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RESEARCH PAPER

Wnt5a induces Ryk-dependent and -independent effects on callosal axon and dendrite growth

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Abstract

The non-canonical Wnt receptor, Ryk, promotes chemorepulsive axon guidance in the developing mouse brain and spinal cord in response to Wnt5a. Ryk has also been identified as a major suppressor of axonal regrowth after spinal cord injury. Thus, a comprehensive understanding of how growing axons and dendrites respond to Wnt5a-mediated Ryk activation is required if we are to overcome this detrimental activity. Here we undertook a detailed analysis of the effect of Wnt5a/Ryk interactions on axonal and dendritic growth in dissociated embryonic mouse cortical neuron cultures, focusing on callosal neurons known to be responsive to Ryk-induced chemorepulsion. We show that Ryk inhibits axonal growth in response to Wnt5a. We also show that Wnt5a inhibits dendrite growth independently of Ryk. However, this inhibition is relieved when Ryk is present. Therefore, Wnt5a-mediated Ryk activation triggers divergent responses in callosal axons and dendrites in the *in vitro* context.

Keywords

Axon outgrowth, dendrite outgrowth, Ryk, Wnt5a

History

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Introduction

In the embryonic nervous system nascent axons navigate towards their specific targets to establish the intricate network of axonal connections within the mature nervous system. Attractive and repulsive guidance cues, secreted from intermediate or final targets, establish concentration gradients along the pathway of the migrating axon. Axon-bound receptors detect these gradients and determine the trajectory of the growth cone (Bashaw & Klein, 2010; Dickson & Zou, 2010; Kolodkin & Tessier-Lavigne, 2010). In addition to directing axon navigation, attractive guidance cues promote axon growth, whereas repulsive cues cause growth cones to stall or collapse (Bashaw & Klein, 2010; Dickson & Zou, 2010; Kolodkin & Tessier-Lavigne, 2010). These molecular guidance systems are also redeployed to direct growth and navigation of dendrites (Kirszenblat et al., 2011; Polleux et al., 2000; Salinas, 2012; Teichmann & Shen, 2011). In contrast to their positive role in developing nervous system, repulsive guidance receptors are detrimental to the axon's ability to regrow after injury in the adult. After spinal cord injury, repulsive cues are re-expressed at the injury site, creating a deleterious environment for regeneration (Giger et al., 2010; Harel & Strittmatter, 2006). Concurrently, the cognate guidance receptors are upregulated on the severed axons, preventing axonal growth. Therefore, a comprehensive understanding of how growing axons and dendrites respond to guidance receptor activation may suggest novel strategies with which to enhance regrowth after injury or in neurodegeneration.

The Wnt signaling pathways play a critical role in neural specification, axon growth and guidance, dendrite extension and synapse formation (Clark et al., 2012; Salinas, 2012; Zou & Lyuksyutova, 2007). Exploration of Wnt signaling in axon guidance has revealed essential roles for Wnt/Frizzled-mediated chemoattraction in the formation of major axon tracts in vertebrates. In this context, the Wnt/planar cell polarity (PCP) pathway has been identified as the relevant signaling pathway and controls cytoskeletal dynamics through the induction of polarized filopodial and lamellipodial activity (Shafer et al., 2011). Wnts and Frizzleds have also been shown to elicit dendrite growth and guidance (Kirszenblat et al., 2011).

Ryk is a non-canonical Wnt receptor known to modulate core components of the PCP pathway (Andre et al., 2012; Macheda et al., 2012). Ryk promotes chemorepulsive axon guidance in the developing mouse brain and spinal cord (Keeble et al., 2006; Li et al., 2008; Liu et al., 2005; Schmitt et al., 2006). Ryk-dependent repulsion is required for the posterior-directed guidance of corticospinal tract axons down the postnatal spinal cord in response to Wnt1 and Wnt5a gradients (Liu et al., 2005). Wnt5a/Ryk-mediated repulsion is

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also essential for the formation of the corpus callosum, the largest interhemispheric commissure in the mammalian forebrain (Keeble et al., 2006). In the mouse, the earliest callosal axons approach and cross the midline between embryonic days 16 and 17 (E16 to E17) (Paul et al., 2007). From E18 onwards, callosal axons project away from the midline into the contralateral hemisphere. *Ryk* is expressed on callosal axons throughout this period, whereas Wnt5a surrounds the callosal midline. Wnt5a/Ryk interactions repel E18 post-crossing axons away from the midline into the contralateral hemisphere (Keeble & Cooper, 2006; Keeble et al., 2006). Conversely, Ryk is not required for the guidance of E16–E17 pre-crossing axons as they approach the midline on the ipsilateral side. Therefore, the spatiotemporal activity of Ryk is tightly regulated. Recent studies have also identified Ryk as a major suppressor of axonal regrowth after spinal cord injury (Hollis & Zou, 2012a,b; Li et al., 2008; Liu et al., 2008; Miyashita et al., 2009). After spinal cord hemisection, the Ryk ligands, Wnt1 and Wnt5a, are acutely upregulated at the injury site, whereas *Ryk* is re-expressed on the lesioned axons (Hollis & Zou, 2012b; Liu et al., 2008). Notably, injection of Ryk inhibitory antibodies into the injury site prevents axon retraction, permitting axonal sprouting (Liu et al., 2008; Miyashita et al., 2009).

