

WHISKING AS A “VOLUNTARY” RESPONSE:
OPERANT CONTROL OF WHISKING PARAMETERS AND EFFECTS OF WHISKER
DEAFFERENTATION

P. Gao¹, B. O. Ploog² and H. P. Zeigler³
Biopsychology Program, Hunter College, CUNY^{1, 3}
Department of Psychology/SAS, College of Staten Island, CUNY²

ABSTRACT

The rat's ability to vary its whisking “strategies” to meet the functional demands of a discriminative task suggests that whisking may be characterized as a “voluntary” behavior --an operant--and like other operants, should be modifiable by appropriate manipulations of response-reinforcer contingencies. To test this hypothesis we have used high-resolution, optoelectronic “real-time” recording procedures to monitor the movements of individual whiskers and reinforce specific movement parameters (amplitude, frequency). In one operant paradigm (N=9) whisks with protractions above a specified amplitude were reinforced (Variable Interval 30-s) in the presence of a tone, but extinguished (EXT) in its absence). In a second paradigm (N=3), rats were reinforced on two different VI schedules (VI20-s/VI120-s) signaled, respectively, by the presence or absence of the tone. Selective reinforcement of whisking movements maintained the behavior over many weeks of testing and brought it under stimulus and schedule control. Subjects in the first paradigm learned to increase responding in the presence of the tone and inhibit responding in its absence. In the second paradigm, subjects whisked at significantly different rates in the two stimulus conditions. Bilateral deafferentation of the whisker pad did not impair conditioned whisking or disrupt discrimination behavior. Our results confirm the hypothesis that rodent whisking has many of the properties of an operant response. The ability to bring whisking movement parameters under operant control should facilitate electrophysiological and lesion/behavioral studies of this widely used “model” sensorimotor system.

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To whom correspondence should be addressed:

Professor H. Philip Zeigler,

Dept of Psychology, Hunter College, 695 Park Ave, New York, NY 10021

Phone: 212.772.5363 Fax: 212.772.5629 E-mail: hzeigler@shiva.hunter.cuny.edu

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The rodent vibrissae function as “mobile sensors” during active touch, generating whisking movements over a wide range of frequencies, velocities, and amplitudes (Carvell and Simons, 1990). During exploratory whisking, these movements may occur without any obvious feedback from the environment (*ex-afference*), although movement-generated input (*re-afference*) is present. During discriminative whisking, movement parameters are modulated by inputs generated during contact and palpation of object surfaces, and their topography and kinematics are significantly correlated with the stimulus properties to be encoded (Carvell and Simons, 1995; Harvey, Bermejo and Zeigler, 2001). These observations suggest that whisking movements may function as tactile *observing responses* (Zeigler and Wyckoff, 1961), modulated by recursively generated patterns of somatosensory input and maintained by association with reinforcing events in the environment. In this respect, the “active touch” behavior of rats resembles that of humans, who, when asked to discriminate object properties e.g., hardness, volume, or shape, solely by touch, typically use hand movements specific for each property (Lederman and Klatzky, 1987).

The observation that rats modulate discriminative whisking movement parameters to meet task demands suggests that whisking may be characterized as a “voluntary” behavior --an operant--and like other operants, should be modifiable by appropriate manipulations of response-reinforcer contingencies. Preliminary support for this hypothesis was provided by a previous study demonstrating operant conditioning of individual whisker movements. (Bermejo, Harvey, Gao and Zeigler, 1996). However, that study had two significant limitations. First, isolation of individual vibrissae required clipping of adjacent whiskers, a procedure which produces immediate disinhibition of cortical neurons (Kelley, Carvell, Kodger and Simons, 1999), complicating interpretation of behavioral effects. The second, and methodologically more significant limitation was the inability of our response-transducing device to provide information on the kinematics or temporal organization of

whisking movements, or to permit real-time reinforcement of specific whisking movement parameters.

In the present study we have used an optoelectronic detector system to monitor the movement trajectories of an individual whisker with high spatio-temporal resolution in a head-fixed preparation in which all other whiskers were intact. This has allowed us to (a) explore the role of reinforcement contingencies in maintaining exploratory whisking, (b) assess the effectiveness of operant conditioning procedures in bringing whisking movement parameters under stimulus control, (c) compare the kinematics and temporal organization of conditioned and unconditioned whisking movements and (d) examine the effects of sensory and motor denervations upon operantly conditioned whisker movements. We now report (1) that whisking movements may be brought under stimulus control using operant conditioning procedures, (2) that operant control of whisking involves modulation of the species-typical whisking pattern by the properties of the reinforcement schedule, and (3) that performance on an operant whisking task is maintained after either sensory or motor denervation of the whiskers. In addition to their methodological implications, our results suggest an unexpected degree of plasticity in the kinematics and temporal organization of rodent whisking patterns. [470]

MATERIALS and METHODS

Subjects: Four male and eight female Long-Evans rats, aged six to 12 months, were housed individually under a 12:12 h reversed light-dark cycle. Testing was carried out under a 23-h water deprivation schedule adjusted to maintain body weight at 85-90% of free-feeding weight. Subjects were adapted to handling before and after placement of a dental cement head-mount. Experimental procedures were conducted in accordance with NIH guidelines for the care and use of experimental animals and were approved by an institutional animal use and care committee.

Surgical Procedures: Rats were anesthetized with a mixture of Ketamine (100 mg/kg of body weight, ip) and Xylazine (5.5 mg/kg of body weight, im). A midline incision (about 2.5 cm long) was made in the scalp, beginning at about 1 cm anterior to bregma. Six self-tapping stainless steel screws (Small Parts #Q-TX0-2) inserted to a depth of 1 ½ turns were used as anchors for a dental cement platform, using the location of bregma as a reference point. A mounting screw (Small Parts #Q-TSB-632-12) was stereotaxically embedded in the central portion of the dental cement platform caudal to bregma. The incision was closed with a single suture at its caudal portion to minimize irritation of the incision edges. Bilateral whisker deafferentation (infraorbital nerve section) was carried out as described in a recent paper from our laboratory (Gao, Bermejo and Zeigler, 2001). Sectioning of whisker motor nerves was accomplished using conventional procedures (Semba and Egger, 1986). Postoperative testing was preceded by a recovery period of from 72 hrs to one week.

