The Development of Movement Patterns During Swimming in the CNS Myelin Deficient Jimpy Mouse

by

Valerie J. Bolivar

Submitted in partial fulfillment of the requirements for the Ph.D degree

at

Dalhousie University

Halifax, Nova Scotia

ISBN 0-612-16015-7

Canada

Name Valerie Bolivar

Dissertation Abstracts International and *Masters Abstracts International* are arranged by broad general subject categories. Please select the one subject which most nearly describes the content of your dissertation or thesis. Enter the corresponding four-digit code in the spaces provided.

Psychology

SUBJECT TERM

0	3	4	9

SUBJECT CODE

UMI

Subject Categories

THE HUMANITIES AND SOCIAL SCIENCES

COMMUNICATIONS AND THE ARTS
Architecture 0729
Art History 0377
Cinema 0900
Dance 0378
Fine Arts 0357
Information Science 0723
Journalism 0391
Library Science 0399
Mass Communications 0708
Music 0413
Speech Communication 0459
Theater 0465

EDUCATION
General 0515
Administration 0514
Adult and Continuing 0516
Agricultural 0517
Art 0273
Bilingual and Multicultural 0282
Business 0688
Community College 0275
Curriculum and Instruction 0727
Early Childhood 0518
Elementary 0524
Finance 0277
Guidance and Counseling 0519
Health 0680
Higher 0745
History of 0520
Home Economics 0278
Industrial 0521
Language and Literature 0279
Mathematics 0280
Music 0522
Philosophy of 0998
Physical 0523
Psychology 0525
Reading 0535
Religious 0527
Sciences 0714
Secondary 0533
Social Sciences 0534
Sociology of 0340
Special 0529
Teacher Training 0530
Technology 0710
Tests and Measurements 0288
Vocational 0747

LANGUAGE, LITERATURE AND LINGUISTICS
Language
- General 0679
- Ancient 0289
- Linguistics 0290
- Modern 0291

Literature
- General 0401
- Classical 0294
- Comparative 0295
- Medieval 0297
- Modern 0298
- African 0316
- American 0591
- Asian 0305
- Canadian (English) 0352
- Canadian (French) 0355
- English 0593
- Germanic 0311
- Latin American 0312
- Middle Eastern 0315
- Romance 0313
- Slavic and East European 0314

PHILOSOPHY, RELIGION AND THEOLOGY
Philosophy 0422
Religion
- General 0318
- Biblical Studies 0321
- Clergy 0319
- History of 0320
- Philosophy of 0322

Theology 0469

SOCIAL SCIENCES
American Studies 0323
Anthropology
- Archaeology 0324
- Cultural 0326
- Physical 0327

Business Administration
- General 0310
- Accounting 0272
- Banking 0770
- Management 0454
- Marketing 0338

Canadian Studies 0385
Economics
- General 0501
- Agricultural 0503
- Commerce-Business 0505
- Finance 0508
- History 0509
- Labor 0510
- Theory 0511

Folklore 0358
Geography 0366
Gerontology 0351
History
- General 0578
- Ancient 0579
- Medieval 0581
- Modern 0582
- Black 0328
- African 0331
- Asia, Australia and Oceania 0332
- Canadian 0334
- European 0335
- Latin American 0336
- Middle Eastern 0333
- United States 0337

History of Science 0585
Law 0398
Political Science
- General 0615
- International Law and Relations 0616
- Public Administration 0617

Recreation 0814
Social Work 0452
Sociology
- General 0626
- Criminology and Penology 0627
- Demography 0938
- Ethnic and Racial Studies 0631
- Individual and Family Studies 0628
- Industrial and Labor Relations 0629
- Public and Social Welfare 0630
- Social Structure and Development 0700
- Theory and Methods 0344

Transportation 0709
Urban and Regional Planning 0999
Women's Studies 0453

THE SCIENCES AND ENGINEERING

BIOLOGICAL SCIENCES
Agriculture
- General 0473
- Agronomy 0285
- Animal Culture and Nutrition 0475
- Animal Pathology 0476
- Food Science and Technology 0359
- Forestry and Wildlife 0478
- Plant Culture 0479
- Plant Pathology 0480
- Plant Physiology 0817
- Range Management 0777
- Wood Technology 0746

Biology
- General 0306
- Anatomy 0287
- Biostatistics 0308
- Botany 0309
- Cell 0379
- Ecology 0329
- Entomology 0353
- Genetics 0369
- Limnology 0793
- Microbiology 0410
- Molecular 0307
- Neuroscience 0317
- Oceanography 0416
- Physiology 0433
- Radiation 0821
- Veterinary Science 0778
- Zoology 0472

Biophysics
- General 0786
- Medical 0760

EARTH SCIENCES
Biogeochemistry 0425
Geochemistry 0996
Geodesy 0370
Geology 0372
Geophysics 0373
Hydrology 0388
Mineralogy 0411
Paleobotany 0345
Paleoecology 0426
Paleontology 0418
Paleozoology 0985
Palynology 0427
Physical Geography 0368
Physical Oceanography 0415

HEALTH AND ENVIRONMENTAL SCIENCES
Environmental Sciences 0768
Health Sciences
- General 0566
- Audiology 0300
- Chemotherapy 0992
- Dentistry 0567
- Education 0350
- Hospital Management 0769
- Human Development 0758
- Immunology 0982
- Medicine and Surgery 0564
- Mental Health 0347
- Nursing 0569
- Nutrition 0570
- Obstetrics and Gynecology 0380
- Occupational Health and Therapy 0354
- Ophthalmology 0381
- Pathology 0571
- Pharmacology 0419
- Pharmacy 0572
- Physical Therapy 0382
- Public Health 0573
- Radiology 0574
- Recreation 0575
- Speech Pathology 0460
- Toxicology 0383

Home Economics 0386

PHYSICAL SCIENCES
Pure Sciences
Chemistry
- General 0485
- Agricultural 0749
- Analytical 0486
- Biochemistry 0487
- Inorganic 0488
- Nuclear 0738
- Organic 0490
- Pharmaceutical 0491
- Physical 0494
- Polymer 0495
- Radiation 0754

Mathematics 0405
Physics
- General 0605
- Acoustics 0986
- Astronomy and Astrophysics 0606
- Atmospheric Science 0608
- Atomic 0748
- Electronics and Electricity 0607
- Elementary Particles and High Energy 0798
- Fluid and Plasma 0759
- Molecular 0609
- Nuclear 0610
- Optics 0752
- Radiation 0756
- Solid State 0611

Statistics 0463

Applied Sciences
Applied Mechanics 0346
Computer Science 0984

Engineering
- General 0537
- Aerospace 0538
- Agricultural 0539
- Automotive 0540
- Biomedical 0541
- Chemical 0542
- Civil 0543
- Electronics and Electrical 0544
- Heat and Thermodynamics 0348
- Hydraulic 0545
- Industrial 0546
- Marine 0547
- Materials Science 0794
- Mechanical 0548
- Metallurgy 0743
- Mining 0551
- Nuclear 0552
- Packaging 0549
- Petroleum 0765
- Sanitary and Municipal 0554
- System Science 0790

Geotechnology 0428
Operations Research 0796
Plastics Technology 0795
Textile Technology 0994

