Age-dependent changes in prefrontal intrinsic connectivity

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The prefrontal cortex continues to mature after puberty and into early adulthood, mirroring the time course of maturation of cognitive abilities. However, the way in which prefrontal activity changes during peri- and postpubertal cortical maturation is largely unknown. To address this question, we evaluated the developmental stage of peripubertal rhesus monkeys with a series of morphometric, hormonal, and radiographic measures, and conducted behavioral and neurophysiological tests as the monkeys performed working memory tasks. We compared firing rate and the strength of intrinsic functional connectivity between neurons in peripubertal vs. adult monkeys. Notably, analyses of spike train cross-correlations demonstrated that the average magnitude of functional connections measured between neurons was lower overall in the prefrontal cortex of peripubertal monkeys compared with adults. The difference resulted because negative functional connections (indicative of inhibitory interactions) were stronger and more prevalent in peripubertal compared with adult monkeys, whereas the positive connections showed similar distributions in the two groups. Our results identify changes in the intrinsic connectivity of prefrontal neurons, particularly that mediated by inhibition, as a possible substrate for peri- and postpubertal advances in cognitive capacity.

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The prefrontal cortex, the brain area associated with the highest-level cognitive operations, is known to undergo a protracted period of development (1–3). A virtually linear increase in performance with age has been observed in tasks that assess visuospatial working memory, executive control, and resistance to distraction, a process that continues well after puberty and into early adulthood (4, 5). The accrual of cognitive capacities during this period parallels structural changes of the prefrontal cortex in humans and nonhuman primates (6–10). Imaging studies in humans suggest that patterns of brain activation associated with working memory tasks undergo distinct changes between childhood and adulthood, supporting the idea of prolonged prefrontal maturation (11–14). However, how the patterns of prefrontal activation change during cortical maturation remains unclear. A possible mechanism that could account for variations in prefrontal responses—and which could have a significant functional impact—is an overall change in the distribution of intrinsic functional connections, i.e., those between neurons within the prefrontal cortex. The intrinsic connectivity of a network is directly related to the correlation structure of neuronal responses, and this determines in a fundamental way the information-coding properties of the network and its ability to sustain activity on its own (16–18). In this study, we sought to determine if the strengths of functional connections inferred from multisite neurophysiological recordings differ between peripubertal and adult monkeys.

Results

We obtained neurophysiological recordings from eight male Rhesus monkeys (Macaca mulatta) while they performed working memory tasks. Three monkeys were tested at approximately the time of puberty (heretofore referred to as young monkeys); five were adult. We conducted quarterly morphometric, hormonal, and radiographic measurements to assess development. Representative developmental data for the three young monkeys are shown in Fig. S1. All measures showed an upward trend between the three quarters preceding and three to five quarters after onset of neuronal recordings as expected for developing monkeys; values in all cases were indicative of monkeys that are entering puberty but have not reached full maturity (19, 20). These three monkeys had a median age of 4.1 y at the last measurement before the onset of neurophysiological recordings (range, 4.0–5.2 y). The mean age during actual recordings used for this analysis was 4.7 y. Mean ages of the adult monkeys during the recordings used for analysis were 7.4 y, 8.7 y, 9.4 y, 10.0 y, and 11.7 y for the five subjects, respectively (range, 6.8–14.0 y).

We estimated the effective intrinsic connectivity by cross-correlation analysis of neurons recorded simultaneously from different microelectrodes, separated laterally by 0.5–1 mm, in areas 8 and 46 of the dorsolateral prefrontal cortex (Fig. 1A). Recordings were obtained from the crown of cortical gyri, allowing us to focus on the effect of horizontal connections between neurons; electrode penetrations that descended into the principal or arcuate sulcus were omitted from the main analysis. We thus relied on three inclusion criteria, as we have done previously (21): that (i) both neurons of a pair were recorded from the exposed surface of a cortical gyrus, (ii) electrodes had entered the cortex at equivalent angles, and (iii) the two neurons were not recorded at depths more

Significance

The prefrontal cortex, the brain area associated with higher cognitive operations such as working memory and executive function, undergoes a protracted period of development. How the activity of prefrontal neurons changes during peri- and postpubertal cortical maturation is largely unknown. To address this question, we recorded neuronal activity from the prefrontal cortex of peripubertal and adult monkeys and compared the functional connectivity between pairs of neurons in each group. The magnitude of connectivity measured between neurons was lower overall in the prefrontal cortex of peripubertal monkeys compared with adults, as inhibitory interactions were generally stronger in young animals. Our results identify changes in intrinsic connectivity between prefrontal neurons as a possible substrate for peri- and postpubertal cognitive maturation.


