Temporal precision and reliability in the velocity regime of a hair-cell sensory system: the mechanosensory lateral line of goldfish, *Carassius auratus*

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FISH HAVE DEVELOPED A UNIQUE SENSORY system that enables them to detect minute water motion, i.e., the mechanosensory lateral line. This sensory system enables them to navigate their environment efficiently, to localize prey, and/or to prevent predation (for review see: Bleckmann 1994). As a short-range sensory system, it enables animals to detect objects within a distance of one body length (Čurčič-Blake and van Netten 2006). Contrary to other sensory systems, we at present have no detailed understanding on the encoding capability of the lateral line system.

Among teleost fishes, the lateral line organs can be subdivided into two subsystems that are composed of hair-cell-based receptors called neuromasts (Webb 1989a). Neuromasts are grouped in two oppositely oriented hair cell populations that are each innervated by a primary afferent (Flock and Wersäll 1962; Nagiel et al. 2008). A given afferent may innervate multiple neighboring neuromasts (Münz 1979), while maintaining the orientation selectivity (Nagiel et al. 2008). Neuromasts occur freestanding on the skin as superficial neuromasts (SN) and in subdermal canals that communicate with the surrounding water through pores, i.e., the canal neuromasts (CN) (Webb 1989b). For either subsystem, the stimulus driving the receptors is the relative motion between the water surrounding the animal and its surface. These motions translate into spatially and temporally varying flow and pressure fields across the fish’s body.

The anatomical differences between SNs and CNs are associated with physiological differences. Most importantly, CNs are insensitive to low-frequency perturbations in a flow-field (Engelmann et al. 2002; Engelmann et al. 2000; Kroese and Schellart 1992). Consequently, CN afferents show unmasked responses to a dipole stimulus presented in a bulk water flow, while the responses to local oscillations of the water as induced by an oscillating sphere (dipole) are heavily masked in SN afferents (Engelmann et al. 2000; Engelmann et al. 2002).

To understand the physical nature of a given stimulus acting on the neuromasts, the transition between the water and the skin of the animal needs to be considered. In this region, the viscosity of the water causes it to move slower the closer one gets to the skin, until no flow occurs at the skin itself. The region within which the velocity is reduced by >1% is the so-called boundary layer (Schlichting and Gertsen 2003). While the boundary layer can be neglected for the encoding capability of the CNs (Čurčič-Blake and van Netten 2006; Goulet et al. 2008), the extent to which the boundary layer influences the stimulus transfer to the SNs is a matter of current debate (Rapo et al. 2009).

SNs are involved in different behaviors, including near-range orientation (Hassan et al. 1992). The ability to utilize SN information for near-range orientation, however, recently has been challenged (Rapo et al. 2009). By means of hydrodynamic simulations, these authors show that the intensity of the stimulus diminishes rapidly within the boundary layer. How-
ever, to which degree this may influence the ability to detect hydrodynamic stimuli, especially the flow profile parallel to the sensory surface, has not been investigated yet. To gain a basic understanding of the level of information SN can provide about a given stimulus, information theory approaches are needed to determine how well SNs can actually encode water motions of varying frequency contents.

Here, we investigated the spiking-response properties of SNs primary afferents of goldfish (Carassius auratus) to sinusoidal and various noise stimuli using a linear reverse correlation approach and covariance analysis. Our aim is to understand the transformation from the physics of simple hydrodynamic stimuli to their neuronal representation at the afferent level. Previous studies mainly focused on spike-rate-based encoding, which we here want to contrast to the hypothesis that spike-time-based encoding might shed new insights on the features of this system. With this covariant matrix approach justication, we show that two stimulus features can be relevant, velocity and acceleration, of which velocity is most limited, and that a linear-model does not capture all the number of stimuli a neuron is responsive to is a priori not neglected for the interpretation of physiological data. We further detail the encoding capability of SNs and link these to potential encoding mechanisms.

**MATERIAL AND METHODS**

*Recording and stimulation.* All experimental procedures were carried out in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources (National Institutes of Health), the European Directive 86/609/EEC concerning the protection of animals used for experimental and scientific purposes, and the Treaty of Amsterdam Protocol on Animal Welfare (1997). The experiments comply with the current animal protection law of the Federal Republic of Germany (TierSchutzgesetz), and protocols were reviewed and approved. For details regarding animal handling and recordings see Chagnaud et al. (2006). In short, goldfish (Carassius auratus) were anesthetized, immobilized, and fixed in a flow tank; the posterior lateral line nerve was exposed close to its entry at the brain. Following the preparation, animals were allowed to recover from the MS-222 anesthesia, and single afferents were recorded using high-impedance electrodes.

As a global hydrodynamic stimulus, we used bulk water flow of 10 cm/s (for details see Chagnaud et al. 2006). Local stimuli consisted of ramped sine waves of 2-s duration and band-pass filtered white noise signals, which were used to drive a sphere (6 mm diameter) parallel to the long axis of the fish. The sphere was placed 5–8 mm laterally to the neuromasts, presumably innervated by the afferent recorded from. For the range of frequencies tested (10–150 Hz), the boundary-layer thickness ranges from roughly 500 to 50 μm (Bleckmann 1994).

Two different types of noise stimuli were presented: 1) noise stimuli of 1-s duration were repeated 100 times (frozen noise); and 2) continuous noise of 120 s duration. Both stimuli had zero mean amplitude. Continuous stimuli had a linear frequency range between 10 and 150 Hz, based on the power spectra of the actual displacement of the sphere. Sphere motion of the continuous noise stimulus was recorded simultaneously to the neuronal responses (gauging sensor 2804, 4810 amplifier, AD Technologies). The nonlinearity of the shaker used to drive the sphere was compensated as best as possible by adjusting the power spectral composition of the input to the shaker to obtain a flat power spectrum between 10 and 150 Hz. Frozen noise stimuli were flat from 10 Hz to the chosen cutoff frequencies (70, 100, or 150 Hz) and presented at 0-, 10-, and 20-dB attenuation (0 dB = 450 μm peak-to-peak displacement at 50 Hz). Only cells with stable firing rates were analyzed.

