Intersegmental coordination of the central pattern generator via interleaved electrical and chemical synapses in zebrafish spinal cord

Lae Un Kim1 · Hermann Riecke1

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Abstract
A significant component of the repetitive dynamics during locomotion in vertebrates is generated within the spinal cord. The legged locomotion of mammals is most likely controled by a hierarchical, multi-layer spinal network structure, while the axial circuitry generating the undulatory swimming motion of animals like lamprey is thought to have only a single layer in each segment. Recent experiments have suggested a hybrid network structure in zebrafish larvae in which two types of excitatory interneurons (V2a-I and V2a-II) both make first-order connections to the brain and last-order connections to the motor pool. These neurons are connected by electrical and chemical synapses across segments. Through computational modeling and an asymptotic perturbation approach we show that this interleaved interaction between the two neuron populations allows the spinal network to quickly establish the correct activation sequence of the segments when starting from random initial conditions, as needed for a swimming spurt, and to reduce the dependence of the intersegmental phase difference (ISPD) of the oscillations on the swimming frequency. The latter reduces the frequency dependence of the waveform of the swimming motion. In the model the reduced frequency dependence is largely due to the different impact of chemical and electrical synapses on the ISPD and to the significant spike-frequency adaptation that has been observed experimentally in V2a-II neurons, but not in V2a-I neurons. Our model makes experimentally testable predictions and points to a benefit of the hybrid structure for undulatory locomotion that may not be relevant for legged locomotion.

Keywords Central pattern generator · Spinal cord · Waves · Gap junctions · Neuronal networks · Zebrafish · Computational model

1 Introduction
Animal locomotion requires the repetitive activation of muscles over a range of speeds that is adaptively varied to achieve a variety of goals including steady motion, turns to avoid obstacles, sudden escapes, and the like. Substantial portions of the motor programs performing these motions are generated within the spinal cord itself, with central pattern generators playing a key role.

In animals like lamprey, leech, and fish the basic motion consists of an undulation wave traveling along the body. A striking experimental observation in the undulatory motion of various species like lamprey, and larvae of xenopus and zebrafish is that the timing of the activity of the different segments of the animal depends only weakly on the frequency of the motion (Wallen & Williams, 1984; Tunstall & Roberts, 1991; Williams, 1992; Masino & Fetcho, 2005). More precisely, the mean delay in the oscillatory motion from one segment to the next is not given by a fixed time delay as it would result from synaptic time constants or a fixed axonal propagation speed but rather by a fixed fraction of the period that is quite independent of the frequency of the wave. Thus, the time delay grows linearly with the period of the oscillation. Importantly, this enables the mode of the physical motion of the animal’s body, i.e. the undulation wavelength, to stay almost the same across a range of frequencies (Wallen & Williams, 1984; Masino & Fetcho, 2005). A fundamental question that arises in the study of the spinal cord locomotor control is how the circuit is able to
limit the dependence of the intersegmental phase difference (ISPD), i.e. the phase lag between successive segments, on the swimming frequency.

Fish and fish larvae often swim in spurts. The initiation of such a spurt requires that the spinal cord quickly establish the correct phase relationships between the adjacent segments, independent of their previous activity. If the organization of the phases were to take multiple oscillation periods, the swimming motion would initially be quite disorganized and ineffective.

The steady-state phase relationship of spinal-cord models has been studied extensively and comprehensively in the context of coupled-oscillator theory, where each segment corresponds to an individual oscillator that is coupled to its nearest and possibly further neighbors (Kopell & Ermentrout, 1986; Kopell et al., 1991; Cohen et al., 1992). This has been obtained explicitly in some models that were formulated in terms of firing rates (Williams, 1992; Varkonyi et al., 2008). In general, however, the frequency-independence turns out not to be robust, but can be improved by long-range connections across multiple segments (Varkonyi et al., 2008).

The duration of the transient that is required to establish steady swimming seems not to have been addressed much so far. Within the weak-coupling framework, in which - due to the averaging over the period - the coupling between segments depends only on the phase differences (Kopell & Ermentrout, 1986), the ordering of the oscillator phases proceeds very slowly: the phases reach their final order only on a time scale that is much longer than the period of the individual oscillations. This framework can therefore not be used reliably to investigate how coherent swim motion is established quickly within just a few oscillation cycles. Instead, one needs to use an approach that does not rely on this averaging procedure. In fact, fast ordering is achieved, in particular, if the caudal neurons fire only upon receiving input from their rostral neighbors, i.e. if they do not oscillate on their own. In this regime they cannot be considered as weakly perturbed oscillators that would be amenable to a phase description.

Classically, two classes of models have been proposed for the organization of spinal cord circuits (McLean & Dougherty, 2015). Particularly for legged locomotion, e.g. in mammals, a multi-layer hierarchical structure has been proposed in which the timing of the motion is controlled by neurons that exclusively make higher-order connections to other interneurons and neurons in the brain stem, while the last-order projections to the relevant motor pools are exclusively made by neurons in a separate layer (McCrea & Rybak, 2008). This structure is especially motivated by the observation of so-called non-resetting perturbations in which the suppression of last-order neurons leads to a brief failure in the activation of the motor neurons but has no impact on the timing of subsequent activations, since the timing is exclusively controlled by the higher-order neurons. For simpler, axial locomotor circuits, as they arise, e.g., in lamprey and zebrafish larvae, a single-layer structure has been proposed in which the last-order neurons driving the motor pool also make higher-order connections to other interneurons, which control the timing of the activations (Kozlov et al., 2009).

Based on recent anatomical (Menelaou et al., 2014) and physiological studies (Menelaou & McLean, 2019) in zebrafish larvae a new, hybrid model has been proposed in which the excitatory last-order neurons not only drive the motor pool, but also project to other excitatory interneurons, as would be the case in a single-layer architecture. As in a multi-layer model, however, these neurons are comprised of two populations, termed V2a-I and V2a-II, which have analogs in the spinal cord of mouse (Hayashi et al., 2018). They differ in their anatomical and physiological properties, particularly in the range of their projections (Menelaou et al., 2014), the relative strengths of their last-order vs higher-order connections, and their mode of spiking (Menelaou & McLean, 2019).

Moreover, it has been shown that the V2a neurons are connected via chemical synapses as well as electrical gap junctions. Interestingly, the gap junctions that connect neurons of the same type (V2a-I) in different segments and that provide fast coupling appear to operate highly asymmetrically, with neurons essentially affecting only neurons that are located more caudally (Fig. 3a in (Menelaou & McLean, 2019)). Asymmetric coupling by gap junctions has been observed in other systems, albeit to a smaller degree (Nolan et al., 1999; Apostolidis & Trussell, 2013; Sevetson & Haas, 2015). Thus, the coupling coefficient, which characterizes the voltage change induced in one V2a-I neuron by a voltage change in another V2a-I neuron, is different when going from neuron 1 to neuron 2 than vice versa. In contrast, the connections between V2a-I and V2a-II neurons in different segments are dominated by chemical synapses. So far, the function of these two populations of neurons and the role of the different types of connections are only poorly understood.

Here we investigate the role of the two neuron populations and the two types of connections in supporting swimming motion, with particular emphasis on the possible role of the connections between the two populations, which are characteristic of the hybrid model. We focus, in particular, on two aspects: on the ability of the system to quickly initiate...
the appropriate wave motion when swimming activity is ini-
tiated from random initial conditions and on the dependence
of the inter-segmental phase differences on the swimming
frequency. We find that in chains of V2a-I neurons that are
only connected via gap junctions the requirement of fast ini-
tiation of waves that propagate reliably can only be satisfied
for unphysiologically strong gap-junction coupling. Includ-
ing, however, the appropriately timed additional excitatory
input from V2a-II neurons via excitatory chemical synapses
reduces the required gap-junction coupling to realistic val-
ues. Moreover, the spike-frequency adaptation of the V2a-II
neurons provides a simple mechanism to limit the change
of the intersegmental phase difference with the frequency
of swimming.

