

Three-dimensional kinematic analysis of frog hindlimb movement in reflex wiping

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Abstract. The three-dimensional kinematics of the hindlimb back-wipe were examined in spinal frogs. The component movements were identified and the relationship between stimulus position and hindlimb configuration was assessed. The planes of motion of the hindlimb were examined throughout the movement. The back-wipe comprises three essential phases: a placing phase (I), in which the foot is drawn over the back of the frog and placed in a position near to the stimulus; a pre-whisk phase (II), in which the endpoint of the foot moves *away* from the stimulus; and a whisk/extension phase (III), in which the stimulus is removed. The pre-whisk phase contributes to force production for the whisk/extension (III). In the placing phase a systematic relationship was found between limb endpoint position and stimulus position in the rostral-caudal direction. The hip, knee and metatarsal joint angles were related to the position of the endpoint in the rostral-caudal direction. However, different frogs tended to adopt different strategies to remove the stimulus. In one strategy, when the knee angle was strongly related to the rostral-caudal stimulus position, the metatarsal angle was weakly related and vice versa. Other strategies were observed as well. There was no adjustment in limb endpoint position for stimulus placement in the medial-lateral direction. Consistent with this finding, the point on the foot at which stimulus contact occurred changed systematically as a function of medial-lateral stimulus placement. Thus, in order to remove the stimulus in different medial-lateral positions, the frog used a different part of the foot rather than moving the foot in the direction of the stimulus. In two frogs a relationship was observed between the elevation of the femur and the medial-lateral stimulus position. The motion planes of the hindlimb were studied by examining the instantaneous plane of motion of the endpoint and the planes of motion of adjacent limb segments. The motion of the endpoint was found not to be planar in any phase of the wipe. In contrast, planar motion of the femur and tibia was observed for all phases. Systematic changes in the

orientation of these planes characterized the different phases. The position of the hindlimb was found to be variable prior to the placing phase. This variability was not related to stimulus position. However, in trials with multiple wipes, once an initial limb configuration was assumed, the limb returned to this configuration before each wipe in the sequence. Evidence for motor equivalence was sought in two ways. The pattern of hindlimb joint angles corresponding to a fixed position of the limb endpoint was examined, and the variability of the endpoint positions was examined for fixed stimulus positions. It was found that for a given endpoint position there was little variation in joint angles. However, for a fixed stimulus position there was greater variation in the endpoint position at the end of the placing phase.

Key words: Movement – Spinal reflex – Kinematics – Frog

Introduction

Studies of spinal reflexes in amphibians and reptiles have identified basic movement elements which can be elicited alone or in combination. These include reflex withdrawal, crossed extension, rhythmical stepping and scratching, and target-directed wiping (e.g., Fukson et al. 1980; Gray and Lissman 1940; Ostry et al. 1991; Shotland et al. 1989; Stein 1983). In the present paper we focus on the wipe reflex of the spinal frog (Berkinblit et al. 1989; Fukson et al. 1980; Giszter et al. 1989; Shotland et al. 1989). The aim is to partition the complex wiping sequence into component behaviours and characterize the basic features of these motions. A further aim is to identify the aspects of hindlimb motion which are specifically dependent on placement of the stimulus.

Previous research has shown the wipe reflex to occur in different forms depending upon where on the frog's body the stimulus is placed. If the stimulus is placed on the back, the animal performs a "rostral back-wipe"

(henceforth, simply back-wipe) in which the hindlimb is brought up and over the back, and the foot removes the irritant. If the stimulus is placed on a hindlimb the frog displays a "hindlimb wipe", in which the non-stimulated hindlimb flexes and wipes the stimulus off with the medial surface of the ankle. An irritant on or around the cloacal fold causes one or the other hindlimb to flex and wipe using the ankle in what may be termed the "caudal back-wipe". Lastly, if the stimulus is on the belly of the frog, it performs an "under-wipe", in which the hindlimb is flexed and rotated, and the foot brushes along the underside of the animal. Wipes to the forelimb are observed in high-spinal preparations. In these preparations, the animal is able to remove a stimulus from a specific position on the forelimb even when the orientation of the arm is changed relative to the body (Fukson et al. 1980). Thus, the frog's spinal nervous system is able to integrate changes in stimulus and body position into coordinated patterns of joint motion. Different patterns of coordination are observed depending on the spatial location of the target.

The back-wipe can be divided into a sequence of basic components (Berkinblit et al. 1984; Fukson et al. 1980; Giszter et al. 1989). Fukson et al. (1980) identify an initial aiming phase followed by a "ballistic" movement in which the stimulus is removed. Berkinblit et al. (1984) identify five phases: flexion, placing, aiming, whisk and extension. Placing and aiming were reported to be stimulus dependent. Giszter et al. (1989) report four phases to the back-wipe: flexion, placing, aiming and whisk/wipe.

Although the hindlimb position clearly depends upon stimulus placement, only Giszter et al. (1989) have carried out detailed analyses relating components of limb and stimulus position. They report a relationship between stimulus position and hindlimb configuration in the rostral-caudal direction alone. The relationship between hindlimb endpoint position and limb configuration remains uncertain. Whereas Berkinblit et al. (1986) report multiple configurations corresponding to a given endpoint position, Giszter et al. (1989) report a relatively inflexible relationship.

There is some evidence that planar hindlimb motion is characteristic of the spinal preparation. Qualitative descriptions of the planes of hindlimb motion have been reported. Based on two-dimensional analyses, Berkinblit et al. (1984) and Giszter et al. (1989) report that the hindlimb motion in the back-wipe is essentially planar. In the present paper, we show that individual phases of the back-wipe are planar and that the plane of motion changes from one phase to the next (see Soechting and Terzuolo 1987a,b for a demonstration that human subjects are unable to produce movements in which the plane of wrist motion changed smoothly. Abrupt changes in the plane of motion led to the proposal that arm movements were organized in a piecewise planar fashion.)

In the present study, the back-wipe reflex in the spinal frog is elicited as stimulus placement is systematically varied in both the medial-lateral and rostral-caudal directions. Hindlimb position and joint motions are recorded in three dimensions. The analyses focus on the identification of phases of movement during the wipe. Within each

phase the relationship between limb configuration and stimulus position is assessed. The variability of endpoint positions and joint angles is examined and the planes of hindlimb motion are identified and compared quantitatively.

Materials and methods

Surgery

Seven adult *Rana catesbeiana* were anaesthetized using inhaled ether or tricaine (MS-222) injected into the dorsal lymph sac at a dosage of 0.2 mg/g body weight. A bur hole was drilled through the spinal column at the level of the first vertebra and the exposed spinal cord was fully transected. The transection was verified visually using a light microscope. Following surgery, the frog was placed in an open tray of water and allowed to recover under refrigerated conditions (4°C) for 1–2 days, or until full wiping behaviour could be elicited.

Movement recording

Hindlimb wiping movements were recorded in three dimensions using a Watsmart motion tracking system. The sampling frequency was 400 Hz. Infrared light-emitting diodes (IREDs) were sutured along the midline of the back, on the coronal line midway between the knee and ankle joints, at the ankle, at the tarsal-metatarsal joint and at the metatarsal-phalangeal joint (Fig. 1). The positions of the IREDs were used to calculate hip, knee, ankle and tarsal-metatarsal joint angles. Light-weight low torsion wires were used to reduce perturbations due to the IREDs. Movement trials were also recorded in two dimensions using a monochrome video camera (Panasonic model WV-BD400). The camera was oriented at approximately 90° to the principal plane of motion. The video record was used to locate stimulus and limb endpoint positions only. All other measures were obtained from Watsmart recordings.

