# Behavioral response inhibition and maturation of goal representation in prefrontal cortex after puberty

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**Executive functions including behavioral response inhibition mature after puberty, in tandem with structural changes in the prefrontal cortex. Little is known about how activity of prefrontal neurons relates to this profound cognitive development. To examine this, we tracked neuronal responses of the prefrontal cortex in monkeys as they transitioned from puberty into adulthood and compared activity at different developmental stages. Performance of the antisaccade task greatly improved in this period. Among neural mechanisms that could facilitate it, reduction of stimulus-driven activity, increased saccadic activity, or enhanced representation of the opposing goal location, only the latter was evident in adulthood. Greatly accentuated in adults, this neural correlate of vector inversion may be a prerequisite to the formation of a motor plan to look away from the stimulus. Our results suggest that the prefrontal mechanisms that underlie mature performance on the antisaccade task are more strongly associated with forming an alternative plan of action than with suppressing the neural impact of the prepotent stimulus.**

prefrontal | antisaccade | monkey | neurophysiology | adolescence

Behavioral response inhibition, and cognitive task performance more generally, improves substantially between the time of puberty and adulthood (1–4). Risky decision-making peaks in adolescence, the time period between puberty and adulthood that is most closely linked to delinquent behavior in humans (5–7). Performance in tasks that assay response inhibition, such as the antisaccade task, improves into adulthood, reflecting the progressive development of behavioral control (3). This period of cognitive enhancement parallels the maturation of the prefrontal cortex (8–11). Anatomical changes in the prefrontal cortex continue during adolescence, involving gray and white matter volumes and myelination of axon fibers within the prefrontal cortex and between the prefrontal cortex and other areas (8–15). Changes in prefrontal activation, including increases (12, 16–20) and decreases (21, 22), have been documented in imaging studies for tasks that require inhibition of prepotent behavioral responses and filtering of distractors.

Much less is known about how the physiological properties of prefrontal neurons develop after puberty. Similar to the human pattern of development, the monkey prefrontal cortex undergoes anatomical maturation in adolescence and early adulthood (23, 24). Male monkeys enter puberty at ∼3.5 y of age and reach full sexual maturity at 5 y, approximately equivalent to the human ages of 11 y and 16 y, respectively (25, 26). By some accounts, biochemical and anatomical changes characteristic of adolescence in humans occur at an earlier, prepubertal age in the monkey prefrontal cortex (27, 28), so it is not known if cognitive maturation or neurophysiological changes occur in monkeys after puberty. The contribution of prefrontal cortex to antisaccade performance has also been a matter of debate, with contrasting views favoring mechanisms of inhibiting movement toward the visual stimulus or enhancing movement away from it (29–31). Potential maturation of behavioral response inhibition may therefore be associated with a more efficient suppression of the stimulus representation in neural activity (weaker visual responses to stimuli inside the receptive field), stronger motor responses (higher activity to saccades), or enhancement of the appropriate goal representation (stronger activity for planning a saccade away from the stimulus). To examine the mechanisms that facilitate the mature ability to resist generating a response toward a salient stimulus, we used developmental markers to track transition from puberty to adulthood in monkeys and sought to identify neural correlates of changes in antisaccade performance within the visual and saccade-related activations of prefrontal neurons.

## Results

**Developmental Profiles.** Four male macaque monkeys (*Macaca mulatta*) were used in this study. Times of puberty and full sexual maturity can vary considerably between individuals, so we used morphometric, radiographic, and hormonal measures to determine the onset of puberty in each (*SI Materials and Methods*). Behavioral and neural experiments were performed at two stages of development: after the onset of puberty and in adulthood.

Measures such as body mass, femur length, and testis size were all rapidly increasing at the first stage of experiments, consistent with individuals in a growth trajectory (Fig. S1). Canines had not erupted in three of four monkeys, and epiphyseal plates of extremities were open in all four, also signs of continued growth. We refer to this as the "young" stage. Initial behavioral training was performed around this time, and neurophysiological recordings were obtained beginning at a median age of 4.3 y (last measurement before the onset of neurophysiological recordings; range, 4.0–5.2 y). Recordings lasted one to two quarters of a year. After that time period, recordings ceased for ∼1 y, a period during which the monkeys received no further exposure to the task or training of any kind. They remained housed in the same animal colony.

The monkeys were then briefly reintroduced to the behavioral tasks, 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 y (range, 5.6–7.3 y; range of intervals from young stage, 1.6–2.1 y). We refer to this as the "adult" stage.

### Significance

The ability to resist impulsive responses matures late in life, after puberty. This longitudinal study of the prefrontal cortex in monkeys shows that behavioral response inhibition improves not because the adult prefrontal cortex is better able to inhibit the effects of a prepotent stimulus but rather because it can more readily form an alternative plan of action. The finding is revealing about the nature of cognitive maturation and the conditions in which it is impaired that have clinical and social implications.