2 Waves in gap-junction Coupled Chains
of V2a-I neurons

We are interested in the wave-like activation of succes-
sive segments of a long spinal cord. Motivated by the
work of Menelaou and McLean (2019) we focus on the
two types of excitatory V2a neurons. In this section we
consider only the V2a-I neurons and for simplicity assume
there is only a single such neuron in each segment. V2a-I
neurons in different segments are coupled by gap junc-
tions or by mixed connections comprised of an electri-
cal gap-junction component and a glutamatergic chemi-
cal component (Fig. 3c in (Menelaou & McLean, 2019)).
The strengths of these two types of connections seem to
be comparable and the temporal signature of the electro-
cal and chemical synapses appear readily distinguishable
(Fig. 4 in (Menelaou & McLean, 2019)). Interestingly,
these gap junctions appear to be unidirectional (Fig. 3a
in (Menelaou & McLean, 2019)) with the pure gap junc-
tions strongly dominating in number. We therefore focus
here on the gap junctions (Fig. 1). In Sect. 3 we include
the V2a-II neurons, which receive input from upstream
V2a-I neurons - predominantly via glutamatergic chemical
synapses - and which also excite their downstream V2a-I
neighbors via such synapses. We will refer to the most
rostral V2a-I neuron as the leading neuron and the rest as
follower neurons. Here we will ignore the lateralization of
the spinal cord and omit the inhibition between neurons on
its left and right side (Kozlov et al., 2009).

Both types of neurons receive input from the reticular
formation in the brain stem (Gahtan & O’Malley, 2003).
These brainstem neurons have varying projection lengths,
such that the shortest ones only reach the first couple of
segments, while the longest ones reach up to two thirds of
the whole spinal cord (Thiele et al., 2014). Thus, the total
amount of input that the V2a neurons receive is likely not
uniform along the spinal cord and depends on their loca-
tion in the spinal cord. For the animal to go into an organ-
ized swimming motion quickly it is important that it reli-
ably establish a stable phase-locked state within very few
oscillations (Fig. 2). Moreover, for the swimming motion
to be consistent across different swimming speeds the
intersegmental phase difference (ISPD) should not vary
substantially with frequency.

![Network structure of the model](image_url)

**Fig. 1** Network structure of the model. The full model is comprised
of an interleaved network of V2a-I and V2a-II neurons. The initial
analysis in Sect. 2 focuses on the network of V2a-I neurons only,
which are unidirectionally coupled by gap-junctions (pink). Each of
the V2a-I receives reticulospinal input.
To gain insight into the mechanism allowing these dynamics and to obtain guidance for numerical simulations we choose very simple models for the neurons, which allow an analytical perturbation approach. At the same time, the prominent presence of gap-junction coupling requires that the voltage evolution during the action potential is approximated reasonably well. We therefore do not use a simple integrate-fire model for the voltage \( v_k \) of the V2a-I neuron in segment \( k \), but the piecewise linear neuron model put forward by Chow and Kopell (2000), written in dimensionless form by absorbing the membrane capacitance and the leak conductance into the time \( t \) and into the definition of the reticular current \( I_{1k} \) injected into that neuron,

\[
\dot{v}_k(t) = I_{1k} - v_k(t) + \sum_{j=-\infty}^{\infty} A_t(t - t_{jk}) + g_k (v_{k-1}(t) - v_k(t)) H_k(t),
\]

\( 1 \leq k \leq N \) (1)

\[
\dot{v}_0(t) = I_{10} - v_0(t) + \sum_{j=-\infty}^{\infty} A_t(t - t_{0j}).
\]

(2)

Here the action potentials are not produced by a nonlinearity but by the inhomogeneous term \( A(t) \). It consists of the explicitly time-dependent exponential spiking current \( A_k(t) \), which is triggered whenever the voltage \( v_k(t) \) reaches the threshold \( v_T \) from below, and the recovery current \( A_R(t) \), which resets the voltage by \( \Delta_v \), at the end of the spike,

\[
A(t) = \begin{cases} 
0 & t < 0 \\
A_k(t) \equiv v_A e^{\tau t} & 0 < t < w \\
A_R(t) \equiv -\Delta_v \delta(t-w) & w \leq t.
\end{cases}
\]

(3)

In (1) the spike threshold is reached in neuron \( k \) at the times \( t_{ik}^{(j)} \). The spike duration in this model is given by \( w \) and the recovery current is modeled with a Dirac \( \delta \)-function, which leads to an instantaneous relative reset of the voltage by \( \Delta_v \),

\[
v_k(t_{ik}^{(j)} + w + \delta) = v_k(t_{ik}^{(j)} + w - \delta) - \Delta_v, \quad 0 < \delta \ll 1.
\]

(4)

The gap-junction coupling strength is given by \( g_{jk} \). Menelaou and McLean (2019) report that the gap junctions are essentially unidirectional, such that the \( k^{th} \) V2a-I neuron receives input from V2a-I neuron \( k-1 \), but not vice versa. Therefore, in (1) \( v_k \) does not depend on \( v_{k+1} \). The unidirectionality may reflect that the gap junctions are located on the axons projecting caudally from each neuron; an action potential traveling along the axon can then drive current into the soma of the coupled neuron and excite there an action potential, but a somatic action potential in that neuron might not trigger a back-propagating action potential on the axon for lack of a spike-initiation zone on the axon near the gap junction. This suggests that the gap junction is effectively only carrying significant current during the axonal action potential, during which the axial voltage gradients are large. In-between action potentials the axon is essentially passive and does not couple the cells strongly. In our simulations we include this aspect by turning the gap junction on only during the spike in the upstream neuron and for a duration \( w \) thereafter, which is indicated by the function

\[
H_k(t) = \sum_{l=-\infty}^{\infty} \left( H(t - t_{ik}^{(j)}) - H(t - t_{ik}^{(j-1)} - 2w) \right)
\]

(5)

in (1). Here \( H(t) \) is the Heaviside step function with \( H(t \leq 0) = 0 \) and \( H(t > 0) = 1 \). The simulations show that outside that interval the gap-junction current is very small (cf. Fig. 3 below). In our analytical treatment in Sect. 2.1 we omit this detail and have the gap-junction current active at all times \( H_k = 1 \).

In (1) we assume that neurons are only affected by neurons in the adjacent upstream segment. Menelaou et al. (2014) find that in zebrafish the axonal range of the neurons is highly heterogeneous, even among neurons of the same type, with some projections reaching across 20 segments and others only to their immediate neighbors. In this paper we will not consider this more complex connectivity, although it might contribute to the robustness of the network’s function (Varkonyi et al., 2008).
2.1 Asymptotic analysis for weak coupling

