Fig. 4. Wnt1/β-catenin signaling instructs eNCSCs to adopt a sensory fate. (**A** to **D**) The effect of Wnt1 is β-catenin dependent. Wild-type (wt) NCSCs exposed to control monolayers fail to generate Brn-3A–positive sensory neurons (A), whereas wt NCSCs exposed to Wnt1 monolayers form ganglion-like cell aggregates containing Brn-3A/NF-positive sensory neurons [(B) and (C)]. β-catenin–deficient (β-cat$^{-/-}$) NCSCs are unable to generate sensory neurons, despite the presence of Wnt1 (D). Scale bar, 10 μm. (**E** to **J**) Clonal analysis demonstrates responsiveness of wt eNCSCs to different instructive growth factors including Wnt1. Of the p75-positive (red) founder cells, 90.3 ± 0.7% coexpressed Sox10 (green) (E). In the presence of Wnt1, founder cells generated clones of Brn-3A–positive sensory neurons (F) expressing NF (G). BMP2 instructed eNCSCs to generate Mash-1–expressing autonomic cells [arrow in (H)]. TGF-β induced a smooth muscle–like fate in most of the eNCSCs (I). Scale bars, 10 μm. (**J**) Quantification of clone composition (see also , fig. S2). (*) $P < 0.001$. The data are expressed as the mean ± SD of three independent experiments. Fifty to 150 clones were scored per experiment. (**K**) The cell number within individual Wnt1- and BMP2-treated clones was analyzed. Numbers (percentage of all clones per condition) are shown as the mean ± SD of three independent experiments, scoring 50 to 150 clones per experiment. (*) $P < 0.01$.
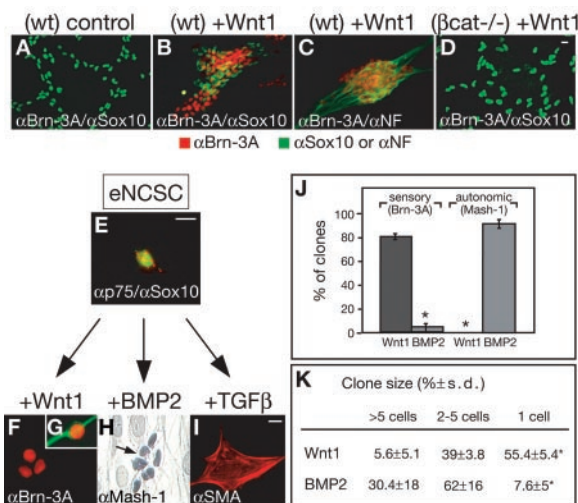
cells (*1–3, 5, 6*) and fate decision processes in NCSCs awaits investigation.

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Anterior Cingulate Conflict Monitoring and Adjustments in Control

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Conflict monitoring by the anterior cingulate cortex (ACC) has been posited to signal a need for greater cognitive control, producing neural and behavioral adjustments. However, the very occurrence of behavioral adjustments after conflict has been questioned, along with suggestions that there is no direct evidence of ACC conflict-related activity predicting subsequent neural or behavioral adjustments in control. Using the Stroop color-naming task and controlling for repetition effects, we demonstrate that ACC conflict-related activity predicts both greater prefrontal cortex activity and adjustments in behavior, supporting a role of ACC conflict monitoring in the engagement of cognitive control.

A major goal of cognitive neuroscience is to understand the precise neural mechanisms that underlie cognitive control (*1*). An important question about the nature of cognitive control is how do the processes involved in implementing control become engaged, or in other words, what controls control (*2*)? One partial answer comes from the conflict hypothesis, which posits that monitoring of response conflict acts as a signal that engages control processes that are needed to overcome conflict and to perform effectively (*3,*

4). Two brain regions that have been associated with cognitive control processes are the ACC and the prefrontal cortex (PFC). Although it is commonly accepted that the PFC is involved in implementing control (*5–7*), there have been differing hypotheses regarding the contribution made by the ACC (*8–10*). One of these, the conflict hypothesis, contends that a function of ACC is the monitoring of processing conflict (*3, 11*). However, this has recently been challenged on two grounds: first, failure to find behavioral evidence for trial-to-trial adjustments in control following conflict when stimulus repetitions

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are removed from the analysis and, second, relative lack of direct evidence of ACC activity predicting subsequent increase in cognitive control (12).