Apparatus: A sound-shielded test chamber (Industrial Acoustics: interior dimensions 80 x 60 x 60 cm) was equipped with a house light, a water delivery system, a tone generator (Radio Shack #273-074A, 2.5 kHz), and a pair of optoelectronic monitoring devices (PAS 11H, laser micrometer, Hama Laboratories, Palo Alto, CA. 94306). For testing, the rat's body was restrained in a V-shaped acrylic enclosure bolted to the chamber floor, with its head fixed to a metal bracket attached to the box by a bolt embedded in a dental cement crown (Figure 1). Water (20-40 µl aliquots) was delivered to the rat's mouth by a gravity-driven, solenoid-controlled water delivery system. A 486 PC was used to control stimulus presentation, reinforcer delivery, and data collection and storage, using customized software written in QuickBasic.

Optoelectronic transduction of individual vibrissa movements: Movements of individual whiskers in the rostro-caudal plane

were monitored using an optoelectronic system (laser-emitter and detector) which has been described in detail elsewhere (Bermejo, Houben and Zeigler, 1998; Gao, Bermejo and Zeigler, 2001: Fig.1). Briefly, interruption of the emitted beam by the shadow of a whisker generates a voltage shift in the subset of shaded sensors (CCDs). Whisker movement trajectories produce successive displacements in the position of that voltage shift, which are linearly related to whisker position. A comparator circuit identifies the successive positions of voltages above a preset threshold and outputs the data to a microprocessor for computation and display of the trajectory. The device has a temporal resolution of 1.4 ms and a spatial resolution of 11 µm. To monitor an individual whisker trajectory with the other whiskers present, a foam marker (3-6-mg/1x1x18mm) is glued along the side of the whisker. This increases its detectability with respect to surrounding vibrissae without affecting whisking kinematics (Bermejo et al., 1998). In the present study, reinforcement was contingent upon movements of the *right* C-1 whisker, which was monitored systematically throughout the course of the experiment, *with all other whiskers present*. In some sessions, we sampled movements of both the right and left C-1 whisker to compare differential effects of reinforcement.

A calibration procedure, which has been described in detail elsewhere (Gao et al., 2001), was carried out at the start of each test

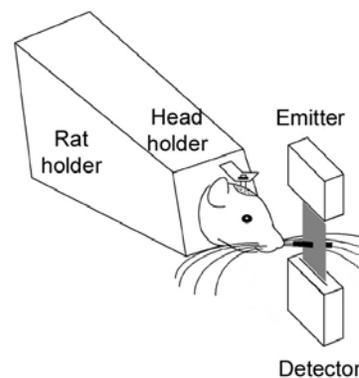


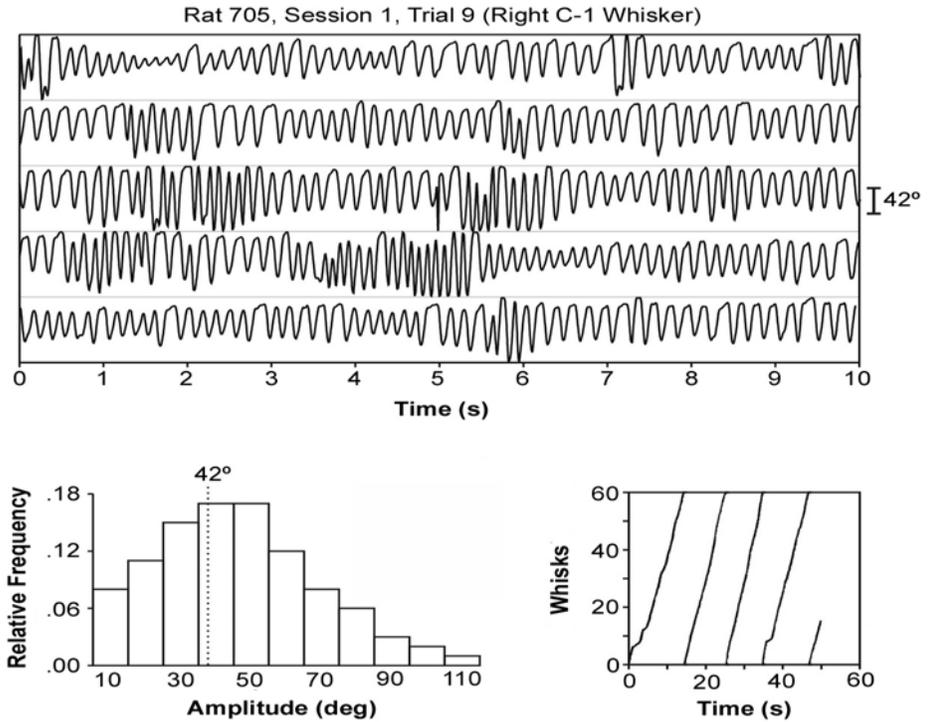
Figure 1. Schematic diagram of the testing situation: Position of the laser emitter/detector with respect to the head-fixed animal. In these experiments, all whiskers were intact on both sides of the face and the right and/or left C-1 whiskers could be marked for monitoring.

session and used to transform data on sensor locations into a record of angular whisker positions. Using the values obtained at calibration, the CCD data were transformed into angular units by a custom-written computer program, written to the hard disk, plotted in angular coordinates and displayed on a computer monitor as a plot of whisker position against time. For data analysis, a custom-written, cursor-driven graphics program was used to scan this plot at various temporal resolutions and to select episodes of whisking for additional analysis. Whisking movements selected for analysis had protraction amplitudes between 1° and 110° (the upper limit of the detector array), with smooth, clearly defined protraction (rising) and retraction (falling) phases and obvious starting and ending points. At the end of each scoring session, the number of whisks selected was calculated, and all data downloaded to a spreadsheet for further analysis.

Experimental Design:

Adaptation: Prior to conditioning, animals underwent a series of adaptation sessions comprising 30 trials, each of 60-s duration, which terminated in 2-s periods during which the house light was turned off and trial data were saved to the hard drive. Water was delivered on a variable-time schedule with a mean of 1 min (VT 60 s) but water delivery was *not* contingent upon the occurrence of a whisking response. In four of the animals (703, 705, L8, L9) nine adaptation sessions were given in order to assess the effects of repeated exposure to the test situation under conditions in which reinforcer delivery was not contingent upon a whisking movement.

