

Contralesional hippocampal spreading depolarization promotes functional recovery after stroke

Corresponding Author: Dr Roger Thompson

This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The paper has much improved and I think that the findings are very interesting and important. However, I still have a number of comments.

„Unilateral hippocampal stroke resulted in atypical mouse behavior coincident with ipsilesional terminal depolarization (sustained increase in GCaMP6f fluorescence).“

The wording should be: „coincident with ipsilesional terminal spreading depolarization“. If the authors are convinced that this terminal depolarization is unique in that it is not a propagating wave, unlike the usual terminal (spreading) depolarization, then they have to prove this with measurements. Otherwise, it can be assumed that the terminal spreading depolarization behaves in the same way in the present model as it does in others, including, for example, human cardiocirculatory arrest (compare e.g. compare video 1 in (Zhao et al., 2021) or (Murphy et al., 2008) or (Farkas et al., 2010) or (Leão, 1947) or (Dreier et al., 2018)) etc. There are exceptions in the literature, (Menyhart et al., 2022) but in the referee's experience, one has to perform rather artificial experimental contortions to obtain non-spreading terminal depolarization. The depolarization that naturally occurs under ischemic conditions usually migrates in the tissue.

Page 3: „we show that unilateral photothrombosis triggered localized terminal depolarization“

Again, it should be: „we show that unilateral photothrombosis triggered localized terminal SD“.

„Results

117 Hippocampal ischemia-evoked terminal depolarization in awake behaving mice correlates with lesion size and increased open field activity“

Again, it should be: 117 Hippocampal ischemia-evoked terminal SD in awake behaving mice correlates with lesion size and increased open field activity“

„Elevated neuronal Ca²⁺ influx is a critical component of excitotoxic cell death and SD in the acute phase of an ischemic stroke“

It should be: „Elevated neuronal Ca²⁺ influx is a critical component of SD and the SD-initiated excitotoxic cell death in the acute phase of an ischemic stroke“

It has been shown by different groups that the massive Ca²⁺ influx follows the onset of SD. The massive glutamate release also follows the onset of SD. (Andrew et al., 2022; Enger et al., 2015; Hinzman et al., 2015).

„Neurons undergoing sustained depolarization in severely ischemic tissue will die within 20 - 30 min, in the absence of timely reperfusion.“

It should be: „Neurons undergoing sustained SD in severely ischemic tissue will die within 20 - 30 min, in the absence of timely reperfusion.“

„Given the persistent sustained increase in neuronal Ca²⁺ recorded through the ipsilesional fiber dorsal to the emerging core (> 30 min post-photothrombosis), we deemed this change in GCaMP6f intensity the terminal depolarization (TD).“

It should be: „Given the persistent sustained increase in neuronal Ca²⁺ recorded through the ipsilesional fiber dorsal to the emerging core (> 30 min post-photothrombosis), we deemed this change in GCaMP6f intensity the terminal SD (TSD).“

From this point on in the manuscript, the abbreviation „TD“ should always be „TSD“ and „terminal depolarization“ should be „terminal SD“. This also includes the figures etc. However, once an abbreviation has been introduced, it should be used. In some places below, „terminal depolarization“ is used. I would no longer write this out, but replace it with the abbreviation TSD throughout the manuscript. Regarding TSD, the full continuum of SD is observed in this single initial wave during focal

ischemia, and also in subsequent spontaneous SDs.(Dreier and Reiffurth, 2015; Hartings et al., 2017; Zhao et al., 2021) The continuum entails changing characteristics of the wave determined by the local conditions of the tissue. Most importantly, the duration of the depolarization and near-complete breakdown of ion homeostasis, as indicated by the negative DC shift, varies from persistent in the ischemic core to short-lasting in the periphery, since repolarization requires activation of energy-dependent membrane pumps such as Na,K-ATPases. Short-lasting DC shifts thus indicate enough ATP at the recording site to fuel repolarization. This feature renders the negative DC shift duration a useful measure for (i) the tissue energy status and (ii) the risk of injury (excitotoxicity) at the recording site.(Dreier et al., 2017; Somjen, 2001)