One prediction of the conflict hypothesis is that the occurrence of conflict and the subsequent engagement of control should result in behavioral adjustments (3). For instance, in the Stroop color-naming task, there is greater conflict for incongruent trials (e.g., naming the color of a word printed in green ink when the word is "RED") than for congruent trials (the word "RED" printed in red ink) (13, 14).

Behavioral adjustments following conflict include being faster on incongruent trials preceded by incongruent (iI) trials than on incongruent trials preceded by congruent (cI) trials, and being slower on iC than on cC trials. The conflict hypothesis explains these behavioral adjustments as the result of high conflict on incongruent trials leading to the recruitment of greater cognitive control on the subsequent trial (3). Thus, iI trials are faster than cI trials because the preceding incongruent trial results in greater cognitive control on an iI than on a cI trial (conversely, iC are slower than cC trials because the greater recruitment of cognitive control on iC trials leads to greater focus on the print color, and therefore a reduction of the influence of the word which, in this case, would have been facilitative) (3, 15). These combined effects should produce a previous trial (c versus i) by current trial (C versus I) interaction.

Such effects have been observed in previous studies (15, 16) using the Eriksen flanker task (17), a spatial analog to the Stroop task. However, the interpretation of these effects in terms of adjustments in control has recently been challenged. Mayr and colleagues (12) reported results suggesting that previous evidence of post-conflict adjustments using the Eriksen flanker task may instead have been due to the effects of stimulus repetitions, so they called into question the interpretation of these findings as support for the conflict hypothesis.

Another important prediction of the conflict hypothesis is that conflict-related activity in ACC should predict a subsequent increase in PFC activity. This is based on the assumption that PFC is responsible for executing cognitive control and producing corresponding adjustments in behavior. Previous research has yet to provide direct evidence for such effects.

To test these predictions we used the Stroop task, which produces greater reaction time (RT) interference and has fewer stimulus repetitions than the Eriksen flanker task used in previous work. First, we examined whether adjustments in behavior were present after removing stimulus repetitions. Then, we examined whether ACC conflict-related activity was associated with greater behavioral

adjustments and increased PFC activity on the subsequent trial, as predicted by the conflict hypothesis. Finally, we examined a closely related set of predictions of the conflict hypothesis, that ACC error-related activity should also be followed by an increase in PFC activity and corresponding adjustments in performance (18, 19). According to the conflict hypothesis, adjustments occur after errors, because error trials involve a high degree of response conflict. Therefore, we examined not only whether ACC conflict-related activity predicted behavioral adjustments, but also whether ACC error-related activity predicted an increase in PFC activity and behavioral adjustments (20).

Participants performed the Stroop color-naming task (n = 23). We analyzed only trials that did not include a repetition of either the color or the word, subjecting these to a two by two analysis of variance [(previous trial: congruent versus incongruent) versus (current trial: congruent versus incongruent)]. There was a significant interaction of previous by current trial type [$F(1, 22) = 5.02$, $P < 0.05$, effect size, $r = 0.43$] (21), reflecting a post-conflict adjustment effect (Fig. 1A). Post hoc contrasts revealed that cI trials were significantly slower than iI trials (mean RT difference = 56 ms, $P < 0.01$). In contrast, iC and cC trials did not significantly differ (mean RT difference = 1.4 ms; $P > 0.85$), presumably because of small or absent facilitation effects, as is common in the Stroop task (14). Virtually identical results were obtained when we removed only trials that included exact stimulus repetitions (that is, both color and word repeated; in addition, the amount of post-error behavioral adjustment was significant; for these results, see supporting online material).