Data collection

Wipes to the back and cloacal area were elicited using blotting paper (approximately 2 mm²) soaked in 5% hydrosulphuric acid. Stimuli were randomly placed in eight different positions, covering an area from the scapula to the cloaca and from the midline to the coronal line (Fig. 1). Stimulus positions were chosen in order to cover a wide range of locations on the back and to enable the separation of the effects of rostral-caudal stimulus placement from medial-lateral stimulus placement. Approximately ten trials were collected from each stimulus position for each frog. Extensive data were gathered from four frogs. All testing was carried out at room temperature (22°C).

Data processing

The data were low-pass filtered between 8 and 12 Hz. The cutoff frequency was chosen using Fourier analysis and direct comparison of raw and filtered marker trajectories. A signal to noise ratio of at least 30 dB was used as the criterion for choosing the cutoff frequency. Joint angles were calculated from the filtered data. Ankle and metatarsal joint angles were computed using the scalar product of the vectors defining the joints. The hip and knee joint angles could not be calculated directly. Since markers placed directly at the hip and knee joints would have been obscured from the cameras for portions of time during the wipe, it was necessary to find hip and

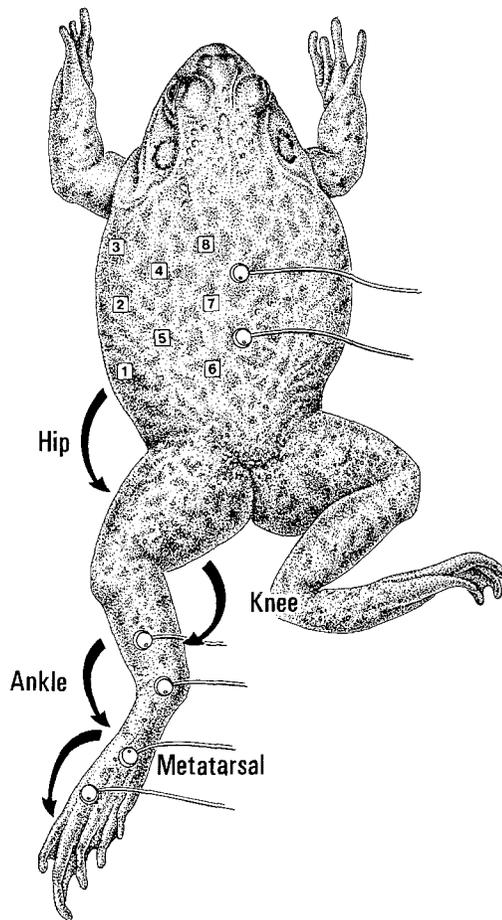


Fig. 1. A frog showing placement of infrared-emitting diodes. Arrows indicate the direction of increasing joint angle for the hip, knee, ankle and metatarsal joints. Numbered squares correspond to the position of stimuli

knee joint coordinates by extrapolation. The point of hip rotation was identified by manipulation of the limb. Using the two back markers and the measured distance to this point, the hip joint coordinates were computed. Similarly, the point of knee rotation was identified by manipulation of the limb. The tibial markers and the measured distance to this point were used to find the knee joint coordinates. Anatomical hip and knee angles were then computed as above using the scalar products of the vectors defining the joints.

Control trials were carried out to test the accuracy of the extrapolation process used to determine joint coordinates. Markers were placed directly over the joints whose positions were estimated, and extrapolated hip and knee angles were compared to corresponding angles computed using actual markers on the joints. The correspondence was good. Small ($5-7^\circ$) but constant differences in the two methods of estimating joint angle were observed in certain instances. However, there were no significant differences in the form of the joint angles over time between the two methods. Ideally, the calculation of joint angles should be based on the coordinates of joint centres. The placement of IREDs over the joints rather than directly at joint centres introduces additional error in computing joint angles. However, since all measurements are based on markers placed on the surface of the limb, the resulting error is presumably small and relatively constant throughout the movement.

In addition to the anatomical hip angle, the motion at the hip was decomposed into pitch, roll and yaw angles. This enabled an examination of relationships between hip motion in different degrees of freedom and the position of the stimulus on the back. Yaw angles (flexion and extension) were computed by projecting onto

the horizontal plane the vector associated with the femur and taking the angle between this projection and the rostral-caudal body axis. A yaw angle of 0° corresponds to femur alignment with the rostral-caudal body axis. Pitch angles (abduction and adduction) were computed by projecting the vector associated with the femur onto the mid-sagittal plane and again calculating the angle between this projection and the rostral-caudal axis. A pitch angle of 0° corresponds to the femur lying in the horizontal plane. Roll of the femur was determined using the projection on the mid-sagittal plane of the vector normal to the plane defined by the femur and tibia. A roll angle of 0° occurs when the femur and tibia lie in the horizontal plane.

The analyses presented here are based, in all cases, on data from individual animals. There is no data pooling either in the statistical tests or in the figures.

Results

Identification of the phases of the wipe reflex

The wipe reflex was found to have three phases. The phases were established on the basis of function and identified through patterns of joint motion. Pauses in the movement trajectory and/or changes in the direction of joint motion were used to demarcate the beginning or end of a phase (Fig. 2). The phases were classified separately for each joint. This resulted in some temporal overlap between phases of motion at different joints.

Figure 2 displays the pattern of joint angles over the course of a wipe as well as the three orientation angles at the hip. The phases are shown in Fig. 3, using a stick figure reconstruction. The first phase involved limb positioning or placing (phase I). In this phase, motion at the hip consisted of abduction and flexion; the ankle and metatarsal joints flexed, and the knee extended. This acted to bring the foot over the back and place it in the region of the stimulus. At the beginning of this phase the phalangeal joints flexed and then straightened. The phalanges then remained fully extended for the remainder of the wipe, such that the foot, consisting of both the phalanges and the metatarsus, could be treated as a single unit. Prior to the placing phase, an initial flexion or an extension of all joints was sometimes observed. The duration of the placing phase ranged from 250 to 500 ms.

The second phase (phase II) comprised a brief flexion, abduction and forward roll at the hip, along with flexion of the knee, and occasionally, the ankle joint. This flexion was present approximately 80% of the time and acted to bring the foot forward and towards the midline. This phase has been previously designated "aiming" (Berkinblit et al. 1986; Giszter et al. 1989). In the present paper, we label this phase "pre-whisk", as we demonstrate that it does not improve the accuracy of limb placement with respect to the stimulus. The duration of the pre-whisk phase ranged from 100 to 250 ms.