*Phase analysis.* Responses of afferents to sine-wave stimuli were analyzed for their phase angles and the strength of the phase coupling by constructing phase histograms (Batschelet 1981). Phase angles, that is, the phase of the sine that the response was coupled to, were represented with respect to maximum displacement of the sphere (see, for example: Kroese and Schellart 1992; Kroese et al. 1978). Phase angles were used to investigate responsiveness of the afferents with respect to the physical nature of the stimulus. For full derivatives of the displacement, we expected coupling 90° in advance (velocity) or 180° in advance (acceleration).

Similarly, we used phase coupling to white noise stimuli to confirm the results based on sine-wave stimuli. Any function of time, say x(t), can be transformed into the frequency domain and back to the time domain by Fourier transforms

\[ X(\omega) = \int_{-\infty}^{\infty} e^{-i\omega t} x(t) dt \]

\[ x(t) = \frac{1}{2\pi} \int_{-\infty}^{\infty} e^{i\omega t} X(\omega) d\omega \]

(1)

with ω = 2πf, and a capital, here X, being the Fourier transform of the function (x). Fourier transformation means that an operator in the time domain has its equivalent in the frequency domain. The αth derivative of x(t) can therefore be written

\[ \dot{x}^{(\alpha)}(t) \Leftrightarrow (i\omega)^{\alpha} X(\omega) \]

(2)

where its Fourier transform X(ω) is on the right. In general, the output of a time-invariant system that filters an input x(t) through a filter h(t) can be expressed by a convolution. By the convolution theorem (Dym and McKeen 1972), the output r(t) in the time domain is obtained by a multiplication in the frequency domain; hence, as Eq. 2 tells us, the input/output relation in the frequency domain is through multiplication by iα raised to some power α. As pointed out above, we denote the system’s response by r(t) with Fourier transform R(ω) and assume the system to be linear. We can always write \[ X(\omega) = IX(\omega)\exp[i\theta_\omega] \]

with \( \theta_\omega \) being the phase of X(ω). If the transfer function is a derivative of power α, where α may be any real number (Sokolov et al. 2002), the input/output relation can be expressed in the frequency domain as

\[ R(\omega) = (i\omega)^{\alpha} X(\omega) \]

\[ R(\omega) = (\omega)^{\alpha} X(\omega) \exp[i\theta_\omega] \]

(3)

(4)

We see that in the frequency domain the phase of the \( \omega^\alpha \) derivative is shifted by a phase of \( \pi/2 \), where α need not be an integer but can be any real number. In the frequency domain, the phase of the signal therefore informs us about the order of the derivative leading to that signal; for example, for a derivative of order \( \alpha = 1 \), it will be \( \pi/2 \).

**Modeling of the receptive field.** To model the activity at the afferent fibers, we extend our previously developed theory (Goulet et al. 2008)
to include the effect of the external water flow superimposed on a
local stimulus:
\[
F(x) = \left| 1 + A \frac{2(x - x_0)^2 - D^2}{(x - x_0)^2 + D^2}\right|^\theta
\]
(4)

Here \( I \) is the experimentally determined instantaneous firing rate, \( A \) denotes a scaling parameter, \((x_0, D)\) is the position of the sphere, and \( x \) is the position on the fish body parallel to the vibrating sphere. The variable \( \theta \) is 1 when the neuronal response is in phase with the vibrating sphere and \(-1\) when there is a 180° phase difference. To include the effect of external water flow, we have adjusted the parameter \( I \) to the experimentally determined discharge rates.

**Variability.** The intertrial variability was computed as the inter-spike interval (ISI) distances (Kreutz et al. 2007). In contrast to more common distance-related measures (e.g., van Rossum 2001), the ISI distances is parameter free. ISI distance was evaluated based on the spike-train synchrony in response to 10 repetitions of the frozen noise. To do so, we took the discrete series of spikes (each spike being a \( \delta \)-function) \( x(t) = \sum_{i=1}^{N} \delta(t - t_i) \) with \( t_i \) and \( t_s \) being the series of spike times with the number of spikes denoted as \( M \). To obtain a time-resolved measure of the firing rate of the spike train \( \{t_i^\prime\} \), the value of the current ISI is assigned to each time instant
\[
x_{ISI}(t) = \min(t_i^\prime | t_i^\prime > t) - \max(t_i^\prime | t_i^\prime < t)
\]
(5)
where \( \min(t_i^\prime | t_i^\prime < t) \) is the time to the next spike, and \( \max(t_i^\prime | t_i^\prime < t) \) is the time before the previous spike, with \( x \) labeling the specific run. This has been calculated for all pairs of spike trains (denoted as \( x \) and \( y \), respectively), while the ratio of \( y_{ISI}(t) \) to \( x_{ISI}(t) \) has been normalized (6).
\[
I(t) = \begin{cases} 
\frac{x_{ISI}(t)}{y_{ISI}(t)} - 1 & \text{if } x_{ISI} \leq y_{ISI} \\
\frac{-x_{ISI}(t)}{y_{ISI}(t)} - 1 & \text{else} 
\end{cases}
\]
(6)

The measure is zero in the case of two identical spike trains and approaches 1 and \(-1\), respectively, if the firing rate of the first (or second) train is high and the other low. \( I(t) \) is calculated for every spike and spike train, and the ISI-distance (\( D \)) of two spike trains is then evaluated as
\[
D^2 = \sum_{i=1}^{N} |I(t_i)|
\]
(7)
To obtain the overall variability, we averaged \( D^2 \) over 10 consecutive segments of frozen noise stimuli.

Burstiness of spike trains has been evaluated by calculating the burst fraction. We have determined the upper ISI bound for bursting events based on the autocorrelation function as described by Eyherabide and colleagues (2008). There, this count of intervals below this burst-threshold normalized by the total count of spikes is defined to be the burst fraction.