PSYCHOLOGY
General 0621
Behavioral 0384
Clinical 0622
Developmental 0620
Experimental 0623
Industrial 0624
Personality 0625
Physiological 0989
Psychobiology 0349
Psychometrics 0632
Social 0451

dedicated with love to my parents and husband

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

Neurological mutant mice provide an imperfectly explored and potentially important way to examine the effects of single gene mutations on motor behavior. This thesis research concerns the swimming behavior of one neurological mutant, the dysmyelinating jimpy mouse. The jimpy mutation (*jp*/Y) results from a single base change within the proteolipid protein gene and causes severe dysmyelination throughout the central nervous system while not affecting the peripheral nervous system. Examination of this mutant with a refined swimming test during the early postnatal period allowed the study of developmental effects of dysmyelination on a basic rhythmical motor behavior. Male jimpy mouse pups and their littermate controls were videotaped every second day from postnatal day 3 to 21. Detailed examinations of the movements made during swimming were accomplished by frame-by-frame analyses of the videotaped swimming sessions. Swimming behavior was examined in terms of general swimming style (limb usage), individual limb timing and interlimb coordination. These combined measures of swimming ability were used to provide a richer picture of swimming behavior in these mutants than previously provided.

Although generalized swimming style measures did not show significant differences between jimpy and control groups, more fine-grained analyses did. Jimpy mice displayed stroke duration and velocity deficits in hindlimb movements. However, these deficits did not become apparent until postnatal days 11 and 13. When examining the coordination between pairs of limbs it became evident that jimpy mice were less able to maintain a coordinated swim involving both the forelimbs and hindlimbs. Coordination distinctions may reflect imperfect neural transmission in the jimpy mutant. Further, messages that have to travel the full length of a dysmyelinated spinal cord become temporally delayed, thereby resulting in a lack of normal swimming coordination. My data confirm the importance of detailed behavioral analyses to improve our understanding of how single gene mutations affect motor behavior.

ACKNOWLEDGMENTS

I would like to thank my supervisor John Fentress for introducing me to neurological mutant mice. Little did either of us know that what started as a comprehensive project would change the direction of my academic interests and career. I thank all my thesis committee members, John Barresi, Richard Brown, Jim Clark and John Fentress, for their helpful comments and criticisms on drafts of my thesis. I would especially like to thank my external examiner Richard Sidman for his supportive comments on my thesis research.

Vern Fraser and the other animal care staff deserve special thanks, especially Ross Burgess for taking special care of my "little guys". Thanks go to Wanda Danilchuck for help with everything from filming to proofreading, and especially for her assistance in that last minute rush to get the thesis off to the external examiner.

I thank my parents, who have helped so much both financially and emotionally over the past few years. Yes, Dad I did "finish what I started". Special thanks go to Tigger for always knowing just when to be a welcome distraction. Last, but *absolutely* not least, I thank my husband Kevin, for his hundreds (or was it thousands?) of hours of technical support. Your hours of technical help are surpassed only by your hours of emotional support. In return all I can say is, **"It is finally over!!"**

INTRODUCTION

Nothing in neurobiology makes sense except in the light of behavior.

Gordon M. Shepherd, 1994

This thesis concerns the behavioral (motor) development of a neurological mutant mouse strain (jimpy), on the conviction that behavior provides the fundamental assay of integrated nervous system action (Fentress, 1991). Movement reveals many problems and patterns of organization that can help link issues that are otherwise often separate in traditional neurobiological and behavioral disciplines. The potential for linking genes and behavior has received increasing appreciation in recent years, as symbolized by volume *264* of *Science* (June,1994) on the subject. However, for both invertebrate and vertebrate species reviewed in that issue, the lack of quantification of developmental details in overt behavior remains disappointing. As noted in the introduction of this special issue (Mann, 1994), behavioral traits have for the most part been "inexactly defined" (p. 1688).

Movement provides the most obvious assay of the integrated nervous system operations that make adaptive behavior possible (Cordo & Harnad, 1994). Developmental analyses provide a natural dissection of these individual operations as well as their rules of combination over time (Fentress & Bolivar, 1996). Neurological mutant mice in turn allow the investigator to trace these events in animals that have previously been characterized in detail at cellular and biochemical levels (Sidman, 1983). It is hoped that the

quantitative assessments of mutant movement profiles in development can reveal principles of organization that will forge essential bi-directional communication between several levels of behavioral science and more molecularly oriented research at the neurobiological level (Churchland & Sejnowski, 1992). The potential for combining multiple assays of mutant phenotypes in mice has been reviewed by Takahashi, Pinto and Vitaterna (1994).

Neurological Mutant Mice

Neurological mutant mice with single-gene mutations provide a powerful tool for linking genes with their cellular, neuroanatomical and biochemical effects. The techniques used to determine deficits at any of these levels of analysis have become sophisticated and enable precise measurements (e.g., Dautigny et al., 1986; Fannon, Mastronardi & Moscarello, 1994; Skoff & Knapp, 1990). Some neurological mutants have also been categorized as rodent models of human genetic disorders (e.g., the jimpy mouse for Pelizaeus-Merzbacher disease, the twitcher mouse for infantile Krabbe's disease) and emphasis is placed on how well the mouse disorder matches the human one. As outlined by Sidman (1983), it is important to recognize that due to the developmental and organizational differences between species, these models may have limited generality. Instead, the critical importance of these neurological mutant mice to date has been the insight they provide at more fundamental levels of analysis. These include insights into formal genetics (intragenetic structure and neighboring genetic loci and chromosomal map position), homologous gene products and their target cells and

developmental and physiological time courses given the specific deficits involved (Sidman, 1983). When considered in this more fundamental framework, even those mutations that do not correspond with known human disorders are invaluable in terms of shedding light on basic physiological and developmental principles. One only has to examine Lyon and Searle's (1989) catalogue of neurological mouse mutants to get an appreciation of the vast body of research examining these animals and the degree to which the linkages between genes and physiological deficits have been established.

Neurological mutant mice also have the potential to be a powerful tool for linking genetic mutations to behavior, enabling the investigation of the elationship between behavior, nervous system and genes. Despite the fact that the first indication that a neurological mutation has spontaneously arisen in a mouse colony is often the presence of behavioral (generally locomotor) abnormalities, strong evidence to support the connection between a specific genetic mutation and its related central nervous system structure and behavioral function is often lacking. Part of this problem is due to the complexity of the system being investigated, but another issue is the reflection of the sophistication of the behavioral techniques generally used in behavioral analyses. Goldowitz and Eisenman (1992) note that the potential of the neurological mutant mouse as a way to connect genes and central nervous system structure to function is dependent upon refined behavioral measures. As the behavioral measures become more carefully refined one will be able more accurately to connect central nervous system structure (resulting from both genetic and non-genetic factors) and behavior.

The single-gene mutant can be considered a powerful means of evaluating gene and environment interaction in ethogenesis (Guastavino,

Larsson, & Jaisson, 1992). The literature reviewed below on staggerer (*sg*), weaver (*wv*) and jimpy (*jp*) neurological mutant mice indicate not only the potential richness of single-gene alterations in the study of central nervous system function, but also the need to attain more refined measures combined with systematic developmental analyses.