Separate analyses of conflict- and error-related activity identified activity of overlapping regions within the ACC (Fig. 2A). Using the conflict ACC area as a region of interest (ROI), we found significant error-related activity in that area. Similarly, we found significant conflict-related activity in the error ACC region. This replicated findings from a previous study comparing conflict- and error-related activity within the ACC (11). In our subsequent analyses, we used these regions as ROIs to examine whether ACC activity was associated with behavior and PFC activity in ways predicted by the conflict hypothesis.

First, we tested the prediction that ACC activity on incongruent trials should be less for iI than cI trials. This is because, for iI trials, conflict on the previous trial should recruit additional control and thus reduce conflict on the current trial. As predicted, we found significantly less ACC activity for iI trials than for cI trials (Fig. 1B). This corroborates previous findings using the Eriksen

flanker task, which suggested that behavioral adjustments in response to conflict on a prior trial reduce conflict and associated ACC activity on the current trial (15).

Next, we tested whether ACC activity predicted behavioral adjustments on the subsequent trial. To do so, we divided trials into high-adjustment and low-adjustment trials (22). As predicted by the conflict hypothesis, high-adjustment trials were associated with greater ACC activity on the previous trial than low-adjustment trials (Fig. 2B). In other words, greater ACC activity was followed by faster responding on iI trials (23). Furthermore, greater ACC activity on error trials was associated with greater post-error adjustment on the subsequent trial (24) (Fig. 2B). Thus, as predicted by the conflict hypothesis, greater ACC activity on high-conflict correct trials and error trials was associated with adjustments in behavior on the subsequent trial that reflect augmented control.

Finally, we tested two critical predictions regarding the involvement of the PFC in control and its interaction with the ACC. First, we examined whether PFC activity was associated with behavioral adjustments, as would be predicted if these were mediated by PFC execution of control. As predicted, trials exhibiting the greatest adjustments in behavior following conflict were associated with increased activity in the dorsolateral PFC [right
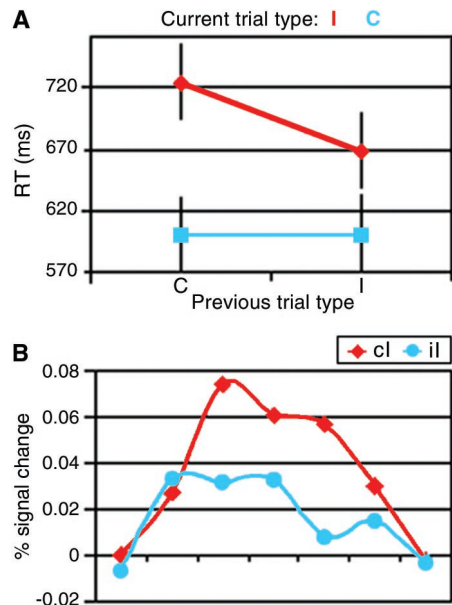
Fig. 1. (A) Post-conflict behavioral adjustments without stimulus repetitions. C, congruent; I, incongruent. (B) ACC activity associated with adjustments in behavior. Percent signal change is the difference from correct congruent trials. Each scan (s) was 1.5 s in duration, with the current incongruent stimulus presented at the beginning of s1. Note that the peak of the BOLD response occurs during s4, which, as expected, is between 4.5 and 6.0 s after the onset of the incongruent stimulus.

middle frontal gyrus, Brodmann areas (BAs) 9 and 8; Fig. 2C]. There was a second PFC region, in the right superior frontal gyrus (BAs 9 and 10), that was also more active on trials with the greatest post-conflict behavioral adjustments. A similar analysis for post-error adjustments of behavior did not reveal any areas of significant activity. However, using the right dorsolateral PFC area associated with post-conflict adjustments as an ROI, we found that an increase of activity in this area was significantly associated with slower responding on post-error trials. Thus, a region that is associated with post-conflict adjustments in behavior also appears to be associated with post-error adjustments in behavior.