Given the detrimental outcome of Wnt/Ryk interactions on axonal growth after injury, it is important that we fully understand the functional consequences of Ryk activation. In the current study, we undertook a detailed analysis of the effect of Wnt5a/Ryk interactions on axonal and dendritic growth in dissociated cortical neuron cultures from wildtype ($Ryk^{+/+}$) and *Ryk* null ($Ryk^{-/-}$) E18.0 embryos. We focused on the callosal neuron subtype within these cultures as their axons are known to be responsive to Ryk-mediated chemorepulsion (Keeble et al., 2006).

Materials and methods

Cortical cultures

Mice were generated from timed matings of $Ryk^{+/-}$ males and females (C57Bl/6j × 129/Sv). All animal procedures were approved by The University of Queensland Animal Ethics Committee. PCR genotyping was performed from genomic DNA extracted from tail tissue as previously described (Halford et al., 2000). Embryos were collected and anesthetized by inducing hypothermia on ice and then sacrificed by decapitation. Cortical tissue was removed from each cerebral hemisphere, the meninges removed and the tissue incubated for 20 min at 37 °C in 500 µl 0.05% trypsin-EDTA (Invitrogen, Carlsbad, CA) after which time 500 µl Trypsin inhibitor and DNase I (Invitrogen) was added. Cells were pelleted at 1500 × g for 5 min at room temperature and then cultured in complete growth medium (Neurobasal medium (Invitrogen), 1 mM L-glutamine (Invitrogen), 2 mM B27 supplement (Invitrogen) and 100 U/ml penicillin and streptomycin (Invitrogen)) on poly-L-ornithine (0.015%, Invitrogen) and Laminin (20 µg/ml, Invitrogen) coated glass coverslips in 24-well plates at a density of 5×10^4 cells/well. Recombinant mouse Wnt5a was obtained from R&D Systems (Minneapolis, MN).

Immunohistochemistry

Cells were fixed with 4% paraformaldehyde (in PBS, pH 7.4, 15 min), blocked (2% normal serum, 1% bovine serum albumin, 0.25% Triton-X 100 in PBS, 1 h), incubated with mouse anti-Satb2 (Abcam, Cambridge, UK, ab51502, 1:100) and rabbit anti-βIII-Tubulin (TUJ1, Covance, Princeton, NJ, MRB-425P, 1:1000) (diluted in blocking solution, 4 °C, overnight) and then incubated with the appropriate Alexa Fluor-conjugated secondary antibodies (Molecular Probes, Eugene, OR, 1:500) and 4′,6-diamidino-2-phenylindole (DAPI, 1:1000, 1 h). Images were captured on an AxioImager upright microscope equipped with a 20× Plan-APOCHROMAT objective, Axiocam high-resolution monochrome camera and AxioVision software (Zeiss, Jena, Germany).

Neurite tracing and statistical analysis

Images were imported into the ImageJ plugin, NeuronJ (National Institutes of Health, Bethesda, MD), and all axons and dendrites manually traced. Data were extracted using MATLAB code (R2011b, Natick, MA) and analyzed using GraphPad Prism 6 (La Jolla, CA). The following parameters were analyzed: axon length (the length of the longest neurite, Cáceres et al., 2012), the total number of axon branches arising from the longest neurite, the total number of neurites (the axon and all dendrites) and dendrite length (the length of all neurites excluding the axon). For continuous data (axon and dendrite length), box and whisker plots were generated showing the median axon or dendrite length (central line) surrounded by the interquartile range (boxes) and the 5th to 95th percentiles (whiskers). Cumulative distribution plots were generated for axon and dendrite length data. For discrete data (number of neurites, axon branches) frequency distribution curves were generated.