The rationale for including these three particular neurological mutants needs to be clarified. The staggerer mouse is reviewed because of the vast behavioral literature available on this mutant, including research both directly and indirectly related to motor behavior. The weaver mouse is included because of the double nature of its deficit (cerebellar and striatal) and because the behavioral measures used in this thesis were perfected in pilot work with this mutant. The jimpy, which has been well described genetically and anatomically, is the subject of this thesis research. Given that the cellular deficits of both the weaver and jimpy mouse were known, when the thesis research was being planned both the weaver and jimpy were considered for study. However, at the time, of the two neurological mutants, only the jimpy mouse had been described genetically. This detailed description allows for potential definitive conclusions relating to structure and function. Recently the weaver mutation has been mapped to the *GIRK2* gene (Patil et al., 1995).

This selective review does not imply that researchers have not examined the behavior of other neurological mutants. In fact, behavioral studies on cerebellar mutants (such as lurcher, nervous, Purkinje cell deficient, hot-foot, and dystonia musculorum) and myelin deficient mutants (such as quaking, shiverer, and twitcher) have provided insights into structure/function relations (e.g., Goodlett, Hamre, & West, 1992; Inagawa, Watanabe, Tsukada, & Mikoshiba, 1988; Lalonde, Botez, & Boivin, 1986; Lalonde, Botez, Joyal, &

Caumartin, 1992; Lalonde, Filali, Bensoula, & Lestienne, 1996; Lalonde, Joyal & Cote, 1993; Oliverio & Messeri, 1973; Olmstead, 1987).

Staggerer Mouse

One of the neurological mutants most extensively studied behaviorally is the staggerer mouse, the result of an autosomal recessive mutation occurring on chromosome 9 (Lyon & Searle, 1989). The homozygous staggerer can be readily identified by its staggering gait, mild tremor and hypotonia (Sidman, Lane, & Dickie, 1962). Although the primary site of action is thought to be the Purkinje cell population, the effects are also evident in the death of granule cells and inferior olive neurons (Blatt & Eisenman, 1985a; Crepel, Delhaye-Bouchaud, Guastavino, & Sampaio, 1980; Herrup, 1983; Herrup & Mullen, 1979; Mariani & Changeux, 1980; Shojaeian, Delhaye-Bouchaud, & Mariani, 1985; Shojaeian Zanjani, Herrup, Guastavino, Delhaye-Bouchaud, & Mariani, 1994; Shojaeian Zanjani, Mariani, & Herrup, 1990).

Studies of the staggerer mouse cover a variety of behaviors, some of which are more obviously related to known or hypothesized functions of the olivocerebellar system than others. For instance these mice show characteristic deficits in general motor ability that are consistent with cerebellar atrophy including reduced activity in exploratory tasks and in a T-maze, difficulties maintaining balance in equilibrium tests (wooden beam and grid) and reduced rearing relative to littermate controls (Lalonde, 1987b; Lalonde, Manseau, & Botez, 1988a). In several tests of motor coordination (rotorod, rotating grid, wooden beam and coat-hanger) staggerer mice showed performance deficits relative to littermate controls and despite practice training of up to seven days did not show any improvement (i.e., they showed no

evidence of sensorimotor learning) (Lalonde, Bensoula, & Filali, 1995; Lalonde, Filali, Bensoula, & Lestienne, 1996).

Motor deficits have also been found in the heterozygous staggerer mouse. Caston, Delhaye-Bouchaud and Mariani (1996) compared heterozygous (+/*sg*) staggerer and control (+/+) mice in terms of their ability to maintain equilibrium on the rota rod, and found that although performance scores decreased for both groups over the two year period studied, scores were consistently lower for the heterozygous mice relative to the controls when tested at three, six and twelve months of age. However, at eighteen months of age and beyond performance differences disappeared. Caston et al. suggest that early (three months of age) performance differences indicate a deficit in motor control which appears prior to the massive neuronal death in the cerebellar cortex (which occurs over the first year of life in the heterozygote) and, therefore, may be due to subtle structural or neurochemical disorders.

Behavioral deficits have also been observed during maze learning and spontaneous alternation which cannot be explained simply by motor deficits and have been linked to visuo-spatial organization deficits (Lalonde, 1987a; Lalonde, Manseau, & Botez, 1988a). Although, in general, staggerer mutants displayed deficits in water maze performance, the relative difference between mutants and controls does not increase with age (Bensoula et al., 1995). Homozygous staggerer mice display deficits relative to control mice when tested in a radial arm maze and active avoidance tasks (Goldowitz & Koch, 1986). In a water filled Z-maze, staggerer mutants make more errors and have longer escape latencies than normal mice (Lalonde, Filali, Bensoula, Monnier, & Guastavino, 1996).

Although Lalonde, Botez and Boivin (1987) reported no difference in general locomotor activity (measured by the number of section crossings) between staggerer and littermate control groups, they found that mutants explored a novel object longer than did control mice. The latter result is in agreement with the findings of Misslin, Cigrang and Guastavino (1986) who reported that staggerer mutants displayed less neophobic behavior when confronted with a novel object in a familiar environment and explored a new environment more quickly than normal mice. It has been suggested that this may result from a deficiency in the inhibitory process and that the cerebellum may play a role in these sorts of complex behaviors (Misslin et al., 1986). Goodall and Gheusi (1987) examined the abnormal ambulatory patterns of staggerer mutants in complex grid-marked mazes and concluded that a lower level of place-to-place movement in relation to littermate controls could be explained by either abnormal novelty reactions to new cells or a tendency to return to cells already encountered.

The swimming behavior of these mutants has been described as relatively normal given their severe disruption in terrestrial locomotion (Sidman et al., 1962). In agreement with Sidman et al., Goodall, Guastavino and Gheusi (1986) found no significant difference in the speed or distance traveled, during the initial stage of the swimming period, between staggerer and littermate controls. The differences they found lay in the style of the swim, including more circling and less time spent immobile by mutant animals. As time in the water increased, controls showed decreased activity, due to increased time spent immobile, whereas staggerers maintained a constant level of activity. This led to exhaustion in the mutants earlier than in the controls. Additionally, unlike the control mice who stayed close to the walls, staggerer mutants tended to spend equal amounts of time in all areas of the pool.

The effects of environment and the issue of plasticity have been explored in the staggerer mutant. In an environmental enrichment study, Guastavino (1984a) subjected young staggerer mutants with daily tilted rotation stimulation from birth to 21 days of age and found stimulated staggerers were significantly better at avoiding holes when ambulating on a holed floor than non-stimulated mutants. In fact, stimulated mutants were able to achieve the same level of hole avoidance as unstimulated littermate control mice. Guastavino and Goodall (1985) emphasize the importance of the experimental conditions when evaluating the success or failure of the enriched environment on the motor performance of these mice. Their examination of the permanency of gait improvement revealed no clear cut conclusions as the results differed based on the measures used (Guastavino & Goodall, 1985). For instance, when tested two months after the stimulation ended, the stimulated groups were no better at avoiding the holes than were the unstimulated mutants, but the stimulated mutants remained permanently more susceptible to subsequent environmental enrichment situations.