In the "whisk/extension phase" (phase III), there was an initial extension of the metatarsal joint (while the hip and knee were still flexing) and then an extension and adduction of the hip combined with extension of the knee, and ankle joints. This served to remove the stimulus from the body. This was followed by the flexion of several joints which, in the case of multiple wipes, repositioned

PHASES OF THE BACKWIPE

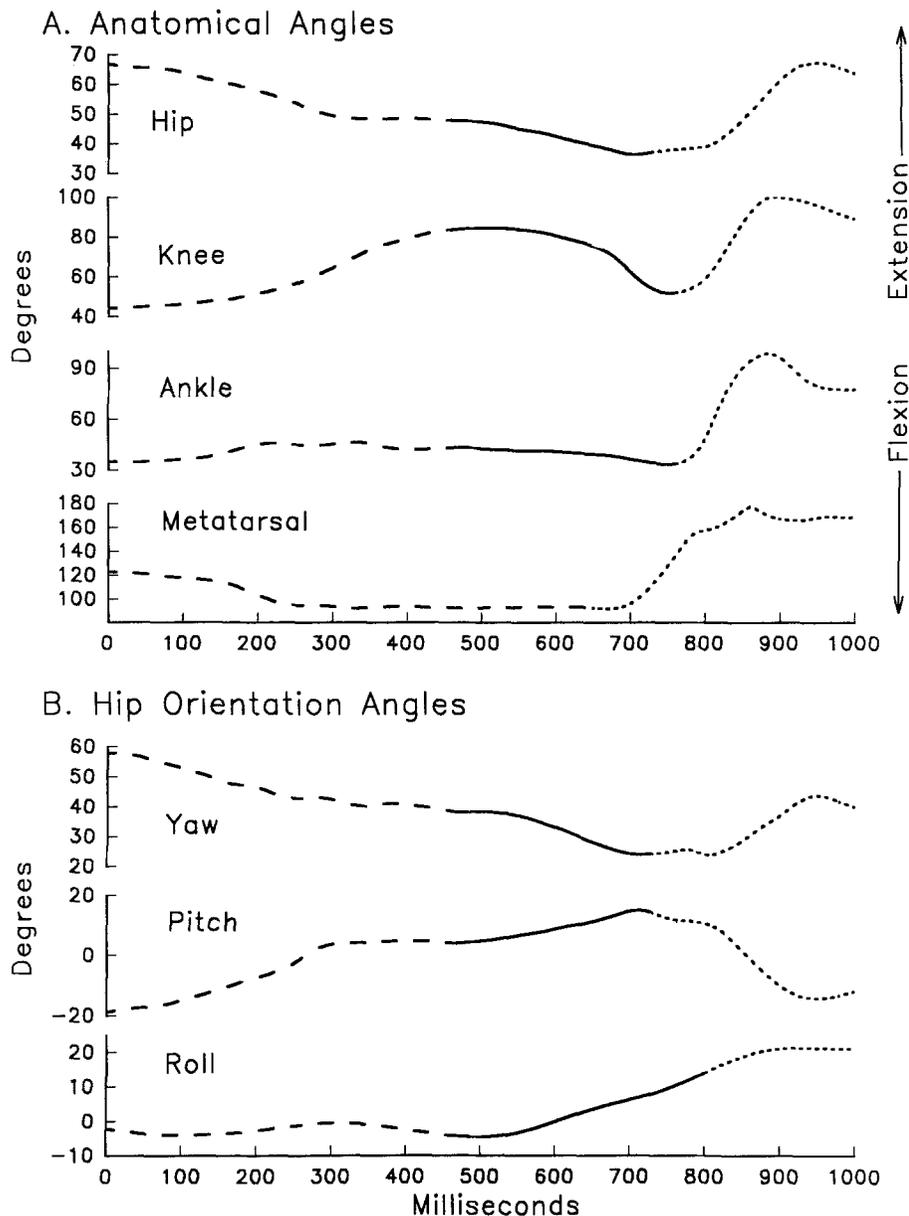


Fig. 2A,B. Phases of the back-wipe. **A** Anatomical angles at the hip, knee, ankle and metatarsal joints for one wipe. Flexion and extension directions are indicated. The *dashed line* indicates the placing phase, the *solid line* indicates the pre-whisk phase, and the *dotted line* indicates the whisk/extension phase. The duration of the record is 1000 ms. **B** Hip orientation angles during the wipe shown in panel A. The yaw angle increases with increasing hip extension. The pitch angle is positive for abduction and negative for adduction. The roll angle is positive for forward rotation; a roll angle of 0° occurs when the femur and tibia are both in the horizontal plane

tioned the limb for the next cycle. The duration of the whisk/extension was 200–300 ms.

The order of these individual joint movements remained unchanged over different back-wipes. That is, the hip always flexed twice in succession and then extended. The knee extended, then flexed and finally extended again. The ankle displayed a small flexion or extension, and then extended, and the metatarsal joint first flexed then extended. Neither the ankle nor the metatarsal joints moved very much during the pre-whisk phase.

Figure 4 shows the basic patterns of joint motion (based on anatomical angles) for selected stimulus positions along the back. Note that direction of joint motion for a given phase does not depend on stimulus position. The placing and pre-whisk phases are distinguished by a change in the direction of knee motion and, as can be seen in Fig. 2, a pause in the movement of the other joints.

Whisk/extension is characterized by a reversal of movement direction at all joints.

Relationship between stimulus position and hindlimb position

The relationship between stimulus position and hindlimb position was examined at the end of phase I and phase II using video recordings. The hindlimb endpoint (the position of the tip of the longest phalange) and stimulus coordinates were obtained in two dimensions from the video record. Orthogonal body axes were used to define a coordinate system for the video analysis. A rostro-caudal axis ran along the midline from the snout to the cloaca. A medial-lateral axis ran through the cloaca and was orthogonal to the rostro-caudal axis. In the analyses which follow, hindlimb position in phase I was measured at the

