

A

**SENSORIMOTOR DEVELOPMENT IN THE NEONATAL WHISKER  
SYSTEM**

by

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A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment  
of the requirements for the degree of Doctor of Philosophy, The City University of  
New York

2005

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## ACKNOWLEDGEMENTS

This work was supported by NIH NS37263 and CUNY awards to the lab and awards given to me by C.U.N.Y. and Hunter College, specifically the Gileece fellowship. I am grateful to Dr. Phil Zeigler, my mentor, for guidance and supervision. I appreciate the beneficial input from my committee members: Dr. Gordon Barr, Dr. Susan Brunelli, Dr. Jim Gordon, Dr. Regina Sullivan and Dr. Gerry Turkewitz. I thank Dr. Roberto Bermejo and Dr. Puhong Gao for invaluable assistance. Thanks to Dr. Asaf Keller, Dr. Phil Waite, and Dr. Jonathan Polan for manuscript reviews. I express appreciation to Drs. Roseann Flores, Peter Moller, Myron Hofer, and Howard Topoff, and to Ellen Breheny and Harriet Moss. Thank you to faculty, students, and colleagues at Hunter College. Thank you to International Society of Developmental Psychobiology for excellence.

I would like to acknowledge Victoria Lawson, Dov Winkleman, Abbe MacBeth, Luis Gomez, and Jamila Hussein for assisting in whisker measurements, to Lisa Ferrandino, Runa Khair, Jessica Schwartz, Terrissa Martin and Helen Martirosova for video analysis, and Barbara Wolin and Sonia Acevedo for animal care. Thanks much to Natrum Baboolaal. Thanks to experts in Audio/Visual.

I thank my colleagues and friends in Developmental Psychobiology at P.I., Zoology Dept. at OU, and the Physics Depts. at Northwestern Univ. and Turin Univ. I express appreciation to my parents, Joanna Newhard and Howard Klein, for expansive guidance and support. Thank you always to Roy Newhard, and to Anna Newhard for inspiration. Grazie sempre to my husband, Paolo Rumerio, for being involved in this, time and energy, support, and illumination in every sense.

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## CHAPTER 1

### INTRODUCTION

#### Sensorimotor integration during development

The control of movement involves a continuous interaction between the movement and sensory inputs generated by the movement. There is now considerable evidence that such sensorimotor interactions play a critical role in the development of behavior (Held and Hein, 1964;). Early sensory inputs during motor development may influence the formation and development of early motor patterns (Walton, Lieberman, Llinas, Begin and Llinas, 1992; Norreel, Pflieger, Pearlstein, Simeoni-Alias, Clarac and Vinay, 2003; Metcalfe, McDowell, Chang, Chen, Jeka, and Clark, 2005). There are two different types of sensory inputs: passive inputs elicited by environmental interactions, and sensory inputs generated by the movements themselves. Motor-generated inputs may lay a foundation for behavioral development (Khazipov, Sirota, Leinekugel, Holmes, Ben-Ari and Buzsaki, 2004). For example, in song acquisition in oscine songbirds, the male zebra finch learns its mating song from adult (conspecific) tutors. Early in life the infant zebra finch generates a plastic song in which the syllables are louder and more variable than the tutor song. The learning process concludes with crystallization of the song into a copy of the adult song. Reversibly disrupting syringial muscle activity during the sensorimotor phase using Botox (which blocks Acetylcholine transmission at the neuromuscular junction) resulted in abnormal adult song. Thus, if the immature birds hear the tutor's song, but do not produce the syllables themselves, they have permanent deficits in learned aspects of songs at adulthood (Pytte and Suthers, 2000). Similar conclusions were drawn in forelimb reaching in kittens. Depriving movement of the

forelimb using Botox from 3-8 weeks of age disrupted kittens' forelimb reaching and permanently reduced myelinated projections within the corticospinal system (Meng, Li and Martin, 2004).

The developmental link between movements and the inputs they generate is likely to be particularly important for such "mobile sensors" as the eyes and hands of primates and the whiskers of rodents. These structures function as both sensory organs and effectors, generating scanning movements used to extract information about the animal's environment. For example, vision involves continuous scanning movements of the eyes in order to localize and foveate the stimulus; in the somatosensory system, scanning movements of the hand may be used to detect, identify, or discriminate an object. An interesting feature of such somatosensory 'observing responses' is that they may be modulated to the properties of the object under examination. Humans, when asked to identify object properties e.g., texture, hardness, volume, or shape, solely by touch use hand movements specific for each property, such as lateral scanning, probing, enclosure, etc. (Lederman and Klatzky, 1987). Ahissar and Zacksenhouse (2001) have suggested that, "since most receptors respond primarily to changes in the sensed signal, the endogenous movements of a sensory organ ... may have evolved to provide sufficient changes in the sensed stimulus even when the environment is stationary". Moreover, Ahissar has demonstrated that haptic scanning is essential for human's texture discrimination of gratings that varied in spatial frequency (Gamzu and Ahissar, 2001). Such "haptic" scanning patterns are among the most complex movements generated by the somato-sensorimotor system. However, little is known about the role and timing of sensory inputs in the ontogeny of "active sensing" behaviors (Bushnell and Boudreau,

1993). Rodent whisking behavior has long been recognized as an ideal model system for the neurobehavioral analysis of this problem.

#### The rodent whisking system.

The rodent whisker system is exquisitely sensitive to inputs, with sensory capacities comparable to those of human somatosensation (Carvell and Simons, 1990). Whisking is a complex movement important for many aspects of such behaviors as exploration, orientation, localization, and discrimination in rodents (See Figure 1.1).

The whiskers are an array of mobile receptors, forming a three- dimensional (row x arc) grid extending beyond the perioral area that protracts and retracts horizontally. (See Figure 1.2; Bermejo et al., 2002). Whisking is the continuously alternating protraction and retraction of the whiskers (Welker, 1964) over a range of amplitudes (10-120 degrees) and frequencies (2-25Hz) and at a predominant frequency of 5-7Hz. These scanning movements function to transform spatial patterns of environmental input into temporal patterns of signals in the receptor array (Ahissar and Arieli, 2001). Whisker inputs are used to modulate its subsequent whisking movement parameters and (as in humans) such modulation is an important feature contributing to successful discrimination. For example, rats make large, stereotypically 5-7 Hz whisks when exploring in free air and localizing objects, and low amplitude, high frequency movements when further examining objects and discriminating textures (Carvell and Simons, 1996). The whisker vibrates at a detectable frequency that depends on whisking and on the shape of the whisker (Hartmann, Johnson, Towal and Assad, 2003). Position of the whisker array may have functional importance (Brecht, Preilowski and Merzenich, 1997).

At least two different studies suggest that adult competence in this system requires an association between whisker movements and their inputs during development. Carvell and Simons (1996) reported that neonatal whisker clipping impaired adult performance on a tactile discrimination task and that the impairment was associated with atypical whisking patterns. Similarly, Nicolelis, Chapin and Lin (1991b) found that whisker motor nerve section in rat pups produced significant disruptions in the organization of the thalamic whisker representation in adults, including reduced and distorted receptive fields. Because the whisking blockade produced by motor nerve section is irreversible, the functional significance of the effects for adult whisking behavior could not be assessed. Nevertheless, such findings support the hypothesis that “normal tactile perception in rats depends upon establishing a close relationship between the somatosensory and motor system beginning in early postnatal life” (Nicolelis et al., 1996, p.2194).

#### Whisking: anatomy and physiology.

Individual whiskers are “sinus hairs” set into individual follicles that are sensory organs with a rich blood supply and composition. Sensory innervation of the follicles is by the infraorbital branch of the maxillary division of the trigeminal nerve. Muscles form a sling surrounding the follicle. Motor innervation of the whisker muscles is by the upper marginal mandibular and buccal branches of the facial nerve (cranial VII, motor; Dorfl, 1982; 1985; see Figure 1.3). When the facial nerve activity causes the intrinsic muscles to contract, the whisker is protracted forward.

Although the anatomy and physiology of the *adult* whisker system have been well characterized, little is known about mechanisms mediating the emergence of whisking or

its subsequent functional development. The trigeminal system is among the earliest system to develop in vertebrates, and is very highly developed in all mammalian species. The vibrissal trigeminal neuraxis begins to develop during gestation and continues to develop postnatally (in Killackey et al., 1990). Fine whiskers appear in follicles early (at embryonic day E12; Yamakato and Yohro, 1979) and specialization of follicle sensory mechanoreceptors precedes the maturation of trigeminal nerve ganglia and brainstem nuclei (by the first day of life, Forbes and Welt, 1981). Somatotopy is well preserved in the whisker system at brainstem, thalamic and cortical levels (Figure 1.4). Vibrissa-related pattern is evident prior to birth in brainstem “barrellettes”, by P (Postnatal day) 1 in VPM thalamus “barreloids”, and after thalamic afferents invade the upper tier of the cortical plate, the “barrels” – thalamocortical cell aggregates in layer IV- begin to form by P3 and are completed by P5 (Killackey and Bedford, 1979; Figure 1.4) This suggests that information from the periphery directs the development of central structures (Woolsey, 1990). Stimulation-evoked functional activity appears in the barrel cortex after P7-8 and prior to whisking. (See Figure 1.5).

Whisker movements begin around P12, and gradually increase in amplitude until the stabilization of the species-typical whisking pattern (5-7 Hz frequency, 12-20 degrees amplitude; Welker, 1964). This developmental timeline coincides with the observation of synaptogenesis in cortical layer 2/3 (at P12; Lendvai, Stern, Chen and Svoboda, 2000; Miller, Blake, Erinjeri, Reistad, Sexton, Admire and Woolsey, 2001), and with cortical thickening (by P20; Rice, 1985). Deafferentation from P9-11 disrupts whisker receptive fields and results in diminished spines in the cortical layers 2/3 (Lendvai et al., 2000).

This timeline coincides with the initial appearance of whisker movements in response to microstimulation of cortical vibrissal motor area by P16 (Keller, 1999, pers. com).

Vibrissal inputs during development may be of two different sources. Vibrissal inputs are passively elicited in neonates during interactions with siblings or dam (Alberts and May, 1984; Larson and Stein, 1984; Distel and Hudson, 1985; Hofer, Shair and Singh, 1976; Hofer, Fisher and Shair, 1981). The use of passive whisker stimulation as a conditioned stimulus suggests a functional integration of early whisker inputs as early as P1 (Landers and Sullivan, 1999). Early interactions are of adaptive value to the rat pup, and whiskers may function as a proximal cue to localize objects (nipple, siblings) and regulate behavioral activity (Sullivan, Landers, Flemming, Vaught, Young Polan, 2003). The emergence of whisker movements during the second postnatal week (Welker, 1964) provides an additional source of afference, *actively* generated afference, that may contribute to the initial organization of rodent behavior.

In the present studies I hope to clarify the contribution of neonatal and perinatal whisking experience to the initial emergence of whisking, the refinement of the whisking pattern during the first perinatal month, the morphology of the developing whiskers and the eventual integration of whisking movements into the behavioral repertoire of the developing and adult rodent. Surgical and pharmacological treatments were used to manipulate the amount, source and timing of sensory inputs available to the developing pup and the effects of these treatments upon whisking were examined. In Chapter 2, whisking was videotaped from the age whisking begins to throughout the first month of life. Fine grained analysis of whisker movements was done frame by frame. Because

analysis of treatment effects requires comparison with normal developing animals, this Dissertation provides the first systematic analysis of the development of whisking.

### METHODOLOGICAL CONSIDERATIONS

The methods currently used to manipulate whisking experience (sensory or motor nerve section) are limited in clarifying the contribution of each different source of whisker afference to the development of the rat's "active touch" system. Trigeminal nerve section removes all potential sources of afference to the whiskers. The nerve regenerates quickly in young animals, however this treatment is permanent in that it produces a disordered and unpredictable pattern of re-innervation in adult central maps (Florence, Garraghty, Wall and Kaas, 1994) that complicates interpretation of effects upon whisking map-related behaviors in adult rats. Neonatal facial motor nerve section may be used to remove *motor-generated* afference to the whiskers, however it is irreversible, making it impossible to examine treatment effects upon the behaviors of the adult animal (Aldskogius and Thomander, 1986; Nicoletti, Oliveira, Lin and Chapin, 1996). Developmental studies of whisking would be facilitated by treatment, which selectively and reversibly block movement-generated inputs. Therefore, pharmacological methods (Botulinum toxin=Botox®) have been utilized to produce a reversible paralysis in whisking muscles by inhibiting acetylcholine release from presynaptic membrane by cleaving binding protein SNAP-25 at syntaxin protein complex (Brin, 1997; Angaut-Petit, Molgo, Faille, Juzans and Takahashi, 1998). Botox is functionally reversible and presynaptic events such as axonal sprouting, activity at motor endplates, and calcium channel release facilitate new neuromuscular contacts and reactivate muscle fibers (Duchen, 1971; De Paiva et al., 1999; Santefe, Urbano, Lanuza and Uchitel, 2000).

Whisking in the recovering animal was measured using opto-electronic recording. (See Figure 1.6, Bermejo et al., 2001.)

Landers et al., (2002) demonstrated that Botox may be used to block whisking in infant and adult rat. Neonatal whisking blockade is functionally reversible. Botox does not impair either the kinematics or the rhythmic patterning of adult whisking. (See Figure 1.7.) In both adult and developing animals the duration of the blockade is dose dependent (Figure 1.8).

The modular organization of the vibrissa system, with each whisker linked functionally to its central representation in a well-established topographic circuitry, facilitates *controlled* manipulations of whisking inputs. For these reasons, the rodent whisker system has become a powerful model for studies of neural and behavioral development. Such studies have shown that deprivation of sensory inputs during development has consequences for the organization of adult central sensory maps (e.g., Erzurumlu and Kind, 2001), sensorimotor representations (e.g., Huntley, 1997) and behavior (e.g., Carvell and Simons, 1996). Behavioral studies using reduction of whisking inputs by Botox treatment during development are needed to clarify the processes by which movement-related inputs contribute the acquisition of “active touch” behaviors.

Although deprivation has proven instrumental in studying neural circuitry underlying behavior, our analysis would be strengthened considerably by using a treatment that increased whisking to provide a comprehensive understanding of the behavior and adaptations involved in manipulating experiential variables. Experience dependent plasticity has been demonstrated in the somatosensory system with enrichment

(Greenough, Black, and Wallace, 1987; Seo, 1992), long-term passive whisker stimulation (Diamond, Armstrong-James, and Ebner, 1993) and neonatal blinding (Rauschecker, Tian, Korte, and Egert, 1992). For example, Rauschecker et al. (1992) have shown in cats that neonatal enucleation produced increased growth of the whiskers and improved performance on an auditory localization task. He later extended this finding to mice demonstrating an increase in the size of a subpopulation of whiskers correlated with an expansion in barrel size (Figure 1.9) and inferred that the effects were due to increased *use* of the whiskers. Welker (1964) suggested that early blind animals have enhanced exploratory behavior that included sniffing and whisking. These data suggested that bilateral neonatal enucleation might be an effective way to increase whisker use (and whisker input). To assess the utility of BEN as a mechanism for generating increased whisker input beginning in infancy, the initial findings were repeated and confirmed and the results are discussed in relation to the development of the whisker system. Animals were purchased through an approved vendor and all procedures on animals were in accordance with Hunter College Institutional Animal Care and Use Committee, National Institutes of Health, and NY State Dept. of Health.

