1 **TITLE**

2 V3 Interneurons Regulate Locomotor Vigor by Recruitment of Spinal Motor
3 Neurons During Fictive Swimming in Larval Zebrafish

4

5 **ABBREVIATED TITLE**

6 V3 Interneurons Regulate Locomotor Vigor

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41 **ABSTRACT**

42 Survival for vertebrate animals is dependent on the ability to successfully
 43 find food, locate a mate, and avoid predation. Each of these behaviors requires
 44 fine motor control, which is set by a combination of kinematic properties. For
 45 example, the frequency and amplitude (vigor; strength) of motor output combine
 46 to determine features of locomotion such as distance traveled and speed.
 47 Although there is a good understanding of how different populations of excitatory
 48 spinal interneurons establish locomotor frequency, there is not a mechanistic
 49 understanding for how locomotor vigor is established. Recent evidence indicates
 50 that locomotor vigor is regulated in part by subsets of identified excitatory spinal
 51 interneurons (INs), such as the V2a neuronal population in adult zebrafish. Here
 52 we provide evidence that the majority of V3 interneurons (V3-INs), which are a
 53 developmentally and genetically defined population of ventromedial
 54 glutamatergic spinal neurons, are active during fictive swimming. Further, that
 55 targeted ablation of V3-INs reduces the proportion of active MNs during fictive
 56 swimming, but ablation does not affect the locomotor frequencies produced.
 57 These data are consistent with a role of V3-INs in providing excitatory drive to
 58 spinal motor neurons during swimming in larval zebrafish, which suggests that
 59 locomotor vigor (but not locomotor frequency) may be regulated, in part, by V3-
 60 INs.

61 **SIGNIFICANCE STATEMENT**

62 Currently, there is a fundamental lack of knowledge about the cellular and
 63 spinal network properties that produce locomotor vigor in vertebrates. Here we
 64 show, directly for the first time, that V3 interneurons in zebrafish larvae are active
 65 during *in vivo* fictive locomotion, and that targeted ablation of the spinal V3
 66 interneuron population reduces the probability of motoneuron firing during fictive
 67 swimming. In contrast to V2a interneurons, ablation of V3 interneurons does not
 68 affect locomotor frequency, the fictive neural correlate of speed, which clarifies
 69 their role in motor control rather than rhythm generation. Thus, we propose that
 70 the V3 interneuron subpopulation is a source of excitation in the vertebrate
 71 locomotor neural circuitry that regulates locomotor vigor independently of speed.

72 INTRODUCTION

73 Efficient locomotion is set by a combination of kinematic properties,
 74 including the frequency and vigor of rhythmic motor output. These properties
 75 combine to determine various locomotor features, such as distance traveled and
 76 speed (Webb, 1975; Jayne and Lauder, 1995; Donley and Dickson, 2000; Tytell,
 77 2004). Locomotor frequency (e.g. limb or tail-beat rhythmic activity) is a well-
 78 studied kinematic variable, which has been shown to increase linearly with speed
 79 (Bainbridge, 1958; Hunter and Zweifel, 1971; Webb et al., 1984; Müller and van
 80 Leeuwen, 2004). Locomotor vigor is defined as the strength of the motor output
 81 and measured as the amplitude of limb displacement in limbed animals during
 82 locomotor events (walking, trotting, galloping, hopping (Heglund et al., 1974)) or
 83 the amplitude of tail-bend in non-limbed animals (fishes) during undulatory
 84 swimming (Wardle et al., 1995; Videler et al., 1999). There is a fundamental
 85 understanding of how identified subsets of excitatory spinal interneurons help to
 86 establish the frequency of motor output (Crone et al., 2009; Zhong et al., 2011;
 87 Ausborn et al., 2012; Eklöf-Ljunggren et al., 2012; Kimura et al., 2013; Ampatzis
 88 et al., 2014; Ljunggren et al., 2014; Menelaou et al., 2014; Bellardita and Kiehn,
 89 2015). A critical gap in our knowledge, however, is a mechanistic understanding
 90 of the cellular and network properties that regulate locomotor vigor in vertebrate
 91 animals.

92 Across vertebrate species from fish to mammals, the neural circuitry that
 93 controls locomotion is located in the ventromedial spinal cord (Grillner and
 94 Jessell, 2009; Kiehn, 2011). In vertebrates, developmental gene expression

95 specifies at least five ventral classes of neuronal progenitor cells. These
 96 progenitors give rise to motor neurons (MNs) and four classes of ventral
 97 interneurons, referred to as V0 to V3 neurons (Ericson et al., 1997; Pierani et al.,
 98 1999). Over the past 10 years, a subset of identified excitatory spinal
 99 interneurons (V2a-INs) has been shown to control the speed of locomotion in a
 100 variety of vertebrates (Zhong et al., 2011; Ausborn et al., 2012; Ampatzis et al.,
 101 2014; Gosgnach et al., 2017). A recent study indicated that V2a-INs in adult
 102 zebrafish were involved in both regulating locomotor speed and vigor (Song et
 103 al., 2018). To date, however, a subpopulation of spinal interneurons that
 104 selectively regulates locomotor vigor has not been identified. In the mammalian
 105 spinal cord, a genetically defined subset of pre-motor glutamatergic neurons,
 106 called V3 interneurons (V3-INs), contribute to limb coordination, motor burst
 107 duration, and muscle spasms, but do not have a clear role in locomotor pattern
 108 generation (Chopek et al., 2018; Danner et al., 2019; Lin et al., 2019).

109 This work shows, directly for the first time, that V3-INs in zebrafish larvae
 110 are active during *in vivo* fictive locomotion and, in part, contribute exclusively to
 111 locomotor vigor and not to locomotor frequency, which clarifies their role in fine
 112 motor control rather than rhythm generation. This is conceptually important
 113 because our results support that the V3-IN subpopulation regulates locomotor
 114 vigor independently of speed. This difference is a major functional distinction
 115 between the V3-INs and V2a-INs since the latter regulate both speed and vigor
 116 (Zhong et al., 2011; Ausborn et al., 2012; Ampatzis et al., 2014; Gosgnach et al.,
 117 2017; Song et al., 2018).

