JOURNAL OF Neuroscience Research

Mimicking Partial to Total Placental Insufficiency in a Rabbit Model of Cerebral Palsy

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Editor 1: Eric Prager
Editor 2: Cristina Ghiani
Editor 3: David McArthur
Reviewer 1: Katharina Quinlan
Reviewer 2: Steven Levison

1st Editorial Decision

Decision letter
Dear Dr Shi:

Thank you for submitting your manuscript to the Journal of Neuroscience Research.

Your manuscript has been reviewed by the editors, and we have found a series of problems and concerns, importantly, we feel that your work is not ready for publication and is missing some important data and controls for full validation of the new model. We regret to disappoint you but we feel that in the present form the manuscript is not ready for peer-review.

If you feel that you can adequately address the concerns of the reviewers, you may revise and resubmit your paper within 90 days. It will require further review by the editors. Please explain in your cover letter how you have changed the present version. If you require longer than 90 days to make the revisions, please contact Dr Cristina Ghiani (cghiani@mednet.ucla.edu). To submit your revised manuscript: Log in by clicking on the link below https://wiley.atyponrex.com/submissionBoard/1/13cb2e83-f7c5-4053-88cf-c6cf5bd0bc89/current

(If the above link space is blank, it is because you submitted your original manuscript through our old submission site. Therefore, to return your revision, please go to our new submission site here (submission.wiley.com/jnr) and submit your revision as a new manuscript; answer yes to the question "Are you returning a revision for a manuscript originally submitted to our former submission site (ScholarOne Manuscripts)? If you indicate yes, please enter your original manuscript's Manuscript ID number in the space below" and including your original submission's Manuscript ID number (jnr-2021-Jan-9435) where indicated. This will help us to link your revision to your original submission.)

Thank you again for your submission to the Journal of Neuroscience Research; we look forward to reading your revised manuscript.

Best Wishes,

Dr Eric Prager
Associate Editor, Journal of Neuroscience Research

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Dr Cristina Ghiani
Editor-in-Chief, Journal of Neuroscience Research

Associate Editor: Prager, Eric
Comments to the Author:
This study develops a new rabbit model mimicking the partial-to-total placental insufficiency and compared it with previous models of total uterine ischemia. The introduction has a very important statement describing the development of a model of CP after HI at preterm gestation but note that they were not sure it mimicked the actual clinical course, specifically as it relates to an immediate and complete cessation of blood supply to the uterus. Therefore, developing a new model that is more translational, in that there is a gradual increase in ischemia is needed. Critically, they note that the new model was compared to the entire dataset of experiments done since 2004/2005 when the model was first characterized. While the potential to publish this paper is there, there are a lot of critical errors throughout the manuscript that must be addressed prior to review. My comments are below and did not include the additional comments from the EiC:

1) In general, what are the randomization and blinding procedures?
2) What is the rationale for developing a partial abruption vs. total? This is necessary in the abstract, which should also be reduced in size and only the most critical information presented
3) Why in rabbits? Is this a normal model?
4) While Kendall et al., 2019 provide important information about the 3Rs and reducing animal numbers, I am concerned about the conditions in which the study took place. Were the housing/husbandry conditions the same, how did they combine datasets from previous studies? What were the procedures for that and what were the inclusion/exclusion criteria? What studies specifically did they use data from?
5) What were the housing/husbandry procedures for the current animals used in this study?
6) How many litters, from how many females? What were the breeding procedures?
7) The experimental groups appear to have only consisted of Full H-I and Partial+Full H-I. Why is there not a partial H-I only group and a naïve control group? These should be included. Also, why did they choose a partial+full H-I? Where is the rationale for this? How did they determine the timeframe for balloon inflation?
8) If you used Full-HI from previous studies, then this study only used the partial-full. This is a critical error since you do not have a new control to compare. While it is fine to use previous datasets, you also need to collect a new set of data to ensure that the outcomes will be the same as previously found.
9) The authors note that with the partial H-I, there was low BP to "about half of that before inflation". Do the authors have data to demonstrate that it was half and how consistent were they? Were any animals from this group excluded?
10) How many animals did you start with and how did you determine the sample size? How many died and how did that change the power?
11) What were the "minor revisions'' for the neurobehavioral assessments
12) Don't use the word gender, use sex, but how many of each sex were used?
13) How did authors determine the brain regions to assess?
14) Student's T-tests are likely incorrect since the sample sizes are so different. This will be a violation of the assumption of normality. I recommend that all data are assessed by a biostatistician, especially to compare the differences in power. Along those lines, the authors need to assess for differences between the groups of data collected from different studies.
15) Correlational analyses were not described in the statistical approaches section
16) Fig 1 appears as a longitudinal design, when it in fact is not. This figure needs to be redone. Again discuss with a biostatistician, but given that this is a 3x2 between subject design, the figures and data will need to be revised accordingly.
17) Fig 2 doesn't have error bars. Also, do not use bar graphs. Please also check the organization of the graphs. They do not appear to align with the figure legends.

Overall, I cannot allow this paper to go for review yet. There are a lot of factors that concerned me, especially with the experimental design and the fact that no additional controls were assessed throughout. I also did not see data regarding the partial HI to confirm that it was indeed partial. I highly recommend a biostatistician review all this information. In addition, because you are comparing a new model to an already existing one, you will need to discuss this in significantly more detail. I believe that if you can take care of all the comments, then the paper will be sufficiently improved to send out for review.

Additional Comments:

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To echo Dr Prager's comments, I am not fully convinced by the rationale and value for developing a partial + full model vs or in addition to a partial only, as the authors state "Uteroplacental insufficiency states also may take time to develop. " hence, a partial model would be valuable to investigate a milder insult that may trigger similar outcomes in the offspring, Since a partial ischemia can eventually trigger and result in a total uteroplacental insufficiency.

Since this is a new model, a more extensive description and study of the effects on brain structures should be provided, the manuscript completely lacks of any histomorphological characterization. A correlation between the neurobehavioral deficits and brain regions abnormalities should be added, the data in figure 7 are not sufficient.

Furthermore, since these kits were delivered by c-section, could the authors determine if they presented with any neurobehavioural deficit, so that the loss in cell viability can be correlated with normal, mild or severe deficits?

