PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Visual-motor integration and fine motor skills at 6.5 years of age and associations with neonatal brain volumes in children born extremely preterm in Sweden: population-based cohort study
AUTHORS	Bolk, Jenny; Padilla, Nelly; Forsman, Lea; Brostrom, L; Hellgren, Kerstin; Åden, Ulrika

VERSION 1 – REVIEW

REVIEWER	Maya Weinstein Functional Brain Center, Wohl Institute for Advanced Imaging, Tel-Aviv Sourasky Medical Center, Israel
REVIEW RETURNED	19-Nov-2017

GENERAL COMMENTS	Reviewer Comments This is a prospective study following extremely preterm infants aiming to correlate regional brain volume at term equivalent age with VMI and motor abilities at 6.5 years. This approach is useful in terms of using neonatal imaging to predict future outcome, since it can be used to identify infants at risk and enable applying clinical interventions at an early age. Introduction: 1. It would be useful to shortly review previous imaging findings of brain regions associated with visual-motor integration and manual dexterity at the introduction, so the basis for choosing specific regions of interest in this study will be addressed. 2. In the introduction the authors relate to the contribution of visual perceptual skills and motor abilities in VMI and state that "an inductive exploration of brain areas associated with VMI is a useful approach" (page 5 lines 28-33). Please explain how this approach will be useful in elucidating to the contribution of visual perceptual skills and motor function on VMI and relate to the study's findings regarding this issue also in the discussion. 3. Page 5 (line 10): please clarify/ rephrase sentence Method: 4. Please provide more detail on the MRI setup for neonates. Where they scanned in natural sleep or under anesthesia? 5. Please clarify if the data first underwent automatic segmentation (step 1) to extract the CSF, GM and WM and later underwent the atlas based segmentation (step 2) to conduct the regional segmentation, because it's not clear whether these are two steps in the analysis of the data or two separate analyses. Results: 6. Please provide a figure of the neonate's brain after coregistration with the atlas template so the reader will be able to view the

	segmented brain and more specifically the regions of interest used in this study. This is especially important as there is reduced gray to white matter tissue intensity contrast in neonates which complicates segmentation at this early age. Discussion: 7. It is not clear enough whether this is an exploratory study, looking at various brain regions or a hypothesis driven study targeting specific brain regions based on previous findings. 8. The authors state that the children included were without major brain lesions or CP, where there any other co-morbidities found in these cohort at 6.5 ages such as ADHD, ASD etc.? If so, this should be detailed and added to the limitations as there are specific volumetric findings pertaining to these syndromes. 9. Please discuss the limitations of using a template in the preterm infant's brain- the problems in co-registration and possible biases; as the current analysis does not extract brain regions based on their anatomical contrast as usually performed in adult volumetric studies but uses an atlas template on the neonatal whole brain to indirectly deduce the region's volume. 10. Almost 50% of the data was discarded due to low imaging quality –please refer to the challenges in scanning this population and suggest what measures may be recommended to improve data quality. 11. The discussion is overall well written and provides possible explanations and interpretations of the study's findings.

REVIEWER	Dustin Scheinost Assistant Professor of Radiology and Biomedical Imaging and in the Child Study Center Yale School of Medicine, New Haven, CT, USA
REVIEW RETURNED	22-Nov-2017

GENERAL COMMENTS	Bolk et al presents a manuscript investigating associations between neonatal brain volumes and visual-motor integration and fine motor skills in children born preterm at 6 years of age. 66 preterm children without brain injury were included for study. Neonate MRI, the Movement Assessment Battery for Children-Second Edition, and the Beery-Buktenica Developmental Test of Visual-Motor Integration - Sixth Edition were the main measures of interest. The volume of the precentral gyrus was significantly correlated with visual motor integration and motor abilities. Motor skills were also associated with cerebellum, and brainstem volumes. This work is timely and builds on other recent work exploring motor/VMI deficits in preterms. The strength of the study is the use of neonatal MRI to predict later neurodevelopment as opposed to concurrent neurodevelopment and MRIs in preterms. Limitations include the lack of controls and lack of multiple comparisons corrections. Both limitations are acknowledged and are not fatal flaws. I have some minor comments below. I'm a little confused on how two different image analysis methods were used. Can the author's clarify? It seems like DARTEL was used to calculate jacobian modulated VBM images and the neonatal AAL atlas was used to define regions to extract volume (defined by jacobian modulated vbm values) from. I would add that the results are not corrected for multiple correction in the figure and table captions (ie Figure 3/Table S3) in addition to the text already in the manuscript. This help avoid any confusion to a reader who may skim the methods and focus on the figures/tables.

