We thank the reviewers for their comments and have tried best to address their concerns. We are encouraged by the comments of the reviewers, indeed reviewer#1 though that our study " shows, beautifully, how one can go from neuronal function in an intact zebrafish larva to detailed structure and connectivity in the brain". It is for precisely this reason that we feel our study will be useful to the broader neuroscience community.

Reviewer 2 expressed concerns regarding (1) the lack of comparison between integrator neuron time constants and morphologies, (2) potential ambiguity in the correspondence between our anatomically defined "stripe" of somata and the *alx*-expressing stripe defined by Kinkhabwala et al., and (3) the lack of reconstruction of abducens neurons. To address concern (1) we have added further statistical analyses to Supplementary Fig. 1 (see also Discussion, line number 288). In brief, the time constants of contralaterally projecting neurons are significantly different from those of the unknown projecting group of neurons. We also found that neural time constant is weakly correlated with mediolateral somatic location, though the correlation is not quite statistically significant.

Regarding concern (2), Reviewer 2 was worried that our anatomically defined stripe of somata might correspond with the *engrailed-1* stripe, which could potentially be confused with the *alx* stripe. The potential for confusion indeed exists above the level of the Mauthner axon, where both stripes are next to each other. However, our study was conducted below the level of the Mauthner axon, where the *engrailed-1* stripe is virtually nonexistent (see Supplementary Fig. 3, location of somata).

In concern (3) Reviewer 2 suggested the great idea of reconstructing abducens neurons that are postsynaptic to integrator neurons. This is interesting, and we would do it if we could. Indeed, it would be possible for us to reconstruct postsynaptic neurons, but the reconstructions would be fragmentary because the neurons are at the ventral most extreme of the imaged volume. Therefore it would not be possible to conclusively identify whether reconstructed postsynaptic neurons are actually abducens neurons. For this reason, we did not reconstruct these neurons.

In summary, we have performed statistical analyses to correlate integrator neuron properties with their morphologies. We feel strongly that the inferred neurotransmitter identities as reported are accurate and that any further reconstructions would not lead to a more complete understanding of the circuit. More detailed answers to reviewer comments follow below.

Reviewer #1:

1. *Page 3: line below... the EM was not done in a behaving fish We combined two-photon calcium imaging and serial electron microscopy in a behaving fish to identify.*
   (a) In this sentence we used the word 'behavior' in the context that the functional recording were performed in an immobilized fish, which was free to move its eyes. To prevent this confusion, we have removed the word behaving (line number 47).
2. *Page 4 line below is worded wrong... just read it to see. , where as for neurons with small dendritic arbors were completely reconstructed and did not have any dendrites that exited the imaged volume.*
   (a) We have now corrected this sentence.
3. *page 5... wording issue again copied below. Both neurons contained another neurite crossed the midline.*
   (a) We have corrected this sentence.
4. *Page 6... This process not processes This processes revealed that the distribution of the presynaptic sites along the axons among the two groups ('ipsi-only' and 'ipsi-contra') were not different (p = 0. 01, kstest).*

(a) We have corrected this sentence.

5. *get rid of the second "group of" in this sentence on page 7 The second major group of neurons that we reconstructed were the eight caudally located, contralaterally projecting group of neurons.*

   (a) We have corrected this sentence.

Reviewer #2:

Major points

1. *Registration of EM-LM volumes (Fig. 1C): How do the authors assess that the functionally imaged cells are successfully re-identified in the EM volume? Fig.1C shows the correspondence between LM and EM images, but it would be more convincing if they showed an example plane where the functional calcium imaging was performed and integrator neurons were identified (as in Fig 1A).*

   (a) We thank the reviewer for the comments. We agree with the reviewers' comments that it will be convincing if we were able to show an example plane where the functional imaging was performed along with the corresponding EM plane. To address this concern, we have added a panel showing integrator neurons from two functionally imaged planes and the same neurons from registered EM planes in Supplementary Fig. 1C.

2. *What is missing throughout the paper is the comparison with integrator property and their ultrastructural morphologies. They have measured time constants of the integrator neurons, but these metrics are not correlated with morphology.*

   (a) We thank the reviewer for the comments. We performed statistical analyses to compare the distribution of integrator time constants between the three morphological groups. We noticed that the 'Contra' and 'Unknown' projecting groups of neurons had statistically different time constants. The remaining distributions do not show any statistically significant differences. We added an extra panel to Supplementary figure 1 (Sup. Fig. 1D, box plot of time constants) and have commented in the main text (line number 288).