Other researchers examining the staggerer mutant have reported deficits in behaviors not generally associated with the cerebellum, for example abnormalities in reproductive behavior. The reproductive behavior research arose in part out of practical considerations (Larsson, Guastavino, & Ly, 1986). With the difficulties in phenotypically identifying the mutant animals prior to postnatal day nine, research examining earlier brain structure and related function was not possible. It thus became advantageous to be able to breed homozygous pairs of these animals to ensure that the offspring would all be homozygous and, therefore, testable at any age. The problem was two- fold. First, as more than fifty percent of these mice generally died by weaning age (Sidman et al., 1962), it was necessary to develop techniques to prolong life,

including placing moistened food on the cage floor and having a long spout on the water bottle (see Larsson, Guastavino, & Ly, 1986). Second, it was necessary to determine normal reproductive behavior for these animals and ways to maximize reproductive fitness if necessary.

Guastavino (1982) was the first researcher to systematically examine the conditions under which homozygous staggerers would breed. Homozygous staggerers housed with other mutants did not normally breed. Prior sexual experience with a normal mate was necessary for mating. Even after prior experience, males had fewer successful matings than females, which may be due to the motor abnormalities associated with this mutation. Females may have had less difficulty in mating than males because lordosis was less affected by balance difficulties than male mounting. Staggerer males also displayed less social interest and less sniffing and sexual behaviors when presented with estrous or non-estrous females than did normal males (Baudoin, Feron, & Magnusson, 1991). Social experience with normal females after weaning increased anogenital sniffing and decreased stereotypic scratching behavior in male staggerers, whereas experience with staggerer females did not have the same effect on staggerer males (Feron, Baudoin, & Magnusson, 1991). Moreover, sexually experienced but not sexually naive staggerer males preferred the odour of estrous over anestrous females (Feron & Baudoin, 1993). In a study of the effect of social isolation on the mating behavior of male staggerer mice, Feron and Baudoin (1995) found that after two months of isolation more male staggerers were able to sire pups with normal females, then non-isolated staggerer males.

Guastavino and Larsson (1992) have suggested that the staggerer gene has a direct effect on reproductive activity, as the female displays delayed puberty and irregular vaginal estrous cycling and a very short reproductive life

span of only three months after puberty. Although neonatal vestibular stimulation has a beneficial effect on the mating success of staggerer mutants, it does not eliminate all of the reproductive abnormalities of the female staggerer, suggesting that not all of the reproductive deficiencies of this mutant are secondary to postural and locomotor abnormalities (Guastavino, Larsson, Allain, & Jaisson, 1993). As these types of deficits do not readily relate to known cerebellar function, Guastavino and Larsson (1992) have suggested that the staggerer mutant should no longer be considered exclusively a cerebellar mutant.

In addition to difficulties in mating, staggerer mutants show deficits in parental behavior. Under normal breeding conditions, the staggerer female does not suckle, retrieve or clean her pups (Guastavino, 1983). After being exposed to foster pups with well developed suckle reflexes and subsequently being constrained in a small box with her own litter, the female will give much better maternal care (Guastavino, 1983, 1984b). It is also critical that the real litter of the staggerer female be given foster care by a normal female during the first twenty-four hours of life (Guastavino, 1984b). Even under the best conditions, approximately one third of the litter die as the staggerer female does not lick the pups, nurses in a sideways position which limits access to her teats, fails to build a nest, and does not retrieve pups which crawl or are pushed away from her body (Guastavino, 1984b). Nest building appears to be problematic for staggerer mutants. Both sexes fail to build proper nests for their own use (Bulloch, Hamburger, & Loy, 1982). It has been suggested that this failure to build a nest may be directly related to the action of the staggerer gene. Unlike the staggerer, other cerebellar mutants are able to build nests. In addition, Boufares, Guastavino and Larsson (1993) found reproductive differences between two lines (breeding populations) of staggerers in the

C57BL/6 strain, despite the fact that both lines displayed the same deficits in locomotor behavior. Once again this supports the notion of co-action of the staggerer gene.

The behavioral findings suggest a much more complex picture of the staggerer mouse than would be assumed given the known cellular deficits. Goodall and Guastavino (1986) have suggested three generalizations that one has to consider when examining the behavior of neurological mutants such as the staggerer: i) the behavioral effects that can be directly related to the known physiological consequences of the mutant gene's action, ii) those effects that are secondary and arise because of deficiencies in some component of a more complex behavior (e.g., mating behavior due to male's problems with mounting) and iii) those behaviors that appear unrelated to the known physiological and anatomical effects, which may be the result of unexpected functions of the known locus of the gene's effect or undiscovered physiological effects of the gene on another part of the nervous or endocrine system.

Although cerebellar deficits in staggerer mice are compatible with the hypothesis of specific disorders in fine motor control, the majority of behavioral studies do not examine movement disorders in a fine-grained manner. Thus, the data on staggerer mice would benefit from refined measures of movement performance such as motor timing, amplitude and interlimb phase relations in basic rhythmic activities. This is especially true given the well established role of the cerebellum in fine motor control (Bloedel, 1992). End point measures of performance do not in themselves clarify the underlying processes in performance. Similar methodological limitations can be found in the existing behavioral literature for other mutant strains (see Goldowitz, Wahlsten & Wimer, 1992 for a review). In addition systematic developmental analyses

might further clarify progressive changes in motor performance in comparison between mutant and control animals.

Weaver Mouse

The weaver mouse is of interest because both cerebellar and striatal central nervous system regions are affected. As shown in pilot work in our laboratory (Bolivar, Danilchuk & Fentress, 1996; Bolivar, Manley & Fentress, 1996; Coscia & Fentress, 1993) specific parameters of movement performance are likely to be dissociable along neuro-anatomical and more subtle circuit properties.

The weaver mutation is now recognized as resulting from a point mutation in the *GIRK2* gene (Patil et al., 1995). The gene codes for a potassium ion pore molecule in the cell membrane. Homozygous weaver mice have abnormalities of granule cells, Purkinje cells, and Bergmann glia in the cerebellum (Blatt & Eisenman, 1985b; Herrup & Trenkner, 1987; Rakic & Sidman, 1973a, 1973b; Rezai & Yoon, 1972; Sidman, 1968; Smeyne & Goldowitz, 1990; Sotelo, 1975), a loss of dopamine neurons in the midbrain and reduced tyrosine hydroxylase activity, dopamine levels and dopamine uptake in the striatum (Gupta, Felten, & Ghetti, 1987; Richter, Stotz, Ghetti, & Simon, 1992; Roffler-Tarlov & Graybiel, 1984, 1987; Schmidt et al., 1982; Simon & Ghetti, 1992; Triarhou, Norton, & Ghetti, 1988). Weaver mutants can easily be distinguished from their normal littermates by the presence of ataxia, fine tremor, and hypotonia by the end of the second postnatal week (Sidman, 1968).

Behaviorally, weaver mutants display less activity in exploratory tests, balance difficulties in equilibrium tests, deficits in spontaneous alternation in a

T-maze, navigational deficits in the water maze and swim with more "vigor" (length of time spent swimming per minute) but with less "ability" (position of the head relative to the water surface) than do controls (Lalonde, 1986a, 1986b, 1987c; Lalonde & Botez, 1986; Lalonde, Manseau, & Botez, 1988b). Triarhou, Norton and Hingtgen (1995), were able to ameliorate some of these locomotory deficits by grafting fetal dopamine cells into the weaver mutants. Weaver mutants that received bilateral transplants of fetal dopamine cells were able to cling to a rotorod longer, toppled onto their sides less, and crossed more squares in an open field matrix than did wild-type mice. As some of the cerebellar and dopaminergic deficits can be traced to the first few days of postnatal life (Simon, Richter, & Ghetti, 1994; Smeyne & Goldowitz, 1989), it is indeed possible that behavioral abnormalities should correlate with these early cellular abnormalities.