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Morphometric measures had plateaued at the time of recordings in the adult stage (Fig. S1 *B*, *D*, and *F*), as expected of mature adults. Serum testosterone level, which fluctuates hourly and is therefore a less reliable indicator, was nevertheless also higher around the time of the adult-stage recordings compared with that of the young stage (Fig. S1*G*).

**Behavioral Performance.** The antisaccade task (32) requires an eye movement to a location diametrically opposed to a salient visual cue, and we observed performance on this task to improve significantly between the time of puberty and adulthood (Fig. 1). We assessed performance for three temporal variants of the antisaccade task: the "overlap" variant (Fig. 1*A*, *Left*), in which the visual cue and fixation point overlapped for 100 ms before both turning off; the "zero-gap" variant (Fig. 1*A*, *Middle*), in which onset of the visual cue and offset of the fixation point occurred simultaneously; and the "gap" variant (Fig. 1*A*, *Right*), in which the visual cue was presented 100 or 200 ms after the fixation point was extinguished. The latter represented the most difficult condition, as no fixation point was present to hold the gaze at the time of stimulus onset. We manipulated the gap in this fashion because we did not know at the outset of the experiments if behavioral improvements would only be evident for the most difficult conditions of the task. Additionally, the cue could appear at any of eight locations in these experiments (rather than two locations often used in antisaccade paradigms; Fig. 1*A*, *Inset*), again making the task more difficult for the animals. On average, asymptotic performance in the young stage was 56.6% correct for the overlap variant of the task (chance performance corresponds to 12.5%). When the same animals were tested in the adult stage, performance improved to an average of 79.6% correct responses (Fig. 1*B*). Similar improvements were observed for the zero-gap and gap variants (Fig. 1*B*). The effect of developmental stage was highly significant (three-way ANOVA of performance with factors young/adult stage, task variant, and individual monkey, $F_{1,912} = 545.6$; $P < 10^{-10}$). The improvement was evident across task variants; no significant interaction was present between the young/adult stage factor and task variant ($F_{2,912} = 1.96$; $P > 0.1$). On the contrary, a significant three-way interaction was present between the young/adult stage, task variant, and individual monkeys ($F_{6,912} = 2.95$; $P < 0.01$), suggestive of individual differences in maturation.

From the perspective of the saccade endpoint alone, most of the adult-stage improvement could be attributed to a reduced propensity to look directly toward the visual cue, or to a location other than the visual cue or the correct antisaccade goal, which are "e2" and "e3" error types, respectively (Fig. 1*C*). Young animals were also more likely to commit a less commonly observed nonspatial error of failing to maintain gaze for sufficient duration on the antisaccade goal (Fig. 1*C*, *e1*).

Performance on the antisaccade task may have improved in adulthood via at least two mechanisms (not mutually exclusive). First, monkeys may have delayed their responses to have more time to view the cue and plan the saccade. The benefit of longer reaction times can be demonstrated by the lower performance in task conditions associated with shorter reaction times (Fig. 1 *C* and *D*). Alternatively, the adult-stage performance gains could have been the result of an increase in the speed at which the antisaccade planning was carried out. Our findings (Fig. 1*D*) were more consistent with the latter explanation, as reaction times were significantly reduced in adulthood, across all task conditions (three-way ANOVA, $F_{1,30442} = 1{,}413.6$; $P < 10^{-10}$). A significant three-way interaction was present between the young/adult stage, task variant, and individual monkeys ($F_{6,30442} = 82.04$; $P < 10^{-10}$), suggesting different patterns of reaction time improvement across tasks for individual animals.

