Sex and Sensitive Period Differences in Potential Effects of Maltreatment on Axial Versus Radial Diffusivity in the Corpus Callosum

Kyoko Ohashi, Ph.D., Carl M. Anderson, Ph.D., Alaptagin Khan, MBBS , Michael L. Rohan, Ph.D., Elizabeth A. Bolger, M.A., Cynthia E. McGreenery, Martin H. Teicher, M.D., Ph.D.

Supplemental Material

Subject Recruitment

Participants were medically healthy, right-handed, unmedicated (except for birth control pills, hormonal replacement therapy or as needed use of asthma inhaler, non-sedating antihistamines or NDSAIDs) and between 18-25 years of age. Individuals with a history of neurologic disease, or who had experienced concussion or head trauma resulting in loss of consciousness for more than 5 minutes were excluded, as were individuals who were born prematurely (less than 37 weeks or 2 kg), had fetal exposure to alcohol or drugs, were consuming alcohol more than 15 days per month or ever use of heroin, cocaine or methamphetamine. Individuals were also excluded if they experienced multiple forms of adversity unrelated to maltreatment (e.g., motor vehicle accidents, natural disaster, muggings, near drowning). Potential participants completed a detailed online assessment and they were selected based on history of maltreatment to increase the number of participants exposed to three or more types of maltreatment, to help ensure that information could be extracted on consequences of exposure to all types of maltreatment at nearly all ages. Maltreated participants

were enrolled without regard to psychiatric history to constitute a representative sample since selecting subjects for any specific disorder or for none could bias the results by including the most affected or resilient subjects. Subjects received $25 for completing the online assessment, $100 per interview and assessment session and $100 for a one-hour MRI protocol.

MRI Data Acquisition and Image Preprocessing

MRI scans were acquired using 3T Siemens TIM Trio (Erlangen, Germany) using previously reported methods (32, 33). Briefly, Multiple diffusion-weighted images were acquired in 72 directions. Scan parameters were: b=1000 sec/mm2; echo time (TE)/repetition time (TR)=81 msec/6sec; matrix=128 x 128 on 240 mm x 240 mm field of view (FOV); slices 3.5 mm without gap. A high- resolution three-dimensional T1-weighted image (TR time = 2,100 ms, TE = 2.25 ms, flip angle = 12°, 1.0 × 1.0 × 1.3 mm voxel size, 3D matrix 256 x 256 x 170 mm field of view, 128 repetitions) was also collected for anatomical reference.

Statistical analysis of sensitive exposure periods

The most important cross-validated type and time risk factors were identified associated with diffusivity measures using random forest regression (RFR) with conditional inference trees (cforest in R package party; http://party.r-forge.r-project.org/), a form of artificial intelligence analytics that has been reported to be resistant to collinearity, which we have used in prior sensitive period studies (44, 46, 50-55).

Selection of this analytical approach was based on a large series of Monte-Carlo simulations using actual exposure data and simulated outcomes (predicting 5-10% of the total variance) which showed that RFR with conditional inference trees (RFR-CIT) most accurately identified the type and timing of maltreatment used to generate the outcomes. For these analyses, the random forest was trained using data from 63.3% of the

participants and evaluated on the withheld test set (36.7%). At least 5% of the sample needed to report some degree of exposure to the type / time risk factor for it to be included as a predictor variable. We use a variant of Brieman's approach with conditional inference trees (57) that rectifies a problem in the estimation of importance of predictors with many versus few levels or categories (57). This process was repeated 50 times with different splits between training and test sets to derive mean measures of VI for each variable. To gauge significance, the overall process was then repeated 1000 times using reshuffled diffusivity values to calculate chance mean and SD importance levels for each variable. The significance of the z test difference between observed and chance VI measures for each variable was calculated and adjusted using Bonferroni correction to control for multiple comparisons.