The focus of this dissertation is to study the influence of sensory inputs on motor development. Chapter 2 presents studies of the development of whisking and the effects of sensory and motor manipulations. Results have generated hypotheses for experiments on neural correlates to whisking. Chapter 3 studies the effects of neonatal binocular enucleation upon whisker size and whisking frequency during development. All of these studies were also designed to study integration of whisking into the behavioral repertoire of the animal, and these analyses will appear in future reports.

## CHAPTER 2

### INFLUENCE OF SENSORY INPUTS ON MOTOR DEVELOPMENT.

The rat's whisking behavior emerges during the second postnatal week, gradually increasing in frequency and amplitude, and reaching adult form by the end of the third week (Welker, 1964). Because whisking persists after whisker deafferentation (Gao, Bermejo and Zeigler, 2001), cortical ablation, or decerebration (Semba and Komisaruk, 1984; Lovick, 1972) this sequence of events is assumed to be independent of peripheral input and reflect the output of a central pattern generator (CPG). However, the studies on which this hypothesis is based were carried out in adults, and little is known about mechanisms mediating the emergence of whisking or its subsequent development. The anatomical organization of the whisker system is sensitive to neonatal manipulations of whisker input (Woolsey, 1990). Carvell and Simons (1996) have shown that reduction of normal vibrissa input during development disrupts both adult performance on a tactile discrimination task and control of the discriminative whisking pattern. These reports suggest a contribution of whisker input to the functional development of whisking. As noted, such input is available early in life.

To examine the development of whisking and develop tools that are used to clarify the contribution of two forms of early sensory inputs to the initial emergence and subsequent refinement of the whisking pattern, perinatal surgical treatments producing varying sources, and durations of whisker deafferentation were done. The infraorbital (IO) branch of trigeminal nerve (V) was sectioned alone or in combination with motor nerve sections (VII; Figure 3.1). Using videographic analysis (30 frames per second=fps) whisking kinematics was measured in pups across the first month of life.

## METHODS

*Subjects.* A total of 159 rat pups of the Long-Evans strain from 33 litters were born in our breeding colony and housed with the mother until weaning at P21. Postnatal developmental events are timed with reference to the date of birth as P0. At P2, litters were culled to 8-10 when possible with equal distribution to males and females.

*Experimental design.* Observations on the initial appearance of whisking movements and eye opening were carried out in six normal litters (N=50 pups).

A group of 109 animals from 27 litters was used in the next series of studies on effects of deafferentation. (See Table 1.)

At birth, pups in a litter were randomly assigned to treatments with equal distribution to males and female (2-4 pups per litter). Pups were tested longitudinally. Each day of life pups were weighed and monitored for whisking qualitatively and eye opening by experimenters blind to treatment. Some of these pups were videotaped and scored by blind observers (30fps). The age whisking emerges was computed for each animal, averaged for litter and compared across treatments. For fine-grained analysis, video analysis was done to measure whisking frequency frame by frame. No more than 1 male and 1 female per litter was used for whisking frequency.

There were five variations of deafferentation treatments: (1) *Unilateral or bilateral deafferentation*: section of the infraorbital nerve was used to abolish all sensory inputs from one or both sides of the face at the age whisking appeared (P10-14). Fifty pups were randomly assigned to treatment groups:

**Uni-IOx:** (n= 20 pups) infraorbital nerve section on one side of the face, as it exits the infraorbital foramen, while the contralateral whiskers are left untreated and used as normal controls.

**Bi-IOx:** (n=8 pups) infraorbital nerve section on both sides. **Normals** (n=3) were used for comparison.

**Surgical controls** (unilateral n=6, and bilateral n=4) sustained all procedures except for nerve section.

For an additional control group, in nine pups (IOx n=6, sham n=3) the section was carried out using an intraorbital approach, in which the infraorbital nerve was sectioned at the base of the eye orbit (Kleinfeld, pers.comm.).

(2) *Developmental timing.* Unilateral section of the infraorbital nerve was carried out at ages prior to, during, or after the emergence of whisking. Eighteen pups from 8 litters were randomly assigned to treatment groups:

**P7-8:** n = 10 pups, prior to whisking

**P10-14:** n=20, at whisking emergence

**P15-21:** n =8, following appearance of adult whisking

(3) *Deafferentation combined with contralateral motor denervation.* Contralateral motor nerve section (VIIx) abolished actively generated input on the side contralateral to the IOx section, further reducing total sensory input to the brainstem. Nine pups from 3 litters were randomly assigned to treatment groups:

**IOx + VIIx**(n=6) Uni-IOx with facial nerve section on the opposite side were done at the age whisking began (P12), the Uni-IOx side was assessed.

A control group was **IOx+ipsi VIIx** (n=3) in which Uni-IOx with facial nerve section was done on the same side, and the untreated side was assessed.

(4) *Effects of repeated sections.* Waite (1984; pers. comm., 2002) reports evidence of functional activity in the trigeminal neuraxis of developing animals a week after infraorbital nerve section. A second section was done to assess whether regeneration is important to resumption of normal whisking. This group also provides a within animal control for age of surgery.

**IOx + resection** (n=4) in Uni-IOx pups treated at P12 the infraorbital nerve was sectioned a second time after whisking had reached normal level at P26-31. Whisking was measured daily.

**IOx + VIIx + resection** (n=3) In IOx+VIIx pups the infraorbital nerve was sectioned a second time upon the appearance of normal whisking.

(5) *Effects of blocking regeneration of the nerve.* Since the nerve has been shown to regenerate and functional activity restored quickly, in a subgroup of animals *regeneration* was prevented using cyanoacrylate adhesive in order to examine its role in mediating the eventual emergence of whisking in deafferented animals. Histoacryl has been used to physically prevent rejoining of the sectioned nerve by forming a glue barrier between the stubs that is stable over long periods of time (Nicoletis et al., 1996). In consideration of general health, litters were culled to 6 and all pups in a litter received the same treatment. Fourteen pups were randomly assigned to treatment groups and monitored daily for weights and whisking.

**P7 Uni-IOx “blocked”**: n=3; IOx sectioned unilaterally at P7 with cyanoacrylate blockade. The contralateral side remained untreated.

**P12 Uni-IOx “blocked”**: n=3; IOx section occurred unilaterally at P12 with acryl blockade. The contralateral side remained untreated.

**Bi-IOx “blocked”**: n=4; at P12 on the “blocked” side of the face the IO was sectioned with the acryl blockade. On the contralateral side, the IO was sectioned without any acryl blockade (IOx “unblocked”).

**IOx “blocked” + VIIx+WC**: n=4; at P12 one side of the face received unilateral infraorbital section with cyanoacrylate blockade and the contralateral side received a motor nerve section and whisker clipping. Whisker clipping was done to provide maximum reduction of input contralateral to the section.

*Responses to stimulation.* Eighteen pups from 3 litters were randomly assigned to treatments: [Normal (n=7), Bilateral IOx (n=9) and Unilateral VIIx (n=2)] at birth. All pups per litter received one treatment. Pups were assessed for whisker head and body movements in response to whisker stimulation at P12-13, on the first post-operative day.

*Surgical Procedures.* Figure 3.1 shows the sensory and motor innervation of the rodent whiskers. Pups were weighed and numbered (using paw tattoo of black India ink; Higgins No. 44201; Bellwood, IL 60104). Pups were anesthetized with isoflurane inhalant (2.5%) and kept warm on a heating pad. For deafferentation, the infraorbital nerve was visualized under a dissecting microscope, lifted with a probe and all branches were sectioned with micro scissors at their exit from the infraorbital fissure.

Motor denervation involved section of the buccal and the upper division of the marginal mandibular branch of the facial motor nerve above the digastric muscle (Semba and Eggers, 1986).

Each incision required one suture (Vicryl from Henry Shein, New York). Post-operative care included applying antibiotic, keeping pups warm, and weight and health monitoring thereafter. The litter was immediately returned to the mother following surgery.

Over the next month, behavioral observations were recorded daily. Following completion of the behavioral observations the area of the nerve section and nerve stub were examined for signs of regeneration. Evidence for nerve regrowth was seen in animals in which regeneration was not blocked.

For blocking regeneration, a needle (31 g) deposited cyanoacrylate (.02ml; Nexabande; Henry Schein; 135 Duryea Road Melville, NY 11747) to form a physical barrier between the nerve stubs in the infraorbital foramen. Following behavioral assessments, the incision was reopened and adhesive removed to view whether the nerve stubs had rejoined. The section was visible with no signs of regeneration. In P7 sectioned animals, there was a partial connection of fibers observed at P26.

*Qualitative measurements of whisking behavior.* Pups were held gently in the hand and whisking was assessed against a white background with head forward for about 10 sec by two observers blind to the treatment based upon amplitude and frequency criteria and relative to control whiskers. Movement level= 0- no whisking, 1-less than 10 degrees amplitude, 2-whisking at reduced frequency and amplitude, 3-normal whisking. [Inter observer-reliability coefficient of correlation ( $r=.86$ )].

*Quantitative measurements of whisking.* Kinematic data on whisker movements was obtained using videography. Pups were videotaped twice weekly in 30 min sessions.

Litters were removed from the dam 10 min before testing and kept in a warmed cage. Five minutes prior to testing pups were restrained in a cloth sac and container made of quick drying Das Pronto modeling clay (2 Industrial Blvd, Plattsburgh NY 12901) leaving the head free to move. All whiskers were clipped at the base (<2mm) except row C and lightweight foam markers were attached bilaterally to the C3 whiskers. The clay container was placed inside a Plexiglas holder fixed on a platform. Animals were coded so that there was no reference to age or treatment. Following a 5 min adaptation period, head and whisker movements were filmed against a white Plexiglas background tilted at a 45 degree angle (illuminated from above by a 5W fluorescent bulb), using a tripod-mounted Canon digital video camera (Canon XL1 3CCD digital video camcorder NTSC) situated above and perpendicular to the animal. To maintain arousal throughout the 30 min session, a drop of warm milk was delivered with a disposable pipette every 3-4 min.

Videotapes were scored by an experimenter blind to treatment group on a Sony Trinitron editing monitor. Ten continuous seconds of whisking defined an "epoch" of whisking and an epoch was obtained for each third of the session. This was done because it was noted that episodes of whisking decreased across the session in both number and interval. Whisking was defined as a protraction of the marked whisker in 2 or more frames. For whisking frequency, only whisks emitted when the head was directed forward were measured.

In a subsequent analysis of head movements from these tapes, displacement of the head either to the left or right of midline was recorded for every second, and number of whisks on right and left sides occurring in the same second as the head displacement was recorded.

*Stimulation responses.* Ten minutes prior to testing, litters were removed from the mother and pups were weighed. Pups were placed individually in a 15 X 10 cm white Plexiglas chamber for 2 min. Then, for 10 sec the occurrence of whisker, head and body (including: pivot, locomotion, lateral head turns) responses sustained for at least 2 sec was measured as a baseline score. Mystacial vibrissae on one side of the face were stimulated for 10 sec (15 back and forth sweeps) using a wooden rod. Stimulation on each side of the face was counterbalanced. Behaviors were recorded in response to whisker stimulation (10 sec). Each movement was counted once, and activity in response to stimulation was computed based on the number of body elements (head, limb, or torso) moved (Hall, 1979; Landers and Sullivan, 1999). Mean cumulative activity scores prior to and following stimulation were recorded, and percent of the time each movement occurred was computed.

In a second stimulation period, the snout was probed with a clean wooden rod on one side of the face (10 sec) and responses were measured and calculated as above prior to and following stimulation.

*Statistical analysis.* Stat view (SAS Institute, Inc., SAS Campus Dr., Cary, NC 27513), Excel (Microsoft, Corp.) and SPSS 12.0 (www.spss.com) were used to compute statistics. Statistics on unilateral treatments were compared within animal between left and right sides. Statistics on bilateral treatments were compared between animals. For each study, 1-way ANOVAs (i.e., treatment) or 2-way ANOVAs (i.e., age in days and treatment) were based on the means (i.e. number of days before whisking emerges). In an additional analysis, litter and sex effects were addressed. If more than 1 male and 1 female animal was used per litter, the average number for that litter was used. ANOVAs

were then done for treatment and sex by age in days. Repeated measures ANOVAs were done on all longitudinal measurements (i.e., weights, whisking frequencies, re-sections post-pre data). For coordination analysis correlations for each second of whisking on the left and right side were computed for each pup, averaged for age and compared across treatment. For stimulation analysis, stimulation difference scores (post-pre stim) were compared using ANOVA (age X treatment).

## RESULTS

*Emergence and development of whisking in normal pups.* Small, uncoordinated movements of the vibrissae were evident as early as P10-14 developing into characteristic rodent whisking movements by P17-21. Figure 2.2 (top) plots the proportion of pups in a litter observed whisking at each postnatal day. The mean age of whisking onset was P12. [There was a tendency for the onset of whisking to be delayed in larger litters. Age of whisking onset was mildly positively correlated with litter size ( $r=.57$ ) and litter size was strongly negatively correlated with body weight ( $r = -0.82$ .)] The onset of whisking always preceded eye opening by a few days (mean difference=2.8 days).

Figure 2.2 (bottom) presents data on changes in whisking frequency over the first postnatal month. The increases in frequency, though gradual, are significant across age [repeated measures ANOVA  $F(1, 3)=126.42$ ;  $p<.0001$ ] reaching a maximum of about 5 Hz under the present testing conditions (i.e., with the body restrained.) Differences between sides are not significant ( $p>.05$ ).

*General condition of normal and deafferented pups: post-operative care and behavioral responses to stimulation.* Weights were taken preoperatively, and there was no weight difference among pups of all treatment groups prior to surgery ( $p>.05$ ). As

Figure 2.3 (top) indicates bilaterally deafferented subjects had lower body weights during the period between the end of nursing (P18) and the beginning of independent feeding (repeated measures ANOVA treatment by weights  $F(2,7) p<.05$ ). Weights had recovered to normal before the end of the first month (P25, Fisher post hoc,  $p>.05$ ). For infraorbital surgery done bilaterally, these data useful for experimental design show that it is important to treat all animals in a litter and limit the number of pups in a litter thereby decreasing competition.

Figures 2.3 (middle and bottom) indicate, deafferented pups emitted few whisker movements in the periods following whisker or snout stimulation [ANOVA  $F(1,26) =97.8, p<.0001$ ] and had lower body activity in response to stimulation of the whiskers (ANOVA  $F(1, 14) =12.149, p<.01$ ) and snout (ANOVA  $F(1, 14)=4.5, p<.05$ ). IOx pups also emitted fewer head movements compared to Normals (ANOVA treatment effect  $F(1,314) = 5.9, p<.05$ ; 3 min time sample).

*Effects of whisker deafferentation upon the emergence and refinement of whisking.* Unilateral section of the infraorbital nerve significantly delayed emergence on the denervated side of the face. Figure 2.4 (Top) compares the time course of whisking emergence across treatment groups. (Middle) Data indicate that in the surgical controls whisking emerges at its normal time. In both deafferented groups, the emergence of whisking occurred at a later postnatal day (ANOVA treatment by age,  $F(2, 33)=21, p<.0001$ ) In the bilaterally deafferented group whisking had not reach normal levels until P30. This effect of deafferentation was also seen in the intraorbitally deafferented pups (ANOVA  $p<.05$ ). A second analysis done to address litter and sex effects and showed that whisking emergence occurred at a later postnatal day in IOx treated pups (unilateral,

bilateral) across litters (ANOVA  $F(2,27) = 46.5$ ,  $p < .0001$ ). Sex effects were not significant ( $p > .05$ ).

