

RESEARCH ARTICLE*Neural Circuits*

Laminar pattern of adolescent development changes in working memory neuronal activity

Junda Zhu,¹ Benjamin M. Hammond,¹  Xin Maizie Zhou,^{2,3} and  Christos Constantinidis^{1,2,4}¹Program in Neuroscience, Vanderbilt University, Nashville, Tennessee, United States; ²Department of Biomedical Engineering, Vanderbilt University, Nashville, Tennessee, United States; ³Department of Computer Science, Vanderbilt University, Nashville, Tennessee, United States; and ⁴Department of Ophthalmology and Visual Sciences, Vanderbilt University Medical Center, Nashville, Tennessee, United States**Abstract**

Adolescent development is characterized by an improvement in cognitive abilities, such as working memory. Neurophysiological recordings in a nonhuman primate model of adolescence have revealed changes in neural activity that mirror improvement in behavior, including higher firing rate during the delay intervals of working memory tasks. The laminar distribution of these changes is unknown. By some accounts, persistent activity is more pronounced in superficial layers, so we sought to determine whether changes are most pronounced there. We therefore analyzed neurophysiological recordings from the young and adult stage of male monkeys, at different cortical depths. Superficial layers exhibited an increased baseline firing rate in the adult stage. Unexpectedly, we also detected substantial increases in delay period activity in the middle layers after adolescence, which was confirmed even after excluding penetrations near sulci. Finally, improved discriminability around the saccade period was most evident in the deeper layers. These results reveal the laminar pattern of neural activity maturation that is associated with cognitive improvement.

NEW & NOTEWORTHY Structural brain changes are evident during adolescent development particularly in the cortical thickness of the prefrontal cortex, at a time when working memory ability increases markedly. The depth distribution of neurophysiological changes during adolescence is not known. Here, we show that neurophysiological changes are not confined to superficial layers, which have most often been implicated in the maintenance of working memory. Contrary to expectations, substantial changes were evident in intermediate layers of the prefrontal cortex.

*adolescence; cerebral cortex; layer; prefrontal cortex; working memory***INTRODUCTION**

Working memory, the ability to maintain and manipulate information in the mind over a timescale of seconds, improves during the course of adolescence (1–4). Anatomical and functional changes in a network of cortical areas including the dorsolateral prefrontal cortex take place during this time and are predictive of performance gains in working memory tasks (5–10). Most importantly, cortical volume and thickness of the frontal lobe change considerably during adolescent development, with an overall pattern of decreased thickness evident in adulthood, thought to represent the pruning of unwanted synapses (11, 12). Neurodevelopmental

disorders that manifest themselves in late adolescence, most notably schizophrenia, are characterized by deficits of working memory and corresponding aberrations in dorsolateral prefrontal cortex function and thickness (13–16).

Over the past few years, it has become possible to study the development and maturation of neuronal responses in the prefrontal cortex of nonhuman primates between the time of adolescence and adulthood (17, 18). Male rhesus monkeys (*Macaca mulatta*) enter puberty at ~3.5 yr of age and reach full sexual maturity at age of 5 yr, aging roughly three times faster than humans (19, 20). However, some debate exists on whether developmental changes at earlier monkey ages correspond to human adolescent maturation markers (21, 22).



Behavioral and neurophysiological recordings obtained two years apart around this time have revealed substantial differences in activity and response properties of neurons between the adolescent and adult prefrontal cortex (23, 24). Most importantly, these include a higher persistent firing rate in the delay periods of working memory tasks (23).

The laminar pattern of neurophysiological response changes has not been investigated until now. Strong anatomical (25, 26) and physiological evidence (27) suggest that superficial layers of the prefrontal cortex play a critical role in the generation of persistent activity and maintenance of working memory. Many previous studies of working memory have focused predominantly on superficial layers for this reason (28). However, there has also been evidence that deep-layer neurons contribute to this activity (29). We were therefore motivated to examine neuronal responses obtained at different cortical depths and investigate the laminar maturation of the prefrontal cortex between developmental stages in a nonhuman primate model of working memory.

MATERIALS AND METHODS

Four male rhesus monkeys (*M. mulatta*) were used in this study. All surgical and animal use procedures were reviewed and approved by the Wake Forest University Institutional Animal Care and Use Committee, in accordance with the U. S. Public Health Service Policy on humane care and use of laboratory animals and the National Research Council's Guide for the care and use of laboratory animals.

Developmental Profiles

We tracked the developmental measures of monkeys on a quarterly basis before, during, and after neurophysiological recordings, as we have documented in detail previously (23, 24). We obtained morphometric measures including body weight, crown-to-rump length, chest circumference, ulna and femur length, testicular volume, and eruption of canines. We additionally measured bone maturation by X-rays of the upper and lower extremities and assayed serum concentration of circulating hormones including testosterone and dihydrotestosterone. Using these measures, we determined the approximate onset of puberty, characterized by a spurt in growth, eruption of canines, and sexual hormonal changes (23, 24). Behavioral and neurophysiological recordings were collected at two stages: mid-adolescence ("young stage") and adulthood ("adult stage"), with a 1.6- to 2.1-yr gap. Median ages at the mid-adolescent and adulthood stages were 4.3 and 6.3 yr, respectively.

Behavioral Tasks

The monkeys were trained to perform an oculomotor delayed response (ODR) task during the young stage (Fig. 1). Once the animals had reached asymptotic performance, neuronal recordings were obtained (described in *Surgery and Neurophysiology*). At the conclusion of these recordings, the animals were returned to their colony and were no longer tested or trained in any task for a period of ~1 yr. A new period of testing and recording was performed in the adult stage.

The ODR task is a spatial working memory task requiring subjects to remember the location of a cue stimulus flashed

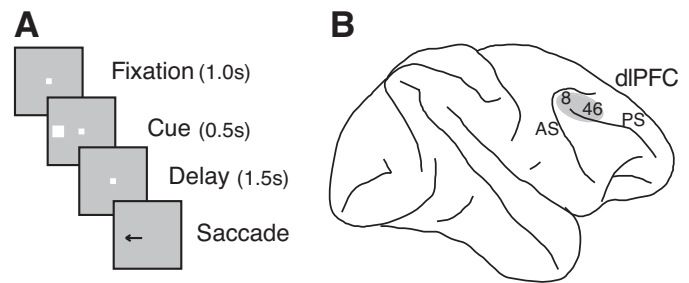


Figure 1. A: sequence of events in the oculomotor delayed response (ODR) task. B: schematic diagram of the monkey brain with approximate location of neuronal recordings in areas 8 and 46 of the dorsolateral prefrontal cortex (dlPFC) highlighted. AS, arcuate sulcus; PS, principal sulcus.

on a screen for 0.5 s. In this study, the cue was a 1° white square stimulus that could appear at one of eight locations arranged on a circle of 10° eccentricity. After a 1.5-s delay period, the fixation point was extinguished, and the monkey was trained to make an eye movement to the remembered location of the cue within 0.6 s. The saccade needed to terminate on a 5°–6° radius window centered on the stimulus (within 3°–4° from the edge of the stimulus), and the monkey was required to hold fixation within this window for 0.1 s.

Animals were rewarded with fruit juice for the successful completion of a trial. Eye position was monitored with an infrared eye tracking system (ISCAN, RK-716; ISCAN, Burlington, MA). Breaking fixation at any point before the offset of the fixation point aborted the trial and resulted in no reward. Visual stimuli display, monitoring of eye position, and the synchronization of stimuli with neurophysiological data were performed with in-house software (30) implemented on the MATLAB environment (MathWorks, Natick, MA).