Brain weights should be supplied as well as kits' weights at various ages, especially at birth and then when the neurobehavioural deficits become evident. The only data available are on acute brain cell viability after the H-I insult, figure 7.

The methods needs extensive re-writing as they are not clear and there are missing details.
How many kits did on average each dam carried/give birth to?

Gender and Sex are not synonym, but have a very different meaning. Sex should be used as the authors are describing biological differences. The lack of sex differences is surprising, as I would have expected that females might be more protected.
The tables should include data for the females as well.

Authors' Response

Comments to the Author:
This study develops a new rabbit model mimicking the partial-to-total placental insufficiency and compared
it with previous models of total uterine ischemia. The introduction has a very important statement describing
the development of a model of CP after HI at preterm gestation but note that they were not sure it mimicked
the actual clinical course, specifically as it relates to an immediate and complete cessation of blood supply
to the uterus. Therefore, developing a new model that is more translational, in that there is a gradual
increase in ischemia is needed. Critically, they note that the new model was compared to the entire dataset
of experiments done since 2004/2005 when the model was first characterized. While the potential to publish
this paper is there, there are a lot of critical errors throughout the manuscript that must be addressed prior
to review. My comments are below and did not include the additional comments from the EiC:
1) In general, what are the randomization and blinding procedures?
The study represents accumulated data from the beginning of the experiments in order to get enough
numbers to reach statistical power. Full H-I numbers have been supplemented from historical data. When
the Partial+Full H-I was started, we compared it to block randomized Full H-I done at the same time in a
ratio of 1:2. But, for the overall study, there is no true randomization because of the addition of historical

controls for Full H-I. We have now included a figure showing how many dams (litters) are divided between
the Same Time Period and Historical Data.
2) What is the rationale for developing a partial abruption vs. total? This is necessary in the abstract, which
should also be reduced in size and only the most critical information presented
All available models of H-I in animals that use a single insult consist of a sudden onset of 100% anoxia to
the fetal brain. In real life, these cases are rare and even rarer with placental abruption, which is the clinical
entity that our model mimics. All cases of total placental abruption start off with partial abruption and change
over time to total abruption. Thus, the rationale for developing a new model was to study the transition from
partial to total abruption. The abstract has been revised and reduced.
3) Why in rabbits? Is this a normal model?
Translational rationale for our rabbit models – motor development:
Rabbits are **peri**natal motor developers akin to humans (Harel et al., 1978). Naïve rodent motor development starts only in the **post**natal period. In rodents, there is a lack of a behavioral phenotype
resembling CP in diverse injury models (H-I, inflammation, trauma, chemical, radiation injury). Pig and nonhuman
primates are also inappropriate for the study of perinatal origins of cerebral palsy (CP) because their
motor development is **pre**natal and is almost complete at birth, unlike humans. The timeline of brain growth
illustrates the difference between mammals that are prenatal, postnatal, and perinatal (Fig 1R). Development of the oligodendrocyte progenitor cells (PreOLs) also illustrates the perinatal similarity of the
rabbit to humans (Fig 2R).
Translational rationale – modeling placental abruption:
The uterine ischemia model results in global fetal hypoxia similar to acute placental insufficiency states in
humans, e.g., akin to that found in placental abruption **(Derrick, Drobyshevsky, Ji, & Tan, 2007; Derrick
et al., 2004; Tan et al., 2005; Tan et al., 1998, 1999)**. Placental abruption results in a higher incidence of
death and adverse neurobehavioral outcomes in humans (Ananth, Berkowitz, Savitz, & Lapinski, 1999;
Gilbert, Jacoby, Xing, Danielsen, & Smith, 2010; Kayani, Walkinshaw, & Preston, 2003; Logitharajah,
Rutherford, & Cowan, 2009; Matsuda, Maeda, & Kouno, 2003). Uterine ischemia results in global hypoxia to
the fetus. Almost immediately, a combined hypoxia and ischemic insult occurs in the fetal brain **(Derrick et
al., 2004)**. Hypertonia was observed in ~80% of the newborn survivors after 40 min H-I at E22 and was later
shown to be associated with white matter injury **(Drobyshevsky, Derrick, Wyrwicz, et al., 2007;**

Drobyshevsky, Jiang, Derrick, Luo, & Tan, 2014; Drobyshevsky, Jiang, Lin, et al., 2014). There are
many advantages of our rabbit model over rodents; see Table 1R.
Fig 1R. Prenatal brain growth in pig and monkey**, Postnatal** in rat and **Perinatal**
in rabbit and human.
Fig 2R. Oligodendrocyte progenitor cell.
Rabbits are much closer to humans.
DAMS NOT AFFECTED: The uterine ischemia is performed under spinal/epidural anesthesia, and dams
breathe normally and maintain normal blood pressure and blood gases throughout the procedure.
4) While Kendall et al., 2019 provide important information about the 3Rs and reducing animal numbers, I
am concerned about the conditions in which the study took place. Were the housing/husbandry conditions
the same, how did they combine datasets from previous studies? What were the procedures for that and
what were the inclusion/exclusion criteria? What studies specifically did they use data from?
The data reflects two time epochs. From 2004 to 2016, the rabbit studies were conducted at NorthsShore
University HealthSystem, and from 2016 onwards, at Wayne State University. The physical condition for
housing/husbandry were almost identical, based on same IACUC protocol. Laboratory personnel
conducting the experiments have been unchanged since 2013. We retrieved all available neurobehavioral
data in surgical groups, sham groups, and naïve group. No data was excluded.
Some of the FHI group of newborn kits (as controls to the PHI+FHI model in the present study) have been
reported for MRI diagnosis (Drobyshevsky, Derrick, Wyrwicz, et al., 2007; Drobyshevsky, Jiang, Lin, et al.,
2014; Drobyshevsky, Yu, et al., 2012) and treatment of CP, including nNOS inhibitors (Ji et al., 2009; Yu et
al., 2011) and stem cells (Drobyshevsky et al., 2015). Inclusion criteria was simple as we were using the
same model for these studies. Exclusion criteria was if there were no neurobehavioral studies performed at
P1.
5) What were the housing/husbandry procedures for the current animals used in this study?
Timed-pregnant New Zealand White dams are ordered from Charles River. Single housing for pregnant
female rabbits in order to reduce stress during pregnancy, facilitate monitoring, and allow appropriate
nesting behavior. There is a separate room for rabbits. Dams were allowed 5-7 days to acclimate following
arrival animal facility and prior to the initiation of experiments. After corresponding surgical procedures,
dams and kits were euthanized after neurobehavioral tests of the kits.
6) How many litters, from how many females? What were the breeding procedures?
Each dam only gave birth to one litter. The numbers of pregnant dams and newborn kits are mentioned in

the article.