Results

Wnt5a/Ryk interactions suppress mouse callosal axon growth

To investigate the effect of Wnt5a on mouse callosal axon growth, E18 cortical cultures from $Ryk^{+/+}$ and $Ryk^{-/-}$ embryos were immunolabeled with an antibody against the transcription factor, Satb2, a marker of callosal projection neurons (Alcamo et al., 2008; Britanova et al., 2008). Anti-βIII-tubulin was used to label all neuronal processes. Satb2, a DNA-binding protein, was localized to the nucleus as seen in wildtype ($Ryk^{+/+}$) E18 cortical cultures (Figure 1A and B, arrows). The intensity of Satb2 immunoreactivity was equivalent in $Ryk^{+/+}$ and $Ryk^{-/-}$ Satb2-positive (Satb2+) neurons (compare Figure 1B and D, arrows). The non-callosal (i.e. Satb2-negative: Satb2-) neurons within these cultures (Figure 1A and C, arrowheads) comprise a heterogeneous population containing corticothalamic, corticospinal and intracortical pyramidal neurons and a small number of cortical interneurons (Gilbert & Wiesel, 1979). Throughout this study the cortical cultures were robust and consistent in their growth parameters.

The ability of Ryk to influence callosal axon growth was initially investigated by comparing the mean axon length after

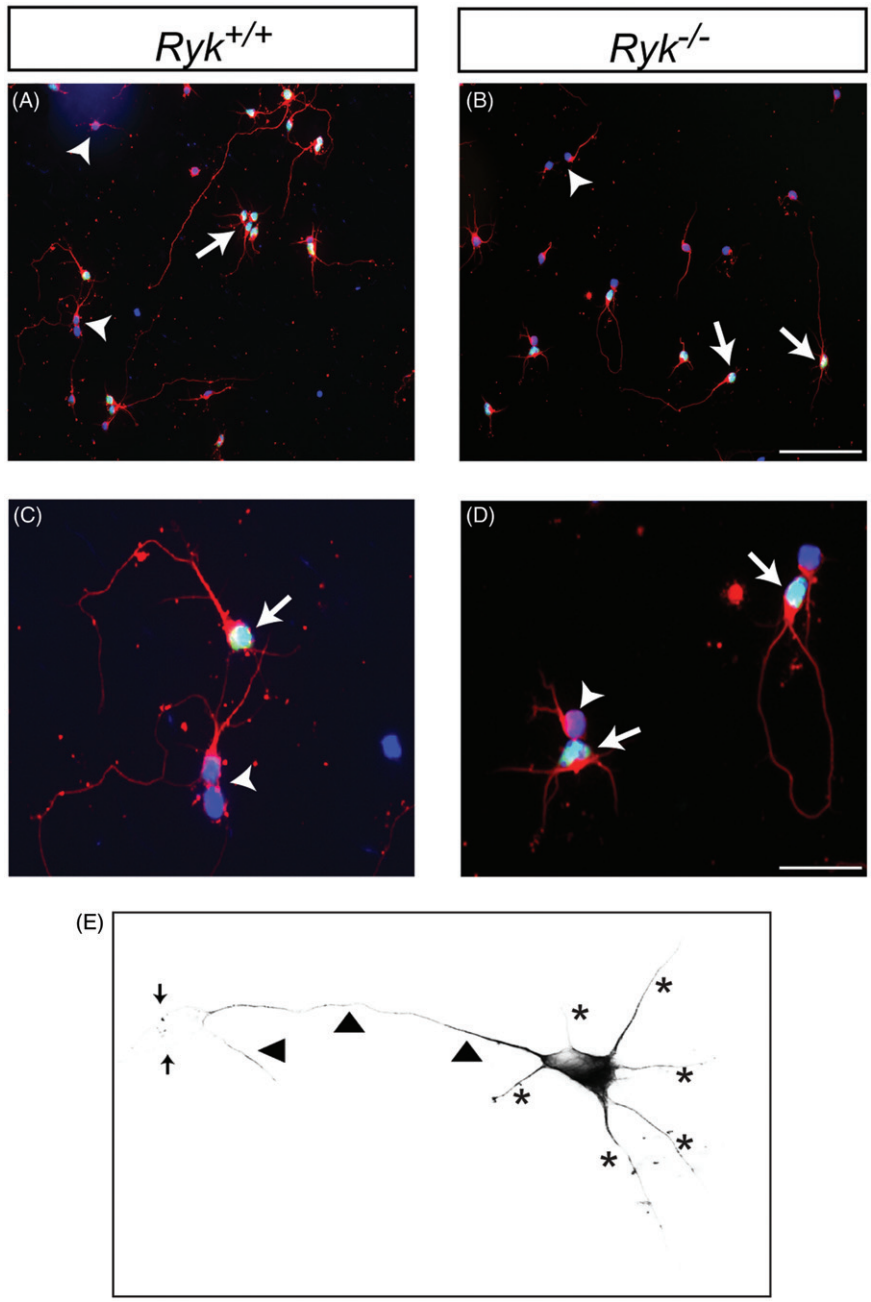

Figure 1. Satb2 labels callosal projection neurons. Satb2 (green) was localized to the nucleus of callosal neurons (arrows) in $Ryk^{+/+}$ (A, B) and $Ryk^{-/-}$ (C, D) E18 cortical cultures. The intensity of Satb2 immunoreactivity was equivalent in $Ryk^{+/+}$ and $Ryk^{-/-}$ neurons. Anti-βIII-tubulin (red) was used to label all neuronal processes. Non-callosal neurons were Satb2-negative (arrowheads). (B) and (D) are higher magnification images of (A) and (C), respectively. (E) A callosal neuron comprising an axon (the longest neurite, arrowheads), axon branches (arrows) and dendrites (asterisks). Scale bars: C, 170 μm; D, 50 μm.