Figure 2.4 (bottom) presents data on whisking frequency over the first postnatal month (P13-28). Whisking frequencies were significantly lower in both unilateral and bilateral groups [repeated measures ANOVA treatment by age for unilateral  $F(1,3) = 21.639$ ,  $p < .0001$ ; bilateral, normal  $F(1, 4) = 63.235$ ,  $p < .0001$ ]. The developmental increases in frequency, though gradual, are significant across age reaching a maximum of about 5 Hz under the present testing conditions. By the end of the 3<sup>rd</sup> week, whisking frequencies in the unilateral IOx rats did not differ significantly from those of normal animals, while the bilaterally denervated animals were still whisking at lower frequencies at the end of the 4<sup>th</sup> week (Fisher post hoc,  $p < .05$ ). Differences between sides were not significant in the bilateral group ( $p > .05$ ). The untreated side of the Uni-IOx emerged normally, with slightly higher frequencies than Normals ( $p > .05$ ).

Deafferentation significantly delayed the time course of bilateral coordination, with the greatest duration of disruption in the unilateral group (Figure 2.5). In normal pups observed at P13, whisking on the two sides of the face was not initially well coordinated ( $r = 0.62$ ,  $p > .05$ , as measured by the value of the correlation between movements of the whiskers on the right and left sides of the face) but gradually became coordinated over the first month ( $r_{P21} = 1.00$ ,  $p < .05$ ). Analysis was done with the head pointed forward.

*Defining the developmental "window"*. Figure 2.6 shows that whisking emerges normally in P7-8 operated pups (P11-12) compared to pups sectioned at P11-13 [2-way ANOVA for treatment and age  $F(2, 35) = 28$ ,  $p < .0001$ ]. Pups deafferented at P15-21

emerged normally at P12, and deafferentation following emergence produced only a transient effect. Analysis averaged across litters show comparable results that IOx at P11-13 showed a significant effect compared to IOx done at earlier or later ages:  $F(2, 23)=.91$ ,  $p<.0001$ , and sex effects were not significant ( $F(1, 23) =.91$ ,  $p>.05$ ).

For P7 IOx group, whisking frequencies were measured at the age of emergence. Data indicated that whisking frequencies on the first day of whisking were not significantly different on the treated and untreated side ( $p>.05$ ).

*Effects of combining unilateral deafferentation with contralateral motor nerve section.* This procedure which abolishes whisking on the side contralateral to the IOx potentiated the effect of the deafferentation. Figure 2.7 (top) plots the mean age in days that whisking emerges for each treatment group and the “whisking scale” value for each phase of whisking development. The first day of whisking is later in the three treatment groups compared to controls [ANOVA treatment by day  $F(2, 31)=23$ ,  $p<.0001$ ]. One striking finding was that IOx+VIIx animals experienced a period of no whisking (whisking scale=0) for about a week. [Additional analysis for litter and sex effects show significant treatment effects: ( $F(2, 20)=.015$ ,  $p<.05$ ) and sex effects are not significant ( $p>.05$ ).]

Figure 2.7 (bottom) presents data on whisking frequency in the surgical controls and all treatment groups during the first postnatal month. Differences between treatment groups were significant across age [normal, bilateral, contra-VIIx  $F(1, 4)=6.64$ ,  $p<.01$ ]. Note that whisking in the Uni-IOx + VIIx group had not developed to normal levels more than one month postnatally (Fisher posthoc,  $p<.01$ ).

In a control group, when the motor nerve section was made on the same side as the sensory nerve section, whisking emerged normally in the untreated side ( $p > .05$ ). This suggests that the effect of VIIx is only present in deafferented whiskers.

*Effects of re-sectioning the infraorbital nerve in previously deafferented pups.* In deafferented pups that began to whisk after a significant delay (repeated measure ANOVA ( $F(1, 5) = .506, p < .0$ ) but re-sectioning the nerve had no effect (ANOVA  $p > .05$ ). The absence of a re-sectioning effect was clear for the IOx+VIIx group. For example, the pups of one litter were initially sectioned on P11, were re-sectioned the morning of their first day of normal whisking (P26) and were whisking normally within 24 hrs. This confirms the results on effects of deafferentation at an older age.

*Effects of preventing nerve regeneration.* Pups were unilaterally sectioned at either P7 or P12 and regeneration on one side was prevented by cyanoacrylate blockade and the other side was untreated. As Figure 2.8 indicates, whisking on the untreated side of the P7 animals emerged at the normal time, 4-5 days post-operatively, while the uni-IOx blocked group emerged at a later age (P11-12; 2-way ANOVA treatment by age;  $F(3,8) = 198, p < .0001$ ). T-tests within animal showed that whisking emerged P11-13 on the untreated side (P7 Uni-IOx,  $t(4) = 14, P < .001$ ; P12 Uni-IOx,  $t(4) = 15, p < .0001$ ) while on the “blocked” side, some animals were not whisking normally more than 40 days after the initial section.

Figure 2.9 presents the effects of blocking regeneration on age of whisking emergence in unilateral and bilateral treatment groups. “Blocked” compared to “unblocked” sides resulted in a significant delay of whisking emergence ( $t(6) = 4.27, p < .01$ ). Again, in an attempt to attain a maximum reduction in whisker afference after

deafferentation we combined unilateral infraorbital section, with section of the contralateral motor nerve and regeneration was blocked by acryl on the deafferented side, and to further reduce input, all whiskers were clipped contralateral to the deafferentation. Results showed a significant delay in whisking emergence comparing IOx+VIIx “unblocked” and “blocked” groups across animals (1-way ANOVA (1, 8) =13.4,  $p < .01$ ). There was not a significant effect of treatment for uni, bilateral and contra-VIIx “blocked” groups ( $p > .05$ ). There was no significant effect for sex ( $p > .05$ ).

*Whisking and head movements.* As Figure 2.10 indicates, the direction of head movements and side of whisking appears to be closely related in pups. Observation of pups at P13 indicates that whisking begins towards the end of a head-turning movement, is likely to be unilateral and on the same side as the direction of the head turn. High speed tracking in adults is needed for comparison (Knutsen, Derdikman and Ahissar, 2005).

## DISCUSSION

These results confirm an earlier description of the development of whisking in rats (Welker, 1964) and fix the period of its initial emergence at P10-14, a few days before eye opening. During the next two weeks there is a gradual increase in whisking frequency. However, these events are not independent of peripheral sensory input, since reducing that input by whisker deafferentation substantially delays the emergence and subsequent refinement of the whisking pattern. These data which demonstrate that whisking relies upon sensory input early in life provides further evidence that this is an emergent behavior

The extent of that delay is proportional to the reduction in whisker input and the impact of whisker deafferentation is dependent upon the perinatal age at which it is sustained, at P12, coincident with onset of whisking. Deafferentation carried out after the age at which the whisking pattern was adult-like has no significant effects on the emergence or subsequent development of whisking.

In adult rodents whisking patterns share modal frequencies (5-7 Hz) with other rhythmic orofacial behaviors (e.g., chewing, sucking, licking) and remain intact after deafferentation (Welker, 1964; Gao, Bermejo and Zeigler, 2001) suggesting the involvement of central pattern generating mechanisms (CPGs). Results suggest that deafferentation interrupts processes involved in central rhythm generation during development. Observations on denervated pups may (a) characterize some the functional properties of a whisking CPG and (b) identify mechanisms involved in its functional development.

Deafferentation early in life delays whisking emergence, however deafferentation in adulthood has no effect on the whisking rhythm. This suggests that sensory input during the period of movement development is important for emergence and refinement of the whisking rhythm but that once established the CPG is independent of that input. The observation that unilateral deafferentation affects whisking only on the treated side suggests that pattern generating mechanisms for the two sides may function independently. On the other hand, the fact that a *contralateral* motor nerve section potentiates deafferentation effects suggests that the CPG receives input from the contralateral whisking side. This effect occurs also in adulthood since *unilateral* sensory denervation is followed by a transient *bilateral* effect upon whisking frequency,

indicating that CPGs on the two sides are coupled. Such coupling occurs over time with increases in correlated whisks on the two sides of the face and bilateral synchrony that often characterizes exploratory whisking in adults (Gao et al., 2001). Deafferentation across development may block the development of coupling, thereby disrupting formation of bilateral connections.

Sensory input may contribute to the *construction* of pattern generating circuitry, to its *timing* properties, to the probability of its *activation* or to its *control* by descending cortical influences (Pearson and Ramirez, 1997; Hattox, Li and Keller, 2003). A role in the *construction* or *timing* properties of the “whisking” CPG seems unlikely since brainstem circuitry is present prenatally and since whisker protraction is produced by the active contraction of a single muscle; retraction is, under most conditions, a passive event, based upon the visco-elastic properties of the follicle muscles (but see Berg and Kleinfeld, 2002). Circuitry required for the production and coordination of more complex motor rhythms such as locomotion uses early postnatal sensory and motor inputs (Nishimaru and Kudo, 2000; Muir and Chu, 2002; Metcalfe, McDowell, Chang, Chen, Jeka and Clark, 2004). In adults and during development, evidence suggests that sensory input from activation of the muscles increases drive and excitability of the system at the level of the brainstem (Nguyen and Kleinfeld, 2005). The development of the oro-facial rhythm coincides with early whisking frequencies (Koslov, Petrov, Kashinsky, Nizhnikov and Spear, 2003). It therefore seems more likely that whisker afference contributes to *activation* of pre-existing circuits, perhaps by generating the input necessary to lower the threshold of those circuits.

If, as has been suggested (Pearson and Ramirez, 1992, p. 226) the effects of sensory input upon a CPG may be similar in principle to the action of neuromodulators, than the disruptive effects of whisker denervation should be related to the reduction in whisker afference which they produce. This is consistent with the development of the somatosensory pathway (D'Amato et al., 1987). This relationship was evident in the present data. Moreover, it is consistent with the observation that combining unilateral deafferentation with section of the contralateral motor nerve produces a greater delay in the emergence and development of whisking than a unilateral deafferentation alone. While nerve regeneration and some return of function is likely to occur after a whisker deafferentation, facial nerve section blocks whisking permanently (Semba and Eggers, 1987), producing a substantially greater reduction in whisker afference. Blocking regeneration in this preparation reflects this greater effect on whisking.

Recent research has identified brainstem serotonergic neurons, as components of a “whisking CPG” (Hattox, Priest and Keller, 2002). Indeed, Keller (2002) has hypothesized that such a circuit could involve, minimally, descending inputs from whisker motor cortex upon one or more of these structures. Serotonin neurons are in close apposition to descending inputs from motor cortex bilaterally (Hattox, Li and Keller, 2003). The finding that in *adult* rats, unilateral motor cortex ablation produces a significant reduction in the frequency of the whisking rhythm is consistent with this hypothesis (Gao, Hattox, Keller and Zeigler, 2004). In adults motor cortex stimulation evokes bursting in serotonergic neurons and elicits whisking (Cramer and Keller, 2004).

Serotonin is implicated in *development* of respiratory CPGs, hypoglossal trigeminal and spinal motor neurons and may also be involved in the development of the

whisking CPG. There are a variety of effects of serotonin on the whisker to barrel pathway until P10 (Hsiao et al., 2002). There is evidence for serotonin in descending whisker pathways during development. 5-HT receptor expression in the facial motor nucleus is at a high level at P8 and increases until P15 then is low at P21 for receptors 2 and 3. These receptors (5-HT 2 and 3) are directly involved in whisking (Hattox et al., 2003). Serotonin depolarizes motor neurons in both neonates and juveniles; however only in juveniles does 5-HT affect membrane resistance (Talley and Bayliss, 2000). Serotonin innervation of facial motor nucleus across age is now being investigated at the University of Maryland, and our preliminary results show a developmental increase in serotonin coincident with age at whisking emergence.

The delayed emergence of whisking in the perinatally deafferented rat presumably reflects effects upon the normal development of function in this circuit. These effects are substantial. Neonatal deafferentation produces an almost 90% reduction in infraorbital nerve fibers proximal to the section, and reductions of 20-30% in the cross-sectional area and cell count of the brainstem trigeminal nuclear complex (Waite, 1982). The procedure also has profoundly disruptive effects upon the functional organization of the whisker representation at many levels of the trigeminal neuraxis presumably reflecting the high proportion of cells in structures normally responsive to whisker inputs. One result is a marked decrease in the responsiveness of trigeminal brainstem regions to whisker stimulation, an effect that is replicated at the somatosensory cortex (Waite, 1982). Finally, neonatal whisker deafferentation reduces 5-HT transporter levels in trigeminal sensory pathways contralateral to the section (Hansson, Cabrera-Vera and Hoffman, 1998). Thus whisker deafferentation during development produces an array of

morphological, physiological and biochemical effects involving many central structures presumed to be involved in activation and modulation of the whisking CPG.

Moreover, the time frame for the emergence of whisking overlaps a period of substantial developmental activity in the anatomy and physiology of the somatosensory cortex, including increases in synaptogenesis (White, 1997; Dunaevsky, Tashiro, Majewska, Mason and Yuste, 1999) as well as changes in the intrinsic membrane properties of the cortical cells resulting in increased excitability (e.g., McCormick and Prince, 1987; Massengill, Smith, Son, O'Dowd, 1997; Brumberg, Nowak, McCormick, 2000). There is considerable overlap in the time frame for the initial emergence of whisking, the time period during which cortical neurons appear to reach their adult physiological phenotype (McCormick and Prince, 1987) and the initial appearance (at about P16) of reliable movement maps to stimulation of the cortical vibrissal motor area (Keller, 1999, pers. com.)

Results suggest that whisker afference may contribute to the normal development of excitability in neural circuitry mediating rhythmic whisking. Early in development that input is generated, not by rhythmic whisking, but by passive stimulation of the whiskers, occurring during interactions with the mother (Sullivan et al. 2002), including suckling behavior. Such interactions accompany postnatal increases in neural activity and may provide a flow of input that contributes to the development of cortical excitability, and modulate the activity of the descending circuits. Reafference as whisking emerges may amplify the developmental increase in excitability in the somatosensory system (Khazipov, Sirota, Leinekugel, Heines, Ben-Ari and Bizaki, 2004). Removal of whisker inputs during this period would disrupt or retard these developmental processes, as well

as diminishing cortical activity in whisker sensory cortex which provides the primary source of afferent drive to whisker motor cortex (Izraeli and Porter, 1995; Farkas, Kis, Toldi and Wolff, 1999; Miyashita, Keller and Asanuma, 1994). The result would be to lower evoked excitability in whisker motor cortex, reducing the drive from descending inputs from whisker motor cortex upon brainstem components of the whisking CPG. The eventual emergence of whisking in this preparation may reflect the re-establishment of function in the regenerating infraorbital nerve.

What remains unclear, however, is the nature of the contribution made by passive and active forms of whisker afference to the development of the whisking rhythm, and its integration into the behavioral repertoire. Preliminary behavioral observations in normal pups suggest that whisker, head, and body movements coincide as they develop. Studies were designed to also examine whisking related behaviors during environmental interactions (using modified tests from Ricceri, Colozza and Calamandrei, 2000), which will be written up in parallel reports. Behavioral adaptations were observed in deafferentated and de-efferented pups. During early development, experience of self-generated movements may enhance the refinement of behavior (Gottlieb, 1997). Thus, these early sensory inputs may help to direct subsequent responses, and refinement of whisking amplitudes and frequencies, used in detection of high resolution stimuli or perception of forms.

