibition of AMPA receptor endocytosis. The defect in LTD is accompanied by impaired targeting of Tiam1, a Rac1 activator, at the synapse. Consistently, the defect in LTD is rescued by overexpression of Rac1. The authors conclude that the MAP1B-Tiam1-Rac1 relay is essential for spine structural plasticity and removal of AMPA receptors from synapses during LTD.

The authors present a convincing set of well-controlled experiments and the manuscript is well written and structured. There are some issues, though, that require attention and should be addressed to improve the manuscript:

1) the authors suggest that MAP1B is a critical factor for the targeting of Tiam1 to synaptic compartments, which may result in impaired Rac1 activation at synapses. The demonstration that Tiam 1 is not correctly targeted to synapses is not convincing. The western blotting in fig. 6A, showing a reduced Tiam1 accumulation in the synaptosomal fraction of MAP1B-deficient animals, lacks loading control and relative normalization. A quantitative western blotting should be performed. An immunocytochemical staining for Tiam1 might also support the author's point.

2) the authors suggest that defective targeting of Tiam1 to synaptic compartments may result in impaired Rac1 activation at synapses. Consistently, they rescue LTD by Rac1 overexpression. Are the authors transfecting a constitutively active form of Rac1? The authors should clarify this issue.

3) The authors convincingly show that LTD is rescued by Rac1 overexpression. Is the removal of AMPA receptors from the spine also rescued by Rac1 overexpression?

4) This study shows that Rac1 in MAP1B het neurons induces AMPA receptor removal from synapses and spine shrinkage. It is known from literature that Rac1 activity plays an essential role in spine enlargement and AMPA receptor clustering during synapse maturation (Tashiro et al., 2000; Tashiro and Yuste, 2004; Wiens et al., 2005). The authors should discuss more in detail their findings in relation to these previous evidences.

5) Fig 7 seems not completely relevant to the message conveyed by this study. Also, although statistical significance is lacking, a clear tendency to reduction in the NMDA response upon RhoDN expression would prompt to perform more experiments. I would suggest to remove this figure or move it to supplementary material.

6) Although the authors demonstrate that spine head diameter is not different between het and wt neurons, in all figures (4A, 4D, 5B) spine dimensions appear larger in MAP1B het neurons. The authors could probably select more representative images.

Referee #3

This study characterizes the role of MAP1B in LTD induced by mGluR and NMDA receptors using in vivo approaches to measure internalization of AMPA receptors and viral infection to modify intracellular signal in hippocampal slices. They show a reduction of the LTD induction in MAP1B defective mice compared to wild types whereas the LTP remains with no significative changes. The lack of LTD response is reflected in both an increase in spine stability as well as the maintenance of GluA2 content in the spines after the activation of NMDA and mGluR receptors. The authors demonstrate that the TIAM1-Rac1 signaling is defective in the MAP1B deficient mice due to a mislocalization of TIAM1. Interestingly, the deficiency in LTD observed in the MAP1B +/- mice is reversed by overexpressing Rac1, demonstrating a direct role of MAP1B-TIAM1-Rac1 cascade in

LTD whether it is independent of Rho activity. These findings help to clarify how the cytoskeleton and associated proteins, together with the actin dynamics, modify the spine plasticity in response to LTD stimulation.
Provided that a few comments (detailed below) are addressed, this manuscript would be suitable for publication in The EMBO Journal.

Major Comments:

1) In some of the experiments (see Fig3E, 3G, 3H), the results are expressed as shRNA versus non-infected cells, when it would be more precise to compare shRNA against a scramble construct. The same can be found in Fig7. It is possible that by "uninfected" the authors mean that the experiment is done with a mock plasmid, but in that case they should clarify what is the meaning of the "uninefected" condition.

2) The authors demonstrate the implication of Rac1 activity in the role of MAP1B in LTD, in Fig6A it would be needed to test whether Rac1 is localized to the synaptosomal fraction in MAP1B+/- or is reduced as it is TIAM1 protein. Are the total levels of Rac1 different between the MAP1B+/- neurons and the MAP1B +/+? Also, some control positive markers from each fraction would be needed in order to compare the amount of the protein of interest.

Minor comments:

1) In the picture shown in fig5, the MAP1B+/- appears to have a higher spine density than MAP1B+/+, however the graphic shown in fig5E shows no differences in spine density between those groups. I would suggest to select a representative image that reflects the same spine density as shown in the graphic after quantification.

2) In the same figure, fig5C, shows a comparison of the actin content in MAP1B+/+ versus MAP1B+/-. My suggestion for expressing this quantification is to include the area of the spine as a parameter in the quantification because again, from the pictures shown it seems the area is bigger in the MAP1B+/- neurons. Fig3E and fig3G should have apart from the number of animals, the number of cells quantified in each condition.