bath application of Wnt5a (0–400 ng/ml) for 24 h. In this analysis, the axon was defined as the longest neurite (Cáceres et al., 2012) (Figure 1E, arrowheads). We observed no significant change in axon growth at 200 ng/ml Wnt5a (Figure 2A). However, there was a significant decrease in wildtype axon length in the presence of 400 ng/ml Wnt5a (Figure 2A) (0 ng/ml Wnt5a, 210.5 ± 13.1 μm; 400 ng/ml Wnt5a 164.6 ± 12.4 μm; $p = 0.006$), whereas this Wnt5a-mediated decrease was not observed for $Ryk^{-/-}$ Satb2+ axons (0 ng/ml Wnt5a, 233.2 ± 11.4 μm: 400 ng/ml, 228.1 ± 10.5 μm). Box and whisker plots revealed a broad distribution of axon lengths (~50–700 μm) within the data set (Figure 2B). Therefore, to test whether the distribution of axon lengths differed significantly between conditions, the cumulative distribution for axon length in the presence of 400 ng/ml Wnt5a was plotted (Figure 2C). Comparisons of the distribution curves indicated that the addition of Wnt5a to wildtype cells significantly decreased axon length ($p = 0.025$, Kolmogorov–Smirnov test). Moreover, the curves for $Ryk^{+/+}$ and $Ryk^{-/-}$ axons in the presence of Wnt5a also differed significantly ($p = 0.001$, Kolmogorov–Smirnov test), confirming that the Wnt5a inhibitory activity required Ryk. Therefore, Wnt5a inhibited callosal axon growth in a Ryk-dependent manner.

To determine whether Wnt5a/Ryk interactions also inhibited growth of non-callosal axons we performed a similar analysis on the mixed Satb2- population in the $Ryk^{+/+}$ and $Ryk^{-/-}$ cultures. We found no significant change in the mean axon length after the addition of Wnt5a for either genotype (Figure 2D) or in the cumulative distributions for each condition (data not shown), demonstrating that Wnt5a and Ryk did not influence axon growth in the mixed cortical neuron population. It should be noted that our cultures contained corticospinal neurons known to respond to