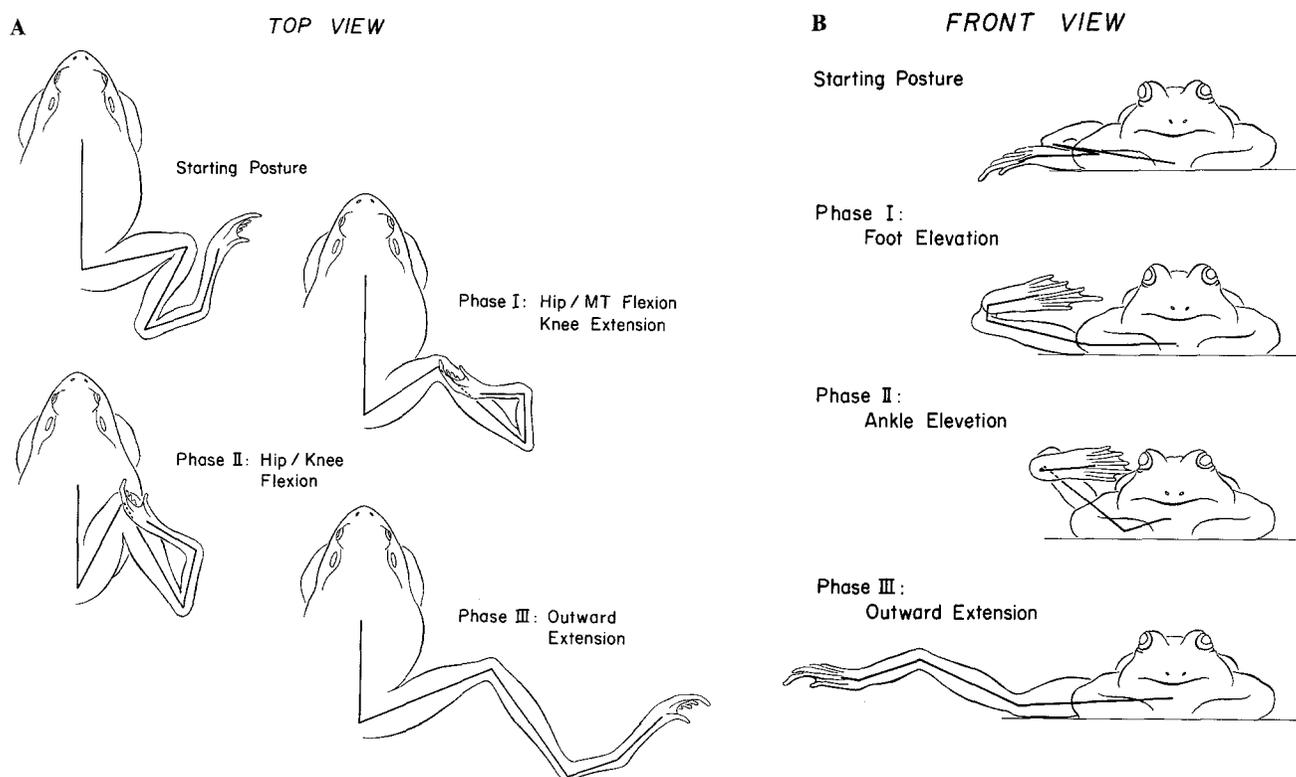


Fig. 3A,B. Stick figure reconstruction of the three essential phases of the back-wipe. *MT*, metatarsal

pause in movement at the end of this phase. Hindlimb position in phase II was measured immediately prior to the start of the whisk (i.e. metatarsal extension).

Rostro-caudal direction. The placing phase was characterized by a positive linear relationship between endpoint position (at the end of the phase) and stimulus position in the rostro-caudal direction ($P < 0.001$ for all frogs). The relationship is shown in Fig. 5. Thus, as the stimulus was moved rostral, the foot was placed further forward. This finding is in agreement with Giszter et al. (1989). A similar but more variable relationship was obtained in phase II just prior to the whisk motion of the foot ($P < 0.01$). The correlation coefficients for these tests are given in Table 1 (endpoint position data for frog D are unavailable). It should be noted that up to this point in the wipe there is little, if any, contact between the foot and the back. Changes to limb position due to contact between the limb and the back are thus minimal.

Medial-lateral direction. Although the frog was successful in removing the stimulus from positions both near and far from the midline, we found that it did not adjust the position of the endpoint for the location of the stimulus in the medial-lateral direction. Systematic relationships were not observed between endpoint position and the stimulus placement in the medial-lateral direction in either the placing or the pre-whisk phase.

In order to explore the strategy used by the frog, we plotted medial-lateral stimulus position against the point on the long axis of the foot at which the stimulus makes contact at the start of the whisk (Fig. 6). We found that as

the stimulus was placed more laterally from the midline, it contacted progressively more proximal positions on the foot (towards the metatarsal-phalangeal joint). Thus the spinal frog does not actively adjust limb endpoint position for stimulus location in this direction but rather contacts the stimulus with a different point on the foot.

Distance to stimulus. A separate examination of the endpoint positions at the end of phases I and II was conducted to determine whether or not limb placement precision improved between placing and pre-whisk. In all frogs, the average absolute distance between the endpoint and the stimulus increased during the pre-whisk phase. In no case did the endpoint come closer to the stimulus during this phase.

In contrast, a reduction in distance between the stimulus and the eventual point of contact was observed between the end of phase I and just prior to the whisk motion. Thus, although the endpoint moves further from the stimulus in preparing for the whisk, the contact point which does not appear to be actively controlled (see above) actually moves closer.

In summary, during the back-wipe, limb endpoint position was related to stimulus position only along the rostro-caudal axis of the body. There was no relationship between endpoint position and stimulus position in the medial-lateral direction. Rather, the frog simply made contact with the stimulus at a different point on the foot. In addition, the average distance between the stimulus and the endpoint increased from phase I to phase II. Thus, the distance between the endpoint and the stimulus was least during the placing phase, not afterwards.

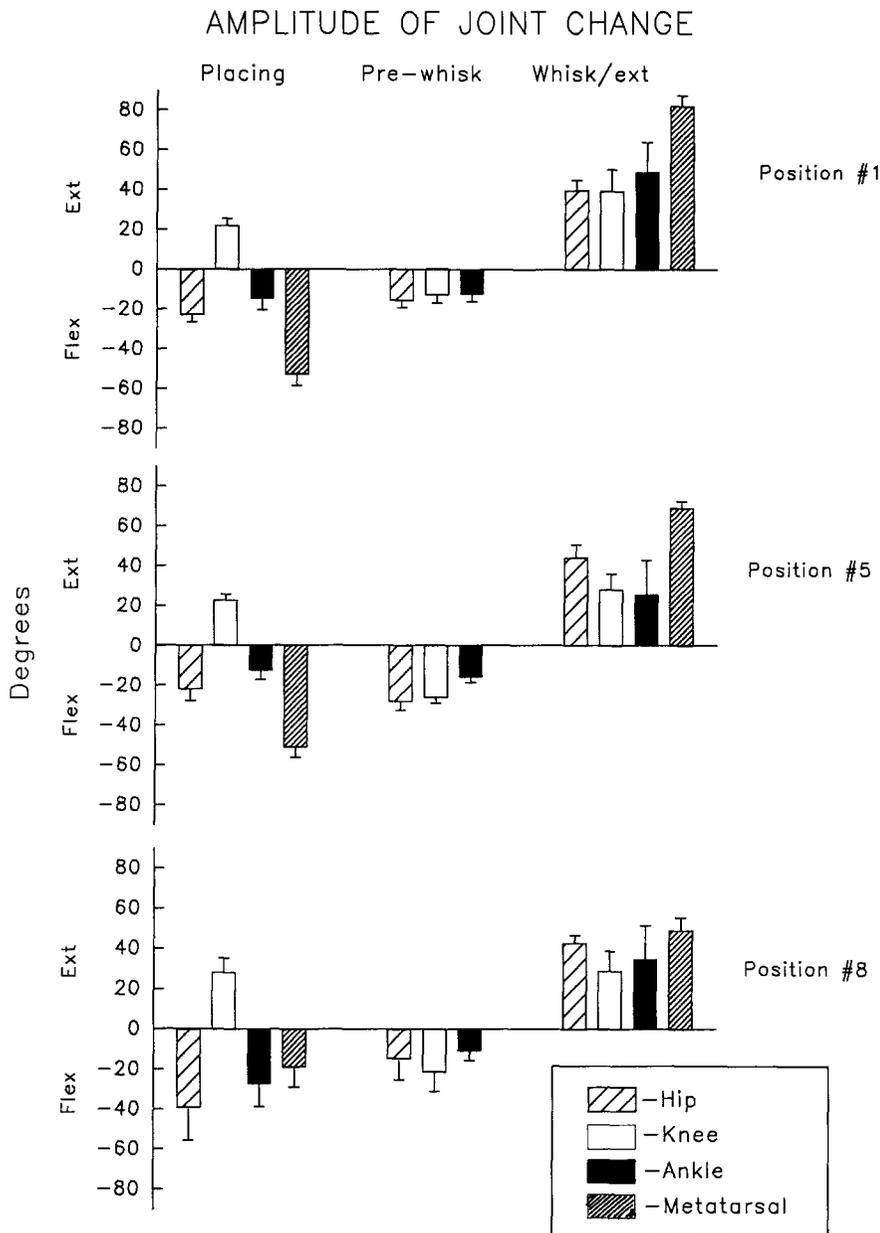


Fig. 4. Average amplitude of joint motion for each phase. Negative amplitudes indicate joint flexion. Data are shown for all joints of one frog over three stimulus positions