SIMILARITY TO HUMANS COMPARISON with OTHER MAMMALS

Oxidant-generating systems, such as xanthine oxidase
are normally low in rabbits, similar to humans.
Rodents have very high circulatory levels of xanthine oxidase
normally that makes the issue of oxidative stress a big confound in
rodent studies.
Global H-I in this animal model, akin to abruption
placentae
Unilateral, ischemic models in rodents
True fetal model. Term gestation 31.5 days. E25
corresponds to 26-30 weeks, and E29 to 34-36 weeks
gestation in humans, from MRI imaging and
oligodendrocyte maturation studies
Most often postnatal models used in rodents. Prenatal uterine
ischemia for 30 min in rats delays acquisition of passive avoidance
and longer escape latency in the Morris water maze test at 28 days
of age.
Dam not affected by uterine ischemia. Antenatal hypoxia in rodent model affects dam
Limited motor development at birth. Sheep and baboon almost fully developed at birth.
H-I results in obvious motor deficits (hypertonia and
postural deficits).
Even with half of a brain gone, H-I and inflammation in rodents
show little hypertonia or postural deficits.

Table 1R.

Breeding is done with a specific protocol which started with our first supplier, Myrtle's of Tennessee. Since
2015, we have ordered our pregnant dams from Charles River, with strict instructions to follow the same
breeding procedure as before. Copulation is witnessed and three attempts are made under observation for
30 min at 30 min intervals starting at 9 AM. We specifically order multiparous dams.

7) The experimental groups appear to have only consisted of Full H-I and Partial+Full H-I. Why is there not
a partial H-I only group and a naïve control group? These should be included. Also, why did they choose a
partial+full H-I? Where is the rationale for this? How did they determine the timeframe for balloon inflation?

There are no neurobehavioral deficits with naive, sham
controls, or partial H-I for 30 min, or even full H-I for 30 min.
There is a progression from partial to total abruption and we
modeled our Partial+Full H-I based on this clinical progression
(Fig 3R). The rationale was also provided in the 3rd paragraph
of the original manuscript. The timeframe for balloon inflation
was determined for Full H-I to give a balance of motor deficits
and deaths and normal kits.

8) If you used Full-HI from previous studies, then this study
only used the partial-full. This is a critical error since you do
not have a new control to compare. While it is fine to use
previous datasets, you also need to collect a new set of data to
ensure that the outcomes will be the same as previously

found.
Actually, that is not true. When the Partial+Full H-I was started at each institution, we compared it to block randomized Full H-I done at the same time in a ratio of 1:2. These dams have been included but because of the lack of numbers, we had to include historical controls to the Full H-I group.
9) The authors note that with the partial H-I, there was low BP to “about half of that before inflation”. Do the authors have data to demonstrate that it was half and how consistent were they? Were any animals from this group excluded?
We did catheter insertion through the left femoral artery, and checked the BP at the right hindlimb throughout the procedure. A baseline BP was obtained before the start of Partial+Full or Full H-I, and the size of balloon to create Partial H-I was determined by BP after the inflation of balloon. For Full H-I, we generally used 0.3 ml saline for the inflation of balloon (BP non-detectable from the other hindlimb). For Partial H-I, we generally used 0.07 ml of saline for inflation, which was slightly adjusted according to the BP after each inflation. The actual blood pressure measurements are provided in Table 2R below. There were no excluded animals.

Table 2R E22 E25

B.p.	**Before Partial**	**After Partial**	**Before Partial**	**After Partial**
Systolic	90.4±2.9	62.0±8.6	99.8±2.7	75.4±3.0
Diastolic	43.8±1.7	29.0±4.2	52.2±5.5	39.9±3.8
MAP	59.3±1.8	40.0±5.6	68.1±4.3	51.7±3.4

Fig 3R: Fetal bradycardia occurs with onset of abruption. Drug to be given after fetal bradycardia both in animals and later in humans.

10) How many animals did you start with and how did you determine the sample size? How many died and how did that change the power?
There may be a misconception by the editor. We have deaths included in the outcome variable. We computed power by running Proc Power (SAS 9.4) using a Wilcoxon-Mann-Whitney Test and estimating originally for 20% delta change in the percentage of Severe and Death categories. Due to the higher numbers of rabbits available, we ran power analysis again using 15% delta change in the Severe+Death%.
In Fig 4R is the curve of the Power vs. the Combined number of animals. With the actual number of animals for both groups, we cross the 0.8 threshold, actually 0.813.
11) What were the “minor revisions’’ for the neurobehavioral assessments
The changes made to the categories in this paper as different from (Z. Shi et al., 2019) is in Table 3R below:

Table 3R	**Added**	**Revisions**	**Removed**
Normal	-	-	

Mild Minor locomotor deficit is now defined: “2.5-3.0”.
Locomotor deficit is defined: “<2.5”
Severe Postural changes removed from definition
for simplicity.
Dead - -

12) Don’t use the word gender, use sex, but how many of each sex were used?
We have now changed gender to sex. The number in each sex category was given in box inserts in Fig 2.

13) How did authors determine the brain regions to assess?
Fig 4R: Power vs total no. of animals.
Brain regions represent the regions that control movement. These four regions have been investigations
before our previous studies.

14) Student’s T-tests are likely incorrect since the sample sizes are so different. This will be a violation of
the assumption of normality. I recommend that all data are assessed by a biostatistician, especially to
compare the differences in power. Along those lines, the authors need to assess for differences between
the groups of data collected from different studies.
We had run Proc Univariate (SAS 9.4) (which gives us mean, median, S.D., SEM, kurtosis, skewness etc.)
on each neurobehavior test data, and even plotted the Q-Q plots to check for normality of distribution. As
can be seen in Fig 5R, most Q-Q plots satisfy normality of distribution. Given the power of parametric
testing and ease of subsequent power calculation, we elected to use Student’s t-test, even though sample
sizes are different. We had elected to show all power calculations to let the reader decide for themselves
whether there was Type I or II error. We have added a sentence to the Statistics section.