**Wiener-filter analysis and stimulus reconstruction.** The activity of a neuron at time \( t \) depends on the stimulus starting a few hundred milliseconds prior to \( t \) and ending shortly before \( t \). Stimulus reconstruction based on the linear filter \( h(t) \) can be used to obtain a linear estimation of the reconstruction \( s_{est}(t) \) for the stimulus \( s(t) \). To perform the linear analysis, we used the algorithm previously established by Bialek et al. (Bialek et al. 1991; Gabbiani et al. 1996; Wessel et al. 1996). In so doing, we used the Dirac delta representation of spikes trains with the mean firing rate \( x_0 \) subtracted: \( x(t) = \sum_{i} \delta(t - t_i) - x_0 \). A reformulation of the Volterra expansion, the Wiener expansion, where all the terms are independent from each other by orthogonalization (Rieke et al. 1997; van Hemmen et al. 2001), gives a linear estimate \( s_{est}(t) \) of the stimulus \( s(t) \), by convolving \( x(t) \) with the filter \( h(t) \)
\[
s_{est}(t) = \int_0^t dt h(t - \tau) x(\tau)
\]
(8)

The optimal filter \( h(t) \) accounts for both the statistics of the stimulus and of the neurons firing (Wessel et al. 1996). All Fourier transforms performed here were done using Bartlett windowing (512-ms window).

The quality of the reconstruction obtained by convolving the spike train with this filter was assessed based on the signal-to-noise measure (Machens et al. 2001). Once \( s_{est}(t) \) is determined, the noise containing the reconstruction is defined as \( n(t) = s_{est}(t) - s(t) \). The signal-to-noise ratio (SNR) then is given by
\[
SNR(f) = \frac{S_{est}(f)}{S_{est}(f)}
\]
(9)
Here \( S_{est}(f) \) denotes the power spectrum (Gabbiani and Koch 1998; Howard 2002) of the noise, and \( S_{est}(f) \) the power spectrum of the stimulus. The SNR hence measures the amount of signal present at a given frequency relative to the noise contained in the reconstructions, such that an absence of correlation between the stimulus and the reconstruction results in \( SNR(f) = 1 \), while correlations yields \( SNR(f) > 1 \) (Gabbiani et al. 1996; Wessel et al. 1996). The least square error \( e^2 \) in the reconstruction is maximal when it equals the variance of the stimulus \( (e^2 = \sigma^2) \). From this, the coding fraction \( \gamma \) is defined as a normalized measure of reconstruction quality:
\[
\gamma = 1 - \frac{e}{\sigma}
\]
(10)

The information about the stimulus \( s(t) \) carried by a spike train per second is given by the mutual information rate. A lower bound of this is calculated as defined by Rieke et al. (1997).
\[
I_{Mut} = \int_0^f df \log_2[1 + SNR(f)]
\]
(11)

**Coherence.** The complex cross-coherence is often referred to as the coherence function (Marmarelis and Naka 1973; Mitra and Bokil 2008; Zeitler et al. 2006) and can be defined as
\[
\gamma(f) = \frac{S_{xy}(f)}{S_{xx}(f)S_{yy}(f)}
\]
(12)
where \( S_{xy} \) represents the cross-spectrum of the two signals, and \( S_{xx} \) and \( S_{yy} \) are the power spectrum of each of the signals. This quantity can be expressed as a complex number of the form \( \gamma(f) = |\gamma(f)| \exp[-i\theta(f)] \). The magnitude of the coherence function reflects how much of the variation in the output signal can be attributed to a linear filtering of the input. It takes a value between 0 (input fully uncorrelated) and 1 (the output is equal to the input after a linear convolution). The time delay between the two processes [the group delay in physics (Mitra and Bokil 2008)] is given by
\[
\tau(f) = \frac{1}{2\pi} \frac{d\Phi}{df}
\]
(13)
For a constant conduction delay \( \tau(f) \) will be constant. However, if the response shifts from acceleration at low frequency to velocity at higher frequencies, we can expect a change in the conduction delay, because a change in the order of the derivative will change the phase between the two signals.

**SR and RR.** To quantify if a single feature of a stimulus is sufficient to explain the neuronal response, we measured the performance of the optimal linear model with respect to the best performance theoretically achievable (Roddey et al. 2000). Ideally, encoding can be described as the sum of a linear reconstruction, a nonlinear contribution, and noise. Here, the nonlinear contribution term is the additional aspect of a stimulus that can be explained by...
the best nonlinear model, whereas the noise is the remaining aspect that cannot be explained at all (e.g., intrinsic noise). Quantifying the suitability of a linear reconstruction thus aims to measure how much of the theoretically achievable reconstruction is related to the linear reconstruction:

\[
\text{Linearity} = \frac{\text{linear reconstruction}}{\text{linear reconstruction} + \text{nonlinear reconstruction}}
\]  

(14)

A comparison between the signal-response coherence and the square root of the RR coherence quantifies the performance of the best linear model with respect to the optimal theoretic performance. Under the assumption that linear encoding occurs, we expected that the signal response (SR) and the square root of the response-response (RR) coherence should closely resemble each other. SR and RR were determined for four repetitions of frozen noise, and their resemblance was quantified by a performance index 100 \times [\text{RS}(f) / \sqrt{\text{RR}(f)}] (Chacron 2006).

Covariance analysis. To determine the nonlinear contribution to the encoding of SNs, we used a covariant matrix approach (Brenner et al. 2000; De Ruyter van Steveninck and Bialek 1988). The stimulus histories preceding every spike \( t_i \) were selected \( S(\tau) = S(t_i - \tau) \) to construct the spike-triggered average (STA) by averaging all stimulus histories \( S(\tau) \) for 125 ms. Next, the covariance matrix of the fluctuations around the average was obtained

\[
C_{\text{SPIKE}}(\tau, \tau') = \langle s(t_i - \tau) \cdot s(t_i - \tau') \rangle - \langle s(t_i - \tau) \rangle \cdot \langle s(t_i - \tau') \rangle
\]

(15)

where \( C_{\text{PRIOR}}(\tau, \tau') = \langle s(t_i - \tau) \rangle \cdot \langle s(t_i - \tau') \rangle \) is the covariance matrix of the stimulus itself.

