which was originally based on structure-activity studies of the memory effects of AVP, is elaborated by the present identification of a preferentially formed metabolite with potent, selective effects on the consolidation of passive avoidance behavior. This metabolite, [pGlu^4, Cyt^6]-AVP-(4–9) may be an active principle mediating central activities of AVP and it could be responsible for one or more of the effects observed after administration of AVP. Recently we found that the peptide is formed by stepwise aminopeptidase cleavage of AVP (*12*). Its accumulation is promoted by the internal cyclization of the NH_2-terminal Gln residue into pGlu, thus protecting the peptide against further aminopeptidase degradation. Preliminary experiments suggest the in vivo presence of vasopressin metabolites in the brain. Using HPLC in combination with radioimmunoassay systems recognizing the COOH-terminal portion of AVP, a peptide with properties of [pGlu^4, Cyt^6]AVP-(4–9) was detected amongst other fragments. The peptidases involved in the proteolytic processing of AVP into neuropeptides with potent and selective central activities may play a key role in the modulation of AVP activity in the brain.

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Which Behavior Does the Lamprey Central Motor Program Mediate?

Abstract. *The isolated lamprey spinal cord, when bathed in 2 millimolar D-glutamic acid, will generate a pattern of motor neuron discharge that has generally been assumed to represent the central motor program for swimming. Motion pictures of behaving lampreys were analyzed by a computer algorithm to estimate undulatory movement parameters that could be directly compared with those generated during D-glutamate–induced undulations. The D-glutamate–induced movement parameters were significantly different from those observed during normal behaviors, including swimming, but accurately predicted the undulations produced by spinally transected adult lampreys.*

The lamprey occupies a strategic evolutionary position in terms of the organization of its nervous system (*1*), and serves as an important cellular model for recovery from spinal cord lesions (*2*) and for the analysis of motor pattern generation (*3*). The lamprey spinal cord has many of the technical advantages of simple invertebrate systems and contains identifiable classes of neurons (*4*). In addition, the lamprey spinal cord can generate a pharmacologically induced motor pattern, "fictive" locomotion, that has been assumed to represent the central motor program underlying swimming (*5*).

The simple motor apparatus of the lamprey allows few behaviors other than those produced by lateral axial undulations. One would therefore expect that the differences between behaviors can be quantified in terms of identifiable movement parameters. To quantify these undulatory behaviors, we developed a computer algorithm that can automatically analyze cinematographic images of freely behaving lampreys (*6*). This algorithm enabled us to estimate such parameters of undulatory movements as repetition frequency, intersegmental delay, phase lag, and curvature and to compare these features with D-glutamate–induced programs generated in situ. Using this algorithm, we were able to analyze the common behaviors of larval and adult lampreys as well as the behaviors of specimens recovering from lesions of the spinal cord (*6*). In addition, we analyzed the movements produced by specimens in which the spinal cord was exposed to a bath containing D-glutamic acid, the normal stimulus of fictive locomotion (*3*). We found that the dynamic organization of the D-glutamate–induced motor program does not closely resemble that of any normal behavior but is similar to the spinal undulations produced spontaneously by spinally transected adult lampreys (*7*).

Recently transformed specimens of the sea lamprey *Petromyzon marinus* were filmed from above with a super 8 movie camera and the films were projected frame-by-frame onto a magnetic digitizing tablet. The digitized images were then rotated to produce the normalized projection shown in Fig. 1A. The arrays of points that delineate each side

of the body were then smoothed and an nth-degree polynomial (usually $N = 5$) was fitted to the data for each side. For each lateral flexion, two parameters were computed, the locus of the flexion, expressed as a percentage of body length from nose to tail, and the curvature of the flexion, expressed as the inverse of the radius of the best-fitted circle to the curvature maximum, normalized to the length of the animal.

When the loci of flexions are graphed as a function of time (Fig. 1B), individual flexions can be sorted into flexion waves progressing from head to tail. The timing parameters of the behaviors can be estimated from such diagrams. The intersegmental delay is the conduction velocity of the wave (slope of the regression line fitted to the locus versus time plot for each flexion wave) multiplied by the allometric constant of percentage of body length per segment. If one extrapolates the regression line for each flexion wave to the nose (locus = 0), the time between the intercepts of successive flexion waves to the same side is the period of the swimming movements.

The degree of flexion during undulatory movements can be characterized from the computation of curvature as a function of locus (Fig. 1B). Our algorithm provides a summary of each behavioral act in the form of a table of the parameters that characterize each flexion wave and the average parameters that characterize the overall motion (Table 1). During this sequence of escape swimming, period, intersegmental delay, and phase lag remain relatively constant, while the curvature can vary by as much as 40 percent. Figure 1C shows the results of a similar analysis of the slowest example of transformer swimming that we observed. Panels B and C of Fig. 1 together provide a good estimate of the dynamic range of normal swimming.