Line 318: „SD is one of several sequelae of ischemia72.“ However, SD is the characteristic change that immediately precedes the dying of the gray matter in ischemia. In other words, compared to all other processes, SD has a prominent position in the event of ischemia. It is not the case that ischemia immediately kills the neurons; rather, the tissue first falls into the SD state and only dies in the SD state after a substantial latency (compare video 1 in (Zhao et al., 2021) for the spread of TSD and to appreciate the SD continuum and figure 4 in (Dreier et al., 2024) for the initial reversibility of (T)SD over the first 15 minutes after the onset of focal ischemia). In addition, other phenomena such as an epileptic seizure may occur in ischemia. However, tissue can die en masse without an epileptic seizure having occurred, but it does not usually die en masse without an SD having occurred first. It does not do justice to the prominent role of SD in cell death in ischemia to write: "SD is one of several sequelae of ischemia." For example, one could write: "In addition to the prominent role of SD in the context of ischemia as an initially reversible state that can progress to cell death, there are also other electrophysiological phenomena associated with ischemia, albeit less frequently, such as epileptic seizures." Alternatively, the authors could find a shorter way of putting it, but it shouldn't come across as if, in ischemia, SD is just one of many phenomena. The SD continuum is the most prominent gray matter phenomenon in ischemia, with the other phenomena coming after it. Nevertheless, SD may have a Janus-faced role (foe or friend) and that is what this paper is about, as far as I understand it. In life in general, and especially in scientific articles, it is more difficult to look at things from both sides than just from one side, but many things and probably also SDs have two sides.

„Seizure and SD can co-occur in both rodents and humans49–54.“

These three studies could also be cited here: (Dreier et al., 2012; Foreman et al., 2022; Revankar et al., 2017)

„Intraluminal middle cerebral artery occlusion (MCAO) induces a large lesion that spreads primarily into superficial neocortex, yet reaches some deeper subcortical regions3, however MCAO requires anesthesia and is highly variable.“

Be cautious here. The structure most at risk after an MCAO is not the neocortex, but the striatum, which is therefore typically affected even when the neocortex shows no signs of infarction, for example in the case of a transient MCAO.

„The hippocampal SD induced hyperactivity observed here was distinct from acute hypo-activity that was triggered by photo-induced MCAO occlusion57.“

„hippocampal SD induced hyperactivity“ should be better explained.

„Increased freezing during the retrieval day (Day 2) correlated with contralesional SD amplitude but had no relationship to ipsilesional TD, contralesional SD in the intact hippocampus in maintaining hippocampal function after a focal unilateral insult.“

This sentence is too complicated. Simplify.

„In peri-infarct tissue, blood brain barrier disruption causes albumin to leak into the brain parenchyma, which can also activate astrocytic transforming growth receptor beta receptor 2 (TGFβR2) signaling, altering the astrocyte transcriptome to disrupt local [K+] and glutamate homeostasis and enhance seizure activity and epileptogenesis107,121,122.“

Better write: „blood-brain barrier dysfunction“ and not „disruption“.

„Ketamine, a less potent uncompetitive antagonist of NMDARs that shares a binding site with MK-801, can also suppress SDs while lowering the seizure threshold14.“

„while lowering the seizure threshold“ should be removed. This is a bit tricky. In the study of Schoknecht et al., the anti-seizure effect of ketamine was smaller than the anti-seizure effect of urethane in the other group. Urethane is a potent anti-seizure drug in animals (, but assumed to be cancerogenic in humans).(Heltovics et al., 1995) The study suggested that ketamine suppresses SDs and reduces the early damage compared with urethane anesthesia, but it did not imply that ketamine further promotes seizures induced by 4-aminopyridine. Ketamine is actually used successfully as a medication in the clinic, for example to treat super-refractory status epilepticus.(Alkhachroum et al., 2020)