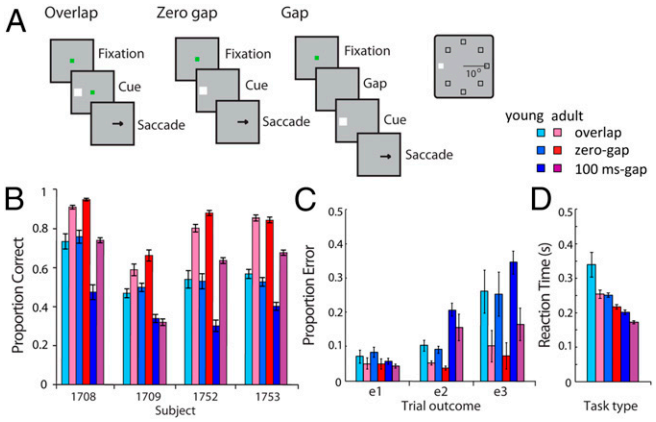


**Fig. 1.** (*A*) Sequence of events in the antisaccade task. (*Left*) Overlap variant: the cue and fixation point overlap for 100 ms before they both turn off and signal the requirement for a saccade away from the cue. (*Middle*) Zero-gap variant: the fixation point turns off simultaneously with the cue. (*Right*) The 100-ms gap variant: the fixation point turns off, and after a 100-ms gap, the cue appears. (*Inset*) Possible locations of the target in the screen. (*B*) Individual performance in the antisaccade task. Mean performance (and SEM) is shown for each monkey ($n = 134$ sessions for young, $n = 179$ for adult). (*C*) Proportions of trials that ended in different types of errors (*e1*–*e3*) for each task variant. Histograms represent means of all sessions during which recordings were obtained. Error bars represent SEM across individual monkeys. (*D*) Mean value of reaction time in correct trials, defined as the interval between the cue presentation and onset of the saccade. Error bars represent SEM across monkeys.

**Overview of Neuronal Activity in the Antisaccade Task.** Neuronal responses recorded during these tasks allowed us to determine the nature of activity changes associated with cognitive development after the onset of puberty. We recorded a total of 607 neurons from areas 8a and 46 of the dorsolateral prefrontal cortex (Fig. 2*A*) in the young stage (33, 133, 158, and 283 neurons from the four monkeys, respectively). We subsequently recorded from 830 neurons in the adult stage from the same monkeys (133, 41, 238, and 418, respectively). To perform a comparison of responses in the antisaccade task when the stimulus appeared in the receptive field and outside it, we distinguished between three categories of neurons: those with visual responses, those with perisaccadic responses (referred to hereafter as "motor" neurons for brevity, even though we did not have direct evidence of influence of these neurons onto eye movements), and those with visuomotor responses. We identified neurons that responded significantly to at least one task epoch of the oculomotor delayed response (ODR) task compared with baseline activity (paired $t$ test, $P < 0.05$). A total of 364 neurons in the young stage and 444 neurons in adult stage were thus selected. The overall pattern of activity did not differ appreciably if we included all neurons recorded in the antisaccade task, breaking down responses based on the ipsilateral and contralateral field (*SI Text*).

Neural activity recorded during correct trials is shown in Fig. 2 *B*–*E*. At the young and adult stages, when the stimulus appeared in the receptive field, activity was highest for the most difficult condition, the gap condition (Fig. 2 *B* and *D*). In other words, increased activation elicited by the stimulus was associated with difficulty in making a correct saccade away from it. When the stimulus appeared out of the receptive field, activity appeared earlier for the gap condition, in which the fixation point turned off before the stimulus appearance, even though peak firing rate differed little between conditions synchronized to the saccade (Fig. 2 *C* and *E*). These effects were even more pronounced for the 200-ms gap variant for the two monkeys that were tested with it (Fig. S2).

To determine the changes between stages, we compared activity in three time periods. Baseline firing rate, before the cue presentation, was significantly higher in the adult stage compared with the young stage ($t$ test, $t_{631} = 7.56$; $P < 10^{-12}$). Firing rate driven by the saccade in the receptive field (above the baseline) was also considerably higher in the adult (Fig. 2*E*, *Inset*). On the contrary, little difference was present between stages for firing rate following the cue in the receptive field, above the baseline (Fig. 2*D*, *Inset*).