To resolve which features of the stimulus relates to the spiking, the distribution of stimuli not related to spiking (the prior distribution) was subtracted from the covariance matrix: \( \Delta C(\tau, \tau') = C_{\text{SPIKE}} - C_{\text{PRIOR}} \). This reveals stimulus features where the variance is altered compared with the prior. Following diagonalization of \( \Delta C \), the eigenvalues were used to estimate the relevance of the eigenvectors.

Analysis of f-I relationship. The STAs and eigenvectors were used to determine the input-output relation of the afferents based on the noise stimulus. The probability of spiking \( P(\text{spike}) \) related to the similarity of the stimulus to a given feature (STA, eigenvector) was measured. Similarity was expressed by the linear projection of feature and stimulus vector. The probabilities \( P(\text{spike|stimulus}) \) were derived from the spike-triggered ensemble (STE) projected onto the STA \( [P(\text{STA|stimulus})] \) and for the joint projection of the first two leading eigenvectors \( [P(\text{Evector,1,2|stimulus})] \).

Taking advantage of these probability distributions, we use Bayes’ law to estimate the f-I relationship for the different features (Brenner et al. 2000; Gussin et al. 2007; Slee et al. 2005).

Fig. 1. A–D: effect of a 10 cm/s water flow on the encoding of a dipole oscillating at \( D = 1.2 \) cm by superficial neuromast (SN) afferents. A and B: peristimulus time histogram (PSTH; bin-width 20 ms) and interspike interval (ISI) distribution (bin-width 5 ms) of a response to a dipole oscillating opposite a SN in still water (A) and in water flowing at 10 cm/s (B). The time that the dipole oscillates is indicated in the PSTHs by the black top-bars. Note that the response to the 50-Hz dipole stimulus is still present in the flow, as can be seen from the peak in the ISI (arrow). AP, action potential. C: receptive field plot of the SN afferent data shown in A and B. The mean (\( n = 5 \) repetitions) experimental firing rates are represented by gray (without flow) and black dots (with flow). Ongoing rates are depicted to the left and highlighted by the gray shading. The predicted firing profile using scaling parameter \( A = 152 \) and the instantaneous firing rates as \( f = 34.2 \) are shown for the still-water condition in gray and for the flow condition with \( f = 40.8 \) in black. D: mean phase coupling to sine-wave stimulation of increasing frequency (\( n = 19 \)). The theoretical prediction with neuronal delays of 6.6 ms (dashed line) is superimposed on the actual measurements (circles, error bars = 1 SE). Note that the change in phase can be completely accounted for by the neuronal delay exerting a linearly increasing influence on the phase with increasing frequency. Thus phase coupling remains constant over the range of frequencies tested here.
\[ P(\text{spike} \cdot \text{stimulus}) = \frac{P(\text{stimulus} | \text{spike}) \cdot P(\text{spike})}{P(\text{stimulus})} \quad (16) \]

Relative contribution of eigenvectors were expressed as the contribution of a given eigenvector to the total variance of the eigenvectors as

\[ \%E_{\text{value}} = \frac{E_{\text{value}}^2}{\sqrt{\sum_{i=1}^{N} E_{\text{value}}^2}} \quad (17) \]

Data analysis. We have analyzed the data by using customized routines in Matlab (version 7.0, The MathWorks). Statistical analysis (SPSS version 12 and Matlab) is based on paired or unpaired tests (t-test, ANOVA, and Kruskal-Wallis test with post hoc testing), depending on the distribution of the data.

RESULTS

Results are based on 51 afferents recorded in 12 goldfish. Eighteen afferents were stimulated with pure sine-wave stimuli (referred to as set 1). Nineteen afferents were stimulated with frozen noise stimuli of different amplitudes (0, −10, and −20 dB) using different cutoff frequencies (70, 100, 150 Hz; referred to as set 2), and an additional 13 afferents were stimulated with continuous (2 min) noise stimuli (referred to as set 3).

Response to a sine-wave stimulus. Former studies (Engelmann et al. 2002; Engelmann et al. 2000; Voigt et al. 2000) have shown that the firing rate of SN afferents increases in response to external water flows, whereas CN afferents are largely unaffected by these stimuli. Hence, an important question is to which extent afferents innervating SNs are responsive to stimuli (like a vibrating sphere) embedded in constant water flow, for example when fish are swimming. The above studies already showed that responses to such stimuli are masked to a large extent. Thus we here address to which degree features previously implied to enable fish to localize prey are affected by external flow.

In line with published data (see, for example, Voigt et al. 2000), external flow does increase the mean firing rate of SN afferents, but the information on the spatial flow pattern is fully preserved. This is shown in Fig. 1 for an exemplary recording. We note that the mean discharge rate increased and the firing pattern changed to a bursting mode, as is evident from the ISI distribution (Fig. 1, A and B). In the example of Fig. 1, the burst-fraction increased from 4.8% in absence of the flow to 21.4% in presence of the flow. This increased burstiness renders the response to the dipole field hardly visible in the peristimulus time histogram (Fig. 1, A and B). However, the mean discharge rate reveals that it is possible to extract the flow velocity of a vibrating sphere from the neuronal discharge under both conditions (Fig. 1C). SN afferent responses are tightly linked to the maximal velocity in the stimulus (see Fig. 1D). This was shown by evaluating the phase coupling to sine-wave stimuli in a range of 10–125 Hz (set 1). Phase angles within the frequency range show a constant slope of their preferred phase. If one accounts for the neuronal delay (derived from STA analysis, see below), phase coupling is constant. As detailed in the MATERIAL AND METHODS section, phase angles were calculated with respect to the maximal displacement in the stimulus; thus the obtained angles are in agreement with a response that is proportional to the first derivative of the stimulus, i.e., with the water velocity. Moreover, a phase of \( \pi/2 \) is in accordance with a velocity filter, but not a fractional derivative, as proposed by Kalmijn (1989). A fractional derivative would result in an angle between \( \pi/2 \) and \( \pi \) in the frequency domain. Therefore, we rejected the hypothesis of a fractional derivative in the frequency range of 10–150 Hz.