We first consider an infinite chain of V2a-I neurons that are coupled by gap junctions only and all receive the same reticulospinal input $I_I$. We are interested in a periodic traveling wave with frequency $f \equiv 1,000/T$. While all quantities are actually dimensionless, we have chosen parameters such that time can be thought of as measured in ms and frequency in Hz. In such a wave the voltage in segment $k$ can be written as

$$v_k(t) = V(t - k\phi T)$$  \hspace{1cm} (6)

with $V$ being a periodic function with period $T$. Here $\phi$ is the intersegmental phase difference (ISPD) between adjacent segments, expressed as a fraction of the period $T$. For simplicity we take the origin in time such that the end of the $l$th spike in segment $k$ occurs at time $t_l = lT + k\phi T$, and consider $V(t)$ only for $0 \leq t \leq T$. Due to the piecewise definition of the time dependence of $A(t)$ and the discontinuity generated by its recovery current (cf. (3)) the periodic solution $V(t)$ satisfies

$$V(T - w) = v_T$$  \hspace{1cm} (7)

| Parameter | Description | Value |
|-----------|-------------|-------|
| $I_{I,0}$ | Reticular current into the leading V2a-I neuron in segment $k = 0$. Determined from frequency $f \equiv 1,000/T$ via (17, 19, 25) |  |
| $v_f$ | Threshold for spiking current (3) | 1 |
| $v_a$ | Amplitude of spiking current (3) | 0.2 |
| $w$ | Spike width (3) | 0.5 |
| $a$ | Adaptation current (28) | \begin{cases} 0 & V2a-I \\ 1 & V2a-II \end{cases} |
| $b$ | Reset of adaptation current (28) | \begin{cases} 0 & V2a-I \\ 0.1 & V2a-II \end{cases} |
| $v_T$ | Threshold for spiking current (3) | 1 |
| $\Delta t$ | Difference between inputs to follower and leader neurons (26) | 9.1 |
| $\xi$ | Growth rate of spiking current (3) | 3.3 |
| $\Delta_v$ | Relative reset of the voltage terminating the spike (3) | 5 |
| $\tau_w$ | Time scale of adaptation current (28) | 5 |
| $a_f$ | Falling exponential rate in chemical synapse (30) | 5 |
| $E_{syn}$ | Reversal potential of the chemical synapse (29) | 10 |
| $g_g$ | Gap junction coupling (1) | 1 |
| $g_c^{(I)}$ | Strength of chemical synapse from V2a-II to V2a-I (if present) (29) | 0.1 |
| $g_c^{(II)}$ | Strength of chemical synapse from V2a-I to V2a-II (if present) (29) | 0.13 |

Fig. 3 Voltages $v_i(t)$ of a chain of electrically coupled V2a-I neurons. a The voltage $v_0(t)$ of the leader neuron (blue) is given by the $O(1)$-solution $V^{(0)}(t)$, while the voltage $v_1(t)$ of the follower neuron (red) has an $O(1)$-contribution $v_1^{(0)}(t) = V^{(0)}(t - \phi T)$ as well as an $O(\epsilon)$-contribution $v_1^{(1)} = V^{(1)}(t - \phi T)$, both delayed by $\phi T$ relative to the leader neuron. The threshold $v_T$ for the onset of the spiking current is indicated by the grey dashed line. b The normalized gap junction current is given to $O(1)$ by $g_g(V^{(0)}(t + \phi T) - V^{(0)}(t))$. In the simulations it is active only during the shaded area (cf. $H_k(t)$ in (1)). Outside that time interval the gap-junction current is quite small in the analytical solution. Parameters: $f = 77.7, \Delta I = 0.04$
\[ V(T + \dot{e}) = V(T - \dot{e}) - \Delta_v, \quad 0 < \dot{e} \ll 1 \]  \\( (8) \)

\[ V(0 + \dot{e}) = V(T + \dot{e}). \]  \\( (9) \)

To make analytic progress we assume the gap-junction coupling to be weak,
\[ g_g = \varepsilon g_{g1}, \quad 0 < \varepsilon \ll 1, \]  \\( (10) \)

and expand the membrane voltage \( V(t) \) also in \( \varepsilon \),
\[ V(t) = V^{(0)}(t) + \varepsilon V^{(1)}(t) + \mathcal{O}(\varepsilon^2). \]  \\( (11) \)

Our goal is to obtain the period as a function of the reticular input current \( I_1 \). This would suggest expanding \( T \) in \( \varepsilon \) for given \( I_1 \). However, because of the threshold and reset conditions \((7, 8)\) it is algebraically preferable to consider the period \( T \) as the independent variable and determine \( I_1(T) \). We therefore expand \( I_1 \),
\[ I_1 = I_1^{(0)} + \varepsilon I_1^{(1)} + \mathcal{O}(\varepsilon^2). \]  \\( (12) \)

At \( \mathcal{O}(1) \) this leads to
\[ V^{(0)} = I_1^{(0)} - V^{(0)}(t) + \sum_{l=-\infty}^{\infty} A(t - t_{l_k}^{(0)}) \]  \\( (13) \)

with
\[ V^{(0)}(T - w) = v_T \]  \\( (14) \)

\[ V^{(0)}(T + \dot{e}) = V^{(0)}(T - \dot{e}) - \Delta_v, \quad 0 < \dot{e} \ll 1 \]  \\( (15) \)

\[ v_0^{(0)} = V^{(0)}(0 + \dot{e}) = V^{(0)}(T + \dot{e}). \]  \\( (16) \)

Considering the interval \( 0 < t < T - w \) for given current \( I_1^{(0)} \) and period \( T \), the initial condition \( v_0^{(0)} \) has to be chosen so that \( V^{(0)} \) satisfies the threshold condition \((14)\). Unlike Chow and Kopell (2000), we consider the relative voltage reset \( \Delta_v \) to be fixed and allow \( v_0^{(0)} \) to vary. The solution that is valid in the interval \( T - w < t < T \) has to satisfy the threshold condition \((14)\) and the periodicity condition \((16)\), including the jump condition \((15)\). This determines \( I_1^{(0)} \) as a function of \( T \),
\[ I_1^{(0)} = \frac{v_T - e^{-T-w}}{1-e^{-T-w}} v_0^{(0)}, \]  \\( (17) \)

resulting in
\[ V^{(0)}(t) = \begin{cases} e^{-t}v_0^{(0)} + e^{t} (1 - e^{-T}) & 0 < t < T - w \\ e^{-t}v_0^{(0)} + e^{t} (1 - e^{-T}) + \frac{v_A}{1 + \varepsilon} \left( e^{\varepsilon(t-(T-w))} - e^{-(t-(T-w))} \right) & T - w < t < T \end{cases} \]  \\( (18) \)

with

\[ \varepsilon \quad \text{Springer} \]
We are interested in a regime in which the follower neurons spike only when they receive also excitatory input from their rostral neighbor via the gap junction. The reticular input \( I_{g,k} \) to the follower neurons therefore needs to be smaller than the input \( I_{g,0} \) to the leader neuron, i.e. \( \Delta I < 0 \).

This interpretation of the perturbation expansion will guide us in the simulations in the remainder of this paper. Along these lines, Fig. 3a shows the voltage traces of the V2a-I neurons in segment 0 and in segment 1. Note that the voltage of segment 1 plateaus at a lower value than that of segment 0, since it receives weaker reticular input current \((I_{g,1}^{(1)}) < 0\). Figure 3b displays the difference in the voltages of segment 0 and 1, which is proportional to the gap junction current.

### 2.2 Direct simulations

To assess the quality of the asymptotic approximation obtained in Sect. 2.1 we performed direct simulations of (1, 3) for finite chains. For computational ease we performed some simulations with relatively short lengths of \( N = 8 \) or \( N = 16 \). For key simulations we used, however, \( N = 32 \) and \( N = 33 \), corresponding to the range observed in zebrafish (Morin-Kensicki et al., 2002; Menelaou et al., 2014). The analytical expansion captured the full numerical simulation quite adequately, even for sizable values of the gap-junction coupling \( g_g \) (Fig. 4a). The fact that in the simulations the gap junction was effective only around action potentials while it was persistently active in the analytical calculation had only minor impact (see also Figs. 13, 14 in Appendix B). In the following we used the asymptotic approximation mostly as a guide for the numerical simulations. We therefore did not limit the values of \( g_g \) to values that were sufficiently small to yield quantitative agreement.