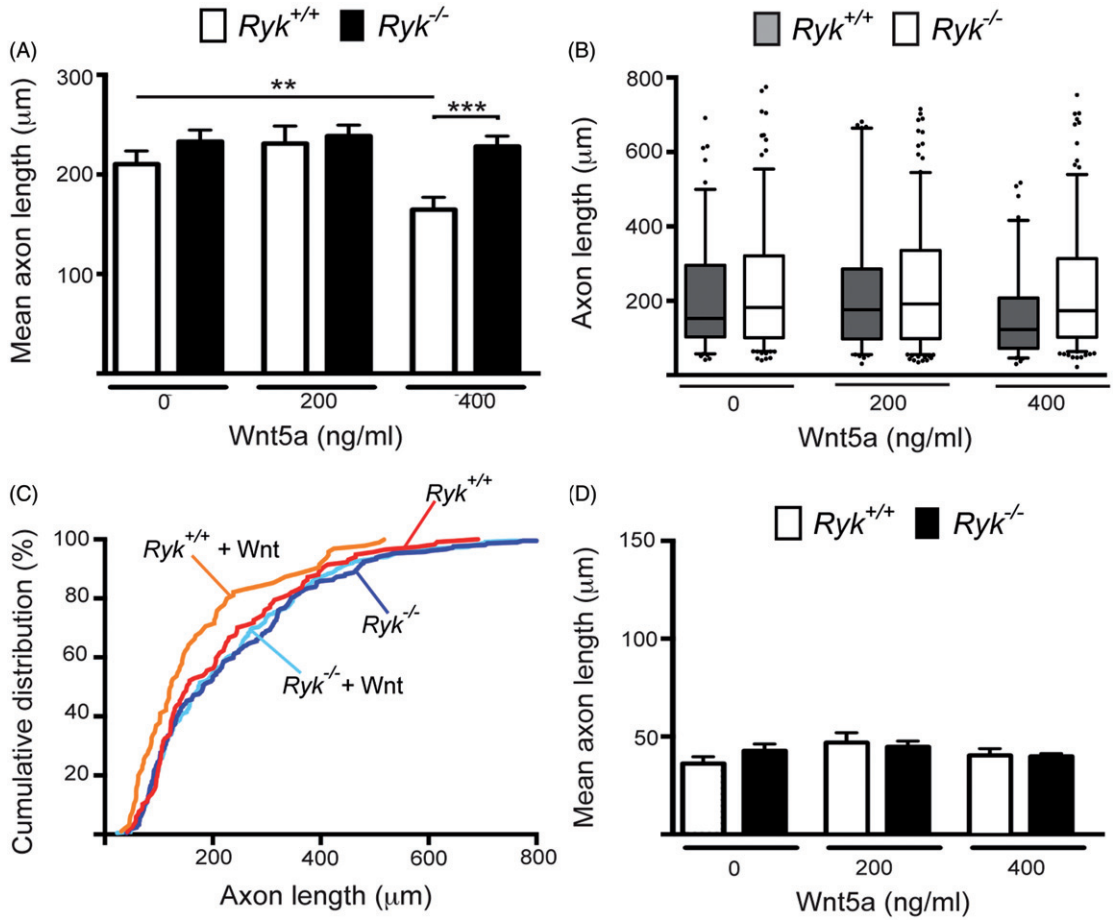

Figure 2. Wnt5a/Ryk interactions suppress callosal axon growth. (A) 400 ng/ml Wnt5a but not 200 ng/ml Wnt5a decreased the mean axon length for $Ryk^{+/+}$ Satb2+ neurons. This decrease was not seen for $Ryk^{-/-}$ neurons. (B) Box and whisker plots revealed a broad distribution of axon lengths for $Ryk^{+/+}$ and $Ryk^{-/-}$ Satb2+ neurons. The median axon length (central line) is surrounded by the interquartile range (boxes) and the 5th to 95th percentiles (whiskers). (C) Cumulative distribution plots for $Ryk^{+/+}$ and $Ryk^{-/-}$ axon lengths ± Wnt5a for Satb2+ axons. (D) There was no significant difference in the mean axon length for non-callosal Satb2- neurons in the presence or absence of Wnt5a. Satb2+ neurons: 0 ng/ml Wnt5a/$Ryk^{+/+}$, $n = 117$; 200 ng/ml Wnt5a/$Ryk^{+/+}$, $n = 100$; 400 ng/ml Wnt5a/$Ryk^{+/+}$, $n = 95$; 0 ng/ml Wnt5a/$Ryk^{-/-}$, $n = 212$; 200 ng/ml Wnt5a/$Ryk^{-/-}$, $n = 245$; 400 ng/ml Wnt5a/$Ryk^{-/-}$, $n = 239$. Satb2- neurons: 0 ng/ml Wnt5a/$Ryk^{+/+}$, $n = 61$; 200 ng/ml Wnt5a/$Ryk^{+/+}$, $n = 23$; 400 ng/ml Wnt5a/$Ryk^{+/+}$, $n = 47$; 0 ng/ml Wnt5a/$Ryk^{-/-}$, $n = 76$; 200 ng/ml Wnt5a/$Ryk^{-/-}$, $n = 117$; 400 ng/ml Wnt5a/$Ryk^{-/-}$, $n = 129$. (A, D) Mann–Whitney test, $^{**}p < 0.01$, $^{***}p < 0.001$.

Wnt5a (Liu et al., 2005). In the mouse, corticospinal axons do not project down the spinal cord until after postnatal day 1. Although Liu et al. (2005) showed that these axons responded to Wnts after post-natal day 1, they also reported that E18.5 corticospinal axons did not respond to Wnt1 or Wnt5a. Thus, we would not expect to see a significant Wnt5a effect on axon growth for this population in our E18.0 cultures.

Wnt5a and Ryk do not affect axon branching

Although Wnt5a has been shown to promote axon branching in the peripheral nervous system (Bodmer et al., 2009), the effect of Wnt5a and Ryk on axonal branching in the central nervous system has not been investigated. Quantification of the number of axon branches elaborated by callosal neurons (Figure 1E, arrows) upon exposure to Wnt5a (400 ng/ml) revealed no significant effect of Wnt5a on $Ryk^{+/+}$ or $Ryk^{-/-}$ axon branch number (Figure 3A and B). Therefore, Wnt5a/Ryk interactions influence callosal axon growth but not axon branching.

