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A developmental stage- and Kidins220-dependent switch in astrocyte responsiveness to brain-derived neurotrophic factor

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MS TITLE: A developmental stage- and Kidins220/ARMS-dependent switch in astrocyte responsiveness to brain-derived neurotrophic factor

AUTHORS: Fanny Jaudon, Martina Albini, Stefano Ferroni, Fabio Benfenati, and Fabrizia Cesca
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers have slightly different opinions on the potential of your manuscript to be accepted at this stage in JCS, raising several substantial criticisms that prevent me from accepting the paper in the present format. They suggest, however, that a revised version might prove acceptable, if you can address their concerns.

In addition to the minor issues highlighted in their reports, testing (at least) one of the proposed mechanism would be required to warrant publication in JCS. if necessary, I would be happy to discuss plans to implement this suggestion after you have discussed the reviewers' reports with your collaborators.

We are aware that you may be experiencing disruption to the normal running of your lab that makes experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

The manuscript 258419-Cesca describes experiments aimed at understanding the role of Kidins220 in the regulation of BDNF-TrkB signaling in both embryonic and postnatal astrocytes.
The authors report that the role of Kidins changes between embryonic and postnatal astrocytes and reveals a novel function of Kidins: regulation of TrkB-
T1 receptor activation of calcium signaling possibly through regulation of Kir4.1 expression. These data add to the field of Trk signaling, particularly that of TrkB-T1 during development and in astrocytes. It is a solid addition.

Comments for the author

The manuscript is clearly written, logically structured, the figures data, and statistics are of high quality and robust. The discussion and conclusions are balanced.

My suggestions are:
1. I found the significance stars got lost amongst the data points in some of the figures. I suggest that you place them level with the top of the Y-axis.

2. In fig 2, the plot of the ratio FL: T1 was hard for me to dissect. The ratio is normalised to 1, but in actual fact it more likely to be 1:10 albeit perhaps 1:20 for the postnatal cells. I feel it would be more obvious if the actual ratios were presented, or provided in the text.

3. Fig 6 does not have a role for Kir4.1, presumably because the relationship is correlative at this stage. If the BDNF-triggered calcium flux was found to be dependent on Kir4.1 (as well as Kidins), this would boost the novelty and significance of the manuscript, as it provides a mechanism.

4. The expression and the possible role of p75 in embryonic and postnatal astrocytes is not considered in the reported experiments. It is appreciated that the study has focused on the Trk receptor, but given Kidins can also interact with p75 it may have a role that should, at least, be discussed. P75 can regulate PIP2 and PTEN levels, and PIP2 is an important activator of Kir4.1. p75 has been reported to regulate Kir3/GIRK channels via this mechanism in neurons with expression regulated by neuronal activity.

5. Please also note/clarify whether Kir4.1 is a homodimer channel or the expression is of the 4.1 subunit that requires another subunit to be an active channel in astrocytes.

Reviewer 2

Advance summary and potential significance to field

The manuscript investigates the responsiveness of embryonic and postnantal astrocytes in vitro to BDNF in Kidins220/ARMS-dependent manner. The authors demonstrate that there are differences in astrocyte responsiveness to BDNF depending on the stage of development - embryonic ones elicit predominantly TrkB RTK-mediated phosphorylation response, whereas postnatal astrocytes respond to BDNF by eliciting Ca2+ transients primarily via truncated form of TrkB receptor. Moreover, these responses are potentiated by Kidins220/ARMS as demonstrated by the use of knock-out cultures in these experiments. In addition, the authors show that Kidins220/ARMS controls the expression of astrocyte-specific genes, e.g., inward rectifier K+ channel, Kir 4.1 and Aquaporin 4. Further, the authors demonstrate that Kidins220/ARMS mediates its modulation of Ca2+ transients primarily through PLCgamma activation regardless of whether it is induced via full length TrkB receptor or the truncated TrkB receptor.

The observations are interesting and provide some novelty in our understanding of Kidins220/ARMS contribution to BDNF-mediated signaling in astrocytes. They provide an initial platform for further studies into more mechanistic aspects of Kidins220-mediated modulation of astrocyte function.