VERSION 1 – AUTHOR RESPONSE

Editorial requests:
- Please revise your title to indicate the research question, study design, and setting. This is the preferred format of the journal.

Reply: The title is now changed to:
"Visual-motor integration and fine motor skills at 6.5 years of age and associations with neonatal brain volumes in children born extremely preterm in Sweden: population-based cohort study"

- On page 6 please state the specific name of the local ethics committee that approved your study along with the approval reference number (if applicable).

Reply: The details of the ethical approval for the study have been added to the text in the last paragraph in Methods-Study Population, on page 7:
"The study was approved by the local ethics committee in Stockholm and Lund (Regionala etikprövningsnämnden i Stockholm, dnr 04-889/2, 2006/1217-32 and 2010/850-31/1; Regionala etikprövningsnämnden in Lund, dnr 42/2004, dnr 2009/9 and dnr 2016/104) and the parents of all the children gave their written, informed consent before the study."
- Along with your revised manuscript, please provide a completed copy of the STROBE checklist (http://www.strobe-statement.org/).
Reply: A completed copy of the STROBE checklist is provided

Introduction:
1. It would be useful to shortly review previous imaging findings of brain regions associated with visual-motor integration and manual dexterity at the introduction, so the basis for choosing specific regions of interest in this study will be addressed.

Reply: We have added a short review of previous imaging findings to the manuscript in the Introduction, page 6:
"It has been reported that several networks in the brain are involved in the mediation of VMI and fine motor skills - the visual, salience, sensory motor and default mode networks (Seculpre 2014). Previous studies in adolescents born preterm have indicated that volumes of the cerebellum and thalamus (Martinussen, Flanders et al. 2009), superior temporal gyrus, insula, medial occipital lobe and temporal lobe (Sripada, Lohaugen et al. 2015) are associated to VMI scores. Also, a study looking at brain growth in preterm children reported that growth of the caudate and globus palldius could predict VMI scores (Young, Powell et al. 2015)"

2. In the introduction the authors relate to the contribution of visual perceptual skills and motor abilities in VMI and state that "an inductive exploration of brain areas associated with VMI is a useful approach" (page 5 lines 28-33). Please explain how this approach will be useful in elucidating to the contribution of visual perceptual skills and motor function on VMI and relate to the study's findings regarding this issue also in the discussion.

Reply: We apologise for being unclear about the purpose of this sentence. We have tried to clarify it in the manuscript, page 5:
"Although problems with VMI can stem from any of the underlying, contributing abilities listed above, when VMI and motor abilities have been investigated together, and perceptual and general cognitive abilities have been held constant, children with lower general motor scores are seen to have lower VMI scores."

“Therefore, a less-defined exploration of brain areas known to be associated with VMI is a useful approach, especially when investigating the neural associations of a multi-modal ability, in groups with potentially atypical brain development trajectories”

3. Page 5 (line 10): please clarify/ rephrase sentence

Reply: We have tried to clarify this sentence in the manuscript text, on page 5:
“On the other hand, visual perceptual deficits in children born EPT are well documented and their role in VMI should not be underestimated even though their contribution to VMI in children born EPT was not investigated in the present study.”

Method:

