Author's Response
to Autism and the amygdala: commentary on Hewitson and coauthors (2010)

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We welcome the opportunity to respond to the issues raised in the commentary of Drs. Novella and Hines regarding our paper examining amygdala growth and opioid ligand binding in infant rhesus macaques (Hewitson et al. 2010). This paper was part of a pilot study performed at the University of Pittsburgh from 2004-2007 that included 16 non-human primate infants, based on the recommendations made by the World Health Organization (WHO) for primate vaccine safety studies (WHO 1994). The pilot study was performed in order to determine the feasibility of undertaking a larger, comprehensive multi-disciplinary study assessing non-human primate infant development in response to different vaccination regimens. Based on the successful completion of the pilot study, the follow-up study now includes a total of 72 animals over 5 years and is currently on-going. We anticipate that the results of this study will be available in 2012.

Drs. Novella and Hines question the number of animals used in the analyses of the neuroimaging data collected as part of this pilot study. The animal numbers were lower than originally designed due to unanticipated equipment failures, a scheduling error, and health problems in two animals in the vaccinated group. Sixteen infants were scheduled to undergo an MRI and PET scan at approximately 4 and 6 months of age, representing a complete longitudinal imaging dataset for each animal. Of the 16 animals, 4 were assigned to the unexposed (unvaccinated) group, with the remaining 12 animals in the exposed (vaccinated) group. Due to a scheduling error, one infant in the unexposed group

inadvertently received a vaccine and had to be removed from the study. Of the remaining 3 animals, one dataset was reduced to a single imaging session due to equipment malfunction (a 5 mCi ^{57}Co transmission source did not retract during collection of the emission scan). Among the 12 animals in the exposed group, complete imaging datasets were only available for 9 animals. Two exposed animals could not undergo scanning procedures at 6 months of age as they had repeated diarrhea that was refractory to antibiotics, causing problems with dehydration. One other exposed animal underwent both scanning procedures but in the second scan, a communication malfunction between the PET scanner and the acquisition console prevented PET list-mode data from being captured. The experience of these unanticipated issues is precisely the rationale for performing pilot studies. Nonetheless, the final availability of only nine complete longitudinal datasets for analyses was clearly specified in the manuscript on page 150, paragraph 2 and was not a major issue in peer review.

With regard to the use of multiple statistical analyses, this study employed Sequential Bonferroni correction which specifically corrects for Type One errors associated with multiple comparisons. This was described in detail in the paper (page 153, paragraph 1). Drs. Novella and Hines suggestion that we placed "selective emphasis on the significant results…..while negative findings tend to be ignored" is curious. Both significant and non-significant findings were reported in detail within the Results section; it is standard practice to discuss any statistically significant results in line with the proposed hypothesis being tested.

Amygdala volume was very similar between exposed and unexposed animals at Time One (4 months of age). By Time Two (6 months of age), amygdala volume in

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exposed animals had increased by 22% compared with a decrease of 25% in unexposed animals, although this difference was not statistically significant. As this was a longitudinal study, it was the trajectory of change over time that was captured by the interaction term revealing a highly significant effect of time when exposure is taken into account. Cross-sectional analyses do not take this into account, which is why it is so important to perform longitudinal analyses for this type of data (Singer and Willet 2003).

In regards to our previously published IMFAR abstract of this work (Hewitson et al. 2008), an error was introduced into the abstract prior to submission, which should have read: "Compared with exposed animals, unexposed animals showed attenuation of amygdala growth and differences in amygdala binding of [¹¹C]diprenorphine." However, the actual data indicating the attenuation of amygdala growth in unexposed animals was presented in the oral presentation at IMFAR. A copy of the relevant slides from this presentation is available upon request. We thank Drs. Novella and Hines for the opportunity to correct this abstract erratum, as no formal mechanism for doing so exists through INSAR, but emphasize that there was in fact no inconsistency in our data presented orally at IMFAR and in Hewitson and coauthors (2010).

Drs. Novella and Hines are incorrect in their commentary that the results from Payne and others (2010) directly contradict our findings. While there is no doubt that, based on modeled developmental trajectories, the size of the primate amygdala increases overall from 1 week to 2 years of age (Payne et al. 2010; Fig. 6), Fig. 3E presents the actual volumetric analyses of amygdala volume in male infants, and this requires further discussion. In their study, Payne and others (2010) performed a total of 29 scans on 5 male infants over two years. However, only 2 males were scanned between 4 and 6 months of age (Table I) representing the same time interval and sample size as our study. Close scrutiny of Fig. 3E shows that amygdala growth was not uniform in the Payne and colleagues study. Thus, it is possible that during early macaque neurodevelopment periods of slowed, and even attenuated, amygdala growth may occur. Clearly, additional longitudinal studies of amygdala volume in a larger group of male macaques will be necessary to determine whether the attenuation of amygdala growth observed by both Payne and coworkers (2010) and Hewitson and her group (2010) occurs during the natural course of infant primate development or whether animal variability or problems with scan segmentation can account for the observed variation in amygdala growth.

In regards to our observation that there was a decrease in opioid ligand binding in unexposed animals with no change in the exposed animals, Drs. Novella and Hines suggest that the lack of change in the exposed animals cannot be interpreted as "abnormal" and that we give "no basis for this assumption". This comment is puzzling – as with any experiment, one typically assumes that statistically significant differences between an 'exposed' and "unexposed" group are driven by the "exposure", regardless of the direction of outcomes observed. Furthermore, we provided several plausible explanations for the apparent absence of change in amygdala opioid ligand binding in exposed versus unexposed animals (page 161, paragraph 5 and page 162, paragraph 1).

The conclusions remain the same as stated in our paper: 1) as a pilot study, the size of the study groups limits the strength of the conclusions that can be drawn; and 2) the results of this pilot study warrant additional research into the potential impact of vaccines on infant brain structure and function. Because of the public health significance, we are optimistic that our continued research, and that of other groups, will in time, provide these answers.

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