Operant Conditioning: Nine animals were assigned to a multiple variable interval 30-s extinction (mult VI 30s EXT) schedule; three animals were assigned to a multiple variable interval 20-s variable interval 120-s (mult VI 20 s VI 120 s) schedule. A variable interval



(VI) schedule arranges reinforcement for the first response that occurs after a mean time interval (e.g., 20-s, 30-s, or 120-s) has elapsed. The more frequent the occurrence of a reinforcement, the richer the reinforcement schedule, the less frequent, the leaner the

Figure 2. Generation of cumulative records of whisking responses from whisking movement data recorded during an adaptation session. Top Panel: Unconditioned movements of a single identified whisker (C-1, right) during 50 s of an adaptation trial displayed at low resolution. (Upward movements are protractions, downwards movements are retractions. Bottom Panels: Left. Frequency distribution of peak protraction amplitudes (degrees) for the entire adaptation session from which the record was taken. The median of the amplitude distribution is indicated by a dashed vertical line and serves as a criterion for selecting responses to be plotted as cumulative records. (See discussion in Materials and Methods). Right: Cumulative record of whisking responses derived from the whisking data presented in the top panel. The cumulative record resets after every 60 responses.

schedule. Under extinction (EXT), reinforcement is never available. A multiple schedule presents two simple schedules of reinforcement successively in random order, with each simple schedule being correlated with its own discriminative stimulus. In the present study, each simple schedule was in effect for a fixed component duration of 60 s. The sequence of the components was

random with two exceptions. First, no more than three successive components with the same schedules occurred. Second, each schedule was presented equally often during each session. Sessions lasted for 30 to 40 minutes.

The house light was on during the entire session except during reinforcement delivery plus two seconds for data storage. Each whisking response that met a specified amplitude criterion (see below) generated auditory feedback (a click). Delivery of a reinforcer was preceded by the sound of the solenoid valve of the water delivery system. For all but one animal, the onset of the rich schedules (VI 20-s or VI 30-s) was signaled by the onset of a 2,500-Hz tone. The onset of the lean schedules (VI 120-s or EXT) was signaled by the absence of the tone. Because of the likelihood that tone onset might generate a burst of whisking, this situation was reversed for one animal (708). For this animal, the tone signaled the leaner (VI 120-s) schedule and the absence of the tone signaled the richer (VI 20-s) schedule.

Since, typically under multiple schedules, the rate of responding is directly proportional to the rate of reinforcement, high response rates were expected in the rich schedules and low response rates in the lean schedules. In order to assess performance on what was essentially a successive discrimination task, a discrimination index was calculated by dividing the number of responses in the rich schedule by the sum of responses in the rich plus lean schedules. Thus, a proportion *above* .50 indicates higher response rates in the rich schedule than in the lean schedule. A proportion of exactly .50 indicates indifference. Discrimination learning was considered to have occurred when the discrimination index had a mean at or above .70 over a block of three successive sessions.

In one animal in which whisking rate had been successfully conditioned using the mult VI 30s EXT schedule, an attempt was made to bring whisking *amplitude* under direct operant control. The amplitude criterion for that animal was progressively increased over successive sessions so that increasingly larger protraction responses were required for reinforcer delivery. All other aspects of the

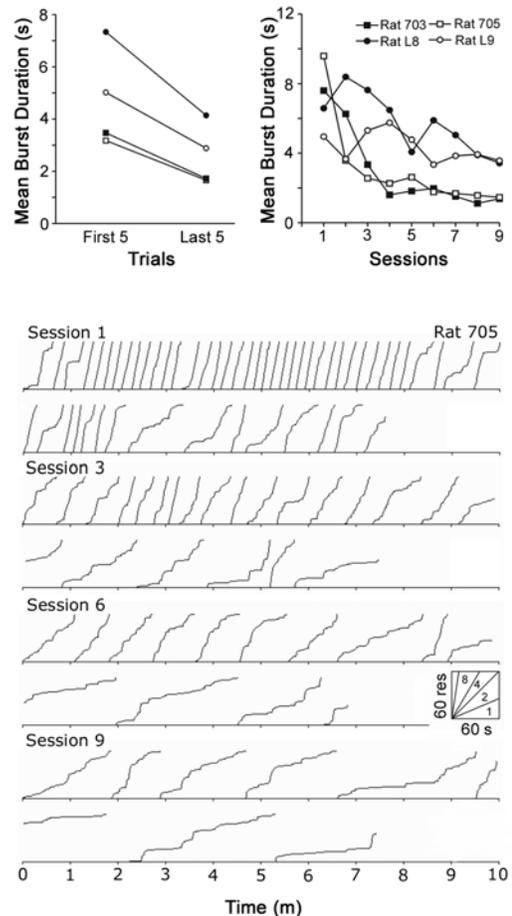


Figure 3. Effect upon whisking behavior of continued testing in the absence of response-contingent reinforcement. Top Panels. Effects of continued exposure to the testing situation upon the amount of whisking (at or above criterion amplitude) recorded within and between adaptation sessions. Data are from the four animals given nine successive sessions of adaptation to the test situation. Left. Within-session effects. Data from the first five and last five trials of each session collapsed across all nine sessions. Right. Between-session effects. Bottom Panels. Effects upon the rate and temporal distribution of whisking responses at or above criterion amplitudes (see Figure 2, above) over the course of adaptation testing (i.e., in the absence of response-contingent reinforcement). Data are plotted as cumulative records for the initial (10 min) trial of each session for the first, third, sixth and ninth successive session. Inset as in Figure 2.

training paradigm were held constant.

Defining the whisking response. In conventional operant conditioning paradigms, measurement of the operant behavior (e.g., pecking, bar pressing, nose poking, etc) is indirect, defined by some property of the transducing device upon whose occurrence reinforcement is made contingent (e.g., force

of the response required to close the switch on the pecking key). In the present study, access to whisking movements was direct, since the optoelectronic device generated a high-resolution, real-time, analog record of individual vibrissa movements (whisks) with clearly distinguishable protraction and retraction phases over an amplitude range of 100° (Figure 2: top). It was therefore necessary to identify a discrete operant “response unit” whose occurrence could be identified (on-line) and whose baseline level could be measured prior to the start of conditioning and differentially reinforced during conditioning with specific schedules. In the present study, a whisking response (whisk) was defined as a protraction whose amplitude was at or above the median amplitude of protractions recorded during the first adaptation session. Identification of whisks was accomplished using specially written computer programs whose functions are described in detail below (Results).

