In a Drosophila genetic screen we have identified a mutant, no commissures (nocs) that lacks midline crossing of commissural axons. Here, I set out to characterize the commissural phenotype of nocs using genetic and cellular approaches:

In Model 1, I hypothesize that nocs induces aberrant guidance of commissural axons toward midline (by the inhibition of netrin). Because netrin-1 is expressed along the D-V axis in the spinal cord and is required for the growth of commissural axons (Serafini, Colamarino et al. 1996), my initial experiments target netrin-1 and its receptor, frazzled (Hiramoto, Hiromi et al. 2000).

While we are analyzing the sequencing results of nocs, I will used antibodies and mRNA to assess netrin and frazzled expression in nocs mutants. This experiment will allow me to deduce if nocs interferes with normal commissural axon molecule expression. Next, I will overexpress netrin-1 or frazzled into nocs mutants, explant the spinal cords and assess midline crossing using axon staining with Fasciclin 2 antibodies. If Model 1 holds, I predict that netrin or frazzled will rescue the phenotype. Alternatively, transplant of fra or descam ventral neurons into nocs mutants will be assessed for other netrin receptors that are potentially involved.

Once we have the sequence of nocs, we will perform in situ hybridization of nocs mRNA in the developing drosophila spinal cord to determine site of action. I will compare staining to netrin and frazzled antibody staining at stage 12-15 and compare to WTs (Harris, Sabatelli et al. 1996). Furthermore, as in Harris et al., I will attempt rescue experiments of the midline crossing phenotype by expression of nocs at the ventral spinal cord. If rescue experiments fail, I can try nocs expression in the midline or overexpress nocs in netrin and frazzled mutants to assess which act downstream of each other.

In Model 2, I hypothesize that nocs is a negative regulator of commissurelss (comm), a sorting receptor to control the intracellular trafficking of Robo1. Because comm has a no commissural crossing phenotype (Seeger, Tear et al. 1993), Model 2 presumes that nocs acts in a similar mechanistic fashion either on comm or on robo1.

While we are analyzing the sequencing results of nocs, I will perform cell transplantation studies of WT pan-GFP neuronal spinal cord cells into nocs mutant spinal cord. I predict that these explants will have normal commissural crossing. Alternatively, I will perform the reverse and transplant nocs GFP spinal cord sections into WT spinal cord to verify that nocs is required for crossing. Additionally, double mutants (comm/nocs or robo1/nocs) should reveal if nocs is required for robo-slit mediated crossing. If Model 2 holds true, then nocs inhibits comm which inhibits robo1 midline repelling.

With the nocs sequence in tow, I will perform in situ hybridization of nocs mRNA in the developing drosophila spinal cord to determine site of action. Next, I will compare nocs staining to comm and robo antibody staining at stage 12-16 in nocs mutatns and compare to WTs (Keleman, Rajagopalan et al. 2002). This regional and genotypic comparison will allow me to sort out if nocs has a regulatory role on comm or robo. Mechanistically, if Model 2 holds true then I will overexpress comm in nocs mutants to assess if this rescues the non-crossing phenotype. Because comm acts through a conserved LPSY sorting motif, I will verify that blacking this motif impairs midline crossing (Keleman, Rajagopalan et al. 2002). Next,

I will overexpress comm with various sorting motif manipulations (Keleman, Rajagopalan et al. 2002) into nocs mutants. I will screen for rescue of midline crossing in spinal cord explants and costain with robo/FasII for axons. This experiment will tell me that nocs may act by interfering intracellular vesicle trafficking of robo by the comm sorting motif.

In summary, two models are proposed by which nocs may cause a midline crossing phenotype. The first hypothesizes that nocs deters normal guidance of commissural axons to the midline while the second hypothesizes that the comm signal (which allows for slit-robo interference) is negatively regulated by nocs.

**References**

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