„Ketamine inhibits SD at anesthetic doses in rodents124 and humans 134,135.“

The following studies could be added for humans: (Carlson et al., 2018; Santos et al., 2019)

Line 323: „In pharmacologically induced preclinical seizure models, SD could terminate focal seizure in the neocortex50,77.“

It has been known for a long time that epileptic seizures can induce SDs and that the electrographic seizure is thereby terminated because the tissue is then in the SD state.(Avoli et al., 1991; Hablitz and Heinemann, 1989; Mody et al., 1987)

Recently, this has been rediscovered. However, it should be kept in mind that the patterns seen when monitoring patients during neurocritical care are highly complex and that the anti-seizure effect is usually short-lived, if at all, and very localized (compare, for example, figure 1 in (Fabricius et al., 2008), figure 5 in (Revankar et al., 2017) and fig. 4 in (Foreman et al., 2022)). Electrographic seizures can also develop from SDs, or induce SDs and then become a seizure again (compare, for example, figure 1 and 2 (human) in (Dreier et al., 2012), figure 6 (human) in (Foreman et al., 2022) or figure 4 (rat) in (Lemale et al., 2022)).

For example, one could write here: "In pharmacologically induced preclinical seizure models, it has been shown that focal seizures can trigger a transient SD, which, after a phase of activity depression, can at least temporarily restore normal spontaneous activity." It should be mentioned somewhere, however, that the patterns of SDs and electrographic seizures in patients during neurocritical care are much more complex and that this is just one possible constellation.

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(Remarks on code availability)

Reviewer #2

(Remarks to the Author)

This is an overall technically excellent paper. It is rigorous, and multi-dimensional in its techniques. The nature of its general conclusions on stroke biology, and sex differences in delayed electrophysiological events after stroke, are limited because of the studies restricted to the hippocampus. This is for two reasons. In humans, stroke in the hippocampus is rare. Second, the findings of spreading depression and contralateral connectivity may derive from the unique structure of the hippocampus. However, the model that is developed and the application of the methods are important. The authors have responded to my comments and the same comments from Reviewer 3, with statements about posterior cerebral artery blood supply, and in separate statements, of their study of subcortical structures. I think that for readers, they need to note directly that hippocampal stroke in humans is rare, so that these readers can interpret the findings in the overall context of their translational applicability. However, the manuscript is a valuable addition to the field, and will provide other labs a model and a set of findings with which to undertake important, further observations.

For me, this is a good paper and would qualify for publication, with that additional note in the introduction or discussion section.

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)

The study by Boyce et al describes a series of well performed experiments where a photothrombotic stroke is induced in the hippocampus of awake mice, and the Ca dynamics within the injured and contralesional hippocampus are monitored through optic fibre implants. Additionally, behavioural deficits induced by the stroke are quantified and differences between males and females noted. Additional experiments were performed to record seizure activity in the hippocampus as well as widefield cortical Ca transients following the stroke. I have several suggestions for how the manuscript may be improved and noted where clarification is required.

Line 91: "we improved upon traditional focal stroke models.."

The study did not improve on an existing model but instead applied an established method to a different brain region. All statements suggesting that this method is an improvement in stroke modeling should be removed from the manuscript as this was not the intention of the study. If really improvement was the intention there could have been much better decisions made about the location and invasiveness of the current study.

Line 221: "thus whether hippocampal ischemia could induce immediate hyperactivity was unknown"

- There are a series of experiments from Pulsinelli and others that reported hyperactivity when forebrain ischemia was induced in unanesthetized rats that should be cited here. (eg. A new model of bilateral hemispheric ischemia in the unanesthetized rat W A Pulsinelli, J B Brierley. Forebrain ischemia in the rat. Relation between duration of ischemia, use of adjunctive ganglionic blockade and long-term recovery B Grøgaard, B Gerdin, K E Arfors)

Does contralesional SD actually stop seizures as indicated in concluding sentence in intro and beginning of discussion?

