1   Multisensory Integration in the Developing Tectum is Constrained by the

2   Balance of Excitation and Inhibition

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9   Working Title: Development of multisensory integration

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20   Conflict of Interest

21   The authors declare no conflict of interest.

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Abstract

26

27   Multisensory integration (MSI) is the process that allows the brain to bind together

28   spatiotemporally congruent inputs from different sensory modalities to produce single

29   salient representations. While the phenomenology of MSI in vertebrate brains is well

30   described, relatively little is known about cellular and synaptic mechanisms underlying

31   this phenomenon. Here we use an isolated brain preparation to describe cellular

32   mechanisms underlying development of MSI between visual and mechanosensory

33   inputs in the optic tectum of Xenopus tadpoles. We find MSI is highly dependent on the

34   temporal interval between crossmodal stimulus pairs. Over a key developmental period,

35   the temporal window for MSI significantly narrows and is selectively tuned to specific

36   interstimulus intervals. These changes in MSI correlate with developmental increases in

37   evoked synaptic inhibition, and inhibitory blockade reverses observed developmental

38   changes in MSI. We propose a model in which development of recurrent inhibition

39   mediates development of temporal aspects of MSI in the tectum.

Introduction

41   Multisensory integration (MSI) is a well-characterized phenomenon of both neural

42   output and behavior where the response triggered by a stimulus of a given sensory

43   modality is altered by the coincident presentation of a stimulus of a different sensory

44   modality (for reviews see Stein and Stanford, 2008; Stein et al., 2009). The result can be

45   either enhancement or suppression of the response relative to the responses evoked by

46   a single unimodal stimulus (Stanford et al. 2005).

47   One central brain area where inputs from multiple sensory modalities first interact is the

48   optic tectum. This midbrain structure, also known as the superior colliculus in mammals,

49   contains laminae that are segregated functionally, as well as anatomically – its

50   superficial layers receive a direct retinal projection that maintains its topographic map

51   of visual space (Graybiel, 1975; Straznicky and Gaze, 1972) and its deep and

52   intermediate layers receive inputs carrying both auditory information (Knudsen, 1982;

53   Lowe, 1986) as well as somato- and mechanosensory information and motor feedback

54   signals (for a review see May, 2006). In a given multisensory neuron in the colliculus, the

55   amount of integration is dependent on both the overlap between spatial receptive fields

56   for the two stimulus modalities and the time window between stimulus presentations in

57   a crossmodal pair (Meredith et al., 1987; Meredith and Stein, 1996). The integrative

58   capabilities of multisensory collicular neurons develop in an activity dependent manner

59   which has been proposed to depend on repeated associations of spatiotemporally

60   aligned stimuli during a critical period (Knudsen, 2002; Knudsen and Brainard, 1991;

61   Wallace et al., 2006; Wallace and Stein, 2007; Xu et al., 2012; Yu et al., 2010).

62   Despite great interest within the field in understanding potential cellular and synaptic

63   mechanisms for multisensory integration and its development (Rowland et al., 2007b),

64   very little experimental work exists on this question (Binns and Salt, 1996; Skaliora et al.,

65   2004). The prevalent experimental models of multisensory integration have been the

66   superior colliculus of cats and rodents and to some degree the optic tectum of the barn

67   owl; their location within the animal makes these structures difficult to access with

68   whole-cell recording techniques in vivo and impossible to isolate in vitro without

69   significantly disrupting the neural networks in which they operate. In Xenopus laevis

70   tadpoles, the optic tectum is superficially located and thus accessible for whole-cell

71   recordings in vivo (Khakhalin et al., 2014) and can be isolated in an intact whole-brain

72   preparation for recordings ex vivo (Pratt and Aizenman, 2007). This is a tremendous

73   advantage over other preparations, where assessing synaptic events during MSI is

74   usually inferred from local extracellular field potentials.

75   In Xenopus, as in other vertebrates, the dominant input to the optic tectum is visual,

76   originating from the contralateral retina, and terminating in the superficial layers of the

77   tectal neuropil (Deeg et al., 2009; Székely and Lázár, 1976). Inputs from the various

78   mechanosensory modalities that ascend via hindbrain nuclei terminate within the

79   deeper layers (Behrend et al., 2006; Deeg et al., 2009; Hiramoto and Cline, 2009; Lowe,

80   1986, 1987). Our laboratory and others have characterized the development of

81   hindbrain mechanosensory projections to the tectum in Xenopus tadpoles, and have

82   shown that these inputs converge in single tectal neurons from very early

83   developmental stages. In this study we use the Xenopus tadpole to begin to examine the

84   cellular and circuit level mechanisms underlying multisensory integration of these

85   hindbrain and visual inputs in the developing midbrain.

86

87   **Results**

88   **Temporal Interactions**

89   Experiments were performed between developmental stages 44-46 and 48-9. During

90   this developmental time span, tectal neurons already have been innervated by both

91   visual and mechanosensory inputs and respond synaptically to activation of either

92   pathway (Deeg et al., 2009; Hiramoto and Cline, 2009). During this timeframe tectal

93   circuits also undergo a period of refinement, both of sensory receptive fields (Dong and

94   Aizenman, 2012; Dong et al., 2009; Tao and Poo, 2005) and of local recurrent circuitry

95   (Pratt et al., 2008). Tectal neurons also undergo dramatic change in their intrinsic

96   excitability (Ciarleglio et al., 2015; Pratt and Aizenman, 2007), and behavioral data

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97 demonstrate that even young Xenopus tadpoles generate behavioral responses to visual

98 and mechanosensory stimuli (Dong et al., 2009; Roberts et al., 2009). We used a whole

99 brain ex vivo preparation which allows us to preserve intact the relevant brain circuits

100 (Wu et al., 1996). We isolated inputs carrying either visual or mechanosensory

101 information by placing stimulating electrodes in the optic chiasm to activate visual

102 pathways (V) and in the contralateral hindbrain to activate mechanosensory pathways

103 (HB; Figure 1A). Previous descriptions of crossmodal integration, evidenced in either the

104 number or temporal distribution of action potentials, reveal that the inter-stimulus

105 interval (ISI) between two crossmodal inputs is a key factor that determines the

106 direction and magnitude of the integrative response (Meredith et al., 1987). This

107 temporal relationship can be further modulated by the relative size of the individual

108 responses, where greater multisensory enhancement is observed if the stronger

109 modality precedes the weaker one (Miller et al. 2015). To assess how ISI influences the

110 ability of Xenopus tectal neurons to integrate crossmodal stimulus pairs, we performed

111 cell-attached recordings from tectal neuron somata to measure individual spikes evoked

112 by stimulation of sensory inputs (Figure 1B). Stimulus intensities were set such that

113 response sizes for each modality were roughly matched. We systematically varied the

114 time interval between the two electrical stimuli delivered to the different sensory

115 pathways. Figure 1C shows examples of spike time raster plots at different ISI's in both

116 developmental groups. From these examples two observations become evident. First

117 that paired responses have a faster onset and can have a greater gain than single

118 modality responses. And second that these changes show a temporal dependence on

119 ISI. The temporal dependence of crossmodal responses on ISI is revealed in the

120 population means of the Multi-Sensory Index (MSIn; see Materials and methods) values

121 from both developmental groups (stages 44–46 and stages 48–49; Figure 1D). In

122 general, greater and more-positive MSIn values were observed as a result of shorter

123 inter-stimulus intervals, whereas negative MSIn values —indicating a suppression of

124 responses due to crossmodal paired stimuli — are produced exclusively by longer inter-

125 stimulus intervals, of up to 1000 ms. Noticeably, the net average MSIn values (both

126 negative and positive) appear to be reduced in the older animals.