15) Correlational analyses were not described in the statistical approaches section
We have added the correlational analysis.

16) Fig 1 appears as a longitudinal design, when it in fact is not. This figure needs to be redone. Again
discuss with a biostatistician, but given that this is a 3x2 between subject design, the figures and data will
need to be revised accordingly.
We are surprised at this comment. The primary outcome variable is a type of ordinal ranked data. The order
matters in this case and is based on how clinicians look at long-term outcome of newborn infants. It would
be incomplete to look at individual outcomes. It is important to note that if deaths are decreased by say
10%, survivors are increased by 10%. A favorable outcome would be if the 10% was distributed between
Normal, Mild and Severe survivors. We have added a reference (Khamis, 2008) to the phrase of “analyzed
by Kendall tau b rank correlation coefficient”.

17) Fig 2 doesn’t have error bars. Also, do not use bar graphs. Please also check the organization of the

graphs. They do not appear to align with the figure legends.
We would beg to disagree. Again, the data is presented as ordinal ranked data, now with two bars indicating
male and female for comparison. There are no “error” bars for this data because these data are percentages of the total number of kits in each group. We have checked the Legend and are perplexed at
the comment that it is not aligned.
Overall, I cannot allow this paper to go for review yet. There are a lot of factors that concerned me,
especially with the experimental design and the fact that no additional controls were assessed throughout.
Fig 5R: Normal distribution checked by Q-Q plots shown for E22.
The controls that the editor is asking for includes naive, sham controls, or partial H-I for 30 min, or even full
H-I for 30 min. We have tested all these groups. All these groups do not manifest neurobehavioral deficits.
I also did not see data regarding the partial HI to confirm that it was indeed partial.
Blood pressure numbers are presented above, show a drop in perfusion. All cases present with fetal
bradycardia just after onset of Partial H-I, which would only happen if the fetus experienced some global
hypoxia from the partial uterine ischemia.
I highly recommend a biostatistician review all this information. In addition, because you are comparing a
new model to an already existing one, you will need to discuss this in significantly more detail.
We have added some sentences to describe the comparison and the historical controls. For our first
publications, we had consulted a biostatistician.
I believe that if you can take care of all the comments, then the paper will be sufficiently improved to send
out for review.
Additional Comments:
To echo Dr Prager's comments, I am not fully convinced by the rationale and value for developing a
partial + full model vs or in addition to a partial only, as the authors state "Uteroplacental insufficiency states also may take time to develop. " hence, a partial model would be valuable to
investigate a milder insult that may trigger similar outcomes in the offspring, Since a partial
ischemia can eventually trigger and result in a total uteroplacental insufficiency.
If one looks at the available models of H-I in animals, the single insult consists of a sudden onset of 100%
anoxia to the fetal brain. In real life, these cases are rare and even rarer with placental abruption, which is
the clinical entity that our model mimics. All cases of total placental abruption start off with partial abruption
(see Fig 3R above). So, the progression of partial to total abruption clinically was what we were translating
in the Partial+Full H-I. Please note that in our hands, Partial H-I alone for 30 min or even Full H-I alone for
30 min do not produce any neurobehavioral deficits.

Since this is a new model, a more extensive description and study of the effects on brain structures
should be provided, the manuscript completely lacks of any histomorphological characterization. A
correlation between the neurobehavioral deficits and brain regions abnormalities should be added,
the data in figure 7 are not sufficient.

We beg respectfully to disagree for various reasons. 1) Cerebral palsy is a disease entity that involve
survivors. Not much is known of the primary, secondary, tertiary phases of brain cell injury in these patients.
2) The brain histopathology most probably recovers by the time of manifestation of hypertonia and postural
deficits. This thesis is confirmed by numerous neuroimaging studies at older ages. 3) In our animal model,
the problem is that histopathological changes are most obvious at the time of secondary and tertiary cell
phases but taking brains 1-3 days after the insult has a) not shown any difference between sham controls
and H-I (blinded analysis showed no difference), and b) we are unable to differentiate between fetuses that
will ultimately become hypertonic (or Severe or Mild) from those fetuses that become normal-looking kits. 4)
Since there were no distinguishing histological lesions between Full H-I and sham controls, we did not
attempt to look at differences between Full and Partial+Full H-I.

Furthermore, since these kits were delivered by c-section, could the authors determine if they presented
with any neurobehavioural deficit, so that the loss in cell viability can be correlated with normal, mild or
severe deficits?

The editor is absolutely right. We cannot distinguish between fetuses that would ultimately become normal,
mild, severe or dead postnatally. This is the most vexing problem studying cerebral palsy. See the response
above. We have developed a fetal MRI biomarker that reliably predicts postnatal hypertonia (Drobyshevsky,
Derrick, Prasad, et al., 2007; Drobyshevsky, Luo, et al., 2012), but only for the E25 fetus and for Full H-I.
Studies are ongoing to extend this MRI biomarker to the Partial+Full H-I and then to investigate cell death
and histopathological changes. These studies are somewhat expensive and are outside the scope of this
paper.

Brain weights should be supplied as well as kits' weights at various ages, especially at birth and then when
the neurobehavioural deficits become evident. The only data available are on acute brain cell viability after
the H-I insult, figure 7.

We did not measure brain or head weights. We have not found brain weights to be of much use

distinguishing between Full H-I and sham controls, even if we do wet/dry weight. Please see the comments
about recovery above. We have kits' body weight in the original Excel sheets next to sex.
The methods needs extensive re-writing as they are not clear and there are missing details.
How many kits did on average each dam carried/give birth to?
We have added an additional reference that provides a detailed description of the animal model (Shi, Luo, &
Tan, 2019) and added some sentences. The averages are mentioned now in Results (it could have been
calculated from the dams and kits' numbers provided in the first paragraph in Results before).
Gender and Sex are not synonym, but have a very different meaning. Sex should be used as the authors
are describing biological differences. The lack of sex differences is surprising, as I would have expected that
females might be more protected.
The tables should include data for the females as well.
We agree and have corrected to sex. We do not find any differences between sex in our animal model. We
had mentioned previously: "We had previously thought that female sex had more deaths but less severely
affected kits in the survivors. With accumulation of enough numbers, we show no statistical difference in
outcomes among males and females. " Even in humans, a severe H-I insult seems to cause the same
amount of injury even if the baby is female.
We have included the male percentage in Tables 1 and 2. We elected not to provide the sex breakdown in
Tables 3 and 4 for two variable correlations.