The support for this statement comes from lines 339 in results section and Fig 4K: "Seizures directly preceding SD were longer than those that did not precede SD (Fig4k)". This does not however indicate that "These waves terminated seizures" as is stated in the discussion. Fig 4K is difficult to interpret and does not actually statistically assess whether SD's shortened seizure duration, but only shows a correlation between LFPs and SD latency.

The number of mice in the seizure detection experiments is low. Only 4 mice in total are reported. This should be reflected in the discussion, or repeated in more animals.

Sentence on line 352 needs to be simplified, restructured as it is not clear in current state: "Surprisingly, electrographic seizure activity that initiated an SD in the contralesional hippocampus preceded seizure observed in the ipsilesional hippocampus (Fig. 5b)"

Line 384 states: "Additionally, slow and small amplitude GCaMP6f transients ($\Delta F/F \gg 10$ a.u.) were observed in contralesional neocortical structures; yet they followed the contralesional hippocampal SD, rather than preceding it (Fig. 5g)." However if the hippocampal and cortical traces are aligned correctly in 5G, the cortical transient seems to precede the HPC recorded event???

Line 83.. in the EVENT of adequate perfusion..., not advent
Change homotopic instead of homotypic throughout manuscript.

(Remarks on code availability)

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We would like to thank all three reviewers for their contributions to improving this study. We have addressed their suggestions point-by-point in bold italic font below.

Reviewer #1 (Remarks to the Author):

The paper has much improved and I think that the findings are very interesting and important. However, I still have a number of comments.

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It should be: „Elevated neuronal Ca²⁺ influx is a critical component of SD and the SD-initiated excitotoxic cell death in the acute phase of an ischemic stroke“

This was updated in the revised manuscript (page 3, line 116-118)

It has been shown by different groups that the massive Ca²⁺ influx follows the onset of SD. The massive glutamate release also follows the onset of SD. (Andrew et al., 2022; Enger et al., 2015; Hinzman et al., 2015).

These references were included with the above changes in the manuscript.

„Neurons undergoing sustained depolarization in severely ischemic tissue will die within 20 - 30 min, in the absence of timely reperfusion.“

It should be: „Neurons undergoing sustained SD in severely ischemic tissue will die within 20 - 30 min, in the absence of timely reperfusion.“

Updated in the revised manuscript (lines 142-143).

„Given the persistent sustained increase in neuronal Ca²⁺ recorded through the

ipsilesional fiber dorsal to the emerging core (> 30 min post-photothrombosis), we deemed this change in GCaMP6f intensity the terminal depolarization (TD).“

It should be: „Given the persistent sustained increase in neuronal Ca²⁺ recorded through the ipsilesional fiber dorsal to the emerging core (> 30 min post-photothrombosis), we deemed this change in GCaMP6f intensity the terminal SD (TSD).“

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We thank the reviewer for their detailed criticism and for highlighting this opportunity to improve our description. Terminal depolarization has been updated to terminal spreading depolarization (or TSD) where applicable in the updated manuscript.

Line 318: „SD is one of several sequelae of ischemia72.“ However, SD is the characteristic change that immediately precedes the dying of the gray matter in ischemia. In other words, compared to all other processes, SD has a prominent position in the event of ischemia. It is not the case that ischemia immediately kills the neurons; rather, the tissue first falls into the SD state and only dies in the SD state after a substantial latency (compare video 1 in (Zhao et al., 2021) for the spread of TSD and to appreciate the SD continuum and figure 4 in (Dreier et al., 2024) for the initial reversibility of (T)SD over the first 15 minutes after the onset of focal ischemia). In addition, other phenomena such as an epileptic seizure may occur in ischemia. However, tissue can die en masse without an epileptic seizure having occurred, but it does not usually die en masse without an SD having occurred first. It does not do justice to the prominent role of SD in cell death in ischemia to write: "SD is one of several sequelae of ischemia." For example, one could write: “In addition to the prominent role of SD in the context of ischemia as an initially reversible state that can progress to cell death, there are also other electrophysiological phenomena associated with ischemia, albeit less frequently, such as epileptic seizures.” Alternatively, the authors could find a shorter way of putting it, but it shouldn't come across as if, in ischemia, SD is just one of many phenomena. The SD continuum is the most prominent gray matter phenomenon in ischemia, with the other phenomena coming after it. Nevertheless, SD may have a Janus-

faced role (foe or friend) and that is what this paper is about, as far as I understand it. In life in general, and especially in scientific articles, it is more difficult to look at things from both sides than just from one side, but many things and probably also SDs have two sides.