Surgery and Neurophysiology

Once the animals had reached asymptotic performance in the behavioral tasks, a 20-mm diameter recording cylinder was implanted over the prefrontal cortex of each animal. Localization of the recording cylinder and of electrode penetrations within the cylinder was based on MR imaging, processed with the BrainSight system (Rogue Research, Montreal, QC, Canada). Recordings were collected with epoxylite-coated Tungsten electrodes with a diameter of 250 μm and an impedance of 4 MΩ at 1 kHz (FHC Bowdoin, ME). Electrical signals recorded from the brain were amplified, band-pass filtered between 500 and 8 kHz, and stored through a modular data acquisition system at 25-μs resolution (APM system, FHC, Bowdoin, ME). Recordings were obtained and analyzed from areas 8a and 46 of the dorsolateral prefrontal cortex. After reaching adulthood, as determined by the developmental indices described earlier, the animals were again tested in the same tasks that they were originally trained. A new phase of recordings was then performed from the same areas using identical recording methods.

Neural Data Analysis

Recorded spike waveforms were sorted into separate units using a semiautomated cluster analysis method based on the

KlustaKwik algorithm. The firing rate of units was then determined by averaging spikes in each task epoch. We identified neurons with significant elevation of firing rate in the 500 ms presentation of the cue, the 1,500-ms delay period, and the 250-ms response epoch, after the offset of the fixation point. The firing rate in this period was compared with the 1-s baseline fixation period, before the presentation of the cue, and neurons with a significant difference in firing rate were identified (paired t test, $P < 0.05$). The location of the receptive field was determined based on responses in the ODR task. Neurons that did not respond with an elevation of firing rate during the ODR task, and for which the receptive field could not be determined, were excluded from the analysis.

Population peristimulus time histograms (PSTHs) were constructed by averaging the responses of multiple neurons. Statistical comparisons in the ODR task involved firing rate distributions recorded during the (baseline) fixation, cue, and delay periods of the task. We used a permutation test using resampling to test the null hypothesis that firing rates of neurons at the young and adult stages came from the same distribution. We randomly reassigned the group labels (young or adult) in 1,000 iterations in each test and compared the observed difference to this distribution of permuted differences. We used a three-way ANOVA to compare the influence of factors of young/adult stage, cue location, and depth group on the neuron's firing rate and spatial tuning.

Receiver operating characteristic (ROC) analysis was performed, comparing the distribution of responses to the best location and the location diametric to it. The area under the ROC curve represents the probability that an ideal observer can discriminate between a stimulus appearing in the overall best and diametric location firing rate in each trial, based on the relative difference in firing rate between the two stimulus conditions. The analysis was performed for spikes recorded during the entire cue period and delay period, and in a time-resolved fashion, in sliding 50 ms bins, stepped every 50 ms. Statistical analyses of neural data were performed in MATLAB (2022b) and Python 3.8.

RESULTS

Four male macaque monkeys (*M. mulatta*) were initially trained to perform the ODR task (Fig. 1A) during adolescence. Neurophysiological recordings were obtained from areas 8a and 46 of the dorsolateral prefrontal cortex after the onset of puberty (which we refer to as the “young” stage) and in adulthood (“adult” stage). The time of puberty was determined based on morphometric, radiographic, and hormonal measures as documented previously (23). One round of neurophysiological recordings was obtained after the onset of puberty, at a median age of 4.3 yr (range: 4.0–5.2 yr). This experimental stage lasted 3–6 mo. Monkeys were then returned to their colony and received no further training or exposure to any behavioral task for ~1 yr. The monkeys were briefly re-introduced to the task and a second round of recordings was obtained. The median age of animals at the onset of the second stage of experiments was 6.3 yr (range of ages: 5.6–7.3; range of intervals from young stage: 1.6–2.1 yr).

Distribution of Recordings

A total of 607 neurons were recorded from areas 8a and 46 of the dorsolateral prefrontal cortex in the young stage, and 830 neurons were recorded in the adult stage from the same monkeys. We identified 309 neurons in the young stage and 322 neurons in the adult stage that responded significantly to at least one visual stimulus during the ODR task compared with baseline activity (evaluated with a paired t test at the $P < 0.05$ level) and selected these neurons for further analysis.

For each electrode penetration, we noted the position where neural activity was first identified (top of the cortex). The depth of each isolated neuron was recorded relative to the top of the cortex, defined in this fashion. We grouped neurons into three groups depending on the depth they were recorded: superficial (0–800 μm), middle (800–1,200 μm), and deep (>1,200 μm). Of task-responsive neurons, 191 neurons at the young stage and 212 neurons at the adult stage were classified as superficial; 71 neurons at the young stage and 54 neurons at the adult stage were classified as recorded at middle depths; and 47 neurons at the young stage and 56 neurons at the adult stage were classified as deep. As this distribution suggests, the superficial layers tended to be over-sampled. However, there was no significant difference in mean depth between the young and adult stage (two-tailed t test, $t_{631} = 1.496$, $P = 0.14$, Fig. 2, A and B).

Firing Rate

We first examined whether there were differences in firing rate between layers. We performed a one-way ANOVA separately for the young and adult stages and for each task epoch (baseline fixation, cue, and delay rate). In the young stage, there was no significant main effect of layer on the baseline firing rate ($F_{2,306} = 1.69$, $P = 0.18$), cue ($F_{2,306} = 0.574$, $P = 0.56$), or delay activity ($F_{2,306} = 1.28$, $P = 0.28$). For the adult stage, however, there was a significant difference in delay activity between groups ($F_{2,319} = 4.60$, $P = 0.01$). No significant difference was found in the baseline and cue firing rate between groups for the adult stage ($F_{2,319} = 2.49$, $P = 0.08$ for baseline, $F_{2,319} = 1.37$, $P = 0.26$ for the cue period).

As we have reported previously, the overall effect of developmental maturation was an increase in firing rate, including for the baseline fixation period, but also for the delay period relative to this baseline (23). We therefore sought to determine whether this increase was confined to superficial layers. A significant increase in pre-cue baseline activity was evident at the adult stage relative to the young stage in the superficial group (Fig. 3A; mean and standard error of the discharge rate in young stage: 6.50 ± 0.4 spikes/s, in adult: 9.3 ± 0.5 spikes/s, permutation test, $P < 0.001$). No significant difference in baseline activity was evident in the middle depth group, though a trend toward an increase was present (Fig. 3B; young stage: 8.35 ± 1.2 spikes/s, in adult: 12.0 ± 1.6 spikes/s, permutation test, $P > 0.05$). Much less of an increase, and no significant difference was present in the deep layer (Fig. 3C; mean discharge rate: young stage: 8.0 ± 1.0 spike/s, in adult: 8.15 ± 1.0 spikes/s, permutation test, $P > 0.05$).

Firing rates during the cue period increased slightly between the adolescent and adult stage, but the difference did not reach statistical significance. The effect was similar

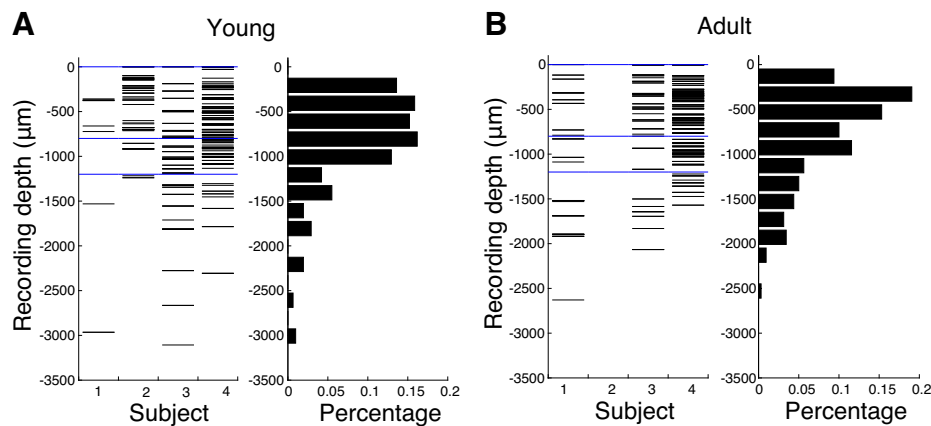


Figure 2. Recording depths. Depths of neurons with significant responses during the task relative to the top of the cortex are shown for the young (A) and adult (B) stages. Each horizontal line represents the depth of one neuron. Histograms summarize the depths of neurons in four monkeys in the young and adult stage. Blue horizontal lines indicate the 0, 800, and 1,200 μm depths that defined the boundaries for the superficial, middle, and deep groups.