Responses to noise stimuli. Using frozen noise stimuli (set 2), we addressed how reliable afferents of SNs respond to external stimuli. Notably, responses were highly reproducible (Fig. 2). The spike-train variability was significantly lower than spontaneous spike-train synchrony at all tested cutoff frequencies (ANOVA: df = 3,58, \( F = 149, P < 0.001 \)). The theoretical range of the spike-train variability is −1 to +1, with 0 indicating no variation between successive responses to the frozen noise stimuli.
noise. Lower stimulus amplitudes resulted in slightly higher spike-train variability.

The high precision found in response to frozen noise raises the question of whether the afferents encode sensory information using a rate or a temporal code. To address this, we investigated the impact of spike-time jitter on stimulus reconstruction (Jones et al. 2004a; Jones et al. 2004b; Sadeghi et al. 2007). Artificial spike-time jitter strongly decreased the quality of the stimulus reconstruction (Fig. 3), with a mean jitter of 3.5 ms being sufficient to reduce stimulus reconstruction by 70%. Spike-time jitter of a similar time scale as the period of the cutoff frequency of the stimulus resulted in a complete failure to reconstruct the stimulus. Thus the temporal precision of the spikes is higher than the shortest timescale contained in the stimulus, reflecting temporal coding (Theunissen and Miller 1995).

Given the constant velocity sensitivity and high precision of encoding, we next ask how well linear stimulus reconstructions of primary afferent responses represent the stimulus over a range of amplitudes (0, −10, and −20 dB) and cutoff frequencies (70, 100, 150 Hz). Stimulus reconstruction based on the individual kernels yielded high coding fractions (range = 0.26–0.52, mean = 0.44, std = 0.07, n = 18, 150-Hz cutoff frequency) and mutual information rates (range = 78–382, mean = 274, std = 67, n = 18, 150-Hz cutoff frequency). Both decreased with decreasing stimulus amplitudes (Kruskal-Wallis test with post hoc Dunn test, P < 0.05). As expected of a linear system, the mutual information rate increased with increasing the cutoff frequency at a given stimulus amplitude, while the coding fraction was less for the highest cutoff frequency (Kruskal-Wallis test with post hoc Dunn test, P < 0.05) (Fig. 4). These results indicate that encoding is linear within the frequency range investigated here, but might saturate toward the high-frequency end of our stimuli, as indicated in the decrease in coding fraction.

The results obtained for sine-wave stimuli indicate that SN afferents respond to the velocity of the stimulus. For any arbitrary input waveform, the filter to obtain such a response must yield the input’s derivative. The hypothetical filter to obtain this response is the difference between two delta functions or, in other terms, a biphasic signal with peaks of opposing sign. Using our noise stimuli, we have obtained two biphasic kernels (Fig. 5), with one being roughly the negative of the other. This is in agreement with the expected biphasic shape of a velocity filter, which transforms a stimulus in such a way that the result represents the velocity in the original stimulus as a function of time. Finding two kernels is to be expected, given the two populations of hair cells of opposing directional sensitivity within each neuromast (Flock and Wersäll 1962). Comparable velocity filters were reported in several other sensory systems (Gussin et al. 2007; Theunissen et al. 1996; Wessel et al. 1996).

The above can be further substantiated using coherence analysis of the power in the noise-stimulus and the evoked neuronal response. This is demonstrated in Fig. 6 (set 3), where the average coherence and the phase in response to a 2-min continuous noise stimulus is shown. With increasing frequency, the coherence increases almost linearly, while the phase of the response shows a linear decline with frequency (Fig. 1D). This indicates that the afferents are sensitive to a derivative of the displacement, since a mere displacement sensor would show constant coherence (and phase) over the frequency range. The best fit between phase and frequency (f) is

\[ y = -0.037 f + 0.15 \]

This linear dependency allows us to determine the conduction delay \( d(t) = -5.99 \text{ ms} \) \( [2\pi f dt = -37.7 \text{ ms}, \text{ therefore, } d(t) = -5.99 \text{ ms}] \). This is consistent with the delay obtained using the STA (Fig. 5), and the linear relationship of the phase further confirms that, over the frequency range investigated here, no evidence of fractional order sensitivity can be found. Thus, based on the phase relationship obtained using the sine-wave stimuli, we infer that SN afferents are responsive to the velocity of the stimulus.

It should be noted, however, that the coherence is low for frequencies <40 Hz, as is reflected in the noisy phase of the response obtained at low frequency. This was likely due to a lack of power in the stimulus at these frequencies in conjunction with the high-pass property of the boundary layer (McHenry et al. 2008).

The high-pass characteristic of the coherence function (Fig. 6A) is directly related to the velocity tuning (Fig. 1) of the afferents. After normalizing the power-spectral density of the spike train and the stimulus using the displacement (Fig. 7A) or the velocity (Fig. 7B), one can directly compare the neuronal response \( n = 13, \text{ set } 3 \) to the power spectra of the two stimuli. The match between the neuronal response and the power spectra of the stimuli is higher for the velocity of the stimulus.

![Fig. 3](http://jn.physiology.org/)

**A** Exemplary original spike train (top, blue) and the same train after a jitter (random Gaussian distribution with a mean of 3.5 ms) was applied (middle, red). The three traces at the bottom show the actual stimulus driving the neuronal response (black), the reconstructed stimulus based on the original spike train (blue), and the reconstruction based on the jittered spike train (red).

**B** Coding fractions (CF) obtained for the three conditions are indicated in the corresponding color to the right of the figure. **B**: mean (±SD) effect of the introduction of spike-time jitter on the stimulus reconstruction (coding fraction). Data are normalized with respect to the reconstruction quality obtained using the original (unjittered) spike train.
This was confirmed by a regression analysis of the normalized power spectra of the stimuli against the normalized power spectrum of the response over a frequency range of 10–150 Hz (displacement: $r^2 < 0.01$, velocity: $r^2 = 0.7$). Again, this reflects that SN afferents are efficiently driven by the velocity component over the full frequency range tested here. Furthermore, it shows the reason for the high-pass appearance of the response-coherence function (see Fig. 6).