118

119 MATERIALS AND METHODS

120 *Fish Lines and Maintenance*

121 All procedures were approved by the Institutional Animal Care and Use
122 Committee of the University of Minnesota Twin Cities. Wild type (Segrest Farms,
123 Gibsonton, FL) and transgenic adult zebrafish (*Danio rerio*) were maintained in
124 the University of Minnesota Zebrafish Core Facility. The transgenic lines used in
125 these experiments were: *Tg(nkx2.2a:mEGFP)^{vu17}* (Kirby et al., 2006),
126 *Tg(vglut2a:DsRed)^{nns9}* (Miyasaka et al., 2009), *Tg(vglut2a:Gal4ff)^{nns20}* (Satou et
127 al., 2013), *Tg(UAS:GCaMP6s)^{nk13a}* (Muto et al., 2017), *Tg(UAS:Kaede)^{s1999t}*
128 (Scott et al., 2007), and *parga^{mn2Et}* (Balciunas et al., 2004). In-crossed and out-
129 crossed transgenic larvae at 4 – 6 days post fertilization (dpf) were used in these
130 experiments. Larval zebrafish were maintained in petri dishes filled with embryo
131 water (60 µg/ml Instant Ocean® salt mix, Cincinnati, OH) and 0.00005%
132 Methylene Blue in a 28.5°C incubator with a 14:10 light:dark cycle.

133

134 *Kaede Photoconversion*

135 *In vivo* photoconversion of genetically encoded Kaede was performed in
136 *Tg(vglut2a:Gal4ff)^{nns20};Tg(UAS:Kaede)^{s1999t}* larvae using a fixed-objective, upright
137 microscope (Olympus FV-1000, Center Valley, PA). Larvae were anesthetized in
138 zebrafish ringers solution (Wiggin et al., 2012, 2014) with 0.02% Tricaine-S (MS-
139 222; Western Chemical, Ferndale, WA), embedded laterally in a fluorodish
140 (World Precision Instruments, Sarasota, FL) filled with 1.5% low-melting point

141 agarose in zebrafish ringers solution and covered with additional saline
 142 containing anesthetic. Photoconversion of Kaede was performed using the
 143 bleach function of the FV-1000 to direct a 405 μm laser spot onto a Kaede-
 144 expressing cell targeted for conversion (5% laser intensity, 7 s, single pulse). The
 145 embedding procedure resulted in small variations in the orientation of the spinal
 146 cord such that the larvae were not precisely level and square with respect to the
 147 imaging plane. Following confocal stack acquisition, Fiji (Schindelin et al., 2012)
 148 was used to rotate the image in order to correct these errors.

149

150 *V3-IN Soma Distribution Analysis*

151 Quantification of the distribution of V3-IN cell bodies was performed using
 152 Fiji and custom Matlab code (Mathworks, Natick, MA). Confocal microscopy was
 153 used to collect overlapping stacks of images documenting the expression of
 154 *Tg(vglut2a:DsRed)^{nns9}* and transmitted light morphology. Images were acquired
 155 at high magnification (40X / NA 0.8 objective) and a z-axis resolution of 1.3 μm .
 156 The fields of view in each stack overlapped with their neighbors by at least 10%
 157 of the width to permit accurate stitching, and included the full width of the spinal
 158 cord and dorsal root ganglia (DRG) in the field of view. The collection of stacks
 159 was stitched into a single 3-D image of the caudal hindbrain and spinal cord
 160 (Preibisch et al., 2009). The Fiji Cell Counter tool was used to identify the
 161 coordinates of all V3 neurons in the rostral 2 mm of the larval spinal cord (out of
 162 a total length of ~3 mm), the coordinates of the DRG neuron clusters, and the
 163 position of the dorsal and ventral edges of the spinal cord. The markers tracing

164 the dorsal and ventral edges of the spinal cord were used to calculate local
 165 tangent lines to the dorsal and ventral edges of the spinal cord at the
 166 rostrocaudal position of each V3-IN. The equation of a line perpendicular to the
 167 ventral tangent and intersecting the V3-IN was calculated, referred to as the “V3
 168 plumb line.” The rostrocaudal distance of each V3 neuron was calculated as the
 169 distance along the ventral surface of the spinal cord between the spinal
 170 cord/hindbrain junction (Segment 2/3 boundary) and the V3 plumb line
 171 intersection with the ventral surface. The dorsoventral height of the spinal cord at
 172 the location of each V3-IN was calculated as the distance between the
 173 intersections of the V3 plumb line with the dorsal surface and the ventral surface,
 174 respectively. The distance of the V3-IN from the ventral surface of the spinal cord
 175 was also measured along the plumb line. The z-axis position of DRG neurons on
 176 each side of the spinal cord were used as boundary points of the lateral edges of
 177 the spinal cord, and a linear fit to the DRG neurons on each side of the animal
 178 was used to calculate the edges of the spinal cord at each rostrocaudal position
 179 in the imaging area. The midline slice and spinal cord width were calculated at
 180 the rostrocaudal position of each V3-IN, along with the distance from midline of
 181 the V3-IN.

182

183 *Whole-mount Immunohistochemistry*

184 Immunohistochemical labeling was conducted using established
 185 procedures (Montgomery et al., 2016). Five dpf *parga*^{mn2Et} larvae were sacrificed
 186 with 0.2% Tricaine-S and fixed overnight in 4% paraformaldehyde at 4°C. Whole-

187 mount larvae were permeabilized with Proteinase K, post-fixed, and blocked with
188 0.2% BSA, 10% normal donkey serum, 0.5% Triton X-100 in PBS. Goat
189 polyclonal anti-choline acetyltransferase (ChAT; 1:50; AB144P) was added to the
190 blocking solution and incubated overnight at 4°C. Fixed larvae were incubated
191 with Alexa Fluor 633 donkey anti-goat secondary antibody (1:500; A-21082,
192 Thermo Fisher Scientific Inc., Waltham, MA) in secondary blocking solution
193 (0.2% BSA, 1% normal donkey serum, 0.5% Triton X-100 in PBS) for three
194 nights at 4°C.

195 Anti-ChAT labeled *parga*^{mn2Et} larvae were embedded laterally in low
196 melting-point agarose. An Olympus Fluoview FV1000 confocal microscope was
197 used to collect images through a 60x objective centered on body segment 15.
198 Confocal stacks contained the entire dorsoventral extent of the spinal cord and
199 spanned approximately three body segments (285 µm). Images were processed
200 in Fiji (open source image processing package) and labeled cells from a single
201 side of the spinal cord (approximately three hemisegments) were quantified using
202 the Cell Counter plugin. Photoshop CS5 (Adobe Systems, San Jose, CA) was
203 used to adjust brightness and contrast across entire z-projected images.