for all depth groups (Fig. 3). For the superficial depth group, the mean discharge rate increased from 18.55 ± 0.9 spikes/s in the young to 21.3 ± 0.9 spikes/s in the adult; in the middle depth group from 21.2 ± 2.2 spikes/s in the young and 26.5 ± 2.8 spikes/s in the adult; and in the deep group from 19.7 ± 1.8 spikes/s in the young to 21.4 ± 1.8 spikes/s in the adult (permutation test, $P > 0.05$ for all depth groups). The mean cue period rate relative to the baseline fixation (evoked rate) between stages was not significantly different in any depth group, either. The evoked cue firing rates in the superficial depth group were 12.05 spikes/s in the young and 12.0 spikes/s in the adult; in the middle depth group were 12.8 spikes/s in the young and 14.6 spikes/s in the adult; and in the deep group were 11.7 spikes/s in the young and 13.3 spikes/s in the adult (permutation test, $P > 0.05$ for all depth groups).

In contrast, we found that the delay period discharge rate was significantly higher at the adult stage (Fig. 3, D–F). A significant increase for the firing rate was observed in the superficial depth group: mean discharge rate rose from 12.9 ± 0.7 to 17.9 ± 0.8 spikes/s (permutation test, $P < 0.001$). The mean evoked delay period rate similarly rose from 3.8 ± 0.5 spikes/s in the young to 6.9 ± 0.6 spikes/s in the adult (permutation test, $P < 0.001$). An even greater absolute increase was evident in the middle depth group (young: 14.7 ± 1.9 , adult: 22.3 ± 2.6 spikes/s, permutation test, $P < 0.05$, Fig. 3E). The difference in evoked delay period rate was also significant in the middle depth group (young: 3.8 ± 1.1 ; adult: 8.6 ± 2.0 spikes/s; permutation test, $P < 0.05$). In contrast, no significant difference was observed in the deep group for either the absolute firing rate (young: 11.9 ± 1.6 ; adult: 13.8 ± 1.5 spikes/s; permutation test, $P > 0.05$) or the evoked firing rate (young: 3.8 ± 0.9 ; adult 5.3 ± 1.0 spikes/s, permutation test, $P > 0.05$). Although not a question we identified a priori, it was notable that firing rate around the time of the saccadic response was also elevated in the adult stage (Fig. 3C).

An important caveat for this analysis is that due to the three-dimensional geometry of the monkey prefrontal cortex, electrodes on sulcal banks likely entered the cortex at a non-perpendicular angle to the surface, leading to potential inaccurate laminar profile of these neurons. We therefore identified sulcal locations and localized penetrations that descended into sulci based on MRI imaging, as also confirmed by the depth pattern of electrode penetrations before reaching the

surface of the cortex. We used sulcal locations to identify sulcal bank neurons, defined as those recorded from electrodes within 1 mm width from the center of a sulcus, which may have encountered superficial-layer neurons at longer depths (17). In total, we identified 166 sulcal bank units (112 in young and 54 in the adult). There was no significant difference in the mean depth between the two stages after excluding these neurons (two-tailed t test, $t_{463} = -0.31$, $P = 0.76$). We repeated the firing rate analysis, after omitting these sulcal bank units (Fig. 3, G and H). Most importantly, the firing rate in the middle layers was significantly elevated even after sulcal bank units were excluded (young: 16.1 ± 2.2 , adult: 20.8 ± 1.8 spikes/s, permutation test, $P < 0.05$, Fig. 3H).

Tuning and Selectivity

We next sought to test whether changes in firing rate also translated into an improvement in the discriminability between stimuli. To analyze the discriminability between the best location and its opposite, we used a receiver operating characteristic analysis. We compared area-under-the-receiver operating characteristic curve values (auROC) across depth groups. Mean auROC values computed during the stimulus presentation period of the ODR task (Fig. 4) were indistinguishable between the young and adult stages in superficial and middle layers (auROC_{superficial} = 0.799 ± 0.009 in adult and 0.807 ± 0.011 in young; auROC_{middle} = 0.802 ± 0.022 in adult and 0.804 ± 0.016 in young; permutation test, $P > 0.05$ in both layers). Discriminability increased in the deep group (auROC_{deep} = 0.846 ± 0.014 in adult and 0.798 ± 0.016 in young; permutation test, $P < 0.05$). Mean auROC values for the delay period were higher at the adult stage than young stage in all depth groups, with the larger differences observed in deeper layers (auROC_{superficial} = 0.668 ± 0.014 in adult and 0.656 ± 0.016 in young, permutation test, $P > 0.05$; auROC_{mid} = 0.686 ± 0.027 in adult and 0.625 ± 0.024 in young, permutation test, $P > 0.05$; auROC_{deep} = 0.737 ± 0.028 in adult and 0.649 ± 0.030 in young; permutation test, $P < 0.05$). For the deeper layers, this improvement in discriminability continued through the end of the delay period and reached its maximum near the time of saccadic preparation (Fig. 4C). The improvement in discriminability across all layers was the result of larger percentages of neurons in the adult reaching higher ROC values at each time point of the delay period (Fig. 4, D–F).