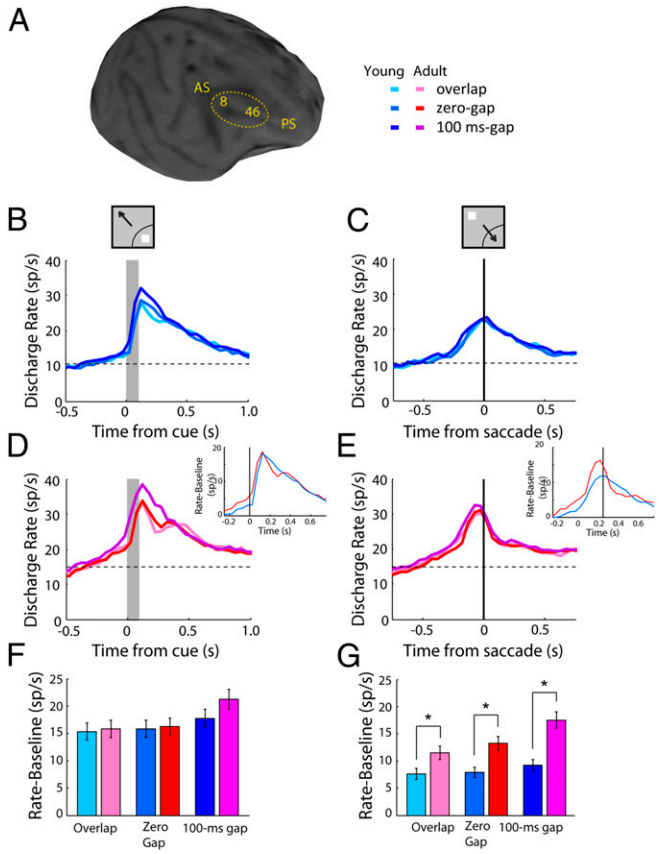


**Fig. 2.** (*A*) MRI image of one young monkey with areas of recording indicated. (*B*) Average population peristimulus time histogram for neurons recorded during the three variants of the antisaccade task in the young stage ($n = 364$) when a stimulus appeared in the receptive field. Activity is synchronized to the cue (gray bar). (*Insets*) Schematic illustration of the stimulus and saccade location relative to the receptive field (arc), which varied for each neuron. (*C*) As in *B*, for a stimulus appearing away from the receptive field, requiring an eye movement toward it. Activity is synchronized to the onset of the saccade (vertical line). (*D* and *E*) As in *B* and *C*, for neurons recorded in the adult stage ($n = 444$). (*Insets*) Average discharge rate minus baseline rate for the zero-gap condition, plotted in the same axes for the young and adult stages. (*F*) Average activity during the stimulus presentation in the receptive field, after subtracting the baseline firing rate, from neurons recorded in sessions matched for behavioral performance ($n = 89$ for the young, $n = 118$ for the adult stage). (*G*) As in *F*, for stimulus presentation out of the receptive field.

**Comparison Between Stages.** Differences between stages may be influenced by the mixture of response properties present in each population sample, so we examined activity separately for neurons with visual, motor, or visuomotor activity in the ODR task and well-defined receptive and motor fields that did not encompass the location diametric to the best response location (Fig. 3 and Fig. S3). Visual neurons selected in this fashion ($n = 53$ in the young stage, $n = 38$ in the adult) exhibited stimulus-driven activity in the antisaccade task that was increased in adulthood compared with the young stage (Fig. 3 *C* and *D*). Some of these neurons also exhibited elevated activity in the antisaccade task even without a stimulus in the receptive field (Fig. 3 *G* and *H*). Such activity would be expected by neurons mediating vector inversion, the planning of an eye movement away from the trigger stimulus (33). Importantly, across the population, this activity was significantly higher in the adult stage vs. the young stage (Fig. 3 *E* and *F*). A two-way ANOVA for firing rate elicited by the stimulus out of the receptive field after subtracting the baseline rate revealed a significant effect of stage (factors young/adult stage and task variants, $F_{1,264} = 29.93$ for stage; $P < 10^{-6}$). In contrast to the dramatic changes observed in visual neurons, the activity of motor neurons was virtually identical between the young and adult stages ($n = 55$ in the young stage, $n = 68$ in the adult). Analysis of activity synchronized to the onset of the saccade (Fig. S3 *E* and *F*) revealed no significant difference in mean firing rate between stages (two-way ANOVA, $F_{1,519} = 0.1$ for main effect of stage; $P > 0.7$). Finally, activity of visuomotor neurons ($n = 121$ in the young stage, $n = 188$ in the adult) mirrored the changes of visual neurons, with an increase in firing rate for the stimulus (Fig. S3 *I* and *J*) and saccade in the receptive field (Fig. S3 *K* and *L*).

The observed differences in firing activity may be related not only to age but also to behavioral performance. To distinguish the impact of these factors, we modified the analysis in three ways. First, we excluded neurons with purely motor activity, which did not differ between stages. This yielded a sample of 309 neurons in the young stage and 324 neurons in the adult stage. Second, we compared firing rates between stages in sessions matched for behavioral performance by selecting neurons recorded in the highest young sessions and lowest adult ones. Performance in this subset of sessions was 71% for the young and 70% for the adult. Third, we subtracted the baseline firing rate from the activity recorded before the stimulus presentation. Mean firing rates computed in this manner were then compared by using a two-way ANOVA with factors young/adult stage and task variant (Fig. 2 *F* and *G* and Fig. S4). In the condition involving a stimulus