Second, we tested whether ACC activity on conflict and error trials predicted activity in our PFC ROI on the following trial. This correlation was significant ($P < 0.01$), even after partialling out shared variance with another task-responsive brain region outside of the frontal lobes (in the left temporal lobe) (25) (Fig. 2D). These findings suggest a direct and specific relation between ACC and PFC that is not explained by widespread fluctuations in brain activity unrelated to the task or a generalized effect of task engagement, although it remains possible that the correlation between these areas reflects the common influence of some other as yet unidentified area(s).

In summary, we found evidence for a direct relation between ACC activity on high-conflict and error trials and behavioral adjustments, reflecting a recruitment of control on subsequent trials. These behavioral adjustments were associated with increases in PFC activity, which was itself directly related to ACC activity on the preceding trial. These findings are all consistent with predictions made by the hypothesis that ACC implements a conflict-monitoring function and that engagement of this function leads to the recruitment of cognitive control. Additionally, these findings provide further support for the hypothesis that PFC is responsible for the execution of cognitive control. They are also consistent with the hypothesis that the ACC itself is not responsible for the allocation of control. Although they cannot rule out a role for the ACC in the execution of control, previous studies have provided evidence against this view (15, 26).

Within the PFC, primarily right-sided regions were associated with behavioral adjustments in behavior. Two other studies have reported similar findings within the PFC, but with primarily left-sided activations associated with the allocation of control. Garavan and colleagues reported greater left inferior PFC activity after errors were made (1), and MacDonald et al. reported left middle frontal gyrus activity during preparation to overcome conflict (27). The different PFC regions activated by these studies could reflect differences in the control processes engaged by the different task configurations used in these studies. Garavan et al. used a Go–No Go task in which participants only responded to a particular alternating sequence of letters (e.g., "X-Y-X-Y"). MacDonald et al. used a cued, task-switching version of the Stroop task in which a verbal cue indicated whether to name the color or read the word. This cue was followed by a 12.5-s interval, which provided participants with considerable time to prepare for the next trial, including implement adjustments in control. This differed from the current study, which involved only color naming, and stimuli that appeared every 3 s requiring that trial-to-trial adjustments in control be made much more rapidly. These differences between studies may have engaged different PFC control processes.

With regard to the region of ACC that we have identified with conflict monitoring (in this study and others), it is important to emphasize that this area makes up a small portion of a large structure. Monitoring response conflict may represent one instance of a more general family of functions subserved by ACC, which is to monitor internal states for signs of breakdowns in processing and performance that require adjustments in control (28). This is consistent with other observations that have been made about ACC function, including its response to pain (29).