   (b) We also now report correlation coefficients between integrator time constant and the somatic locations alongside the figures (Sup. Fig. 1). This analysis revealed that there is a weak correlation between time constants and mediolateral somatic location which was not statistically significant. The remaining correlation along the rostrocaudal and dorsoventral axis were not too weak. We suspect the correlations in our study are weak due to the small sample size of functionally identified cells in this study.

3. *The authors infer the neurotransmitter identity of integrator neurons based on soma position and the pattern of neurotransmitter markers that another group has shown. This assumption underlies one of the strongest conclusions of the paper. However, the correspondence of the stripes shown here with the neurotransmitter stripes shown previously is highly speculative. They define soma stripes as peaks (termed S1-3, Fig. 1D) of cell bodies that are projected along the dorso-ventral axis in this volume, whereas Kinkhabwala et al. (2011) defined stripe organization using transcription factors and neurotransmitter-specific transgenic lines. For example, the authors state that "the medial most stripe S1 aligns with a group of neurons that express the alx transcription factor and are most often glutamatergic". However, S1 is also close to another, engrailed-1 positive stripe, which contains neurons with different properties. Also, previous studies reported glycinergic neurons in the hindbrain, but the authors do not discuss this possibility.*

   (a) The *engrailed-1* stripe which contains glycinergic neurons is very close to the *alx* stripe and that neurons in each of these stripes have different properties. The reviewer is concerned that we may have incorrectly assigned the neurotransmitter identity to some of the cells in the medial most stripe. In our study, all the cells that were imaged, were below the plane of the Mauthner axon (MA). At this level the *engrailed*

-1/glycinergic neurons are virtually not present, which is consistent with the fact that Lee M.M. et al., did not observe any glycinergic integrator neurons below the level of the MA. Regarding the presence of glycinergic neurons in the hindbrain, Lee M M et al., found that only 2% of all integrator neurons were glycinergic, and that the vast majority were glutamatergic or GABAergic. For these reasons, we feel confident that the cells that were imaged here would not have contained potential integrator neurons that were glycinergic. We have clarified the above points in the main text as well (line number 87).

4. *The abducens motor nucleus is the target of integrator neurons in goldfish and zebrafish. Fig. S3 shows that axons of some integrator neurons project near the abducens nerve, but the authors do not show that the axons make direct synapses with abducens neurons. This is a missed opportunity.*
   (a) As the reviewer has noted, the abducens motor nucleus is the target of integrator neurons. In the main text we had mentioned that only 2 of the 6 axons from ipsilaterally projecting integrator neurons projected close to the abducens. Although there are direct synapses made onto other neurons in that area, to determine if the postsynaptic target neurons are indeed abducens neurons, we would have to trace the entire cell. These synapses are at the ventral extent of the imaged volume, we feel that most if not all the neurites of these potential abducens neurons will be incomplete. This would make is impossible to determine if these neurons were indeed abducens neurons. Thus, although we would have liked to have this information, it is not in the scope of the imaged data of the study.

Minor points:

1. *In the Discussion (third paragraph on page 7), the authors discuss that the most laterally located group of neurons in S3, which they speculate to be glutamatergic, have been shown in the previous study by Kinkhabwala et al. to have both ipsilateral and contralateral projections. However, such data are not shown in the Kinkhabwala et al. paper.*
   (a) The reviewer correctly pointed to a mistake in discussion that was made by the authors. In Kinkhabwala et. al. the lateral most class of cells corresponds with the *Barhl2* stripe of glutamatergic cells. The morphology of these cells, their axonal projections are yet to be determined. We have corrected the text to reflect this (line number 281)
2. *In Fig S2B, the caption states "tight junctions" but the main text mentions "gap junctions"?*
   (a) This has now been corrected. We have now consistently used ‘cell-junctions’ in the main text and supplementary material. A reference to gap junctions is mentioned, but we make it clear that in our dataset we are not sure if these are gap junctions.
3. *The format of the current manuscript does not seem to conform to the guidelines of Current Biology.*
   (a) We have now reduced the number of supplementary figures to conform with the guidelines.
4. *The manuscript needs to be edited extensively for typos and grammatical errors (e. g., singular/plural of cilium: "We also observed a primary cilia on all 22 integrator neurons that were reconstructed. Primary cilium are know to be present in most, if not all mammalian cells.")*
   (a) These issues have been corrected.