204

205 *In Vivo Rhod-2 AM Dye Loading of Motor Neurons*

206 For *in vivo* characterization of MNs in *parga*^{mn2Et} larvae, a 0.25% Rhod-2
207 AM (Life Technologies, Grand Island, NY) stock was made by dissolving 50 µg of
208 dye in 20 µL Pluronic F-127:DMSO (1:4; Life Technologies, Grand Island, NY).
209 Next, the skin was removed from the midbody region and a 1:50 dilution of the

210 Rhod-2 AM stock was bath applied to the exposed muscles to permit
211 sequestration of indicator dye by MNs via the peripheral motor axons. Sensory
212 neurons were not strongly loaded by this procedure. Following one-hour
213 incubation, the Rhod-2 AM solution was thoroughly washed-out using zebrafish
214 ringers solution. Confocal images were collected and cells were quantified as
215 described for whole-mount immunohistochemistry (above).

216

217 *Peripheral Nerve Recordings*

218 Peripheral nerve (PN) recordings were performed and analyzed as
219 previously described (Masino and Fetcho, 2005). In brief, the larvae were
220 anesthetized, the skin was carefully removed from the recording region, and
221 larvae were paralyzed with of 0.1 mM α -bungarotoxin (Tocris, Ellisville, MO) to
222 prevent muscle contractions. The anesthetic was removed to allow the larvae to
223 perform fictive behavior, and a suction electrode (tip diameter: 9-15 μ m) was
224 placed over the intermyotomal cleft to record MN axons. Fictive motor bursts
225 were identified using custom Matlab code (Wiggin et al., 2012).

226

227 *Calcium Imaging and Analysis*

228 For calcium imaging of V3-INs,
229 *Tg(vglut2a:Gal4ff)^{nns20};Tg(UAS:GCaMP6s)^{nk13a}* zebrafish larvae were used. For
230 calcium imaging of MNs, Calcium Green-1 AM (Life Technologies, Grand Island,
231 NY) dye was loaded into MNs using the protocol for Rhod-2 AM dye loading
232 described above. Larvae were transferred to a fixed-stage Olympus BX51 WI

233 upright microscope with either a 20x / NA 0.5 or a 40x / NA 0.8 water dipping
 234 objective lens. A field of view containing *GCaMP6s*-expressing V3-INs in
 235 transgenic larvae or Calcium Green-1 AM-loaded MNs in wild type larvae was
 236 selected for calcium imaging and a simultaneous peripheral nerve (PN) recording
 237 was acquired. Calcium indicator fluorescence was excited with an E-Cite Series
 238 120 Q lamp (Excelitas Technologies, Waltham, MA) and acquired at 20 Hz with a
 239 Retiga EXi camera and QCapture Pro 6.0 software (QImaging, Surry, BC). Data
 240 were acquired during spontaneous fictive swimming in 10-60 s durations for both
 241 V3-IN and MN trials. Multiple trials (typically 4) were acquired for each field of
 242 view, with at least five min between trials. Movement artifacts during data
 243 acquisition were corrected in Fiji by registering frames from all trials to a common
 244 reference image (Preibisch et al., 2009). Optical and electrophysiological data
 245 acquisition were synchronized by using the sync output of the stimulator to
 246 trigger acquisition of the PN recording.

247 For all calcium imaging experiments, raw fluorescence values were
 248 determined by drawing a region of interest around each identified V3-IN or MN
 249 soma and using the Plot Z-axis Profile command in Fiji. Fluorescence from a
 250 region of interest containing background signal was subtracted from raw soma
 251 fluorescence measurements to reduce the effect of photobleaching. Baseline
 252 fluorescence (F_0) was defined as the mean fluorescence in the first 10 frames of
 253 the recording. To calculate $\Delta F/F$, F_0 was subtracted from fluorescence for each
 254 frame ($F_{(t)}$), then divided by F_0 ; $\Delta F/F = (F_{(t)} - F_{(0)}) / F_{(0)}$. A neuron was considered
 255 active if $\Delta F/F$ was 10% or greater.

256

257 *Laser Ablation of V3-INs*

258 Acute ablation of the V3-INs was performed using a Micropoint pulsed
259 nitrogen pumped dye laser (Andor Technology, Belfast, Northern Ireland). Light
260 ($\lambda = 435 \text{ nm}$) was delivered via an Olympus BX51 WI microscope using a 60x /
261 NA 1.0 water dipping objective lens. The laser was focused to apply maximal
262 light intensity to the imaging plane of the microscope, the intensity of the laser
263 was calibrated to produce a 4.5 – 5.0 μm hole in a mirror slide (this optimal
264 intensity was determined empirically), and the x-y coordinates of the laser point
265 in the field of view were indicated on a digital display. Three to six dpf
266 *Tg(vglut2a:DsRed)^{nns9}* zebrafish larvae were anesthetized in 0.02% Tricaine in
267 zebrafish ringers solution and embedded laterally in a gel composed of 3%
268 methylcellulose and 0.02% Tricaine in zebrafish ringers saline. A coverslip was
269 placed over the gel and secured at the edges with agarose to stabilize the
270 position of the larvae.

271 Targeted, bilateral laser ablation of DsRed⁺ V3-INs in the ventral spinal
272 cord was performed by positioning the microscope objective such that a neuron
273 was in the x-y position at which the laser was focused. Laser pulses were applied
274 to each V3-IN soma at 33 Hz for 10 s. Laser intensity was empirically determined
275 *a priori* such that >95% of the targeted V3-INs were not present 24 h post-
276 ablation. Following the train of laser pulses, the microscope was repositioned to
277 focus on another identified V3-IN, and the protocol was repeated. Larvae were
278 embedded for approximately 50 min in order to perform bilateral ablation of ~140

279 identified V3-INs between bod segment (S) 5 and S20. To confirm that laser
280 ablation of targeted V3-INs was effective and persistent, the lack of DsRed
281 fluorescence in the lesioned spinal cord of *Tg(vglut2a:DsRed)^{nns9}* larvae was
282 confirmed ~24 hours post-ablation. Sham larvae were treated identically to V3
283 ablation larvae, but the x-y position of the laser was offset by 5 – 20 µm from
284 each V3 cell, producing non-specific damage to neurons other than the V3
285 population.