Comments for the author

I have some questions relating to the data presented.

Fig. 1A – in panel C, it appears to me that both 5min and 30 minute points are quite different between control and Kidins220-/- BDNF treated cultures, but apparently significance is only reached at 30 minutes. Is this because the intensity was summed up for both P-MAPK bands? How do levels of P-MAPK look at later time points? Does activation of MAPK or any other components ever reach a level seen in controls? In other words, is this a question of kinetics of activation response in KO cultures or is the magnitude of the response affected?

Fig. 2B - the authors state that there is time dependence in of MAPK phosphorylation in control and Kidins220-/- cultures (right hand side graphs?), but I don't see where the two sets are shown. It appears that these measurements were done in control cultures only in this experiment.
I am intrigued by the developmental switch in astrocyte responsiveness to BDNF and its modulation by Kidins220. It is interesting that the embryonic astrocytes express higher levels of TrkB-FL and activate the canonical RTK signalling cascade whereas the predominant form found in postnatal astrocytes is the truncated version of TrkB and the response elicited is the induction in Ca2+ transients. Is there a switch in the predominant form of metabolism in these cells during development? Do they switch from primarily glycolytic metabolism to OxPhos as they mature? Is the induction of Ca2+ transients potentially related to such a switch? Is anything known about Kidins220 function and cellular metabolism? Given that mutations in Kidins220 are associated with quite severe neurological disorders, many of which are linked to dysfunctions in mitochondrial metabolism, it would be an interesting aspect of Kidins220's contribution to astrocytic responsiveness to BDNF at different stages of development. I would welcome more discussion on such possibilities.

At the moment, the manuscript provides and initial characterisation of Kidins220's role in modulating BDNF responsiveness by different isoforms of TrkB receptor in astrocytes. While it is important, I do not feel that at this stage the manuscript provides enough mechanistic insight into how Kidins220 modulates BDNF responsiveness in the developing astrocytes to warrant its publication in JCS.

First revision

Author response to reviewers' comments

Reviewer 1

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Reviewer 1 Comments for the Author:
The manuscript is clearly written, logically structured, the figures data, and statistics are of high quality and robust. The discussion and conclusions are balanced.

We thank the Reviewer for the positive comments on our manuscript and for the constructive

criticism. We have performed additional experiments and hope that the revised version of our work is now considered suitable for publication in JCS.

My suggestions are:
1. I found the significance stars got lost amongst the data points in some of the figures. I suggest that you place them level with the top of the Y-axis.

We followed this advice and modified accordingly all figures.

2. In fig 2, the plot of the ratio FL: T1 was hard for me to dissect. The ratio is normalized to 1, but in actual fact it more likely to be 1:10 albeit perhaps 1:20 for the postnatal cells. I feel it would be more obvious if the actual ratios were presented, or provided in the text.

In Fig. 1A, Fig. 2A, Fig. 3A we now show the FL:T1 ratio with real values, in separate graphs.

3. Fig 6 does not have a role for Kir4.1, presumably because the relationship is correlative at this stage. If the BDNF-triggered calcium flux was found to be dependent on Kir4.1 (as well as Kidins), this would boost the novelty and significance of the manuscript, as it provides a mechanism.

The reduced expression of Kir4.1 in the absence of Kidins220 is indeed an interesting finding. In the revised version of the manuscript, we expanded the qRT-PCR results with western blotting analysis, which confirmed the reduction of Kir4.1 protein in Kidins220$^{lox/lox\text{-}Cre}$ samples (new Fig. 5B). We would rather not add Kir4.1 to the model (now in Fig. 6B) because, as the Reviewer rightly says, findings at this stage are still correlative and more work is needed to address the functional relevance of Kir4.1 reduction in KO cells, which however goes beyond the scope of the present work.
For what concerns the functional interaction between Kir4.1 and BDNF, there is limited evidence that Kir4.1 channels mediate Ca^{2+} currents at low (<2 mM) extracellular K^+ (Hartel et al., 2007), however under our experimental conditions this is unlikely, as the extracellular K^+ concentration in the recording buffer during Ca^{2+} imaging is 3 mM. On the other hand, recent evidence demonstrates that downregulation/inhibition of Kir4.1 in astrocytes induces BDNF expression through MEK1/2 signaling (Kinboshi et al., 2017). Thus, albeit there are data supporting a functional link between Kir4.1 and BDNF, we would exclude a direct involvement of Kir4.1 in the observed BDNF-mediated Ca^{2+} transients.
Of note, the model in Fig. 6B has been updated to indicate a shift in metabolism from embryonic to postnatal astrocytes, which leads to increased production of lactate (see also the answer to the last point raised by Reviewer#2).

