Sensitive periods for white matter plasticity in human

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Abstract

As a child matures, some brain circuits stabilize while others remain plastic. However, the literature on maturational changes in the brain's capacity for experience-dependent plasticity is primarily based on experiments in animals that mature over dramatically different time-scales than humans. Moreover, while principles of plasticity for sensory and motor systems might be conserved across species, the myriad of late-developing and uniquely human cognitive functions such as literacy cannot be studied with animal models. Here we use an intensive reading intervention program, in combination with longitudinal diffusion MRI measurements in school-aged children with dyslexia, to model the sensitive period for white matter plasticity and literacy learning.

Introduction

Younger brains are more plastic. That the brain's capacity for plasticity diminishes with age is commonly held as an axiom of development, and carries important implications for education and the treatment of developmental disorders. For example, developmental dyslexia is rooted in neuroanatomical differences within well-characterized brain circuits [1–6] and interventions intended to remediate these deficits are presumed to be more effective in younger, compared to older, children [7,8].

The theory that plasticity diminishes over development takes different forms, ranging from strict critical periods which define windows of development during which specific experiences can shape the structure of a neural circuit [9–11], to sensitive periods in which circuits exhibit varying degrees of plasticity and propensity for experience dependent change [12,13]. Many aspects of cognition may be subject to broad sensitive periods (e.g., pre-puberty), during which large swaths of cortex remain malleable to environmental demands until rising hormone levels stabilize the relevant circuits [14] (reviewed in [15]). A wealth of research in model organisms including mice and non-human primates has identified cellular changes (e.g., the refinement of ocular dominance columns in primary visual cortex [11,16,17]) that only

occur during isolated developmental windows, and mechanisms (e.g., perineuronal nets [18,19]) that govern the transition from plasticity to stability for a circuit. A wealth of research in humans has identified aspects of learning (e.g., the sound inventory of language [12,20]) that become more difficult with age. However, to our knowledge, there is no data directly linking changes in the human capacity for learning high-level cognitive functions to changes in the brain's capacity for experience-dependent structural plasticity. While it is appealing to assume that the timing of critical/sensitive periods discovered in animal models might generalize to human, there are dramatic differences in the time-course of maturation across species. For example the myelination process is prolonged by more than a decade in humans compared to other primate species, even after adjusting for developmental milestones such as puberty [21]. Therefore, plasticity in humans might not be subject to the same constraints as other species, highlighting the importance of understanding the principles governing plasticity in the human brain.

The two principal challenges to studying experience-dependent plasticity in humans are, first, establishing an experimental paradigm that is appropriate for human research subjects and capable of inducing large-scale structural changes in the brain and, second, developing non-invasive measurements that are sensitive to changes in cellular properties of human brain tissue. Our previous work demonstrated that combining an intensive reading intervention program with longitudinal diffusion-weighted magnetic resonance imaging (dMRI) measurements in children with dyslexia is a powerful paradigm for studying experience-dependent changes in the white matter [22]. In a sample of 24 children between 7 and 13 years of age, eight weeks of targeting training in reading skills caused large-scale changes in tissue properties for multiple anatomical tracts (Cohen's d = 0.5-1.0 across different white matter tracts), that were coupled to large improvements in reading skills (Cohen's d = 0.5-1.0 across different reading tests). Here we capitalize on this paradigm, and a larger sample of subjects (N=34), to test the hypothesis that there is a sensitive period for this circuit and that the amount of intervention-driven plasticity measured in the white matter depends on the age of the subject. Specifically, we consider three hypotheses:

1. Younger subjects (<9 years of age) will show larger intervention-driven changes in diffusion properties compared to older subjects (>9 years of age). The magnitude of change for each subject will be computed as (1) the difference between the pre- and post-intervention measurement sessions and (2) a linear fit summarizing the rate of change in diffusion properties as a function of intervention hours. Baseline reading scores will be considered as a covariate in case there is a difference in plasticity between more and less impaired readers.
2. Younger subjects will show more rapid changes compared to older subjects, irrespective of the absolute magnitude of change. The timescale of change for each subject will be computed as (1) the percentage of overall change that occurs within the first three weeks of the intervention and (2) the growth rate observed between the first 2 sessions.
3. Alternatively, we may observe the same magnitude and time-course of experience-dependent white matter plasticity over this age range (7-13 years of age). Such a finding would imply that, for the training paradigm and age range studied here, age is not a major factor in determining a child's response to intervention, and that the sensitive period for these white matter networks extends from early elementary-school into young adulthood.