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than 1 mm apart from each other. This selection process produced a main sample of 855 neuron pairs recorded from the young monkeys (597, 126, and 132 pairs from three subjects, respectively) and 465 pairs from the adult monkeys (188, 26, 187, 20, and 44 pairs from five subjects, respectively). In addition, a sample of recordings obtained from the principal sulcus was analyzed separately. These measurements also involved electrode penetrations separated by 0.5–1.0 mm, and neurons recorded at depths no more than 1 mm apart from each other. This sample comprised 266 pairs from the young monkeys (69, 99, and 98 from three subjects, respectively), and 203 pairs from the adult monkeys (25, 30, 115, and 33 from four subjects, respectively). Considering the uncertainty of the cortical layers of the neurons recorded in the sulcus, the analyses presented here relied mostly on the recordings obtained from surface cortex, unless stated otherwise. Note, however, that all results based on the smaller sample from the sulcus were consistent with those from the surface.

To estimate the connection strength between two neurons, we constructed cross-correlation histograms (CCH) corrected by the number of expected coincidences. The correction was based on surrogate spike trains in which the time of each spike in a trial was sampled randomly from a uniform distribution spanning 50 ms around the time of the original spike, thus destroying temporal structures beyond 50 ms (21). The percentage of spikes from the two neurons occurring within a few milliseconds of each other, in excess of the expected number assuming that the two neurons discharged independently, represents the effective strength of their connection (21, 22). We focused on the cross-correlation strength for the central 5 ms of the CCH, which represents synchronized discharges in the time scale of synaptic interactions (±2.5 ms). Mean cross-correlation strength was significantly lower (two-tailed permutation test, \( P < 0.005 \)) in the young monkeys than in the adult monkeys (Fig. 1B). Similarly, connectivity strength for the young compared with the adult monkeys was also lower (\( P < 0.05 \)) based on the sulcus recordings (Fig. S2A).

The overall higher connectivity we observed in adult monkeys could be caused, in principle, by an increase in the strength of positive (excitatory) interactions, a decrease in the strength of the negative (inhibitory) interactions, or some combination of the two. In fact, analyses of the distributions of young and adult cross-correlation strengths (Fig. 1C) revealed that the difference in connectivity strength between young and adults was solely the result of a decrease in negative correlation strengths. When only the positive correlation strengths were compared across the two groups of monkeys, no significant difference was observed in their means (\( P = 0.68 \)); in contrast, when only the negative correlation strengths were compared the difference was large (−0.40 ± 0.03% in young vs. −0.20 ± 0.02% in adults, mean ± SEM) and highly significant (\( P = 0.00006 \)). Virtually identical results were obtained when the analyses of positive and negative correlations were based on the neuronal pairs sampled from the principal sulcus (Fig. S2 B and C). In view of these findings, we also tested whether the proportion of negative correlations (regardless of their magnitude) also varied in the two age groups, and found that indeed it did: 38% of the pairs recorded from young animals had negative correlations, vs. only 25% of the pairs from adult animals (permutation test, \( P < 10^{-5} \)). In other words, negative functional connections in young monkeys were more common and stronger in magnitude than in adults, which suggests a selective decrease of inhibitory interactions after puberty.