Participants

Table S1

General demographic information on participants

N	345
Age (years)	21.6 ± 2.5
Subjects Education (years)	14.7 ± 2.1
Parental Education (years)	15.7 ± 3.2
Financial sufficiency during childhood	
Much less than enough money	5%
Less than enough money	20%
Enough money	42%
More than enough money	29%
Much more than enough money	3%
Race	
White	68%
Asian	15%
Black	9%
American Indian/Alaska Native/Hawaiian	2%
Other	5%
Hispanic Ethnicity	16%

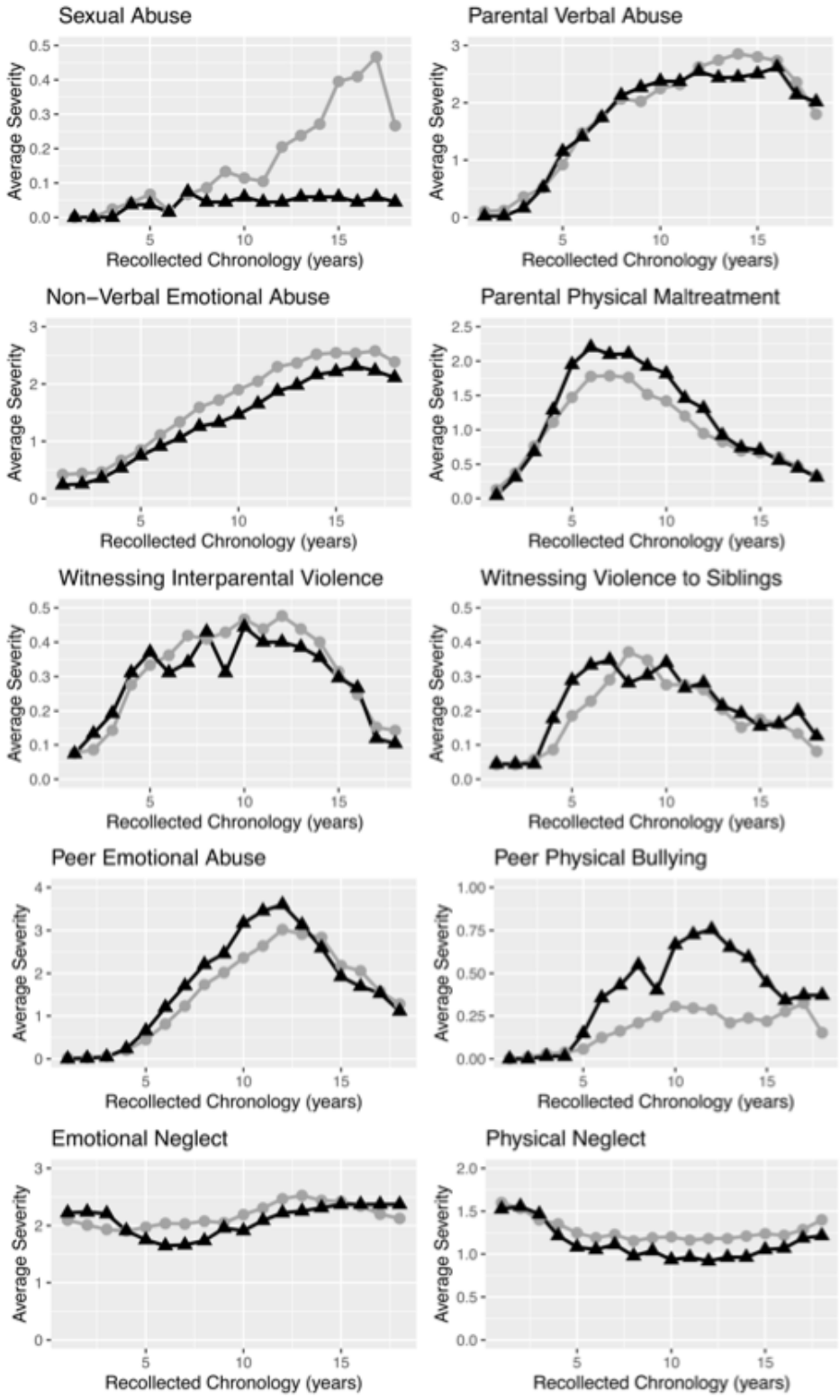

Figure S1. Type and timing of childhood maltreatment.

