We thank the reviewers for their comments and have tried best to address their concerns. Although, one of the reviewers had noted that the results that we have presented were incomplete and would be of interest to a specialist, we think that the approach in this study will appeal to the broader neuroscience community. 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.

The main concerns of the reviewers were regarding (1) the lack of correlation between integrator neuron properties to the morphologies of these neurons, (2) the interpretation of the neurotransmitter identity of these neurons and (3) the reconstruction of abducens neurons. To address the reviewers concerns we have now included results from statistical analysis that we had not presented reported. Regarding the lack of correlation between integrator neuron properties and morphology of integrator neurons, we computed the correlation coefficients and have now reported these results in the appropriate figures. We observed weak linear correlation between integrator neuron properties and their respective morphologies. Similarly, the distributions of integrator time constants were compared across the different morphological groups. Here we noticed that the contralaterally projecting neurons had significantly different distribution as compared to the unknown projecting group of neurons. The remaining distributions were found to be not significant. These results have been added to the discussion.

The neurotransmitter identities of these cells were inferred from the stripe like distribution of the somata. Since our study was not performed in a transgenic line with labeled neurons, we relied on the highly specific description of these stripes and their spatial locations from previous studies. As an example, we report that the medial most stripe of cells most likely expresses the *alx* transcription factor. This is consistent with the fact that both a previous study and our study report these neurons as having ipsilaterally projecting axons. Similarly the reviewers correctly note that the *engrailed-1* stripe which abuts the *alx* stripe is known to have neurons with different properties. However, below the level of the Mauthner axon, the stripe with *engrailed-1* cells virtually nonexistent. Our study was conducted below the level of the Mauthner axon. Finally, the reviewer would have liked for us to validate that these integrator neurons made direct synapses onto abducens neurons. For this we would have to reconstruct these potential abducens neurons. The reconstruction of these neurons would likely not be accurate as these neurons are at the ventral most region of the imaged volume, leading to, at-best, partial reconstructions. Such a partial reconstruction would make it near impossible to assign these neurons as abducens neurons. For this reason, we did not reconstruct these neurons.

In summary, we have performed statistical analysis to correlate integrator neuron properties to 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.
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 their comments. We agree with the reviewers comments that it will be more convincing if we were able to show an example plane where the functional imaging was performed along with the corresponding EM plane. In order to do that, the orientation of the animal during functional imaging and during subsequent EM sectioning should be exactly the same. This however is not the case, and it very hard to achieve. Instead, we digitally transformed the functionally imaged planes onto the EM volume using landmarks. To address the reviewers comments, we have added a panel with images that show the functionally imaged areas and their corresponding EM planes in the supplementary figures (Sup. 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 their comments. The correlation coefficients between integrator time constants and the morphological properties have now been reported along side the figures (Sup. Fig. 1). This analysis reveal that there is a weak correlation between integrator time constants and spatial location of the neuron. Similarly, there are also weak correelations between the size of the neurons and its time constant (this was reported previously). These trends are consistent with a previous study (Miri et. al. 2011). We suspect the correlations in our study are weak due to the small sample size of functionally identified cells in this study. Previous studies where similar correlations were made had sample sizes in the hundreds of imaged neurons.

   (b) Regarding the comparison of integrator time constants to the assigned morphological group, we have now added an extra panel to Sup. Fig. 1D (box plot of time constants from each group). Here we compared the distribution of integrator time constants between the three morpological groups. We noticed that the 'Contra' and 'Unknown' projecting groups of neurons had statistically different distributions of integrator time constants. The remaining distributions do not show any discernible differences. We have added these points to the discussion in the main text.

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) We thank the reviewer for this comment. As the reviewer noted, 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). Below the level of the MA, *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 are glycinergic. We have clarified the above points in the main text as well.

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 were indeed abducens neurons, we would have to trace the entire cell. These synapses are at the ventral most extent of the imaged volume, and 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 the intended interpretation.

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.