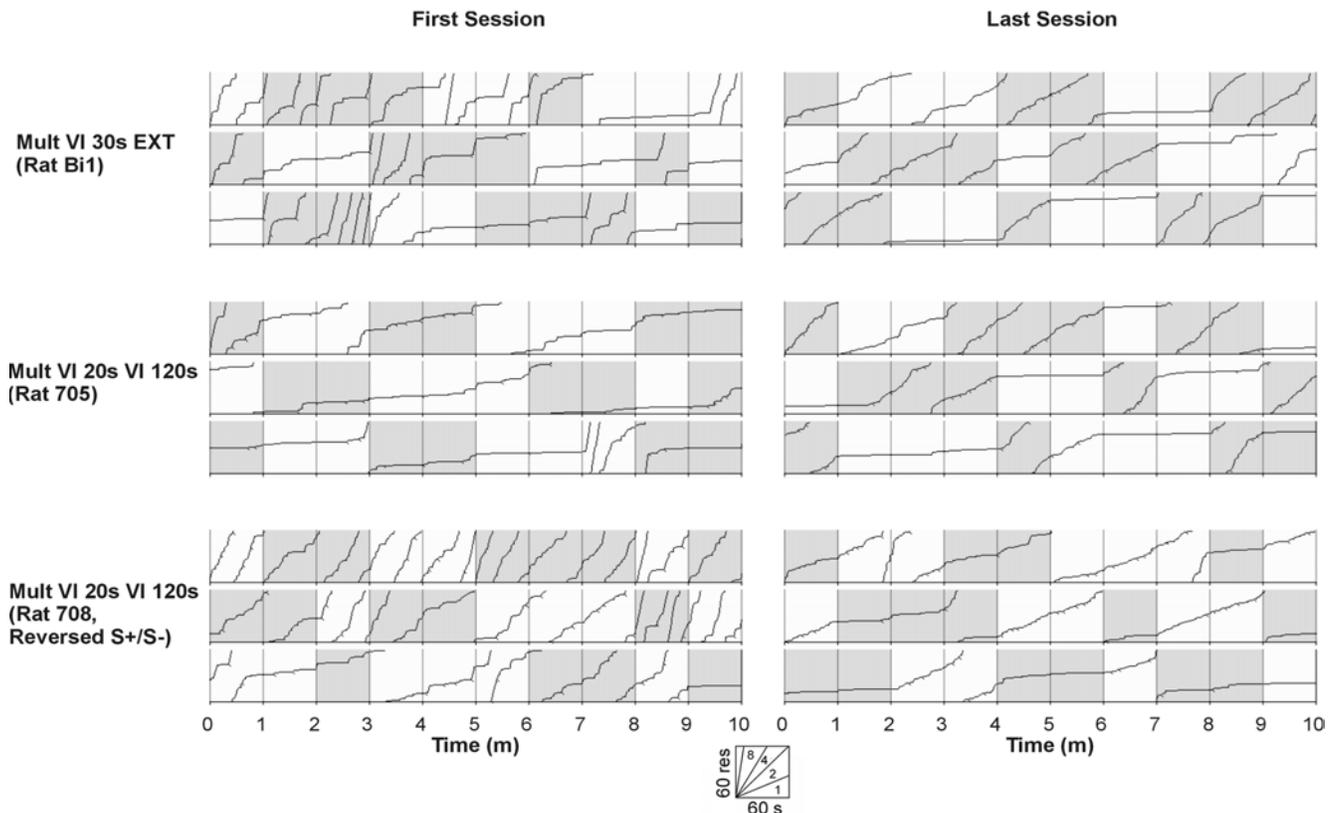
Another program was used to identify the start and end of discrete bursts of whisking and to calculate burst durations. A whisking burst was defined as any period during which the rat whisked at a rate greater than 4 responses/s and its duration was defined as the interval between the start and end of such

a period. The temporal organization of whisking responses was characterized using a Finite Fourier Transform (FFT) analysis of the whisking waveforms.

RESULTS

Figure 2 illustrates the procedures by which operant whisking response rates were derived from the raw movement data generated during a session. The top panel presents, for a representative animal, a record of *all* the whisking movements made during a single, 50-s trial recorded during the first adaptation session. A custom-written program identified “critical points” (movement onset, peak and termination) in the trajectory of each whisking movement and extracted the peak protraction amplitude. A relative frequency

Figure 4. Operant conditioning of the rat’s whisking response under multiple schedules (mult VI 30-s EXT; mult VI 20-s VI 120-s): Effects of manipulating stimulus and reinforcement variables. Cumulative records of whisking during the initial (left panels) and final (right panels) sessions of operant conditioning under each of the multiple component schedules. The presence of the discriminative stimulus (tone) is indicated by shading. For Rat’s Bi1 and 705, the presence of the tone is associated with a “rich” reinforcement schedule (VI 30-s VI 20-s, respectively). For rat 708 this relationship is reversed and the tone is associated with the lean schedule (VI 120-s). Inset as in Figure 2.



distribution of protraction amplitudes for the entire session is plotted in the bottom left hand panel. For this animal, the median protraction amplitude was 42°. During conditioning, protractions at or above this amplitude level were treated as whisks and were eligible for reinforcement. The rate at which protractions of this amplitude were emitted prior to conditioning defined the operant level of whisking. To display data on whisking response rates, a second program scanned the whisking record and, for each protraction meeting amplitude criterion, generated an output to a virtual cumulative recorder. The resulting cumulative record is shown in the bottom right hand panel of Figure 2, which plots cumulative whisks against time. The result of this process is to transform the analog data present in the whisking record into a cumulative plot whose slope is directly proportional to the rate of responding while preserving the analog properties of each whisk for later analysis if required. This mode of presentation permits comparison of data on rodent whisking behavior with the traditional rate data obtained

for such operant responses as key-pecking by pigeons and lever-pressing by rats (Ferster and Skinner, 1957).

The top panel of Figure 3 illustrates the effects upon whisking rates of continued exposure to the test situation during adaptation sessions (i.e., when reinforcer delivery was *independent* of the occurrence of a whisking response). Repeated exposure to the test situation did not produce systematic effects upon the *amplitudes* of unconditioned whisking movements (not shown) but did reduce the *amount* of whisking, as measured by the duration of whisking bursts. This reduction was evident both within (left panel) and between (right panel) sessions. In the bottom panel of Figure 3 the data for one of these animals (705) are re-plotted as cumulative records to illustrate the within-and-between session effects of repeated exposure to the test situation upon whisking response rates. For all sessions, the rate of whisking (as indicated by the slope of the record) is highest at the start of the session and declines within the session. In addition to this within-session reduction there is also a trend towards reduced responsiveness across the nine adaptation sessions. This is evident even at the start of each session, when whisking is usually at its highest level.