4. The expression and the possible role of p75 in embryonic and postnatal astrocytes is not considered in the reported experiments. It is appreciated that the study has focused on the Trk receptor, but given Kidins can also interact with p75 it may have a role that should, at least, be discussed. P75 can regulate PIP2 and PTEN levels, and PIP2 is an important activator of Kir4.1. p75 has been reported to regulate Kir3/GIRK channels via this mechanism in neurons, with expression regulated by neuronal activity.

Following the Reviewer's suggestion, we studied p75 expression, and how it is affected by the lack of Kidins220, in embryonic and postnatal cells; these data are shown in new Figure S4. We first checked p75 expression in WT and KO embryonic cells and found comparable levels of the protein (**Fig. S1A**). We subsequently compared p75 levels in embryonic and postnatal wild type cells and found no differences linked to the developmental stage (**Fig. S1B**). In this respect, it is interesting to note that p75 behaves differently compared to TrkB-f.l., whose expression declines in postnatal astrocytes (**Fig. 2A**). Finally, we compared p75 levels in postnatal cells in the presence and absence of Kidins220, and again found no appreciable differences in receptor expression (**Fig. S1C**). These results are commented in the relevant sections of the Results. Reference to p75 has been made also in the introduction (p.4) and discussion (p.10).
As mentioned by the Reviewer, besides the well-studied interaction with Trk receptors, Kidins220 interacts also with p75 and therefore alterations of the p75 system are expected in Kidins220 KO cells. Albeit we cannot exclude that Kidins220 does modulate the expression and signaling

properties of astrocyte-expressed p75 in vivo, our data seem to exclude a prominent role of Kidins220 on this pathway in our in vitro experimental system.

5. Please also note/clarify whether Kir4.1 is a homodimer channel or the expression is of the 4.1 subunit that requires another subunit to be an active channel in astrocytes.

In astrocytes, Kir4.1 channels exist both as homo-tetramers of Kir4.1 subunits and as hetero-tetramers of Kir4.1-Kir5.1 subunits (Hibino et al., 2004; Patterson et al., 2021). In our RT-qPCR and WB analysis we quantify the monomer Kir4.1, which likely reflects the abundance of both homo- and hetero-tetramers. This is now mentioned in the discussion (p. 11).

Reviewer 2
Advance Summary and Potential Significance to Field:
The manuscript investigates the responsiveness of embryonic and postnatal astrocytes in vitro to BDNF in Kidins220/ARMS-dependent manner. The authors demonstrate that there are differences in astrocyte responsiveness to BDNF depending on the stage of development - embryonic ones elicit predominantly TrkB RTK-mediated phosphorylation response, whereas postnatal astrocytes respond to BDNF by eliciting Ca2+ transients primarily via truncated form of TrkB receptor. Moreover, these responses are potentiated by Kidins220/ARMS as demonstrated by the use of knock-out cultures in these experiments. In addition, the authors show that Kidins220/ARMS controls the expression of astrocyte-specific genes, e.g., inward rectifier K+ channel, Kir 4.1 and Aquaporin 4. Further, the authors demonstrate that Kidins220/ARMS mediates its modulation of Ca2+ transients primarily through PLCgamma activation regardless of whether it is induced via full length TrkB receptor or the truncated TrkB receptor.
The observations are interesting and provide some novelty in our understanding of Kidins220/ARMS contribution to BDNF-mediated signaling in astrocytes. They provide an initial platform for further studies into more mechanistic aspects of Kidins220-mediated modulation of astrocyte function.

We thank the Reviewer for the positive comments on our manuscript and for the constructive criticism. We have performed additional experiments and hope that the revised version of our work is now considered suitable for publication in JCS.