Recent work in our laboratory has used detailed movement analyses to study motor development of the weaver mouse. One of the behaviors that we have studied in detail is grooming, a complex behavior involving whole body movements as well as postural adjustments (Berridge, 1994; Berridge, Fentress, & Parr, 1987; Golani & Fentress, 1985). With both cerebellar and striatal abnormalities, we expected grooming deficits in the weaver pups. Homozygous weaver mice displayed fundamental abnormalities in grooming style and engaged in more frequent but shorter grooming bouts than did control mice (Bolivar, Danilchuk, & Fentress, 1996; Coscia & Fentress, 1993). However, these grooming differences were both context and age dependent (Bolivar, Danilchuk, & Fentress, 1996). Littermate control mice spent more time grooming, and displayed longer grooming bouts than mutants (in both pre-swim and post-swim contexts). After postnatal day thirteen, during the

post-swim condition, mutants reached the performance level shown by controls in the pre-swim test (Bolivar, Danilchuk, & Fentress, 1996).

As grooming differences did not become evident until postnatal day thirteen, the details of swimming, a rhythmic behavior that is relatively unaffected by gravity, were also examined. Weaver mutant mice displayed a developmental lag in terms of swimming style and a generalized slowness in limb movements during the swim relative to controls (Bolivar, Manley, & Fentress, 1996). Specific movement alterations could be traced to as early as postnatal day three, a time at which the earliest biochemical and neuroanatomical deficits in these animals have been established (Bolivar, Manley, & Fentress, 1996; Simon et al., 1994; Smeyne & Goldowitz, 1989). Surprisingly, however, limb movement patterns during swimming continued to exhibit good overall coordination throughout the third postnatal week, even though terrestrial locomotor ataxia has become pronounced by this time. This indicates that swimming is not simply another measure of terrestrial locomotion but a valuable assay in its own right.

The measures that we developed for weaver mice hold considerable promise for the study of numerous forms of neurological disorder that cross central nervous system regions. Since integrated behavior at the intact organism level involves the collective cooperation of numerous central nervous system regions (Posner & Raichle, 1994), mutations that affect this communication are of particular interest. At the same time, specific and genetically based developmental defects that involve multiple central nervous system regions can have important consequences on a variety of forms of motor behavior (Weiner & Lang, 1989).

Jimpy Mouse

Jimpy is a sex-linked mutation resulting in dysmyelination throughout the central nervous system but having no effect on the peripheral nervous system (Phillips, 1954; Sidman, Dickie & Appel, 1964). Myelin, in the central nervous system, is an extension of the cell membrane of the oligodendrocyte that becomes spirally wrapped around nerve axons and functions as an insulator that increases the speed of nerve impulses along the axon. Each site that is wrapped by a single oligodendrocytic process is termed an internode and each oligodendrocyte is capable of maintaining 30-50 internodes of myelin (Peters & Proskauer, 1969). The process of extending the cell membrane of the oligodendrocyte to form a myelin sheath around an axon is complex and involves the production, and incorporation into the membrane, of a large number of lipid and protein constituents. A variety of lipids are incorporated, including galactolipids, cholesterol, cholesterol esters and phospholipids. The major proteins involved include myelin-associated glycoprotein, myelin basic proteins and proteolipid protein. Most of the myelination process, in rats and mice, occurs postnatally (Jacobson, 1963; Miller, 1992).

Myelin acts as an insulating material, separating the axon from the extracellular conducting medium. Nodes of Ranvier, which separate the internodes of myelin, however, remain in contact with the electrolytic medium. The action potential, as it moves down the axon, jumps quickly from node to node. These nodes permit the rejuvenation of full amplitude action potentials. Further, since the axon has to be electrically activated only at the nodes, then the speed of transmission along a myelinated axon occurs much faster than along a nonmyelinated axon.

In the hemizygous jimpy (*jp*/Y) mouse, a small number (5%) of the oligodendrocytes have normal lifespans and lay down myelin sheaths, which

are devoid of proteolipid protein (Duncan, Hammang, Goda, & Quarles, 1989; Skoff & Knapp, 1990). It has been hypothesized that proteolipid protein may act as a spacing mechanism during compaction of the myelin sheath (Hudson, Friedrich, Behar, Dubois-Dalcq, & Lazzarini, 1989). In addition, the jimpy mutant shows deficits in other myelin proteins, lipids and associated enzymes. The hemizygous jimpy mouse has reduced levels of myelin-associated lipids, in particular sulfatide and cerebrosides (Galli & Galli, 1968; Hogan, Joseph, & Schmidt, 1970; Matthieu, Widmer, & Herschkowitz, 1973; Sidman et al., 1964). In 20-day-old jimpy mice, myelin basic protein and myelin-associated glycoprotein levels are reduced to 1.9% and 5.3% of control brain levels, respectively (Yanagisawa & Quarles, 1986). Deficits are also evident in the tissue content of cholesterol, monogalactactosyl diglyceride, and long-chain (C_{22}-C_{24}) fatty acids (Deshmukh, Inoue, & Pieringer, 1971; Joseph & Hogan, 1971; Nussbaum, Neskovic, & Mandel, 1969). The enzymatic activities of cholesterol ester hydrolase and 2'-3'-cyclic nucleotide 3'-phosphohydrolase (CNPase) are also reduced (Eto & Suzuki, 1973, Kurihara, Nussbaum, & Mandel, 1969; Matthieu, Quarles, Webster, Hogan, & Brady, 1974; Sarlieve, Farooqui, Rebel, & Mandel, 1976; Yanagisawa & Quarles, 1986).

In the case of female heterozygotes (carriers), there is an early deficit in the number of oligodendrocytes but this appears to be compensated for by an increase in their proliferation rate during myelination (Bartlett & Skoff, 1989; Rosenfeld & Friedrich, 1986). Skoff and Ghandour (1995) have demonstrated 21% fewer oligodendrocytes in heterozygotes versus normal adults yet there are normal amounts of myelin in these animals.

Jimpy mice show a hindquarter tremour when attempting movement which is absent during rest (Phillips, 1954). This tremour becomes evident by the

end of the second postnatal week, and by the end of the third postnatal week many of the mutant animals display tonic-clonic seizures during which the head is arched back and extremities extended with contracted distal joints (Phillips, 1954; Sidman et al., 1964). These mutants usually die by thirty days of age and often following a seizure (Sidman et al., 1964).

In the jimpy mouse, a point mutation in the proteolipid protein gene results in exon five of the gene not being included during mRNA transcription (Dautigny et al., 1986; Hudson, Berndt, Puckett, Kozak, & Lazzarini, 1987; Macklin, Gardinier, King, & Kampf, 1987; Morello, Dautigny, Pham-Dinh, & Jolles, 1986; Moriguchi et al., 1987; Nave, Bloom, & Milner, 1987; Nave, Lai, Bloom & Milner, 1986). The protein constructed from this aberrant mRNA results in an abnormal Cys-rich C terminus and misfolding of the protein (Fannon et al., 1994; Nave et al., 1986). It has been suggested that this aberrant form of proteolipid protein could be cytotoxic, leading to the premature death of oligodendrocytes (Nadon, Arnheiter, & Hudson, 1994; Schneider, Griffiths, Readhead, & Nave, 1995). Evidence of cytotoxicity of aberrant proteolipid protein in oligodendrocytes has been provided by Roussel, Neskovic, Trifilieff, Artault, & Nussbaum (1987) and Gow, Friedrich, Lazzarini (1994).