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2nd Editorial Decision

Decision Letter

Dear Dr Shi:

We've now received the feedback from two reviewers and have appended those reviews below along with the editorial comments. The reviewers are overall very enthusiastic and supportive of the study. They did raise some concerns and made some suggestions for clarification, but I expect that these points should be relatively straightforward to address. If there are any questions or points that are problematic, please feel free to contact me. I am glad to discuss.

We ask that the authors carefully revise the manuscript to include all the changes/additions that were requested during the first round of reviews by the editors.

We ask that you return your manuscript within 30 days. Please explain in your cover letter how you have changed the present version and submit a point-by-point response to the editors' and reviewers' comments. If you require longer than 30 days to make the revisions, please contact Dr Cristina Ghiani

JOURNAL OF Neuroscience Research

(cghiani@mednet.ucla.edu). To submit your revised manuscript: Log in by clicking on the link below https://wiley.atyponrex.com/submissionBoard/1/06f46767-4920-47cc-aeed-10f82581c669/current

(If the above link space is blank, it is because you submitted your original manuscript through our old submission site. Therefore, to return your revision, please go to our new submission site here (submission.wiley.com/jnr) and submit your revision as a new manuscript; answer yes to the question "Are you returning a revision for a manuscript originally submitted to our former submission site (ScholarOne Manuscripts)? If you indicate yes, please enter your original manuscript's Manuscript ID number in the space below" and including your original submission's Manuscript ID number (jnr-2021-Jan-9435.R1) where indicated. This will help us to link your revision to your original submission.)

The journal has adopted the "Expects Data" data sharing policy, which states that all original articles and reviews must include a Data Availability Statement (DAS). Please see https://authorservices.wiley.com/author-resources/Journal-Authors/open-access/data-sharing-citation/data-sharing-policy.html#standardtemplates for examples of an appropriate DAS. Please include the DAS in the manuscript as well.

Thank you again for your submission to the Journal of Neuroscience Research; we look forward to reading your revised manuscript.

Best Wishes,

Dr Eric Prager
Associate Editor, Journal of Neuroscience Research

Dr Cristina Ghiani
Editor-in-Chief, Journal of Neuroscience Research

Associate Editor: Prager, Eric
Comments to the Author:

We have now received the recommendations from each of the two reviewers and statistical editor. I'm happy to say that they found the manuscript to be well conducted and described, though they each have some minor comments. Importantly, some of the comments described in your response to my original review were not added to the manuscript. Please ensure that if it wasn't added that you do so, when possible. It's important that the conversation be open for all the readers as well. Thank you again and we look forward to receiving your revised manuscript.

Additional Editorial comments:
The introduction would benefit by a bit more background, for instance, the authors may want to expand on the rationale of using rabbits.
Please make sure to read all the files attached and to format the manuscript following the JNR guidelines to the authors, in particular, the abstract.

DATA ACCESSIBILITY
To enable readers to locate archived data from Journal of Neuroscience Research papers, we require authors to include a 'Data Accessibility' section just before the References. This should list the database(s) and URL(s) or dataset DOIs for all data associated with the manuscript. Data deposit repositories might include unstructured repositories such as Dryad, FigShare, NeuroMorpho or centralized repositories from the institutions in which the research was conducted. We also strongly recommend depositing data in the Open Science Framework. JNR will also allow small data sets to be included as Supplementary Files with the article.

The language of the manuscript still need some refinements, some examples from the methods, but there are more throughout :

"Neurobehavior data...." would read better as "Neurobehavioral data...."
"ordinal data was also" would read better as "ordinal data were also...."

"During the conduct of the newer model, we had conducted a block randomized..." this sentence would read better as ""During the developement of the newer model, we had conducted a block randomized....."
or
"While developing the newer model, we had conducted a block randomized
or
"During the characterisation of the newer model, we had conducted a block randomized..."

"in a ratio of 1:2. For this study, we added historical controls of Full H-I kits to have sufficient power (Fig 1)."
"in a ratio of 1:2. In the present study, we added historical controls of Full H-I kits to have sufficient power

(Fig 1).

Statistics Editor: McArthur, David
Comments to the Author:
Nowhere in the narrative are any actual t-test or anova results presented. For anova we would expect "F(df1,df2) = x.xx, p = 0.yyy" for example, e.g., standard notation. Kindly supply these findings everywhere such test results are appropriate. The statistics cannot be checked without this.

Note please that the "effect of sex" is only appropriate if you are in the business of assigning sex to study participants (which, regrettably, is one possible interpretation for the nonspecialist of your phrase "sex assignments were done"). Instead this is better referred to as "differences by sex" and, for the general readership, "sex assignments" might better be replaced by "assessments of sex".

The exact duplication of values in the upper and lower triangles of the correlation matrices is of no intellectual value. Choose one of the other of the two triangles and leave the opposite side blank.

Minor note: Pink lettering on white background as in Figure 3 is a poor color choice.

Reviewer: 1

Comments to the Author
review is contained in the attachment

Reviewer: 2

Comments to the Author
Summary
This study compares a new rabbit model of fetal hypoxia-ischemia that introduces 30 minutes of partial placental insufficiency followed by 40 minutes of total placental insufficiency. The currently used model induces 40 minutes of total uterine insufficiency, only. The rationale for producing this more gradual model of placental insufficiency is that it is more clinically relevant than the abrupt, full abruption model and that with this new model, neuroprotective drugs can be introduced at the onset of bradycardia, which occurs during the 30 minutes of partial placental insufficiency. This new model is more translational and will be more appropriate for preclinical drug testing than the current model. Overall this is a well written manuscript that clearly presents the results of a significant amount of work. The manuscript has been improved after responding to the Editor's suggested revisions. Below are additional suggestions for revisions and clarifications.