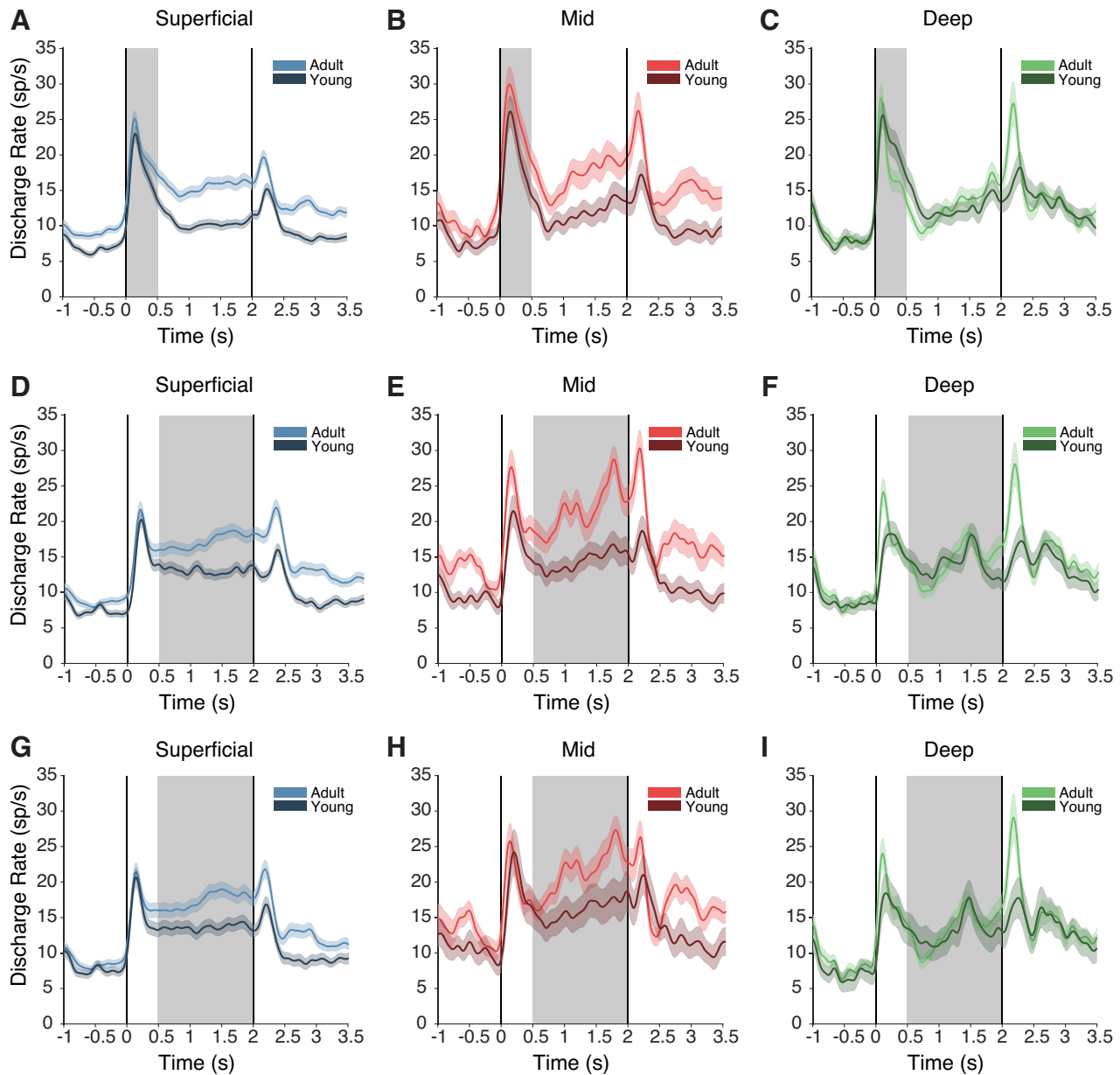


Figure 3. Firing rate in different depth groups and developmental stages. **A:** average, population peristimulus time histogram for neurons that responded to the visual stimulus and were recorded during the oculomotor delayed response (ODR) task from the superficial layer at adult and young stages. Responses are shown for a stimulus in the neuron's receptive field and aligned to the stimulus onset of each trial. Vertical lines represent stimulus onset and fixation offset ($n = 191$ for the young, $n = 212$ for the adult stage). **B:** as in **A**, for the middle layer ($n = 71$ for the young, $n = 54$ for the adult). **C:** as in **A**, for the deep layer ($n = 47$ for the young, $n = 56$ for the adult). **D–F:** as in **A–C**, for trials with the best delay activity of each neuron. **G–I:** as in **D–F**, after excluding sulcal bank units ($n = 126, 46, 25$ for the young, $n = 176, 43, 49$ for the adult, in **G, H, I**, respectively).

The changes in ROC values between depth groups and stages did not correspond to a proportional increase in firing rates for the best stimulus locations (Figs. 3 and 4). Thus, we proceeded to analyze the tuning of the units in different layers, both during the cue presentation and delay period. A three-way ANOVA of firing rate, with factors of cue location, layer, and stage, revealed a significant main effect of depth group, for both the cue presentation period and for the delay period ($F_{2,5014} = 19.60$, $P = 3.31 \times 10^{-9}$ for the cue period; $F_{2,5014} = 21.93$, $P = 3.31 \times 10^{-10}$ for the delay period). Similarly, the test revealed a significant main effect of stage,

and this was present both in the cue period and in the delay period ($F_{1,5014} = 15.43$, $P = 8.69 \times 10^{-5}$ for the cue period; $F_{1,4998} = 143.93$, $P = 1.02 \times 10^{-32}$ for the delay period). A significant interaction between group and stage was also present ($F_{2,5014} = 6.22$, $P = 0.002$ for the cue period; $F_{2,5014} = 17.63$, $P = 2.35 \times 10^{-8}$ for the delay period). These results suggest significant differences in firing rate between depth groups, and differential effects of developmental stage on firing rate. On the other hand, there was no significant interaction between cue location and depth group ($F_{14,5014} = 0.32$, $P = 0.99$ for the cue period; $F_{14,5014} = 0.22$, $P = 0.99$ for the

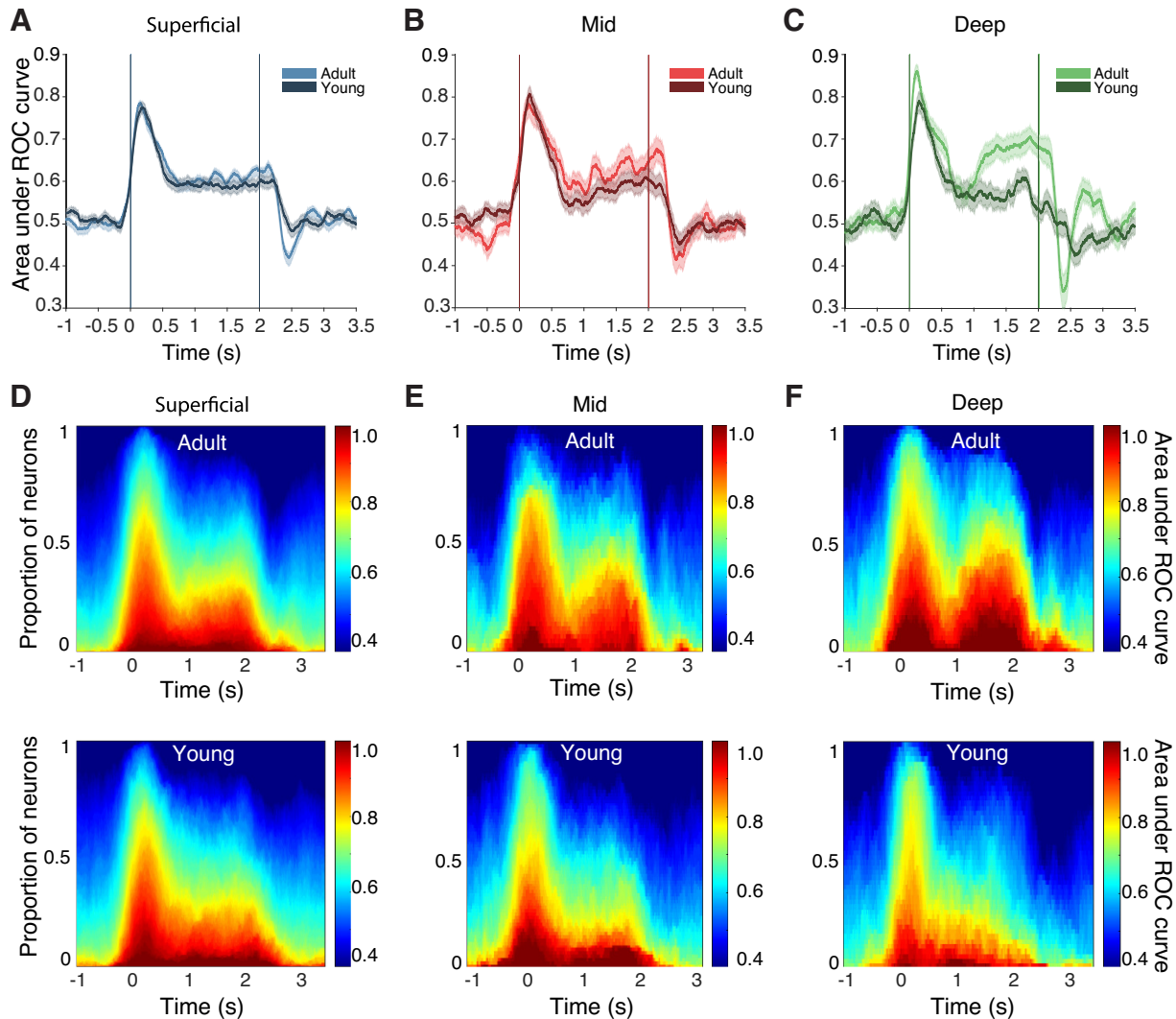


Figure 4. Receiver operating characteristic (ROC) analysis in each layer. **A:** mean area under the ROC curve in successive 100-ms windows is plotted as a function of time during the oculomotor delayed response (ODR) task, for superficial layer neurons at adult and young stages ($n = 191$ for the young, $n = 212$ for the adult stage). **B and C:** as in **A**, for the middle and deep layer, respectively ($n = 71$ for the young, $n = 54$ for the adult in middle; and $n = 47$ for the young, $n = 56$ for the adult in deep layers). **D:** percentages of neurons at adult (top) and young (bottom) stages reaching different levels of ROC values at each time point of the ODR task. **E and F:** as in **D**, for the middle layer and deep layer, respectively.

delay period). The result suggests that the shape of tuning curves was similar in different depth groups.