The jimpy mutation results in abnormalities in both of the central nervous system macroglial cell populations (i.e., astrocytes and oligodendrocytes). The cell cycle of jimpy astrocytes in vitro is 5-6 hours longer than that of normal mice (Knapp & Skoff, 1993) and the intracellular pH of astocytes in jimpy is higher than in normal cultures (Knapp, Booth, & Skoff, 1993). The central nervous system of these mutants contains only half of the normal number of oligodendrocytes and the cell cycle of the remaining cells is irregular (Knapp & Skoff, 1987; Privat, Valat, Lachapelle, Baumann, & Fulcrand, 1982). The

number of proliferating glial precursor cells is elevated as is the rate of oligodendrocyte cell death (Knapp & Skoff, 1987; Knapp, Skoff, & Redstone, 1986; Skoff, 1982). Knapp, Skoff & Redstone (1986) have described the developmental pattern of oligodendrocyte death in detail. By postnatal day four, myelination has already begun in the spinal cord of littermate controls, whereas in the hemizygotes there is already a significant level of oligodendrocyte cell death. In the hemizygotes oligodendrocyte cell death in the spinal cord peaked at postnatal day twelve. In the case of the corpus callosum the first myelin sheaths were seen at day eleven and there was no difference between mutant and control groups in terms of the number of dying oligodendrocytes. However, by postnatal day sixteen oligodendrocyte cell death in the hemizygotes had become significantly higher than in controls and this difference increased up to postnatal day twenty-two, the last day studied.

Based on transplantation studies it has been proposed that an as yet unidentified, soluble factor, which is under the control of the proteolipid protein gene, is required during embryonic development and it is at this point that the effect of the jimpy mutation really begins (Knapp, Benjamins, & Skoff, 1996; Lachapelle, Gumpel, & Baumann, 1994; Lachapelle, Lapie, & Gumpel, 1992). Evidence from a study with double mutant mice (quaking*jimpy) supports the theory that the proteolipid protein gene codes for an oligodendrocyte signaling factor in addition to the structural protein (Billings-Gagliardi, Karthigasan, Kirschner, & Wolf, 1990). While proteolipid protein is virtually absent in the double mutant, as in the jimpy, the number of oligodendrocytes is intermediate between that of the jimpy and quaking mutants. Since the two major defining features of the jimpy mouse - virtual absence of proteolipid protein and severe loss of oligodendrocytes - seem to be affected independently in the double mutant, this could be taken as evidence that something in the quaking

mutation is ameliorating the jimpy toxicity to oligodendrocytes, but is not able to affect the jimpy inability to manufacture proteolipid protein.

Little research, other than the original papers describing the mutation (Phillips, 1954; Sidman et al., 1964), has been directed toward the behavioral manifestations of this genetic defect. In an examination of the development of the righting reflex and geotaxis, the strength of jimpy mouse responses were moderate at postnatal days 3-4, and strong by days 11-14 (Fox, 1965a). In addition, the hyperkinesias observed in young mice (less than one postnatal week of age) were exaggerated in the jimpy mutants and persisted in the caudal region after that time (Fox, 1965a).

Bolivar and Brown (1994) examined the development of jimpy mutants (*jp*/Y) from postnatal days 2-20, comparing them to littermate controls. Using several assays of motor behavior we found that *jp*/Y mice spent less time engaged in coordinated activities such as locomotion and grooming than their control littermates beginning at the second week postpartum. The amount of time spent in slight movement (defined as any movement of the body extremities or head that does not result in a change in body location), however, revealed earlier differences between the two groups. As early as postnatal day two, the mutant pups spent less time engaged in slight activity than did the littermate controls. As more coordinated motor activities became apparent in the controls, the *jp*/Y group lagged behind, spending more time in slight movement. This could well be a result of their inability to perform the more coordinated activities. Weight differences were also evident between the two groups, the *jp*/Y mice weighing less from day six onward. The *jp*/Y group was also delayed in reaching developmental milestones including eye opening and displaying auditory startle. Bolivar and Brown (1994) also noted that as early as postnatal day two differences were evident between *jp*/Y and

normal littermates in terms of the number of ultrasonic vocalizations produced by pups when separated from their mother, with the mutant pups producing fewer of these cries. Paradoxically, even though it has generally been assumed that ultrasonic vocalizations of rodent pups serve as a signal to attract maternal attention (see Allin & Banks, 1972), the mothers of jimpy pups selectively retrieved mutant pups over control littermates when given a choice between them (Bolivar & Brown, 1995).

Given our findings of behavioral deficits in the jimpy mouse, together with our work on the weaver mouse, one would expect that by utilizing detailed assays of movement one might be able to find measurable motor behavioral differences between neurological mutants and littermate controls, earlier in development than has previously been shown. As has been seen, the genetics and the cellular deficiencies of the jimpy mouse have been defined anatomically, and chronologically. While the actual mechanism underlying oligodendrocyte cell death has yet to be confirmed, one should in principle be able to correlate the known timetable of myelination with the results of an ontological examination of motor coordination (e.g., swimming movements). Furthermore, as the jimpy is a rodent model for the human sex-linked myelin deficiency (Pelizaeus-Merzbacher disease), one may be able to translate earlier behavioral markers from these animals to humans, bearing in mind the caution made by Sidman (1983) on the imperfectness of animal models for human disease.

Movement Analysis

To move things is all that mankind can do; ... for such the sole executant is muscle, whether in whispering a syllable or in felling a forest.

Charles Sherrington, 1924

Movement has been described as the most fundamental expression of the central nervous system (Lashley, 1951). In spite of the richness offered in Sherrington's description, detailed analyses of limb movement are generally not pursued by behavioral scientists (cf. Cordo & Harnad, 1994; Golani, 1992). Individual movements involved in a particular behavior are often assumed to be present by investigators even when they are not specifically examined by them. For instance, the frequency or duration of grooming and locomotor behaviors in rodents are often used to assay the effects of drugs, diseases or lesions, but the spatiotemporal and sequential nature of the individual components of the movements are not examined.

The study of movement, at detailed levels, is essential both as a foundation for behavioral research and as a means of examining the form and content of the movement itself. Furthermore, it is important to examine movement patterns at complementary levels of analysis to avoid missing out on valuable higher-order pattern information (Fentress, 1992; Fentress & Bolivar, 1996). As Fentress (1992) has stated on the subject of frameworks or taxonomies of movement analyses: "My prejudice is that there is not one best framework but that complementary frameworks can yield complementary insights" (p. 1537). Of course integrating the information from various levels of analysis is in itself

not always easy. What can be done, however, is to dissect individual properties of movement for a given limb and then ask how these properties are coordinated across limbs. Such methodology provides an objective framework from which more abstract models of movement organization are most appropriately derived.

Lorenz (1981) argued that movement patterns provide a morphology of behavior, that permits detailed phylogenetic and ontogenetic comparisons (cf. Golani, 1992). Pioneering neurological studies by von Holst (1937; translated in Gallistel, 1980) demonstrated dynamic and level dependent rules in the coupling of individual limb movement properties in various vertebrate species. From these careful observations of behavior, von Holst derived such fundamental principles as relative coordination among individual central nervous system oscillators.