We performed this analysis on several other behaviors, such as burrowing and crawling mediated by nose-to-tail or tail-to-nose lateral undulations. The results of this analysis are shown in Table 2. A striking feature is that intersegmental delay is highly correlated with period, so that intersegmental phase lag is essentially constant for all lamprey behaviors (*8*). The differences between behaviors in this phase-constant system result from variation in either the range of frequencies or the curvature that the body achieves during the flexion wave (Table 2).

To compare this analysis of normal behaviors with the motor pattern produced by pharmacological stimulation, we used the same algorithm to analyze the undulatory movements produced in situ in specimens whose spinal cord was surgically exposed to a bath containing 2 m*M* D-glutamic acid (*5*). The graph of locus versus time for this behavior is shown in Fig. 1D. The period and intersegmental delay of the resulting behavior are beyond the range characteristic of normal swimming (Table 2).

One might argue that the differences between normal behaviors and the D-glutamate–induced undulations are due to a lack of the proprioceptive feedback normally present in vivo (*9*). Unlike mammals, cyclostomes do not possess

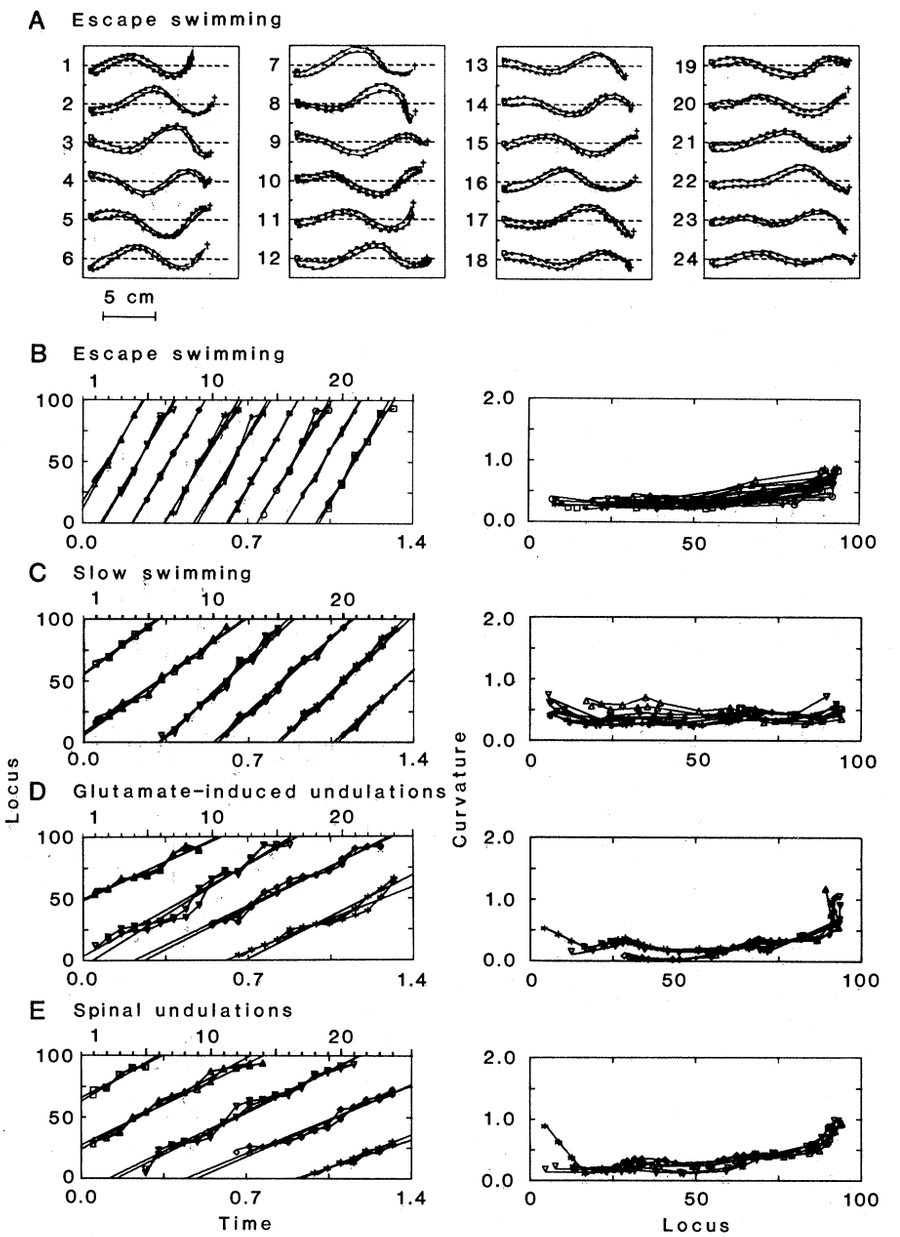

Fig. 1. Analysis of lamprey behaviors. (A) Graph of the normalized digitized images of escape swimming in a recently transformed lamprey. A least-squares regression line is fitted to the set of points outlining the body; the line is rotated to become the *x*-axis. The number to the left of each image is the movie frame number. The interframe interval is 55 msec. (B) Analysis of the images presented in (A). The left panel is a plot of the locus of flexion waves versus time, going from nose (0) to tail (100). A least-squares regression line is fitted to the points for each side of the specimen. The right panel shows the curvature of lateral flexions (1/radius of best-fitted circle to curvature maximum) plotted as a function of locus. Note that curvature stays fairly constant for the length of the specimen. The computed parameters for this sequence are given in Table 1. (C) Analysis of the slowest example of transformer swimming we have observed. (D) Analysis of the lateral undulations produced by bath application of 2 m*M* D-glutamic acid to the exposed spinal cord of an adult. (E) Analysis of spinal undulations produced spontaneously by an adult lamprey whose spinal cord had been surgically transected at nine myotomes posterior to the last gill arch.

muscle receptors and the major source of proprioceptive feedback is stretch receptors intimately associated with the spinal cord (*10*). Grillner *et al.* (*11*) demonstrated that phase-modulating reflexes mediated by these receptors are functional in highly dissected preparations and are probably not affected by the less invasive dissection used in the present experiments. Thus the proprioceptive feedback that operates in vivo certainly operates during D-glutamate–induced behavior.

We have, however, found one behavior that resembles the undulations evoked by D-glutamate. After complete spinal cord transection, recently transformed adult lampreys soon exhibit spontaneous and continuous undulatory movements when attached to the aquarium by their oral sucker (*7*). An analysis of these spinal undulations is presented in Fig. 1E. Notice that the dynamic organization of these movements produced spontaneously in vivo is similar in both timing and amplitude to that of movements produced by D-glutamate in situ (Fig. 1D and Table 2).