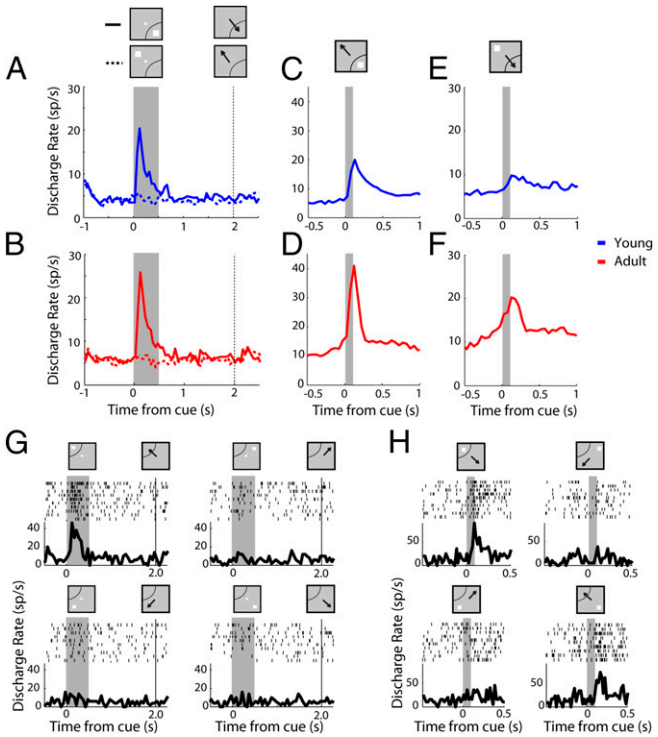


**Fig. 3.** (*A*) Average population peristimulus time histogram for neurons with visual but no motor activity, tested with the ODR task in the young stage ($n = 53$). Activity is synchronized to the stimulus presentation (indicated as a gray bar). Dotted vertical bar represents the time point when the fixation point turns off, which cues the monkey to perform an eye movement. (*B*) As in *A*, for the adult stage ($n = 38$). (*C* and *D*) Activity for the same neurons as in *A* and *B* during the appearance of the stimulus in the receptive field in the antisaccade task. Responses from all antisaccade task variants have been averaged together. (*E* and *F*) Average activity for the same neurons as in *A* and *B* during the appearance of the stimulus out of the receptive field in the antisaccade task. (*G*) Rasters and peristimulus time histograms for a neuron with visual but no motor activity in the ODR task. The neuron responds only to a stimulus in the receptive field (*Top Left*). (*H*) Responses of the same neuron in the antisaccade task. The neuron responds strongly to a stimulus in its receptive field (*Top Left*), but also to a diametric stimulus that instructs an eye movement toward the receptive field (*Bottom Right*).

out of the receptive field, requiring a saccade into the receptive field, the effect of stage was highly significant ($F_{1,615} = 32.98$; $P < 10^{-7}$). A post hoc Tukey test confirmed a significant increase for each of the task variants ($P < 0.05$, $P < 0.005$, and $P < 0.0005$ for the overlap, zero-gap, and gap conditions, respectively; Fig. 2*G*). In contrast, when the stimulus appeared in the receptive field, there was no significant main effect of stage in the two-way ANOVA ($F_{1,615} = 1.14$; $P > 0.2$). These results identify changes in firing activity that are likely a result of maturation.

An important consideration for a longitudinal study of this sort is that differences between the young and adult monkeys may reflect the cumulative exposure to the task, rather than developmental stage itself. To test for this possibility, we separated sessions in the young stage between early and late phases of recordings, relying on a median split (Fig. S5). A two-way ANOVA revealed no significant differences in firing rate between early and late sessions for the condition involving the stimulus in the receptive ($F_{1,743} = 2.62$; $P > 0.1$) or for the saccade in the receptive field ($F_{1,729} = 0.49$; $P > 0.4$).

Finally, we saw consistent results across individual monkeys. In two monkeys, we collected sufficient recordings in the young and adult stages to make possible a comparison in sessions matched for performance between stages (monkey 1752, young performance, 71%; adult performance, 69%; and monkey 1753, young performance, 69%; adult performance, 70%). In both monkeys, a significant increase in firing rate was present when the stimulus appeared out of the receptive field (two-way ANOVA, $P < 0.01$ and $P < 10^{-7}$ for the two animals) but not when the stimulus appeared in the receptive field ($P > 0.4$ and $P > 0.2$, respectively).

**Relationship Between Performance and Firing Rate.** Task performance improved greatly between the young and adult stages, so it was important to identify aspects of activity associated with high and low levels of performance. We first examined the young stage by splitting the recording sessions down the median (Fig. 4 *A* and *B*). In the condition of a stimulus appearing in the receptive field (Fig. 4*A*), a two-way ANOVA of firing rate relative to baseline, with factors high/low performance and task variant, revealed a significant effect of performance ($F_{1,921} = 9.92$; $P < 0.005$). In contrast, we found no effect in the condition involving the saccade in the receptive field ($F_{1,921} = 0.2$; $P > 0.9$). The higher cue-driven activity in trials with lower performance is also consistent with the overall trend observed earlier between firing activity and task difficulty: when the stimulus was in the receptive field, higher activity was observed for the gap condition vs. the zero-gap and overlap conditions (Fig. 2).