Reviewer 2 Comments for the Author:
I have some questions relating to the data presented.
Fig. 1A – in panel C, it appears to me that both 5min and 30 minute points are quite different between control and Kidins220-/- BDNF treated cultures, but apparently significance is only reached at 30 minutes. Is this because the intensity was summed up for both P-MAPK bands? How do levels of P-MAPK look at later time points? Does activation of MAPK or any other components ever reach a level seen in controls? In other words, is this a question of kinetics of activation response in KO cultures or is the magnitude of the response affected?

For quantification of MAPK phosphorylation levels we always summed the intensity of both bands, but results are the same when quantifying separately top and bottom bands. Indeed, in Figure 1C there is a trend reduction of P-MAPK at 5 min in KO vs WT cells, however the values are quite variable so statistical significance is not reached.
Following the Reviewer's suggestion, we performed BDNF stimulation in WT and KO cultures for longer times, i.e., 1 and 2 h, and found that at these time points both genotypes go back to control levels. Actually, phosphorylation of MAPK and Akt dropped to lower levels in KO samples compared to WT at 2 h (new **Fig. S2A** and main text p.5). Altogether, we conclude that in KO cells the magnitude of BDNF response is affected, not its kinetics.

Fig. 2B - the authors state that there is time dependence in of MAPK phosphorylation in control and Kidins220-/- cultures (right hand side graphs?), but I don't see where the two sets are shown. It appears that these measurements were done in control cultures only in this experiment.

Indeed, the experiments were performed only in wild type cells. We clarify this better in the main text (p. 6).

I am intrigued by the developmental switch in astrocyte responsiveness to BDNF and its modulation by Kidins220. It is interesting that the embryonic astrocytes express higher levels of

TrkB-FL and activate the canonical RTK signaling cascade whereas the predominant form found in postnatal astrocytes is the truncated version of TrkB and the response elicited is the induction in Ca^{2+} transients. Is there a switch in the predominant form of metabolism in these cells during development? Do they switch from primarily glycolytic metabolism to OxPhos as they mature? Is the induction of Ca^{2+} transients potentially related to such a switch? Is anything known about Kidins220 function and cellular metabolism? Given that mutations in Kidins220 are associated with quite severe neurological disorders, many of which are linked to dysfunctions in mitochondrial metabolism, it would be an interesting aspect of Kidins220's contribution to astrocytic responsiveness to BDNF at different stages of development. I would welcome more discussion on such possibilities.

We thank the Reviewer for this interesting suggestion. To assess the glycolytic vs OxPhos metabolism, we quantified the amount of lactate in the medium of embryonic and postnatal cultures, in the presence and absence of Kidins220 (new **Fig. 6A**). Interestingly, we found that:
(i) In WT postnatal cells, the production of lactate is higher compared to embryonic cells, suggesting a predominant glycolytic mechanism;
(ii) KO embryonic cells produce three times more lactate compared to WT, suggesting an impaired mitochondrial metabolism, while lactate levels are comparable to WT cells in the postnatal stage.
For what concerns WT cells, our findings are in line with evidence showing that in mature astrocytes the production of lactate through anaerobic glycolysis plays a predominant role in astrocyte metabolism (reviewed in (Magistretti and Allaman, 2015)).
For what concerns KO cells, the present findings, taken together with our previous data showing a reduction of secreted ATP in embryonic KO vs WT cells ((Jaudon et al., 2019) – Fig. 5C), suggest that in KO embryonic astrocytes the balance between glycolysis and OxPhos is switched toward lactate production, which could be indicative of mitochondrial dysfunctions. The mechanisms underlying this effect are presently unknown, and the role of Kidins220 in mitochondria physiopathology (and in general, in cellular metabolism) is completely unexplored. As suggested by the Reviewer, the induction and the modulation of Ca^{2+} transients could be a major player, especially given the strict crosstalk between ER and mitochondria in controlling intracellular Ca^{2+} homeostasis (Verkhratsky et al., 2018). We believe these data will be instrumental to better understand the pathogenic mechanisms underlying Kidins220-dependent neurodevelopmental diseases (i.e., SINO syndrome and associated pathologies). This is extremely interesting and is the object of our current investigations. We updated the model in Fig. 6B highlighting these new data. We also discuss this in the 'results' (p. 8) and 'discussion' (p. 11) sections.