Results highlight the shifting importance of sensory input in the functional development of behavior and define a sensitive period to prior to P14 in the development of rhythm- generating mechanisms. Evidence from whisker denervation studies indicates that vibrissa inputs contribute to whisker system function, but that the nature of that

contribution shifts over the course of development. Three pieces of evidence suggest this shift across age. During the immediate postnatal period, whisker denervation disrupts barrel formation (Van der loos and Woolsey, 1973), suggesting an “instructive” role for vibrissa input in this process. Whisker deafferentation disrupts suckling behavior in pups (Hofer, Fisher and Shair, 1981) but has no effect upon independent feeding in young rats (Klein, Duffin and Kraje, 1994) nor adult rat ingestive behavior (Jacquin and Zeigler, 1983). Whisking may occur in the absence of afference in adult rats, however our data show whisking emergence requires afference. The mechanisms mediating these developmental effects remain to be clarified.

## CHAPTER 3

DEVELOPMENT OF WHISKERS AND EFFECTS OF NEONATAL ENUCLEATIONS

Developmental crossmodal visuo-tactile plasticity is well documented (see Sathian, 2000, 2005) and reflects adaptation of a developing animal to an altered environment. Binocular neonatal enucleations results in an expansion of the whisker-barrel system in mice (Rauschecker et al., 1992). The authors suggested that the increase in whisker size in neonatally enucleated mice and kittens was related to *increased use of the whiskers* but presented no direct evidence. Because no data on whisker size were available for the rat, a preliminary study of the effects of bilateral neonatal enucleation (BEN) in rats was done in collaboration with Dr. S. Haidarlu of the Weizmann Institute, Israel. Measurements of whisker size (length and diameter) and barrel size were carried out in a group of rats. Observations were restricted to a moveable subset of the mystacial vibrissae based on preliminary measurements of all whiskers. (See Figure 3.1.)

Results clearly demonstrated (as in Rauschecker et al., 1992) a significant correlation between whisker length and diameter (Figure 3.2: top) and whisker and barrel size (bottom) in *Normal* rats. In addition, we found that body weight strongly correlated with whisker size (Figure 3.2 middle). Body weight was dependent upon the sex of the animal.

Thus, this preliminary study provided variables needed for further investigation of effects of BEN on the rat whisker system. Additionally, to verify method of whisker measurement, Rauschecker et al., (1992) was replicated in a litter of mice. Results showed that the largest differences were for the top 12 longest whiskers, which are in the

most lateral and caudal positions (Figure 3.3; top). (Below) Distributions were skewed to the right, though it narrowly missed significance ( $p=.05$ ). This suggested that the enucleated mice had longer and thicker whiskers.

To determine the utility of the BEN paradigm for experiential manipulations of the whisker system (for use in future analyses of behavioral studies), the following experiments were done: whisker size was measured in normally reared adult rats (P60) and adult rats that had sustained bilateral enucleation shortly after birth. Neonatal sensory and motor nerve sections were used to provide a comparison to BEN. Second whisker development of over the course of the first postnatal month was measured. Third, whisking frequencies were assessed in P21 BEN animals in an open field.

## METHOD

*Subjects.* Fifty-two female Long Evans rat pups from five litters were used to examine the differential effects of bilateral neonatal enucleation (BEN) and sensorimotor denervation.

*Experimental Design:* Twenty-five female rats from 5 litters were assigned to treatment groups. All pups in a single litter received one treatment: BEN ( $n=9$ ), Normal ( $n=13$ ), or IOx+VIIx ( $n=3$ ; in which sensory and motor nerve sections were carried out on opposite sides within the same animal.)

For the study of normal whisker development in rats,  $n=2-3$  normal subjects from 4 litters were sacrificed at one of the following ages P8, 17, 25, 60 and whiskers were measured at each age.

For the study of whisking, one female pup from each litter treated with BEN; (n=2) or Normal (n=2) was randomly sampled and measured for whisking frequency in an open field.

*Surgeries.* Neonatal Binocular Enucleation (BEN). At P2-3 pups were anesthetized using Isoflurane (2.5%). An incision was made through the eyelids and the cornea. The vitreous, lens and then entire eye were exposed and aspirated. Deafferentation (IOx) and contralateral motor nerve (VIIx) section procedures have been described in Chapter 2.

*Measurements of whisker size.* Subjects were anesthetized with isoflurane (2.5%; at P60) or sacrificed (younger than P60), and the whiskers were clipped using micro scissors. Position specific analysis revealed that the most significant differences between groups in the cat and mouse were present for the longest whiskers, which are in the most lateral and caudal positions (Rauschecker et al., 1992). In agreement, our preliminary data showed the largest effects in lateral and caudal whiskers. For this reason, measurements were restricted to a subset of whiskers Row C, D, E and Columns 1-3. Then, each whisker was straightened out on card stock using tape, labeled by row and column, and placed in waxed envelopes that were later coded. Using an electronic caliper, whisker lengths and diameters were measured by two experimenters that were blind to treatment.

*Statistical Analysis.* Frequency distributions were computed for whisker lengths of BEN and Normal rats. Then individual whiskers were aligned based on position in row and column. Difference distributions (BEN-Normal) were calculated for each individual whisker. Difference distributions were centered on zero difference and symmetry was

plotted. A t-test determined significance for whether there was a skew. For development of whisker size, mean for each whisker at each position was calculated and compared across ages. A difference distribution between whiskers at each position across ages was computed to determine rate of growth. Further, size proportions comparing smallest to largest row were computed and compared across ages.

*Open field arena.* The open field was a cleaned, white 10x10 cm Plexiglas arena set in a dark room under infrared light. The video camera was situated above the chamber perpendicular to the floor. A white dot of non-toxic removable paint was placed on the animal 10 min prior to testing. Five minutes prior to testing lightweight foam markers (1.4 mm) were placed bilaterally on C1 whiskers.

*Measurements of whisking in open field.* Whisking frequency was measured frame by frame (30fps) by video analysis in Normal and BEN rat pups (P21) that were unrestrained in an open field arena. The first 10 seconds of video was sampled in which the head was pointed forward. Whisking was defined as a displacement of the whisker relative to head and neck markers in 2 or more consecutive frames.

*General condition of the animals.* BEN pups (P12, n=6 or P21, n=8) or uni-VIIx pups (P12; n=2) were tested for whisker and body responses to stimulation of the whiskers and snout. If more than 1 pup was used per litter, the average for that litter was recorded.

Mystacial vibrissae or snout on one side of the face were stimulated for 10 sec. (See Chapter 2.) Behavior was recorded prior to stimulation (10 sec) and in response to stimulation (10 sec). Specific movements of the whisker, head and body sustained for 2 sec were recorded (Hall, 1979; Landers and Sullivan, 1999). Number of movements

(post-pre stimulation) was computed. ANOVA was done to compare effects of treatment (normals were from Ch 2) and age.

## RESULTS

*Effects of Bilateral Neonatal Enucleation in rats:* Rats blinded from birth have longer and thicker whiskers as adults. The length change in BEN rats is not equally distributed among the entire whisker array. In normal animals a full range of sizes are present (Figure 3.4 top). Whiskers in blinded animals were longer than in Normals. The BEN animals have significantly more whiskers over 20mm length. BEN whiskers were also more uniform in size as seen in frequency distributions. (Bottom) The shift towards larger whiskers is significant, (t-test,  $t(61) = 2.198$ ;  $p < .05$ ) with the maximum increase at 10 mm. There were no significant differences between litters of the same group.

Because of the observed relationship between body weight and whisker size we tested the hypothesis that the increased whisker size in the BEN animals reflects increased body weights. However, body weights between Normals and BENs in adults were not significantly different ( $p > .05$ ).

*Effects of infraorbital nerve section upon whisker morphology.* The whiskers of adult rats that had sustained either neonatal sensory or neonatal motor denervation were significantly smaller than Normals, for both sensory and motor nerve section [ANOVA treatment by length  $F(2,234) = 199$ ,  $p < .001$ ; by diameter  $F(2, 234) = 20.06$ ;  $p < .0001$ ; See Figure 3.5.] Further analyses showed that for diameter (but not length) IOx and VIIx were significantly different (Fisher post hocs,  $p < .05$ ). Observations of whiskers of deafferented pups revealed that they were bent and brittle in appearance.

*Development of the macrovibrissae.* All of the above results were measured in the adult rat, however there are no systematic data on whisker size across development. Our results in Figure 3.6 (top) indicate that whisker size increases, though at a diminishing rate, over the first postnatal month. The growth occurs among all whiskers proportionally. Columns 6 and 7 are not seen until after P30. As Figure 3.6 (bottom) indicates, the rate of whisker growth is most rapid at the first two postnatal weeks.

*Behavioral development in BEN rat pups.* At P12 BEN pups responded with whisker and body movements to stimulation of the whiskers (Figure 3.7 middle) and snout (Figure 3.7 bottom) more than at P21 [ $F(1, 13)=7.8, p<.05$ ], once rodents are at the age of normal vision function (P20; Fagliolini, Pizzorusso, Berardi, Domenici and Maffei; 1994). However, the decrease in responsiveness across age is consistent with normal responses to whisker stimulation (Landers et al., 1999). The whisker stimulation responses at P21 were higher in BEN pups compared to Normals [ $(F1, 15)=4.565 p<.05$ ]. Responses to whisker stimulation were higher in BEN pups than in VIIx and normals [ $F(2, 14)=7.4, p<.01$ ].

*Whisking in BEN rats.* Preliminary data, based on video analysis of rats in an open field, suggest that P21 BEN young rats whisk at significantly higher frequencies than p21 Normals (t-test  $t(89)=2.731; p<.001$ ; See Figure 3.8).

## DISCUSSION

The present studies, in addition to characterizing the time course of whisker growth in developing pups, have demonstrated that manipulations that increase or decrease input to the whiskers may have an effect upon one aspect of their morphology—

whisker size. Data show that rats sustaining either sensory or motor denervation of the whiskers as neonates have significantly smaller and thinner whiskers. Data have replicated in mice and extended to rats an earlier finding (by Rauschecker et al., 1992) that bilateral neonatal enucleation leads to correlated increases in the size of both individual whiskers and of their cortical (barrel) representation in the somatosensory area.

The effect was restricted to a subset of the longest whiskers, primarily those defining a ventral portion of the whisker array (row D). In this respect our findings differ from an earlier report by Bronchti, Schonenberger, Welker and Van der Loos (1992) in which the greatest effects were seen in whiskers, which were located within the animal's visual field.

The mechanism mediating the effects of these treatments upon whisker growth are currently unknown. It has been suggested that in both the BEN and denervated animals they involve variations in the blood supply to the whiskers. The blood vessels serving the whisker sensory and motor nerves lie in close proximity to the nerves themselves. Although following motor nerve section whiskers did not appear bent or disarrayed, this could account for bent, disorganized appearance of the whiskers in deafferented animals.

The relation between whisker size and cortical barrel size—though not the focus of this report—is clearly in agreement with the many reports suggesting considerable plasticity in the whisker-to-barrel system and the close link between neonatal sensory input and barrel development (Woolsey, 1990). It is likely that effects presented in this study are linked to developmental mechanisms—the central focus of my Dissertation. Denervation does not have similar effects when carried out in adult rats (Lillessar and

Hildebrand, 1999). Comparable controls are not yet available on bilateral enucleation in adult rats.

Later developing brain areas are generally the most vulnerable to manipulations (i.e. postnatal maturation of cortical response properties; Hainline, 1998). It has been suggested that sensory inputs from motor activity (i.e. in locomotion) may serve to shape somatosensory cortical responses, anchor somatotopic representation to motor maps, strengthen long range motor-sensory connections and contribute to the establishment of sensorimotor coordination (Khazipov et al., 2004). The mechanisms that link somatosensory and motor inputs in the whisker system are unknown.

In agreement with previous studies of whisker sensorimotor integration (Benedetti, 1995), whisker size in deafferented pups may coincide with behavioral compensations (such as increased use of perioral, body tactile areas) used in orientation in the perinatal environment. Preliminary data reported above, indicating that BEN animals with larger whiskers also whisk at higher rates, are more sensitive to stimulation and spend more time engaged in whisking and rearing are certainly consistent with this account.

Sensory input and motor experience play a critical role in the development of sensory and motor systems, coordinating them and building the foundation for later developing systems. In infants, sensory and motor system development allows movement through and exploration of an expanding environment (in Michel and Moore, 1995). The infant's reliance upon sequential development of sensory systems (vision; Turkewitz and Kenny, 1985) and upon interactions among vibrissae and vision (in kittens: Turkewitz, Gilbert, and Birch, 1974) for development of exploratory behaviors and perceptual

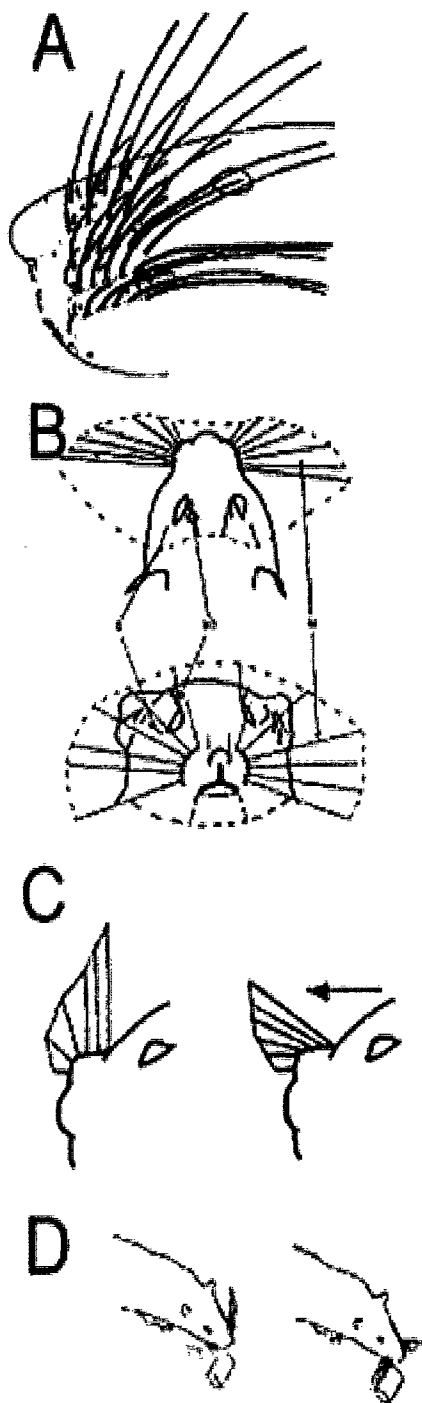
abilities has been elucidated. Afference from whisking at emergence may be integrated in the behavioral repertoire early in life during this developmental transition to active exploration of the environment.

Further analyses of behavior and their neural correlates are needed. Predictions may include: motor inputs from whisking may function to modulate the whisking pattern during environmental interactions to enable detection of higher spatial and temporal resolution and changes in patterns. Loss of early inputs may effect motor patterns later in life during fine-grained discriminations of texture and form, while early *stimulation* may enhance these behaviors.