Finally, it should be noted that neither our study (nor any others of which we are aware) have produced insights into the specific mechanisms by which detection of conflict in ACC engages the recruitment of control. One possibility is that ACC issues specific signals that govern the recruitment of control, activating relevant representations in PFC. In contrast, we have favored the view that ACC signals modulate the strength of PFC representations, whereas other mechanisms determine their content. This modulation could be produced by a direct influence of ACC on PFC, or by way of other neuromodulatory systems, such as the locus coeruleus (2).
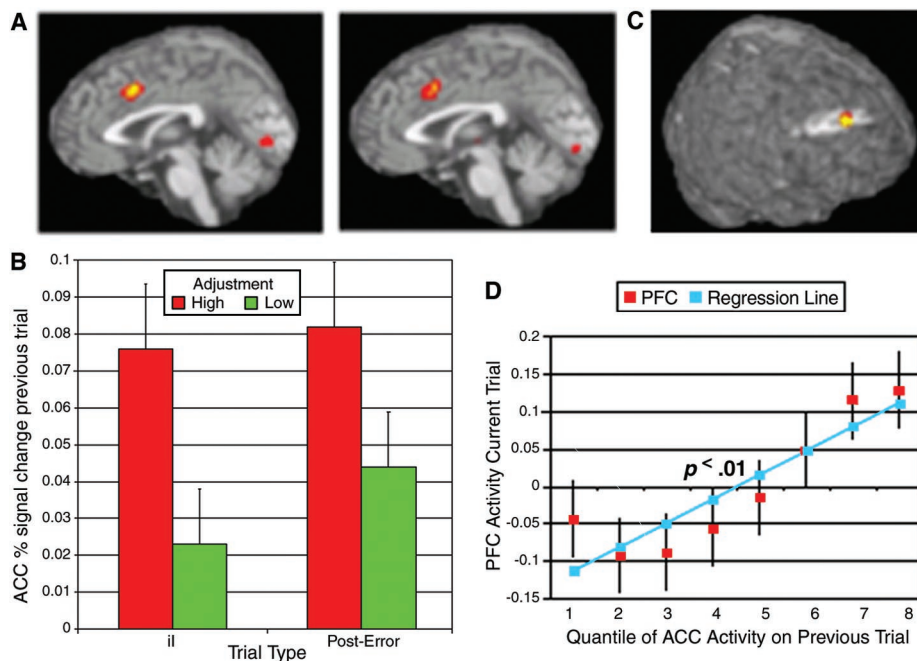

Fig. 2. (**A**) Overlapping regions of ACC activity on conflict (incongruent) trials (left side of figure, Talairach coordinates: 1, 10, 40) and error trials (right side of figure, coordinates: 3, 14, 41). (**B**) ACC activity on the previous incongruent trial predicted behavioral adjustments on the next trial. High adjustment stands for fast RT on iI trials and slow RT on post-error trials. Low adjustment stands for slow RT on iI trials and fast RT on post-error trials. ACC percent signal change on previous trial (abscissa) is the percent difference from congruent trials averaged over scans s3 to s7 (i.e., time window of 3 to 10.5 s). (**C**) Area of right middle frontal gyrus, BAs 9 and 8 (Talairach coordinates: 30, 34, 37), more active during high-adjustment post-conflict and post-error trials than during low-adjustment post-conflict and post-error trials (see text for further information). (**D**) ACC activity on previous conflict (incongruent) and error trials predicts PFC activity on the current trial. Conflict and error trials were divided into eight quantiles based on ACC activity (percent signal change from correct congruent trials). For each quantile, the mean activity on the subsequent trials is plotted for the right dorsolateral PFC ROI (see text).

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22. High-adjustment trials were defined as all iI trials with an RT faster than their median. Conversely, low-adjustment trials were slow iI trials.
23. Although there was not a significant adjustment effect for iC trials, we performed a similar analysis, with high-adjustment iC trials being the third slowest iC trials and low-adjustment trials being the third fastest iC trials. Again, increased ACC conflict activity predicted greater adjustment, with increased ACC activity preceding slow iC trials. (We used a median split for iI trials because of their fewer number.) The pattern of results for iI and iC trials indicates that ACC activity on the previous trial was not associated with overall faster or slower responses on the current trial, but rather that there was an interaction between speed and current trial type [$F(1, 22) = 9.67$, $P < 0.005$].
24. This analysis was performed only on post-error congruent trials, as there were too few post-error incongruent trials (30% of all trials were incongruent—see Supporting Online Material) to support a reliable analysis. For this reason, post-error adjustments were expected to produce a slowing of response.
25. To conduct this analysis, we examined associations of ACC and PFC with a third region, in the left temporal lobe, that showed the largest response to incongruent trials of any nonfrontal regions, and then controlled for these relations in assessing the correlation between ACC and PFC. Specifically, for each participant, we partialed out the variance in the relation between ACC (on conflict and error trials) and the temporal region (on post-conflict and post-error trials), and similarly for PFC (on post-conflict and post-error trials) and the temporal region (on conflict and error trials). We then performed a *t* test to determine whether the residual correlation between ACC and PFC activity across the group of participants was significant (see Supporting Online Material).
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28. In addition, there may be other processes associated with the ACC that were not detected in this study since functional magnetic resonance imaging (fMRI) is sensitive to population-level activity.
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Cdh1-APC Controls Axonal Growth and Patterning in the Mammalian Brain