Methods

Rationale for pre-registration

Understanding maturational changes in plasticity that occur over the course of elementary school is an important scientific challenge with practical implications for education practice. Our previous work established an experimental paradigm (intensive reading intervention program) and measurement protocol (longitudinal diffusion MRI measurements) for quantifying experience-dependent plasticity in human white matter (Huber et al., 2018*, Nature Communications*, [22]). Based on these data, a power analysis confirms that we have the statistical power to detect meaningful maturational differences in experience-dependent plasticity, if such differences exist. However, if the data indicate that older (middle-school aged) children show the same large-scale changes in white matter as younger (1st and 2nd grade) children, it would substantially revise our preconceived notions about brain maturation, plasticity, and learning in an academic setting. Therefore, the outcome of the proposed investigation will answer fundamental scientific questions irrespective of the results. Hence, we wish to pre-register our work in order to obtain a thorough review of our methodology and reasoning **before analyzing the age-dependence of our previously reported effects**.

We believe that pre-registration is important since our conclusions will depend on how we operationalize our hypotheses and process our data. Hypothesizing after knowing the results, or "HARKing", has recently been highlighted as a major issue contributing to a publication bias in the biomedical and social sciences [23]. Munafo and colleagues maintain that practices such as HARKing are often subconscious and unintentional, reflecting well-known psychological biases that are difficult to avoid. Hence, in line with their perspective, we acknowledge the possibility that our approach to data analysis might change in a manner that is more likely to confirm a statistical relationship between age and plasticity, rather than a lack thereof. While registration prior to data collection is optimal, there are many cases (such as the present one) where it is not feasible. In our case, studying maturational changes in the brain's capacity for plasticity first required establishing the sensitivity of our paradigm, and determining the pattern of changes that are induced by the intervention. Our previous work optimized our analysis pipeline to reliably measure white matter plasticity in an individual, and made pre-registration possible for the present study. Thus, the data acquisition and analysis methods are identical to those reported in Huber et al., (2018), with the addition of 10 new intervention subjects that were run as a replication cohort.

Modeling sensitive periods

A sensitive (or critical) period is a window of development during which a circuit is particularly responsive to environmental inputs [18]. As the balance between excitatory and inhibitory signaling changes, the capacity for plasticity within a circuit increases, marking the onset of a sensitive period. The sensitive period remains open until molecular brakes close the window for plasticity. However, this developmental period need not have a strict boundary, and may instead gradually close, leading to diminshed plasticity over a period of months or years. We therefore model this time-course as a Gaussian function where the mean of the Guassian (μ) marks the age (x) of peak plasticity, the height of

the Gaussian (β) denotes the magnitude of plasticity that is possible, and the width of the Gaussian (σ) corresponds to the window of development during which the sensitive period is open.

$$\beta e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$

If we assume that for literacy the sensitive period is near its peak at the beginning of elementary school when children begin formal reading instruction, then a narrow sensitive period (e.g., σ=1, black curve in **Figure 1a**) would imply that the window for plasticity in the reading circuitry is shut by 3rd or 4th grade, wheras a broad sensitive period (e.g., σ=6, red curve in **Figure 1a**) would imply that the circuit remains plastic through young adulthood. Based on these hypothetical sensitive periods, ranging from σ=1 to σ=6, we simulate our precision for estimating model parameters (**Figure 1b,c**) and detecting a significant correlation between plasticity and age (**Figure 1d**), based on effects reported in Huber et al., (2018).