To ensure that this difference in effective connectivity was not merely the result of systematic differences in other properties of neuronal pairs recorded from young and adult monkeys, we tested the effect of three factors known to affect correlation strength: electrode separation, firing rate, and signal correlation. Although we limited our neuron selection to electrode pairs within 0.5–1 mm, we observed a general trend toward decreased effective connectivity as a function of increasing distance (Fig. 2A), in agreement with prior studies (21, 23). However, cross-correlation strength was consistently lower in young vs. adult monkeys for subsets of neurons recorded at equal distances (Fig. 2B). We also sought to determine the effect of firing rate on cross-correlation strength in the two groups (Fig. 2B), as overall strength is expected to increase as a function of increasing firing rate (24). However, the overall firing rate of the neuronal samples was higher in the young rather than the adult monkeys [two-tailed \( t \) test, \( t_{2,638} = 3.22, 3.18, \) and 3.33 (\( P < 0.005 \)) for the fixation, stimulus, and delay periods; Fig. S3A]. Furthermore, the effect of firing rate was greater in the adult than in the young monkeys (\( F \) test, \( F_{1,1316} = 24.51; P < 10^{-5} \)). Last, we compared the signal correlation, a measure of similarity in the tuning properties of the two neurons of a simultaneously recorded pair, between the samples of neurons recorded from the young and the adult monkeys. There was no significant difference between the mean signal correlation of the two samples (two-tailed \( t \) test, \( t_{1,318} = 1.85; P = 0.065 \)), and cross-correlation strength was consistently lower in the young monkeys for groups of neurons equalized for signal correlation (Fig. 2C).

We examined with similar methods two other variables that might have conceivably affected our analysis: recording depth and behavioral performance. The young and adult monkeys varied slightly in terms of average cortical depth of recordings (mean and SD of young group, 0.74 ± 0.49 mm; adult, 0.28 ± 0.34 mm), but the effect of recording depth was to inflate the estimated connectivity of the young group and could not explain the observed results. To further ensure that lower levels of correlation were not associated with a specific layer that was over-sampled in the young group, we also compared subgroups of recordings obtained at depths <0.5 mm in both groups. For these samples (\( n = 172 \) pairs for the young, \( n = 332 \) for the adult), the mean strength of effective connectivity was again significantly lower in the young group and could not explain the observed results.
lower in the young than in the adult animals (one-tailed \( t \) test, \( t_{502} = -4.79; P < 10^{-3} \)).

As a way to equalize behavioral performance between groups, we performed this initial analysis on data from more complex behavioral working memory tasks in adult monkeys (delayed match to sample and match/nonmatch tasks), whereas we relied on a simpler, oculomotor delayed response task for the young monkeys. Behavioral performance was thus very similar in the two groups (88% correct in young, 87% in adults, excluding breaks in fixation) and the difference was not significant (two-tailed \( t \) test, \( t_{510} = 0.35; P > 0.3 \)). Nevertheless, we carried out additional analyses to determine whether the observed differences in connectivity could have been caused by differences in the behavioral tasks used (as detailed later).

To test the combined effect of these factors on effective connectivity, we performed an analysis of covariance (ANCOVA) including all the aforementioned variables (firing rate, interelectrode distance, signal correlation, recording depth, and performance). When this was done, we found a highly significant difference between the young and adult groups (\( F(1,1311) = 45.2; P < 10^{-3} \)). A significant difference between groups was also present for the equivalent analysis of the sulcus recordings, excluding recording depth, which no longer indicated cortical layer (\( F(1,463) = 10.6; P < 0.005 \)). The outcomes of all these control analyses relied on the full distribution of cross-correlation strengths, which includes positive and negative values. Similar results were obtained when these controls were restricted to the negative cross-correlation strengths only, consistent with their dominant role in driving the differences between young and adult animals.

To examine the potential effect of the task itself on the estimates of cross-correlation strength, we performed three controls. First, we repeated the analysis restricting the spike selection for cross-correlation to time epochs common to all tasks used: the fixation interval, the presentation of the visual stimulus that needed to be remembered, and the initial delay period that followed it (Fig. S3B). By using firing rate, distance, signal correlation, recording depth, and performance variables as covariates in the linear model, the difference in CCH strength was highly significant for all three task epochs (ANCOVA, \( F(1,894) = 13.3, P < 0.0005; F(1,753) = 21.5, P < 10^{-5}; F(1,595) = 10.8, P < 0.005 \) for the stimulus, delay, and fixation epochs, respectively). Second, we analyzed data from three adult monkeys that were tested in a working memory task and in a task involving presentation of the same stimuli displayed passively. In the passive task, no response was required and the monkeys were rewarded simply for maintaining fixation. Data from 47 neuron pairs that were tested with the passive task met the criteria for analysis; 42 of these were tested in both tasks (Fig. S3C). We found no significant difference between mean effective connectivity in the working memory and passive tasks (0.25% for the working memory, 0.27% for the passive task; two-tailed \( t \) test, \( t_{42} = 1.03; P > 0.3 \)). When we used the linear model with all covariates to compare the group of 47 pairs from the adult monkeys tested with the passive task, with the entire group of 855 pairs recorded from the young animals with the oculomotor delayed response task, the difference between groups was still significant (ANCOVA, \( F(1,896) = 6.88, P < 0.01 \)). Third, we analyzed data from three adult monkeys before training in any working memory task, when stimuli were presented passively. Data from 475 neuron pairs were available for analysis from this condition. Average cross-correlation strength was 0.72%, which was higher than the 0.37% in the adult monkeys performing the working memory tasks reported earlier; however, the groups varied considerably in firing rate and other variables. We again used the linear model with all covariates to compare the group of 47 pairs recorded before working memory training from the adult monkeys with the group of 855 pairs recorded from the young animals. The difference between groups was diminished, but still highly significant (ANCOVA, \( F(1,1323) = 11.72; P < 0.001 \)). We conclude that group differences between the young and adult monkeys are robust and detectable across a range of task conditions, when variables such as the firing rate and signal correlation are accounted for.