4. Please provide more detail on the MRI setup for neonates. Where they scanned in natural sleep or under anesthesia?

Reply: The children were fed before the MRI examination and given a low dose of chloralhydrate (30 mg/kg) orally or rectally. The infants received individually moulded ear plugs (Affinis Dental Putty Soft; Forsberg Dental, Stockholm, Sweden) and neonatal (Mini-Muffs; Natus Medical Inc, San Carlos, CA, USA) and paediatric ear muffs (Bilsom Junior; Bacou-Dalloz Nordic, Sweden). We also used a sound dampening hood that had been custom-made, and was attached to the upper half semicircle of the magnet bore and thereby we could reduce the level of noise by up to 24 dB. The time to scan an infant was around 30 minutes. There was a physician in the scanner room during the whole procedure (Skiöld B et al, White matter changes in extremely preterm infants, a population-based diffusion tensor imaging study, Acta Pediatrica 2010).
We have added information on sedation to the manuscript in the Methods-MRI data and acquisition section, page 8:
“All the children were scanned using a Philips Intera 1.5 Tesla MRI system (Philips International, Amsterdam, The Netherlands) at term equivalent age.The child was fed before the scanning procedure and given a low dose of chloralhydrate (30 mg/kg) orally or rectally, as previously described (Skiöld et al, 2010)”

5. Please clarify if the data first underwent automatic segmentation (step 1) to extract the CSF, GM and WM and later underwent the atlas based segmentation (step 2) to conduct the regional segmentation, because it's not clear whether these are two steps in the analysis of the data or two separate analyses.

Reply: We apologise for not being clear about this. We used two separate analyses: atlas-based segmentation and automatic segmentation.
- Atlas-based segmentation to conduct the regional segmentation of specific regions (Shi et al PlosONe 2011; Infant brain atlases from neonates to 1- and 2-year-olds).
- Automatic segmentation to extract the mean volumes of the grey matter white matter, cerebrospinal fluid, basal ganglia, brainstem and cerebellum (Kuklisova-Murgasova et al Neuroimage. 2011; A dynamic 4D probabilistic atlas of the developing brain).

We have clarified this in the manuscript in the Methods, page 8-9:
“MRI data went into two separate analyses, atlas based segmentation and automatic segmentation; atlas-based segmentation to conduct the regional segmentation of specific regions (Shi 2011) and automatic segmentation to extract the mean volumes of the grey matter white matter, cerebrospinal fluid, basal ganglia, brainstem and cerebellum (Kuklisova-Murgasova 2011).”

Results:

6. Please provide a figure of the neonate's brain after coregistration with the atlas template so the reader will be able to view the segmented brain and more specifically the regions of interest used in this study. This is especially important as there is reduced gray to white matter tissue intensity contrast in neonates which complicates segmentation at this early age.

Reply: We have added a supplementary figure (Supplementary Figure 1) with the following footnote: "Atlas overlaid on the sagittal, coronal and axial T1-weighted images from a single preterm child. Individual anatomical regions are color coded."

Discussion:
7. It is not clear enough whether this is an exploratory study, looking at various brain regions or a hypothesis driven study targeting specific brain regions based on previous findings.

Reply: We consider this study exploratory in nature, even though it is based on selected regions. To clarify this, we have added "exploratory" to the first sentence in the Discussion, page 13:
"In this exploratory study, the associations between brain volumes at term equivalent age and VMI and fine motor skills at 6.5 years in children born EPT without major brain lesions or CP were explored in regions of the brain previously reported to be involved in those functions."

8. The authors state that the children included were without major brain lesions or CP, where there any other co-morbidities found in these cohort at 6.5 ages such as ADHD, ASD etc.? If so, this should be detailed and added to the limitations as there are specific volumetric findings pertaining to these syndromes.

Reply: In this study, we did not investigate or adjust for any ADHD or ASD. We have added this information as a limitation in the Discussion (page 17):
"We did not adjust for any diagnosis of autism or ADHD, which are reported to be common in children born extremely preterm (D'Onofrio, Class et al. 2013) and have been linked to altered brain development in preterms (Bora, Pritchard et al. 2014, Padilla et al. 2015)".

9. Please discuss the limitations of using a template in the preterm infant's brain- the problems in co-registration and possible biases; as the current analysis does not extract brain regions based on their anatomical contrast as usually performed in adult volumetric studies but uses an atlas template on the neonatal whole brain to indirectly deduce the region's volume.

Reply: We have added the following paragraph in the manuscript, in the Discussion page 16-17:
"Segmentation of cerebral tissues at term-equivalent age in children who were born extremely preterm is challenging due to the characteristics of the developing preterm brain. The segmentation can be limited in small structures of the brain since the volumes are smaller and there is also a lower signal-to-noise ratio in preterm children. To minimize this we used only high quality MRI. To guide segmentation we used a larger number of tissue probability maps from preterms (Kuklisova-Murgasova 2011) with an extraclass tissue map for background, to provide a better modelling of the cerebrospinal fluid and other non-brain voxels and also ta aid further tissue classification."