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The anaphase-promoting complex (APC) is highly expressed in postmitotic neurons, but its function in the nervous system was previously unknown. We report that the inhibition of Cdh1-APC in primary neurons specifically enhanced axonal growth. Cdh1 knockdown in cerebellar slice overlay assays and in the developing rat cerebellum in vivo revealed cell-autonomous abnormalities in layer-specific growth of granule neuron axons and parallel fiber patterning. Cdh1 RNA interference in neurons was also found to override the inhibitory influence of myelin on axonal growth. Thus, Cdh1-APC appears to play a role in regulating axonal growth and patterning in the developing brain that may also limit the growth of injured axons in the adult brain.

The ubiquitin ligase anaphase-promoting complex (APC) is essential for coordination of cell cycle transitions, including mitotic exit (*1*–*3*). APC activity is stimulated by the regulatory proteins Cdc20 and Cdh1 in a cell cycle–dependent manner. Cdc20 association with APC is required for APC activity during early mitosis, but Cdh1 association is required for APC activity during late mitosis and G1 (*4*, *5*). Cdh1 and core components of APC are also expressed in postmitotic neurons in the mammalian brain (*6*, *7*).

To characterize Cdh1 function in postmitotic neurons, we used a DNA template–based RNA interference (RNAi) method to acutely knock down the expression of Cdh1 in primary cerebellar granule neurons (*8*). The expression of cdh1 small hairpin RNAs (shcdh1) reduced Cdh1 expression in COS cells and in primary neurons (fig. S1, A and B). The shcdh1-expressing granule neurons did not reenter the cell cycle, failing to incorporate bromodeoxyuridine or undergo cytokinesis (*9*). Cdh1 knockdown had no effect on the survival of the granule neurons (fig. S1C). However, Cdh1 knockdown altered the morphology of neuronal processes, raising the possibility that Cdh1 might regulate neuronal morphogenesis.

To determine the role of Cdh1 in neuronal morphogenesis, we transfected primary cerebellar granule neurons from postnatal day 6 (P6) rat pups with the control U6 or U6/shcdh1 plasmid at the time of plating, when these neurons just begin to grow axons (*8*). Each day for 6 days after transfection, we measured the length of axons and dendrites in the same transfected granule neurons. Axons and dendrites of transfected neurons were identified on the basis of their morphology and by the expression of the axonal marker Tau and the somato-dendritic marker MAP2 (Fig. 1A). While the expression of shcdh1 in granule neurons had little effect on the generation and growth of dendrites, Cdh1 knockdown led to a dramatic increase in axonal length (Fig. 1, B and C), both accelerating the rate of growth and significantly augmenting the final axonal length (Fig. 1C). The vast majority of transfected cells expressed the neuron-specific β-tubulin type III (Tuj1), a marker of postmitotic granule neurons that was not affected by Cdh1 RNAi (Fig. 1E). Thus, Cdh1 specifically inhibits axonal but not dendritic growth in postmitotic granule neurons.

Cdh1 RNAi lowered the numbers of neurons with short axons and increased the numbers of neurons with long axons, which supports the idea that Cdh1 knockdown did not operate in a subpopulation of neurons (Fig. 1D). In other experiments, Cdh1 knockdown increased the total length but not the number of branches of granule neuron axons (*9*), which suggests that Cdh1 knockdown specifically enhances the growth of the major axonal fibers in neurons. A second construct encoding small hairpin cdh1 RNAs (shcdh1-b) that reduced the expression of Cdh1 also promoted the growth of axons (Fig. 1F). shRNAs to several other genes encoding proteins unrelated to Cdh1, including the transcription factor MEF2A (*10*), failed to promote axonal growth (Fig. 1F).

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