In both invertebrate and vertebrate neurobiology, coupled "central pattern generators" are now widely accepted as the basis for many essential properties of motor expression (Davis, Jacobs & Schoenfield, 1989; Grillner, 1990). Central pattern generators are networks of neurons within the central nervous system that play an important role in generating and controlling rhythmic behaviors (cf. Collins & Stewart, 1993). Numerous rhythms in mammalian movement, such as seen in locomotion and grooming, involve a hierarchically ordered set of commands and presumed pattern generators. The intrinsic operations of these central pattern generators have allowed investigators to examine a number of dynamic processes within the central nervous system, and have provided an important corrective to simple reflex models of brain-behavior relations (Grillner, 1990). Central patterns generators are normally triggered and can often be modulated by processes

extrinsic to the basic pattern generating circuits, but once initiated patterning circuits exhibit a high degree of autonomous organization.

These pattern generators are only partially isolable in that they interact with both sensory inputs and supraspinal mechanisms (Cordo & Harnad, 1994; Pearson, 1987). Together these mechanisms become incorporated within dynamic systems that defy full analysis by reductionistic methods (Churchland & Sejnowski, 1992). This is because the full system properties depend equally upon isolable individual operations and their combined actions (Fentress, 1990). This leads to a perspective of movement organization that must rest upon the evaluation of individual mechanism properties within the context of often complex dynamic systems (Kelso, 1995; Thelen & Smith, 1994). In operational terms one can ask when individual processes are independent or coupled in their operation. The importance of the idea for developmental studies is that systems must express both progressive differentiation (refinement) and integration (sophisticated rules of combination). Interestingly, no systematic developmental studies of these sorts of pattern generators and their potential combinations exist. Thus, it is impossible with current knowledge to predict whether the pattern generators remain stable after their appearance or improve with age. More complex rules of motor coordination are even more ambiguous. It would be of considerable interest to know whether myelin deficiency can slow down basic timing properties within central pattern generators as well as the coupling between limbs.

While much progress has been made in the neurobiological analysis of movement, evaluation of integrated movement patterns in intact animals remains in many respects an enigma. The importance of movement analysis for setting essential questions in neurobiology is, however, gaining greater acceptance. As pointed out by Churchland and Sejnowski (1992), the

computational problems of extracting circuit properties from even a small number of neural elements is formidable. For this reason circuit constraints must be evaluated at their own level. The same applies to models of movement. For example, Churchland and Sejnowski (1992), offer this question of perspective:

> *"How does the brain control the immense array of muscle cells so that the whole body moves in the right way? This problem is probably the most basic one a nervous system evolved to solve" (p. 331)*

Limits on the quantification of natural movement properties and their rules of combination, have limited much traditional research. In recent years investigators have become increasingly sensitive to the necessity of precise description of movement as a prelude to successful model building. There has also been increased appreciation for the basically relationistic nature of movement (Golani, 1992), as well as the frequent sensitivity of movement details to the broader contexts within which these details are expressed. As illustration Bolivar, Danilchuk and Fentress (1996) have shown that relativisticly defined relations among limb properties in rodent grooming can vary in their apparent stability as a function of the descriptive perspective employed, and also vary as a function of the particular test conditions (e.g. pre-swim versus post-swim grooming). Clearly these properties of movement have a developmental history. Analysis of their assembly, and potential disassembly, during ontogeny may thus provide a powerful analytical tool that can synthesize current neurobiological and behavioral research.

The Ontogeny of Movement Patterns

The task of describing and understanding a particular movement pattern is not easy when studying individuals of any age, but it is especially difficult when studying very young animals. Depending upon the environmental conditions and the motor behavior being studied, movement elements in young animals may appear sporadic, be presented in a different order than in the adult, or be incomplete (Fentress & Bolivar, 1996). Therefore, to study the ontogeny of movement patterns one must perform detailed analyses using multiple descriptive levels so that one can determine underlying similarities and differences between the adult and non-adult forms (Fentress, 1990). Also it is critical that the behavioral task not be confounded by other physiological factors such as balance or neuromuscular strength. There have been few attempts to provide testing frameworks during early stages of development which allow the organism to move freely while at the same time avoiding these kinds of confounds. Studies of movement ontogeny in rodents have concentrated on three basic movement patterns; terrestrial locomotion, grooming and swimming.

Terrestrial Locomotion

Investigations of rodent locomotion have traced the ontogeny of limb use and coordination. Although quadrupedal locomotion would seem the most obvious type of locomotion for investigation, it presents problems for those wanting to make observations during the early postnatal period. The development of the quadruped stance takes approximately two weeks and proceeds in a rostral-caudal direction, with head, forelimb and shoulder

elevation occurring before elevation of the hindlimbs and pelvis (Altman and Sudarshan, 1975).

In an in-depth investigation of posture and body part participation during the acquisition of locomotion in the rat, Altman and Sudarshan (1975) provided time frames for each part of the process. Due to the rostral-caudal development the first type of locomotion does not really involve all four legs. Pivoting involves the movement of the forelimbs while the pelvis remains anchored to the ground, resulting in a circling type of locomotion. It becomes common by the end of the first postnatal week, but by the end of the second week it is occurring infrequently. The next locomotor pattern they observed was crawling, in which the elevation of the front part of the body is more controlled than that of the hind portion. Finally, walking with the completely raised quadruped posture becomes evident toward the end of the second postnatal week. A similar progression, with pivoting and crawling preceding walking, is evident during the postnatal development of mice (Fox, 1965b). Therefore, for anyone studying interlimb coordination in rodents, this particular type of locomotion is difficult to assess until toward the end of the second postnatal week.

Eilam and Golani (1988) studied the ontogenetic progression of movement types in the rat pup. Young pups (postnatal days 0-1) only move along the lateral dimension, older pups (postnatal days 5-6) move along both lateral and forward dimensions and older pups (postnatal days 11-14) move along lateral, forward and vertical dimensions. Body and limb segments are incorporated in movements along each dimension in a rostral-caudal order and a particular segment moves laterally first, followed by forward and vertical movements, respectively (Eilam & Golani, 1988). This ontogenetic progression uses the same order (lateral-forward-vertical) of movements as

that seen in the rat pup during 'warm-up' after a period of immobility (Eilam & Golani, 1988; Golani, 1992; Golani, Bronchti, Moualem, & Teitelbaum, 1981).

In contrast to the more qualitative methodology of many investigators (e.g., Altman and Sudarshan, 1975) Westerga and Gramsbergen, (1990) examined locomotor development in the postnatal rat (days 10-20) using quantitative measures such as stride length (distance between the position of the third toe at two consecutive footstrikes), displacement (distance between the position of the hip joint at two consecutive footstrikes), mean duration of the step cycle, stance duration and swing duration (from the first sign of flexion of any joint until footstrike with the hindpaw). There appears to be a transformation in locomotion development around postnatal day fifteen, from a swimming-like movement (abduction, rotation and hyperextension of the paw) to the digitigrade adult pattern without marked rotation. There is a sudden increase in the speed of the pup's locomotion around postnatal day fifteen accompanied by a decrease in step cycle, stance phase and swing phase durations. Only stride length shows a more gradual increase which was evident beginning at the first day of testing. It appears, therefore, that adult style locomotion is not a gradual process, but is characterized by abrupt changes at the beginning of the third week after birth. This transition to adult locomotor style is both delayed and prolonged in rat pups undernourished during gestation and lactation, and a longer-lasting abnormality in fine movement coordination is also evident (Gramsbergen & Westerga, 1992).