286

287 *Statistical Analysis*

288 Statistical analysis of data was performed using two-sample *t*-test,
289 ANOVA test, Holm-Sidak post-hoc test, Kolmogorov-Smirnov test, and least-
290 squares linear regression. Statistical tests were carried out using SigmaPlot 12
291 and 14 software (SyStat Software, San Jose, CA), Matlab (Mathworks, Natick,
292 MA), or Microsoft Excel (Microsoft, Seattle, WA). An α level of 0.05 was used to
293 determine statistical significance. Data are reported as means and standard
294 deviation (Mean, SD).

295

296 **RESULTS**

297 *V3-INs are Individually Identifiable in the Larval Zebrafish Spinal Cord*

298 To characterize the functional roles of V3-INs *in vivo*, it was necessary to
299 reliably target the neurons for recording and perturbation. While there are no
300 published transgenic zebrafish lines that specifically label neurons expressing
301 *sim1a* (the zebrafish V3-IN marker), it is possible to infer V3-IN identity from their

302 other characteristics. V3-INs proliferate ventral to the central canal and are
303 glutamatergic (Zhang et al., 2008; Yang et al., 2010). In zebrafish, there is >90%
304 overlap of *vesicular glutamate transporter 2* (*vglut2a*) and *sim1a* expression in
305 the ventral spinal cord at the embryonic stage (1.5 dpf) (Yang et al., 2010).
306 However, it is possible that the embryonic ventral glutamatergic neurons migrate
307 or are replaced by a non-V3 population between 1.5 dpf and the free-swimming
308 larval stage (>3 dpf).

309 To test the hypothesis that the ventral DsRed⁺ neurons observed in 4 – 6
310 dpf *Tg(vglut2a:DsRed)^{nns9}* larvae are V3-INs, we crossed this line to
311 *Tg(nkx2.2a:mEGFP)^{vu17}*, which marks p3 progenitor neurons (Briscoe et al.,
312 2000; Schäfer et al., 2007) (Fig. 1A). At 4 – 6 dpf, *nkx2.2a:mEGFP* expression
313 was restricted to the region ventral to the central canal (Fig. 1C). The p3 domain
314 of the zebrafish spinal cord also produces Kolmer-Agduhr (KA) neurons (Yang et
315 al., 2010), and likely other neuronal types, so it was expected that there would be
316 more *nkx2.2a:mEGFP*-expressing neurons than *vglut2a:DsRed*-expressing
317 neurons (Fig. 1B). Approximately 94% of *vglut2a:DsRed*-expressing neurons
318 ventral to the central canal also expressed *nkx2.2a:mEGFP* ($n = 6$ larvae, 162
319 neurons), and were therefore confirmed to be V3-INs (Fig. 1D). There was not a
320 significant difference between the dorsoventral position of the *vglut2a:DsRed*-
321 expressing neurons that co-expressed *nkx2.2a:mEGFP* and those that did not
322 (two-sample t-test; $t = 1.6$; $p = 0.10$), so there was not a way to reliably segregate
323 the identified V3-INs, from those that did not express *nkx2.2a:mEGFP*. However,
324 the overwhelming majority of the DsRed⁺ neurons ventral to the central canal

325 were confirmed to be V3-INs. The lack of confirmed transgene overlap in the
326 remaining cells may be due to variegated transgene expression rather than the
327 presence of a non-V3-IN ventral glutamatergic neuronal population. Based on
328 this experiment, we treat the ventral *vglut2a:DsRed*-expressing neurons as the
329 V3-IN population, with the caveat that a small fraction (~6%) may be
330 misidentified.

331

332 *V3-INs are Located in the Ventromedial Spinal Cord and are Distributed Along*
333 *the Rostrocaudal Axis of the Spinal Cord*

334 In mice, the V3-INs migrate following differentiation to form spatially
335 segregated dorsal and ventral populations that have different cellular properties
336 and may be necessary for different behaviors (Borowska et al., 2013). To
337 determine if there are multiple populations of spatially segregated V3-INs in
338 zebrafish larvae, we used confocal microscopy to create 3-D reconstructions of
339 the spinal cords of 4 – 6 dpf *Tg(vglut2a:DsRed)^{nns9}* zebrafish larvae (Fig. 2A-B).
340 The center of each V3-IN soma in the most rostral 2 mm of the spinal cord was
341 tagged, and the distribution of the neurons within the spinal cord was calculated
342 based on the imputed borders of the spinal cord (see *Methods*). Neurons in the
343 far caudal spinal cord were not counted because the dimensions of the spinal
344 cord taper to the extent that it was difficult to determine the location of the central
345 canal and Mauthner axons. All of the neurons ($n = 4$ larvae; 797 neurons) were
346 located medial of the Mauthner axon (Fig. 2C). V3-IN density was highest in the
347 rostral spinal cord but remained close to 1 cell per 10 μm throughout the cord

(Fig. 2D). The V3-IN somas were approximately 5 μm in diameter in the rostrocaudal axis, so a density of ~ 1 cell per 10 μm thoroughly tiled the ventral spinal cord. The majority of V3-INs were located in the most medial and most ventral 25% of the spinal cord (Figs. 2E & F). There was no parasagittal plane through the medial spinal cord that was unoccupied by V3-INs (Fig. 2E), which suggested that the V3 population may be considered a midline population. However, the density of neurons was not highest at the sagittal midline of the spinal cord, and when cell density was plotted along the population axis of the V3-INs, the point of highest density was adjacent to the spinal cord midline (Fig. 2G). Therefore, we propose that distinct left and right populations of V3-INs are present, but that there are not multiple ipsilateral populations of spatially segregated V3-INs in larval zebrafish.