Results from these experiments suggest that early visual deprivation enhances whisker morphology and early whisking frequencies. Selective manipulations of sensory and motor experience in perinatal animals can be a powerful tool for clarifying the contribution of sensory inputs to normal development in the whisker system, one of the most widely used models for the study of CNS patterning and sensorimotor integration.

<b>Experimental design</b>										
	<b>IOx</b>			<b>IOx+VIIx</b>			<b>Block IOx regeneration</b>		<b>Sham</b>	
	Uni	Uni	Uni	Bi	Contra	Ipsi	Uni	Uni	Contra	Control
<b>Age (P)</b>	7-8	10-14	15-21	11-13	11-13	11-13	7	11-13	11-13	11-13
<b>Litter n</b>	4	9	4	4	2	1	1	2	2	5
<b>Pup n</b>	10	20	8	8	6	3	3	7	4	22

Table 1. The number of animals and number of litters used in each treatment group (bilateral, unilateral or contralateral nerve section) and age at surgery (P=postnatal days).



**Figure 1.1. Schematic illustration of the whisker system in rats. (A)** Whiskers are an array of mobile receptors surrounding the perioral area (B) with trajectories of specific amplitudes and frequencies. (C) Protraction is a horizontal sweep of the whiskers. (D) Whiskers are used during exploration of objects in the environment.

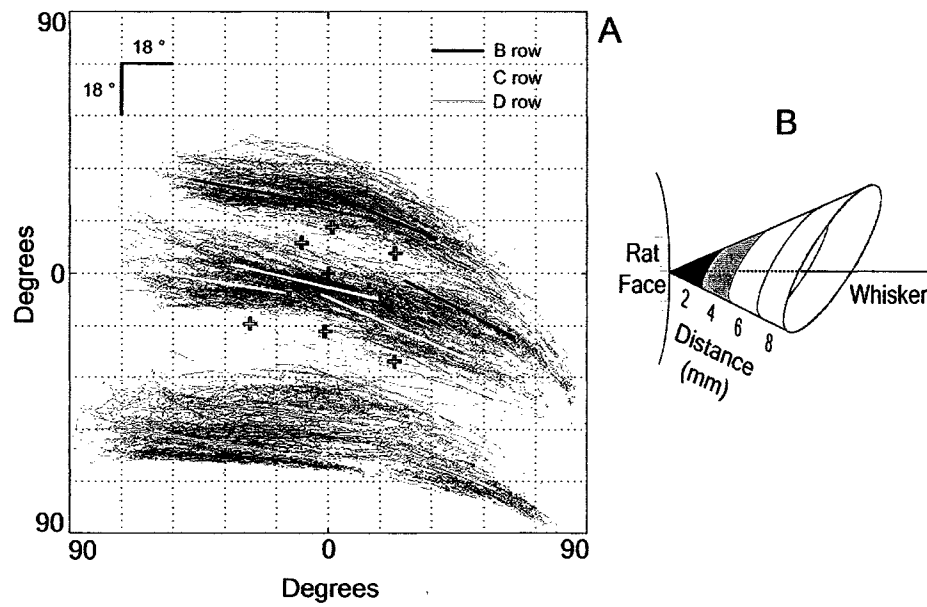
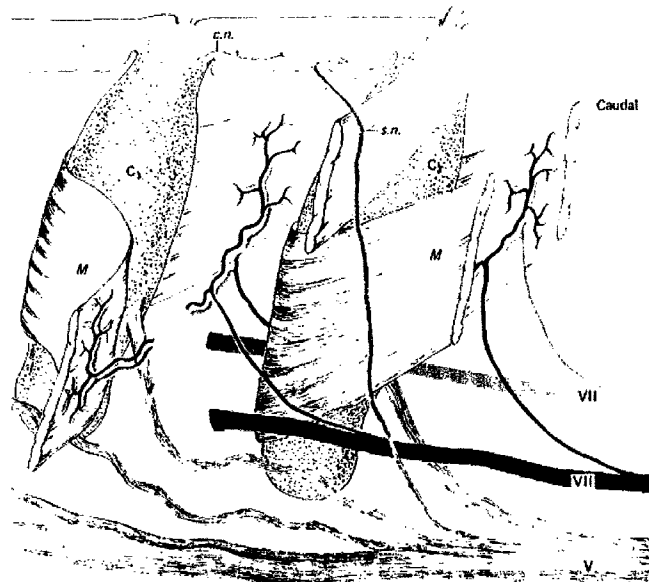
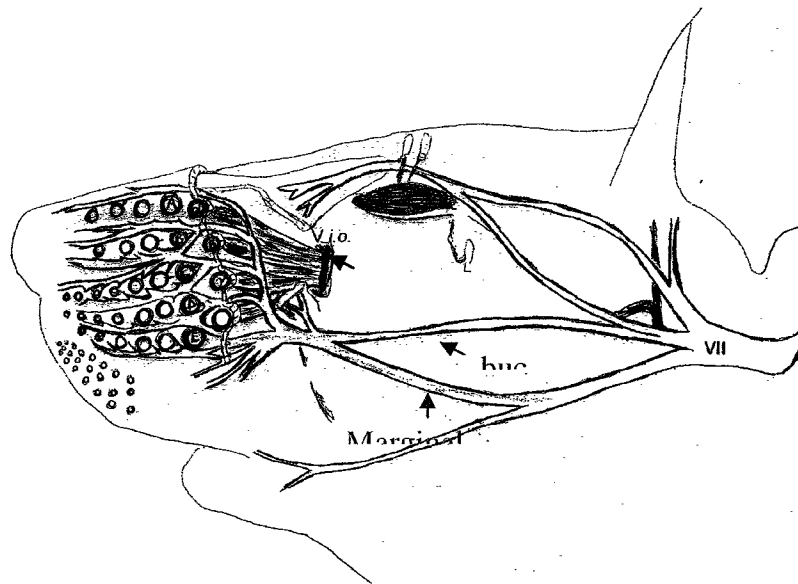


Figure 1.2. Diagram of the two-dimensional topography of an idealized whisk, and views of the “whisking space” of an individual whisker at a distance from the face. (A) Two-dimensional space (“domains”) for whiskers in columns 1 to 3, in rows B to D (B) Representation of trajectory at different distances from the face for the whisker at the center of the 9 whisker grid (courtesy of Bermejo et al., 2002)



**Figure 1.3. (Top) Sensory and motor innervation of the whiskers.** Sensory nerve (black arrow) is a branch of the trigeminal cranial nerve V and the motor nerves (buccal, upper marginal mandibular in gray) are branches of the facial nerve VII. (Bottom) Medial aspects of 2 whisker follicles and their motor and sensory nerves. The extremity of a follicular muscle (M) was divided to show interfollicular space. Branches of facial nerve VII send nerves into the muscles. The trigeminal nerve V gives off follicular nerves (f) perforating the follicles and a skin nerve (sn) reaching the skin and sending off a nerve to the conical body of the follicle (cn; from Dorfl, 1985).

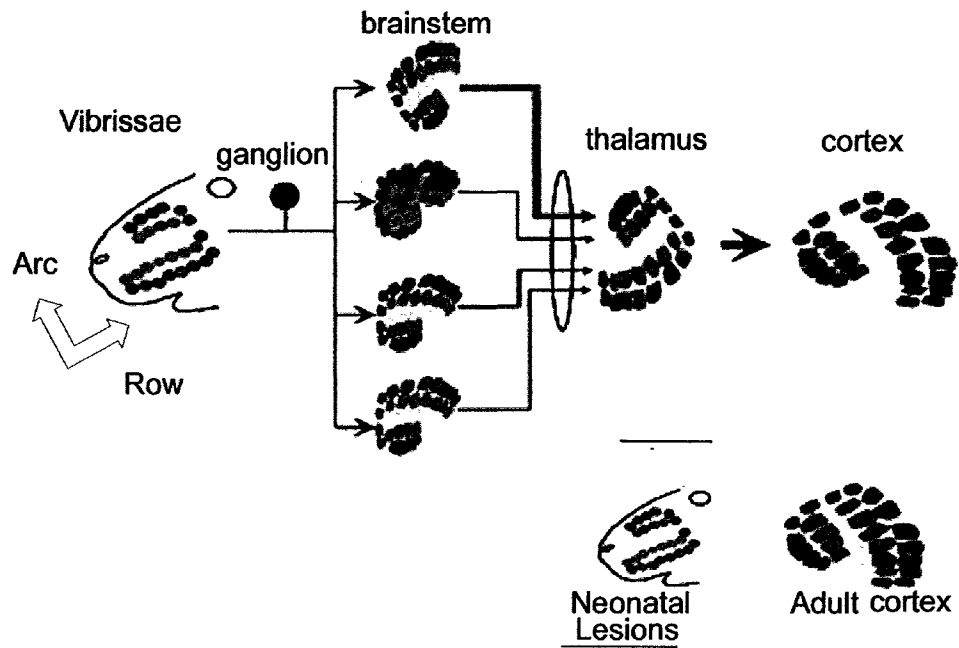


Figure 1.4. Schematic illustration of the whisker to barrel pathway. Representations of whiskers at brainstem, thalamus and cortical levels, and (bottom right) effects of neonatal whisker lesions. Neonatal whisker lesions (in Row C) if done prior to P5 delete corresponding cortical barrels (from Van der loos and Woolsey, 1973; Woolsey, 1990).

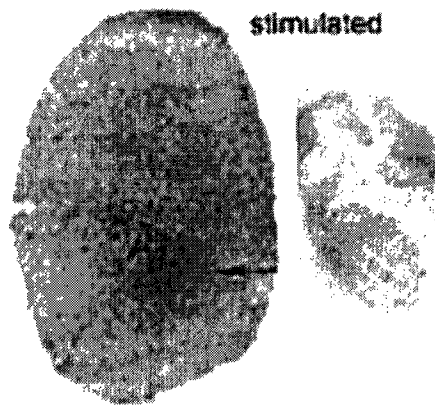


Figure 1.5. Whiskers are functional in the neonatal rat pup. (Left to Right) Photomicrographs of whisker stimulation-evoked 2-deoxyglucose (2-DG) activity in tangential section of barrel cortex at postnatal day 8. Somatotopic map of body surface is indicated by cytochrome oxidase activity (Landers and Sullivan, 1999).

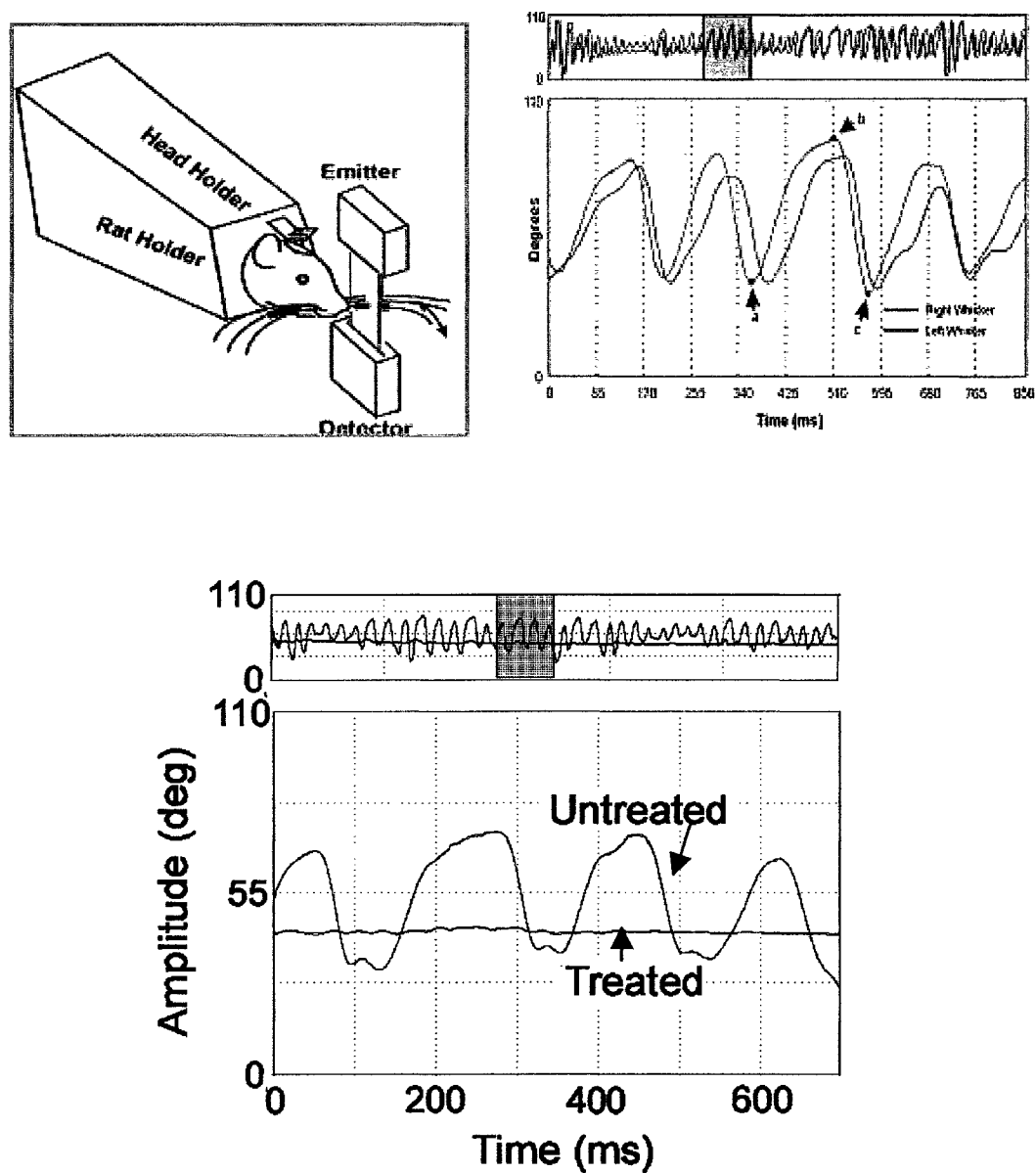


Figure 1.6. Optoelectronic monitoring of whisking in rats. Movements of the right and left C-1 vibrissa of a normal rat are recorded during whisking (Upper left). Interruption of the emitted beam (laser curtain) by a whisker produces a voltage shift in a subset of shaded sensors (CCDs). Whisking results in successive displacements in the position of that voltage shift which are linearly related to whisker position (right; courtesy of Bermejo, Houben & Zeigler, 1998). (Below) The top portion represents the trial in seconds. The shaded portion of the record highlights a sample which is displayed at higher temporal resolution below. Bilaterally recorded whisking in an adult animal that was given Botox unilaterally.