The best cue location exhibited the greatest increase in firing rates in all layers, and the relative difference was more pronounced in the delay than in the cue period (Fig. 5, **A** and **B**). Unlike in superficial and middle layers, firing rates of locations away from the neurons' best locations were lower in deep layer (Fig. 5), although the difference did not reach statistical significance. A two-way ANOVA was performed on each layer individually. We observed significant effects of the stage on cue firing rate and delay activity in superficial and middle layers with greater increases in the superficial depth group ($F_{1,3208} = 15.35$, $P = 9.12 \times 10^{-5}$ for the cue period, $F_{1,3208} = 154.78$, $P = 9.80 \times 10^{-35}$ for the delay period in superficial group; $F_{1,984} = 7.62$, $P = 0.006$ for the cue period, $F_{1,984} = 30.16$, $P = 5.06 \times 10^{-8}$ for the delay period in the middle depth group), and no significant effect of stage in the deep group ($F_{1,808} = 1.88$,

$P = 0.17$ for the cue period; $F_{1,808} = 0.33$, $P = 0.57$ for the delay period).

DISCUSSION

Changes in the activity of the prefrontal cortex have been thought to underlie the maturation of cognitive abilities during adolescence, including working memory (31, 32) and other executive functions (33, 34). These in turn are associated with structural brain changes, including changes in cortical volume and thickness that occur during this period (11, 35). Neurophysiological studies in nonhuman primates have begun to uncover the nature of these changes at the level of single-neuron responses (23, 24). Our current study provides a comprehensive analysis of the developmental changes in the firing rates and selectivity of neurons in the dorsolateral prefrontal cortex of macaque monkeys at different cortical depths. We found that increases in firing rate

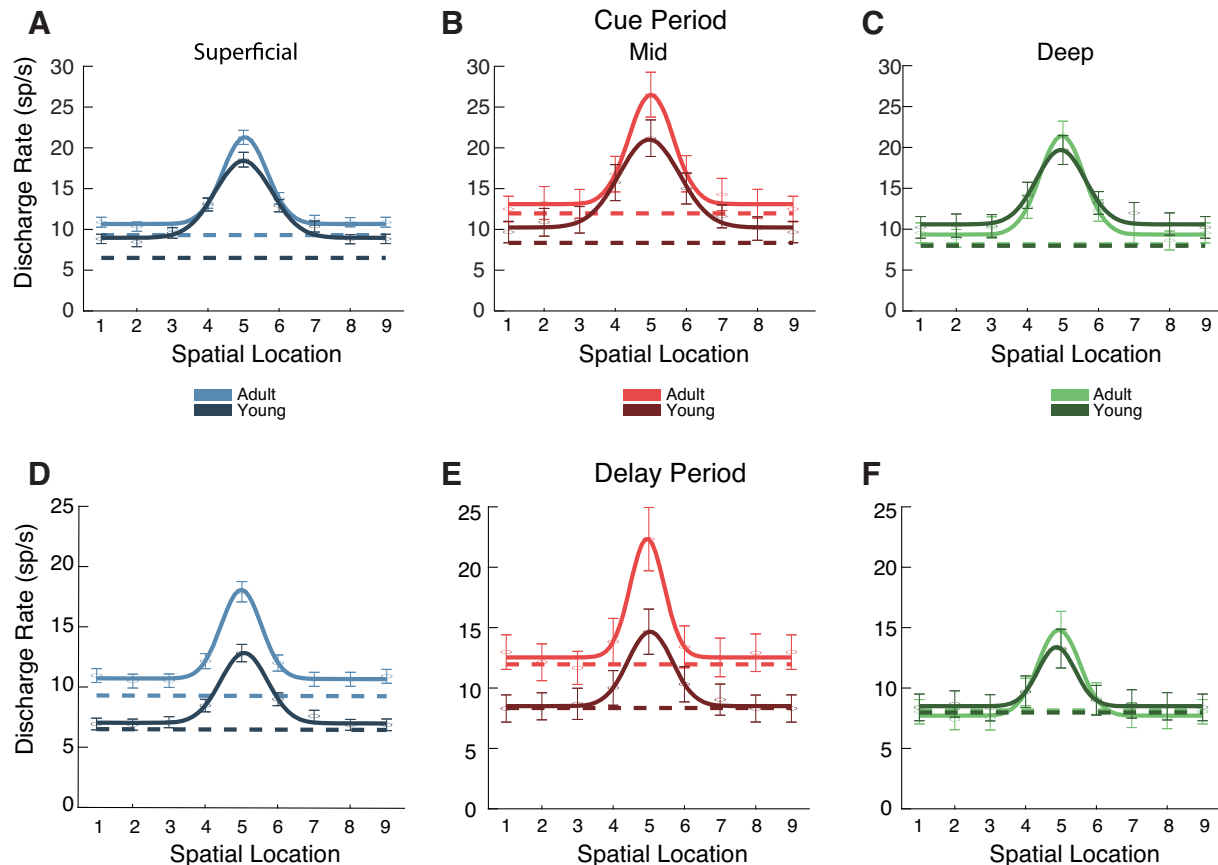


Figure 5. Neuronal tuning in each layer. **A:** average activity (and means \pm SE) during the cue period of the oculomotor delayed response (ODR) task in neurons recorded from the superficial layer at adult and young stages. Locations have been rotated, so that the best location of each neuron is represented in location 5. Location 9 is the same as location 1 ($n = 191$ for the young, $n = 212$ for the adult stage). Solid lines represent the best Gaussian fit of the population average. **B** and **C:** as in **A**, for the middle and deep layer, respectively ($n = 71$ for the young, $n = 54$ for the adult in middle; and $n = 47$ for the young, $n = 56$ for the adult in deep layers). **D:** average activity (and means \pm SE) during the delay period of the ODR task from the superficial layer at adult and young stages. **E** and **F:** as in **D**, for the middle and deep layer, respectively.

between adolescence and adulthood were not uniform across all layers of the cortex, but changes were not confined to the superficial layers, either, which are most commonly thought to mediate working memory responses (25, 27, 28). Our findings reveal a complex picture of neural maturation in the prefrontal cortex during adolescence, characterized by layer-specific changes in firing rate and stimulus discriminability. This layer-specific maturation pattern suggests that different cortical layers may play different roles in working memory processing, which matures differently over the course of development.

Monkey Adolescent Development

Similarly to human development, the monkey's prefrontal cortex continues to mature during adolescence and into early adulthood (21, 36, 37). Monkeys undergo a similar pattern of structural brain changes, as well (38, 39). Some brain morphological changes, however, appear to occur earlier in monkeys. For example, pyramidal neuron spike density peaks at an age of 3.5 mo and pruning appears to have been completed by the age of 4 yr (40). Some biochemical and anatomical changes (such as expression of ankyrin, parvalbumin, and GABA receptor subunits) have also been shown to occur in the monkey prefrontal cortex at a much earlier age

than equivalent changes in human adolescence, leading to the suggestion that the monkey adolescent period begins as early as 1.5–2 yr of age (21, 22). Our results are more in line with the age range predicted by the time of sexual maturity and a 1:3 factor of aging time (19, 20). We show that between the ages of 4–6 yr, substantial changes in prefrontal baseline and delay period activity and stimulus discriminability take place, and that these have a specific laminar pattern.