V3-INs Project Bilaterally and Have Descending Processes

To characterize the morphologies and putative synaptic connectivity of the V3-INs, we examined the 3-D morphology of individual V3-INs using confocal microscopy (Fig. 3). The density of DsRed fluorescence is too high in the *Tg(vglut2a:DsRed)^{nns9}* transgenic line to accurately trace the processes of a single neuron, so we expressed a photoconvertible protein (Kaede) in *Tg(vglut2a:Gal4)^{nns20};Tg(UAS:Kaede)^{s1999t}* larvae to identify and photoconvert single V3-INs (Fig. 3A-B). Following photoconversion, it was possible to obtain morphological images of the V3-INs ($n = 5$ neurons, 4 larvae; Fig. 3C). Based on this sample of V3 morphologies, it was evident that variation in the projection

371 patterns of the V3-INs was present. For example, the V3-IN imaged in S10 had
 372 minimal projection density dorsal or rostral to the cell body, while the V3-IN in
 373 S18 predominantly projected rostrally and dorsally (Fig. 3C, Lateral z-Projection).
 374 Despite these differences, there were some common features of this sample of
 375 V3-IN morphologies. First, the imaged V3-INs possessed bilateral projections,
 376 but had a higher density of processes ipsilateral to the cell body (Fig. 3C,
 377 Horizontal z-Projection). Second, V3-INs had the greatest density of processes
 378 close to the cell body and longer caudal projections than rostral projections (Fig.
 379 3C). Third, while the full extent of the descending processes of the S9 and S14
 380 neurons is not presented in Fig. 3C, V3-INs had a maximum detectable
 381 projection distance shorter than 300 μm . The limited projection length we
 382 observed may be due to the Kaede photoconversion method used and should be
 383 considered a lower bound on the full extent of the projections of the V3-INs. The
 384 projection pattern of the V3-INs appeared to innervate the ventral spinal cord,
 385 which is populated by MNs, V2a-INs (Eklöf-Ljunggren et al., 2012; Menelaou and
 386 McLean, 2013), other V3-INs, KA neurons, and serotonergic neurons (McLean
 387 and Fetcho, 2004; Yang et al., 2010).

388

389 *The Majority of V3-INs are Active During Spontaneous Fictive Swimming*

390 In mice, there is only indirect evidence supporting temporal correlation
 391 between V3-IN activity and locomotion (Borowska et al., 2013). To test the
 392 hypothesis that activity in the V3-INs is appropriately timed to provide excitatory
 393 drive to MNs during locomotion, we monitored the activity of the V3-INs during

394 spontaneous fictive swimming using a genetically-encoded calcium indicator
 395 (GCaMP6s; Fig. 4). The activity of V3-INs and fictive motor output during
 396 spontaneous swimming was measured via simultaneous calcium imaging and
 397 peripheral nerve recording using 4 - 6 dpf
 398 *Tg(vglut2a:Gal4ff)^{nns20};Tg(UAS:GCaMP6s)^{nk13a}* larvae (Fig. 4A-B). We found that
 399 the majority, 86 of 93 (92%), of V3-INs were active ($\Delta F/F > 10\%$) during
 400 spontaneous fictive swimming episodes ($n = 6$ larvae, 15 trials). Therefore, this is
 401 the first direct evidence that V3-INs are active during fictive locomotion, which
 402 supports the participation of V3-INs in producing locomotor activity.

403

404 *Ablation of V3-INs Does Not Affect Episodic or Burst Properties of Spontaneous* 405 *Fictive Swimming*

406 The previous experiments demonstrated that the V3-INs have
 407 morphology, neurotransmitter phenotype, and activity consistent with providing
 408 excitatory drive to motor neurons but did not demonstrate the function of the V3
 409 neurons or their necessity for behavior. To determine whether canonical features
 410 of fictive swimming (episode duration, burst (tail-beat) frequency, and burst
 411 duration) are modulated by V3-IN activity, we performed laser ablation of a large
 412 percentage of the V3-IN population ($>95\%$; ~ 140 V3-identified somas across
 413 body segments 5 to 20; Fig. 5). Three experimental groups were used: 1) The V3
 414 ablation group, in which all V3-INs on both sides of the spinal cord between S5
 415 and S20 were laser ablated ($n = 6$ larvae; 145 (SD 9.6) neurons per larva).
 416 Representative examples of targeted laser ablation efficacy within a restricted

417 region (Fig. 5A-B) and spatial localization (Fig. 5C-D) are shown. 2) The sham
418 ablation group, in which laser pulses were delivered 5 – 20 μ m rostral to each
419 identified V3-IN cell body ($n = 6$ larvae; 130 (SD 10.8) sham targets per larva). 3)
420 The unmanipulated control group was not embedded or exposed to laser pulses
421 ($n = 6$ larvae).

422 The day following laser ablation or sham ablation, fictive swimming was
423 measured using PN recordings. Episode duration, burst frequency, and burst
424 duration were not significantly different between unmanipulated, sham and
425 ablation experimental groups during spontaneous swimming (one-way ANOVAs;
426 all $F < 1.3$; all $p > 0.30$; Table 1). These results indicate that the population of
427 spinal V3-INs do not play a significant role in establishing episode duration, burst
428 frequency (a proxy for tail-beat frequency), or burst duration during fictive
429 locomotion in larval zebrafish.

430

431 *Ablation of V3-INs Reduces the Proportion of Active Motor Neurons but Does Not* 432 *Affect Peak Locomotor Frequency During Spontaneous Fictive Swimming*

433 Since the ablation of spinal V3-INs in zebrafish larvae did not affect
434 episode duration, burst frequency, or burst duration (Table 1), we tested the
435 hypothesis that ablation of V3-INs reduces locomotor vigor by decreasing the
436 number of active MNs during spontaneous fictive swimming. In order to ablate
437 V3-INs without also photo-damaging or bleaching motor neurons, we used a two-
438 step neuronal targeting strategy. First, V3-INs were identified and ablated using
439 the *Tg(vglut2a:DsRed)^{nns9}* transgenic line. Following recovery from laser

440 ablation, motor neurons were loaded with a cell-permanent calcium indicator,
 441 Calcium Green-1, AM. To verify that spinal neurons loaded with AM dye (Calcium
 442 Green-1 and/or Rhod-2) were MNs, we first confirmed motor neuronal specificity
 443 of GFP expression in the *parga^{mn2Et}* enhancer trap line (Balciunas et al., 2004).
 444 *Parga^{mn2Et}* larvae immunolabeled for the cholinergic marker choline
 445 acetyltransferase (ChAT) revealed that the majority of GFP-expressing neurons
 446 co-labeled with ChAT antibodies ($n = 5$; 91.3% (SD2.1); Fig. 6A-A''). A small
 447 percentage of dorsally-positioned ChAT+ cells did not colocalize with GFP (4.1%
 448 (SD2.8); Fig. 6A-A'', arrowheads); these cells were considered members of a
 449 subset of cholinergic spinal interneurons that modulate MN excitability recently
 450 identified in zebrafish (Bertuzzi and Ampatzis, 2018). Thus, GFP expression in
 451 the *parga^{mn2Et}* enhancer trap line was specific to MNs.