Thus far our analysis was restricted to linear methods. To analyze if this approximation is valid, we used the covariance approach, which allows one to directly illustrate which features of the stimulus give rise to a spike. Thus the covariance approach reveals if there is more than one such feature in the stimulus, i.e., whether the afferents were responsive to the stimulus velocity and additional features like acceleration.

The importance of the features was evaluated based on their eigenvalues. Since the first two eigenvectors do account for 84% of the total variance, we restrict our presentation to the two most important eigenvectors. In all cells, the dominant eigenvector was very similar to the STA (Fig. 8). In the few cases where a second eigenvector of strong eigenvalue was recovered ($n = 3$), this second feature always resembled the first derivative of the STA (Fig. 8C). This is shown for two exemplary recordings in Fig. 8. Here we plot a two-dimensional representation of the stimulus segments preceding spiking (the STE) projected (dot-product) onto the two most dominant eigenvectors. This distribution is superimposed over the distribution obtained by projecting the segment not preceding a spike (the prior). In both cases, the projection values of the eigenvectors with the STE are shifted toward positive values for the first eigenvector and are mainly symmetric around the projection between the STAs with the STE.

The distributions of projections of the STE onto the dominant eigenvectors give an intuitive readout of their ability to drive spiking (see Fig. 8, C and F). Again, the distribution of the first eigenvector’s projection value is clearly distinct from
the prior distribution. It has a positive mean with a reduced standard deviation compared with the prior distribution and is almost similar to the distribution based on the projection between the STA and the STE. The projection distribution of the second eigenvector usually was very similar to the prior distribution. Thus the second eigenvector did not contribute significantly to the spiking (Fig. 8C). Only in three cells did the second eigenvector show a significant contribution to spiking (Fig. 8F), with the distribution being shifted to negative values with larger width than those of the first eigenvector.

Based on the high contribution of the first eigenvector, it is reasonable to stick to the linear encoding assumption, as further substantiated by a SR and RR analysis based on frozen-noise stimuli (data not shown). The linear performance index in the frequency range from 10 to 150 Hz was 70.7 ± 13.5 (n = 13 cells and 21 measurements), which again indicates that the optimal linear encoding model would capture roughly two-thirds of the possible information contained in the spike train (set 3).

One advantage of the covariant matrix method is the possibility to extract the input-output relation (f-I function) for the afferents from a stimulus varying rapidly. In that way, one can circumvent nonlinearity effects that can be induced by adaptation at the detector level. While adaptation has not been studied systematically in the lateral line system, unpublished data suggest similar rapid adaptation in the lateral line, as has been found for other hair-cell systems (S. M. van Netten, personal communication). It is interesting to see that the f-I function we obtain is linear over a wide range (the data were fit from SD 0–3, Fig. 9), and the residuals between linear and nonlinear sigmoid fitting are quite small.

Given that both hair-cell populations and their afferents have opposing polarities, this means that the coding range of the system is fairly linear. Combining information from hair cells of different orientations will thus give a reliable estimate of the local flow velocity and direction.

DISCUSSION

To the best of our knowledge, this is the first time that the mechanosensory lateral line system has been investigated using information theoretic approaches. Although we have restricted the present analysis to the SNs of this sensory system, the general approach will be highly useful for all future studies in the field. Our results demonstrate that lateral line primary afferents of SNs reliably encode noise stimuli with high coding fractions, and that the boundary layer for the frequency range investigated (10–150 Hz) does not significantly affect the neuronal responses of SN afferents. In goldfish, SNs outnumber CNs by at least 12 times. Based on the location on the skin where afferents were recorded, we expect that all of our recordings have been obtained from SNs. This is further corroborated by the flow sensitivity of those recordings where flow stimuli were tested.

SN afferents encode flow velocity. As previously discussed by other authors (Engelmann et al. 2002; Engelmann et al. 2002; Engelmann et al. 2002), the flow sensitivity of SNs is much greater than that of CNs. This is consistent with the fact that SNs are innervated by hair cells with a large range of basolateral orientations, which allows for a much wider range of flow directions to be encoded. In contrast, CNs are innervated by hair cells with a more limited range of basolateral orientations, which limits the range of flow directions that can be encoded.

Fig. 6. Mean coherence (A) and phase angle (B) of all (n = 13) SN afferent responses to stimulation with a band-pass filtered white noise of 10–150 Hz. Note that the low coherence at low frequency is related to a lack of power in this range (see Fig. 7 for power spectrum of the stimulus). For frequencies where power-spectral intensity is constant, the phase declined in a linear fashion with increasing frequency. The linear fit represented by the solid line in B is $y = -0.037f + 0.1496$.

Fig. 7. Mean (n = 13) normalized power spectral density of the neuronal response (thick black line) and the normalized power spectral density of the stimulus (gray area). In A, stimulus displacement was used to compute the power spectra, while in B the velocity content of the stimulus was used. The inset in A shows an exemplary neuronal response (raster, top trace) to a small excerpt of the stimulus (bottom trace).
our data of SN afferent recordings show that SNs are being driven by the velocity of the external flow at the neuromast. In addition, we can rule out that the optimal stimulus is of fractional order (Sokolov et al. 2002). Fractional order responsiveness would mean that SNs are sensitive to a mix between velocity and acceleration (Jielof et al. 1952; Kalmijn 1989) due to the influence of the boundary layer on the water flow (see below) for frequencies $f < 10$ Hz. However, a previous theoretical study predicted that fractional derivation may play a role for frequencies $< 5$ Hz (Bretschneider et al. 1985; Kroese et al. 1978; McHenry et al. 2008). Evidence for a sensitivity to such fractional derivatives in other sensory system have been found in the peripheral passive electrosensory system of catfish and weakly electric fish (Bretschneider et al. 1985; Engelmann et al. 2010). An influential study by Kalmijn (1989) proposed that the mechanosensory lateral line should be sensitive to a fractional derivative of the displacement. With respect to encoding natural hydrodynamic stimuli, the question of fractional-order sensitivity in the lateral line is likely negligible. Based on this assumption, we expect that the ability to extract distance and location of a dipole source should remain largely unaffected.