This introduction has been modified to the reviewers suggestions in the updated manuscript: “In addition to the prominent role of SD in ischemic tissue, ischemia can evoke several other electrophysiological phenomena including epileptic seizures⁷⁷.” (Line 318, page 11).

„Seizure and SD can co-occur in both rodents and humans^{49–54}.“

These three studies could also be cited here: (Dreier et al., 2012; Foreman et al., 2022; Revankar et al., 2017)

These references have been added to the text (lines 323-324).

„Intraluminal middle cerebral artery occlusion (MCAO) induces a large lesion that spreads primarily into superficial neocortex, yet reaches some deeper subcortical regions³, however MCAO requires anesthesia and is highly variable.“

Be cautious here. The structure most at risk after an MCAO is not the neocortex, but the striatum, which is therefore typically affected even when the neocortex shows no signs of infarction, for example in the case of a transient MCAO.

„The hippocampal SD induced hyperactivity observed here was distinct from acute hypo-activity that was triggered by photo-induced MCAO occlusion⁵⁷.“

„hippocampal SD induced hyperactivity“ should be better explained.

This sentence has been expanded to clarify what is meant by hyperactivity, describing an increase in ambulation and rearing behaviour linked to the onset of hippocampal SD (pages 17-18, lines 442-444).

„Increased freezing during the retrieval day (Day 2) correlated with contralesional SD amplitude but had no relationship to ipsilesional TD, contralesional SD in the intact hippocampus in maintaining hippocampal function after a focal unilateral insult.“

This sentence is too complicated. Simplify.

This sentence was simplified and split into two sentences. “Increased freezing during the retrieval day (Day 2) correlated with contralesional SD amplitude but had no relationship to ipsilesional TSD. This suggests contralesional SD in the intact hippocampus plays a unique role in maintaining hippocampal function after a focal unilateral insult.” (see page 19, lines 517-521).

„In peri-infarct tissue, blood brain barrier disruption causes albumin to leak into the brain parenchyma, which can also activate astrocytic transforming growth receptor beta receptor 2 (TGFBR2) signaling, altering the astrocyte transcriptome to disrupt local [K⁺] and glutamate homeostasis and enhance seizure activity and epileptogenesis^{107,121,122}.“

Better write: „blood-brain barrier dysfunction“ and not „disruption“.

This was addressed in the updated manuscript.

„Ketamine, a less potent uncompetitive antagonist of NMDARs that shares a binding site with MK-801, can also suppress SDs while lowering the seizure threshold¹⁴.“

„while lowering the seizure threshold“ should be removed. This is a bit tricky. In the study of Schoknecht et al., the anti-seizure effect of ketamine was smaller than the anti-seizure effect of urethane in the other group. Urethane is a potent anti-seizure drug in animals (, but assumed to be cancerogenic in humans).(Heltovics et al., 1995) The study suggested that ketamine suppresses SDs and reduces the early damage compared with urethane anesthesia, but it did not imply that ketamine further promotes seizures induced by 4-aminopyridine. Ketamine is actually used successfully as a medication in the clinic, for example to treat super-refractory status epilepticus.(Alkhachroum et al., 2020)

This is resolved in the manuscript, where the suggested omission was made (page 21, lines 592-594).

„Ketamine inhibits SD at anesthetic doses in rodents¹²⁴ and humans ^{134,135}.“

The following studies could be added for humans: (Carlson et al., 2018; Santos et al., 2019)

The suggested studies were added (page 21, line 597).