Relationship between stimulus position and anatomically defined joint angles

The relationship between anatomical joint angles and stimulus position was examined at the end of each movement phase. In the placing phase there was a systematic decrease in the knee angle as the stimulus was placed further forward. In addition, the metatarsal joint angle increased reliably with more rostral stimulus placement. In contrast, the ankle angle did not vary significantly with rostro-caudal stimulus position in any of the frogs tested, and the hip angle varied with rostro-caudal position in only two ($P < 0.01$; see Fig. 7 and Table 1).

In the pre-whisk phase, there was no systematic relationship between joint angles and rostro-caudal stimulus position. Similarly, in phase III there was no systematic relationship between final joint angles and stimulus position. Thus, adjustments to stimulus position were made

only in the placing phase. There were no systematic relationships observed between anatomically defined joint angles and stimulus position in the medial-lateral direction in any phase of the wiping motion.

Different frogs tended to adopt different strategies to remove the stimulus. For example, in two frogs, when the knee angle was strongly related to the rostro-caudal stimulus position, the metatarsal angle was weakly related or vice versa. Specifically, in Frog A, the correlation between the knee angle and the rostro-caudal stimulus position was -0.74 , while the correlation coefficient between the metatarsal angle and the stimulus position was 0.20 (see Table 1). In Frog D, the opposite pattern was observed. The correlation between the knee angle and the stimulus position was -0.26 , while correlation between the metatarsal angle and the stimulus position was 0.61 . Thus, for Frog A, as the stimulus was placed in a more rostral position, the knee angle decreased and the

RELATIONSHIP BETWEEN STIMULUS POSITION AND ENDPOINT POSITION IN THE ROSTRO-CAUDAL DIRECTION

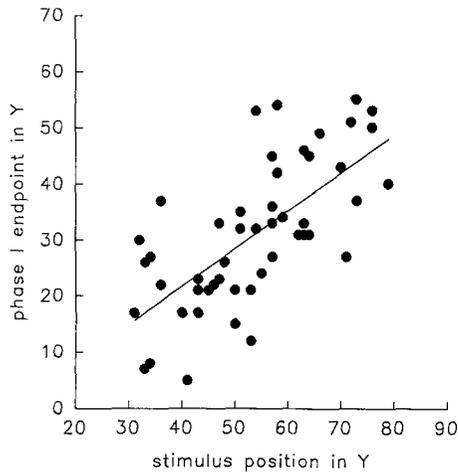


Fig. 5. Relationship between the stimulus position and endpoint position along the rostro-caudal (Y) body axis at the end of phase I. Distances of the endpoint and the stimulus from the cloaca are shown in millimeters. Data are shown for frog C

metatarsal angle remained constant. For Frog D, the knee angle remained constant and the metatarsal angle increased. Hence, while for these frogs both the knee and metatarsal angles were related to rostro-caudal stimulus position, each favoured a particular joint to adjust the hindlimb position to the location of the stimulus. Different strategies in which adjustments to stimulus position involved multiple joints were adopted by frogs B and C. In none of the frogs tested was there a completely unadjusted strategy (cf. Giszter et al. 1989).

In summary, in the placing phase, different joints were used to adjust hindlimb position to stimulus position in the rostro-caudal direction. Two animals achieved this adjustment to stimulus position using either the knee or the metatarsal joint but not both. The two remaining animals used more than one joint.

Relationship between stimulus position and hip orientation angles

There were few reliable relationships between hip orientation angles and stimulus position (Table 1). Two frogs

Table 1. Pearson product-moment correlation coefficients relating stimulus position to endpoint and joint angle

Relationship	Frog			
	A	B	C	D
Stimulus position to endpoint position along rostro-caudal body axis				
Phase I	0.63**	0.81***	0.70***	—
Phase II	0.55**	0.78***	0.15	—
Rostro-caudal stimulus position to phase I anatomical joint angle				
Hip angle	−0.39	0.44**	−0.39**	−0.19
Knee angle	−0.74***	−0.73***	−0.38*	−0.26
Ankle angle	−0.45	−0.52	−0.18	0.07
Metatarsal angle	0.20	0.39**	0.39**	0.61***
Rostro-caudal stimulus position to phase II anatomical joint angle				
Hip angle	0.18	−0.26	−0.12	0.08
Knee angle	−0.49**	−0.18	0.19	−0.16
Ankle angle	0.01	0.42	0.20	0.45
Rostro-caudal stimulus position to phase I hip orientation angle				
Pitch	−0.38*	0.03	−0.15	−0.18
Roll	0.37*	0.15	0.24	0.42
Yaw	−0.38*	−0.06	−0.31*	−0.25
Medial-lateral stimulus position to phase I hip orientation angle				
Pitch	0.09	0.23	0.27*	0.60*
Roll	0.28	0.09	0.13	0.51
Yaw	−0.15	−0.11	0.22	0.61*
Rostro-caudal stimulus position to phase II hip orientation angle				
Pitch	0.18	0.15	0.06	−0.21
Roll	0.34	0.13	0.21	0.44
Yaw	0.12	0.26	−0.33*	−0.08
Medial-lateral stimulus position to phase II hip orientation angle				
Pitch	0.05	0.60***	0.24	−0.20
Roll	0.30	0.11	0.20	0.56
Yaw	−0.18	0.20	0.16	−0.43

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

POINT OF CONTACT OF STIMULUS ON FOOT

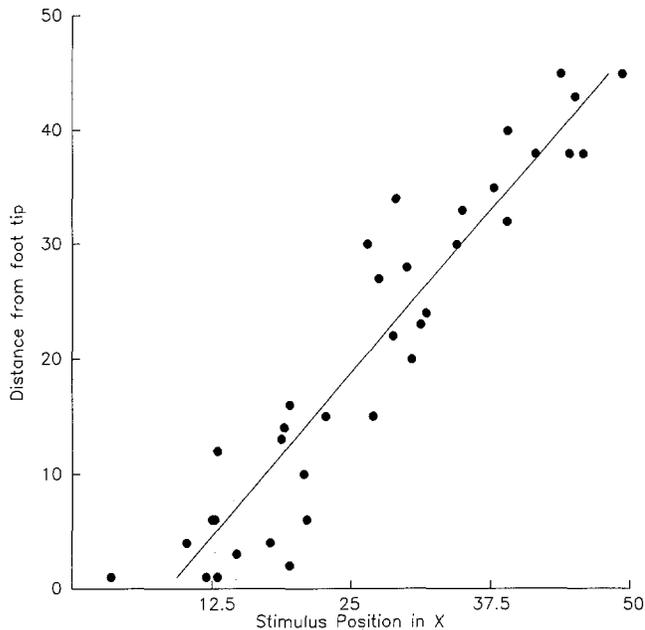


Fig. 6. Relationship between the stimulus position along the medial-lateral (X) body axis and the point along the long axis of the foot at which stimulus contact is made during the whisk. Distance of the stimulus from the foot tip and the midline is given in millimetres. Data for frog A are shown

showed a significant relationship between hip yaw angle (flexion/extension) and stimulus position in the rostro-caudal direction. A similar pattern is present between rostro-caudal stimulus placement and anatomical hip angle. A significant relationship was also observed for two frogs between hip pitch angle (adduction/abduction) and stimulus position in the medial-lateral direction. This latter finding may reflect adjustment of the hindlimb to differences in stimulus elevation which arise due to the curvature of the frog's body.