Time course of the severity of exposure to different types of maltreatment in males (black) and females (gray)

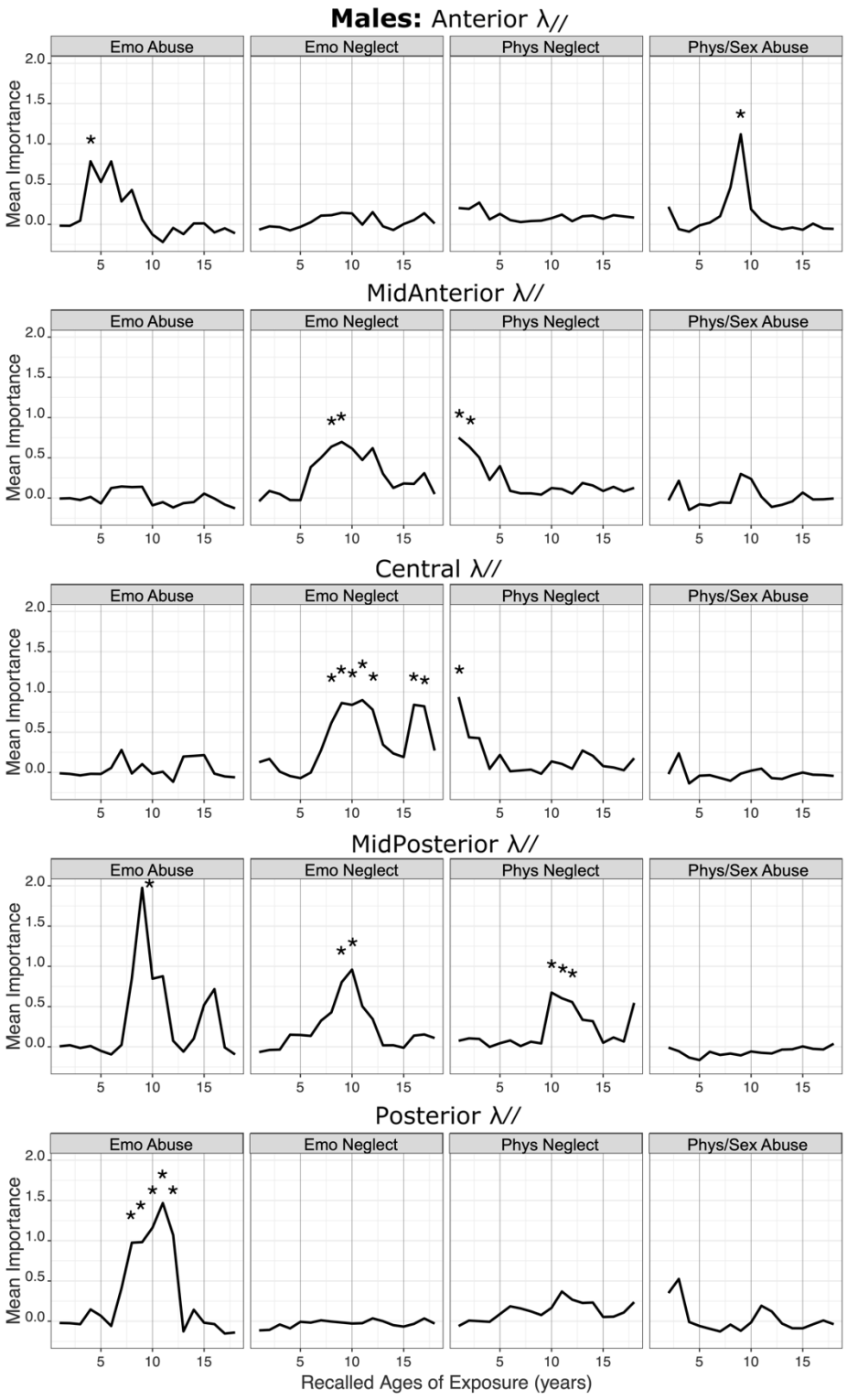

Figure S2. Results of the random forest regression analysis for measures of $\lambda_{//}$ with 4 predictors in males. Emotional (Emo) abuse consisted of exposure to parental non-verbal emotional abuse, parental verbal abuse, witnessing interparental violence, witnessing violence to siblings and peer emotional abuse. Phys/Sex abuse included parental physical abuse, peer physical bullying and sexual abuse.