At the moment, the manuscript provides and initial characterization of Kidins220's role in modulating BDNF responsiveness by different isoforms of TrkB receptor in astrocytes. While it is important, I do not feel that at this stage the manuscript provides enough mechanistic insight into how Kidins220 modulates BDNF responsiveness in the developing astrocytes to warrant its publication in JCS.

We hope that with the new data we have added, the manuscript is now considered suitable for publication in JCS.

References:

Hartel, K., K. Singaravelu, M. Kaiser, C. Neusch, S. Hulsmann, and J.W. Deitmer. 2007. Calcium influx mediated by the inwardly rectifying K+ channel Kir4.1 (KCNJ10) at low external K+ concentration. *Cell Calcium*. 42:271-280.

Hibino, H., A. Fujita, K. Iwai, M. Yamada, and Y. Kurachi. 2004. Differential assembly of inwardly rectifying K+ channel subunits, Kir4.1 and Kir5.1, in brain astrocytes. *J Biol Chem*. 279:44065-44073.

Jaudon, F., M. Chiacchiaretta, M. Albini, S. Ferroni, F. Benfenati, and F. Cesca. 2019. Kidins220/ARMS controls astrocyte calcium signaling and neuron-astrocyte communication. *Cell death and differentiation*.

Kinboshi, M., T. Mukai, Y. Nagao, Y. Matsuba, Y. Tsuji, S. Tanaka, K. Tokudome, S. Shimizu, H. Ito, A. Ikeda, A. Inanobe, Y. Kurachi, S. Inoue, and Y. Ohno. 2017. Inhibition of Inwardly Rectifying Potassium (Kir) 4.1 Channels Facilitates Brain-Derived Neurotrophic Factor (BDNF)

Expression in Astrocytes. *Front Mol Neurosci*. 10:408.
Magistretti, P.J., and I. Allaman. 2015. A cellular perspective on brain energy metabolism and functional imaging. *Neuron*. 86:883-901.
Patterson, K.C., U. Kahanovitch, C.M. Goncalves, J.J. Hablitz, A. Staruschenko, D.K. Mulkey, and M.L. Olsen. 2021. Kir 5.1-dependent CO2 /H(+) -sensitive currents contribute to astrocyte heterogeneity across brain regions. *Glia*. 69:310-325.
Verkhratsky, A., M. Trebak, F. Perocchi, D. Khananshvili, and I. Sekler. 2018. Crosslink between calcium and sodium signalling. *Exp Physiol*. 103:157-169.

Second decision letter

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AUTHORS: Fanny Jaudon, Martina Albini, Stefano Ferroni, Fabio Benfenati, and Fabrizia Cesca

ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.

Reviewer 1

Advance summary and potential significance to field

The authors have made excellent revisions to the manuscript in line with the suggestions by the reviewers. It will make an important contribution to the literature on astrocytes and neurotrophins in development as it highlights the signaling complexity and important role in metabolism in astrocytes in response to BDNF.

Comments for the author

My only remaining question/issue is regarding Fig 6 "Kidins220 contributes to the sustained activation of the MAPK pathway in embryonic cells, whereas it modulates Kidins220 BDNF-induced [Ca2+]i transients in postnatal cells" doesn't make sense (how can it modulate itself), and the diagram is not intuitive.
I'm not sure why Kidins is placed beside T1 where it is not having any effect in embryonic cells. Should the arrow to MAPk be from a kidins and TrkB FL interaction not just from FL; maybe a plus sign is required. On the RHS why is kidins below the curly bracket of lactate and glucose? What does the bracket signify?
In postnatal, kidins +T1 -> calcium transients - assisted by FL+Kidins, and resulting in gene transcription of K4.1?

Reviewer 2

Advance summary and potential significance to field

I like the manuscript now - it is interesting to see lactate measurement data and these observations will hopefully lead to a lot of new and exciting findings.

Comments for the author

See above.