127 One possibility that may explain the apparent decrease in MSIn in older animals is that

128 that at stages 48–49 individual cells could be strongly selective for one or only a few

129 adjacent ISI's, but vary widely with respect to the ISI to which they are most sensitive. As

130 a result, these differences could be drowned out in the population averages. Thus, to

131 investigate the possibility that tectal neurons become tuned to respond best to different

132 crossmodal ISI's over development, we measured the ISI's at which each cell's maximal

133 MSIn values were generated and plotted these ISI values as a histogram for both

134 developmental stages (Figure 2A,B). We found that in the stage 48–49 group, maximum

135 MSIn responses occur at a relatively broad range of ISIs (Figure 2B) across the

136 population of cells, in contrast to the younger tadpoles, where maximal MSI mostly

137 occurs at short ISIs (Figure 2A). Another way to describe this is that in the older

138 tadpoles, tectal neurons, as a population, are tuned to respond optimally to a wider

139 range of crossmodal ISIs (Figure 2D; median ISI, st 44-6 = 200 ms, IQR = 300 ms (n=26),

140 st 48-9 = 400 ms, IQR = 775 ms (n=40), P <0.05, Mann-Whitney). However, despite

141 these differences in temporal tuning, the peak MSIn value that could be achieved by a

142 given cell was still roughly twice as large in the younger developmental group (Figure

143 2C; mean max MSI, st 44-6 = 1.15±0.31 (n=26), st 48-9 = 0.55±0.10 (n=40), P <0.05,

144 unpaired T-test).

145 Next we tested whether any systematic differences between H-V and V-H pairings could

146 be hidden in these combined data. Thus we compared the peak MSIn values at the same

147 ISI, across the two stimulus sequences, and consistently we found that both sequences

148 were equally effective in evoking multisensory responses (see Table 1). Likely this is a

149 result of the fact that in these experiments stimulus intensities were adjusted so that

150 response magnitudes were similar across modalities. As a result, we do not distinguish

151 between different stimulus sequences for the remainder of this study.

152 Independent of the magnitude of a cell's responses to its preferred stimulus, another

153 measure of developmental refinement is the degree of its selectivity for that particular

154 stimulus combination. Because responses to paired stimuli peak at different ISIs in

155   different cells, to study the tuning of ISI selectivity across cells it is necessary to align

156   their MSIn-versus-ISI distributions at the peak MSIn value in each neuron. This

157   alignment of all 'ISI tuning curves' at their maximal MSIn values should provide a

158   common reference point for comparing the shapes of the entire curve, across cells.

159   Tuning curves of individual cells, representing different combinations of H and V offsets,

160   were aligned at their central peak in the X axis to allow for direct comparison of average

161   temporal tuning curve shapes as shown in Figure 2E. In the stage 44-6 tadpoles it was

162   evident that across cells, crossmodal response enhancement was present over a wider

163   range of ISIs surrounding the central peak, and that this tuning narrowed significantly

164   over development. To quantify this difference we determined the best curve fit for each

165   data, and the resulting curves were compared statistically using an F test, which tests

166   whether the dataset is best fit by two separate curves or by a single curve. We found

167   significant differences between the tuning curves between developmental stages (F-

168   test: F=9.966 (2,716), p<0.00001), with the curve being narrower in the older group.

169   Taken together, these data suggest that over development, peak multisensory

170   responses decrease in terms of absolute gain, however cells become preferentially

171   tuned to a greater diversity of temporal intervals, yet also become more sharply tuned

172   around their preferred interval.

173

174   **Deviations from Linearity**

175   While the MSI metric reveals how the response to a single stimulus is changed by a

176   preceding input, it does not quantitatively address the manner in which the two inputs

177   interact. The method described by Stanford et al. (Stanford et al., 2005) allows us to

178   compare the actual output of multisensory responses to what is expected from a purely

179   additive combination of responses to individual, single modality inputs (for details of

180   how the predicted sum is determined, see Materials and methods). Evoked multisensory

181   responses could either result in supralinear, linear or sub linear summation, examples of

182   sublinear and supralinear summation are shown in Figure 3A. When we compared the

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183   maximal evoked crossmodal response to the response predicted by a linear sum of both

184   inputs, we found that for both developmental stages data points fall below the line of

185   unity indicating a tendency for sub-linear summation (i.e. predicted > actual, Figure 3B).

186   This tendency toward sublinearity was evident by plotting a cumulative probability

187   distribution of Z-scores across cells (Figure 3C), and by comparing the relative

188   proportion of cells showing sublinear, linear and supralinear responses (Figure 3D).

189   Although the cumulative probability curve shifts towards the right (supralinear) over

190   development (Figure 3C), we found no statistical differences in the distribution of Z

191   scores across developmental groups (Kolmogorov-Smirnov test, $D = 0.260$, $P = 0.230$),

192   indicating that the overall distributions of integrating responses remains stable during

193   this developmental period. However there was a shift, such that the proportion of cells

194   showing sublinear responses decreased and the proportion showing linear responses

195   increased over development (Figure 3D).

196

197   **Comparison to Unisensory Pairs**

198   One possible explanation for the observed developmental decrease in the net gain of

199   multisensory responses and the shortening of the temporal window for multisensory

200   integration could be that these both reflect developmental changes intrinsic to tectal

201   neurons and the tectal network. Between stages 45 and 49, the temporal dynamics in

202   tectal network activity change, such that evoked recurrent excitation in the tectum

203   decreases, and becomes more temporally compact (Pratt et al., 2008). Furthermore,

204   tectal neurons become less excitable over this developmental time window, generating

205   fewer action potentials when depolarized (Ciarleglio et al., 2015; Pratt and Aizenman,

206   2007). If the effects on the temporal properties and raw magnitude of multisensory

207   integration are simply due to changes overall in tectal circuitry and excitability,

208   respectively, then these changes should also be manifested in all paired interactions,

209   including responses to pairs of unisensory stimuli. To test this, we performed paired

210   stimulation of either V or HB inputs over the same range of ISIs we used for the

211   crossmodal pairs. We found that at both developmental stages paired unimodal

212 responses showed significant response enhancement over a wide range of ISIs (Figure

213 4A,B), in contrast to what we had observed for crossmodal responses (Figure 1D, 2A).

214 Moreover, there was significantly more enhancement in response to unimodal pairs

215 than there was for crossmodal pairs, and no developmental decrease was observed in

216 the gain of the paired responses (Figure 4C, compare to 2C; mean max PPE, st 44-6 =

217 2.25±0.44 (n=29), st 48-9 = 2.57±0.33 (n=26), P = 0.56, unpaired T-test). Similarly, there

218 was also no developmental difference in the range of ISIs that produced the maximal

219 enhancement (Figure 4D, compare to 2D; median ISI, st 44-6 = 400 ms, IQR = 300 ms

220 (n=29), st 48-9 = 400 ms, IQR = 300 ms (n=26, P <0.34, Mann-Whitney). Finally, when we

221 looked at the temporal tuning around the maximal ISI, there was no narrowing of the

222 temporal tuning window for unimodal pairs over development as both tuning curves

223 were not significantly different from each other (Figure 4E, compare to 2E, F=1.394

224 (2,544), P=0.249).