Other suggestions:

The authors show that Rac1 overexpression restores the LTD in MAP1B+/- neurons, it would be informative too to see whether the levels of surface GluA2 are reduced as expected.

1st Revision - authors' response 04 June 2013

Reviewer 1

1. Following the observation of the reviewer, we have replaced the representative spine examples, so that the initial signal from wild-type and MAP1B +/- is more comparable, and the spines do not disappear after LTD induction. In addition, as requested by the reviewer, we now present plots of fluorescence ratios (SEP-GluA2/Tomato) for the LTD experiments (Suppl. Fig. 2C; page 11, first paragraph). Nevertheless, we need to point out that these plots are not very straightforward, because the changes in SEP-GluA2 (receptor internalization) and Tomato (spine shrinkage) are not synchronic, and therefore, changes in the ratio will depend on both parameters varying independently. Thus, in the case of wild-type neurons, the ratio SEP-GluA2/Tomato increases right after NMDA application. This is because spine shrinkage (reduction in Tomato fluorescence) is faster than receptor internalization (reduction in SEP-GluA2 fluorescence) (Fig. 4C and E). In the case of MAP1B +/- neurons, both receptor internalization and spine shrinkage are impaired, but there is a residual and gradual decrease in spine size (Fig. 4E), which results in a slow increase in SEP-GluA2/Tomato ratio. Given the complications to interpret these plots, we prefer to show them as Supplementary Material, to make the information available, but not distract readers from the main flow of the results.
2. As requested by the reviewer, we have now used scrambled shRNA as control for the MAP1B shRNA (Fig. 3E-H; page 9, second paragraph), and a GFP expression vector as control for Rac

and Rho-DN expression (Fig. 7A, B and Suppl. Fig. 5; page 13, second paragraph, and page 14, first paragraph).

3. We agree with the reviewer in that we cannot conclude that the effects of MAP1B deficiency are exclusively on LTD, and that the enhancement of LTP is a consequence of the reduction in LTD. This is a plausible scenario, but our data do not demonstrate it. Elucidating the potential effects of MAP1B on LTP is outside of the scope of this work. Therefore, we have changed the wording of the document (page 17, end of first paragraph) to avoid implying that the exclusive effects of MAP1B are on LTD, as indicated by the reviewer.

4. We appreciate the recommendation of the reviewer to emphasize the novelty of our study. We have modified the Discussion (beginning of page 18) to more clearly state that our results show for the first time a specific mechanism by which MAP1B controls AMPA receptor endocytosis during LTD. This is an important point, considering that new publications are starting to equate MAP1B levels with AMPA receptor internalization (for example Chen and Shen, J Neurosci 33:9013-20, 2013), even though the mechanism for this coupling has not been reported yet.

Minor comments:

1. Actually, on page 10, first paragraph, we did refer to Fig. 5G-H, as the quantification of spine head diameter carried out with dye-filled neurons on apical dendrites. But the reviewer is right that Supplementary Fig. 3 (basal dendrites) should also be cited here. This has been modified accordingly.
2. If space limitations allow it, we would prefer to keep Fig. 1 in the main text (not as supplemental data). Although it contains essentially negative data (electrophysiological parameters that do not change in MAP1B +/- animals), we consider these are important results. Previous reports with other models of MAP1B deficiency showed multiple deficiencies in synaptic function and plasticity, probably as pleiotropic consequences of altered neuronal development. By using a heterozygous MAP1B animal that is devoid of detectable neurological problems, we have been able to dissect a distinct function of MAP1B in LTD, separable from its role during neuronal developmental. To strengthen this point, we consider it is important to show in the main text that multiple parameters of synaptic function are normal in these animals.

Reviewer 2

1. As requested by the reviewer, we now include quantitative western blots of synaptosomal and whole-slice homogenates, including PSD95 as a synaptic marker. As shown in revised Fig. 6, the decrease in synaptic enrichment is specific for Tiam1, and does not occur with Rac1 or PSD95 (page 12, last paragraph).

2. We have indeed trying transfecting a constitutively active form of Rac1 for the rescue experiments, as indicated by the reviewer. However, we have consistently found that overexpression of constitutively active Rac1 was very toxic in our system (organotypic hippocampal slice cultures). We have been able to bypass this problem by overexpressing the wild-type protein. We suspect that in this manner we are supplying enough active Rac1 protein, without reaching toxic values. This point is now clarified in the revised version of the manuscript (page 13, first paragraph).

3. This is a critical new experiment, which is now added in the revised manuscript. We generated a new viral vector for the co-expression of SEP-GluA2 and wild-type Rac1 fused to Tomato. As shown in new Fig. 7C, AMPA receptor endocytosis (monitored by the decrease in SEP-GluA2 fluorescence) is restored in MAP1B +/- slices upon Rac1 overexpression (page 15). To note, this manipulation did not alter AMPA receptor internalization in wild-type slices. This important result strengthens our conclusion that MAP1B has a distinct role in LTD by controlling AMPA receptor endocytosis via Rac activation.