Line 323: „In pharmacologically induced preclinical seizure models, SD could terminate focal seizure in the neocortex^{50,77}.“

It has been known for a long time that epileptic seizures can induce SDs and that the electrographic seizure is thereby terminated because the tissue is then in the SD state.(Avoli et al., 1991; Hablitz and Heinemann, 1989; Mody et al., 1987) Recently, this has been rediscovered. However, it should be kept in mind that the patterns seen when monitoring patients during neurocritical care are highly complex and that the anti-seizure effect is usually short-lived, if at all, and very localized (compare, for example, figure 1 in (Fabricius et al., 2008), figure 5 in (Revankar et al., 2017) and fig. 4 in (Foreman et al., 2022)). Electrographic seizures can also develop from SDs, or induce SDs and then become a seizure again (compare, for example, figure 1 and 2 (human) in (Dreier et al., 2012), figure 6 (human) in (Foreman et al., 2022) or figure 4 (rat) in (Lemale et al., 2022)). For example, one could write here: “In pharmacologically induced preclinical seizure models, it has been shown that focal seizures can trigger a transient SD, which, after a phase of activity depression, can at least temporarily restore normal spontaneous activity.“ It should be mentioned somewhere, however, that the patterns of SDs and electrographic seizures in patients during neurocritical care are much more complex and that this is just one possible constellation.

Both points are addressed in the revised text (see lines 323-327, page 11-12).

References

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Reviewer #2 (Remarks to the Author):

This is an overall technically excellent paper. It is rigorous, and multi-dimensional in its techniques. The nature of its general conclusions on stroke biology, and sex differences in delayed electrophysiological events after stroke, are limited because of the studies restricted to the hippocampus. This is for two reasons. In humans, stroke in the hippocampus is rare. Second, the findings of spreading depression and contralateral connectivity may derive from the unique structure of the hippocampus.

However, the model that is developed and the application of the methods are important. The authors have responded to my comments and the same comments from Reviewer 3, with statements about posterior cerebral artery blood supply, and in separate statements, of their study of subcortical structures. I think that for readers, they need to note directly that hippocampal stroke in humans is rare, so that these readers can interpret the findings

in the overall context of their translational applicability. However, the manuscript is a valuable addition to the field, and will provide other labs a model and a set of findings with which to undertake important, further observations.

For me, this is a good paper and would qualify for publication, with that additional note in the introduction or discussion section.

We thank the reviewer for these suggestions. We have now acknowledged the relatively rarity of hippocampal strokes in humans in the discussion. While hippocampal stroke is rare in humans, there is a recent preprint (Mitlasoczki et al., bioRxiv, October 12th 2024) posted identifying hippocampal seizure-induced SD as a trigger to post-ictal wandering in viral encephalitis (a murine model for temporal lobe epilepsy) and in humans with refractory focal epilepsy with a history of post-ictal wandering, suggesting our findings might have a wide-reaching impact outside of stroke. This is described in the discussion, page 18, lines 469-475.

Reviewer #3 (Remarks to the Author):

The study by Boyce et al describes a series of well performed experiments where a photothrombotic stroke is induced in the hippocampus of awake mice, and the Ca dynamics within the injured and contralesional hippocampus are monitored through optic fibre implants. Additionally, behavioural deficits induced by the stroke are quantified and differences between males and females noted. Additional experiments were performed to record seizure activity in the hippocampus as well as widefield cortical Ca transients following the stroke. I have several suggestions for how the manuscript may be improved and noted where clarification is required.

Line 91: “we improved upon traditional focal stroke models..”

The study did not improve on an existing model but instead applied an established method to a different brain region. All statements suggesting that this method is an improvement in stroke modeling should be removed from the manuscript as this was not the intention of the study. If really improvement was the intention there could have been much better decisions made about the location and invasiveness of the current study.

This introductory sentence was modified to remove mentions to improvement. Any other similar language was also removed in subsequent sections.