Of particular interest is the dependence of the ISPD \( \phi \) on the difference between the input to the leader neuron and that to the follower neurons,

\[
\Delta I = I_{g,k} - I_{g,0},
\]

and on the frequency \( f \) of the wave. For sufficiently negative \( \Delta I (\Delta I \gtrsim -0.02 \text{ in Fig. 4b}) \) and not too high frequencies \( f \) the ISPD \( \phi \) increases monotonically with \( f \) (Fig. 4b). Since \( \phi \) is defined as the spike-time difference relative to the period, this is the case when the time it takes a spike in neuron \( k-1 \) to push the voltage in neuron \( k \) across the spiking threshold does not decrease rapidly with increasing frequency. The gap junction current driving neuron \( k \) is proportional to the voltage difference between the two neurons. For fixed difference in the spike times of the two neurons that voltage difference increases with increasing slope of the voltage trace \( v(t) \). Since the slope increases with frequency, the gap-junction current increases, as well, decreasing the spike-time difference. If \( \Delta I \) is not too negative, this leads to a decrease in \( \phi \) for large frequencies (\( \Delta I \gtrsim -0.02 \text{ in Fig. 4b} \)) and a non-monotonic \( f \)-dependence. In the following, we will be mostly concerned with the frequency regime in which \( \phi \) increases with \( f \).

Ideally, the ISDP \( \phi \) would not depend on the frequency of the wave at all in order to keep the undulation wavelength constant. This would require that \( \Delta I \) be tuned quite precisely in parallel with any frequency changes. To avoid such a biologically unlikely complex control task we first investigate the dependence of \( \phi \) on the frequency of the wave in two simple scenarios (Fig. 4b, c):

![Fig. 4 Intersegmental phase difference \( \phi \). a) The numerical solution (yellow asterisks) converges to the asymptotic approximation (line) as \( \epsilon \to 0 \). \( f = 127.2 \), \( g_g = \epsilon \), \( \Delta I = \epsilon I_{g,0}^{(1)} \). b) Analytical (lines) and numerical (symbols) results for \( \Delta I(\phi) \) for \( f = 59.3, 77.7, 112.8, 127.2 \) (blue line as in (a)). 145.7, 170.7, 186.7, 206.7 (increasing to the right.). Scenario 1 (crosses): the base input for the whole chain, \( I_{g,0}^{(1)} \), is increased with frequency, while \( \Delta I \) is kept constant. Scenario 2 (circles): only the base input of the leader neuron is increased, implying that \( I_{g,1}^{(1)} \) is reduced to keep \( I_{g,k} \) constant. c) Same data as in (b), plotted as a function of the frequency for scenario 1 (crosses) and scenario 2 (circles) with chain length \( N = 8 \). The ISPD varies less with frequency for scenario 1.](image-url)
1. When changing the frequency, the synaptic reticulo-spinal input is adjusted uniformly for the whole chain, \( k \geq 0 \), while the leader neuron receives additional, frequency-independent input. Thus, \( \Delta I < 0 \) is kept fixed, while \( I_t^{(0)} \) is increased to increase the frequency.

2. When changing the frequency, the synaptic reticulo-spinal input \( I_{k0} \) to the leader neuron is changed. In this scenario \( \Delta I \) is reduced to more negative values as \( I_{k0} \) is increased in order to keep \( I_{k1} \) fixed for \( k \geq 1 \) (cf. (25, 26)).

For low frequencies both scenarios result in a very similar dependence of the ISDP on the frequency. For higher frequencies, however, the ISDP increases substantially faster with frequency in scenario 2, where only the input to the leading rostral neuron is increased (Fig. 4c). From this perspective scenario 1 would be preferable.

However, in scenario 1 the total input to the follower neurons is increased with increasing frequency. For high frequencies this can bring them above threshold, \( I_{t0} + \Delta I > v_T \), allowing them to fire on their own without being triggered by their rostral neighbor. As a result, when swimming motion is initiated from random initial conditions by a step in the input current, neurons in different segments fire out of order initially and it takes quite a few oscillation periods for the firing of the chain to become ordered in a rostral to caudal manner (Fig. 5, top right). In fact, during the transient quite a few segments exhibit negative phase lags, \( \phi < 0 \), reminiscent of experimental observations (Masino & Fetcho, 2005). Thus, for \( I_{k1} > v_T \), the initial swimming motion is expected to be disorganized. Indeed, the fraction of random initial conditions for which the correct firing sequence is established in simulations within at most 2 oscillation periods (color-coded in Fig. 5, top left) drops precipitously when \( \Delta I \) is increased above \( \Delta I > v_T - I_{k0} \) (corresponding to the red dashed line). We are therefore mainly interested in the regime in which the follower neurons constitute ‘potential oscillators’ (Tunstall et al., 2002) and spike only when triggered by their rostral neighbor. For weak gap-junction coupling the time to establish the correct firing sequence also becomes long.

When \( \Delta I \) is too negative for a given gap-junction coupling \( g_g \), not all spikes of the leader neuron trigger a wave that propagates all the way to the caudal end (black down arrows in Fig. 5, bottom right). In that case the wave propagating caudally has a lower frequency than the intended swim frequency or the wave may fail to reach the caudal end altogether. The minimal \( \Delta I \) that allows wave propagation at the leader frequency depends on the gap-junction strength (solid red line in Fig. 5, center, \( f = 263, N = 32 \)). For low \( g_g \), it increases rapidly with decreasing gap-junction strength to values for which the ordering is slow. For shorter chains and lower frequencies the relevant range of \( \Delta I \) extends further.

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**Fig. 5** Range of relevant \( \Delta I \) in scenario 1 without V2a-II neurons.
Left: Fraction of random initial conditions that reach the correctly ordered steady state within at most 2 oscillation cycles. The dashed line corresponds to \( I_{k21} = I_{t0} + I_t = v_T \) (\( f = 263, N = 33, 21 \) runs). Right top: Space-time diagram of the voltage for \( \Delta I = -0.035, \ g_g = 0.7, \ N = 32 \). Proper spike ordering is only obtained after \( \approx 17 \) cycles (star). Right bottom: For strongly negative \( \Delta I \) not all leader oscillations trigger a wave that reaches the caudal end (arrows, \( f = 263, N = 32 \)). Center: Above the dashed lines the spiking order is established too slowly, below the solid lines (with symbols) waves do not propagate properly. (red: \( f = 263, N = 32 \); blue: \( f = 77.5, N = 8 \)). The dotted lines indicate the minimal value of \( \Delta I \) if \( g_g \) is restricted to \( g_g \leq 1 \).
towards negative values, particularly for large $g_g$ (solid blue line for $f = 77.5$, $N = 8$).

For the chain to support waves that propagate along its full length and that establish the proper firing order quickly, the current difference $\Delta I$ has to lie below the dashed line and above the dotted line in Fig. 5 (center). For low frequencies (blue lines) this allows a significant range of parameters. With increasing frequency, however, the upper limit is shifted downward and for $f = 263$ the relevant range of parameters shrinks to a small region with $g_g$ near 1 and for very strong gap junctions, which are most likely unphysiological.

The range of physiologically sound and therefore relevant gap junction conductance can be estimated from the coupling coefficients measured between V2a-I neurons. The coupling coefficients between V2a-I neurons that are 2 to 4 segments apart were recorded to be in the range 0.005-0.02 (Menelaou & McLean, 2019). This suggests a coupling coefficient up to $\sqrt{0.02} \approx 0.38$. In our model the coupling coefficient for directly connected neurons can be derived as $\frac{g_0}{g_0+1}$.

The measurements therefore suggest that the maximal gap junction conductance $g_0$ is less than 1 and we will accordingly focus in the following on that range in the gap junction conductance.

A characteristic feature of gap junctions compared to chemical synapses is that the current they provide can change sign and be depolarizing or hyperpolarizing. To assess the impact of this aspect we simulated also the case of rectified synapses, which are only depolarizing. We found that the hyperpolarization that arises when the presynaptic voltage is lower than the postsynaptic one is a major factor limiting the hyperpolarization that arises when the presynaptic voltage is lower than the postsynaptic one is a major factor limiting the hyperpolarization that arises when the presynaptic voltage is lower than the postsynaptic one is a major factor limiting the hyperpolarization that arises when the presynaptic voltage is lower than the postsynaptic one.