We reached the same conclusions when we analyzed error responses (Fig. S6), considering trials specifically involving incorrect saccades toward the stimulus (e2 errors). We identified neurons with such error trials in each of the spatial configurations involving a stimulus in the receptive field and saccade in the receptive field. In the condition requiring a saccade toward the receptive field (Fig. S6*B*), we found no significant difference between correct and error trials (paired *t* test, $t_{107} = 0.15$; $P > 0.8$). However, error trials were associated with increased activity in the condition involving a stimulus in the receptive field (paired *t* test, $t_{107} = 2.62$; $P < 0.01$). This difference between correct and incorrect responses was also evident when quantified with choice probability (*SI Text*) after the stimulus appeared in the receptive field (Fig. S6*C*). No equivalent differences were present for a saccade toward the receptive field (Fig. S6*D*). In other words, trials in which stimulus-driven activity was higher than average tended to result in errors (Fig. S6*A*).

The difference in activity between low- and high-performance sessions displayed a qualitatively different pattern in the adult stage (Fig. 4 *C* and *D*). Adult high-performance sessions were now primarily characterized by increased responses in the condition involving the saccade into the receptive field (Fig. 4*D*). A two-way ANOVA of firing rate after subtracting the baseline with factors high/low performance and task type revealed a significant effect of performance ($F_{1,948} = 10.7$; $P < 0.005$). No significant effect of performance was now present for responses

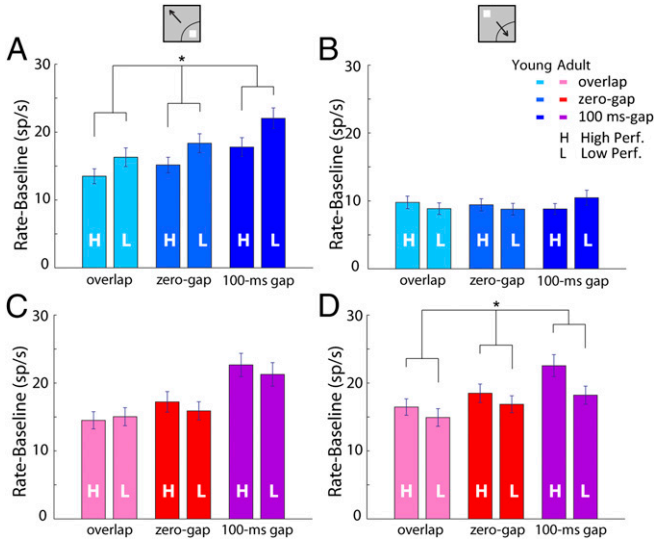


**Fig. 4.** (*A*) Average firing rate in each of the variants of the antisaccade task in the young stage for correct trials in behavioral sessions that exhibited above-average (labeled "H", $n = 153$ neurons) or below-average performance (labeled "L", $n = 156$ neurons). All responses involve the stimulus appearing in the receptive field. Asterisk indicates significant effect on two-way ANOVA. (*B*) Average firing rate for the same neurons as in *A*, when the stimulus appeared away from the receptive field. (*C*) Average firing rate in each of the variants of the antisaccade task in the adult stage for correct trials in behavioral sessions that exhibited above-average ($n = 155$) or below-average performance ($n = 163$). All responses involve the stimulus appearing in the receptive field. (*D*) Average firing rate for the same neurons as in *C* when the stimulus appeared away from the receptive field.

to the cue in the receptive field (two-way ANOVA, $P > 0.5$). Analysis of errors in the adult stage, in which a smaller sample of trials was available for direct comparison of the two conditions, was inconclusive; no significant difference was present for the condition with the stimulus in the receptive field (paired *t* test, $t_{50} = 0.15$; $P > 0.8$) or the condition with the stimulus out of the receptive field (paired *t* test, $t_{50} = 0.85$; $P > 0.4$).

These results suggest that sensory-driven and goal-related responses may contribute to variability in behavioral performance. Ultimately, the monkey's choice is likely to be determined by the relative difference between these two representations. For this reason, we quantified the difference in activity evoked by the stimulus inside the receptive field vs. the stimulus outside by a receiver operating characteristic (ROC) analysis (Fig. 5). Values greater than 0.5 indicate higher activity for the stimulus inside the receptive field, and values lower than 0.5 indicate higher activity for the stimulus outside. This measure showed that the stimulus representation dominated early in the trial. In the young stage, ROC values peaked at 120 ms and then decreased toward 0.5 to signal a somewhat weaker representation of the visual stimulus (Fig. 5, blue curves). In the adult stage, the representation of the stimulus peaked at ~90 ms (Fig. 5, red curves), a significantly earlier time point (evaluated with a bootstrap test at the $\alpha = 0.001$ significance level). After that, the signal decreased sharply, and, in the overlap and zero-gap conditions, even dipped below the 0.5 value to signal a stronger representation of the goal relative to that of the stimulus (Fig. 5). The relative strength of the goal-related activation decreased with increasing task difficulty for both age groups (Fig. 5 *A*–*C*). This greater reversal in favor of the goal in correct trials was mediated mostly by the visual and visuomotor neurons (Fig. S7). For the motor neurons, little difference in the timing or peak (minimum) of the ROC curves was observed (Fig. S7*B*). Importantly, in error trials, reaction times occurred before this reversal, and no difference between young and adult groups was present (Fig. S8). These results show that the representation of the saccadic goal overcomes the stimulus-related signal earlier and more strongly in the adult monkeys than in the young monkeys.