Callosal dendrite growth is regulated by Wnt5a and Ryk

As Wnts and Frizzleds have been shown to promote both dendrite growth and guidance (Kirszenblat et al., 2011), we also investigated whether Wnt5a/Ryk interactions influenced callosal dendrite development (Figure 1E, asterisks). This analysis revealed a Ryk-independent effect of Wnt5a. Wnt5a (400 ng/ml) significantly suppressed $Ryk^{-/-}$ dendrite growth (Figure 4A) (0 ng/ml Wnt5a, 126.2 ± 6.6 μm; 400 ng/ml Wnt5a, 109.0 ± 5.6, $p = 0.045$), suggesting that in the absence of Ryk, Wnt5a/Frizzled interactions were inhibitory. As observed for axon length, box and whisker plots revealed a broad distribution of dendrite lengths (~20–400 μm) (data not shown). Analysis of cumulative distribution plots for dendrite length (Figure 4B) confirmed that Wnt5a inhibited dendrite growth in a Ryk-independent manner ($p = 0.016$, Kolmogorov–Smirnov test). Conversely, we found that the addition of Wnt5a had no significant effect on wildtype ($Ryk^{+/+}$) dendrite growth (Figure 4A and B), indicating that in the presence of Ryk, wildtype dendrites did not respond to

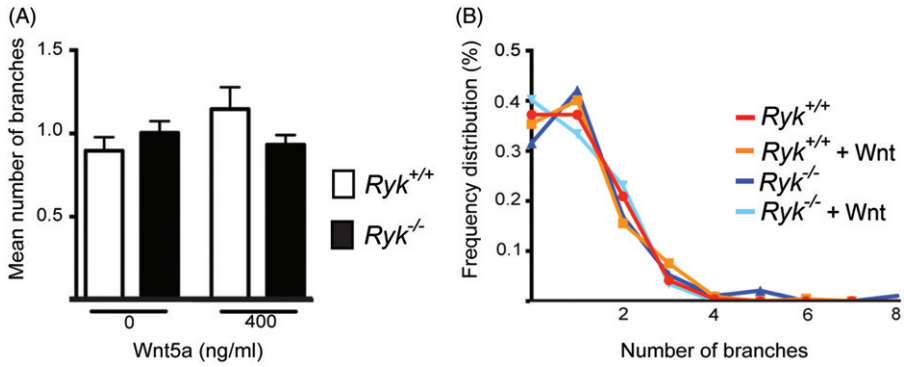

Figure 3. Ryk and Wnt5a do not influence axon branching. (A) Wnt5a (400 ng/ml) had no significant effect on the mean number of branches on $Ryk^{+/+}$ or $Ryk^{-/-}$ Satb2+ axons (Mann–Whitney test). (B) Frequency distribution plots of the number of axon branches ± Wnt5a (400 ng/ml) on $Ryk^{+/+}$ and $Ryk^{-/-}$ axons. 0 ng/ml Wnt5a/$Ryk^{+/+}$ (●), $n = 117$; 400 ng/ml Wnt5a/$Ryk^{+/+}$ (■), $n = 95$; 0 ng/ml Wnt5a/$Ryk^{-/-}$ (▲), $n = 212$; 400 ng/ml Wnt5a/$Ryk^{-/-}$ (▼), $n = 239$.

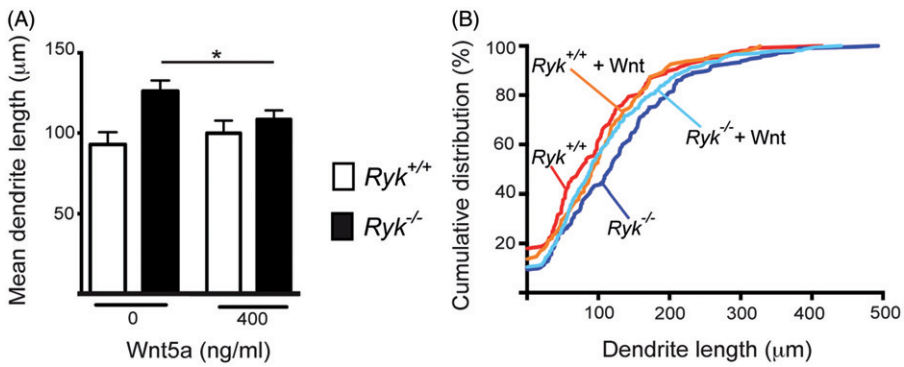

Figure 4. Ryk represses Wnt5a-mediated dendrite growth inhibition. (A) Wnt5a (400 ng/ml) decreased the mean dendrite length for $Ryk^{-/-}$ Satb2+ neurons (Mann–Whitney test, $^{*}p < 0.05$). This effect was not seen for $Ryk^{+/+}$ neurons. (B) Cumulative distribution plots of $Ryk^{+/+}$ and $Ryk^{-/-}$ dendrite lengths ± Wnt5a (400 ng/ml) for Satb2+ neurons. 0 ng/ml Wnt5a/$Ryk^{+/+}$, $n = 117$; 400 ng/ml Wnt5a/$Ryk^{+/+}$, $n = 95$; 0 ng/ml Wnt5a/$Ryk^{-/-}$, $n = 212$; 400 ng/ml Wnt5a/$Ryk^{-/-}$, $n = 239$.