225 Taken together, these data suggest that even at Stage 49, tectal cells can still exhibit

226 robust enhancement to paired stimuli, and thus are not constrained by developmental

227 changes in network dynamics and cellular excitability. In light of this observation, the

228 temporal changes in multisensory integration observed during development likely occur

229 through a different mechanism.

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231

232 **Inhibition underlies developmental changes in multisensory integration**

233 A second hypothesis for explaining developmental changes in multisensory integration

234 is related to the development of inhibition. In the superior colliculus it has been

235 proposed that maturation of inhibitory feedback correlates with the emergence of

236 multisensory integration. In the tectum, between developmental stages 45 and 49 there

237 is a large increase in evoked inhibitory drive, as well as a negative shift in the Cl-

238 equilibrium potential (Akerman and Cline, 2006; Miraucourt et al., 2012). Inhibition is

239 known to both narrow the tuning of sensory neurons to their preferred responses and

240   to alter the timing and reliability of sensory driven spike output (Shen et al. 2011). Thus,

241   in the tectum, this increased inhibitory drive could constrain multisensory interactions

242   by decreasing the amount of enhancement and narrowing the temporal window for

243   interaction.

244   If inhibitory inputs are constraining multisensory responses in stage 48-49 tadpoles,

245   then we can make three experimental predictions to test this hypothesis. First, we

246   would predict that blocking inhibition should broaden the temporal tuning for

247   multisensory integration and increase the overall levels of multisensory enhancement.

248   Second, inhibitory blockade should alter the spike timing of multimodal responses, and

249   third, the balance of excitation to inhibition of multisensory responses should decrease

250   over development.

251   We first tested the prediction that inhibitory blockade in stage 48-49 tadpoles broadens

252   the temporal window for multisensory integration, and increases the overall

253   enhancement in multisensory responses. To block inhibition we performed recordings in

254   the presence of 100 µM picrotoxin, a GABAA receptor blocker. We found that inhibitory

255   blockade resulted in an overall increase in MSIn values over a wide range of ISIs (Figure

256   5A). The ISI values at which the maximum MSIn was recorded were also shorter and

257   became less variable across cells (Figure 5B,D; median ISI, PTX = 100 ms, IQR = 425 ms

258   (n=22), no drug = 400 ms, IQR = 775 ms (n=40), P = 0.029, Mann-Whitney) and the

259   overall maximum MSIn per cell increased significantly (Figure 5C; mean max MSIn, PTX =

260   1.96±0.25 (n=22), no drug = 0.55±0.100 (n=40), P = 0.0001, unpaired T-test). Inhibitory

261   blockade also caused a significant broadening of the temporal tuning of the

262   multisensory integration window (Figure 5E, F=28.72(2,678), p<0.0001). These effects

263   are all similar to what we had observed in the younger, stage 45 tadpoles without

264   inhibition blocked. Inhibitory blockade also affected the linearity of crossmodal

265   responses. In the presence of picrotoxin, there was an increase in the relative

266   proportion of neurons showing supralinear responses (Figure 5F), significantly shifting

267   the cumulative distribution of Z-scores to the right (Figure 5G, D=0.595, p<0.0001,

268   Kolmogorov-Smirnov test). Interestingly, inhibitory blockade did not have any effect on

269 response enhancement caused by unimodal stimulus pairs, suggesting that its effects

270 are not due to general enhancement of network excitability (Max PPR, Control:

271 2.57±0.32, n=26, PTX: 2.17±0.3, n=12, p=0.39; Difference in Z-scores, D=0.267, p=0.413,

272 Kolmogorov-Smirnov test).

273 In mammals, spiking responses to crossmodal stimulus pairs always have a more rapid

274 onset, than responses to single stimuli of either modality (Rowland et al., 2007a). In our

275 preparation we observe the same effect at both developmental stages and across

276 multiple ISIs. Figures 6A and B show the median onset and IQR of spiking responses to

277 both single stimuli and paired stimuli at multiple ISIs for all cells, and we consistently

278 find that paired crossmodal responses are significantly faster than single stimulus

279 responses (Figure 6D; st 44-6: p<0.0001, n=25, st 48-49: p=0.04, n= 36, PTX: p<0.0001,

280 n=19, paired T-test). Inhibition in the tectum is known to affect the precision of the

281 spike output pattern of tectal neurons (Shen et al., 2011). We find that in picrotoxin,

282 while paired responses still have a faster onset than single responses (Figure 6C, D), the

283 onset of paired responses is significantly delayed when compared to stage 48-9 controls

284 (Figure 6E; p<0.05). This suggests that inhibition not only regulates the temporal tuning

285 and gain of multisensory integration, but also regulates the timing of tectal cell output.

286 Finally we tested the third prediction stemming from the hypothesis that enhanced

287 inhibition constrains multisensory responses in older tadpoles: that we would expect to

288 observe differences in the relative amount of inhibition to excitation during cross modal

289 responses at the different developmental time points. We performed whole-cell voltage

290 clamp recordings excitatory and inhibitory synaptic responses during crossmodal

291 stimulation at different ISIs. To isolate excitatory from inhibitory conductances, we

292 recorded at the reversal potential for Cl- (-45 mV) to isolate excitatory currents and at

293 the reversal potential for ionotropic glutamate receptors (+5 mV) to isolate inhibitory

294 currents (Akerman and Cline, 2006; Khakhalin and Aizenman, 2012). We then converted

295 the synaptic currents into conductance values (see Materials and methods). Sample

296 excitatory and inhibitory conductances are shown in Figure 7A, at different ISIs. Overall,

297 we found that inhibitory conductances were much greater in general than excitatory

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298   conductances and that the ratio of excitation to inhibition varied greatly across cells,

299   consistent with prior observations (Akerman and Cline, 2006; Khakhalin and Aizenman,

300   2012). Based on our previous studies, it is hard to predict a tectal cell's spike output by

301   simply looking at the raw values of individual conductances. Thus it would be more

302   informative to look at the relative enhancement of both excitation and inhibition

303   separately in response to crossmodal pairs. We calculated the MSIn of evoked

304   crossmodal responses to assess the amount of response enhancement of both

305   excitation and inhibition at the different ISIs (see Materials and methods). At both

306   developmental stages we found on average an overall enhancement of both excitatory

307   and inhibitory multisensory responses across all ISIs (Figure 7B,C). However we found

308   proportionally greater enhancement across ISIs of excitatory responses in the stage 44-

309   46 tadpoles, compared to stage 48-49 (p=0.037, 2-way ANOVA). In contrast we found

310   that enhancement of inhibitory conductances, and its dependence on ISI, did not

311   change over this developmental time window. We also compared data from individual

312   cells by plotting the maximal Excitatory MSIn vs. the corresponding Inhibitory MSIn for

313   both developmental stages (Figure 7D). In both cases maximal Excitatory and Inhibitory

314   MSIn values were positively correlated (St 44-46: r=0.758, p<0.0001; St 48-49: r=0.767,

315   p<0.0001). However the ratio between excitatory enhancement and inhibitory

316   enhancement was greater in younger tadpoles than in older tadpoles, overall, consistent

317   with the observation that excitatory currents show proportionally greater MSIn values

318   than inhibitory currents (test for differences in slopes of linear fits: p=0.023,

319   F=5.56(1,45).

320   Taken together, these data indicate that in younger tadpoles, the ratio of excitation to

321   inhibition, in response to crossmodal stimulus, is greater than in the older group, which

322   may in part explain the greater amount of multisensory enhancement of spike output

323   observed in the younger tadpoles. This change in the excitation to inhibition ratio may

324   also explain the sharpening of the temporal tuning of multisensory responses. However,

325   these data do not directly account for the dependence of multisensory integration on

326   ISI. One possibility is that the temporal dynamics of concurrent excitation and inhibition

327   may vary as a function of ISI, thus resulting in different patterns of spike output. In order

328   to test this hypothesis we would need to record excitation and inhibition simultaneously

329   during a single response, due to the trial-to-trial variability in response shape,

330   something that was not done in the present study.

