We thank the reviewers for their comments and tried as best to explain 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 thing that the approach that we have demonstrated in this study will have a wide 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". We have carefully gone over the comments of the reviewers in oder to best answer their question. To do this, we first re-traced the neurons in this study using a semi-automated 3D approach to make sure that our reconstructions were accurate. This process added a few additional details to the existing claims, and secondly it helped identify some potential ambiguous traces that have now been removed.

As part of the new evidence, one the previously reported cells had a segment of the axon that was under-reconstructed. This has now been fixed. This addition changed some of the anatomical facts of the neuron, like lengths, size etc. It also revealed new synaptic partners to the network of cells, increasing the number from previously reported 4 synapses to now 6. The second part of the new evidence was the identification and removal of a previously over-reconstructed branch. This has now been removed from the analysis. This resulted in the reclassification of one cell from the 'ipsi-contra' group to the 'contra-only' group. All figures have been updated accordingly and the comments of the reviewers are answered in line.

Reviewer #1:

We thank the reviewer for their comments and have tried to address all the concerns that were raised.

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) Corrected, by removing 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) Grammar was corrected to read correctly now.
3. *page 5. . . wording issue again copied below. Both neurons contained another neurite crossed the midline.*
   (a) Similar to above
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) Similar to above
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) Similar to above

Reviewer #2:

We thank the reviewer for their comments.

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 more convincing if we were able to show an example plane where the functional imaging was performed. 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 shown parts, but not the entire plane, of the functionally imaged areas and their corresponding EM planes as supplementary figure (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) The reviewer has a very great point here. These time contacts were computed with the purpose to correlating them to the morphologies. In Sup. Fig 1, we show some of the time constant properties of the cells as a function of spatial location, somata size. Performing statistical analysis of the these time constants based on their assigned morphological group, did not reveal any significant trends. We have added an extra panel to Sup. Fig1D (box plot of time constants), to show that there is no discernible trends. We suspect this is 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) 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. In our study, all the cells that were imaged below the plane of the Mauthner axon (MA). In a previous study by (Lee MM et.al., 2015) a detailed analysis of the neurotransmitter identity of integrator neurons was performed and we have listed some of the key points

      i. Immunofluorescence imaging in transgenic fish that express reporters for both glutamate and glycine show that below the plane of the MA, there are very few glycine positive neurons.

      ii. Of the 273 functionally imaged cells, it was found that only 2% of all integrator neurons were glycinergic.

      iii. lastly, many of cells that were neither glutamatergic nor glycinergic were in fact found to be GABAergic.

   For these reasons, we feel confident that the cells that were imaged here would not have contained potential integrator neurons, that are glycinergic and expressing the *engrailed-1* transcription factor. We have reflected some of the above ideas 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 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 like 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) Corrected
3. The format of the current manuscript does not seem to conform to the guidelines of Current Biology.
   (a) Once accepted, we can make a decision to reduce the word limit.
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) Corrected.