Suggestions for Revisions

1. For Figure 2, it would be helpful to have a legend on the graph to define the colors used for the two groups so that a reader can better comprehend the graph simply by looking at it, and without having to consult the figure legend.
2. For Figure 7. There is a statistically significant difference between the two models for suck/swallow as depicted in 7E as stated in the legend; however there is no indication on the graph that this is statistically different, thus it would be helpful to add an asterisk to demarcate statistical significance.
3. For Figure 8. I cannot determine what the individual lines represent. Does each line represent a biological replicate? Please clarify the figure legend.
4. For Figure 8. Please provide additional information about the assay used in the Methods (incl. catalog number). It is not clear if this is a cell death or a cell viability assay. If the latter, then precaution is urged in interpreting the results as what these data may be showing is reduced cell proliferation in the partial to full H-I group vs. the Full H-I group, which is an entirely different interpretation than concluding that there is ongoing cell death in the partial to full H-I group.
5. The authors introduce the concepts of preconditioning and pre-acclimatization, but then dismiss these as irrelevant for explaining why the partial to full abruption group fared better in terms of fewer male kit deaths and fewer behavioral abnormalities than the full abruption group. However, it seems entirely reasonable to suppose that a physiological adaptation to the H-I is triggered by the partial reduction in blood flow that then reduces the extent of damage when the full H-I condition occurs. While such a scenario is not supported by rat and mouse H-I pre-conditioning models, there are sufficient physiological variables between the rat and mouse models and this new rabbit model such that dismissing such adaptations may be premature. If the authors agree, they should consider discussing this possibility in the Discussion.
6. In this reviewer's opinion the last sentence of the manuscript does not belong in the conclusions. The last paragraph can be re-written to echo the ideas presented in the Statement of Significance.
7. Table 1. The first row heading should be number of dams.
8. Tables 3 and 4. The legends state that $\alpha<0.005$. Is this correct or should it read $\alpha<0.05$?

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Authors' Response

Associate Editor: Prager, Eric

Comments to the Author:

We have now received the recommendations from each of the two reviewers and statistical editor. I'm happy to say that they found the manuscript to be well conducted and described, though they each have some minor comments. Importantly, some of the comments described in your response to my original review were not added to the manuscript. Please ensure that if it wasn't added that you do so, when possible. It's important that the conversation be open for all the readers as well. Thank you again and we look forward to receiving your revised manuscript.

We thank the editor and reviewers' insightful comments. We have added most of the salient points in our response to the original review now in the manuscript.

Additional Editorial comments:

The introduction would benefit by a bit more background, for instance, the authors may want to expand on the rationale of using rabbits.

Added.

Please make sure to read all the files attached and to format the manuscript following the JNR guidelines to the authors, in particular, the abstract.

We have revised accordingly.

DATA ACCESSIBILITY

To enable readers to locate archived data from Journal of Neuroscience Research papers, we require authors to include a 'Data Accessibility' section just before the References. This should list the database(s) and URL(s) or dataset DOIs for all data associated with the manuscript. Data deposit repositories might include unstructured repositories such as Dryad, FigShare, NeuroMorpho or centralized repositories from the institutions in which the research was conducted. We also strongly recommend depositing data in the Open Science Framework. JNR will also allow small data sets to be included as Supplementary Files with the article.

Data will be available from the corresponding upon reasonable contact.

The language of the manuscript still need some refinements, some examples from the methods, but there are more throughout :

"Neurobehavior data...." would read better as "Neurobehavioral data...."

"ordinal data was also" would read better as "ordinal data were also...."

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"in a ratio of 1:2. For this study, we added historical controls of Full H-I kits to have sufficient power (Fig 1)."

"in a ratio of 1:2. In the present study, we added historical controls of Full H-I kits to have sufficient power (Fig 1).

We have revised accordingly.

Statistics Editor: McArthur, David

Comments to the Author:

Nowhere in the narrative are any actual t-test or anova results presented. For anova we would expect "F(df1,df2) = x.xx, p = 0.yyy" for example, e.g., standard notation. Kindly supply these findings

everywhere such test results are appropriate. The statistics cannot be checked without this.
We have entered the information. We have chosen not to depict the actual P values that are not significant, for brevity.
Note please that the "effect of sex" is only appropriate if you are in the business of assigning sex to study participants (which, regrettably, is one possible interpretation for the nonspecialist of your phrase "sex assignments were done"). Instead this is better referred to as "differences by sex" and, for the general readership, "sex assignments" might better be replaced by "assessments of sex".
Revised accordingly.
The exact duplication of values in the upper and lower triangles of the correlation matrices is of no intellectual value. Choose one of the other of the two triangles and leave the opposite side blank.
We were following depiction of a colored table done before by others to give the reader a quick feel of the strength of the correlations. Has now been modified following the wishes of the reviewer.
Minor note: Pink lettering on white background as in Figure 3 is a poor color choice.
Changed to purple.
Reviewer: 1
Comments are transferred from PDF to the following:
Summary: This manuscript presents clinically relevant data on the outcomes of prenatal hypoxiaischemia in a rabbit model of cerebral palsy with and without a period of partial ischemia preceding the total ischemia. There is also a very interesting data set contained in this paper on the outcomes of hypoxia ischemia at 70% gestation vs ~80% gestation. Although some portions of this paper are difficult to interpret, the results are relevant and important to the community of researchers investigating the relationship between developmental insults and cerebral palsy.
Minor:
Data in figure 1 would be much more appropriate for a pie chart to show distribution.
Changed to a pie chart.
Heart rate: it is mentioned in the paper that fetal heart rate is increased within minutes of onset of partial H-I. Please include the data on heart rate for full vs partial H-I.
We did not record the extent of deceleration in fetal heart rate (not acceleration) as heart rate was monitored by an acoustic signal coming from ultrasound doppler. We also are not able to measure every fetus's heart rate in a litter. When we originally started our model, we would try sequentially measuring fetal heart rate of every fetus across the uterine wall after a laparotomy, using the handheld doppler instrument. The science of heart rate signal analysis has advanced beyond just reporting a heart rate number. To do justice to heart rate comparisons would require some sort of non-invasive imaging and electrocardiographic measurement at the same time, which is simply not feasible in the present *in vivo* rabbit model.
Figure 4: it is stated in the methods that the olfaction tests are all based on aversive response, but it could be stated more clearly in the methods and results again what "better" and "worse" means in this context. Is it possible for an animal with severe motor deficits to show a strong aversive response?
The response to odors is based on an ordinal scoring system from 0-4 at 0.5 increments. In this context, "worse" refers to a low score and "better" refers to a higher score. Interestingly, we have previously observed that even rabbit kits with severe motor deficits sometimes had an aversive response with high score. We have added a sentence to the legend.
This paper provides enough numbers to look at the question raised by the reviewer and one can see from Tables 3 and 4 that the response to odor has a weak negative correlation (if any) in the Full H-I group at both ages. Only for Partial+Full H-I at E25 (but not at E22) is the strength of negative correlation >0.7. This interesting observation is worthy of further study and confirms our suspicion that the Partial+Full H-I may result in slightly qualitatively different injury from that following Full H-I.