331

332   **Discussion**

333   In this study we provide novel insight into the synaptic and circuit-level mechanisms

334   that underlie developmental changes in the temporal rules mediating multisensory

335   integration in the optic tectum. We found that optic tectal neurons exhibit enhanced

336   responses to crossmodal stimulus pairs, compared to single modality responses, and

337   that this enhancement is heavily dependent on the ISI. The temporal window for

338   multisensory enhancement becomes narrower between developmental stages 44-46

339   and 48-49, but across the tectal population neurons become preferentially tuned to a

340   wider range of ISIs. We also find that the net gain of multisensory responses decreases

341   between these stages. Across both developmental stages we also consistently observed

342   a speeding up of response latency in paired compared to single responses. Inhibitory

343   blockade in older tadpoles results in a broadening of the integration window, increased

344   gain, and delays in response latency. Furthermore, the balance of excitation to inhibition

345   during crossmodal responses decreases over this developmental period.

346   Taken together our data are consistent with a model in which crossmodal inputs to the

347   tectum recruit local networks of excitatory neurons, resulting in enhanced tectal cell

348   responses. Crossmodal inputs also recruit local inhibition, and the relative enhancement

349   of inhibition, versus excitation, increases over development. This change in relative

350   enhancement sculpts the temporal sensitivity of the tectal network such that individual

351   neurons become more narrowly tuned to specific ISIs, thus enhancing the diversity of

352   responses to different temporal combinations of inputs. Local inhibition also serves to

353   constrain the timing of evoked multisensory responses, by limiting the temporal window

354   in which spikes can occur during a response, and thus shortening response latencies.

355   Our data are consistent with this model in several ways. First we show that

356   pharmacologically blocking inhibition results in a broadening of the window for

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357 multisensory integration in the older tadpoles. During inhibitory blockade, tectal cells

358 are no longer narrowly tuned to a specific ISIs, respond with a greater gain, and

359 preferentially respond to shorter ISIs. All of these characteristics are similar to what we

360 observed in the younger tadpoles. Second, we find that the relative amount of

361 multisensory enhancement (as measured by higher MSIn values) of inhibitory to

362 excitatory responses is greater in the older tadpoles, indicating that they receive more

363 inhibition relative to excitation. There is ample evidence throughout the nervous system

364 that suggests that the inhibition to excitation ratio can sharpen the tuning of sensory

365 neurons to their preferred stimulus (Pouille and Scanziani, 2001; Priebe and Ferster,

366 2008; Shen et al., 2011; Wehr and Zador, 2003). Having more inhibition can limit the

367 range of stimuli that can effectively drive a cell above spike threshold. Our observation

368 that the balance of excitation and inhibition changes developmentally for multisensory

369 responses is consistent with this view.

370 Inhibitory blockade also results in alterations of how crossmodal inputs summate, by

371 causing an increase in the proportion of neurons that show supralinear summation. Our

372 data show that at both developmental stages tectal neurons tend to either mostly show

373 linear or sublinear summation, with a small percentage of cells showing supralinear

374 responses. Thus it is likely that inhibition is also constraining, to a degree, the amount of

375 multisensory integration in the younger tadpoles. Finally, blocking inhibition delays the

376 median onset latency of multisensory responses, which is consistent with a role for

377 feedforward and feedback inhibition in controlling the temporal output of neuronal

378 responses of sensory neurons (Pouille and Scanziani, 2001; Shen et al., 2011). Although

379 inhibitory feedback has been proposed to be a central mechanism for regulating

380 multisensory integration in the superior colliculus (Rowland et al., 2007b), to our

381 knowledge ours is the first experimental evidence directly supporting this model.

382 Recent work by Miller et al. (2015) shows that the temporal tuning of crossmodal

383 responses can be strongly dependent on input size disparity, such that if the stronger

384 modality is presented first, there will be a greater amount of multisensory enhancement

385 than if the weaker modality is presented first. When both modalities are matched in

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386 their response sizes, then the temporal order becomes less important. This 'stronger-

387 first' rule is thought to result from differential recruitment of inhibition. In our findings

388 we did not observe any differences between the order in which visual or hindbrain

389 inputs were activated, likely because we used responses of similar size in our

390 experiments. It will be interesting to see in future studies if the 'stronger first' rule is

391 preserved in the tectum, and whether this rule can be disrupted by inhibitory blockade.

392 Our findings in the optic tectum also share other fundamental similarities and

393 differences with other animal models of multisensory integration. (i) In the superior

394 colliculus of mammals (Meredith et al., 1987) as well as in the optic tectum of owls

395 (Bergan and Knudsen, 2009), there are clear demonstrations of dependency of

396 multisensory integration to the temporal interval between different modality inputs.

397 Furthermore, a developmental narrowing of the temporal window underlying

398 multisensory integration has been shown behaviorally in humans (Hillock et al., 2011)

399 and has been proposed to mediate development of important cognitive processes such

400 as temporal binding. (ii) Second, like in the tadpole tectum, in mammals, collicular cells

401 show also differences in the type of integration, having a combination of supralinear,

402 linear and sublinear responses (Alvarado et al., 2007). (iii) Third, another important

403 aspect that underlies the development of multisensory integration in the mammalian

404 colliculus is the presence of cortical feedback (Jiang et al., 2001; Wallace et al., 1993;

405 Wallace and Stein, 2000). While tadpoles do not have a proper cortex, during the

406 developmental stages used in this study tadpoles do have forebrain feedback into the

407 tectum (Zittlau et al., 1988), as well a local recurrent circuits (Pratt et al., 2008) which

408 can provide the necessary excitatory network activity to drive multisensory integration

409 in individual neurons. The relative contributions of both of these feedback circuits on

410 multisensory integration remains to be determined, yet based on the timing of the

411 tectal neuron spiking, it is likely that it is this recurrent activity that drives the majority

412 of spiking activity, rather than the afferent input itself (Pratt et al., 2008). Since the

413 temporal output characteristics of these recurrent circuits has been shown to be highly

414 plastic in response to experience (Pratt et al., 2008), this raises the possibility that this

15

415 network plasticity can help tune the selectivity of different tectal cells over development

416 to specific combinations of crossmodal ISIs. This type of network plasticity may also help

417 explain observations in the mammalian superior colliculus where temporal statistics of

418 cross-modal stimuli during development and in adulthood shape the temporal tuning of

419 multisensory responses (Xu et al. 2012, Yu et al. 2013). Using the Xenopus tadpole

420 tectum preparation it should be possible to study this type of plasticity at the cellular

421 level.