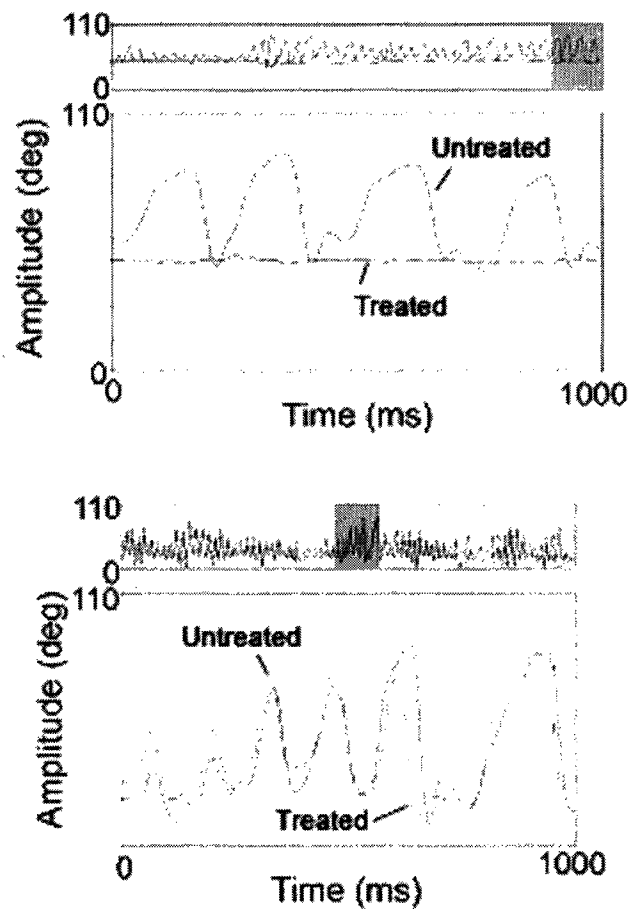


Figure 1.7. Movement trajectories on the treated and untreated sides of adult animals. (Top) Effects of motor nerve section during the first postnatal week upon adult whisking. Note the absence of whisking movements on the treated side. (Bottom) Whisking movement trajectories in an adult animal which had sustained Botox-induced blockade of whisking from day P12-40. A full range of whisking movements is present on both the treated and untreated sides (from Landers, Pytte, and Zeigler, 2002).

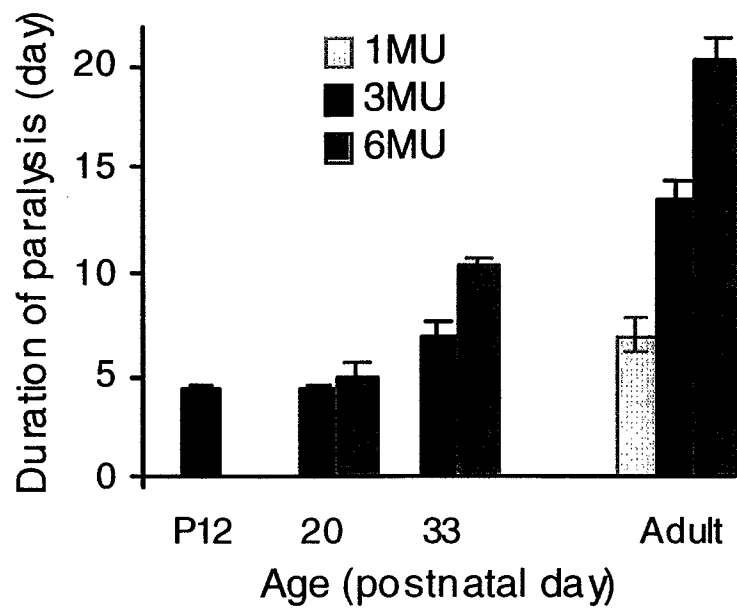


Figure 1.8. Relation between Botox dosage and the duration of whisking paralysis in developing and adult rats (from Landers et al., 2002). Notice the duration of blockade was lower in pups than in adults, presumably due to axonal sprouting in the whisker pad (De Paiva et al., 1999).

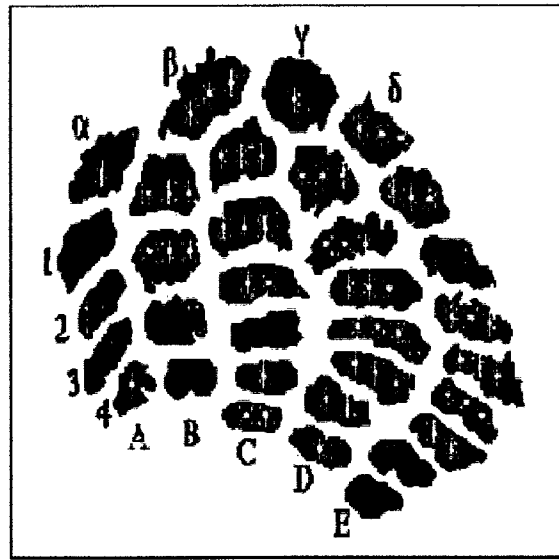


Figure 1.9. Barrel field expansion in neonatally enucleated adult mice: \*,\*\*,\*\*\* indicates increasingly significant expansion ( $p < .05$ ,  $.005$ ,  $.0005$ ) of single barrel size in the blind as compared to normal controls, based upon distributions of size differences (courtesy of Rauschecker et al., 1992).

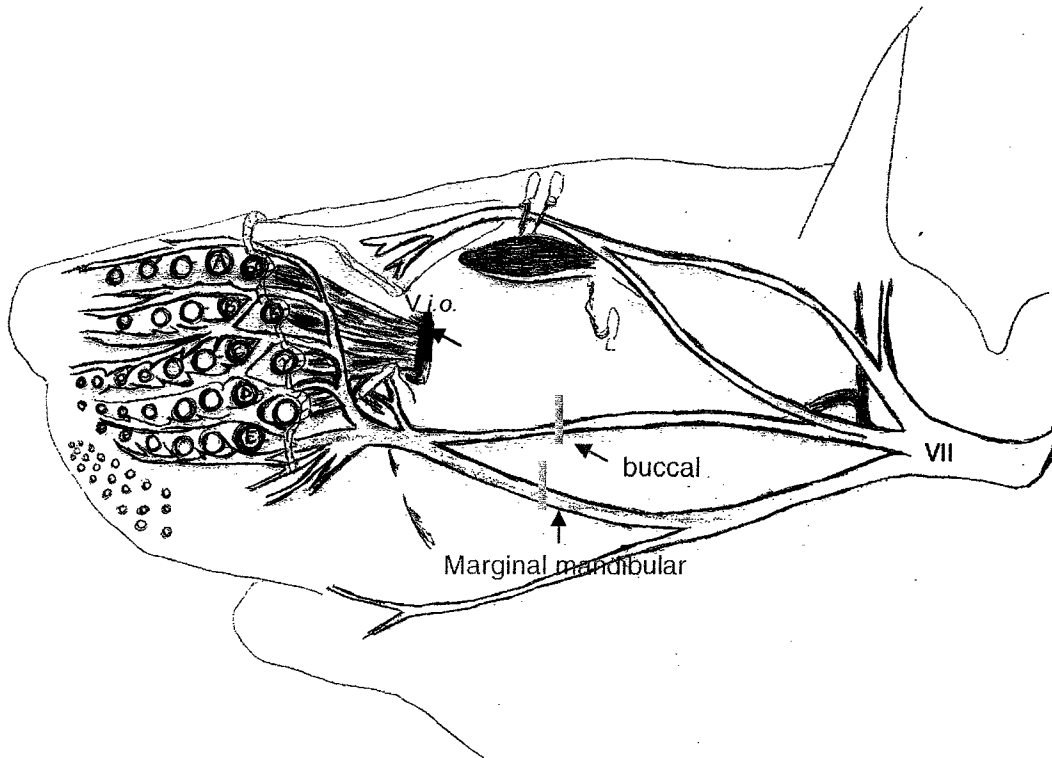


Figure 2.1. Sensory and motor innervation of the rodent whiskers (drawing based on Dorfl, 1985). Site of sensory nerve section (infraorbital i.o.; black line) and motor nerve section (VII; buccal and marginal mandibular nerves; gray line).

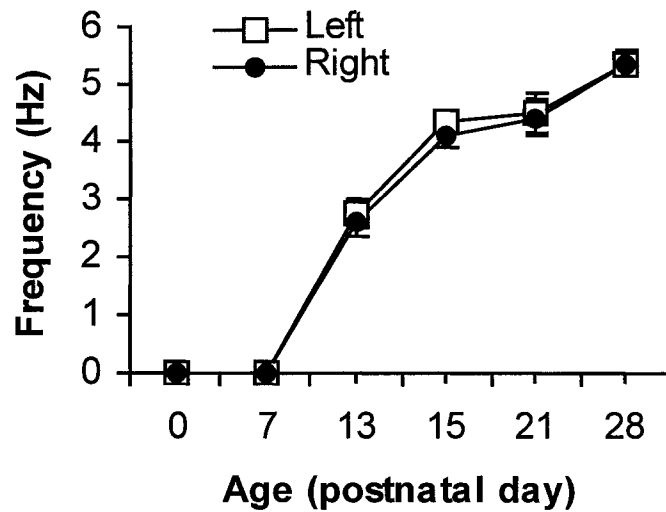
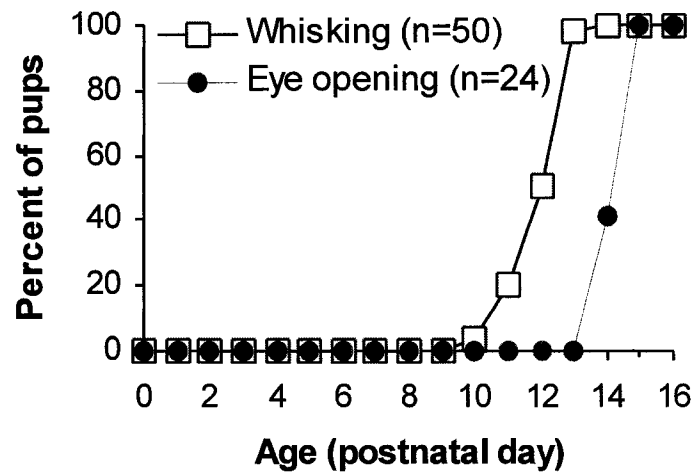


Figure 2.2. Emergence of whisking in normal Long-Evans pups. (Top) Percent of subjects observed whisking at each postnatal day. (Bottom) Changes in whisking frequency over the first postnatal month. N (pups) = 3; n (whisking movements) = 192. Means $\pm$ SE.

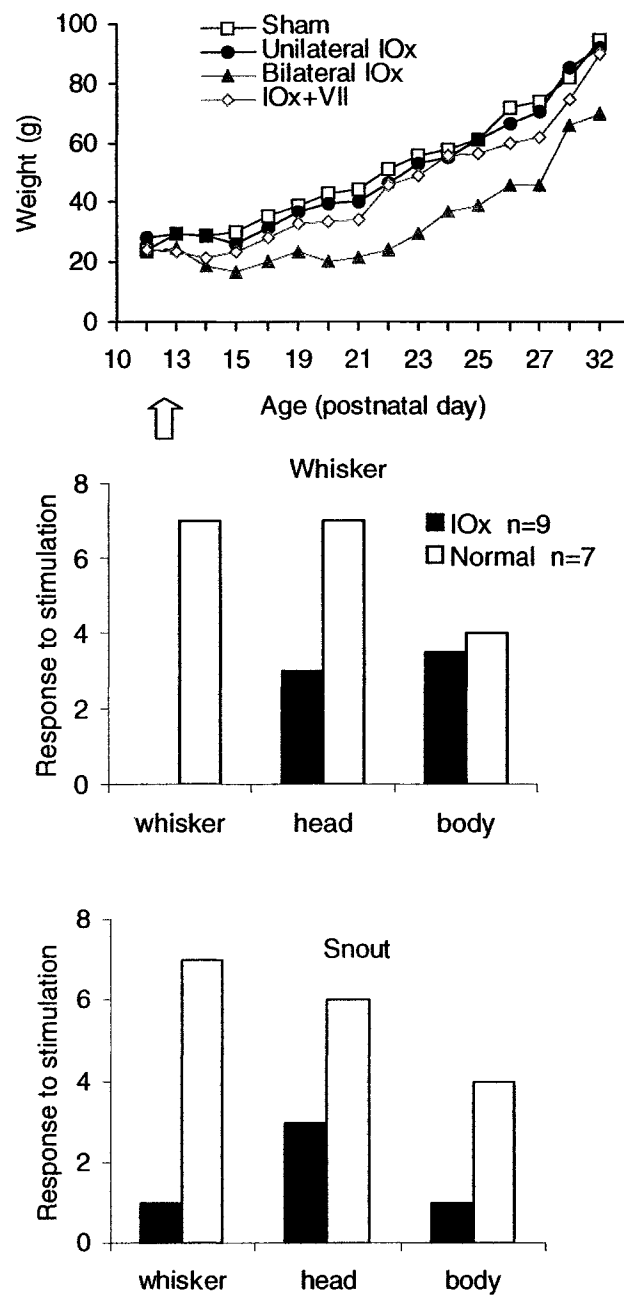


Figure 2.3. General condition of the pups. (Top) Mean body weight of the animals across age. Note: there was no weight loss preoperatively (arrow indicates age at section). (Middle) Mean cumulative number of whisker, head and body responses to stimulation of the whiskers (10sec; post-pre). (Bottom) Mean number of whisker, head and body responses to stimulation of the snout (10sec; post-pre). Mean $\pm$ SE.

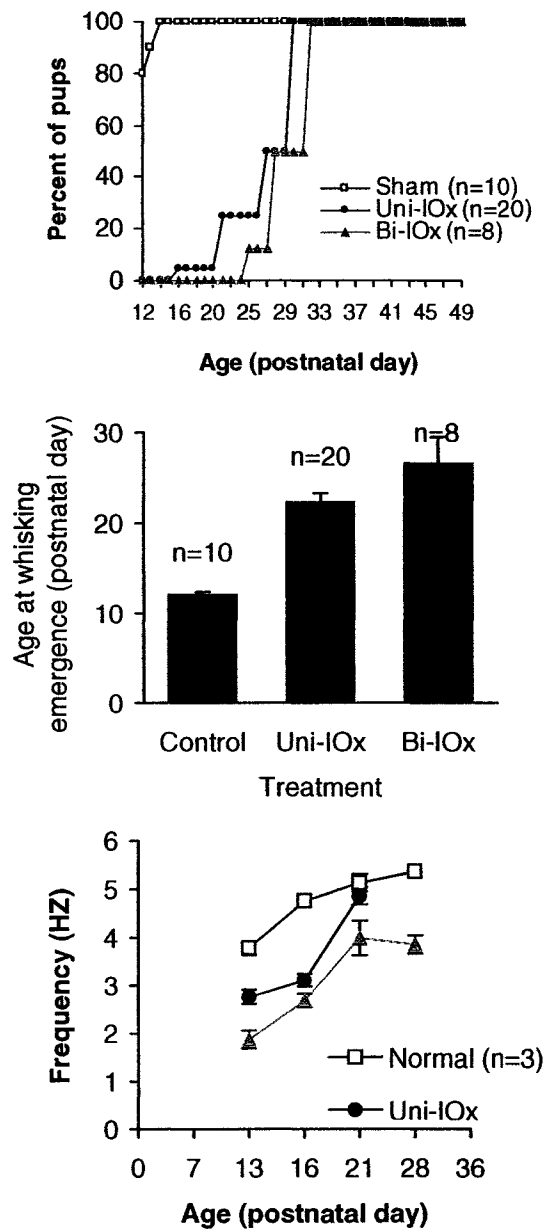


Figure 2.4. Effects of whisker deafferentation upon the initial emergence and subsequent refinement of the whisking pattern. (Top) Population of animals (%) observed whisking normally at each day of life. (Middle) Mean age at whisking emergence for each treatment. (Bottom) Treatment effects upon whisking frequency over the first postnatal month. Note that while the emergence curves for the three groups are similar, there are significant differences in initial and final frequency values. Mean $\pm$ SE.