Superficial Layers in Working Memory Maintenance

Horizontal connections between pyramidal neurons in layers II and III of the prefrontal cortex have been thought to provide the anatomical substrate through which neuronal discharges reverberate and persist during working memory (25, 41). The prefrontal cortex appears unique in the pattern of these connections, in that prefrontal neurons have the most extensive dendritic trees and the largest number of spines among cortical neurons (42, 43). Functional correlates of this anatomical connectivity are reflected in patterns of discharges of distant prefrontal neurons (44, 45). Biophysical models of working memory have simulated precisely this type of connection between neurons to create recurrent networks of units that continue to excite each other (46, 47). Indeed, neurons with persistent activity are

more readily identified in the superficial layers of the prefrontal cortex, comprising the top $\sim 800\ \mu\text{m}$ of the cortical volume (27). Other types of neural activity that have been associated with working memory, such as synchronized γ -band oscillations in the local field potential, have also been localized in the superficial layers (48). Recent advancements in layer-specific functional MRI techniques have also allowed for the dissociation of activity in superficial and deeper cortical layers during different periods of a working memory task in the human dorsolateral prefrontal cortex (49).

Given this evidence, it appeared plausible that improvement in working memory ability would manifest itself with improved persistent activity generation, focused on the superficial layers. A systematic difference in firing rate between the adolescent and adult stages was indeed present for the superficial layers; however, this involved an overall increase in fixation period rather than delay-period activity. This “baseline” activity, rather than being passively generated, has been increasingly recognized as a preparatory process that has important implications for the execution of cognitive tasks (50, 51). The generation of persistent activity during working memory is directly dependent on this baseline; computational studies reveal that networks with high baseline activity are more likely to be able to sustain activation and persistent activity may die off in networks with lower baseline activity if neurons fail to generate sufficient action potentials as a result of synaptic activation (52). In that sense, the increased baseline activity we observed in the superficial layers of the adult prefrontal cortex may be indirectly related to the improved working memory ability. It was also notable that this baseline firing rate increase was less pronounced as a function of cortical depth; the middle depth group showed only a slight, nonstatistically significant increase, whereas the deep group showed the least change, with the mean discharge rate remaining almost the same from the young to the adult stage.

Delay Period Activity

Neurons in the prefrontal cortex (53, 54) and other brain areas interconnected with it (55, 56) continue to generate persistent discharges that exceed the neuron’s baseline during the delay period of spatial working memory tasks. In our study, a big increase in delay period activity relative to the baseline was observed for the middle layers; superficial layers exhibited a modest increase in this measure. This result was unexpected, as middle layers have been more implicated in the representation of sensory stimuli (27), driven by afferent inputs to the prefrontal cortex. The result raises the possibility that much of the increase in delay period activity is a distributed effect, contributed by areas connected to the prefrontal cortex (57), as much as it is dependent on intrinsic changes within the prefrontal cortex. The improved representation of the saccadic target also suggests that the output of the prefrontal cortex was enhanced. These results suggest a change of computations occurring within the prefrontal cortical column after adolescence. Changes in synaptic strengths appear to modify prefrontal cortical activity.

There are several caveats in this analysis. Layer determination was only approximate, with three depth ranges

identified based on fixed depths, rather than precise histological identification. Depth was also determined approximately, based at the point that spiking was first detected. However, it should be noted that this measure of depth was conservative; it is much more likely for the true top of the cortex to be missed during penetration than to be falsely identified prematurely. In this sense, our results may even underestimate the adolescent changes in nonsuperficial layers. An inherent limitation in our analysis was that it relied on recordings at different depths obtained from different electrode tracks. Future studies with laminar arrays will be in a position to more precisely ascertain cortical depths—and neuronal activity differences—in the same electrode penetrations.

Another caveat is that adult monkeys had more cumulative exposure to the task than young monkeys, and it is well established that training can increase persistent activity during working memory (58, 59). In this sense, the changes we observed in the middle layers may be primarily driven by training rather than by developmental maturation. We have shown changes in firing rate cannot be fully attributed by training or improved performance attained in the task (23). Nonetheless, it would be no less surprising that the effects of training would disproportionately affect the middle layers rather than the superficial ones during the generation of persistent activity.

Stimulus Representation and Discriminability

In yet another unexpected finding, the increase in delay firing rate did not lead to a proportional improvement in stimulus discriminability in the superficial and middle layers. In contrast, it was the deep layers that exhibited the greatest improvement in stimulus discriminability from the young to the adult stage despite the lack of a significant increase in delay firing rate. Deep layers are thought to reflect the output of the maintenance operation for the guidance of motor action (27), and in this sense, increased precision may be most critical there. This layer-specific improvement in discriminability may reflect a refinement of neural circuits in the deep layer, which enhances the precision of neural tuning and contributes to the maturation of cognitive abilities. In fact, evidence exists that a multitude of signals is communicated from frontal cortical areas to subcortical structures such as the superior colliculus during the maintenance of working memory (60). Thalamic nuclei, and particularly the mediodorsal nucleus also receive input from deep cortical layers and exhibit persistent activity during the delay period of working memory tasks, whose disruption impairs performance (61, 62).

Our observations of layer-specific changes in firing rate and stimulus discriminability underscore the importance of considering the structure-function coupling at a fine spatial scale. Previous studies have uncovered laminar fractionation of other cognitive functions as well (63, 64). Future studies should consider the heterogeneity of neural development across different cortical layers and aim to elucidate the underlying mechanisms and functional implications of these layer-specific changes.

DATA AVAILABILITY

Data will be made available upon reasonable request.

ACKNOWLEDGMENTS

We thank Stanley Vinet and Chrissy Suell for technical assistance and Rye Jaffe for helpful comments on the manuscript.

GRANTS

Research reported in this paper was supported by the National Institute of Mental Health under Award Number R01 MH116675.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

Christos Constantinidis is an editor of *Journal of Neurophysiology* and was not involved and did not have access to information regarding the peer-review process or final disposition of this article. An alternate editor oversaw the peer-review and decision-making process for this article.

AUTHOR CONTRIBUTIONS

X.M.Z. and C.C. conceived and designed research; J.Z., X.M.Z., and C.C. performed experiments; J.Z., B.M.H., X.M.Z., and C.C. analyzed data; J.Z. and C.C. interpreted results of experiments; J.Z. and C.C. prepared figures; J.Z. and C.C. drafted manuscript; J.Z., B.M.H., and C.C. edited and revised manuscript; J.Z., B.M.H., X.M.Z., and C.C. approved final version of manuscript.