422 Our findings also show some important differences with the mammalian literature. (i) In

423 the present study we find that pairs of unimodal stimuli result in greater integration

424 over a wider range of intervals than crossmodal pairs. This finding is opposite to what is

425 observed in mammals, where crossmodal inputs are more effective than unimodal ones

426 (Alvarado et al., 2007). There are likely two mechanisms underlying this difference. The

427 first is due to presynaptic interactions that lead to paired pulse facilitation, which has

428 been described for both visual and hindbrain inputs in the tadpole tectum (Deeg et al.,

429 2009). The second may be due to localization of inputs in the dendritic arbor of tectal

430 neurons. Unisensory pairs thus, might be more effective in driving local dendritic

431 depolarization since they are not spatially segregated, and therefore activate non linear

432 response elements such as NMDA receptors or voltage gate Ca++ channels, thus

433 enhancing the amount of integration. (ii) A second key difference is that the

434 developmental decrease in pair-driven enhancement of excitatory conductances and

435 spike output appears to contradict developmental studies in mammals, where the

436 progressive refinement of superior colliculus receptive fields is associated with the

437 capacity for response enhancement, after paired stimulation (Wallace and Stein, 1997).

438 Indeed, an analogous developmental refinement of visually-driven receptive fields,

439 specifically, is found for both excitatory and inhibitory conductances — recorded in the

440 same neuron — in the X. laevis optic tectum over the same developmental stages

441 examined here (Tao and Poo, 2005). One possible explanation for this discrepancy is the

442 use of bipolar stimulating electrodes in our preparation to evoke action potentials

443 throughout an afferent pathway. While this stimulation paradigm provides excellent

16

444 temporal control of stimulus delivery, this stimulation method by its nature has no

445 selectivity for spatiotopic organization. Each stimulus is therefore considered "whole-

446 field," and loses its spatial specificity. Furthermore, in vivo stimuli are likely to also have

447 more temporally complex patterns that may result in somewhat different multisensory

448 interactions. It will be important in future studies to compare the rules underlying

449 multisensory integration using in vivo visual and mechanosensory stimuli.

450 A second possible explanation to this discrepancy between tadpole tectum and

451 mammalian colliculus, is that in tadpoles tectal neurons are innervated by sensory input

452 from very early developmental stages (Gaze et al., 1974), and thus the developmental

453 stages of the circuits in this study may correspond to a much younger stage than what is

454 typically looked at in mammalian studies.

455 Overall, our findings use a reduced preparation to describe, for the first time at the

456 cellular level, the development the temporal properties underlying multisensory

457 integration in the Xenopus tadpole optic tectum. We have used this preparation to

458 uncover some of the basic phenomenology of multisensory integration in this

459 preparation, and have shown that recruitment of recurrent inhibition is a central

460 mechanism in mediating this process. We have also shown that some of the basic

461 features and computations of multisensory integration observed in higher order

462 mammalian preparations are conserved in the developing amphibian tectum, indicating

463 that these are likely to be fundamental and evolutionarily conserved processes. These

464 studies further open the door to follow up studies focusing on the behavioral

465 consequences of these processes, as well as on the role of early sensory experience in

466 determining the temporal properties of multisensory integration. Several

467 neurodevelopmental disorders including autism, are often accompanied with deficits in

468 sensory integration (Baum et al., 2015; Markram and Markram, 2010; Foxe et al. 2015).

469 Thus, these results will not only enhance our general understanding of the development

470 and merging of neural circuits and systems, but they will better allow us to understand

471 how these processes may go awry in neurodevelopmental disorders.

472

17

473

Materials and methods

Experimental Animals

Wild-type Xenopus laevis tadpoles were raised on a 12 hour light/dark cycle at 18°C in 10% Steinberg's solution. In our laboratory, tadpoles reach the Nieuwkoop and Faber (Nieuwkoop and Faber, 1956) developmental stages 44–46 at 7–10 dpf, and stage 48 - 49 at 14 dpf. Xenopus laevis tadpoles between developmental stages 44 and 49 were utilized for this study. The Brown University Institutional Animal Care and Use Committee (IACUC) approved all handling of animals in accordance with National Institutes of Health (NIH) guidelines.

Whole-Brain Preparation

The whole-brain preparation is as follows, after Wu et al. (1996): animals were first anesthetized in 0.01% tricaine methane sulfonate (MS-222) in 10% Steinberg's, the dorsal surface of skin was then removed to expose the brain, the dorsal midline was cut at all levels from base of spinal cord through the olfactory bulbs, and the brain dissected out. The preparation was transferred to a recording chamber with room temperature HEPES-buffered extracellular saline (containing: 115mM NaCl, 4mM KCl, 3mM $CaCl_2$, 3mM $MgCl_2$, 5mM HEPES, and 10mM glucose; pH 7.2, 255mOsm) and positioned on top of a block of Sylgard, with the exposed walls of the ventricle facing upwards. Shortened insect pins were inserted through the caudal extent of hindbrain and through one or both olfactory bulbs. For stimulation of retinal ganglion cell axons, a bipolar stimulating electrode consisting of two adjacent 25-μm platinum leads (CE2C75; FHC, Bowdoin, ME) was placed at the optic chiasm (OC), and for stimulation of mechanosensory projections, a second bipolar stimulating electrode was placed in the rostral hindbrain (HB) contralateral to the recording site (see Figure 1). Individual neurons in the optic tectum were visualized through a light microscope with a 60× water-immersion objective, in combination with an infrared CCD camera. To achieve access to the tectal cells at the recording site the jagged tip of a broken glass micropipette was used to lift away the periventricular membrane, with the aid of a micromanipulator. These recording sites

502 were selected consistently from within in the middle third of the optic tectum's rostral-

503 caudal dimension, to avoid introducing variability in the maturational state of neurons

504 studied at a given stage of tadpole development, given that the tectal circuit matures

505 along a rostral-to-caudal gradient in individual animals (Pratt et al., 2008; Wu et al.,

506 1996).

507

508 *Electrophysiology*

509 Glass micropipettes were pulled for tip resistances of 8–12 MΩ. For whole-cell recording

510 micropipettes were filled with filtered Cs^+-methane sulfonate/TEA intracellular saline

511 (containing: 80mM Cs^+-methane sulfonate, 20mM TEA, 5mM $MgCl_2$, 20mM HEPES,

512 10mM EGTA, 2mM ATP, and 0.3mM GTP; pH 7.2, 255mOsm), and for loose cell-attached

513 (LCA) recording of action potentials, the same micropipettes were filled with filtered

514 extracellular saline. In experiments where blocking $GABA_A$-receptor mediated inhibition

515 was required, 0.1mM picrotoxin was added to the extracellular solution.

516 Electrophysiological signals were detected with an Axopatch 200B amplifier, digitized at

517 10kHz by a Digidata 1322A analog-to-digital converter, and formatted for recording by

518 pClamp 9 acquisition software. Leak subtraction was performed on-line, in real-time by

519 the acquisition software. In our recording conditions the junction potential is predicted

520 to be 12mV, but was uncorrected in the recorded traces. To detect changes in access

521 resistance over the course of a recording, a 5mV depolarizing square wave was applied

522 at the start of each trace. In all experiments, only cells demonstrating responses to both

523 optic chiasm and hindbrain stimulation were chosen for recording.