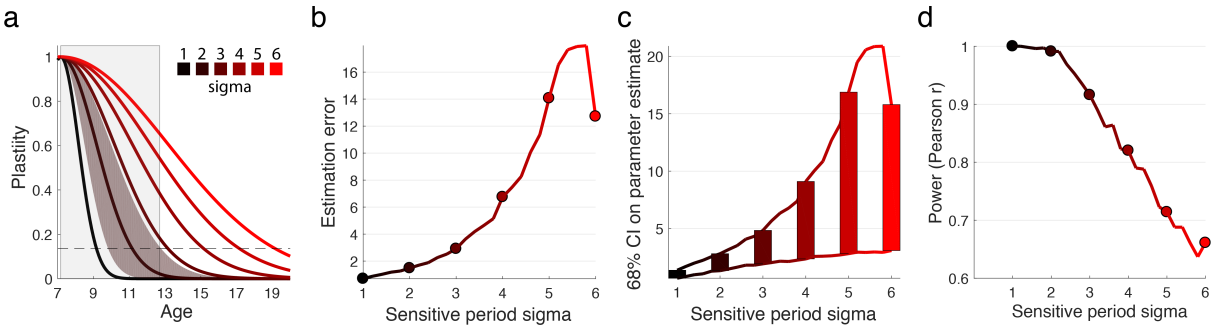

Figure 1: Simulation of sensitive periods. *(a) Sensitive periods are modeled as a Guassian function where the width of the Gaussian (sigma) defines the window of plasticity (see simulateAgeEffect.m). The light gray box denotes the age range of subjects in our study. Simulated data was generated from the Gaussian model by adding noise, where the amount of noise was calibrated based on the session-to-session variability of the measurements in the absenence of intervention (estimated from data in control subjects). Six curves are illustrated for potential models ranging from sigma=1 to sigma=6 and the standard error of the model fit to the simulated data is illustrated for one curve (sigma=2). (b) The average error on the sigma parameter estimate is shown for different potential models given the signal to noise ratio of our data. (c) Confidence intervals are displayed based on models with different sigma parameters. (d) Based on simulating data from different sensitive period models, we calculate the probability of detecting a significant correlation (p < 0.05) between plasticity and age. Simulation code is available at: https://github.com/yeatmanlab/plasticity*

Huber et al., (2018) reported the amount of change measured in the white matter over the course of a tightly controlled and intensive reading intervention program delivered to elementary school children with dyslexia. If we operationalize white matter plasticity as the magnitude of change in mean diffusivity (MD) measured over the course of the intervention program, then we can scale each curve such that the mean value over the measured age range (gray shading **Figure 1a**) is equal to the average MD change reported in the sample. The standard deviation of MD change measured in the control group (which did not undergo the intervention and did not, on average, show any change over the measurement period) is used as an estimate of noise. We then: (1) simulate 10,000 datasets coming from sensitive periods with different σ values ranging from 1 to 6, and add Gaussian noise to each simulated dataset based on the noise estimate in the control group, (2) fit the sensitive period model to each simulated dataset based on non-linear optimization, (3) calculate the Pearson correlation between age and MD change for

each dataset and, (4) calculate the reliability of the fitted parameters and correlation coefficients. This simulation demonstrates that if the sensitive period for plasticity in the reading circuitry closes before 14 years of age, then we would have a high liklihood of detecting a significant correlation (>90%), and would be able to accurately estimate parameters of the sensitive period model. If the sensitive period extends through adolecence, then the upper bound on our parameter estimates would be unreliable. For example, if the true sensitive period is σ=5, meaning that the window for plasticity closes around 17 years of age, then we would still be able to infer that the sensitive period is open through elementary school (lower bound on confidence interval), but could not reliably infer the upper bound.

Participants

The intervention group included 34 children (14 female), ranging in age from 7 to 12 years, who participated in an intensive summer reading program. Subjects were recruited based on parent report of reading difficulties and/or a clinical diagnosis of dyslexia. A total of 132 behavioral and MRI sessions were carried out with this group.

An additional 66 behavioral and MRI sessions were conducted in a non-intervention control group with 25 participants, who were matched for age but not reading level. These subjects were recruited as a control group to assess the stability of our measurements over the repeated sessions and this stability estimate was used as an estimate of noise in our simulations (**Figure 1**). Control subjects participated in the same experimental sessions, but did not receive the reading intervention. Twelve of these control subjects had typical reading skills (5 female), defined as a score of 85 or greater on the Woodcock Johnson Basic Reading composite and the TOWRE Index. All subjects completed a battery of reading tests prior to the intervention period to confirm parent reports and establish a baseline for assessing growth in reading skill.

All participants were native English speakers with normal or corrected-to-normal vision and no history of neurological damage or psychiatric disorder. We obtained written consent from parents, and verbal assent from all child participants. All procedures, including recruitment, consent, and testing, followed the guidelines of the University of Washington Human Subjects Division and were reviewed and approved by the UW Institutional Review Board.

Reading intervention

24 intervention subjects reported in Huber et al., 2018 were enrolled in 8 weeks of the *Seeing Stars: Symbol Imagery for Fluency, Orthography, Sight Words, and Spelling*[24] program at three different *Lindamood-Bell Learning Centers* in the Seattle area. An additional replication cohort of 10 subjects was run using the exact same training protocol at the University of Washington campus. The intervention program consists of directed, one-on-one training in phonological and orthographic processing skills, lasting four hours each day, five days a week. The curriculum uses an incremental approach, building from letters and syllables to words and connected texts, emphasizing phonological decoding skills as a foundation for spelling and comprehension. A hallmark of this intervention program is the intensity of the training protocol (4 hours a day, 5 days a week) and the personalized approach that comes with one-on-one instruction.