For simplicity, we will assume that these chemical synapses (Menelaou & McLean, 2019). $V_{2a-I}$ and $V_{2a-II}$ are, however, predominantly chemical (glutamergic) synapses. $V_{2a-I}$ gap-junction connections, the connections between $V_{2a-I}$ and $V_{2a-II}$ neurons project caudally to the respective other type of neurons (Fig. 7). Unlike the $V_{2a-I}$ to $V_{2a-I}$ gap-junction connections, the connections between $V_{2a-I}$ and $V_{2a-II}$ are, however, predominantly via chemical (glutamergic) synapses. $V_{2a-I}$ and $V_{2a-II}$ neurons receive reticulospinal input, which we denote by $I_{\text{sp}}$.

$V_{2a-I}$ and $V_{2a-II}$ neurons project caudally to the respectively other type of neurons (Fig. 7). Unlike the $V_{2a-I}$ to $V_{2a-I}$ gap-junction connections, the connections between $V_{2a-I}$ and $V_{2a-II}$ are, however, predominantly via chemical (glutamergic) synapses (Menelaou & McLean, 2019). For simplicity, we will assume that these chemical synaptic projections extend only to the neighboring caudal segment. Thus, the $V_{2a-I}$ and $V_{2a-II}$ neurons form an interleaved network (Fig. 7). Importantly, in this network the $V_{2a-I}$ neurons of the spinal cord network and add $V_{2a-II}$ neurons to the model described above.

$V_{2a-II}$ neurons show significant spike-frequency adaptation; for current-step stimulation their spike frequency rapidly drops from 400Hz to 100Hz (Menelaou & McLean, 2019). To capture this behavior we extend the neuron model (1) and include an adaptation current $u$,

$$v_k = I_{\text{ill}} - v_k + \sum_{l=-\infty}^{\infty} A(t - t_k^{(l)}) - u_k,$$  \hspace{1cm} (27)

$$r_u u_k = av_k - u_k + b \delta(t - (t_k^{(l)} + w)),$$  \hspace{1cm} (28)

which hyperpolarizes the cell and is increased with each spike. The Dirac $\delta$-function in (28) incorporates a shift of $u$ by an amount $b$ at $t = t_k^{(l)} + w$, i.e. at the end of the $l$-th action potential. Due to the adaptation, the initial spike frequency is twice as large as the tonic frequency (Fig. 6a). In the model it contributes substantially to the early spiking of the $V_{2a-II}$ neurons compared to the $V_{2a-I}$ neurons (Fig. 6b) when the input current is stepped up above threshold, as is observed experimentally (Menelaou & McLean, 2019). In the spiking and recovery current $A(t)$ (cf. (3)) $t_k^{(l)}$ is the $l$-th time that the $V_{2a-II}$ neuron in segment $k$ reaches the spike threshold $v_r$.

Similar to the $V_{2a-I}$ neurons, the $V_{2a-II}$ neurons receive reticulospinal input, which we denote by $I_{\text{sp}}$.

In the following section, we therefore investigate how the feedforward synaptic connections mediated by $V_{2a-I}$ and $V_{2a-II}$ neurons can expand the relevant range of $\Delta I$ and discuss how to keep the ISPD relatively constant over a range of frequency.

### 3 Interleaved chains of $V_{2a-I}$ and $V_{2a-II}$ neurons

The two types of $V_{2a}$ interneurons exhibit quite distinct morphology and electrophysiology. The resulting functional differences are not yet fully understood. Here, we explore the possible role of $V_{2a-II}$ neurons in the rhythmic control

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**Fig. 6** Spike-frequency adaptation of $V_{2a-II}$, but not of $V_{2a-I}$ neurons. (a) Instantaneous frequency defined via the inter-spike intervals for step-current input for $V_{2a-I}$ and $V_{2a-II}$ neuron. (b) Voltage traces for $V_{2a-I}$ (blue, cf. (1)) and for $V_{2a-II}$ (red, cf. (27, 28)) in response to a step in the input current at $t = 5$.
receive input from the V2a-II neuron of their rostral neighbor segment and the relative timing of these two inputs, which is a major determinant of the wave propagation and its ISPD, can be controlled by changing, e.g., the bias current $I_{II}$. To model the chemical synapse on the V2a-I neuron we include for each spike at time $t_{I}^{(l)}$ of the presynaptic V2a-II neuron a conductance-based synaptic current with reversal potential $E_{syn}$ to (1),

$$I_{syn}(t) = g_{c}^{(l)}(v_{k}(t) - E_{syn}) \sum_{I} s(t - t_{II}^{(l)}).$$  

(29)

Here $E_{syn}$ is the synapses’ reversal potential. The current is assumed to have a fixed waveform given by the difference between two exponential functions,

$$s(t) = H(t) s_{0}(e^{-\sigma_{f}t} - e^{-\sigma_{r}t}),$$  

(30)

with $H(t)$ denoting the Heaviside step function. With the normalization factor $s_{0} = \sigma_{r} - \sigma_{f}$ the maximal conductance is $g_{c}^{(l)}$. Analogously, we model the chemical synapses driving the V2a-II neurons based on the spiking of the V2a-I neurons. Its strength is taken to be $g_{c}^{(II)}$. For both types of synapses we use the same time constants.

While the gap junction results in depolarizing and in hyperpolarizing input, the excitatory chemical synapse ($E_{syn} > v_{T}$) provides only depolarizing input to the V2a-I neurons (Fig. 8). This input has a different effect than increasing the tonic base input $I_{T}$, since it is active only over a short time window, the timing of which is controlled by the upstream V2a-II neuron. The additional excitation allows to reduce $\Delta I$ substantially without jeopardizing the propagation of the wave. At the same time, since the excitation is only triggered when the rostral neighbor spikes, the maximal value of $\Delta I$ that allows sufficiently fast ordering is hardly affected (compare Fig. 9, left with Fig. 5, left). Thus, there is a wide range of $\Delta I$ for which reliably propagating waves are established within 1 or 2 oscillations (Fig. 9, right center). In fact, while without V2a-II input almost no values of $\Delta I$ are relevant for fast swimming at physiological gap-junction strengths, with chemical synapses there is a substantial relevant range of $\Delta I$ for all values of the gap-junction strength that we are interested in (Fig. 9, center).

Fig. 7 Model of interleaved network of V2a-I and V2a-II neurons. V2a-I neurons drive V2a-I neurons directly via gap junctions and indirectly via chemical synapses through the V2a-II neurons. The bias current into the V2a-II controls the relative timing of the two inputs. Unlike the other V2a-II neurons, the V2a-II neuron in segment 0 receives input from the V2a-I neuron in the same segment since there is no rostrally neighboring segment. Both types of neurons receive reticulospinal input. V2a-I neurons are connected unidirectionally via gap junctions.

Fig. 8 Inputs to the V2a-I neurons via chemical synapses and gap junction. Top: voltages $v_{k-1}$ and $v_{k}$ of V2a-I neurons $k$ (solid) and $k - 1$ (dashed). Bottom: total synaptic input current (red, thick) arising from a chemical synapse (blue), which is driven by a spike in a V2a-II neuron that is triggered by the spike of V2a-I neuron $k - 1$, and a gap junction (green) between the V2a-I neurons $k - 1$ and $k$. 