Figure 4 illustrates for three representative subjects the effects of the different multiple-schedules upon whisking rates during single conditioning trials early and late in training. The data are plotted as conventional cumulative records. Plots on the left are records taken from the first conditioning session; plots on the right are from the last session. Rat Bi1 (top) was tested under the VI 30s EXT schedule. The VI 30-s (reinforced) component was signaled by the presence of a tone (shaded). Note that during the initial session moderate to high response rates are seen in both (shaded and unshaded) components. During the final session, there is very little responding in the EXT (unshaded) component (except at the start of the session), while tone onset (shaded) is followed by a period of rapid responding. Rats 705 (middle) and 708 (bottom) were trained on the mult VI 20-s VI120-s schedule;

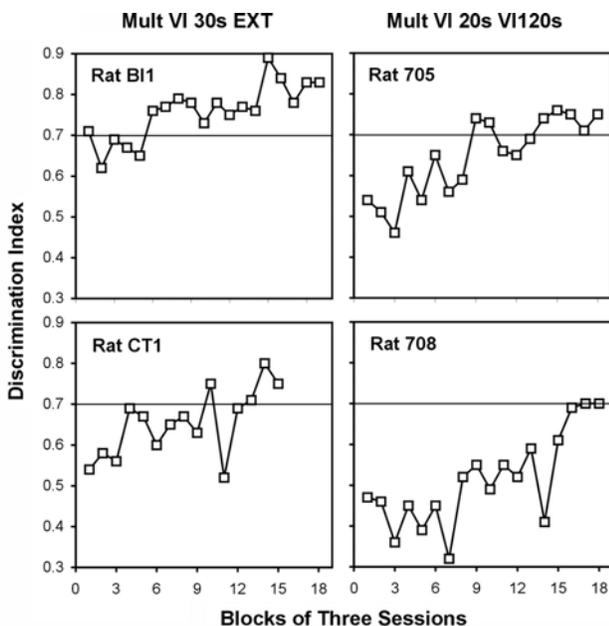


Figure 5. Acquisition of a discrimination between signaled components of a mult VI schedule by four representative animals. Left panels. Performance on the mult VI 30-s EXT schedule. Right panels. Performance on the mult VI 20-s VI 120-s. In three of the animals the tone signals the richer schedule; in the fourth (Rat 708) this relationship is reversed. Each data point represents the mean of three sessions.

i.e. both components were reinforced but at different rates. In Rat 705 the (VI 20-s (rich) schedule is signaled by tone onset (shaded); in Rat 708, the stimulus-reinforcer relationship is reversed; i.e., the shading signals the VI 120-s (lean) schedule; the unshaded panels indicate control by the VI 20-s (rich) schedule. In the initial sessions of training, similar whisking rates were observed in the two schedule components. With continued training, rates on the two components became differentiated, with the higher rates occurring in the richer (VI 20) schedules.

Figure 4 also illustrates another important feature of the operant conditioning processes: the gradual increase in the stability of the whisking patterns in the reinforced components. In the initial sessions, there may be considerable variation in the slopes of the cumulative records during the S+ periods, with periods of little or no whisking alternating with bursts of activity lasting for many seconds. These whisking bursts, though they occurred most frequently early in the session, could occur at any point, and were seen in both reinforced and unreinforced schedule components. With continued training, such occurrences became increasingly rare. During the last sessions, whisking patterns were generally quite stable, with relatively constant slopes in all reinforced components.

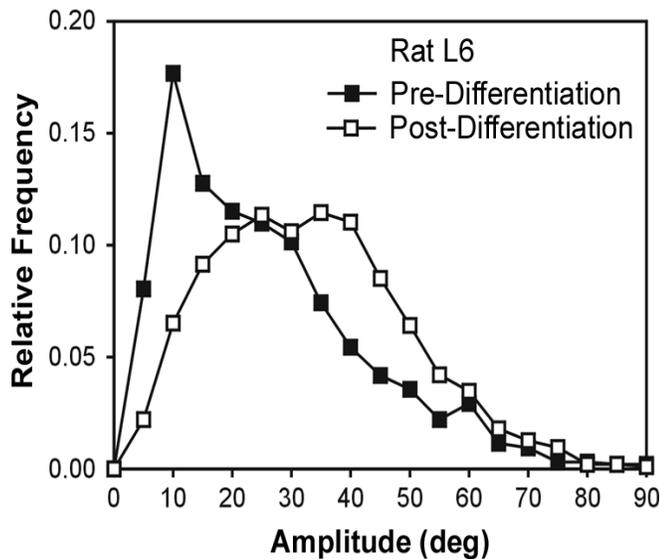
To illustrate individual differences in training time to reach criterion performance, Figure 5 presents learning curves for four subjects (two for each schedule). Note the relatively slow acquisition of differential responding for Rat 708 in which the tone stimulus signals the onset of the leaner component. For some animals, training was continued even after criterion performance was achieved in order to explore the stability and persistence of performance. Increased training tended to enhance performance. Training time (sessions to criterion) for the entire group of 12 animals is presented in Table 1.

The previous figures illustrated the effects of manipulating reinforcement schedules upon whisking response *rate*. In one animal (L6) we attempted to bring whisking response *amplitude* under experimental control by differentially reinforcing increasing larger amplitudes in successive sessions. During its

initial operant training on the VI 30s EXT schedule (Sessions 1-66) we reinforced whisking response with protraction amplitudes at or above the median amplitude of protractions made during the adaptation period. During the differential reinforcement sessions (Sessions 67-102) the amplitude criterion was increased in small steps over successive sessions, without changing any other property of the procedure. Figure 6 compares the relative frequency distribution of protraction amplitudes on the last session of the initial training (pre-Differentiation) with the distribution obtained in the last session of amplitude differentiation training (post-Differentiation). There is an obvious shift towards larger amplitudes in the mode of the post-differentiation distribution. This difference is highly significant (Kolmogorov-Smirnov test, $p < .0001$).

The data presented in the previous figures were plotted as cumulative records and provide no information about either the temporal distribution or the kinematics of conditioned whisking movements. Figure 7 presents records of the actual whisking movements (Right C-1, whisker) emitted early and late in learning by an animal trained under the VI 30-s EXT schedule. The top and bottom panels illustrate whisking responses made during the first and last training sessions, respectively and compare performance during the reinforced (Left: VI 30-s: S+) and the non-reinforced (Right: EXT: S-) components of the mult VI 30-s EXT schedule. Data from this specific animal were selected for presentation because they illustrate both within and between-session trends which were present, to varying degrees, in the data of all the animals. Several of these trends are noted below.