Planes of motion in the back-wipe of the spinal frog

Several authors have suggested that the wiping movement is largely planar (Berkinblit et al. 1984; Giszter et al. 1989). We have looked at this claim in a number of ways. Using three-dimensional stick figure reconstructions of the wiping movement which could be rotated to visualize the wipe from any perspective, we observed that the plane of motion assumed by the hindlimb was not constant throughout the wipe. However, the plane of motion of the hindlimb appeared to remain constant within a phase and changed planes between phases.

The planes of motion assumed by the frog during the back-wipe were studied quantitatively (a) by examining the variability of the vectors normal to the plane of motion within each phase and (b) by determining if there was a statistical difference in the direction of these vectors when grouped by phase. For both tests, the vector normal to the plane of motion was found by calculating the

cross product of the vectors formed by the hip and knee markers, and by the knee and ankle markers. This cross product thus represented the vector normal to the plane formed by the femur and the tibia. A normal vector was computed every 2.5 ms. If the motion of the hindlimb was confined to a single plane, one would expect to see all the normal vectors pointing in the same direction. Similarly, if the plane of motion remained constant during a phase, but changed between phases, one would expect to see three groups of vectors, each pointing in a different direction.

An extension of the statistical technique of Fisher et al. (1987), developed by Gilles Ducharme of the University of Montreal, was employed to test the hypothesis that the vectors in three-dimensional space were pointing in the same direction within a phase and pointing in different directions between phases. To determine whether motion was planar within a phase, for each phase of each trial the standard deviation of normal vector directions about the mean direction was computed. Table 2 gives the average of these standard deviations for each animal. The standard deviations reflect the variability in the orientation of the plane of motion. It can be seen that the data are characterized by small standard deviations about the mean within each phase. This suggests that motions within a phase are planar.

To test for whether or not the planes of motion changed between phases, the average of the vectors normal to the plane of motion for each phase was computed, \mathbf{R}_j ($j=1,2,3$). The average of the normal vectors for all three phases combined was also computed (\mathbf{R}_w). A chi-square test was employed to determine whether the individual group mean normal vectors were significantly different from the combined group mean normal vector. The formula for the observed value of chi-square is:

$$\chi_{\text{obs}}^2 = 2 \cdot N (\hat{\rho} - \mathbf{R}_w)$$

where $\hat{\rho}$ is the average of \mathbf{R}_1 , \mathbf{R}_2 , and \mathbf{R}_3 and N is the number of vectors in the data set. The critical value of χ^2 was calculated using $2 \cdot N - 2$ degrees of freedom.

Pairwise comparisons between the average normal vectors for each phase were carried out when the overall test of significance was reliable. For example, to test whether or not the plane of the first phase was significantly different from the plane of the second phase, the average normal vectors in the first and second phases (\mathbf{R}_1 and \mathbf{R}_2) and the average normal vector for the combined set (\mathbf{R}_{12}) were computed. The observed value of χ^2 , again based on $2 \cdot N - 2$ degrees of freedom, was calculated as

$$\chi_{\text{obs}}^2 = 2 \cdot N [(\mathbf{R}_1 + \mathbf{R}_2/2) - \mathbf{R}_{12}]$$

Tests for differences in the plane of hindlimb motion were carried out separately for each animal. For all four frogs there was a significant difference between the means of the three phases (Table 3), indicating that hindlimb motion was not confined to a single plane throughout the wipe.

Pairwise comparisons revealed different planes of motion for different phases. Specifically, the planes of motion differed between phases I and II for frogs A, B and

RELATIONSHIP BETWEEN ROSTRO-
CAUDAL STIMULUS POSITION AND
PHASE I JOINT ANGLE

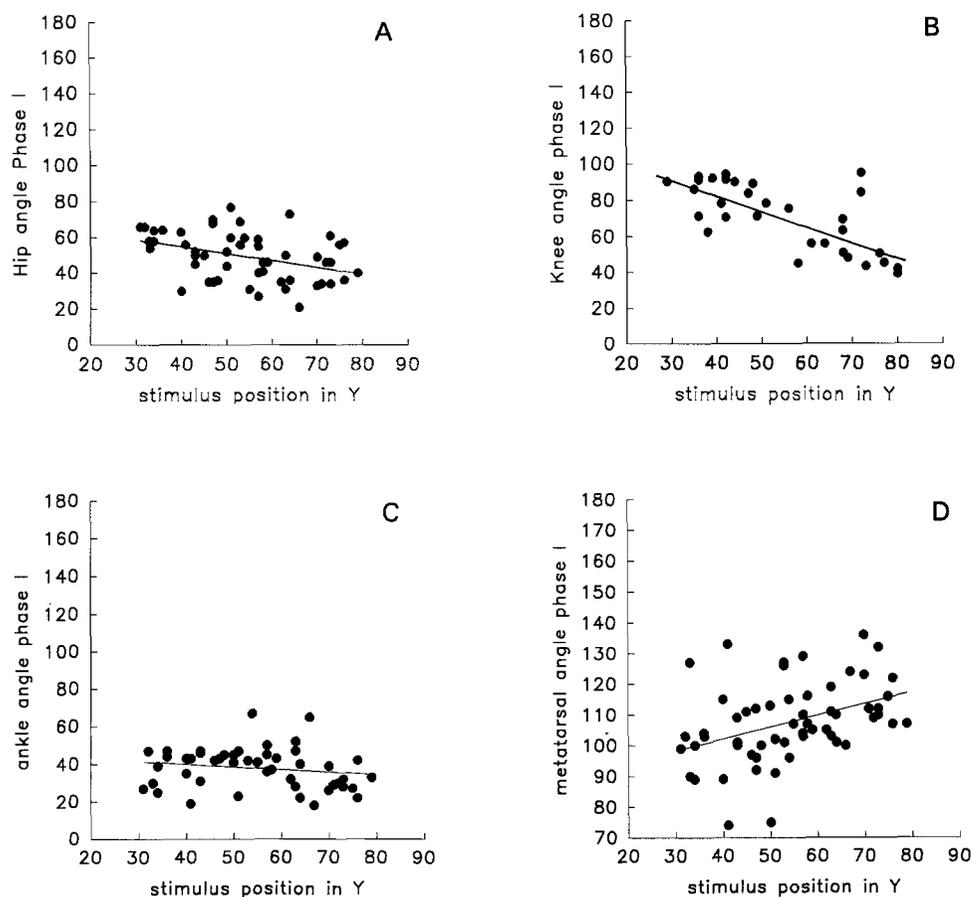


Fig. 7A–D. Relationship between stimulus position in the rostro-caudal (Y) direction and anatomical joint angle at the end of phase I for hip (A), knee (B), ankle (C) and metatarsal (D) joints (frog C). Distances of the stimulus from the cloaca are in millimetres; joint angles are in degrees. The relationships shown in panels B and D are statistically reliable