## Discussion

Our findings demonstrate that cognitive development in nonhuman primates mirrors the progression of response inhibition observed in humans during adolescence (1, 2, 34). We relied on the antisaccade task, used widely in the human literature for its simplicity, as performance of the task does not require mastery of complex rules requiring extensive instruction but rather the ability to resist a prepotent stimulus and plan a movement away from it (3, 4, 35–37). Our longitudinal study was designed to track the same individuals at different stages to minimize interindividual variability, which is considerable around puberty (38). Inevitably, this means that our subjects had more experience in the task as adults. However, we should note that we allowed the monkeys to reach asymptotic performance before the onset of recordings and that experiments at each stage were separated by 1–1.5 y of no exposure to the task. No appreciable differences in neural activity were observed in early and late recordings in the young stage (Fig. S5), even though the 3–6-mo period of our recordings represents a significant period for monkey development during which a continuous improvement in performance would be expected. In contrast, prominent differences in firing rate were present between the young and adult stages when comparing across sessions equated for performance (Fig. 2 *F* and *G* and Fig. S4) and across behavioral outcomes (Fig. 4). This suggests that the observed changes in prefrontal activity between the young and adult stages were a result of developmental maturation. Our results, most importantly, revealed little evidence that the adult prefrontal cortex improves in its ability to suppress the effects of a prepotent stimulus; instead, it appears to form a stronger plan of action toward the appropriate goal, consistent with its broader functional role (39).

**Response Inhibition in Adolescence.** Performance on the antisaccade task exhibits significant improvements in adolescence in humans (3, 4) and is impaired in childhood conditions such as attention deficit/hyperactivity disorder (35) and mental illnesses such as schizophrenia, which typically manifest in early adulthood (36, 37). Young monkeys are able to master tasks that require response inhibition, such as the stop signal task and the object retrieval detour, and performance has been shown to improve with age around the time of puberty (38). The present findings show that performance in the antisaccade task also improves markedly between puberty and adulthood. A relatively uniform increase in performance was observed for several variants of the task, which differed in absolute difficulty. Performance benefits were observed for all types of errors, including the ability to resist making an eye movement toward the cue. This enhanced control was not achieved through a general slowing of reaction times in the adult stage; to the contrary, adult monkeys needed less time to process the cue and plan a correct saccade.

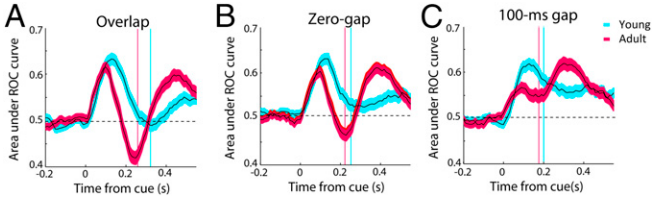


**Fig. 5.** (*A*) Area under ROC curve comparing the distribution of firing rates for the conditions with the stimulus in the receptive field and saccade in the receptive field in the overlap variant of the antisaccade task. Average ROC area values are shown for neurons in the young ($n = 309$) and adult stages ($n = 324$) in successive 100-ms windows, stepped every 10 ms, synchronized to the onset of the cue (time 0). Values greater than 0.5 are indicative of neurons generating a greater response for a stimulus in the receptive field; values lower than 0.5 indicate greater response for a saccade toward the receptive field. Vertical lines represent mean reaction times in the task. (*B*) Average ROC area values for the same neurons as in *A* in the zero-gap condition. (*C*) Average ROC area values in the 100-ms gap condition.

**Neural Changes in Antisaccade Task.** The adult stage was characterized by a number of changes in neuronal activity. We first observed an increase in the baseline activity, even before the appearance of the cue (Fig. 2). This is important because low levels of baseline activity were predictive of errors (Fig. S6), as found in other studies (40), and because baseline activity is likely related to response preparation, which has been identified as a critical parameter for the developmental improvement of inhibitory control (41, 42). Baseline activity in our data may represent preparation for the task by virtue of representing the task rules ahead of the stimulus presentation.