524

525 Loose-cell attached recordings were used to measure action potentials without breaking

526 through the cell membrane and without electrical access, and were defined as having

527 seal resistances in the 40–200MΩ range. The pipette tip was dirtied prior to cell contact

528 to prevent formation of a tight seal. Action potentials were detected off-line by

529 importing the digitized traces into the AxoGraphX analysis environment and by using an

530 amplitude threshold to identify events and determine post-stimulus onset times.

531

532   Experiments to examine the temporal characteristics of excitatory and inhibitory

533   synaptic conductances were performed with whole cell recordings. Voltage-clamp mode

534   was used to isolate synaptic conductances mediated by excitatory neurotransmitter

535   receptors and those mediated by inhibitory neurotransmitter receptors. By using the

536   voltage-clamp to hold the cell's membrane potential at the reversal potential of a given

537   synaptic current, it is possible to eliminate the driving force on the ions mediating that

538   current and thus "zero" the amplitude of that particular type of synaptic event. Previous

539   work in the laboratory has shown that the reversal potential of excitatory AMPA and

540   NMDA receptor-mediated currents is +5mV and the reversal potential of synaptic

541   currents mediated by inhibitory $GABA_A$ receptors is –45mV (Bell et al., 2011).

542

543   *Stimulus Properties*

544   Electrical stimulation was initiated automatically by the acquisition software. At pre-

545   specified time points, ISO-Flex stimulus isolators (A.M.P.I., Jerusalem, Israel) were

546   activated for 0.2msec by an ON-OFF command signal from the digitizer. The output of

547   each stimulus isolator (one for each stimulus electrode) was manually set, based on the

548   responsiveness and dynamic range of each cell, to deliver between 10µA and 800µA

549   across the poles of it's bipolar electrode, for the duration of the command signal.

550   Stimulus strength was set to ensure that spike output from both inputs was closely

551   matched.

552

553   *Data Analysis*

554   All analyses were performed offline, using AxoGraphX software and the MATLAB

555   programming environment. Prism software (GraphPad) was used for curve fitting and

556   statistical tests. Sample sizes were based on power analyses and known variability from

557   prior work in our experimental system. Statistical tests used, P and N values are

558   indicated within the results. Non-parametric tests were used when appropriate. No

559   outliers were removed from the data, and all multiple comparisons were corrected

560   using a Bonferroni correction. Determination of the Multisensory Index was performed

561   as described by Meredith and Stein (Meredith and Stein, 1983), using the equation:

562   MSIn = (CM – SM)/SM, where SM is the average number of action potentials evoked by a

563   single-stimulus presentation (Single Modality), CM is the average number of action

564   potentials evoked by the paired-stimulus presentation (Combined Modality), and MSIn

565   is the Multisensory Index. This measure indicates the difference (or "gain") in a cell's

566   response (positive or negative) resulting from the addition of a second stimulus,

567   corrected for the cell's maximal single-stimulus response. Because MSIn corrects for

568   each neuron's maximal output in the single-stimulus condition, it is a normalized

569   measure that can be compared across neurons that may vary in overall spike output. In

570   this study MSIs were calculated separately for both sensory modalities and

571   combinations of temporal sequences. Determination of the predicted neuronal

572   response to paired stimuli, both unimodal and crossmodal, was performed in the

573   manner of Stanford and colleagues (Stanford et al., 2005). For each cell, responses after

574   each trial of the individual (baseline) stimulus presentations were collected and, as

575   appropriate for the type of paired responses being predicted, all possible unimodal trial-

576   by-trial combinations or all possible crossmodal trial-by-trial combinations were

577   determined. For each such combination of trials, the sum of spike counts recorded in

578   each was calculated. Thus, with 33 single-stimulus trials delivered through each

579   modality, in each cell, $33 \times 33 = 1089$ possible sums exist. In the actual experiments,

580   however, at each ISI 4 trials of paired stimuli were presented, and the mean response

581   over these trials was determined. To mirror these procedures in the predictive analysis,

582   for each cell and for each type of stimulus pair, 4 of the possible sums were randomly

583   selected (with replacement) and their mean taken. This randomly sampling and

584   averaging was repeated 10,000 times for each pair type, to create an approximately

585   normal distribution of predicted mean sums. In each cell, Z-score comparisons of the

586   actual mean response, at each ISI of each pair type, were then performed against this

587   distribution.

588

589

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735 **Figure Legends**

736 **Figure 1. Multisensory integration in tectal neurons is dependent on interstimulus**

737 **interval (ISI).**

738 **(A)** Diagram showing placement of the "V" and "HB" bipolar electrodes relative to tectal

739 afferents and recording location. **(B)** Raw traces of cell-attached spikes generated during

740 single and paired stimulation. Letters indicate times of stimulus presentation and

741 modality of stimulus. **(C)** Example raster plots from two cells, one from a stages 44–46

742 animal (*left*) and one from a stages 48–49 animal (*right*) as a function of ISI. **(D)** Grouped

743 data from both developmental groups. In a given cell, data are averaged over trials at

744 each ISI, to determine the MSIn ratio. Plotted here is the population means of these

745 trial-averaged MSIn ratios, at the ISI's tested. Error bars show +/– S.E.M. MSIn =

746 (combined response – single response) / single response.

747 **Figure 2. Developmental changes in temporal tuning and gain of MSI**

748 **(A, B)** Histogram bars show the ISI that for each cell, exhibited maximal MSIn values.

749 Notice that values cluster at shorter ISIs in the younger tadpoles. Older tadpoles are

750 tuned to a broader range of ISIs. **(C)** Maximum MSIn ratios are compared across

751 developmental stages, and show decrease in MSIn gain over development. Error bars

752 indicate +/– S.E.M. **(D)** The identity of the ISI's responsible for the maximal response

753 (and thus the maximum MSIn ratio) in each developmental group are compared. Error

754 bars show interquartile range. ∗ : $P < 0.05$. **(E)** MSIn-versus-ISI tuning curves from

755 crossmodal pairs were aligned at their peak values, and then averaged across cells. Solid

756   lines connect the population means. Shaded areas demarcate +/– S.E.M. Stage 48-49

757   cells are more narrowly tuned around their preferred ISI. MSIn = (combined response –

758   single response) / single response.

759   **Figure 3. Interaction between different modalities is mostly linear or sublinear.**

760   (A) Traces show examples of two possible outcomes of interactions between different

761   modality responses. The upper traces show an example of sublinear summation, where

762   the combined response evoked fewer spikes than the sum of the individual responses.

763   The bottom example shows supralinear summation, where the combined response

764   evokes more spikes than the sum of the individual responses. **(B)** For each cell, the

765   maximum raw spike counts after paired stimulation (combined response) is plotted

766   against the spike count predicted by the sum of adding individual modality responses.

767   Dashed line represents the line of unity. Notice that most points cluster to the right of

768   the dashed line, indicating a sublinear interaction. (**C**) Cumulative Frequency

769   Distributions of Z-score values are plotted for the comparison between predicted and

770   actual number of action potentials recorded after paired stimulation, in each cell.