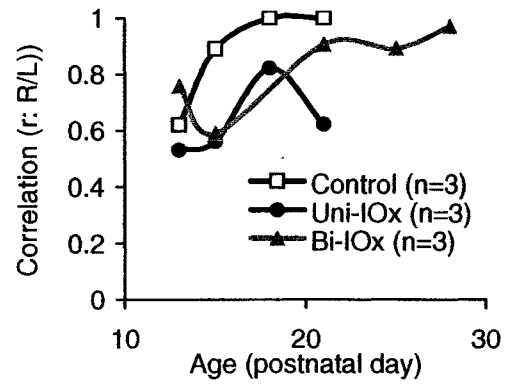


Figure 2.5. Correlation (Pearson's  $r$ ) between the occurrence of whisking on the left (L) and right (R) side of the face within the same second. Data are from whisks made with the head pointed forward.

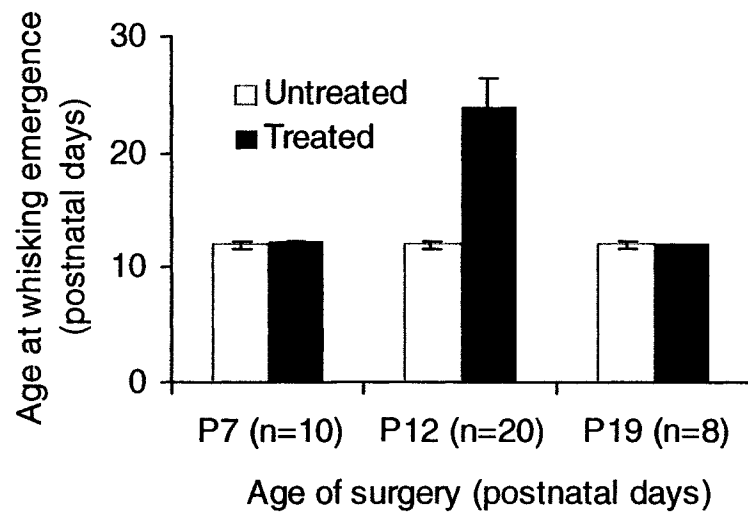


Figure 2.6. Defining the developmental window. Postnatal age that whisking emerges in unilateral IOx animals treated at ages P7, P12 and P19.

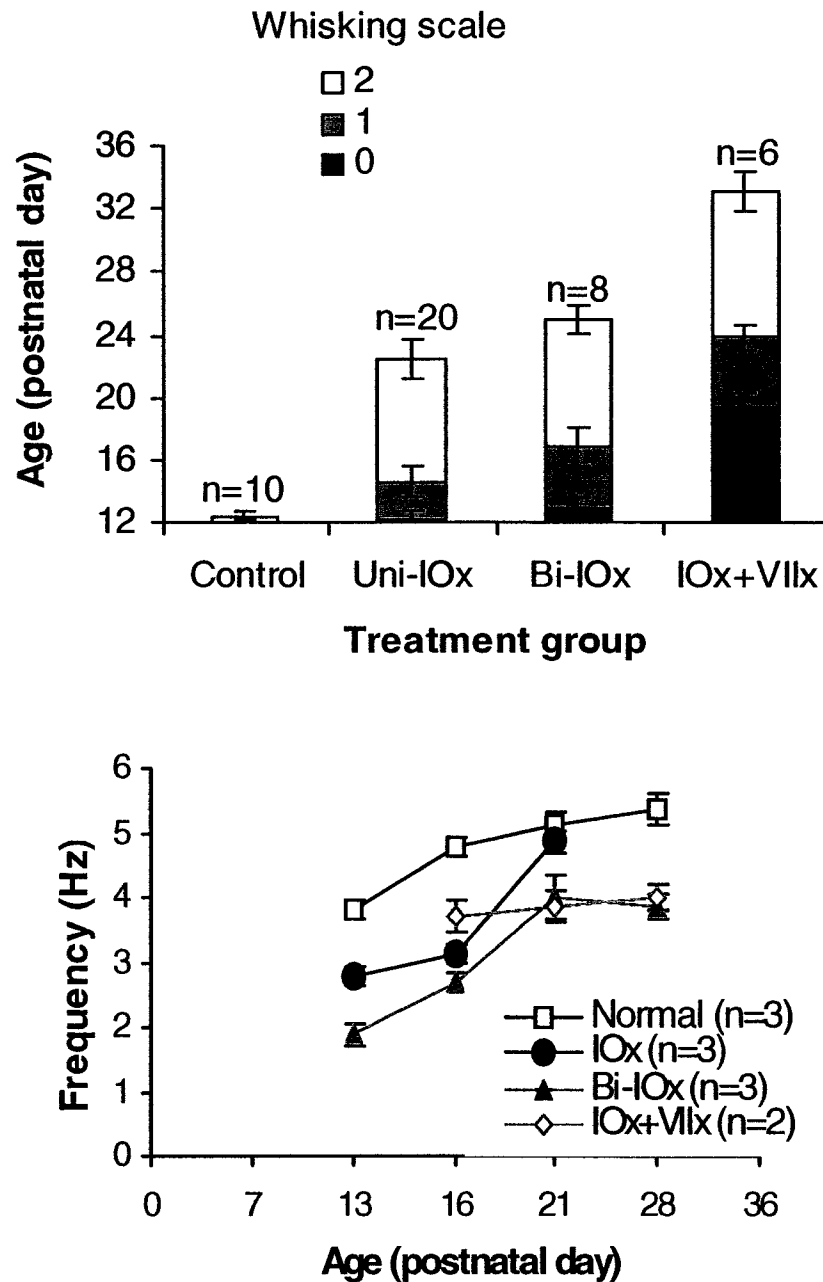


Figure 2.7. Potentiation of unilateral deafferentation effects by the addition of a contralateral motor nerve section. (Top) Age whisking emerges in for each treatment and whisking scale (0=no whisking, 1-2=low whisking amplitude and frequency, 3=normal whisking). (Bottom) Time course for the development of normal whisking frequencies.

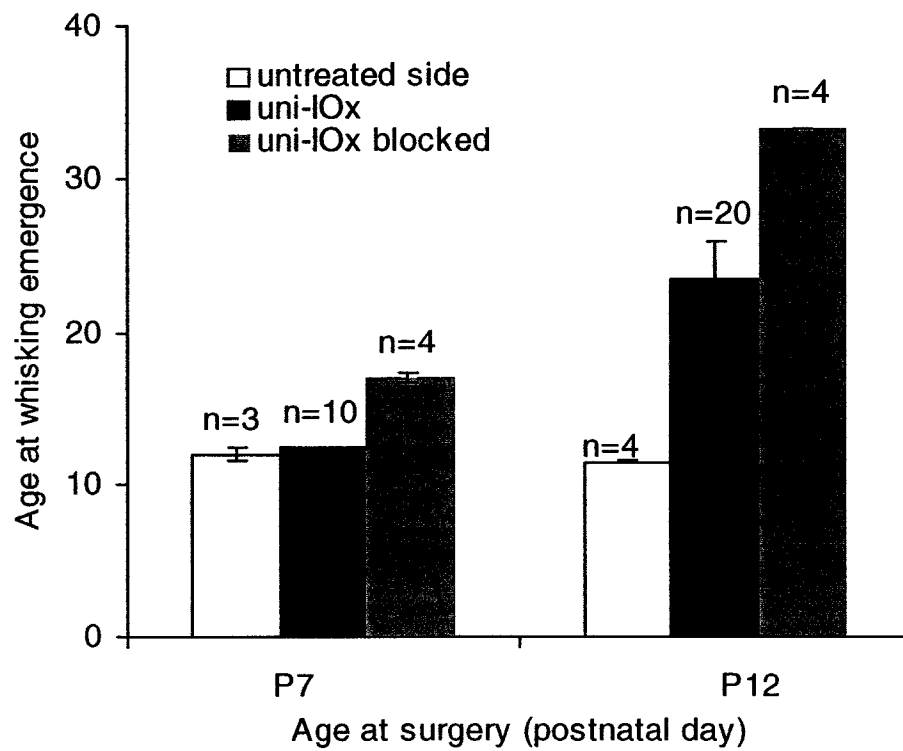


Figure 2.8. Effects of IOx with blockade of regeneration across ages. Mean age at whisking emergence in P7 and P12 Uni-IOx groups with acryl blockade on the treated side while the untreated side was normal. Data for Uni-IOx (unblocked) are replotted. Mean $\pm$ SE.

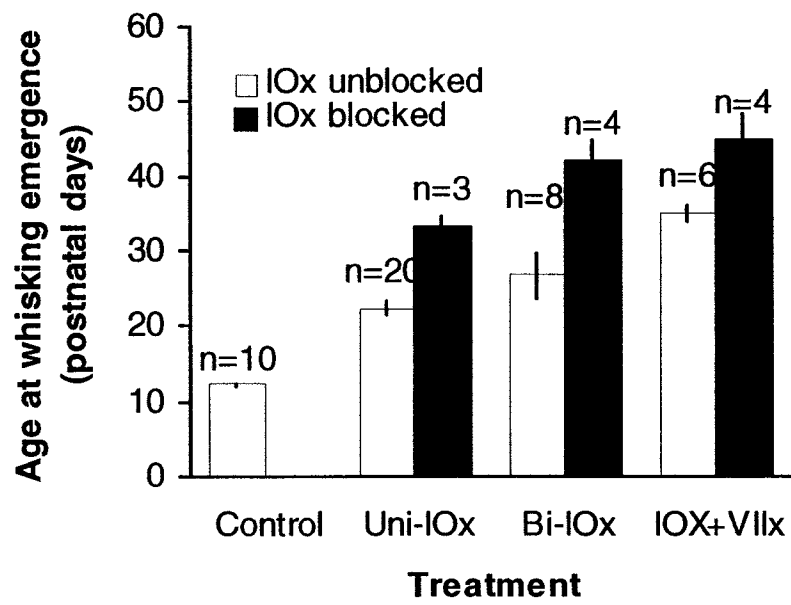


Figure 2.9. Effects of blocking regeneration in IOx pups with sensory and motor denervation. Mean age at emergence of whisking in all IOx “unblocked” and regeneration “blocked” groups. Mean  $\pm$  SE.

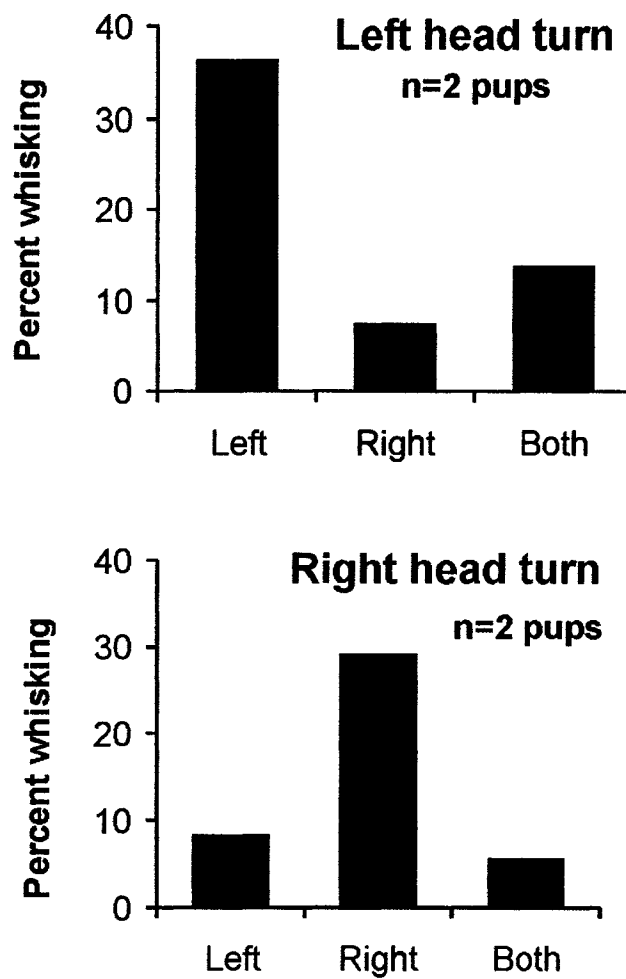


Figure 2.9. Whisking with freely moving head: relation between whisking and head movements in normal P13 pups. Whisking occurs on the side of the face that is the same side as the head turn most of the time. Percent of time (10 sec sample) head turns coincide with whisking.

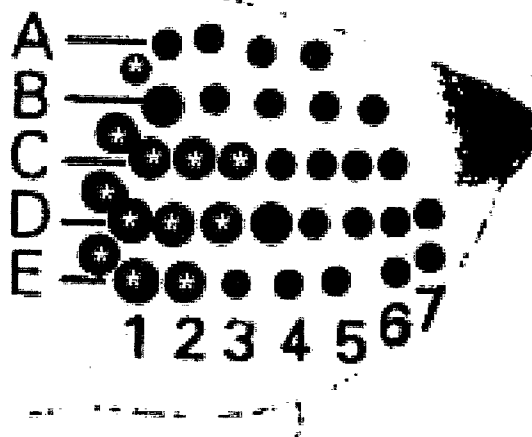


Figure 3.1. Representation of the whisker array in BEN rats. Preliminary data suggested a subset of whiskers showed the largest size difference (straddlers, Row C, D, E in Columns 1-3; asterisks).

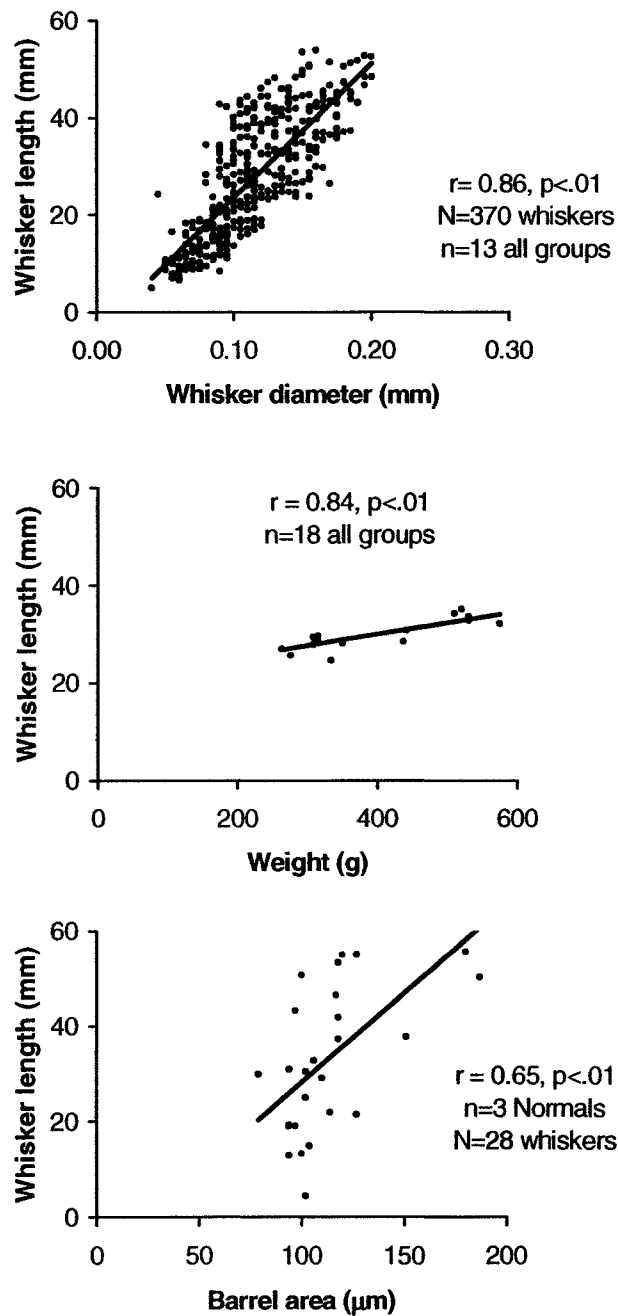


Figure 3.2. Relation between whisker size, body weight and barrel size. In our preliminary study, there was a significant positive correlation between (top) whisker length and diameter and (middle) whisker length and body and (bottom) whisker length and barrel size.