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Fig. 9 Range of relevant values of $\Delta I$ with V2a-II neurons (cf. Fig. 5). Left: Fraction of random initial conditions that reach the correctly ordered steady state with at most 2 oscillation cycles. The dashed line corresponds to $I_{12} = I_{1}^{0} + I_{1} = v_{r}$ ($f = 263, N = 33, 11$ runs). Right panels: space-time diagrams of the voltage for $f = 263, N = 32$. Top: proper spike ordering is interrupted by additional spikes ($\Delta I = -0.04, g_{c} = 0.8$). Center: fast establishment of properly propagating wave ($\Delta I = -0.56, g_{c} = 0.8$). Bottom: not all leader oscillations trigger a wave that reaches the caudal end ($\Delta I = -0.64, g_{c} = 0.8$). Center: Above the dashed lines the spiking order is established too slowly, below the solid lines waves do not propagate properly. (red: $f = 263, N = 32$; blue $f = 77.5, N = 8$). The dotted lines indicate the minimal value of $\Delta I$ if $g_{c}$ is restricted to $g_{c} \leq 0.6$. Other parameters: $I_{II} = 1.2$

3.1 Control of ISPD $\phi$ by V2a-II neurons

To gain insight into the impact of the chemical synapses on the ISPD $\phi$ between the V2a-I neurons we include the synaptic current (29) in our asymptotic analysis and consider it to be of the same order as the gap junction current from the rostral V2a-I neuron. At $O(\epsilon)$ in the asymptotic expansion the equation can then be written as

$$
\dot{v}_{k}^{(1)} = E - V_{k}^{(0)} + g_{c}^{(1)}(v_{k}^{(0)}(t) - v_{k-1}^{(0)}(t)) + \sum_{l = -\infty}^{\infty} g_{c} \epsilon v_{k}^{(0)},
$$

(31)

(cf. (29) and (10) with $g_{c}^{(1)} = \epsilon g_{c}^{(1)}$). Note that the conductance depends on the time $t_{I,k-1}$ at which the V2a-II neuron in segment $k - 1$, which drives the chemical synapse, reaches the spike threshold. This depends in turn on the spike time of the preceding V2a-I neuron in segment $k - 2$. The delay $\tau_{\Delta}$ of the spikes of the V2a-II neuron in segment $k - 1$ relative to the spikes of the V2a-I neuron in segment $k - 2$ that drive it will be seen to play an important role. Focusing on periodic solutions with period $T$ that are phase-shifted by $\phi$ between consecutive segments (cf. (6)), we obtain then

$$
\dot{v}_{k}^{(1)} = E - V_{k}^{(0)} + g_{c}^{(1)}(v_{k}^{(0)}(t + \phi T) - v_{k}^{(0)}(t)) +
\sum_{l = -\infty}^{\infty} g_{c} \epsilon s(t - (IT + \tau_{\Delta} - w - 2\phi T))(E_{syn} - v_{k}^{(0)}),
$$

(32)

As in the case without chemical synapses, these equations can be solved analytically, but the resulting expressions are too involved to display explicitly. Again we use the analytical results as guidance for the direct simulations without aiming for quantitative agreement.

While the frequency range over which waves propagate robustly along the chain of V2a neurons is increased with the addition of V2a-II neurons (cf. Figs. 5, 9), the ISPD $\phi$ still increases significantly with increasing frequency (Fig. 10a, b and c). Thus, for fixed $\Delta I$ the undulating swimming pattern of the animal still changes with swimming speed (cf. Fig. 4b). However, if we allow $\Delta I$ to become less negative with increasing frequency, the range in $\phi$ can be reduced. The minimal and maximal values of $\phi$ are then determined by the range of relevant $\Delta I$ values (cf. Fig. 9), which is marked by the solid portion of the thick lines in Fig. 10a, b, and c. Thus, the minimal variation in $\phi$ is given by the difference between the maximal $\phi$ at the lowest frequency and the minimal $\phi$ at the highest frequency. Without chemical synapses the ISPD varies then at least from 0.029 to 0.041 across the frequency range 77.5 $\leq f \leq$ 204 (blue lines for perturbation results and diamonds for numerical results in Fig. 10a).

Through the chemical synapses the input from V2a-II neurons substantially reduces the lower limit of $\Delta I$ and with it increases the maximal $\phi$. As a result, the $\phi$-values associated with the relevant range of $\Delta I$ can overlap significantly for different frequencies, allowing $\phi$ to be kept independent of frequency by varying $\Delta I$. For instance, for
\( \tau_a = 0.1 \) the ISPD can be held constant over the frequency range \( 77.5 \leq f \leq 204 \) for any value of \( \phi \) within the range \( 0.01 \leq \phi \leq 0.03 \) (Fig. 10b). Such a range in the ISPD exists also for larger \( \tau_a \) (Fig. 10c). Thus, through the chemical synapses the difference \( \Delta f \) in the reticulospinal current allows to adjust \( \phi \) - and with it the wavelength of the wave - over some range and to keep it independent of the frequency.

Since the ISPD \( \phi \) depends significantly on the delay \( \tau_a \) with which the V2a-II spikes relative to the V2a-I in the preceding segment (Fig. 10d), tuning that delay can also render \( \phi \) independent of frequency (bright red solid circles in Fig. 10d, f). This can be achieved by varying the baseline input \( I_{II} \) to the V2a-II neurons, which varies the spike times of the V2a-II neurons. The tuning range is limited by the condition that \( I_{II} \) remain in the range that allows reliable spiking of V2a-II neurons for given frequency \( f \). To the left of the left blue line in Fig. 10d the reticulo-spinal input \( I_{II} \) to the V2a-II neurons would have to be chosen so large that they spike irregularly and more than once per cycle, while to the right of the right blue line they do not spike at all. Keeping \( \phi \) constant requires \( \tau_a \) to become quite small when the frequencies are large. To wit, to the left of the black dotted line \( \tau_a = \phi T \) the V2a-II spikes earlier than the V2a-I in the same segment. Since the chemical synapse that drives the V2a-II neuron introduces a slight delay compared to the gap-junction that drives the V2a-I neuron, the situation \( \tau_a < \phi T \) might be unexpected. However, in experiments the V2a-II neurons are indeed observed to fire on average before the corresponding V2a-I neurons (Menelaou & McLean, 2019).

Thus, the synaptic input from the V2a-II allows the ISPD of the propagating wave to be kept constant by tuning the reticulospinal input to the V2a-I (\( \Delta f \)) or to the V2a-II neurons (\( I_{II} \)). However, such a detailed tuning of these currents when varying the frequency may not be physiologically realistic. We therefore investigate in the next section the simpler,
approximate strategy of having the V2a-II neurons spike significantly only for higher frequencies.

3.2 Spike-frequency adaptation of V2a-II neurons and ISPD control

For low values of $\tau$, the additional input from the V2a-II neurons reduces the ISDP $\phi$ of the wave. Since $\phi$ increases with the frequency of the wave, it is expected that the overall variation of $\phi$ with varying frequency can be reduced by having the V2a-II neurons being active only in the upper frequency range. An interesting question is therefore, whether the activity of the V2a-II neurons can be restricted to higher wave frequencies without any tuning of the reticulo-spinal input $I_{II}$ to the V2a-II neurons with frequency.

In the scenarios considered here the V2a-II neurons spike in response to the inputs they receive from the V2a-I neurons in their rostral neighbor segment. If the V2a-II neurons were simple integrators (cf. (1)) they would start spiking when the number of input spikes they receive within their temporal integration window was sufficiently large. They could therefore turn on at higher frequencies, if in each cycle the V2a-I neurons spiked in bursts and the number of spikes per burst increased with swimming frequencies. Considering the fact that in each segment there are multiple V2a-I neurons, it would also be sufficient if the fraction of V2a-I neurons that spike in each cycle and provide input to the V2a-II neuron increased with swimming frequency.