First, comparison of the right and left panels in the top half of the record with those presented in the top panel of Figure 2 indicates that, early in conditioning, whisking patterns in both the VI 30-s and EXT components are similar to those recorded during adaptation, with prolonged periods of continuous whisking at the characteristic modal frequency of 5-7 Hz. Second, the frequency and temporal distribution of whisking in the S+ and S- components during



the *initial* conditioning session are very similar. In both, extended bursts of relatively high amplitude dominate both records. Note, however, that, even in this first conditioning session, many periods of little or no activity were seen during the EXT component. In both components many of the responses are at or above the amplitude criterion for reinforcement (as indicated by the presence

Figure 6. Differentiation of whisking response amplitude by selective reinforcement of successively larger protraction amplitudes. Frequency distribution of protraction amplitudes for the last session prior to the introduction of differentiation training (session 66) and the final session of differentiation training (Session 102).

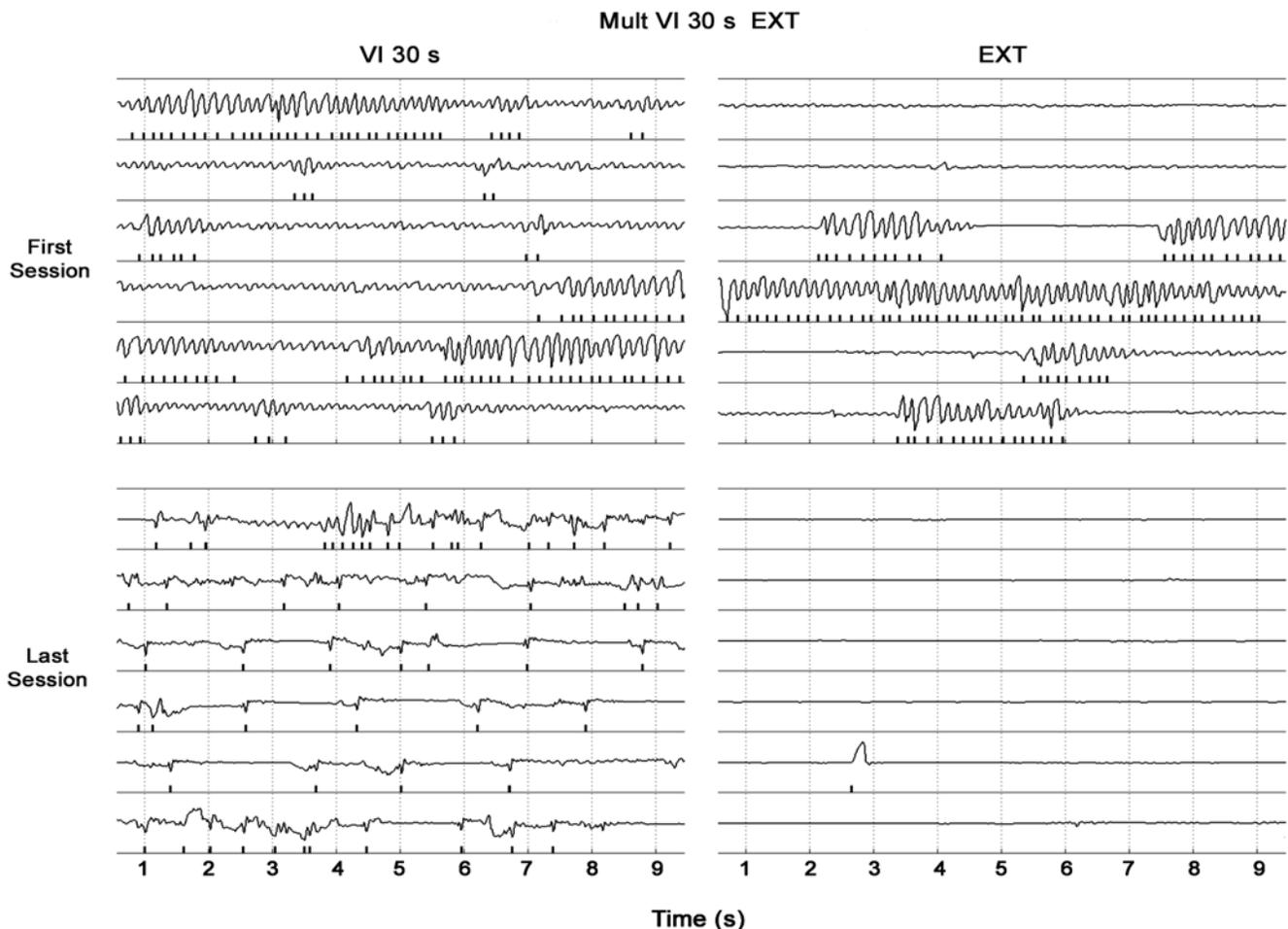


Figure 7. Effects of conditioning manipulations upon the temporal distribution of individual whisking responses. A comparison of responding during the initial (top) and final (bottom) session of training on a mult VI 30-s EXT schedule. Data presented are from a single trial of each session. Data recorded under the VI 30s component (tone present) are on the left. Data recorded under the EXT component (tone absent) are on the right. The presence of a tick in the baseline of each record indicates the occurrence of a whisking movement at or above the criterion amplitude for this animal. Note, in the first session, the presence of relatively prolonged periods of whisking in the VI 30-s condition and the reduction in the frequency of such bursts during the VI 30-s component of the last session. Note also that, in contrast to the first session, only one whisking response occurs during the EXT component of the final session.