771   Vertical dashed lines indicate Z = +/–1.97, the point at which actual responses are +/–2

772   S.D.'s away from the respective predicted response. For greater detail on generation of

773   the predicted responses and calculation of Z-scores, see *Materials and methods*. (D)

774   Distribution of cells by interaction type shows few developmental differences.

775

776   **Figure 4. Developmental changes in MSI do not simply reflect changes in network**

777   **dynamics.**

778   (**A**) Grouped data showing enhancement of unisensory pairs at both developmental

779   groups. In a given cell, data are averaged over multiple trials at each ISI, to determine

780   the PPR. Plotted here is the population means of these trial-averaged PPR ratios, at the

781   ISI's tested. Error bars show +/– S.E.M. (**B**) Histogram bars show the ISI that for each cell,

782   exhibited maximal PPR values. Notice that values tend to cluster at shorter ISIs in both

783   groups. **(C)** Maximum PPR ratios are compared across developmental stages, and show

784   no change in PPR gain over development. Error bars indicate +/– S.E.M. **(D)** The identity

785   of the ISI's at which maximal PPR occurred, separated by developmental group. Error

786   bars show interquartile range. n. s. = not significant. **(E)** PPR-versus-ISI tuning curves

787   from unimodal pairs were aligned at their peak values, and then averaged across cells.

788   Since pairs were unisensory, only one direction is shown. Solid lines connect the

789   population means. Shaded areas demarcate +/– S.E.M. Tuning for unisensory pairs does

790   not change over development. PPR = (paired response – single response) / single

791   response.

792   **Figure 5. Inhibitory blockade broadens tuning window and enhances gain of MSI.**

793   **(A)** Grouped data from stage 48-9 tadpoles in control condition and in the presence of

794   GABAA receptor blocker PTX. In a given cell, data are averaged over trials at each ISI, to

795   determine the MSI ratio. Plotted here are the population means of these trial-averaged

796   MSIn ratios, at each ISI tested. Error bars show +/– S.E.M. **(B)** Histogram bars show the

797   ISI at which each cell exhibited maximal MSIn values in control and PTX groups. Notice

798   that PTX shifts the preferred ISI values to shorter intervals. **(C)** Maximum MSIn ratios are

799   compared across control and PTX conditions, and show increase in MSI gain with

800   inhibitory blockade. Error bars indicate +/– S.E.M. **(D)** The identity of the ISI's

801   responsible for the maximal response (and thus the maximum MSI ratio) in control and

802   PTX groups are compared. Error bars show interquartile range. ∗ : $P < 0.05$. **(E)** MSI-

803   versus-ISI tuning curves from crossmodal pairs were aligned at their peak values, and

804   then averaged across cells. Solid lines connect the population means. Shaded areas

805   demarcate +/– S.E.M. PTX treated cells are more broadly tuned around their preferred

806   ISI. **(F)** For each cell, the maximum raw spike counts after paired stimulation (combined

807   response) is plotted against the spike count predicted by the linear sum of individual

808   modality responses. Dashed line represents the line of unity. Notice that in PTX treated

809   group most points cluster to the left of the dashed line. (**C**) Cumulative Frequency

810   Distributions of *Z*-score values are plotted for the comparison between predicted and

811   actual number of action potentials recorded after paired stimulation, in each cell.

812   Vertical dashed lines indicate $Z = +/-1.97$, the point at which actual responses are $+/-2$

813   S.D.'s away from the respective predicted response. For greater detail on generation of

814   the predicted responses and calculation of Z-scores, see *Materials and methods*. Z-

815   scores in the PTX treated group are significantly shifted toward the right (p<0.0001,

816   Kolmogorov-Smirnov test).

817   **Figure 6. Crossmodal responses have faster onset latencies than single unimodal**

818   **responses across groups, but inhibitory blockade slows response onset times.**

819   (**A,B,C**) Plot of response onset times for single responses and crossmodal responses at

820   multiple ISIs for all experimental groups. For each paired ISI, as well as the appropriate

821   control stimulus (*left axis*), the .25, .50 (median), and .75 quartiles of post-stimulus

822   times for all first spikes are plotted on the bottom axis. Dashed lines connect 0.25 and

823   0.75 quartile values, and solid lines connect .50 quartile (median) values. (**D**)

824   Comparison of response onset times of paired vs. unpaired responses shows that across

825   conditions, cross modal responses occur faster than single modality responses. (**E**)

826   Comparison between paired response onset times across groups shows that while there

827   is no significant speeding up of the response over development, picrotoxin delays onset

828   times. Bars indicate median values, error bars show the IQR. *: $P < 0.05$

829   **Figure 7. Multisensory responses have a greater inhibitory to excitatory ratio in older**

830   **animals.**

831   (**A**) Sample excitatory (red) and inhibitory (blue) synaptic conductances recorded at

832   different ISIs. S1 and S2 show timing of the individual modality responses (V and HB,

833   respectively in this example). (**B**) Plot of crossmodal enhancement of excitatory synaptic

834   conductances as a function of ISI at both developmental stages. Notice that excitation

835   becomes relatively less enhanced in the older tadpoles. (**C**) Plot of crossmodal

836   enhancement of inhibitory synaptic conductances as a function of ISI at both

837   developmental stages. Notice that there is no developmental change in the amount of

838   inhibitory enhancement. P value determined from 2-way ANOVA. (**D**) Plot of maximum

839   excitatory and inhibitory enhancement for each cell. Dotted line represents linear fit for

840   each developmental group. P value determined by an F-Test and compares both fits.

841   Notice that older cells exhibit relatively more inhibitory than excitatory enhancement.

842

843   Table 1.

Table 1

Crossmodal Order	Stage 44-46 (n=13)			Stage 48-49 (n=20)		
	Mean MSIn ± SEM	P	Summary	Mean MSIn ± SEM	P	Summary
VH	0.98 ± 0.57	0.605	n.s	0.50 ± 0.17 (n=20)	0.628	n.s.
HV	1.31 ± 0.26			0.60 ± 0.12		
	Median ISI (ms) ± IQR			Median ISI (ms) ± IQR		
VH	200 ± 250	0.964	n.s.	350 ± 675	0.731	n.s
HV	100 ± 350			500 ± 875		

844

845   **Table 1: Comparisons between presentation order for cross-modal stimuli do not reveal consistent**

846   **differences in MSI ratios, in either developmental group.**

847   Top: Within both developmental groups, maximum MSIn ratios are compared between the two cross-

848   modal sequences. n.s.: not significant (unpaired t-test). Bottom: The identity of the ISI's responsible for

849   the maximal response is compared between the two cross-modal, within each developmental group. * : P