Figure 7: please include some indicator in Figure 7 that the results of panel E are significantly different. Results of the neck movement (time of activity) should be shown if they are significant.
We have added an asterisk in Figure 7. The neck movement (time of activity) results were updated, and the P value (=0.0246) was no longer less than the a priori α = 0.0029. The results have been corrected as well.
Clarity of the results of cell survival assay: In the abstract it is stated that "Partial + Full H-I (n=6) showed less cell viability than Full H-I (n=8) after 24-hr ex vivo in the brain regions studied." Since results were not significantly different until 72 hours, it should be stated that cells showed less viability at 72 hrs or after 60 hrs both in the abstract and in the discussion line 54 p 17. This was expressed more clearly in the statement of significance. Later, in the results section, the numbers are provided for cell viability. Are those cell counts? Please state in the results what these numbers are. For reference, what was cell viability in sham control brain tissue?
We apologize for the inadvertent error. We now mention 72-hour in the abstract.
The numbers are unit of luminescent signal that represents live cells in the well.
We did not collect data on E25 sham controls because of cost considerations.
Discussion: Could the authors describe a bit more how the results here differed from Drobyshevsky et al 2012. Specifically, it appears that death of fetuses / neonates increases when the H-I insult occurs later in gestation. This, along with the need to reduce time of H-I when it is inflicted at E28, would suggest that the severity of an insult is greater later in gestation. Paradoxically, the authors here show a higher percentage of severely injured kits at E22 than E25. Do the authors have any theories on why this would be?
Probably the reviewer meant E29 instead of E28. We have not done any H-I experiments at E28 that we can recall, only inflammation. Yes, we have published previously that the stillbirths and perinatal deaths increases with increasing gestation given the same amount of uterine ischemia (see Derrick et al 2012, Pediatr Res, 72(2), 154-160). There is a simple explanation for more severe kits at E22 than E25. We had alluded to it in the discussion in the trickle-down effect. In the continuum of injury, first there is mild, then severe, then death. It is a well-known clinical fact that when we rescue babies with hypoxic-ischemic encephalopathy, the deaths can decrease resulting in greater numbers of severely affected survivors. That is why most clinical studies use death plus major disability as the clinical endpoint for neurotherapeutic studies. We have added some sentences in the discussion.
Table 1: The numbers should be labeled more carefully. Perhaps "No. of Dead (%)" otherwise the first number is interpreted as a percent not the total. Please apply this to the rest of the categories as well.
Adjusted.
Reviewer: 2
Comments to the Author
Summary
This study compares a new rabbit model of fetal hypoxia-ischemia that introduces 30 minutes of partial placental insufficiency followed by 40 minutes of total placental insufficiency. The currently used model induces 40 minutes of total uterine insufficiency, only. The rationale for producing this more gradual model of placental insufficiency is that it is more clinically relevant than the abrupt, full abruption model and that with this new model, neuroprotective drugs can be introduced at the onset of bradycardia, which occurs during the 30 minutes of partial placental insufficiency. This new model is more translational and will be more appropriate for preclinical drug testing than the current model. Overall this is a well written manuscript that clearly presents the results of a significant amount of work. The manuscript has been improved after responding to the Editor's suggested revisions. Below are additional suggestions for revisions and clarifications.
Suggestions for Revisions

1. For Figure 2, it would be helpful to have a legend on the graph to define the colors used for the two groups so that a reader can better comprehend the graph simply by looking at it, and without having to consult the figure legend.
Done.
2. For Figure 7. There is a statistically significant difference between the two models for suck/swallow as depicted in 7E as stated in the legend; however there is no indication on the graph that this is statistically different, thus it would be helpful to add an asterisk to demarcate statistical significance.
Done.
3. For Figure 8. I cannot determine what the individual lines represent. Does each line represent a biological replicate? Please clarify the figure legend.
Each line represents the line joining the reading from one brain region of one fetus. We have added a sentence: "Lines join points that represent Means of n=8 in Full and n=6 in Partial+Full fetal brains." Also, a color legend has been added to the Figure.
4. For Figure 8. Please provide additional information about the assay used in the Methods (incl. catalog number). It is not clear if this is a cell death or a cell viability assay. If the latter, then precaution is urged in interpreting the results as what these data may be showing is reduced cell proliferation in the partial to full H-I group vs. the Full H-I group, which is an entirely different interpretation than concluding that there is ongoing cell death in the partial to full H-I group.
We confirm it is RealTime-Glo MT Cell Viability Assay. The luminescent signal is produced by live cells and is in proportion to the number of live cells. We added this information in the revision.
5. The authors introduce the concepts of preconditioning and pre-acclimatization, but then dismiss these as irrelevant for explaining why the partial to full abruption group fared better in terms of fewer male kit deaths and fewer behavioral abnormalities than the full abruption group. However, it seems entirely reasonable to suppose that a physiological adaptation to the H-I is triggered by the partial reduction in blood flow that then reduces the extent of damage when the full H-I condition occurs. While such a scenario is not supported by rat and mouse H-I pre-conditioning models, there are sufficient physiological variables between the rat and mouse models and this new rabbit model such that dismissing such adaptations may be premature. If the authors agree, they should consider discussing this possibility in the Discussion.
Conditions for pre-acclimatization or preconditioning were different from those in our new model. While we would like to agree with the reviewer in that the partial uterine ischemia reduces the damage that a full uterine ischemia would cause (mentioned in the sentence in the discussion: "Nevertheless compensatory mechanisms could be triggered …"), we have no clear-cut confirmation of this (see Fig 2). At this present time, we are not convinced either way whether Partial+Full is worse or better than Full H-I. A future study could investigate Partial+Normal+Full (fulfilling preconditioning) or a stepwise prolonged Partial+Full (fulfilling pre-acclimatization) to tease out the biological effects from these two conditions.
6. In this reviewer's opinion the last sentence of the manuscript does not belong in the conclusions. The last paragraph can be re-written to echo the ideas presented in the Statement of Significance.
We have removed the last sentence. We have added some sentences from the Statement of Significance to the last paragraph as suggested.
7. Table 1. The first row heading should be number of dams.
Revised accordingly.
8. Tables 3 and 4. The legends state that $\alpha<0.005$. Is this correct or should it read $\alpha<0.05$?
That is correct, $\alpha<0.005$, because of Bonferroni correction. That is why we are confident of the significance of the correlations.