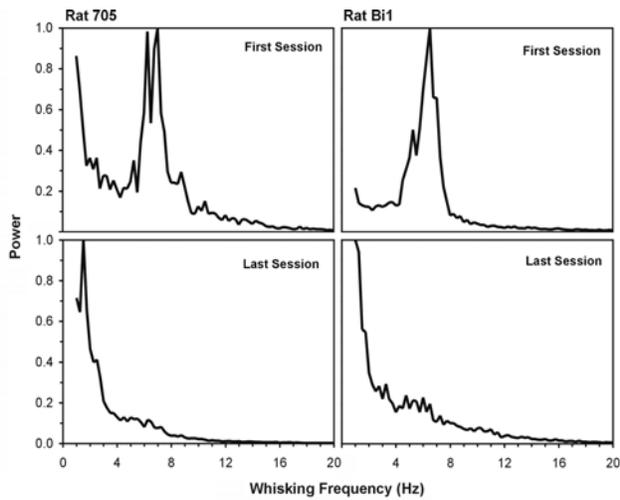


Figure 8. Fourier analysis of conditioned whisking movements. Spectral plots for the first and final sessions of two animals trained under different operant schedules. (Left) Mult VI 20-s 120s (Right) Mult VI 30-s EXT. In both animals the 5-8Hz peaks characteristic of the first session have been replaced by peaks at much lower frequencies.

of a tick in the baseline). Third, in the *final* training session, the animal continues to emit many such responses during the VI 30-s component, although their number is substantially reduced. In contrast, during the EXT component, only a single such response is emitted. Finally, the temporal organization of whisking during the S+ component is quite different in the first and last sessions. In the final session, the whisking were emitted at much lower rates (as low as 2- 4 Hz), and were more likely to occur as isolated events, separated by intervals without whisking activity.

These effects upon conditioned whisking frequency were confirmed by Fourier analyses of data recorded during the first and final conditioning sessions. Figure 8 presents spectral plots based upon this analysis for two representative animals, one of them being the rat whose data are presented in Figure 6 (above). Modal whisking frequencies for the first conditioning session are between 6-8 Hz, comparable to those seen during Adaptation. During the final conditioning sessions, there is little activity at that frequency and modal frequencies between 2-4 Hz dominate the record, confirming the pattern evident in the raw data plot of Figure 6. Finally, although

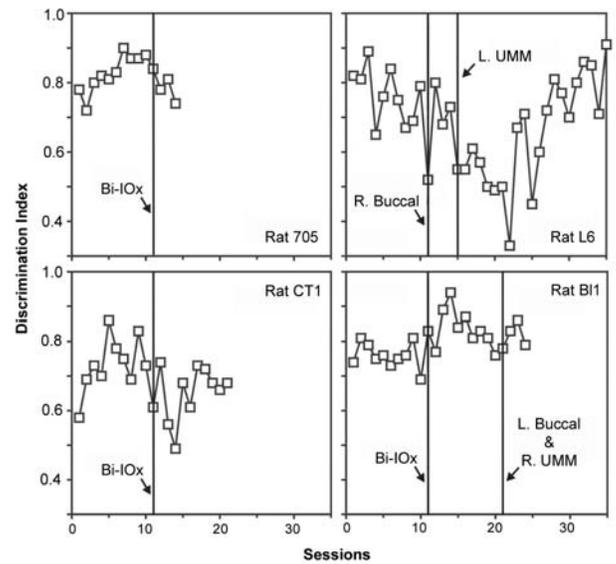


Figure 9. Effects of sensory and/or motor denervation of the whiskers upon performance on the discriminated operant whisking task. (Left) Effects of bilateral deafferentation of the whiskers. (Right) Effects of motor (top) and combined sensory and motor denervation (bottom). Abbreviations: Bi-IOx (Bilateral section of the infraorbital nerve); Buccal/UMM (section of the Buccal and Upper Marginal Mandibular divisions of the VII (facial) motor nerve). In all cases, conditioned whisking data is being monitored from the Right C-1 whisker.

only movements of the reinforced whisker were systematically analyzed in each animal, its homologous whisker tended to show very similar patterns of conditioned activity (not shown).

Effects of sensory and/or motor denervation of the whiskers.

Previous studies have shown that *neither* bilateral whisker deafferentation nor unilateral section of only *one* of the pair of whisker motor nerves disrupts whisking behavior in the adult rat (Gao, et al., 2001; Semba and Egger, 1986). Following completion of testing, four rats were deafferented (bilateral section of the infraorbital nerve—see Methods). Three animals sustained one-or-two stage partial motor denervations in the following combinations: Rat L6: Right Buccal followed by Left Upper Marginal Mandibular; Rat CT-3: Bilateral Buccal; Rat UN-4: Bilateral Upper Marginal Mandibular;. In one rat (BI1) bilateral deafferentation was followed by a period of retesting and then by unilateral section of one

motor nerve on each side of the animal. As in the other animals, movements of both right and left whisker were monitored, but only those of the right whisker were reinforced. Data for four representative animals are presented in Figure 9. Following either sensory or motor denervation, performance on the operant whisking task was either unimpaired or only transiently disrupted. Two of the three deafferented (Bi-lox) animals maintained or improved performance; the third showed a transient disruption followed by a return to preoperative levels. Similar effects were seen in rat L-6 after successive section of a motor nerve on each side of the animal. In rat BI1, neither the initial bilateral whisker deafferentation, nor the addition of a unilateral motor nerve section on each side disrupted performance.

DISCUSSION

Attempts to achieve operant control of whisking raise a number of methodological and conceptual problems. First is the need for a monitoring system which (a) records each occurrence of a whisking movement, (b) represents movement topography with high spatio-temporal resolution and (c) permits reinforcement of specific whisking movement parameters "on-line". Second, is the problem of defining the *response unit*, i.e. the specific behavior to be reinforced. In conventional operant conditioning situations, the behavioral unit is an "arbitrary" event which may be only indirectly related to the target response (e.g., switch closure produced by key pecks or lever presses). Because whisking movements may vary along several response dimensions (frequency, amplitude, velocity) the experimenter must specify precisely which parameters of the movement are to be reinforced. Third, is the problem of *operant level*, i.e. the initial level of responding before conditioning. The operant level provides a baseline from which changes in the rate of responding reflect the effectiveness of the conditioning procedures. The operant level of many target behaviors (e.g., key pecking, lever pressing) is at or very close to zero, while the operant level of rodent whisking may be quite high. Rats in a novel environment

may generate intermittent bursts of whisking for prolonged periods (Gao, et al., 2001). Distinguishing *conditioned* from *spontaneous* whisking under these circumstances presents a significant problem. However, establishment of a stable and predictable response baseline is a prerequisite for a variety of experimental treatments, including unit recording or brain lesions.