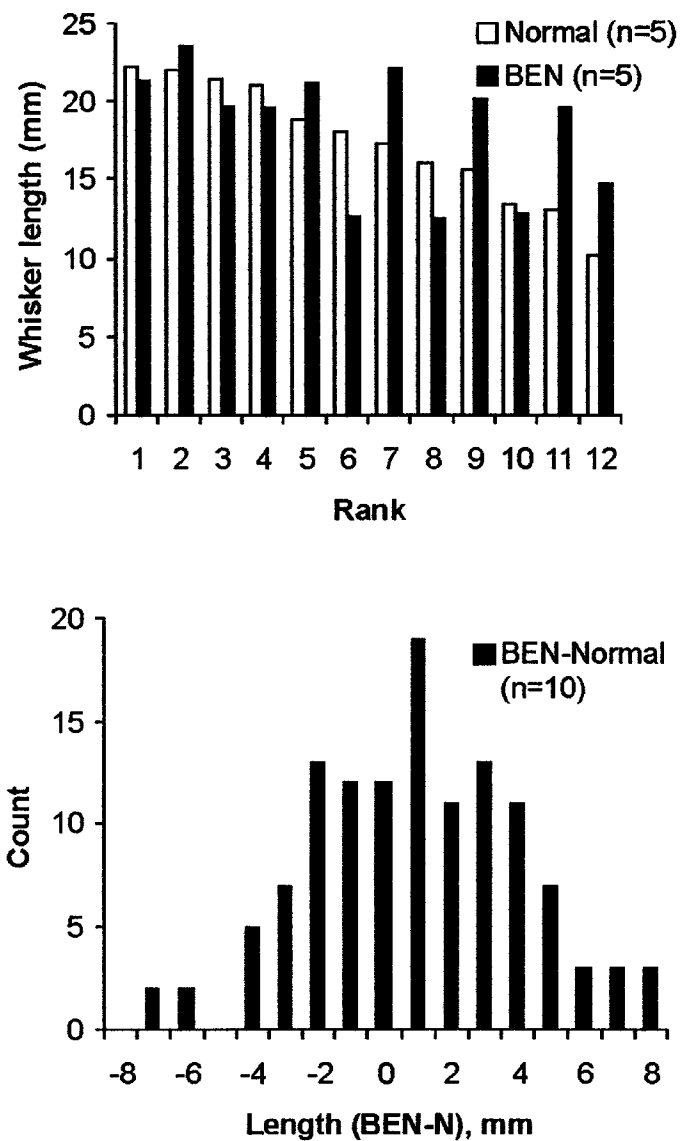


Figure 3.3. Whisker size in mice after long term visual deprivation. (Top) The length distribution of whiskers is shown for the top 12 longest whiskers in normal and BEN mice. (Bottom) The distribution of differences in whisker diameter between BEN and Normals.

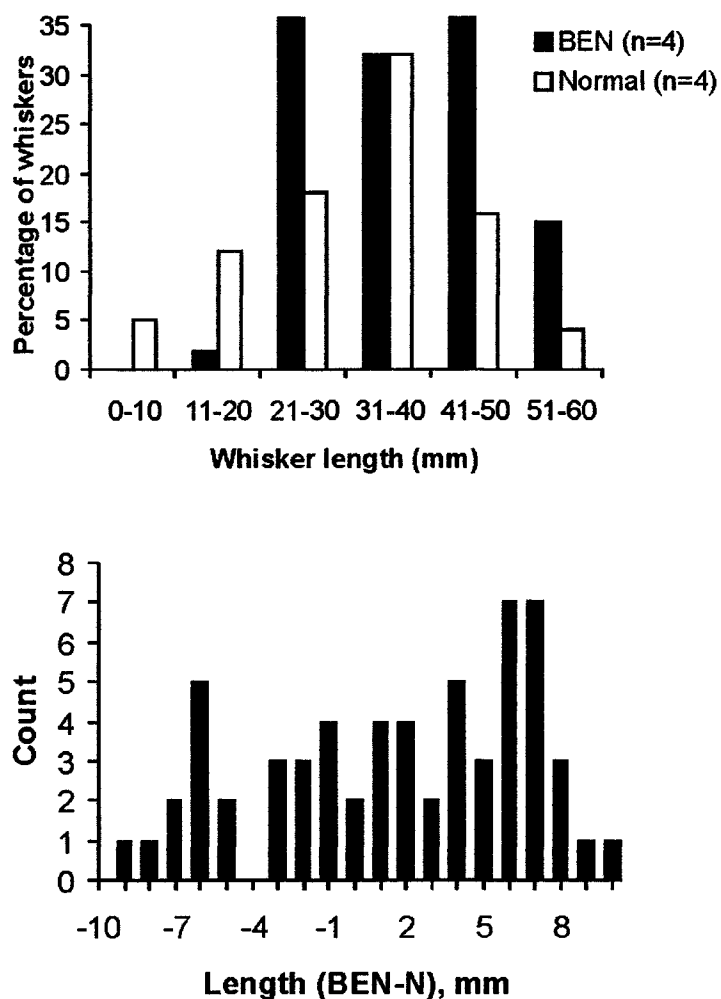


Figure 3.4. Whisker size in rats after long term visual deprivation. (Top) The length distribution of whiskers is shown for normal and BEN rats. (Bottom) Distribution of differences in whisker diameter between BEN and Normals. Distributions were assembled separately for each whisker position and group. Then the distributions from each group and position were compared by calculating the difference between them whisker by whisker. This procedure delivers one distribution for all the differences in all positions between the two groups and eliminates variation due to position.

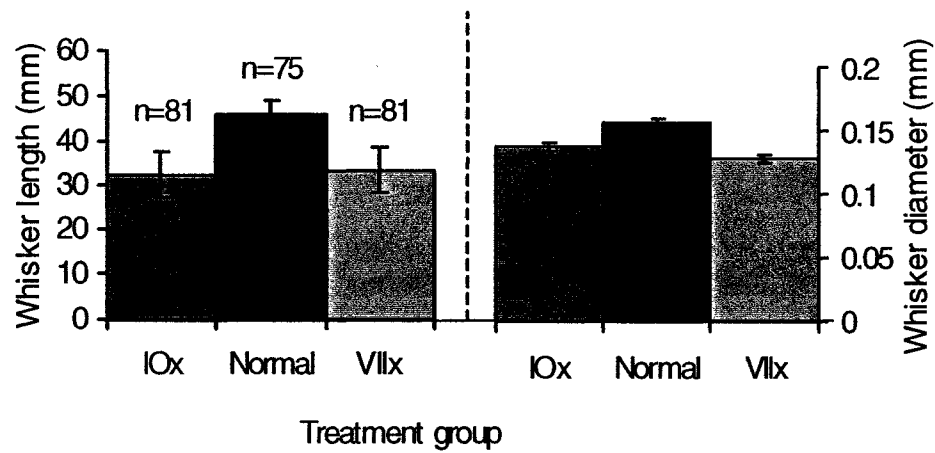


Figure 3.5. Effects of neonatal denervation upon adult whisker size. Both whisker length and diameter are significantly reduced by either sensory (IOx) or motor (VIIx) denervation of the whiskers in pups. Mean  $\pm$  SE.

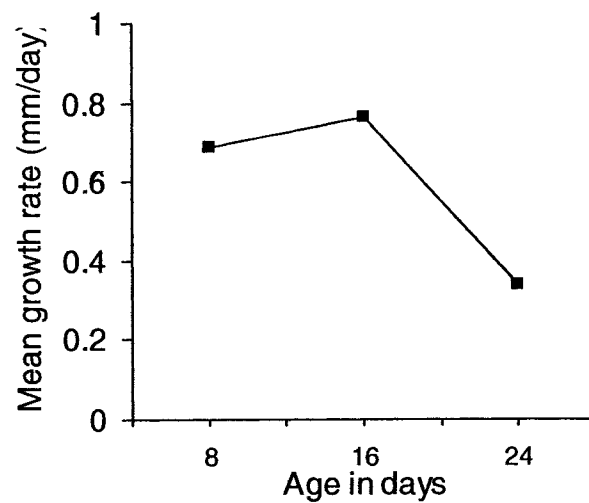
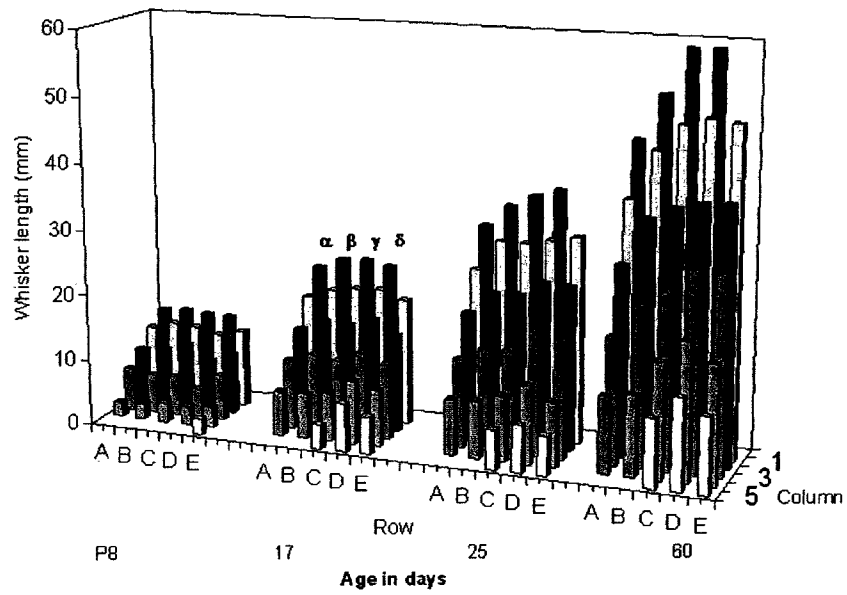


Figure 3.6. Development of the macrovibrissae. (Top) Whisker length over the first postnatal months and in adults (P60). (Bottom) Mean growth rate across age is plotted. Note: diminishing growth rate with age.

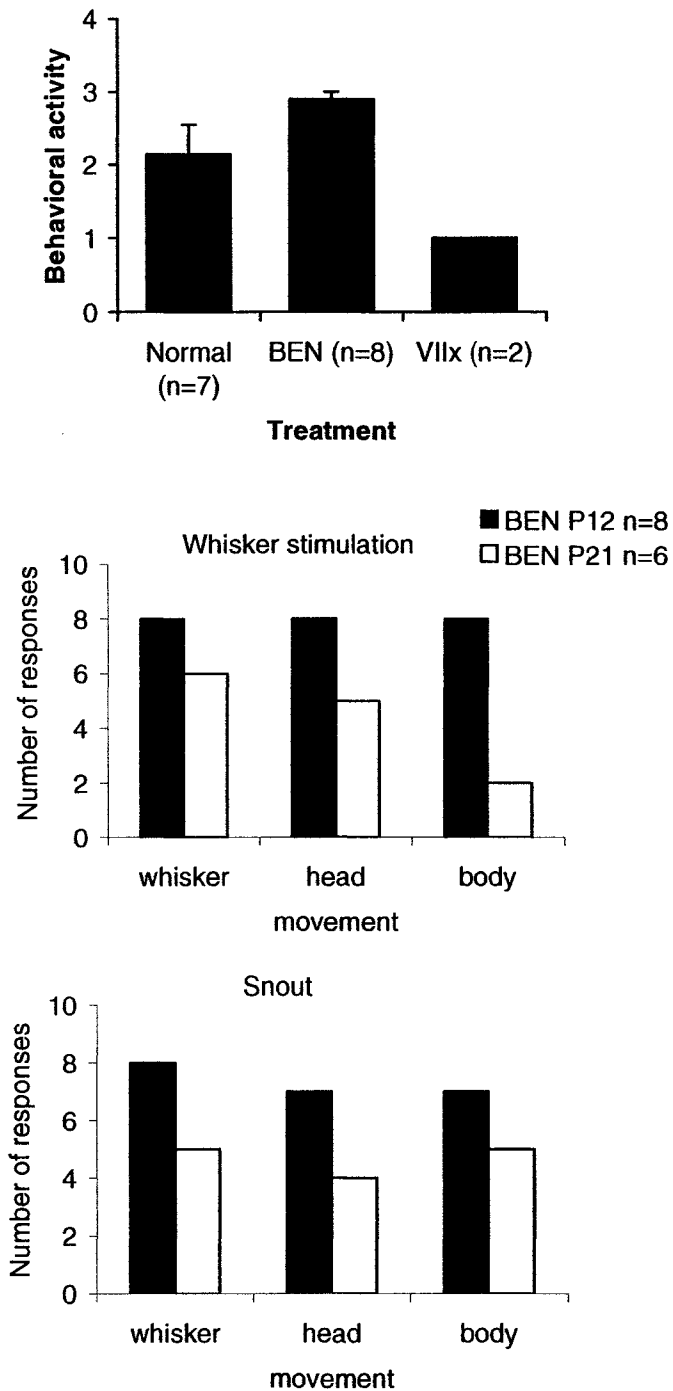


Figure 3.7. Responses of BEN pups to whisker stimulation. (Top) Behavioral activity based on number of body movements was compared across treatments. Mean $\pm$ SE. (Middle) Mean number of behavioral responses to whisker stimulation and (bottom) responses to snout stimulation are shown across age.

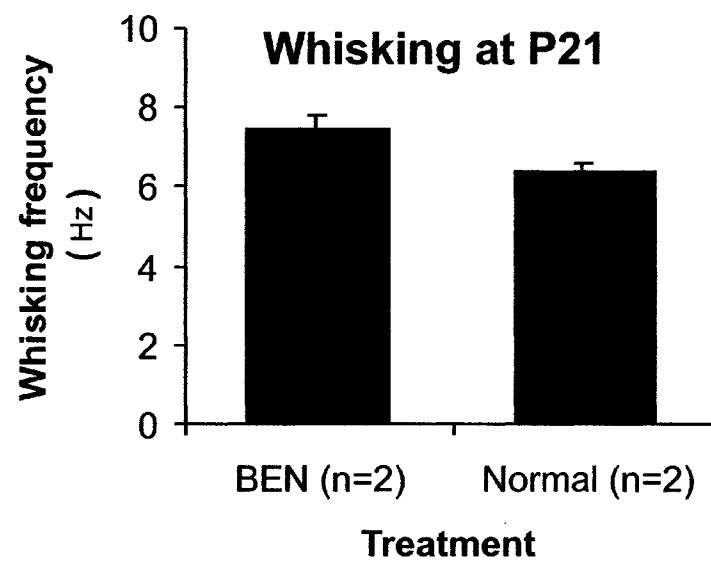


Figure 3.8. Effects of BEN on whisking frequency. BEN young rats whisk at a higher frequency (sampled time: 10 sec).

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