Table 2. Average standard deviations about the mean for vectors normal to the plane of motion

	Placing phase (degrees)	Pre-whisk phase (degrees)	Whisk/ext phase (degrees)
Frog A	2.84	2.96	3.89
Frog B	4.49	2.98	3.35
Frog C	2.92	3.09	3.40
Frog D	1.70	1.92	2.46

Table 3. Results of chi-square test for differences between phases in direction of vectors normal to the plane of motion

Difference between	Frog A	Frog B	Frog C	Frog D
Means	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.05$
Placing vs pre-whisk	$P < 0.01$	$P < 0.05$	$P < 0.01$	$P > 0.05$
Placing vs whisk/ext	$P < 0.01$	$P > 0.05$	$P < 0.01$	$P < 0.05$
Pre-whisk vs whisk/ext	$P < 0.01$	$P < 0.05$	$P < 0.01$	$P > 0.05$

C; the planes differed between phases I and III for frogs A, B, and D, and the planes differed between phases II and III for frogs A, B and C.

The orientation of the planes of motion are shown graphically in Fig. 8, which gives the distribution of vec-

tors normal to the plane of hindlimb motion for each frog. (A normal vector angle of zero radians corresponds to a vector lying in a horizontal plane along the medial-lateral body axis. This would be equivalent to a vertically oriented plane of hindlimb motion.) It can be seen that

Distribution of vectors normal to the plane of hindlimb motion

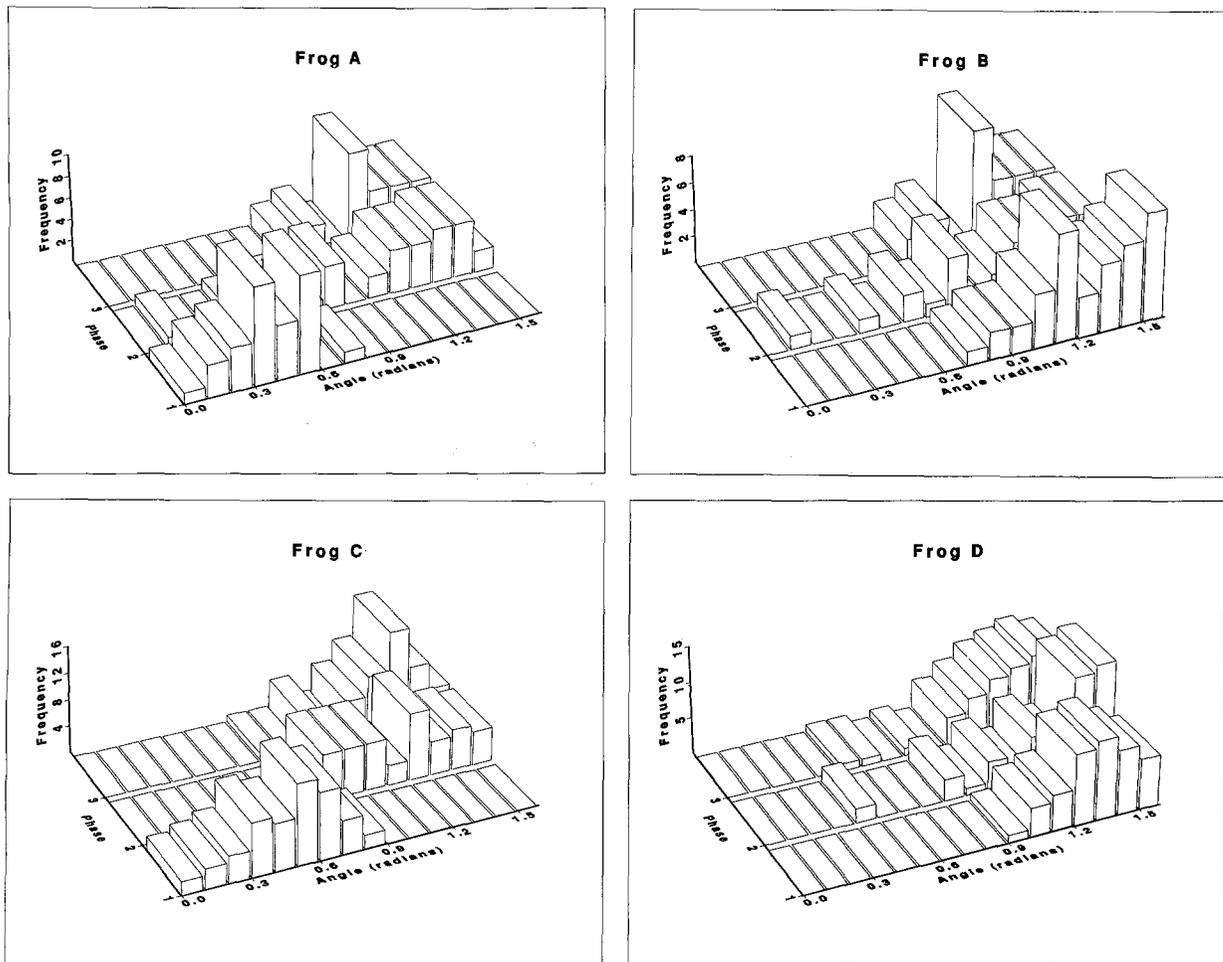


Fig. 8. Frequency distribution of vectors normal to the plane of the hindlimb wiping movement. Vector directions are shown relative to a vector lying in the horizontal plane along the medial-lateral body axis. Bin width is 0.1 radians (approximately 5.8°). Data are shown for all frogs

the phases were characterized by different planes of motion as indicated by the average normal vector directions. Moreover, although the planes of motion for the different phases tended to follow an orderly sequence, in different animals the orientation of the pre-whisk plane tended to be closer to either the placing or the whisk/extension plane.

Starting position of the hindlimb

Several researchers have proposed that the wiping reflex in both amphibians and reptiles is characterized by an invariant initial limb configuration (Berkinblit et al. 1986; Fukson et al. 1980; Stein et al. 1986). We have examined this in two ways by testing (a) whether there is a single invariant initial configuration and (b) whether there is an initial posture which is systematically related to stimulus position. To address the issue of whether a single invariant starting position is assumed, joint angles at the beginning of the placing phase were examined.

(This is the last stationary point prior to the wipe.) In single wipes, we observed either an initial flexion movement and then a brief pause prior to the placing phase or, more often, the frog would move directly into the placing phase from the current rest position of the limb. Thus, if no adjustment of limb position occurred before the placing motion, it was assumed that this position of the limb was an "acceptable" initial configuration for the nervous system. Using this criterion, the initial angles at the hip and knee were not constant and indeed varied over a range of approximately 60° . Moreover, this variability prior to the placing phase was not related to the rostro-caudal stimulus position. Thus, the initial posture of the limb was not invariant over differences in stimulus position either.