REFERENCES

- Luna B, Garver KE, Urban TA, Lazar NA, Sweeney JA. Maturation of cognitive processes from late childhood to adulthood. *Child Dev* 75: 1357–1372, 2004. doi:10.1111/j.1467-8624.2004.00745.x.
- Crone EA, Wendelken C, Donohue S, van Leijenhorst L, Bunge SA. Neurocognitive development of the ability to manipulate information in working memory. *Proc Natl Acad Sci USA* 103: 9315–9320, 2006. doi:10.1073/pnas.0510088103.
- Isbell E, Fukuda K, Neville HJ, Vogel EK. Visual working memory continues to develop through adolescence. *Front Psychol* 6: 696, 2015. doi:10.3389/fpsyg.2015.00696.
- Kwon H, Reiss AL, Menon V. Neural basis of protracted developmental changes in visuo-spatial working memory. *Proc Natl Acad Sci USA* 99: 13336–13341, 2002. doi:10.1073/pnas.162486399.
- Luna B, Thulborn KR, Munoz DP, Merriam EP, Garver KE, Minshew NJ, Keshavan MS, Genovese CR, Eddy WF, Sweeney JA. Maturation of widely distributed brain function subserves cognitive development. *NeuroImage* 13: 786–793, 2001. doi:10.1006/nimg.2000.0743.
- Ordaz SJ, Foran W, Velanova K, Luna B. Longitudinal growth curves of brain function underlying inhibitory control through adolescence. *J Neurosci* 33: 18109–18124, 2013. doi:10.1523/JNEUROSCI.1741-13.2013.
- Bunge SA, Dudukovic NM, Thomason ME, Vaidya CJ, Gabrieli JD. Immature frontal lobe contributions to cognitive control in children: evidence from fMRI. *Neuron* 33: 301–311, 2002. doi:10.1016/s0896-6273(01)00583-9.
- Satterthwaite TD, Wolf DH, Erus G, Ruparel K, Elliott MA, Gennatas ED, Hopson R, Jackson C, Prabhakaran K, Bilker WB, Calkins ME, Loughhead J, Smith A, Roalf DR, Hakonarson H, Verma R, Davatzikos C, Gur RC, Gur RE. Functional maturation of the executive system during adolescence. *J Neurosci* 33: 16249–16261, 2013. doi:10.1523/JNEUROSCI.2345-13.2013.
- Luna B, Velanova K, Geier CF. Development of eye-movement control. *Brain Cogn* 68: 293–308, 2008. doi:10.1016/j.bandc.2008.08.019.
- Darki F, Klingberg T. The role of fronto-parietal and fronto-striatal networks in the development of working memory: a longitudinal study. *Cereb Cortex* 25: 1587–1595, 2015. doi:10.1093/cercor/bht352.
- Bethlehem RAI, Seidlitz J, White SR, Vogel JW, Anderson KM, Adamson C et al. Brain charts for the human lifespan. *Nature* 604: 525–533, 2022 [Erratum in *Nature* 610: E6, 2022]. doi:10.1038/s41586-022-04554-y.
- Gonzalez-Burgos G, Miyamae T, Pafundo DE, Yoshino H, Rotaru DC, Hoftman G, Datta D, Zhang Y, Hammond M, Sampson AR, Fish KN, Ermentrout GB, Lewis DA. Functional maturation of GABA synapses during postnatal development of the monkey dorsolateral prefrontal cortex. *Cereb Cortex* 25: 4076–4093, 2015. doi:10.1093/cercor/bhu122.
- Dienel SJ, Lewis DA. Alterations in cortical interneurons and cognitive function in schizophrenia. *Neurobiol Dis* 131: 104208, 2019. doi:10.1016/j.nbd.2018.06.020.
- Selemon LD, Goldman-Rakic PS. The reduced neuropil hypothesis: a circuit based model of schizophrenia. *Biol Psychiatry* 45: 17–25, 1999. doi:10.1016/s0006-3223(98)00281-9.
- Yoon JH, Minzenberg MJ, Ursu S, Ryan Walter BS, Wendelken C, Ragland JD, Carter CS. Association of dorsolateral prefrontal cortex dysfunction with disrupted coordinated brain activity in schizophrenia: relationship with impaired cognition, behavioral disorganization, and global function. *Am J Psychiatry* 165: 1006–1014, 2008 [Erratum in *Am J Psychiatry* 165: 1359, 2008]. doi:10.1176/appi.ajp.2008.07060945.
- Catts VS, Fung SJ, Long LE, Joshi D, Vercammen A, Allen KM, Fillman SG, Rothmond DA, Sinclair D, Tiwari Y, Tsai SY, Weickert TW, Shannon Weickert C. Rethinking schizophrenia in the context of normal neurodevelopment. *Front Cell Neurosci* 7: 60, 2013. doi:10.3389/fncel.2013.00060.
- Zhou X, Zhu D, Katsuki F, Qi XL, Lees CJ, Bennett AJ, Salinas E, Stanford TR, Constantinidis C. Age-dependent changes in prefrontal intrinsic connectivity. *Proc Natl Acad Sci USA* 111: 3853–3858, 2014. doi:10.1073/pnas.1316594111.
- Zhou X, Zhu D, Qi XL, Lees CJ, Bennett AJ, Salinas E, Stanford TR, Constantinidis C. Working memory performance and neural activity in the prefrontal cortex of peri-pubertal monkeys. *J Neurophysiol* 110: 2648–2660, 2013. doi:10.1152/jn.00370.2013.
- Plant TM, Ramaswamy S, Simorangkir D, Marshall GR. Postnatal and pubertal development of the rhesus monkey (*Macaca mulatta*) testis. *Ann N Y Acad Sci* 1061: 149–162, 2005. doi:10.1196/annals.1336.016.
- Herman RA, Zehr JL, Wallen K. Prenatal androgen blockade accelerates pubertal development in male rhesus monkeys. *Psychoneuroendocrinology* 31: 118–130, 2006. doi:10.1016/j.psyneuen.2005.06.004.
- Lewis DA. Development of the prefrontal cortex during adolescence: insights into vulnerable neural circuits in schizophrenia. *Neuropsychopharmacology* 16: 385–398, 1997. doi:10.1016/S0893-133X(96)00277-1.
- Hoftman GD, Lewis DA. Postnatal developmental trajectories of neural circuits in the primate prefrontal cortex: identifying sensitive periods for vulnerability to schizophrenia. *Schizophr Bull* 37: 493–503, 2011. doi:10.1093/schbul/sbr029.
- Zhou X, Zhu D, Qi XL, Li S, King SG, Salinas E, Stanford TR, Constantinidis C. Neural correlates of working memory development in adolescent primates. *Nat Commun* 7: 13423, 2016. doi:10.1038/ncomms13423.
- Zhou X, Zhu D, King SG, Lees CJ, Bennett AJ, Salinas E, Stanford TR, Constantinidis C. Behavioral response inhibition and maturation of goal representation in prefrontal cortex after puberty. *Proc Natl Acad Sci USA* 113: 3353–3358, 2016. doi:10.1073/pnas.1518147113.
- Kritzer MF, Goldman-Rakic PS. Intrinsic circuit organization of the major layers and sublayers of the dorsolateral prefrontal cortex in the rhesus monkey. *J Comp Neurol* 359: 131–143, 1995. doi:10.1002/cne.903590109.
- Goldman-Rakic PS. Regional and cellular fractionation of working memory. *Proc Natl Acad Sci USA* 93: 13473–13480, 1996. doi:10.1073/pnas.93.24.13473.
- Markowitz DA, Curtis CE, Pesaran B. Multiple component networks support working memory in prefrontal cortex. *Proc Natl Acad Sci USA* 112: 11084–11089, 2015 [Erratum in *Proc Natl Acad Sci USA* 112: E5555, 2015]. doi:10.1073/pnas.1504721112.
- Constantinidis C, Franowicz MN, Goldman-Rakic PS. Coding specificity in cortical microcircuits: a multiple electrode analysis of primate