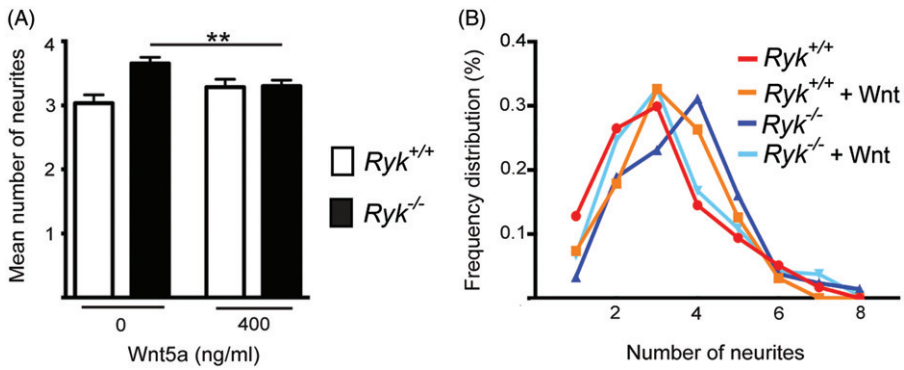

Figure 5. Ryk represses Wnt5a-mediated suppression of neurite outgrowth. (A) Wnt5a (400 ng/ml) decreased the mean number of neurites on $Ryk^{-/-}$ Satb2+ neurons (Mann–Whitney test, $^{**}p < 0.01$). This effect was not seen for $Ryk^{+/+}$ neurons. (B) Frequency distribution plots of the number of neurites induced by Wnt5a (400 ng/ml) in $Ryk^{+/+}$ and $Ryk^{-/-}$ Satb2+ neurons. 0 ng/ml Wnt5a/$Ryk^{+/+}$ (●), $n = 117$; 400 ng/ml Wnt5a/$Ryk^{+/+}$ (■), $n = 95$; 0 ng/ml Wnt5a/$Ryk^{-/-}$ (▲), $n = 212$; 400 ng/ml Wnt5a/$Ryk^{-/-}$ (▼), $n = 239$.

Wnt5a. This suggested that Ryk was able to inhibit Wnt5a-mediated growth suppression. Finally, although Figure 4(A) suggested that in the absence of Wnt5a $Ryk^{-/-}$ dendrite growth was enhanced compared to $Ryk^{+/+}$ growth, this difference was not statistically significant. Cumulative distribution analysis (Figure 4B) confirmed that the dendrite length distribution curves for $Ryk^{+/+}$ and $Ryk^{-/-}$ neurons did not differ significantly (Kolmogorov–Smirnov test). Taken together these data suggest that there is a complex regulatory interaction between Ryk and Frizzled in response to Wnt5a.

Total neurite number is determined by Ryk

As for axon and dendrite growth, the initiation of neurite outgrowth from the neuronal cell body is dependent on the polarization of the actin cytoskeleton within filopodial protrusions (Cáceres et al., 2012). Therefore, we next investigated whether Wnt5a and Ryk influenced the total number of neurites (the axon and dendrites) elaborated from callosal neurons. The neurite number per cell was quantified after 24 h exposure to Wnt5a (400 ng/ml) and frequency distribution curves constructed (Figure 5A and B). Wnt5a decreased the mean neurite number in $Ryk^{-/-}$ callosal neurons (0 ng/ml Wnt5a, 3.7 ± 0.1 neurites; 400 ng/ml Wnt5a, 3.3 ± 0.1 neurites, $p = 0.002$), indicating a Ryk-independent effect. Conversely, there was no significant effect of Wnt5a on wildtype neurite number, indicating that Ryk suppressed this Wnt5a inhibition. In addition, there was no significant effect on neurite number between genotypes in the absence of Wnt5a (Figure 5A and B). In summary, as seen for dendrite growth, Wnt5a inhibition of neurite outgrowth was dependent on the availability of Ryk, again suggesting a complex interplay between Ryk and Frizzled.

Discussion

We have previously demonstrated a key role for Wnt5a/Ryk-mediated chemorepulsion in the guidance of post-crossing callosal axons during mouse cortical development (Keeble & Cooper, 2006; Keeble et al., 2006). In the absence of Ryk,

callosal axons cross the midline but fail to enter the contralateral hemisphere, instead accumulating at the midline (Keeble et al., 2006). Although Wnt5a-mediated repulsion is clearly abrogated in *Ryk*$^{-/-}$ callosal axons, the effect of Ryk on callosal axon growth was not assessed in our previous study. Here, we have taken an *in vitro* approach to determine the influence of Wnt5a/Ryk interactions on E18 post-crossing callosal axon growth. We show that Wnt5a inhibits callosal axon extension in a Ryk-dependent manner, suggesting that in addition to its function as a repulsive guidance receptor, Ryk also suppresses mouse callosal axonal growth in response to Wnt5a. However, Wnt5a and Ryk did not affect axon branching. Recent studies have also identified Ryk as an inhibitor of dorsal root ganglion and corticospinal axon growth in the context of spinal cord injury (Hollis & Zou, 2012b; Li et al., 2008; Liu et al., 2008; Miyashita et al., 2009). Thus, Ryk growth inhibitory activity parallels the activity of many chemorepulsive guidance receptors (e.g. the semaphorin receptors, neuropilins/plexins) (Kitsukawa et al., 1997), which cause axons to stall within the chemorepellent gradient.