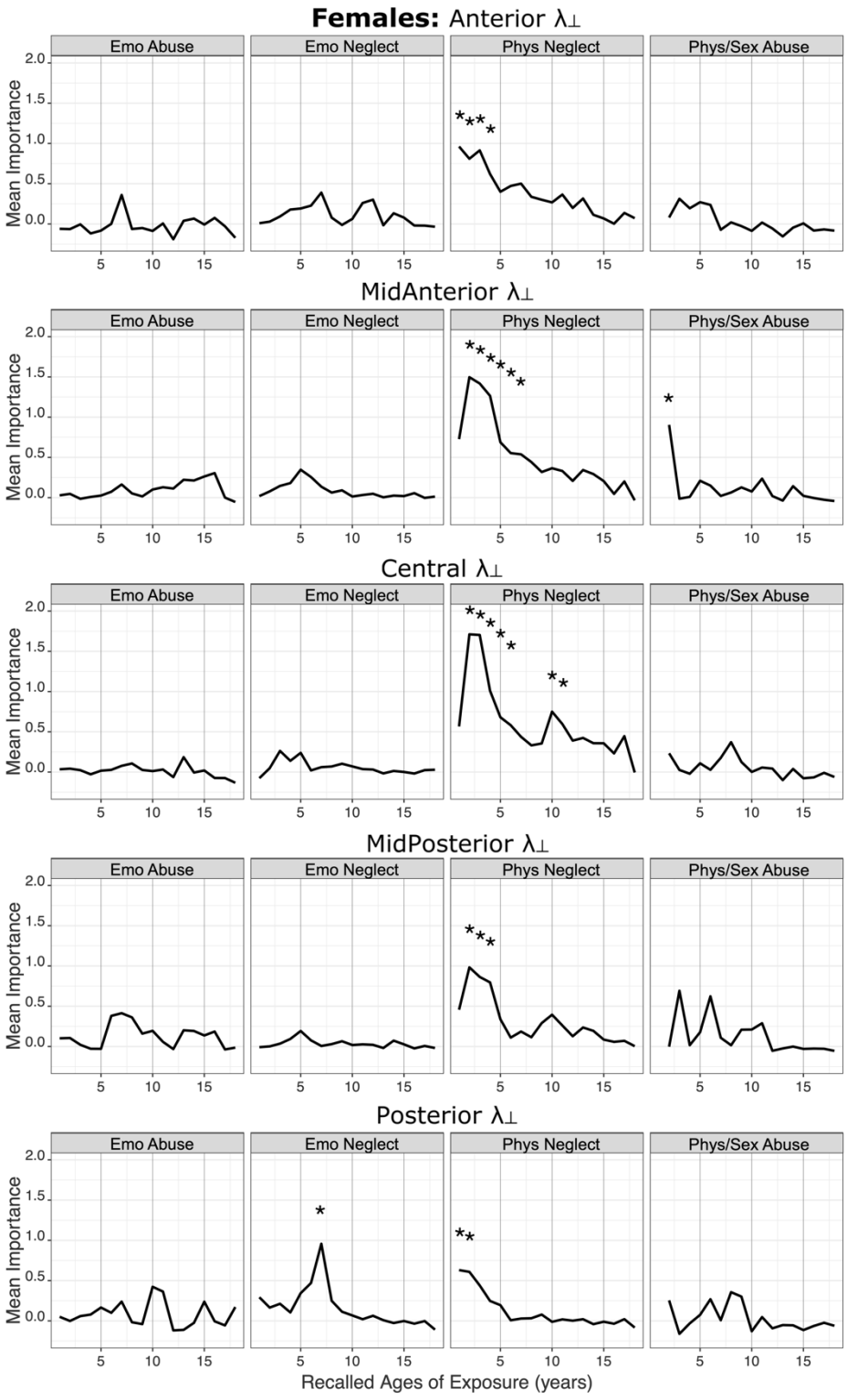

Figure S3. Results of the random forest regression analysis for measures of $\lambda_{\perp}$ with 4 predictors in females.