Experimental Sessions

Subjects participated in four experimental sessions separated by roughly 2.5-week intervals. For the intervention group, sessions were scheduled to occur before the intervention (baseline), after 2.5 weeks of intervention, after 5 weeks of intervention, and at the end of the 8-week intervention period. For the control group, sessions followed the same schedule while the subjects attended school as usual. This allowed us to control for changes that would occur due to typical development and learning during the school year.

In addition to MRI measurements, described in greater detail below, we administered a battery of behavioral tests in each experimental session. These included sub-tests from the Wechsler Abbreviated Scales of Intelligence (WASI), Comprehensive Test of Phonological Processing (CTOPP-2), Test of Word Reading Efficiency (TOWRE-2) and the Woodcock Johnson IV Tests of Achievement (WJ-IV).

MRI Acquisition and Processing

All imaging data were acquired with a 3T Phillips Achieva scanner (Philips, Eindhoven, Netherlands) at the University of Washington Diagnostic Imaging Sciences Center (DISC) using a 32-channel head coil. An inflatable cap was used to minimize head motion, and participants were continuously monitored through a closed circuit camera system. Prior to the first MRI session, all subjects completed a session in an MRI simulator, which helped them to practice holding still, with experimenter feedback. This practice session also allowed subjects to experience the noise and confinement of the scanner prior to the actual imaging sessions, to help them feel comfortable and relaxed during data collection.

Diffusion-weighted magnetic resonance imaging (dMRI) data were acquired with isotropic 2.0mm^3 spatial resolution and full brain coverage. Each session consisted of 2 DWI scans, one with 32 non-collinear directions (b-value = 800 s/mm^2), and a second with 64 non-collinear directions (b-value = 2,000 s/mm^2). Each of the DWI scans also contained 4 volumes without diffusion weighting (b-value = 0). In addition, we collected one scan with 6 non-diffusion-weighted volumes and a reversed phase encoding direction (posterior-anterior) to correct for EPI distortions due to inhomogeneities in the magnetic field. Distortion correction was performed using FSL's *topup* tool [25,26]. Additional pre-processing was carried out using tools in FSL for motion and eddy current correction, and diffusion metrics were fit using the diffusion kurtosis model [86] as implemented in DIPY [27]. Data were manually checked for imaging artifacts and excessive dropped volumes. Given that subject motion can be especially problematic for the interpretation of group differences in DWI data, data sets with mean slice-by-slice RMS displacement > 0.7mm were excluded from all further analyses. Datasets in which more than 10% of volumes were either dropped or contained visible artifact were also excluded from further analysis.

White Matter Tract Identification

Fiber tracts were identified for each subject using the Automated Fiber Quantification (AFQ) software package [28], after initial generation of a whole-brain connectome using probabilistic tractography (MRtrix 3.0) [29,30]. Fiber tracking was carried out on an aligned, distortion corrected, concatenated dataset including all four of the 64-direction (b-value = 2,000 s/mm^2) datasets collected across sessions for each subject. This allowed us to ensure that estimates of diffusivity and diffusion anisotropy across session

were mapped to the same anatomical location for each subject, since slight differences in diffusion properties over the course of intervention can influence the region of interest that is identified by the tractography algorithm.

Quantifying White Matter Tissue Properties

To detect intervention-driven changes in the white matter, we fit the diffusion kurtosis model as implemented in DIPY to the diffusion data collected in each session [31,32]. The diffusion kurtosis model is an extension of the diffusion tensor model that accounts for the non-Gaussian behavior of water in heterogeneous tissue containing multiple barriers to diffusion (cell membranes, myelin sheaths, etc.). After model fitting, diffusion metrics were projected onto the segmented fiber tracts generated by AFQ. Selected tracts were sampled into 100 evenly spaced nodes, spanning termination points at the gray-white matter boundary, and then diffusion properties (mean, radial, and axial diffusivity (MD, RD, AD) and fractional anisotropy (FA)) were mapped onto each tract to create a "Tract Profile".

Statistical Analysis

Data analysis was carried out using software written in MATLAB. To assess change over the course of intervention, we first averaged the middle 60% of each tract to create a single estimate of diffusion properties for each subject and tract. We selected the middle portion to eliminate the influence of crossing fibers near cortical terminations, and to avoid potential partial volume effects at the white matter / gray matter border. Mean tract values were then entered into a linear mixed effects model, with fixed effects of intervention time (either hours of training, or session entered as a categorical variable) and a random effect of subject. Code and data from Huber et al., (2018) can be accessed from the GitHub repository: https://github.com/yeatmanlab/Huber_2018_NatCommun.

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