452 Next, to confirm that loading of AM dye was restricted to MNs, the skin
 453 was removed from both sides of the body from *parga^{mn2Et}* larvae, in which GFP
 454 expression was shown to be limited to MNs (Fig. 6A-A''), and larvae were
 455 incubated in zebrafish ringers solution containing Rhod-2 AM dye ($n = 4$; Fig. 6B-
 456 B''). Rhod2-AM, which was not used for calcium imaging experiments because it
 457 did not produce a strong calcium signal, was used as a proxy for Calcium Green-
 458 1 AM since the excitation/emission wavelengths of Calcium Green-1 AM
 459 overlapped with, and could not be disambiguated from, GFP expressed in
 460 *parga^{mn2Et}* MNs. The majority of GFP-expressing MNs co-labeled with Rhod-2
 461 AM (84.3% (SD4.3); Fig. 6B-B''). A small percentage of GFP-negative cells
 462 contained Rhod-2 AM (5.4% (SD0.5); Fig. 6B-B'', arrowheads). Taken together,

463 these results confirmed that MNs were selectively labeled with AM dye and,
464 therefore, the cell permeable calcium indicator (Calcium Green-1 AM) could be
465 used to monitor *in vivo* calcium activity in spinal MNs.

466 To examine the effects of V3-IN ablation on the production of fictive
467 locomotor vigor, we determined the proportion of spinal MNs that were active
468 during spontaneous fictive swimming episodes. MN activity in
469 *Tg(vglut2a:DsRed)^{nns9}* larvae was measured using *in vivo* calcium imaging by
470 loading MNs with Calcium Green-1 AM, while peak burst frequency during
471 spontaneous fictive locomotor activity was measured using extracellular PN
472 recordings (Fig. 7A-B). We quantified the proportion of active MNs and peak
473 burst frequencies during spontaneous fictive swimming for Control (50 swim
474 episodes, 4 larvae), Sham Ablated (14 swim episodes, 2 larvae), and V3-IN
475 Ablated (59 swim episodes, 3 larvae) groups. Importantly, there were no
476 differences in the properties of the fictive swimming produced by these larvae,
477 consistent with our prior experiments (Table 1). Neither the distribution of peak
478 burst frequencies (Kolmogorov-Smirnov test < 0.33 , $p > 0.14$ for all group
479 comparisons; Fig. 7C), nor the mean peak burst frequencies, were different
480 between Control, Sham, and Ablation Groups (Control = 39.0 Hz (SD8.5); Sham
481 = 36.4 Hz (SD10.7); Ablated = 39.3 Hz (SD8.9); one-way ANOVA, $F = 5.0$, $p =$
482 0.08; Fig. 7D).

483 Assessment of the probability distributions showed that the proportion of
484 active MNs in the Ablated group was significantly reduced compared to both the
485 Control (Kolmogorov-Smirnov test = 0.68, $p < 0.001$) and Sham (Kolmogorov-

Smirnov test = 0.54, $p = 0.001$) groups (Fig. 7E). The proportion of active MNs was not significantly different between the Control and Sham groups (Kolmogorov-Smirnov test = 0.24, $p = 0.50$; Fig. 7E). Further, V3-IN ablation had a main effect of significantly reducing the proportion of active MNs during spontaneous fictive swimming (Fig. 7F; Control mean = 65.2% (SD16.7); Sham mean = 56.6% (SD24.2); Ablated mean = 41.2% (SD15.6); one-way ANOVA, $F = 25.3$; $p < 0.001$). Pairwise multiple comparisons (Holm-Sidak; Fig. 7D) revealed significant differences for the proportion of active MNs between both the Control and Ablated groups ($t = 7.02$, $p < 0.001$) and the Sham and Ablated groups ($t = 2.98$, $p = 0.007$), but significant differences were not found between Control and Sham groups ($t = 1.54$, $p = 0.10$). These results indicate that V3-INs participate in regulating locomotor vigor by recruitment of spinal MNs but do not regulate burst frequency during spontaneous fictive locomotion.

DISCUSSION

The goal of this study was to characterize the potential role of V3-INs in producing locomotor vigor in genetically tractable and optically accessible larval zebrafish. Locomotor vigor, the strength of a locomotor output, is regulated by the recruitment of motor units appropriate to the desired movement: small units for fine behaviors and large units for large movements (Zajac and Faden, 1985; Mendell, 2005). This recruitment pattern of small to large motor units, the so-called size principle, is well established in both mammalian and swimming locomotor systems (Henneman et al., 1965; Gustafsson and Pinter, 1984a,

1984b; Cope and Pinter, 1995; McLean et al., 2007; Gabriel et al., 2011).

However, investigations into the pre-motor neural mechanisms that contribute to the regulation of locomotor vigor have been difficult in mammals for two primary reasons. First, mammalian spinal locomotor circuits are complicated by the requirements to control and coordinate limbs and joints, rather than simply produce a smooth gradient of motion intensity (Grillner, 2003; Grillner and El Manira, 2020). Second, despite recent progress in tool development, *in vivo* functional manipulations of mammalian spinal locomotor circuits using mini-scopes and fiber-optics are both challenging and somewhat limited in use (Montgomery et al., 2015; Samineni et al., 2017; Canales et al., 2018). Zebrafish larvae provide several advantages over mammalian models for *in vivo* studies: the spinal cords of fish are less complicated than mammals, zebrafish are recognized as a powerful neurogenetic model system, and the translucent nature of zebrafish larvae makes them particularly appropriate for *in vivo* optical methods of investigation. In this study, we found that, unlike the mammalian spinal circuit, V3-INs in the larval zebrafish spinal cord are contained within a single, ventral anatomical domain (Fig. 1-2) and are not necessary to fictive locomotor rhythm (Table 1) (Zhang et al., 2008; Danner et al., 2019). Instead, zebrafish V3-INs are swim-active neurons that are necessary for recruitment of motor neurons during fictive locomotion (Fig. 4, 7), and therefore are most likely necessary for the recruitment of motor units during free-moving locomotion.