- prefrontal cortex. *J Neurosci* 21: 3646–3655, 2001. doi:10.1523/JNEUROSCI.21-10-03646.2001.
29. Sawaguchi T, Matsumura M, Kubota K. Catecholaminergic effects on neuronal activity related to a delayed response task in monkey prefrontal cortex. *J Neurophysiol* 63: 1385–1400, 1990. doi:10.1152/jn.1990.63.6.1385.
30. Meyer T, Constantinidis C. A software solution for the control of visual behavioral experimentation. *J Neurosci Methods* 142: 27–34, 2005. doi:10.1016/j.jneumeth.2004.07.009.
31. Montez DF, Calabro FJ, Luna B. Working memory improves developmentally as neural processes stabilize. *PLoS One* 14: e0213010, 2019. doi:10.1371/journal.pone.0213010.
32. Simmonds DJ, Hallquist MN, Luna B. Protracted development of executive and mnemonic brain systems underlying working memory in adolescence: a longitudinal fMRI study. *NeuroImage* 157: 695–704, 2017. doi:10.1016/j.neuroimage.2017.01.016.
33. Larsen B, Luna B. Adolescence as a neurobiological critical period for the development of higher-order cognition. *Neurosci Biobehav Rev* 94: 179–195, 2018. doi:10.1016/j.neubiorev.2018.09.005.
34. Constantinidis C, Luna B. Neural substrates of inhibitory control maturation in adolescence. *Trends Neurosci* 42: 604–616, 2019. doi:10.1016/j.tins.2019.07.004.
35. Gogtay N, Giedd JN, Lusk L, Hayashi KM, Greenstein D, Vaituzis AC, Nugent TF 3rd, Herman DH, Clasen LS, Toga AW, Rapoport JL, Thompson PM. Dynamic mapping of human cortical development during childhood through early adulthood. *Proc Natl Acad Sci USA* 101: 8174–8179, 2004. doi:10.1073/pnas.0402680101.
36. Fuster JM. Frontal lobe and cognitive development. *J Neurocytol* 31: 373–385, 2002. doi:10.1023/a:1024190429920.
37. Malkova L, Heuer E, Saunders RC. Longitudinal magnetic resonance imaging study of rhesus monkey brain development. *Eur J Neurosci* 24: 3204–3212, 2006. doi:10.1111/j.1460-9568.2006.05175.x.
38. Kim J, Jung Y, Barcus R, Bachevalier JH, Sanchez MM, Nader MA, Whitlow CT. Rhesus macaque brain developmental trajectory: a longitudinal analysis using tensor-based structural morphometry and diffusion tensor imaging. *Cereb Cortex* 30: 4325–4335, 2020. doi:10.1093/cercor/bhaa015.
39. Sawiak SJ, Shiba Y, Oikonomidis L, Windle CP, Santangelo AM, Grydeland H, Cockcroft G, Bullmore ET, Roberts AC. Trajectories and milestones of cortical and subcortical development of the marmoset brain from infancy to adulthood. *Cereb Cortex* 28: 4440–4453, 2018. doi:10.1093/cercor/bhy256.
40. Elston GN, Oga T, Fujita I. Spinyogenesis and pruning scales across functional hierarchies. *J Neurosci* 29: 3271–3275, 2009. doi:10.1523/JNEUROSCI.5216-08.2009.
41. Goldman-Rakic PS. Cellular basis of working memory. *Neuron* 14: 477–485, 1995. doi:10.1016/0896-6273(95)90304-6.
42. Elston GN. The pyramidal neuron in occipital, temporal and prefrontal cortex of the owl monkey (*Aotus trivirgatus*): regional specialization in cell structure. *Eur J Neurosci* 17: 1313–1318, 2003. doi:10.1046/j.1460-9568.2003.02552.x.
43. Elston GN. Pyramidal cells of the frontal lobe: all the more spinous to think with. *J Neurosci* 20: RC95, 2000. doi:10.1523/JNEUROSCI.20-18-j0002.2000.
44. Katsuki F, Qi XL, Meyer T, Kostelic PM, Salinas E, Constantinidis C. Differences in intrinsic functional organization between dorsolateral prefrontal and posterior parietal cortex. *Cereb Cortex* 24: 2334–2349, 2014. doi:10.1093/cercor/bht087.
45. Hart E, Huk AC. Recurrent circuit dynamics underlie persistent activity in the macaque frontoparietal network. *eLife* 9: e52460, 2020. doi:10.7554/eLife.52460.
46. Wimmer K, Nykamp DQ, Constantinidis C, Compte A. Bump attractor dynamics in prefrontal cortex explains behavioral precision in spatial working memory. *Nat Neurosci* 17: 431–439, 2014. doi:10.1038/nn.3645.
47. Compte A, Brunel N, Goldman-Rakic PS, Wang XJ. Synaptic mechanisms and network dynamics underlying spatial working memory in a cortical network model. *Cereb Cortex* 10: 910–923, 2000. doi:10.1093/cercor/10.9.910.
48. Bastos AM, Loonis R, Kornblith S, Lundqvist M, Miller EK. Laminar recordings in frontal cortex suggest distinct layers for maintenance and control of working memory. *Proc Natl Acad Sci USA* 115: 1117–1122, 2018. doi:10.1073/pnas.1710323115.
49. Finn ES, Huber L, Jangraw DC, Molfese PJ, Bandettini PA. Layer-dependent activity in human prefrontal cortex during working memory. *Nat Neurosci* 22: 1687–1695, 2019. doi:10.1038/s41593-019-0487-z.
50. Hwang K, Ghuman AS, Manoach DS, Jones SR, Luna B. Frontal preparatory neural oscillations associated with cognitive control: a developmental study comparing young adults and adolescents. *NeuroImage* 136: 139–148, 2016. doi:10.1016/j.neuroimage.2016.05.017.
51. Zhou X, Qi XL, Constantinidis C. Distinct roles of the prefrontal and posterior parietal cortices in response inhibition. *Cell Rep* 14: 2765–2773, 2016. doi:10.1016/j.celrep.2016.02.072.
52. Wang XJ. Synaptic basis of cortical persistent activity: the importance of NMDA receptors to working memory. *J Neurosci* 19: 9587–9603, 1999. doi:10.1523/JNEUROSCI.19-21-09587.1999.
53. Funahashi S, Bruce CJ, Goldman-Rakic PS. Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. *J Neurophysiol* 61: 331–349, 1989. doi:10.1152/jn.1989.61.2.331.
54. Fuster JM, Alexander GE. Neuron activity related to short-term memory. *Science* 173: 652–654, 1971. doi:10.1126/science.173.3997.652.
55. Qi XL, Elworthy AC, Lambert BC, Constantinidis C. Representation of remembered stimuli and task information in the monkey dorsolateral prefrontal and posterior parietal cortex. *J Neurophysiol* 113: 44–57, 2015. doi:10.1152/jn.00413.2014.
56. Chafee MV, Goldman-Rakic PS. Matching patterns of activity in primate prefrontal area 8a and parietal area 7ip neurons during a spatial working memory task. *J Neurophysiol* 79: 2919–2940, 1998. doi:10.1152/jn.1998.79.6.2919.
57. Mejias JF, Wang XJ. Mechanisms of distributed working memory in a large-scale network of macaque neocortex. *eLife* 11: e72136, 2022. doi:10.7554/eLife.72136.
58. Constantinidis C, Klingberg T. The neuroscience of working memory capacity and training. *Nat Rev Neurosci* 17: 438–449, 2016. doi:10.1038/nrn.2016.43.
59. Qi XL, Constantinidis C. Neural changes after training to perform cognitive tasks. *Behav Brain Res* 241: 235–243, 2013. doi:10.1016/j.bbr.2012.12.017.
60. Sommer MA, Wurtz RH. Frontal eye field sends delay activity related to movement, memory, and vision to the superior colliculus. *J Neurophysiol* 85: 1673–1685, 2001. doi:10.1152/jn.2001.85.4.1673.
61. Watanabe Y, Funahashi S. Thalamic mediodorsal nucleus and working memory. *Neurosci Biobehav Rev* 36: 134–142, 2012. doi:10.1016/j.neubiorev.2011.05.003.
62. Bolkan SS, Stujenske JM, Parnaudeau S, Spellman TJ, Rauffenbart C, Abbas AI, Harris AZ, Gordon JA, Kellendonk C. Thalamic projections sustain prefrontal activity during working memory maintenance. *Nat Neurosci* 20: 987–996, 2017 [Erratum in *Nat Neurosci* 21: 1138, 2018]. doi:10.1038/nn.4568.
63. Opris I, Hampson RE, Stanford TR, Gerhardt GA, Deadwyler SA. Neural activity in frontal cortical cell layers: evidence for columnar sensorimotor processing. *J Cogn Neurosci* 23: 1507–1521, 2011. doi:10.1162/jocn.2010.21534.
64. Chandrasekaran C, Peixoto D, Newsome WT, Shenoy KV. Laminar differences in decision-related neural activity in dorsal premotor cortex. *Nat Commun* 8: 614, 2017. doi:10.1038/s41467-017-00715-0.