JOURNAL OF Neuroscience Research

3rd Editorial Decision

Decision Letter

Dear Dr Shi:

Thank you for submitting your manuscript to the Journal of Neuroscience Research. We've now received the reviewer feedback and have appended those reviews below. I'm glad to say that the reviewers are overall very enthusiastic and supportive of the study. They did raise some concerns and made some suggestions for clarification, but I expect that these points should be relatively straightforward to address. If there are any questions or points that are problematic, please feel free to contact me. I am glad to discuss.

We ask that you return your manuscript within 30 days. Please explain in your cover letter how you have changed the present version and submit a point-by-point response to the editors' and reviewers' comments. If you require longer than 30 days to make the revisions, please contact Dr Cristina Ghiani (cghiani@mednet.ucla.edu). To submit your revised manuscript: Log in by clicking on the link below https://wiley.atyponrex.com/submissionBoard/1/5fdc48f8-f8bb-405a-8648-83315e53f587/current

(If the above link space is blank, it is because you submitted your original manuscript through our old submission site. Therefore, to return your revision, please go to our new submission site here (submission.wiley.com/jnr) and submit your revision as a new manuscript; answer yes to the question "Are you returning a revision for a manuscript originally submitted to our former submission site (ScholarOne Manuscripts)? If you indicate yes, please enter your original manuscript's Manuscript ID number in the space below" and including your original submission's Manuscript ID number (jnr-2021-Jan-9435.R2) where indicated. This will help us to link your revision to your original submission.)

The journal has adopted the "Expects Data" data sharing policy, which states that all original articles and reviews must include a Data Availability Statement (DAS). Please see https://authorservices.wiley.com/author-resources/Journal-Authors/open-access/data-sharing-citation/data-sharing-policy.html#standardtemplates for examples of an appropriate DAS. Please include the DAS in the manuscript as well.

Thank you again for your submission to the Journal of Neuroscience Research; we look forward to reading your revised manuscript.

Best Wishes,

Dr Eric Prager
Associate Editor, Journal of Neuroscience Research

Dr Cristina Ghiani
Editor-in-Chief, Journal of Neuroscience Research

Associate Editor: Prager, Eric
Comments to the Author:
Thank you for your revisions. The manuscript is nearly ready to be accepted. The first reviewer did make a mistake in requesting the pie chart for Fig 1 and asked that you replace that with the original figure and make fig 2 a pie chart.

My other concern is your statement you wrote in response to the statistical editor that you chose not to depict that P value that are not significant for brevity. That is not an acceptable response, in my opinion and I ask that you please include all exact p values, whether significant or not. We do not have page/word limits so brevity is a non-issue.

After you fix these two small issues, we will be happy to accept the paper without further review.

Additional Editorial comments:
The manuscript will still benefit by a thorough editing of the language.

Please note that 'data' is the plural form, so the verb should always be plural:

"The data was derived ..." should be "The data WERE derived ...."
"No data was excluded." should be "No data were excluded."

'.....the analysis and interpretation of THE data..."

Reviewer: 2

Comments to the Author
The authors have satisfied my concerns.

Reviewer: 1

Comments to the Author
The manuscript is much improved and almost ready for publication. In my previous review I had erroneously stated that Figure 1 would be more appropriate as a pie chart. I intended to write that Figure 2 would be more appropriate as a pie chart. If this change could be made (figure 1 reverted to previous format and convert figure 2 to a pie chart), I have no further comments.

Authors' Response

Associate Editor: Prager, Eric
Comments to the Author:
Thank you for your revisions. The manuscript is nearly ready to be accepted. The first reviewer did make a mistake in requesting the pie chart for Fig 1 and asked that you replace that with the original figure and make fig 2 a pie chart.
The previous version of Fig 1 is used. The new Fig 2 with pie chart is used.
My other concern is your statement you wrote in response to the statistical editor that you chose not to depict that P value that are not significant for brevity. That is not an acceptable response, in my opinion and I ask that you please include all exact p values, whether significant or not. We do not have page/word limits so brevity is a non-issue.
The exact p values are added in figure legends.
After you fix these two small issues, we will be happy to accept the paper without further review.
Additional Editorial comments:
The manuscript will still benefit by a thorough editing of the language.
Please note that 'data' is the plural form, so the verb should always be plural:
"The data was derived ..." should be "The data WERE derived ...."
"No data was excluded." should be "No data were excluded."
'.....the analysis and interpretation of THE data..."
Revised accordingly.
Reviewer: 2
Comments to the Author
The authors have satisfied my concerns.
Reviewer: 1
Comments to the Author
The manuscript is much improved and almost ready for publication. In my previous review I had erroneously stated that Figure 1 would be more appropriate as a pie chart. I intended to write that Figure 2 would be more appropriate as a pie chart. If this change could be made (figure 1 reverted to previous format and convert figure 2 to a pie chart), I have no further comments.
Revised as above.

4th editorial decision

JOURNAL OF Neuroscience Research

Decision Letter

Dear Dr Shi:

Thank you for the hard work you put into this paper. It is excellent and we are happy to accept it. Congratulations!

In the coming weeks, the Production Department will contact you regarding a copyright transfer agreement and they will then send an electronic proof file of your article to you for your review and approval.

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Dr Eric Prager
Associate Editor, Journal of Neuroscience Research

Dr Cristina Ghiani
Editor-in-Chief, Journal of Neuroscience Research

Author response