4. As pointed out by the reviewer, Rac1 has a clear role in spinogenesis and synapse maturation, probably by modulating actin remodeling during spine enlargement and stabilization. On the other hand, we are showing here that Rac1 is required for AMPA receptor endocytosis and spine shrinkage during LTD. These results are consistent with the requirement of Rac1 activity for LTD (Bongmba et al, Brain Res 1399:79-95, 2011; Martinez & Tejada-Simon, Neuropharmacology 61:305-312, 2011). Therefore, it appears that Rac1 has different functions

for spinogenesis versus synaptic plasticity at established synapses. This probably reflects the complexity of the actin cytoskeleton and its remodeling, where a given molecular mechanism may have different functional outcomes depending on the developmental stage of the structure where it takes place. Similar situations probably apply to other regulators of the actin cytoskeleton. For example, RhoA is thought to act in an antagonistic manner with Rac1 for spine morphogenesis (Tashiro et al, 2000). However, RhoA has also been associated to stabilization of the actin cytoskeleton (Schubert et al, J Cell Biol 172:453-467, 2006). This issue is now further discussed in the revised manuscript, together with the inclusion of the references cited by the reviewer (end of page 18 and beginning of page 19).

5. As suggested by the reviewer, we now present the original Fig. 7 as supplementary material (Suppl. Fig. 5).

6. We have chosen other example images for wild-type and MAP1B +/- spines, which more accurately reflect the average spine dimensions.

Reviewer 3

1. As requested by the reviewer, we have now used scrambled shRNA as control for the MAP1B shRNA (Fig. 3E-H; page 9, second paragraph), and a GFP expression vector as control for Rac and Rho-DN expression (Fig. 7A, B and Suppl. Fig. 5; page 13, last paragraph, and page 14, end of second paragraph).

2. We have now evaluated the levels of Rac1 and a synaptic marker (PSD95) in the synaptosomal fractions from wild-type and MAP1B +/- neurons. As shown in revised Fig. 6, the decrease in synaptic enrichment is specific for Tiam1, and does not occur with Rac1 or PSD95 (page 12, last paragraph).

Minor comments:

1. We have changed the example images in Fig. 5A to more accurately reflect similar spine densities in wild-type and MAP1B +/-, as quantified in Fig. 5E.

2. As the reviewer points out, Fig. 5A-C shows an increase in the amount of actin-GFP in the spines from MAP1B +/-. It would be problematic to quantify spine area from these images, because brighter spines (with more actin) will also appear larger. We believe the quantification of spine size is more accurately carried out with specialized software for morphology analysis on dye-filled neurons, as we did in Fig. 5G, H. Also, we now indicate the number of cells as well as the number of animals used for these quantifications, as requested by the reviewer.

Other suggestion: We have indeed tested whether Rac1 overexpression would also rescue GluA2 internalization, as it does with LTD. This is an important experiment, which was also requested by reviewer #2. As described above (reviewer 2, point 3), Rac overexpression does rescue AMPA receptor internalization upon LTD induction in MAP1B deficient neurons (page 15). This important result strengthens our conclusion that MAP1B has a distinct role in LTD by controlling AMPA receptor endocytosis via Rac activation.

2nd Editorial Decision 18 June 2013

Thank you for submitting your revised manuscript to the EMBO Journal. Your revision has now been seen by referees #1 and 2. As you can see below both referees appreciate the introduced changes and support publication here.

Referee #1 has a few comments regarding fig 7 that seem sensible and easy enough to resolve. I would like to you to take these comments into consideration in a final round of revision. Once we get the revised manuscript back we will proceed with its acceptance.

Thank you for submitting your interesting study to the EMBO Journal!

REFEREE REPORTS

Referee #1

The authors have responded sufficiently to my criticisms and by providing data from new experiments have made the manuscript stronger and more appropriate for publication. My only additional comment is that in the new Fig. 7 the authors could remove the uninfected data from A and B and compare GFP alone to the GFP-Rac transfection. This would be the proper comparison (they compared uninfected with GFP-Rac, p=0.03). It appears the GFP to GFP-Rac comparison is significantly different but having a p value will be important. Also, coloring the labels (GFP, Rac, Rho-DN) the same colors as the circles and squares would make the graphs in Fig. 7A and B easier to interpret.

Referee #2

The Authors have carefully addressed all raised issues. The manuscript is heavily improved, with the new experiments strengthening the conclusion that MAP1B has a distinct role in LTD by controlling AMPA receptor endocytosis via Rac activation.

2nd Revision - authors' response 28 June 2013

Please find enclosed our revised manuscript, with the modified Fig. 7 as indicated by reviewer #1.