To examine the temporal properties of the difference in effective connectivity, we examined a wider range of cross-correlation time scales. The difference in effective connectivity strength we have described was based on the central 5 ms of the CCH (Fig. 3A). Basing the comparison on the central 20 ms of the CCH revealed a difference that was still significant, albeit less pronounced (permutation test, \( P < 0.005 \); Fig. S4A). A difference in the opposite direction was observed for spike-count correlation (noise correlation), with the young group exhibiting higher values than the adult group (Fig. S4B).

As the number of animals tested was relatively small, we wondered how consistent the effect was across individual subjects; in other words, would the result still hold when considering each monkey as one observation? This possibility is important because the overall difference could, in principle, be driven by an outlier individual contributing many extreme correlation values to its pool. This was not the case. When considering only the negative correlations for each monkey, the means of the young animals were all smaller than the means of the adult animals (Fig. 3B). The probability of this outcome happening just by chance is 1 in 56, or 0.018, given that there are three young and five adult monkeys.
To confirm this result, we also considered distance between electrodes, firing rate, signal correlation, behavioral performance, and recording depth as independent variables in each monkey. We performed a regression analysis of cross-correlation strength against these variables, without assuming any difference between the young and adult subjects. Uncorrected results from each monkey are shown in Figs. S5 and S6. We then subtracted from the raw cross-correlation strength (positive or negative) of each pair the predicted effect of these variables. The residuals of this process reflect individual differences, excluding these effects. Averaged for each monkey, these residual cross-correlation strengths were separable and not overlapping for the groups of young and adult monkeys (Fig. 3C). The difference between means, using equal numbers of samples from each monkey, was significant (permutation test, $P < 0.05$). These results indicate that, despite the small number of subjects and noisy nature of cross-correlation strength measurements, the difference in effective connectivity between young and adult monkeys was robust across monkeys.

Discussion

Our study demonstrated an age-dependent difference in intrinsic connectivity in the prefrontal cortex. Specifically, we observed an overall decrease in the incidence and strength of negative correlations in adult animals, suggestive of a selective decline in intrinsic inhibitory connections as an animal matures. Inhibitory interactions have been implicated in sculpting the spatial and temporal selectivity of prefrontal neurons, which is essential for the execution of working memory tasks (25). Although it is generally accepted that the prefrontal cortex and the cognitive functions it supports reach full maturity only in adulthood, the nature of the corresponding neurophysiological changes in the developing prefrontal cortex has not been established. We examined a number of factors that could account for systematic differences in connectivity strength between the two groups, including firing rate, distance between neurons in each pair, and neuronal tuning, but the difference in effective connectivity persisted even after all these factors were accounted for. The decreasing incidence and efficacy of putative inhibitory interactions in adults biased the distribution of correlation strengths toward positive values; however, this result did not correlate with an increase in the strength of excitatory connections. Thus, the overall shift toward more positive cross-correlation strengths in adult animals could be attributed to a selective loss of inhibitory connections and is in keeping with the typical developmental progression of decreasing numbers of anatomical connections (26). The apparent conservation of excitatory interactions is also interesting and could result in part from several factors. For example, synaptic pruning typically involves silent synapses (26), which would not alter the measured effective connectivity between neurons; synapses may be lost between neurons at distances longer (>1 mm) than those sampled in our study; and homeostatic mechanisms may maintain the firing of a neuron at a constant level after the loss of synaptic inputs, presumably by strengthening the efficacy of remaining synapses (27).