10. Almost 50% of the data was discarded due to low imaging quality –please refer to the challenges in scanning this population and suggest what measures may be recommended to improve data quality.

Reply: We have added the following to the limitations in the Discussion, page 16:
"This was due to rigorous entry and data quality criteria, as well as implicit methodological difficulties related to the scanning of preterm infants. Scanning preterm neonates is considered a challenging

task due to their immature physiology and anatomy. Patient motion may occur more often thus patient preparation and image protocols should be modified and be dedicated for neonates. To minimize this limitation the development of novel pulse sequences to increase the speed of image acquisition, and MRI coils tailored to the head size of the subject, would have the potential to further increase success (Smyser 2012; Magnetic resonance imaging of the brain at term equivalent age in extremely premature neonates: to scan or not to scan?).

11. The discussion is overall well written and provides possible explanations and interpretations of the study's findings.

Reviewer: 2
Reviewer Name: Dustin Scheinost
Institution and Country: Assistant Professor of Radiology and Biomedical Imaging and in the Child Study Center, Yale School of Medicine, New Haven, CT, USA
Competing Interests: None

Bolk et al presents a manuscript investigating associations between neonatal brain volumes and visual-motor integration and fine motor skills in children born preterm at 6 years of age. 66 preterm children without brain injury were included for study. Neonate MRI, the Movement Assessment Battery for Children-Second Edition, and the Beery-Buktenica Developmental Test of Visual-Motor Integration - Sixth Edition were the main measures of interest. The volume of the precentral gyrus was significantly correlated with visual motor integration and motor abilities. Motor skills were also associated with cerebellum, and brainstem volumes. This work is timely and builds on other recent work exploring motor/VMI deficits in preterms. The strength of the study is the use of neonatal MRI to predict later neurodevelopment as opposed to concurrent neurodevelopment and MRIs in preterms. Limitations include the lack of controls and lack of multiple comparisons corrections. Both limitations are acknowledged and are not fatal flaws. I have some minor comments below.

I'm a little confused on how two different image analysis methods were used. Can the author's clarify? It seems like DARTEL was used to calculate jacobian modulated VBM images and the neonatal AAL atlas was used to define regions to extract volume (defined by jacobian modulated vbm values) from.

Reply: We apologise for being unclear on this matter.
We have now specified in the paper that we used two separate methodologies: the atlas-based segmentation and the automatic segmentation
In the atlas-based segmentation we used a 90-regions anatomical atlas provided by Shi (Shi et al PlosONe 2011; Infant brain atlases from neonates to 1- and 2-year-olds) to define regions by coregistration methods. After that, the volume of each region was determined by using a proper script written in MATLAB selecting the region of interest via its voxel value.

In automatic segmentation the tissue class images created during segmentation were used to generate a custom template to improve coregistration using DARTEL (AShburner 2007) After this step the images were modulated via SPM 8 software.
We have added information to clarify the methodologies used in the manuscript in Methods, page 9-10:

For atlas-based segmentation:
"The volume of each region was determined by using a proper script written in MATLAB selecting the region of interest via its voxel value."
For automatic segmentation:
"We used the tissue class images created during segmentation to generate a custom template to improve coregistration using Diffeomorphic Anatomical Registration Through an Exponential Lie

algebra algorithm (DARTEL) (Ashburn 2007). After this step the images were modulated via SPM 8 software to improve the inter-subject registration."

I would add that the results are not corrected for multiple correction in the figure and table captions (ie Figure 3/Table S3) in addition to the text already in the manuscript. This help avoid any confusion to a reader who may skim the methods and focus on the figures/tables.

Reply: This has been added in the tables and figures as proposed.
In the footnotes of Suppplementary Table 3 and in Figure 2 we have added:
"Results are presented without correction for multiple comparisons."

VERSION 2 – REVIEW

REVIEWER	Dustin Scheinost Yale School of Medicine, USA
REVIEW RETURNED	02-Jan-2018

GENERAL COMMENTS	The authors have addressed my comments.