Further Discussion

We had previously proposed that sensitive exposure periods emerge because maltreatment tends to target brain structures undergoing rapid developmental change (19, 66). One way to interpret this is that processes undergoing rapid change may be more susceptible to disruption by stress. However, we do not believe that stress is damaging the brain, rather we suspect that it is fostering specific phenotypic adaptations and that these adaptations are occurring primarily through modifications to developmental processes occurring at these times. Consequently, these changes would likely occur in females through alterations in myelination and in males through alterations in axonal diameter.

Another important observation was that PN was often associated with alterations in diffusion parameters that were opposite to the effects of other types of maltreatment. These findings are consonant with a number of studies showing differential effects of neglect (particularly physical neglect or social deprivation) from effects of abuse (or threat) (66-69).

Longitudinal versus Retrospective Neuroimaging Findings

The closest example of a prospective study with annual assessment of some components of maltreatment (i.e., emotional and physical cruelty to child or mother) is the Avon Longitudinal Study in which a birth cohort (N=494) was prospectively assessed for exposure at 8, 21, 33, 47, 61 and 73 months of age and then neuroimaging at 18-21 years of age (73). Briefly, they found that out of 30 preselected brain regions that the right caudal anterior cingulate and the right precuneus were the only regions that showed a significant relationship with overall severity of exposure to early adversity during the first 6 years (73). We found in our

comparably aged sample using the MACE and RFR-CIT that the most important predictor of right caudal anterior cingulate volume was Phys at age 5 while the most important predictor of right precuneus volume was WIPV at age 5, Phys at ages 6 and 7 and peer physical bullying at age 13. In contrast, left caudal anterior cingulate was not significantly predicted by type and timing of maltreatment and left precuneus was most importantly predicted by Peer_E at age 12. This fits with their finding of right but not left sided sensitivity of these structures to adversity between 8 and 73 months. Hence, our retrospective approach appears to yield comparable insights but has the advantage of providing even greater detail and does not require 18 years of annual assessments per sample.

Prediction of CC parameters based on type and timing of maltreatment

In general, the RFR-CIT models fit significantly with observed values both for the within bag samples used to derive the fit and the out of bag (OOB) used to assess how well these models might predict CC parameters in a new sample. This was particularly true for measures of RD, FA and MD in females and L1 in males. However, a substantial portion of the fit could be attributed to age, which was often the most significant predictor variable. Hence, we examined how well the models predicted CC parameters partialling out associations with age. In females the strongest predictions were for RD in midposterior (within bag r = .614, $p < 10^{-22}$; OOB r = .537, $p < 10^{-16}$) and FA in midanterior (within bag r = 0.530, $p < 10^{-15}$; OOB r = 0.290, p = .00002). For males the best predictions were for L1 in posterior (within bag r = 0.802, $p < 10\text{-}^{30}$; OOB r = 0.181, p = 0.037) and midposterior (within bag r = 0.722, $p < 10^{-21}$; OOB r = 0.173, p = 0.047) segments. In short, the type and time risk factors identified for the most strongly influenced CC parameters appeared to have a significant degree of predictive value.

Additional Limitations

Another limitation is that we followed the strategy used by FreeSurfer and divided the CC into 5 equally long segments in the anterior-posterior plane. This approach may make it difficult to identify potential effects of maltreatment on segmental volume as alterations in a specific segment may be obscured by this approach, which limits regionally specific differences in volume to alterations in thickness and width. An alternative strategy would have been to use DTI and/or functional connectivity to divide the CC into more anatomically or physiologically specific segments (74, 75).

There are also fundamental problems in endeavoring to provide insights about potential differences in myelination and axonal parameter using DTI, since the resolutions of images are mostly in the millimeter range versus the micrometer range for axons and myelin sheaths. Recently, various imaging modalities have been developed to estimate myelination and studies have shown that other modalities such as myelin water imaging (76), magnetization transfer (77), or combinations of different modalities (78) may better represent myelination than diffusion weighted imaging alone. Similarly, use of ultrahigh magnetic fields (79) and new theoretical models (80) are being developed to quantify axon markers such as axon density and radii, though currently these sequences require hours of scan time and are performed *ex vivo*. In the future new technologies should provide more detailed information on the possible effects of maltreatment during developmental sensitive periods on the microstructure of the CC.