Fig. 8. Example of the two dominant responses found by the eigenvector analysis. The majority of afferents had a single dominant contribution to the spiking that was characterized by an eigenvector similar to the spike-triggered average (STA; A–C). In few afferents, two eigenvectors of significant contribution were recovered (D–F). In the latter case, the first eigenvector resembled the STA, while the second eigenvector resembled the first derivative of the STA. A and D: scatter plot of the projection of the first and second eigenvector on the STE distribution (gray dots). Each dot represents the x- and y-coordinate obtained from the dot-product of the stimulus preceding a spike with the first and second eigenvector. The black dots represent the projection of the prior distribution with the first two eigenvectors, and the green circle shows the projection between the STA and the eigenvectors. B and E: probability distribution of the STE projected onto the STA (green) and the first two eigenvectors (red and blue). C and F: comparison of the STA (green) to the two eigenvectors. Color code is as in B and E. The relative contribution strength of eigenvectors I and II is indicated in the legend.

Fig. 9. Mean (±SD) input-output function (f-I) based on the dominant eigenvector obtained for 13 recordings. While the relation is best described by a sigmoid fit, the linear fitting (solid line) captures the data extremely well ($r^2 = 0.94$).
The inertial force increases faster with the stimulus frequency than the viscous force, leading to a dependence of the boundary-layer thickness upon frequency; for a recent review, see van Netten (2006). Consequently, the forces acting on neuromasts are attenuated more strongly at low frequencies than at high frequencies (high-pass filter), and the mixed contribution of viscous and inertial forces for changing stimulus frequencies might give rise to a fractional power of the frequency (Kalmijn 1989; McHenry et al. 2008; van Netten 2006).

At the level of the afferents, the data obtained from our analysis of both sine-wave and white noise stimuli indicate an optimal encoding for the first derivative of the stimulus, which is equivalent to a sensitivity of the system to the flow velocity at frequencies $>10$ Hz. It should be noted, however, that, at frequencies $<10$ Hz, which constitute a rich source of biotic information (Bleckmann 1994), additional research is needed to determine whether at these frequencies fractional derivative sensitivity of the cupula (McHenry et al. 2008) affects neuronal encoding at the afferent level. Modeling of cupula dynamics in zebrafish indicates that the frequency response of the cupula motion is in fact influenced by the changes of the hydrodynamic forces acting on the cupula in the different frequency regimes (McHenry et al. 2008).

**Precise temporal encoding by SN afferents.** Our study quantified for the first time the precision, at which lateral line SN afferents encode hydrodynamic stimuli. The high precision of spike timing displayed by primary afferents indicates that temporal decoding is of high importance in the lateral line system, if the animal were to make full use of the information potentially available from the spike train. Timing of each spike driven by a stimulus contains information, and we, therefore, believe that the lateral line heavily relies on a temporal encoding scheme (Dayan and Abbott 2001; Theunissen and Miller 1995).

Using both covariance and coherence analysis, we have addressed the question if responses of SN afferents are sufficiently explained by linear system analysis approaches. Similar to several other studies in other systems (Brenner et al. 2000; De Weille 1983; Gussin et al. 2007; Metzner et al. 1998; Slee et al. 2005), we found a maximum of two relevant eigenvectors for the covariance analysis [but see Fairhall et al. (2006) for more complex examples]. Most of the variance was sufficiently captured based on the first eigenvector, which in itself was similar to the STA. Whenever a significant second eigenvector was found, it resembled the first derivative of the STA. Thus, in line with our coherence analysis, which indicates that a linear approximation of encoding suffices, the covariance analysis also points to linear encoding.

While the first eigenvector clearly resembled the STA, the meaning and relevance for information processing of the second eigenvector are less obvious. One hypothesis as to why the second eigenvector is the derivative of the STA is that temporal jitter can induce additional relevant eigenvectors (with derivative-like structure and significant eigenvalue level), if they are on a similar temporal scale as the feature of the eigenvectors themselves (Gollisch 2006). For the lateral line afferents, we have shown that spike-time jitter of 3.5 ms decreases the stimulus reconstruction by 70%. This is a much shorter time scale than that of the eigenvectors; hence it is unlikely that intrinsic jitter is the cause of the occurrence of eigenvectors that are the first derivative of the STA. On the other hand, it was also proposed (Aguera y Arcas and Fairhall 2003; Aguera y Arcas et al. 2003) that the presence of a second eigenvector similar to the first derivative of the first one is an intrinsic property of the Hodgkin and Huxley equation, making neurons sensitive to a mix of velocity and acceleration.

In summary, we have to emphasize that nonlinear terms were rather marginal. Future studies may well address this issue in more detail. This would then allow a clearer distinction of fractional and nonfractional responsiveness at frequencies below those presently investigated.

**Encoding of biological stimuli.** In line with previously published results, our data confirm that the SNs are responsive to the velocity of the water motion. While this velocity sensitivity results in a strong activation of SN afferents in natural flow conditions, like self-motion of the animal or external flow, these afferents are still able to encode multiple sources of motion. Thus, even in conditions where SN afferents increase their discharge rate due to the external flow, spatial information may still be read out from their responses (Fig. 1).

This conclusion is not contradictory to prior investigations, which showed that responses of SN afferents to a dipole can be masked by such external water flow, as complete masking often occurred at higher flow velocities (Engelmann et al. 2002; Engelmann et al. 2000). Thus encoding of separate stimulus sources will highly depend on the signal-to-signal ratio between the two sources.