Line 221: “thus whether hippocampal ischemia could induce immediate hyperactivity was unknown”

- There are a series of experiments from Pulsinelli and others that reported hyperactivity when forebrain ischemia was induced in unanesthetized rats that should be cited here. (eg. A new model of bilateral hemispheric ischemia in the unanesthetized rat W A Pulsinelli, J B Brierley. Forebrain ischemia in the rat. Relation between duration of ischemia, use of adjunctive ganglionic blockade and long-term recovery B Grøgaard, B Gerdin, K E Arfors)

These references have been included and the text updated to reflect the contribution of these studies: “While there was evidence that forebrain ischemia in unanesthetized

rats could trigger hyperexcitability and behavioral seizures^{69,70}, it was unclear whether focal hippocampal ischemia would induce hyperactivity.”(page 7, lines 218-220).

Does contralesional SD actually stop seizures as indicated in concluding sentence in intro and beginning of discussion?

The support for this statement comes from lines 339 in results section and Fig 4K:

“Seizures directly preceding SD were longer than those that did not precede SD (Fig4k)”.

This does not however indicate that “These waves terminated seizures” as is stated in the discussion. Fig 4K is difficult to interpret and does not actually statistically assess whether SD’s shortened seizure duration, but only shows a correlation between LFPs and SD latency.

Thank you for this suggestion. The language mentioned here has been modified to “these waves followed seizures”

The number of mice in the seizure detection experiments is low. Only 4 mice in total are reported. This should be reflected in the discussion, or repeated in more animals.

The number of mice analysed in each experiment where seizure and SD are detected (n = 5 in Figure 4 and n = 4 in Figure 5) is now mentioned in the discussion. Page 20, lines 547, 548.

Sentence on line 352 needs to be simplified, restructured as it is not clear in current state: “Surprisingly, electrographic seizure activity that initiated an SD in the contralesional hippocampus preceded seizure observed in the ipsilesional hippocampus (Fig. 5b)”

Thank you for identifying this weakness, we have now moved this sentence to follow the description of the analyses and reworded it to: “In each of the bilateral LFP recording where contralesional hippocampal seizure was immediately followed by an SD, suprathreshold rhythmic spiking appeared first in the contralesional LFP before appearing in the ipsilesional LFP (Fig. 5b).” Page 13, line 358-360.

Line 384 states: “Additionally, slow and small amplitude GCaMP6f transients ($\Delta F/F \gg 10$ a.u.) were observed in contralesional neocortical structures; yet they followed the contralesional hippocampal SD, rather than preceding it (Fig. 5g).” However if the hippocampal and cortical traces are aligned correctly in 5G, the cortical transient seems to precede the HPC recorded event???

We have added some clarifying details to this section and included an additional Extended Data Figure (Ext. Data Fig. 10). Firstly, it is true that rhythmic seizure-like GCaMP dynamics appear concurrently with similar activity in the hippocampus, however there is no evidence of pan-neuronal depolarization, nor the subsequent dip in GCaMP below baseline, that would occur during an SD. Instead, there is a transient increase in that is 5-10% of the amplitude of an SD. This suggests that while the seizure may be propagating to nearby cortical brain areas, the SD is not. We have updated the text to better explain these observations: “Rhythmic seizure-like GCaMP6f dynamics were observed in contralesional mesoscale recordings from nearby cortical

brain areas. This suggests that the seizures preceding hippocampal SD could be propagating into neocortex. Given that SD involves pan-neuronal depolarization²⁰, the accompanying slow and small amplitude GCaMP6f transients ($\Delta F/F \approx 10$ a.u.) in contralesional neocortical structures were not indicative of SD entering these structures (Extended Data Fig. 10). Here, the increase in GCaMP6f was between 5-10% of what was observed during cortical SD in these same structures after cortical photothrombosis (Extended Data Fig. 10).” Page 15, lines 385-392.

Line 83.. in the EVENT of adequate perfusion..., not advent
Change homotopic instead of homotypic throughout manuscript.
This has been resolved in the revised manuscript.