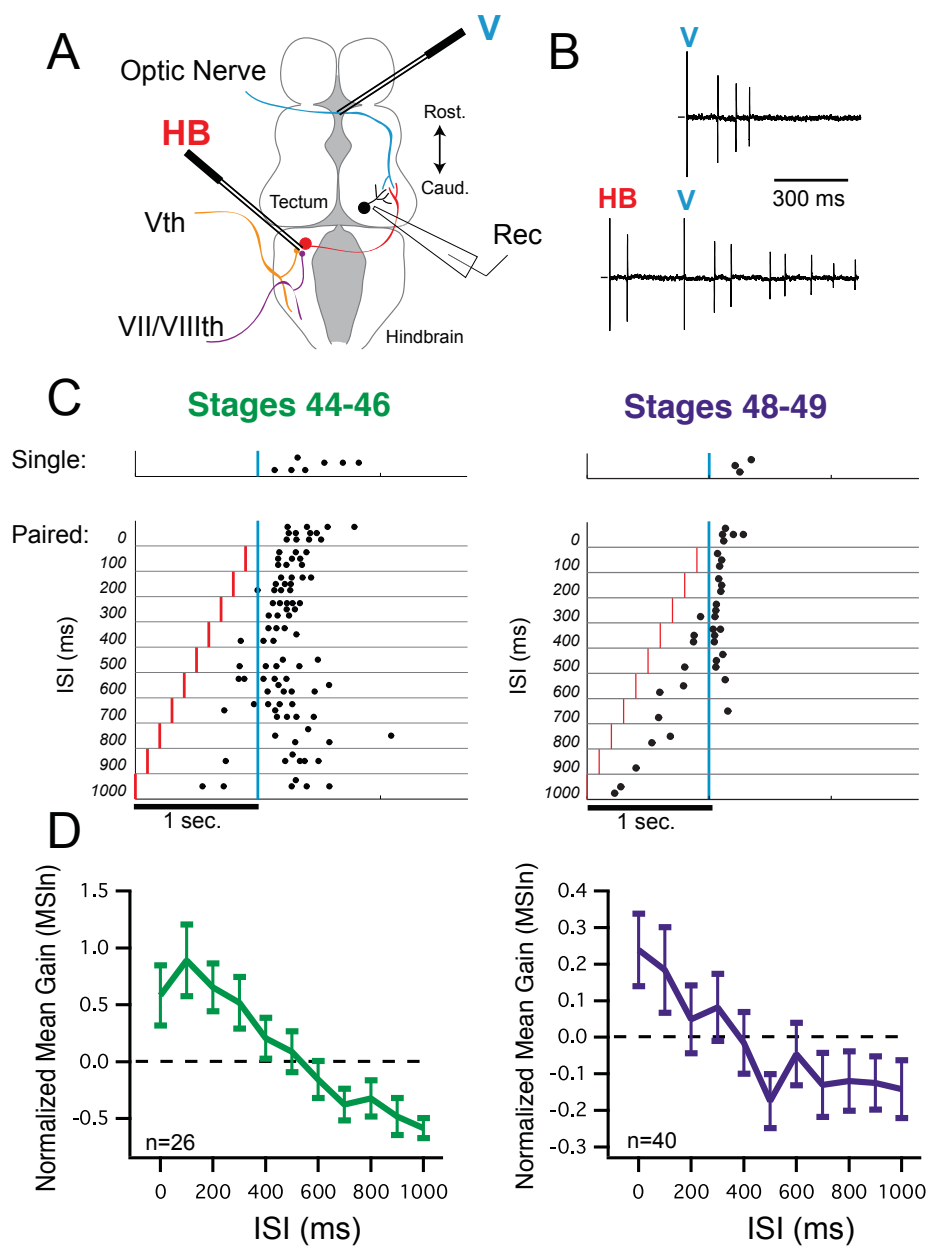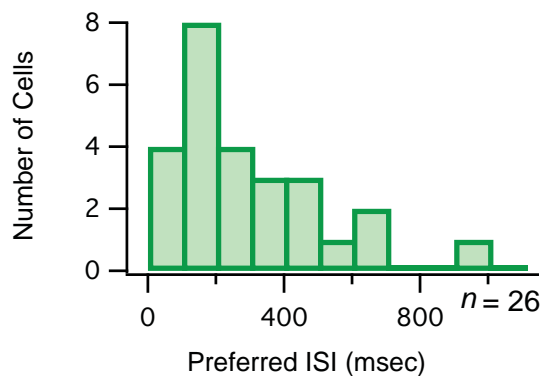850   < 0.05 (Mann-Whitney rank-sum test); n.s.: not significant (Mann-Whitney rank-sum test). V = visual, H =

851   hindbrain

852

A

Optic Nerve
V
HB
Rost.
Caud.
Vth
Tectum
Rec
VII/VIIIth
Hindbrain

B

V
HB V
300 ms

C

Stages 44-46   **Stages 48-49**

Single:

Paired:

ISI (ms)

0
100
200
300
400
500
600
700
800
900
1000

1 sec.   1 sec.

D

Normalized Mean Gain (MSIn)

1.5
1.0
0.5
0.0
-0.5

n=26

0   200 400 600 800 1000
ISI (ms)

0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3

n=40

0   200 400 600 800 1000
ISI (ms)

Fig 1

A **Stages 44-46**

B **Stages 48-49**

C

D

E

A

V H

Sublinear

H

V

V+H

Supralinear

H

V

V+H

B

Actual Paired Spike Count, Max

Stage 44-46 (*n* = 26)
Stage 48-49 (n=40)

Predicted Paired Spike Count

C

Fraction of Total

Z-score

sublinear   supralinear

D

Sublinear
Linear
Supralinear

Stage 44-46

Total=24

Sublinear
Linear
Supralinear

Stage 48-49

Total=39

Fig. 3

A

Normalized Mean Gain (MSI)

HV   Control

PTX

Picrotoxin
No Drug

ISI (msec)

B

Number of Cells

Preferred ISI (msec)

C

Max MSI (each cell)

*

Picrotoxin No Drug

D

ISI of Max MSI (msec)

*

Picrotoxin No Drug

E

Stage 48-49 No Drug
Stage 48-49 Picrotoxin

MSI (CR-SR)/(SR)

Temporal Offset from Max ISI

F

Actual Paired Spike Count, Max

PTX (*n* = 22)
No Drug (*n* = 40)

Predicted Paired Spike Count

G

Fraction of Total

p < 0.0001

Z-score

Fig. 5

A **Stages 44-46**

Single

Cross Modal ISI (ms)

0
100
200
300
400
500
600
700
800
900
1000

Time after stimulus (ms)
0 100 200 300 400 500 600 700 800 900

n = 25

B **Stages 48-49**

Single

Cross Modal ISI (ms)

0
100
200
300
400
500
600
700
800
900
1000

Time after stimulus (ms)
0 100 200 300 400 500 600 700 800 900

n = 39

C **Picrotoxin**

Single

Cross Modal ISI (ms)

0
100
200
300
400
500
600
700
800
900
1000

Time after stimulus (ms)
0 100 200 300 400 500 600 700 800 900

n = 22

D

Median Response Onset (sec)

0.4
0.3
0.2
0.1

*   *   *

S   CM   S   CM   S   CM
St. 44-6   St. 48-9   Picrotoxin

E

Median CM Response Onset (sec)

0.20
0.15
0.10
0.05
0.00

*

St. 44-6   St. 48-9   Picrotoxin

A

Excitatory Conductance ($V_m = -45$mV)
(traces inverted)
Inhibitory Conductance ($V_m = +5$mV)

S_1 — — — → S_2

400 pS
200 ms

B

Excitatory Conductances

% Enhancement (CR/SR)
Normalized Mean Gain (MSI)

Stage 44-46 (n=22)
Stage 48-49 (n=25)

p=0.037

ISI (ms)

C

Inhibitory Conductances

% Enhancement (CR/SR)
Normalized Mean Gain (MSI)

ISI (milliseconds)
ISI (ms)

D

Max Excitatory Enh.

p=0.023

Max Inhibitory Enh.