Despite the chemorepulsive activity of Ryk, callosal axons continue to grow through high levels of Wnt5a as they cross the callosal midline in the developing cortex. In contrast to the above observations, several studies on cortical neurons from postnatal hamster sensorimotor cortex have suggested that Ryk alone promotes callosal axon growth, whereas Ryk and Frizzled cooperate to induce chemorepulsion (Hutchins et al., 2011; Li et al., 2009). Initially, bath application of recombinant Wnt5a was found to increase the rate of axon outgrowth and axon length in dissociated hamster cortical neurons (Li et al., 2009). This effect was inhibited by Ryk siRNA. However, in these experiments callosal neurons were not specifically identified within the mixed cortical neuron population. Live imaging of callosal axon growth in slice cultures of hamster cortex has provided further evidence that Ryk can promote growth (Hutchins et al., 2011). We show here that in the absence of Wnt modulators, Wnt5a/Ryk interactions suppress callosal axon growth. Conversely, Hutchins et al. (2011) demonstrated that in the environment of the developing hamster cortex, Ryk could promote axon growth. Together these studies highlight the complexity of Wnt5a/Ryk signaling in the developing CNS and suggest that the Wnt5a/Ryk effect on axon growth may be reversed by Wnt modulators in the local environment. These may include other Ryk ligands (e.g. Wnt1 and Wnt3) or modulators such as sfrps (secreted-frizzled related proteins). Thus, it seems likely that Wnt5a is not the only factor regulating Ryk activity in the context of callosal axon growth and that unidentified Wnt modulators may act *in vivo* to fine-tune axonal responses as they cross the callosal midline.

Wnts and Frizzleds have been shown to promote both dendrite growth and guidance (Kirszenblat et al., 2011). Here, we demonstrate that Wnt5a also influences dendrite growth via a complex regulatory interaction between Ryk and possibly Frizzled. The Wnt5a-mediated inhibition of dendrite growth in the absence of Ryk (Figure 4) suggests that Wnt5a inhibits growth via a Frizzled receptor. However, wildtype dendrites did not respond to Wnt5a, indicating that Ryk was able to suppress Wnt5a-mediated growth inhibition. Together, these observations lead to the hypothesis that Ryk may act to repress Wnt5a/Frizzled-mediated growth inhibition. Ryk may accomplish this by sequestering Wnt5a, thereby preventing binding to Frizzled. Such context-dependent activity is observed for many guidance receptors. For example, the netrin receptor, DCC, induces chemoattraction in a Netrin-1 gradient but becomes a repulsive receptor when it forms a complex with its coreceptor Unc5 (Bradford et al., 2009; Hong et al., 1999).

The effect of Wnt5a and Ryk on neurite number paralleled that observed for dendrite extension. In the early phase of neuronal differentiation, one neurite sprout is selected to become the axon based on its spatial relationship to the Golgi and centrosome (reviewed in Cáceres et al., 2012). Subsequently, the remaining neurites take up a dendritic identity. As the majority of neurites give rise to dendrites, the effect of Wnt5a on callosal neurite number may reflect the dendritic response. As in growth cone guidance, neurite elaboration is dependent on the polarization of the actin cytoskeleton and filopodial extension (Cáceres et al., 2012). Therefore, it is not surprising that the initiation of neurite growth is also regulated by non-canonical Wnt signaling pathways known to control actin polymerization (Clark et al., 2012; Niehrs, 2012).

The Wnt/Ryk signaling pathway plays an essential and beneficial role in the establishment of major axon tracts in the developing nervous system. The divergent roles of Wnt5a in callosal axon and dendrite growth argue for a context-dependent response in which Wnt5a activity is determined by its interaction with Ryk and Frizzled. That Wnt5a-mediated repulsion of cortical axons also requires both Ryk and Frizzled (Li et al., 2009) further highlights the complex interplay between these Wnt receptors. Establishing how Ryk signaling is integrated with Frizzled-activated pathways will greatly expand our understanding of the basic molecular mechanisms underpinning axon and dendrite growth and guidance in the embryo and may suggest novel strategies with which to enhance neuronal repair after injury or in neurodegenerative disease.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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