We also observed increased activity preceding a saccade into the receptive field (higher activity following a stimulus in the ipsilateral field; Fig. S9). This increase was not driven by the activity of motor neurons (Fig. S3 *E* and *F*). Instead, we found increased activation of purely visual neurons (Fig. 3*F*). It is likely that this change between stages is associated with the neural representation of the goal through processes such as shifting of attention and vector inversion, which correspond to the encoding of a spatial location away from the stimulus (33). Similar activation by stimuli that the monkey is explicitly instructed not to foveate has been previously reported in the prefrontal cortex (43). Activity associated with vector inversion has also been reported in the Lateral Intraparietal Area (44), at least for a memory-guided antisaccade task, which allows the monkey considerable time to plan the response away from the stimulus. We now report that neurons in areas 8a and 46 of the dorsolateral prefrontal cortex (Fig. S10) exhibit vector-inversion–related activity for an antisaccade task that imposes no delay between the stimulus presentation and response. Furthermore, we found that this was enhanced in adulthood, providing a possible substrate for the ability to plan an appropriate response away from the salient stimulus.

In principle, the improved adult performance could have also been the result of more efficient suppression of neuronal responses representing the stimulus. In the young stage, higher levels of visual activity were observed in the most difficult task variants (Fig. 2), in sessions that resulted in lower overall performance (Fig. 4*A*), and in error trials (Fig. S6). However, explicit suppression of visual responses was not observed in the adult stage. In general, evoked visual responses (relative to baseline levels of activity) exhibited very little difference between stages (Fig. 2*D*, *Inset*). Among visual neurons, higher levels of activity were observed in adulthood (Fig. 3*D*). The interpretation of this absolute increase in visual activity between stages is not clear; what matters the most is likely the relative balance between the cue-driven activity and the internal representation of the saccadic goal. Even among purely visual neurons, the goal was represented to a greater extent in adulthood (Fig. 5 and Fig. S7*A*).

The prefrontal cortex was initially thought to inhibit the ipsilateral superior colliculus for generating an eye movement in the contralateral field, which could serve as an inhibitory signal to avert a saccade toward the stimulus (30, 31). However, recent evidence supports the idea that prefrontal cortex exerts a net excitatory effect on the ipsilateral superior colliculus (29). In this context, the prefrontal cortex provides the target of the correct saccade, which is to be directly translated into motor output in the superior colliculus. Our results are consistent with the latter interpretation, as we found that, between puberty and adulthood, there is an increase in the prefrontal activity associated with the internal representation of the correct target location, which could direct or reinforce the appropriate movement. The prefrontal cortex is part of a broader network activated during the antisaccade task, and including the superior colliculus (45), basal ganglia (46), frontal eye fields (47), supplementary eye fields (48), and posterior parietal cortex (49). Our results do not preclude the possibility that developmental changes in neurophysiological activity occur in areas outside the prefrontal cortex, and that these may additionally affect motor or visual-related activity related to the task. It is upon future studies to investigate if this is the case.

## Materials and Methods

All surgical and animal use procedures were 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. We tracked developmental measures of monkeys in a quarterly basis before, during, and after neurophysiological recordings. The monkeys were initially naïve to behavioral training or task execution. They were trained in the ODR task and subsequently in three different variants for the antisaccade task during the young stage. When the animals had reached asymptotic performance in the ODR and antisaccade tasks, a 20-mm-diameter recording cylinder was implanted over areas 8a and 46 of the prefrontal cortex. At the conclusion of these recordings, the animals were no longer tested or trained for a period of ~1 y; they were tested again after reaching adulthood. In the ODR task, visual neurons were defined as having significant elevation of firing rate in the 500-ms presentation of the cue over the 1-s baseline fixation period (paired $t$ test, $P < 0.05$), no saccadic activity in the 250-ms response epoch, and no significant activity in the 1,500-ms delay period (that could be related to saccade preparation). In the antisaccade task, firing rates in a 200-ms window were subjected to a two-way ANOVA, using as factors the three variants of the task (overlap, zero-gap, and 100-ms gap) and the young/adult stage. In some analysis, we subtracted the baseline firing rate (computed in the 1-s fixation period) and then performed the ANOVA. Analysis was also performed on neural responses aligned to the onset of the saccade. In this case, firing rate was calculated in the 200 ms preceding the saccade onset. An ROC analysis was used to compare the distributions of firing rates of a neuron to two stimulus conditions, in a time-resolved fashion, using a 100-ms-long moving window. The stimulus location that elicited the best stimulus response during the ODR task was determined. We then compared responses in the antisaccade task involving a stimulus at the best location and at its diametric location. Detailed methods are provided in *SI Materials and Methods*.

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