In contrast, when there were multiple wipes to a single stimulus position, the initial configuration was invariant for that specific trial. It was observed that when the frog performed multiple wipes in response to an irritant, there was little variability in the starting position. Specifically, during multiple wipes the hindlimb always returned to

within 5° of the initial joint configuration. However, as noted above, this initial configuration was different for different stimulus positions.

Motor equivalence

Two tests for motor equivalence were conducted. We examined the pattern of hindlimb joint angles corresponding to a fixed position of the endpoint. We also examined endpoint positions and associated joint angles corresponding to a fixed stimulus position. Both tests were conducted using limb positions at the end of the placing phase. Positions falling within a 5-mm² radius were treated as equivalent. This criterion was adopted because identical positions were rarely observed.

Joint angles were examined corresponding to a fixed endpoint position. In each frog we found approximately six groups of endpoint positions that satisfied this criterion. For a given fixed endpoint position, joint angles varied little from trial to trial (< 5°). Moreover, the variation which did occur was not the result of any systematic relationship between pairs of joint angles. Such a relationship might have been expected if inter-joint compensation were at work to preserve endpoint position.

Trials in which stimulus positions fell within a 5-mm² radius were also examined. The corresponding variability in both endpoint position and joint angles was assessed. It was found that for a given stimulus position, endpoint positions varied over a range of approximately 10 mm. Joint angles varied as a consequence; however, as noted in the preceding paragraph, any variation observed in joint angles could be attributed almost entirely to differences in endpoint position. Thus, although multiple limb configurations corresponding to a single endpoint position were not observed, there was some variability in the position from which the stimulus could be successfully removed. Note that the variability in limb endpoint position for a given stimulus position (approximately 10 mm) was small relative to the surface area over which stimuli were removed (approximately 1800 mm² for the *Rana catesbeiana* used in this study). The adjustment to stimulus position reported above for the rostral-caudal direction thus reflects a positioning accuracy considerably greater than the trial-to-trial placement variability.

Discussion

The present study identifies a number of techniques used by the spinal frog in performing the back-wipe. We observed that limb position is adjusted to account for the location of the stimulus along only one body axis. Of the four joints of the frog hindlimb, only one or two were used to position the limb relative to the stimulus. The hindlimb motion was partitioned into a small number of phases; within each phase it was planar. In multiple wipes, the limb returned to a constant starting position. For a given endpoint position the frog assumed a fixed set of hindlimb joint angles. The hindlimb wiping movement was thus achieved by a number of elementary behaviours

used in combination rather than by the use of a single simplifying strategy.

The wipe movement was accomplished using four joints of the hindlimb – hip, knee, ankle, tarso-metatarsal – which in total have 8 degrees of freedom. The reflex had three essential components: a placing phase (I), a pre-whisk phase consisting of a quick flexion of the hip and knee (II), and a whisk combined with an extension of the limb (III). Only the placing and whisk/extension phases were present in each wipe. Even when the second phase was absent, the frog was able to successfully remove the irritant from its back.

In the placing phase the spinal frog adjusted the hindlimb position to take account of the stimulus only along a single body axis, and restricted the stimulus-related adjustment to the hip, knee and metatarsal joints, while bringing the ankle into a fairly fixed position. The lack of adjustment of limb endpoint position to stimulus placement in the medial-lateral direction may be related to the length of the foot. In *Rana catesbeiana*, when the foot (from the metatarsal joint to the foot tip) is fully extended, it covers the entire lateral half of the back. Thus, the frog need not move the limb to account for stimulus position in the medial-lateral direction, but rather can remove the stimulus by contacting it with a different part of the foot.

There was also evidence in the placing phase that the elevation of the femur varied with medial-lateral stimulus placement. This may indicate that the spinal frog is able to adjust for stimulus height.

Giszter et al. (1989) suggest that the frog may employ different wiping strategies to remove the stimulus. They propose that in a discrete solution, a number of different stimulus positions elicit a single strategy for the removal of the stimulus whereas in a continuously adjusted solution there is a 1:1 map between stimulus placement and the wiping strategy. In this study we found that the frog incorporated both of these strategies, in that there was a continual adjustment of hindlimb position for stimulus placement in the rostral-caudal direction while there was a single position of the hindlimb associated with different stimulus placements along a medial-lateral axis. This is consistent with the view that the frog uses a number of different basic strategies in reflex wiping.

An initial inspection of phase II seems to indicate that the foot moves towards the stimulus, implying a specific adjustment to stimulus location. However, a number of characteristics of this phase suggest that its primary function is other than aiming at the stimulus (see Berkinblit et al. 1986). Although the hindlimb endpoint does move closer to the stimulus in the rostral-caudal direction, the absolute distance between the endpoint and the stimulus increases. Note, however, that the ultimate contact point is closer to the stimulus just prior to the whisk than at the end of the placing phase. Nevertheless, the lack of adjustment of the foot as a whole to the position of the stimulus in the medial-lateral direction suggests that the animal is not “aiming” the eventual contact point on the foot towards the stimulus. Thus, it appears that the function of this phase is not improvement of the placement precision of the limb.

The combination of metatarsal extension and hip and knee flexion resulted in a "whipping" action. This may increase the force with which the stimulus is cast from the body. In wipes without the second phase, the stimuli typically landed near to the frog's body. In contrast, when the second phase was present, wipes were much more vigorous and the stimulus was often cast more than 25 cm. Hence, phase II may function more to increase the force of the wiping motion than to position the limb with respect to the stimulus.

Previous reports, based both on lower precision recording and multiple two-dimensional views, have suggested that the hindlimb motion in the wipe reflex occurs in a single plane (Berkinblit et al. 1986; Giszter et al. 1989). Our results indicate that while the plane of motion of the hindlimb remains constant within a phase, this plane changes from one phase to another. The pre-whisk phase may be a transition between the placing and whisk/extension phases. That is, only two of the frogs displayed motion planes for the pre-whisk phase which differed from the orientations of both the placing and whisk/extension phases. Thus, the plane assumed by the hindlimb for this phase may be linked to the orientation of the plane of motion of one of the other phases of the wipe.

It has been suggested that the hindlimb may adopt an invariant initial limb configuration prior to the initiation of hindlimb wiping or scratching movements. This was not observed in the present study. When the stimulus was placed on the back a variety of behaviours were observed. In some cases, the hindlimb was observed to flex to an initial posture from which the placing phase (I) began. In other cases, the limb first extended and then began the placing motion. The placing motion was also observed to begin directly without either an initial flexion or an initial extension of the limb (also see Berkinblit et al. 1986). Even when the limb initially flexed, an invariant joint configuration was not assumed prior to the placing phase. The present findings are consistent with those of Fukson et al. (1980), who observed that when stimuli were placed on different areas of the back skin, numerous intermediate postures were assumed.

In multiple wipes, once an initial posture was assumed, this set of joint angles was maintained as a starting position for all remaining wipes. This constant initial configuration observed in multiple wipes may be similar

to the "natural starting point" reported for the scratching movements of spinal turtles (Valk-Fai and Crowe 1978).

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