531 *V3-INs are a population of previously uncharacterized ventral VGlut2a+ neurons*
532 *in larval zebrafish*

533 Here, we report that almost all ventrally-located neurons labeled by the
534 *Tg(vglut2a:DsRed)^{nns9}* and *Tg(vglut2a:Gal4ff)^{nns20}* transgenic lines are V3-IN cells
535 (Figs. 1-5). The V3-INs, a numerous ventromedial population, are distributed
536 across the rostrocaudal axis of the spinal cord (Fig. 2) and project to both sides
537 of the spinal cord, with a richer innervation of the ipsilateral side of the spinal
538 cord (Fig. 3). In this dataset, there are several different V3-IN projection patterns
539 (Fig. 3), but none of these anatomical projection patterns resemble previously
540 characterized ventral neuron cell types, such Kolmer-Agduhr cells (Dale et al.,
541 1987; Bernhardt et al., 1992; Montgomery et al., 2016), ventral serotonergic
542 neurons (Van Raamsdonk et al., 1996; Brustein et al., 2003; McLean and Fetcho,
543 2004; Montgomery et al., 2018), or VeMes (Hale et al., 2001). V3-INs are a
544 morphologically distinct cell type or collection of cell types that have not been
545 characterized through previous experimental approaches.

546 The observed diversity of cell morphology in the V3-IN cell population is
547 similar to the diversity of V3-INs in the mammalian spinal cord (Borowska et al.,
548 2013; Deska-Gauthier et al., 2020). In mammals, the morphology of V3-IN
549 subtypes is correlated with their circuit function (Borowska et al., 2013).
550 However, we have not found evidence for differential recruitment of V3-INs
551 during fictive swimming or functional heterogeneity. It may be that larval
552 zebrafish V3-INs are a primitive class of excitatory spinal INs, as has been
553 previously proposed for *Engrailed1*-expressing inhibitory neurons in zebrafish

(Higashijima et al., 2004). However, analysis of cell morphology in zebrafish larvae is complicated by ongoing development in the nervous system and the rostrocaudal developmental gradient (Cole and Ross, 2001). Further experiments are necessary to clarify if these morphologies represent distinct sub-types of V3-INs, developmental stages of a single morphological family, or regional specializations of the V3-INs.

Zebrafish V3-INs are active during fictive locomotion

Results indicating that V3-IN activity is directly correlated to fictive locomotor activity in vertebrate preparations has not been convincingly presented. In mice, there is indirect evidence indicating temporal correlation between V3-IN activity and locomotion (Borowska et al., 2013). Through simultaneous optical and electrophysiological recordings, we showed that activity in the majority of V3-INs is correlated to fictive swimming (Fig. 4) and is therefore appropriately timed to provide excitatory drive to MNs during locomotion. Thus, this is the first direct evidence in any vertebrate preparation that V3-INs are active during fictive locomotion, which provides supporting evidence that they may participate in locomotor activity.

V3-IN activity is necessary for motor neuron recruitment, but not necessary for rhythm generation

Given that V3-INs have been shown to affect both the regularity and coordination of locomotor activity in mice (Zhang et al., 2008), it was surprising

577 that laser ablation of V3-INs in larval zebrafish had no effect on episode duration,
 578 mean burst frequency, or burst duration during fictive swimming in larval
 579 zebrafish (Table 1). One interpretation of this finding is that despite similarities in
 580 gene expression and neurotransmitter profile, pre-motor interneuron function is
 581 divergent between axial and tetrapod locomotion. The latter argument has been
 582 advanced regarding the V2a interneurons (Dougherty and Kiehn, 2010;
 583 Dougherty et al., 2013); the different effects (or lack thereof) of V3-IN ablation in
 584 zebrafish and mice may be a second example of the trend. An important
 585 difference between the current study and mouse knock-out experiments is the
 586 mechanism of ablation. In the current study, V3-INs were ablated acutely, and
 587 little time (~24 hours) was given for circuit compensation. In the mouse
 588 experiments, on the other hand, V3-INs were ablated or silenced chronically
 589 (Zhang et al., 2008; Rabe Bernhardt et al., 2012), leaving opportunity for
 590 physiological compensation. Acute silencing experiments using optogenetics
 591 could be performed in both species (Arrenberg et al., 2009; Hägglund et al.,
 592 2013), and may demonstrate that this difference between species is due to the
 593 experimental manipulation rather than the underlying neural circuit.

594 Over the last several years, a subset of identified excitatory spinal
 595 interneurons, called V2a interneurons, has been shown to control the speed of
 596 locomotion in a variety of vertebrates (Zhong et al., 2011; Ausborn et al., 2012;
 597 Ampatzis et al., 2014; Gosgnach et al., 2017). Of particular interest, a recent
 598 study also ascribed a role for regulating locomotor vigor to V2a-INs in adult
 599 zebrafish (Song et al., 2018). Taken together, these results indicate that V2a-INs

are involved in regulating both locomotor speed and vigor. To date, however, a subpopulation of spinal interneurons that selectively regulate locomotor vigor has not been identified. Our work provides evidence that, in zebrafish larvae, V3-INs contribute exclusively to locomotor vigor. Specifically, we show that targeted ablation of V3-INs (Fig. 5) reduces the probability of MN firing during fictive swimming (Fig. 7E-F), but does not affect the range of locomotor frequencies produced (Fig. 7C-D). This suggests that vigor, but not frequency, may be determined, in part, by the proportion of active V3-INs. Thus, we propose that V3-INs are a source of excitation in the vertebrate locomotor neural circuitry that regulate the vigor of locomotor output.

Conclusion

The work presented here addressed an important concept regarding motor control; specifically, a fundamental lack of understanding of the cellular properties that produce locomotor vigor in vertebrates. We showed, directly for the first time, that V3-INs in zebrafish larvae are active during *in vivo* fictive locomotion and contribute, at least in part, exclusively to locomotor vigor. Further, targeted ablation of the V3-IN spinal cord population does not affect locomotor frequency (speed), which clarifies their role in fine motor control rather than rhythm generation. Thus, we propose that the V3-IN subpopulation regulates locomotor vigor independently of speed, which is a functional difference between the V3-INs and V2a-INs since the latter regulate both speed and vigor (Zhong et

622 al., 2011; Ausborn et al., 2012; Ampatzis et al., 2014; Gosgnach et al., 2017;
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897 cord. *Nat Commun* 2:274 Available at:
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903 **Figure 1. V3-INs are identified by co-localization of *vglut2a:DsRed* and**

904 ***nkx2.2a:mEGFP* transgene expression in neurons located in the**

905 **ventromedial spinal cord. (A) Whole-mount**

906 *Tg(nkx2.2a:mEGFP)^{vu17};Tg(vglut2a:DsRed)^{nns9}* double transgenic zebrafish larva.