Whisking was recorded in immobilized animals using real-time, opto-electronic monitoring of whisker movement trajectories. Immobilization confines whisker movements to a restricted location and facilitates monitoring of individual whiskers. The high spatio-temporal resolution of the monitoring system provides precise data on whisking kinematics and temporal organization and allows us to distinguish protractions from retractions. Because whisking movements were recorded in real time, delivery of reinforcement (on defined schedules) could be made contingent upon the occurrence of a specific whisking movement parameter (e.g. amplitude, frequency), rather than upon the occurrence of an arbitrary, transducer-defined response. The problem of defining a response unit (whisk) is necessarily linked to the definition of its operant level. Our target response was the protraction component of whisking-- the only component actively generated by the follicular musculature (Dorfl, 1982; Carvell, Simons, Lichtenstein and Bryant, 1991). For each subject, a population of unconditioned whisking movements was recorded during adaptation, the median amplitude of that population was calculated, and whisks were defined as protraction movements at or above this amplitude. The operant level of whisking was defined as the rate at which such responses were emitted prior to the first conditioning session. Finally, the problem of distinguishing "spontaneous" from "conditioned" whisking responses was dealt with by manipulating stimulus-response-reinforcer contingencies and comparing response rates in the presence and absence of the conditional stimulus.

The importance of such contingencies in maintaining responding is demonstrated by the observation that, in their absence, i.e. over a series of sessions in which reinforcement

was independent of whisking, there was a gradual reduction in the probability of movements meeting the amplitude criterion for “whisks”. In contrast, the introduction of specific response-reinforcer contingencies maintained whisking at criterion amplitudes throughout the duration of individual test sessions and produced stable patterns of whisking across many weeks of such sessions. Since any activating effects of the tone should decline with repeated exposure, continued whisking is likely to reflect primarily the effects of contingent reinforcement. [Note: Although only the Right C-1 whisker was reinforced, response patterns on the two sides were similar. This is not surprising since whisking in head-fixed rats is often bilaterally synchronous (Gao, et al., 2001) so that the same whisking patterns would tend to be reinforced on both sides].

Manipulation of reinforcement schedules generated whisking patterns, which like more conventional operants, varied systematically with reinforcement rate (Ferster and Skinner, 1957). During test intervals associated with these schedules, whisking occurred at relatively constant and predictable rates, generating cumulative records whose slopes remained fairly stable within and across sessions. These observations support the conclusion that the whisking patterns seen in these animals reflect a process of operant conditioning. This conclusion is strengthened by the differential stimulus control seen in the two components of the multiple schedule paradigms. The mult VI 30-s EXT schedule is essentially a go/no go discrimination. It produced the predicted high whisking rates in the presence of the discriminative stimulus (S+ component), and cessation or significant reduction of whisking in its absence (S-component). In many subjects whisking was under tight stimulus control—terminating at the offset of the tone, and resuming at its onset (Fig. 4). Similarly, in the mult VI 20-s VI 120-s schedule tone offset and onset were clearly associated with differences in the rate and persistence of whisking. [Note: Stimulus control in this situation was apparent even under conditions when the S+ was the absence of a discriminative stimulus]. Most subjects achieved criterion performance,

(discrimination indices $> .70$) though there were considerable individual differences in acquisition time.

Evidence that increases in the index were not due simply to the activating effect of the tone is of two types. First, increases in the discrimination index reflected reduction or elimination of responding on the leaner schedule; second, the successful performance of rat 708, in which the tone was associated with the leaner schedule. Not surprisingly, this animal required a very large number of trials to achieve criterion performance (Fig. 5)].

Additional evidence for operant control is provided by effects of the experimental manipulations upon the amplitude and frequency of conditioned whisking. Effects upon amplitude were evident in an increased probability of responses meeting the amplitude criterion and in the shift in modal amplitude seen after differentiation training (Fig. 5). Effects upon frequency were evident in the gradual shift in the temporal organization of whisking over the course of training. During adaptation and in the initial sessions of training whisking tended to occur in bursts with a characteristic modal frequency of 6-8 Hz and which was present in *both* S+ and S- schedule components. With continued exposure to the reinforcement contingencies, such bursts were rarely seen in the S-component. In the S+ component, the 6-8 Hz rhythm became less frequent and the whisking pattern increasingly reflected the pattern of reinforcer delivery (Fig. 7). Operant control of whisking thus involved modulation of the species-typical whisking pattern by the properties of the reinforcement schedule.

Unlike feedback-dependent tactile discriminations, the operant whisking task is assumed to be under open-loop control. The finding that *neither* sensory nor motor denervation of the whiskers impairs performance is consistent with that assumption and with the hypothesis that *retention* of the task is independent of re-afferent inputs generated during whisking movements. It is possible that *acquisition* of this task might be dependent upon such inputs. However, previous studies in primates have shown that forelimb deafferentation did

not prevent the acquisition of conditioned visuomotor (pointing) and somatomotor (grasping) behaviors (Taub, Ellman and Berman, 1966; Taub, Goldberg and Taub, 1975).

Our current knowledge of the anatomy of the rodent trigeminal system suggests that acquisition of whisking-mediated *tactile* discriminations involves processing of inputs by sensorimotor circuits linking somatosensory (“barrel”) cortex (Guic-Robles, Valdivieso and Guajardo 1992) to whisker motor cortex (Carvell, Miller and Simons, 1996) and thence to a brainstem CPG for whisking (Hattox, Priest and Keller, 2002). The finding that conditioned whisking may be controlled by an *auditory* stimulus is consistent with observations of its control by other sensory modalities; e.g. olfaction (Welker, 1964). However, the circuitry linking central control of the CPG to inputs other than tactile stimuli is currently unknown.

Recent studies of tactile discrimination in rats have shown that variations in the amplitude and frequency of discriminative whisking movements are correlated with the sensory properties of the discriminanda (Carvell and Simons, 1995; Harvey, et al., 2001). The rat’s ability to vary its whisking “strategies” to meet the functional demands of the discriminative task suggests “voluntary” control over whisking parameters. The finding that whisking movement parameters may be brought under stimulus control using operant conditioning procedures is consistent with that conclusion.

The rat’s whisker system is a widely used model for experimental studies of sensorimotor control (Kleinfeld, Berg and O’Connor, 1999; Sachdev, Jenkinson, Zeigler and Ebner, 2001). Such studies would be facilitated by methodologies which bring the initiation, kinematics and temporal organization of whisking under the control of the researcher, rather than the rat. Operant conditioning paradigms improve control of stimulus and response specification and measurement, including manipulation of response parameters normally under the control of the animal. They are especially important for the study of behaviors, such as whisking, which involve continuous interactions between the

acquisition of information and the control of a mobile sensor. [1448].

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