In view of the substantial spike-frequency adaptation of the V2a-II neurons (Fig. 1h in Menelaou & McLean, 2019) another interesting scenario is conceivable, which would not require burst firing or larger neuron populations. Implementing the spike-frequency adaptation by extending the minimal neuron model (1) to include an adaptation current (27, 28) gives the neurons the character of a resonator (Izhikevich, 2003). Thus, they are most responsive to inputs arriving within discrete, short time windows, and even if the V2a-II neuron always were to receive only a single spike as input, the timing of that spike can determine whether the V2a-II neuron spikes or not. Since the timing varies with the frequency of the wave, this could drive the V2a-II to spike at higher but not at lower frequencies. Thus, without the V2a-II neurons the ISDP $\phi$ varies substantially with frequency (green crosses in Fig. 11). With the V2a-II neurons included, however, the variation in $\phi$ is much smaller (black circles in Fig. 11), since $\phi$ is shifted down significantly when the V2a-II neurons fire driven by correctly timed rostral V2a-I neuron spikes (cf. Fig. 12 in Appendix A). This occurs above a threshold frequency ($f_{th} \approx 91.2$ in Fig. 11).

Thus, the spike-frequency adaptation of the V2a-II neurons can provide a mechanism that selectively provides additional current to V2a-I neurons during fast but not slow swimming. The ISPD then still increases with frequency in both dynamic regimes, but the downshift of the ISPD when the V2a-II neuron becomes active reduces the overall variation of $\phi$ substantially. Based on this model one may surmise that having a variety of V2a-II neuron populations that switch on at different frequencies might decrease the range of ISPD further, as each activated V2a-II neuron will provide additional input that can reduce the time it takes the V2a-I neuron to become activated.

4 Discussion

This paper has been motivated by a recently proposed new model for the organization of the spinal cord of zebrafish larvae (Menelaou & McLean, 2019). Classically, two types of connectivity models for such motor circuits have been proposed. In the hierarchical, multi-layer model two separate interneuron populations make the higher-order connections, which receive control signals from the brain, and the last-order connections to the motorneurons, respectively (McCrea & Rybak, 2008; Rybak et al., 2015). In the single-layer model there is only a single interneuron population that maintains both types of connections (Kozlov et al., 2009). The experimental results of Menelaou and McLean (2019) suggest a hybrid combination of these two models, since it identified two separate, but interacting populations of excitatory interneurons (V2a-II) that each make last- and higher-order connections, albeit to different degrees. In particular, V2a-I neurons make weaker last-order connections to the motor pool than the V2a-II neurons. The roles that these different populations play in generating swimming motion have not been clarified yet.

![Fig. 11 Adaptation of V2a-II neuron and $\phi$ control](image-url)
Our minimal model of such a hybrid axial circuit suggests that the synergetic interaction of the two types of interneurons may serve dual objectives. Fast swimming requires relatively strong input into the leader neuron. At the same time, the input into the follower neurons has to remain weak enough to avoid that they spike without being triggered by their rostral neighbor. Otherwise, it takes too long for the different segments to establish the correct spiking order when starting from random initial conditions. For the wave to propagate along the whole spinal cord for such a large difference in the inputs requires unphysiologically strong gap-junction coupling. Appropriately timed input from the V2a-II neurons to the V2a-I neurons via the chemical synapses can, however, overcome this limitation. It allows propagating-wave solutions in long chains even when the tonic input to the V2a-I neurons is weak enough not to interfere with the rapid establishment of the correct spiking order along the segments.

In addition, the synaptic input from the V2a-II neurons to the V2a-I neurons modifies the ISPD depending on its timing relative to the gap-junction input that the V2a-I neurons receive from their rostral V2a-I neighbor. This timing depends on the tonic input to the V2a-II neurons. The ISPD can therefore be kept constant by a suitable tuning of the tonic input to the V2a-II neurons. Less precise, but more robust and requiring very little tuning is a binary control scheme in which the V2a-II neurons spike only in the upper range of swimming frequencies. This aspect emerges naturally in the model through the spike-frequency adaptation that the V2a-II neurons exhibit (Menelaou & McLean, 2019): there is a limited time window after each spike in which the V2a-II neurons are more excitable than at a later time. If the wave frequency is too low, the rostral inputs come too late and do not elicit any V2a-II spikes.

How does this control scheme compare with further observations of Menelaou and McLean (2019)? Their Fig. 2c shows that the firing reliability of the V2a-II neurons increases quite sharply at a swim frequency of 35Hz. It is tempting to interpret this as the turning-on of the V2a-II neurons that results from the interaction. This becomes, however, significant only for stronger coupling when the current induced by the coupling becomes of the order of the spiking currents themselves. An exact treatment of the gap junction coupling that includes changes in the wave forms is possible in a population description of heterogeneous quadratic-integrate-fire neurons (Pietras et al., 2019).

Multi-layer and single-layer models differ in characteristic ways in their response to the deletion or inhibitory suppression of specific neurons (McCrea & Rybak, 2008). In single-layer models the inhibition of the last-order neurons, which give output to the motor pool, suppresses the whole rhythmic activity since these neurons are also providing the timing information. Brief such suppressions reset the rhythm and are therefore called resetting deletions. In contrast, in multi-layer models the inhibition of the last-order neurons does not affect the higher-order neurons and has no effect on the rhythm (‘non-resetting deletion’). In the hybrid model, the suppression of V2a-II neurons does not suppress or reset the rhythm. However, it suppresses the input to the
V2a-I neurons from the V2a-II neurons via the chemical synapse. The model therefore predicts an increase in the ISPD between the segment in which the suppressed neuron is located and its caudal neighbor. Thus, relative to the most rostral segment the phases of all segments caudal to the suppressed neuron will be shifted by the same amount. If V2a-II neurons are suppressed in multiple segments the phase shift of the more caudally located segments is predicted to be proportional to the number of suppressed neurons. Measurements of the phase shift resulting from suppressing neuronal spikes should therefore allow an experimental assessment of the role of the chemical synapses between V2a-I and V2a-II neurons in the timing control during swimming and would provide a test of the model.

In multi-layer models higher-order neurons are typically associated with timing control, while last-order neurons are assumed to play a key role in controlling the amplitude of the motion. Thus, increasing tonic reticulospinal input to the last-order neurons can increase the number of spikes elicited by each timing pulse emitted by the higher-order neurons, thus increasing the amplitude of the motion. The increased tonic input will also affect the timing of the spikes in the last-order neurons, but if they all receive the same reticulospinal input that phase shift would be the same for all segments and the ISPD would be unaffected. Thus, the wavelength of the undulation would be independent of the amplitude.

This independence is not likely to persist in the hybrid model. Even though it features two different excitatory populations, they interact with each other via chemical synapses. In the hybrid model discussed here a global change in the reticulospinal input leads to a similar change in the spike timing of all V2a-II neurons and with it of the input to the V2a-I neurons via the chemical synapses. Since that input modifies the ISPD between the V2a-I neurons, a global change in the reticulospinal input will induce a change in all ISPDs. Thus, in contrast to the wavelength of the multi-layer model, the wavelength of the hybrid model investigated here varies with the amplitude of the motion. Based on the regime considered here, in which the ISPD decreases with increasing tonic input to the V2a-II neurons, the model predicts that at fixed swimming frequency stronger swimming motions would be associated with longer wavelengths of the undulation.

The experimental observations suggest that in zebrafish the V2a-I neurons resemble more closely first-order neurons, while the V2a-II neurons are more akin to last-order neurons. What distinguishes this network from a hierarchical model are the connections from the V2a-II neurons back to the V2a-I neurons. In our computational model these connections play a key role in enabling the network to support reliably and properly ordered propagating waves that can be established quickly from random initial conditions with ISPDs that do not depend strongly on the swimming frequency. While these requirements are likely to be important for undulatory swimming motion, they are less relevant for legged locomotion. This could explain why the zebrafish spine has this hybrid structure, while the spinal networks of four-legged animals have a hierarchical structure (Rybak et al., 2015).