907 (B-D) A representative single confocal optical section of the spinal cord

908 exhibiting: (B) *nkx2.2a:mEGFP* expression, (C) *vglut2a:DsRed* expression, and

909 (D) overlaid *nkx2.2a:mEGFP* and *vglut2a:DsRed* expression. The boundaries

910 of the spinal cord and central canal are illustrated by solid and dashed white

911 lines, respectively, in panel B. White triangles indicate neurons that co-express

912 both transgenes and the black triangle indicates the only *vglut2a:DsRed* neuron

913 ventral to the central canal that did not co-express *nkx2.2a:mEGFP* in the field of

914 view. Scale bars: 1 mm in A and 10 μ m in B-D.

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Figure 2. Distribution of V3-IN Cell Bodies in the Spinal Cord. (A) Maximum

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intensity projection of a confocal stack of the spinal cord of a 5 dpf

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Tg(vglut2a:DsRed)^{nns9} zebrafish larva. The dashed white outline indicates the

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region of the cord in which cells were quantified. The solid white outline is the

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region of the image shown at higher magnification in (B). (B) A higher

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magnification field of view. White circles indicate V3 neurons, black triangles

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indicate DRG neurons located lateral of the spinal cord. (C) Distribution of

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location of all quantified V3 neurons (red dots) projected in the coronal plane of

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the spinal cord. The schematic outline of the spinal cord (solid grey line), as well

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as the location of the central canal (cc) and Mauthner axon (m), are approximate.

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The heavy black line indicates the line of best fit that best describes the axis of

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the V3 neuronal population. (D-G) The windowed moving average of V3 density

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along the rostrocaudal axis (D; window width: 250 μm), mediolateral axis (E;

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window width: 15% SC radius), dorsoventral axis (F; window width: 15% SC

932 radius), and along the population axis illustrated in panel C (G; window width:
933 15% SC radius). Black lines are the mean, grey area indicates the standard
934 deviation. SC: spinal cord. Scale bar: 100 μm in A and 19 μm in B.

Figure 3. V3-INs are descending, bilaterally projecting cells. Kaede was expressed in *vglut2a*-expressing neurons in *Tg(vglut2a:Gal4ff)^{nns20};Tg(UAS:Kaede)^{s1999t}* double transgenic larvae (A). A single V3-IN was exposed to UV laser light, converting the Kaede protein from green fluorescence to red (B). (C) Inverted contrast projections of Kaede-red fluorescence in 5 neurons. Each row of images is a neuron shown in a parasagittal projection (left) and horizontal projection (right). On the horizontal projections, the center of the cell body is indicated with white triangles to account for optical bloom and contralateral projections are indicated with black triangles.

946 Lateral projections: Rostral left, dorsal up. Horizontal projections: Rostral left,
947 lateral up. Scale bar: 20 μm .

Figure 4. V3-IN activity is temporally correlated to fictive locomotion. (A)

Each V3 neuron in the field of view in

Tg(vglut2a:Gal4ff)^{nns20};Tg(UAS:GCaMP6s)^{nk13a} double transgenic larvae was

assigned a ROI during spontaneous fictive swimming. Panels in (A) show raw

954 GCaMP6s fluorescence (F) and correspond to numbered time-points in (B).
 955 Bottom panel in A shows calculated $\Delta F/F$ between 3 and 7 s time-points. Dashed
 956 lines represent ventral boundary of the spinal cord. (B) Synchronized recordings
 957 of GCaMP6s fluorescence (top) and fictive swimming (bottom) allowed
 958 identification of swimming-related neuronal activity.

Figure 5. Targeted ablation of V3-INs is both efficient and spatially

restricted. (A-B) Confocal stacks of the same *Tg(vglut2a:DsRed)^{nns9}* zebrafish larva before (A) and 12 hours after (B) laser ablation of the V3-INs in the region indicated (red dashed lines). (C-D) A magnified view of two V3-INs before (left) and after (right) a single cell laser ablation. Scale bars: 20 μm in A and B and 5 μm in C and D.

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Figure 6. Spinal neurons loaded with rhodamine-2 AM dye are definitively

identified as MNs. (A) The EGFP-expressing *parga^{mn2Et}* enhancer trap line (A,

green) was labeled with antibodies to choline acetyl transferase (ChAT; A', red).

Colabeling (A'') of EGFP with ChAT antibodies indicated that EGFP expression in

the *parga^{mn2Et}* line is a marker of spinal motor neurons. White arrowheads

976 indicate ChAT-positive cells that do not contain EGFP (non-MNs). (B) Live
 977 *parga^{mn2Et}* larvae were anesthetized, skinned, and incubated in rhodamine-2 AM
 978 dye (Rhod-2, AM). EGFP expression (B, green) and Rhod-2, AM labeling (B',
 979 red), showed that Rhod-2, AM specifically labeled all motor neurons (B"). White
 980 arrowheads indicate two Rhod-2, AM-labeled cells that did not contain EGFP.
 981 Scale bar: 20 μ m.

Figure 7. V3-IN laser ablation reduces the proportion of motor neurons that are active during fictive swimming. MNs in *Tg(vglut2a:DsRed)^{nns9}* larvae were loaded with Calcium Green-1 AM dye and assigned ROIs during recording. Synchronous MN fluorescence and fictive swimming recordings were compared between Control, V3-IN Ablated, and Sham ablated preparations. (A and B) Raw Calcium Green-1 AM fluorescence panels correspond to numbered time-points in aligned $\Delta F/F$ (each gray line represents a MN) and peripheral nerve traces in a Control preparation (A) and a V3-IN Ablated preparation (B). (C-F) Cumulative probability distributions and mean Peak Burst Frequencies (C and D) and percent of MNs active (10% $\Delta F/F$ threshold) during fictive swimming episodes (E and F). Asterisks represent significant differences. Error bars represent SD.

		Episode Duration (msec)	Burst Frequency (Hz)	Burst Duration (msec)
Control (<i>n</i> = 6 larvae)		720, SD713	25.7, SD3.4	10.4, SD2.8
Ablated (<i>n</i> = 6 larvae)		524, SD223	28.3, SD1.9	11.8, SD2.2
Sham (<i>n</i> = 6 larvae)		878, SD642	28.2, SD3.8	11.4, SD2.7
ANOVA	F	0.58	1.30	0.42
	<i>p</i>	0.57	0.30	0.67

996
997 Table 1. Measures of the episodic and burst properties from peripheral nerve
998 recordings during fictive locomotion in zebrafish larvae. All values presented as
999 mean, standard deviation.