The time of puberty in humans marks a period of continued increase in working memory ability (4, 5) as well as structural changes to the prefrontal cortex (28–30). Cortical thickness follows an inverted-U shape, peaking during the onset of puberty, and sex-specific differentiation in cortical structures begins to be observed at that time (30–32). The monkey prefrontal cortex continues to undergo anatomical maturation during adolescence and early adulthood, similar to the human pattern of development (26, 33, 34). From a developmental standpoint, the male rhesus monkey enters puberty (the typical transition point between the juvenile and adolescent state) at ~3.5 y of age, equivalent to 11 y in humans (19, 35). Some developmental studies suggest, however, that the human adolescent prefrontal cortex is more similar to that of the juvenile, 2- to 3-y old macaque (26, 36). If so, the bias toward increased inhibitory connectivity we report here for young monkeys might also be an intrinsic feature of human prefrontal cortex at a comparable stage of development.

Our conclusions regarding the maturation of the prefrontal cortex involve a number of caveats, including that the study relied exclusively on male monkeys, that it was not practical to obtain neurophysiological recordings from a greater number of animals, and that effective connectivity represents a functional rather than anatomical measure of connectivity, which nonetheless allows statistical comparisons at the level of neuronal populations. Additionally, the behavioral tasks differed between the young and adult monkeys, but we believe that the choice of task is unlikely to account for the differences in connectivity strength observed across groups, because these remained significant across a range of tasks and task epochs analyzed.

Our results highlight the nature of changes that occur during the maturation of the prefrontal cortex at the level of single neurons, and these may be related to changes in patterns of prefrontal activation observed with imaging methods (11–14, 37). The finding of an inhibitory bias in the strength of intrinsic connectivity in young animals provides a potential functional basis for the cognitive changes that occur after puberty.

Methods

Eight 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 US 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.

Surgery and Neurophysiology. A 20-mm-diameter recording cylinder was implanted over the dorsolateral prefrontal cortex of each monkey. We performed neuronal recordings by using arrays of two to eight microelectrodes in each cylinder with glass-coated, tungsten electrodes of 250 μm diameter with an impedance of 1 MΩ measured at 1 kHz (Alpha-Omega Engineering) or epoxy-coated tungsten electrodes with a diameter of 125 μm and an impedance of 4 MΩ at 1 KH (FHC). A microdrive system (EPS drive; Alpha-Omega Engineering) was used to position electrodes and advance them into the cortex. The electrical signal was amplified, band-pass-filtered between 500 Hz and 8 kHz, and recorded through a modular data acquisition system at 25-μs resolution (APM System). Anatomical localization of electrode penetrations was determined based on structural MRI of the brain obtained after implantation of the recording cylinders (BrainSight; Rogue Research). Data were collected from areas 46 and 8a in the dorsolateral prefrontal cortex. Most recordings analyzed were collected from the exposed surface of the cortex, at least 1 mm away from the principal sulcus, in the superior convexity of the prefrontal cortex, and in the surface cortex between the principal and acute sulcus (Fig. 1A). We used three additional selection criteria to ensure that our analysis focused on horizontal connections across the surface of the cortex, as described before (21); (i) both neurons of a pair must have recorded at a depth of <2.5 mm from the surface of the cortex; (ii) the two electrode penetrations of a pair must have met the surface of the cortex no more than 1 mm apart from each other (i.e., if one electrode traversed more than 1 mm than the second before entering the cortex, the pair was discarded); and (iii) pairs of neurons that were recorded at depths >1 mm relative to each other were discarded, even if the penetrations otherwise avoided a sulcus. Sulcus recordings were processed separately.

Behavioral Tasks. The monkeys sat 60 or 68 cm away from a monitor in a dark room with their heads fixed, as described in detail previously (38, 39). An IR eye position tracking system (model RK-716; ISCAN) sampled and recorded eye position at 240 Hz. Monkeys were required to maintain their gaze on the fixation target throughout a trial; breaks in fixation aborted the trial. The visual stimulus presentations were controlled by in-house software (40), developed in the MATLAB (MathWorks) computational environment.