Our analysis shows how even in a purely feedforward model the variation of the ISPD with frequency can be reduced by mutual coupling of the two types of neurons by gap junctions and chemical synapses and by exploiting the frequency-dependent response of the adaptive V2a-II neurons. The frequency dependence observed in zebrafish seems, however, yet weaker (Masino & Fetcho, 2005). It is likely that the performance of the full network is enhanced in addition by feedback. This can be provided by ascending connections of the V2a-II neurons (Menelaou et al., 2014), which provide rostral neurons with information about the phase of caudal neurons, and by sensory input from ventral stretch receptors, which are known to modulate the ISPD in leech (Cang & Friesen, 2000). We expect that the feedforward model can provide insight about where and what kind of feedback input should enter the circuit to support robust swimming motion.

While on average the experimentally observed ISPD is remarkably independent of the frequency, the ISPDs are very noisy and can occasionally even be negative (Masino & Fetcho, 2005). Since the magnitude of the ISPDs is quite small in multi-segment animals, even weak perturbations may be sufficient to render some of them negative. For uncorrelated noise the fluctuations in the phase difference between segments is expected to grow with the distance between the segments, but only like the square-root of the distance, in contrast to the mean, which grows linearly with the number of segments. This would leave the overall phase lag across the whole spinal cord relatively robust. Since both V2a-I and V2a-II neurons contribute to the ISPD, an interesting open question is whether their cooperation might reduce the impact of local noise that is not correlated between the two neuron types.

Experimental results strongly suggest that a key role of the V2a-II neurons is the control of the amplitude of the swimming motion (Menelaou & McLean, 2019). In our minimal hybrid model the activity of the V2a-II neurons turns out to be a major determinant of the ISDP $\phi$. Thus, our model predicts that the ISPD and the amplitude are not independent of each other. At this point the encoding of the amplitude in the V2a-II activity pattern is, however, not sufficiently well known to warrant a detailed model capturing this aspect.

In this study we have only considered simple periodic swimming activity. An important component of that motion is its left-right alternation. We expect that this can be captured by a straightforward extension, as it has been done in many previous studies (e.g. Kozlov et al., 2009), in which corresponding populations on the left and right side inhibit each other via interneurons like the V0d neurons (Menelaou & McLean, 2019). The role of the various neuronal populations in other,
more complex motions like turning, orienting, or self-righting remains an interesting open question.

Appendix 1

Adaptation of V2a-II neuron and $\phi$ control

A key feature arising from the adaptation current is the overshoot in the voltage that occurs during the recovery phase after a spike (black arrow near $t = 30.5$ in Fig. 12a, b). Synaptic input from the V2a-I neuron arriving during that overshoot can drive an action potential in the V2a-II neuron (blue triangles in (Fig. 12a), even if it is too weak to elicit a full action potential at a later time (red triangle near $t = 38$). Thus, inputs arriving at a higher frequency will drive V2a-II spikes and reduce the ISDP $\phi$, while low-frequency inputs of the same amplitude will not elicit any spikes (Fig. 12a). The overshoot does not arise in the relevant time window if the previous input to the neuron only

![Graph](image)

**Fig. 12** Adaptation of V2a-II neuron and $\phi$ control. (a-c) Numerical solutions for V2a-II neurons for different input frequencies $f$. (a) Bistability between no-spiking for low-frequency periodic input ($f = 77.5$) and spiking for high-frequency input ($f = 112$). Arrow marks the peak of the overshoot. Dashed grey line: spiking threshold. (b) When switching the input from high to low frequency the V2a-II stops firing (red line). (c) Switching from low to high frequency does not trigger spiking in the V2a-II (blue line). Parameters: $g_{\text{in}}^{(II)} = 0.055$, $\Delta I = -0.04$, $I_{\text{II}} = 1.4$, $N = 8$
depolarized it without triggering an action potential (green arrow in Fig. 12b, c). In that case, even input that arises early does not evoke an action potential (blue triangle in Fig. 12c).

Thus, in this regime the V2a-II neuron exhibits significant hysteresis: once a spike is triggered, inputs with sufficiently high frequency maintain the spiking (Fig. 12b), but they do not initiate a transition to spiking. However, spiking will cease, when the input frequency becomes too low (Fig. 12a).

For the synaptic strength used in most of the paper ($g_{c}^{(II)} = 0.13$) the V2a-II neurons spike over the whole range of frequencies investigated. In Fig. 11 we consider therefore weaker synaptic strengths ($g_{c}^{(II)} = 0.055$) for which the spiking depends on the timing of the inputs and therefore on the frequency of the wave.

For large $f$ the V2a-II spike and reduce the ISPD $\phi$. When the frequency is lowered below the threshold $f_{th} \approx 91.2$, the V2a-II neurons stop spiking and have $\phi$ jump up to the upper branch (blue arrow). Due to the hysteresis in the spiking transition (Fig. 12c) it is not sufficient to merely increase the frequency now above $f_{th}$ to induce spiking and have $\phi$ jump down to the lower branch. However, a brief boost in the base input $I_{ll}$ whenever the frequency is changed would trigger an initial spike of the V2a-II neuron. The resulting overshoot would be sufficient to induce repetitive spiking for high frequencies, but not for low frequencies (Fig. 12a). It would therefore induce a jump to the lower branch (red arrow).

**Wave propagation with rectifying and persistent gap junctions**

A characteristic feature of most gap junctions is that they can be depolarizing and hyperpolarizing, depending on the voltage difference across the junction. In a wave propagating along the segments this difference will be negative during a brief time when the presynaptic cell has spiked already, but the postsynaptic cell is just about to spike (cf. Fig. 3b).

To assess whether this hyperpolarization can suppress the propagation of the wave we have simulated also a version of the model in which the gap-junction current is rectified, i.e. it is set to 0 when it is hyperpolarizing. Indeed, in that case waves can propagate for significantly more negative values of $\Delta I$ (Fig. 13, open symbols vs filled symbols). Moreover, while for non-rectified gap junctions increasing their strength does not substantially enhance the propagation range, that range grows linearly for rectified gap junctions.

For the fast electrical coupling no hyperpolarizing current was observed in (Menelaou & McLean, 2019). Note, however that the currents shown in their Fig. 3 were measured in response to a single action potential in the presynaptic cell and no spike in the postsynaptic cell. In such a configuration a purely depolarizing gap-junction current is obtained also in our model without employing a rectifier, if $\Delta V$ is suitably reduced.

Motivated by the apparent unidirectionality of the gap junctions in (Menelaou & McLean, 2019), we assumed that they are only effective during the strong voltage deflections associated with action potentials. This feature does, however, not have substantial impact on the wave propagation and merely shifts the propagation limits upward by some amount (Fig. 13, red vs blue symbols).

![Fig. 13 Wave propagation without V2a-II. If the gap junctions are only depolarizing, the parameter range in which waves propagate extends to more negative values of the current difference (open symbols). Whether the gap junctions are active only during action potentials (blue symbols) or all the time (red symbols) affects the parameter range for propagation only modestly.](image1)

![Fig. 14 Wave propagation with V2a-II. If the gap junctions are only depolarizing, the parameter range in which waves propagate extends to more negative values of the current difference (open symbols). Whether the gap junctions are active only during action potentials (blue symbols) or all the time (red symbols) has a moderate impact on the parameter range for propagation.](image2)
Note that in (Menelaou & McLean, 2019) gap-junction currents (of both signs) were measured also for small voltage deflections. However, this coupling was attributed to a slow, indirect, electrical coupling between cells (Fig. 7 in (Menelaou & McLean, 2019)) and not the fast direct coupling between V2a-I neurons discussed here.

In the presence of chemical synapses the effect of the hyperpolarization is less pronounced (Fig. 14).

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Declarations

Conflicts of interests/Competing interests The authors have no relevant financial or non-financial interests to disclose.

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