The central motor program evoked by the bath application of D-glutamate is no closer to swimming than it is any of the other behaviors mediated by front-to-rear lateral undulations such as burrowing or crawling. The similarity of the spinal undulations to the D-glutamate–induced undulations suggests that both differ from normal behaviors due to lack of descending input. There are numerous examples of systems in which central motor programs have been modulated by synaptic or humoral input in terms of one or more parameters, such as frequency, intersegmental delay, or amplitude (*12*). In the lamprey such modulation of D-glutamate–induced behavior might be sufficient to produce each of the other behaviors. It is tempting to speculate, therefore, that the central motor program represents a fundamental undulatory pattern that is modulated by different descending systems to produce the complete undulatory behavioral repertoire.

Table 1. Computed parameters resulting from the analysis of escape swimming presented in Fig. 1, A and B. Each row represents the computed parameters for an individual flexion wave to either the left or to the right. Period is the period of swimming movements estimated from the start times of flexions to the same side. Delay is the intersegmental delay computed by multiplying the conduction velocity (percent of body length per second) divided by the allometric constant of the percent of body length per segment. Phase is the intersegmental phase lag (intersegmental delay per period). Curve is the average curvature (Fig. 1B) of the flexion wave. The columns labeled first and last indicate the first and last frames (from Fig. 1A) of the movie in which the flexion wave was recognized by the algorithm.

Side	Period (second)	Delay (msec)	Phase	Curve	First	Last
Left	0.257	3.3	0.013	0.55	2	5
Right	0.266	3.3	0.013	0.54	4	8
Left	0.266	3.3	0.012	0.46	6	10
Right	0.268	3.4	0.013	0.46	8	13
Left	0.260	3.4	0.013	0.46	11	15
Right	0.248	3.2	0.013	0.32	13	17
Left	0.257	3.4	0.013	0.42	15	20
Right		3.4		0.34	18	22
Left		3.4		0.38	20	24
Mean	0.260	3.3	0.013	0.43		

Table 2. Computed parameters resulting from the analysis of different behaviors. Swimming has a broad dynamic range, and we have presented examples representing the extremes of slow swimming and rapid escape swimming, as well as the average of all samples. In addition, swimming can occur in the absence of the resistance provided by water on a wet surface (terrestrial swim). Crawling will also occur on the wet substrate and can be achieved both by front-to-rear and rear-to-front flexion waves. Finally, when provided with a suitable substrate or when brightly illuminated for filming, specimens will attempt to burrow. Each entry represents the mean of each parameter for a representative sequence of the different behaviors in a specimen, or, in the case of swimming average, pooled data from sequences of swimming in 17 different adults.

Behavior	Period (seconds)	Delay (msec)	Phase	Curve
Slow swim	0.541	6.9	0.013	0.40
Average swim	0.324	4.1	0.013	0.37
Escape swim	0.260	3.3	0.013	0.43
Terrestrial swim	0.260	3.0	0.012	0.37
Forward crawl	1.004	15.1	0.012	1.27
Backward crawl	1.809	−14.7	−0.011	0.96
Burrowing	0.965	22.1	0.015	0.65
Glutamate undulations	0.761	7.3	0.010	0.40
Spinal undulations	0.753	9.9	0.012	0.27

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