The monkeys were tested with several behavioral tasks. We chose generally more difficult ones for the adult monkeys as a way to equalize performance rates between the young and adult monkeys. Adult monkeys were tested with the delayed match-to-sample task (39) and match–nonmatch task (38); the young monkeys were trained to perform an oculomotor delayed response task (41). In the delayed match-to-sample task, monkeys were trained to remember the location of a stimulus and to release a lever when a subsequent stimulus appeared at the same location. The stimulus consisted of a 1.5° square in green or red color and was displayed at one of nine locations on a 3 × 3 grid with 15° separation between locations. In half the trials, an array of multiple stimuli was presented, one of which differed in color and constituted the cue. After the monkeys pulled the lever and kept their eye fixated for 0.5 s, a cue was presented for 0.5 s, followed by a delay period of 1.0 s and a pseudorandom sequence of zero to two nonmatch stimulus presentations, each lasting 0.5 s and separated by delay periods of 0.5 s. When a stimulus appeared at the same location as the cue, the monkeys were required to release the lever within 0.5 s after the match stimulus disappeared to receive a reward. The trial was immediately aborted if the monkeys released the lever at any other time during the trial. Variations of the task with only four instead of nine spatial locations used in a block of trials were collected for sessions (39).

In the match–nonmatch task (38), a 2° white stimulus appeared in one of nine locations arranged on a 3 × 3 grid of 10° separation between adjacent stimuli. A trial started with a 1-s fixation interval, followed by a first stimulus presentation lasting for 0.5 s. After the first stimulus presentation, there was a 1.5-s delay period, and then a second stimulus appeared at the location identical or diametrically opposite to the first stimulus, also for 0.5 s. After another delay period (1.5 s), choice targets were presented, and the monkey was required to make an eye movement to the green target if the stimuli matched or to the blue target if the stimuli did not match. We additionally analyzed data from passive presentations of the stimuli used in the match–nonmatch task. Blocks of trials involved presentation of the exact same stimuli, and with the same timing of presentation. However, the choice targets did not appear in these trials, and the monkey was rewarded solely for maintaining fixation (42).

The oculomotor delayed response task required monkeys to remember the location of a stimulus flashed on a screen for 0.5 s. This 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 stimulus within 0.6 s.

Developmental Profiles. As in humans, the age of pubertal onset can vary considerably between individuals, making chronological age an imprecise index. We therefore tracked developmental measures in a quarterly basis over a period before, during, and after the behavioral training and neurophysiological recordings. Morphometric measures obtained included body weight, crown-to-rump length, chest circumference, and ulna and femur length. We ascertained testicular volume with an orchidometer (Prader Orchidometer; ESP). We checked for visible eruption of canine teeth and determined bone maturation by X-rays of the upper and lower extremities. We additionally obtained blood samples and determined serum concentration of circulating hormones including testosterone and dihydrotestosterone through extraction and enzyme-immunoassay (performed at the assay services unit of the Wisconsin National Primate Research Center). Developmental data were plotted relative to the onset of neurophysiological recording. Considerable variability is present in measures of body weight and hormone concentrations, so we smoothed the corresponding data points with a three-point triangular filter.

Neuron Selection. Recorded spike waveforms were sorted into separate units using an automated cluster analysis method based on the KlustaKwik algorithm, relying on principal component analysis of the waveform; this was implemented in MATLAB as described previously (38). Spike sorting may introduce errors in the estimation of correlated firing (43), but this represents a random source of error for our analysis and can only dilute the difference between groups that have otherwise been obtained with identical means of data acquisition and automated spike sorting. To avoid the most serious problems with spike separation, all data analyzed in this paper involves pairs of neurons recorded from separate electrodes. Neurons with high correlations of firing during the period of interest were identified by comparing the firing rate in the 0.5-s interval around a stimulus presentation with the 1- or 0.5-s interval of fixation (paired t test, P < 0.05). Neurons with a significant change in activity in other task epochs were evaluated in a similar way. Only trials from correct behavioral responses to the task were used in these analyses.