Similar to these earlier studies, we can expect that responses to local dipoles will be masked by external water flow, if the SNR is poor (see Fig. 1, A and B). Hence, for weaker signals, complete masking of spatial information can be expected. However, assuming that temporal encoding is needed to extract the information contained in the SN afferent spike trains in an optimal fashion (see Fig. 7), this means that the induction of bursts by local turbulence will deteriorate the encoding of additional stimuli.

Functionally, however, such external flow may turn out to be advantageous by allowing lateral line sensors to function as stochastic resonance detectors, where moderate external noise might actually be beneficial in the detection of subthreshold signals (Russell et al. 1999). Currently, this is speculative, but our finding on the temporal precision with which SNs encode white noise stimuli (Figs. 2 and 3) strongly calls for a reevaluation of the encoding in this sensory system.

**Do bursts encode different features of hydrodynamic stimuli?** While not investigated here, future studies using information theory approaches should focus on the significance of spike bursts. In the lateral line system, bursts have been shown to be associated with the occurrence of local turbulences in large-scale flows (Chagnaud et al. 2007; 2008a; Chagnaud et al. 2008b; Engelmann et al. 2002). Given that spike timing conveys a considerable amount of information (see Results), future studies should investigate if these bursts convey different information than single spikes. Examples for burst-specific encoding have been found in weakly electric fish, where bursts were shown to convey information on subtypes of stimulus features relevant in electrolocation and communication (Metzner et al. 1998) and comparable studies on insect auditory systems (Eyherabide et al. 2009; Marsat and Pollack 2006). In fact, locally synchronized bursting of neurons may be a general mechanism by which salient, but stochastic, signals...
can be transmitted, as proposed by Marsat and coworkers (2009).

For lateral line afferents, it has been suggested (Chagnaud et al. 2008b), that fish may be able to determine flow speed and direction based on cross-correlations of the activity of neighboring neuromasts. This is a first indication that bursts might convey different information than nonbursting sequences in the lateral line system. Given the remarkable temporal precision of SNs, it is conceivable that a similar temporal analysis between pairs of inputs may suffice to determine average flow speeds or the directions on a single spike level, a question that should be addressed in future studies aiming to unravel the relationship between natural signals and their neuronal representation.

Consequences for central processing of lateral line information. In addition to our finding that SN afferents can reliably encode over a wide frequency range, our information theoretic approach further revealed that the information capacity of single SN afferents is considerably high. Thus a low degree of convergence of uncorrelated single afferent input would be sufficient to allow for a full encoding of sensory stimuli. Based on the mean coding fraction determined at a cutoff frequency of 150 Hz (45%), we estimate that, to extract the stimulus at 90% precision, integration of roughly 30 afferents \( N = \left(1 - 0.45/0.1\right)^2 = 30 \) would suffice. Since goldfish, the species studied here, have 1,800–2,000 SNs on each side of their body (Puzdrowski 1989), with a slightly denser distribution on the head (Schmitz et al. 2008), this dense sensory surface is ideal to perform hydrodynamic imaging of the environment at high temporal and spatial resolution.

The estimated number of afferents is expected to be a lower bound, since neuromasts contain two groups of hair cells of opposing orientation, which are innervated separately (Faucerre et al. 2009; Flock and Perssälv 1962). Convergence of such opposite polarities can greatly enhance the encoding capability (electroosensory system: Chacron 2006; somatosen-sory system: Jones et al. 2004a). While we did not explicitly study convergence, our data show that SN afferents can be grouped into afferents tuned to opposite polarity straightforwardly based on their STA. Future studies on central neurons in the first processing area of lateral line input could employ similar strategies, as we have done here to investigate if central convergence is limited to afferent input from SNs of equal orientation, thereby maintaining the directionality information.

Behavioral studies have confirmed that fish can determine and localize objects (Coombs and Janssen 1990; Coombs and Patton 2009). The question is then how central lateral-line units analyze the incoming data so that the animal can obtain an image of its environment. One possibility to solve this task is to use a hydrodynamic topographic neuronal map, for which anatomical as well as weak physiological evidence has been published (Alexandre and Ghysen 1999; Kröther et al. 2002). Although not essential to the decoding of position and distance of a source (Goulet et al. 2008), topographic representations would be beneficial for the extraction of such information in general. The ability to extract this information is directly implied in the anatomy of the sensory system. Hair cells are directionally selective, and, in each neuromast, two opposing hair-cell polarities are found (see above). Thus, using the information of the directionally sensitive subunits, flow directions, as well as location and distance to a source should, therefore, be available. Based on the \( f/I \) function we here constructed from nonstationary responses (Fig. 9), the ability of SNs to precisely convey stimulus amplitude and, provided that afferents of opposite polarity converge on a single target, flow direction is evident. We tested (data not shown) that the discrepancy between our linear approximation for the stimulus transfer does not invalidate our previous findings regarding spatial response profiles (see Fig. 1); our data indicate that the error is negligible, validating our previous comparisons between stimuli and modeled SN responses (Fransesch et al. 2009; Goulet et al. 2008).

Given these findings, it remains enigmatic why a large body of data on SNs shows that SN afferents are not directional sensitive when exposed to bulk water flow (Chagnaud et al. 2008a; Voigt et al. 2000). At the level of the afferents, it appears that they rectify flow directions as to only convey amplitude and phase information. Contrary to this result, directional sensitivity to bulk water flow (V. Hofmann and S. Künzel, personal communication), as well as to moving objects (Engelmann and Bleckmann 2004; Engelmann et al. 2003; Mogdans et al. 1999; Plachta et al. 2003) increases from the medulla to tectal levels. In light of our finding that spike timing is important in peripheral encoding of lateral line stimuli and recent works suggesting an important role of spatio-temporal integration by the lateral line system (Chagnaud et al. 2008b), future research should further investigate the lateral line system, considering temporal coding and information extraction.

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AUTHOR CONTRIBUTIONS

J.E. coordinated the research, analysis and writing; J.G. devised the theoretical framework and was involved in analysis and writing; J.L.v.H. provided theoretical support; S.N.J. participated in analysis; B.P.C. contributed data and was involved in the writing; B.S. provided data.

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