Cross-Correlation Analysis. To estimate the strength of intrinsic neural connections of each brain area, cross-correlation analysis was performed on pairs of neurons recorded simultaneously from separate electrodes spaced 0.5–1.0 mm apart from each other, implemented in MATLAB. Only neuron pairs containing more than 1,000 spikes in total, and at least 100 spikes in each neuron, for the task epoch analyzed, were included. CCHs were constructed from spike trains of each pair of neurons (21) by using all available spikes from all recorded trials and conditions, as well as for separate periods, such as the fixation period. As previous studies have identified CCH peaks of varying widths, we relied on time scales of ±50 ms and ±200 ms with bins of 1 and 4 ms, respectively. CCH peaks almost always were centered at time 0, a finding indicating that the two recorded neurons shared common inputs. We therefore identified peaks at the central 5 and 20 ms of the ±50 ms and ±200 ms CCHs, respectively.

Strength was computed as the area under the peak, subtracting the expected value of the CCH under the assumption that the two spike trains are independent. It was expressed as a percentage of the total number of spike counts, as described in detail elsewhere (21). To account for potential effects of stimulus presentations or other factors covarying during a trial that could simultaneously increase firing rates in both neurons of a pair, we corrected CCHs by creating surrogate spike trains. We randomly varied the time of each spike in a trial by sampling a replacement spike time from a uniform distribution spanning 50 ms around the time of the original spike. This method destroyed temporal structures beyond 50 ms.

Averaged cross-correlation strengths were compared between the groups of pairs recorded from the young and the adult monkeys using a t test and a permutation test. All results were consistent between the two. The analysis was performed for all available spikes, and for spikes recorded during the fixation period, stimulus presentation period, and delay period (for those neuron pairs that satisfied the minimum spike criteria in each task epoch). To test the influence of firing rate on cross-correlation strength, we calculated the
geometric mean of discharge rates for each neuron pair. This was defined as the square root of the product of mean discharge rates of each neuron, across all conditions used to construct the CCH. Cross-correlation strength was then plotted as a function of firing rate. Other factors (behavioral performance in the session in which the data were recorded, depth of recording for each neuron, distance between electrodes, and signal correlation) were examined in similar fashion. The cumulative effect of all these variables was determined by ANCOVA implemented in the SPSS computing environment (version 21; IBM). A linear model was constructed with cross-correlation strength as the dependent variable (average value for each pair of neurons), young or adult group membership as a fixed factor, and firing rate, behavioral performance, recording depth (average value for each pair of neurons), electrode distance, and signal correlation as covariates. This analysis was performed for cross-correlation strength determined from the entire spike train, and separately in each of the fixation, stimulus presentation, and delay periods.

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Fig. S1. Developmental profile. (A) Body mass for each of the three young monkeys as a function of time, evaluated in quarterly assays. Data are aligned to the onset of neurophysiological recordings (Q0). (B) Serum testosterone concentration obtained from each monkey. Data for [T] were run in a single batch for all available samples at the time. (C) Testis size as a function of time. (D) Femur length as a function of time.

Fig. S2. Analysis of principal sulcus recordings. (A) Predictor-corrected average (and SE) of cross-correlation strength for data from principal sulcus recordings in young (n = 266) and adult monkeys (n = 203). (B) Distribution of cross-correlation strengths among all pairs of neurons from sulcus recordings. Vertical lines represent means of the distributions. (C) Average values of cross-correlation strengths for negative (inhibitory) interactions in sulcus recordings, plotted separately for each one of the young and adult monkeys. Horizontal lines represent group means.
Fig. S3. Effects of firing rate, epoch, and task. (A) Mean firing rate from all pairs and all stimulus conditions used to estimate effective connectivity in the young and the adult animals. (B) Cross-correlation strength computed separately at different task epochs—fixation period, stimulus presentation, and delay period following the stimulus—for the same sample of neuron pairs. (C) Cross-correlation strength in the same group of neurons, tested with a working memory task, and with passive presentation of visual stimuli.

Fig. S4. Time scale of connectivity strength differences. (A) Mean cross-correlation strength estimated based on the center 20 ms of the cross-correlation histogram (CCH; predictor-corrected). (B) Mean spike-count correlation (noise correlation) for the same pairs of neurons.

Fig. S5. Variability of cross-correlation strength between individual monkeys. (A) CCHs for pairs of neurons are averaged separately for each animal in the young and (B) in the adult monkeys, plotted with a separate color. (C and D) Same as in A and B for data recorded from the principal sulcus.
Fig. S6. Mean cross-correlation value, computed separately for each subject. Each point represents the average (and SE) of cross-correlation strength for each of three young (blue) and five adult subjects (red).