Grooming

In contrast to locomotion, grooming is a more complex movement pattern, and is mentioned here for the purpose of phenotypic contrast with the more

basic repetitive swimming sequences studied for this thesis. Grooming consists of a set of actions invcl ing the face, limbs and body which are connected in a distinct pattern. Facial grooming includes a number of individual strokes including paw wash, nose strokes, fast elliptical movements around the nose, above nose-below eyes strokes, above eyes-below ears strokes, above ears-to top of head strokes, and arms over-extended strokes (Fentress, 1972; Richmond & Sacks, 1980). During face grooming these strokes tend to occur in ascending order and with increasing amplitude. Body grooming is most often preceded by several components of facial grooming. Facial grooming is a very robust set of movements and even amputation of the forelimbs does not eliminate other aspects (e.g., tongue and shoulder movements) of the behavior (Fentress, 1973). Individual components can be predictably linked together and in this way one can determine the order of movements within a bout of grooming (Berridge et al., 1987; Fentress, 1972; Fentress & Stilwell, 1973). In addition to the overall linkage among components, grooming contains both flexible and stereotypic sequential phases (Berridge & Fentress, 1986; Berridge et al., 1987).

Facial washing has been seen as early postnatally as day two in the rat pup although it has been described as a very rudimentary non-functional version of adult grooming (Bolles & Woods, 1964). In fact face wiping has been identified and described during embryonic development (Robinson & Smotherman, 1991, 1992a, 1992b; Smotherman & Robinson, 1987, 1989). Richmo ıd and Sachs (1980) examined the development of grooming in the Norway rat throughout the first postnatal month. They found that it took about three weeks for the full adult grooming pattern to appear, although the first grooming movements were evident a day or two after birth. At postnatal day two the pup made nose wiping movement with the forepaws, although they

usually did not make contact with the nose. By day three contact was usually made with the nose and by day five forepaw licking became evident. Eye and ear wiping also appeared early (days 6-8), but were not incorporated into an adult like grooming sequence until day eleven. Licking of the more posterior parts of the body came later, during the third postnatal week. The development of the grooming actions of the forepaws and mouth followed a rostral-caudal pattern, with movements around the nose occurring first and those of the tail regions being the last to be displayed.

Berridge (1994) examined the grooming behavior in supported rat pups and reported short bursts of grooming strokes on postnatal day one. These first strokes may be done by one forepaw or both, and after several bouts of these strokes they may be replaced by air swimming or pushing type movements. Accuracy improves for the rest of the first postnatal week and the pups make more contact of paw to face, with 40% of the strokes actually making contact with the face on day one to 70% on day seven. Without support spontaneous grooming is not commonly seen until day eight. The highly stereotypical pattern or 'chain' of adult rat grooming (Berridge, et al., 1987) also develops with age, and during postnatal days 9-15 the transition from immature to adult pattern is most evident. Furthermore, although the component movements forming this grooming 'chain' are evident as early as postnatal days 10-11, they are impoverished and require another day or two to become more adult-like (Berridge, 1994).

In young postnatal mice, as in rats, early grooming strokes may appear to be ineffectual. Fentress (1972) found that pups may not establish contact with the forepaw and tongue during the paw licking stage. Even later in development, although the nine day old mouse is able to effectively bring the forepaw to the mouth and lick it, individual movements may be slow, of short

trajectories or not in adult sequential order (Fentress, 1972). In a detailed analysis of the ontogeny of mouse facial grooming in supported pups over the first two weeks of postnatal life, Golani and Fentress (1985) were able to separate the form of forelimb strokes into three developmental phases. The first phase (0-100 hours) is characterized by isolated strokes or bouts of strokes that vary in amplitude and symmetry. Often these strokes overshoot the target, even missing the face completely. During the second phase (100-200 hours) one sees the emergence of more controlled symmetrical strokes from both forepaws. The forepaw movements have simpler and more predictable paths and more predictably make contact with the face. Patterns tend to be restricted, strokes are double-handed and stereotyped, and bouts disappear. During the final phase (200-300 hours) grooming begins to resemble that of the adult animal. There is also more diversity during the final phase and bouts reappear containing both symmetrical and asymmetrical strokes.

Golani and Fentress (1985) found that during early mouse grooming development there are two kinds of changes: monotonical, for example the gradual disappearance of forepaw strokes that miss the face; and trend reversals, for example the disappearance and reemergence of bouts or strokes. More recently, Coscia and Fentress (1993) and Bolivar, Danilchuk and Fentress (1996) have examined the development of grooming in the neurological mutant weaver mouse and its littermate controls. They used complementary levels of analyses, from the most general (e.g., the amount of time spent grooming during a 150 second period) to detailed analyses of particular grooming strokes. With age, both weaver and control mice have a tendency to increase the number of different strokes incorporated into their grooming bouts (especially up to postnatal day 15) and increase the amount

of time spent grooming. In addition, the importance of context is evident in the Bolivar, Danilchuk and Fentress (1996) study, as differences in the amount of time spent grooming and characteristics of the grooming movements before and after a short period of supported swimming are also evident.

Swimming

When performing developmental investigations with young rodent pups it is important to select a behavioral action that can be quantified and that the young animal is capable of doing. The confound of gravity on the ability to measure neuromuscular strength is often a problem in developmental investigations that start in the early postnatal period. For instance, terrestrial locomotion, although providing an excellent means of examining limb coordination, is not a useful assay for very young rodents, as they simply cannot move their bodies in forward locomotion until they have the neuromuscular strength to overcome gravity. However, that does not mean that they cannot provide coordinated movements of their limbs. In fact, coordinated movements of the limbs are evident in both prenatal and early postnatal life (Bekoff & Lau, 1980; Bekoff & Trainer, 1979; Smotherman & Robinson, 1986) when the animal has been given the appropriate environmental context. By placing the animal in a liquid environment, the effects of gravity are drastically reduced, and in the case of the neonatal rodent pup, this provides a simulation of the *in utero* environment. Swimming behavior is, therefore, useful for studying early motor development as it can provide limb coordination measures much earlier in development than is possible when studying normal terrestrial locomotion. For this thesis research

swimming has been selected as the behavioral action to investigate the development of coordinated motor behavior, in the neurological mutant, jimpy.

Swimming has been used as a behavioral assay of central nervous system function in rodents for many decades, and has been described as an adaptive response integrating coordinated reflex actions like righting, vestibular, and extensor-flexor reflexes (Schapiro, Salas, & Vukovich, 1970). Due to the integration of the reflexes involved in swimming, it is an excellent biological model by which one may examine the development of the neural substrate as well as its adaptability or flexibility (Schapiro et al., 1970). In an early study of reflex and behavioral development in the mouse, Fox (1965b) included swimming as one of his behavioral assays of locomotor patterns. He used very simple descriptions of swimming behavior, describing the newborn mouse as being able to swim well in circles until 6-8 days postpartum, after which time it began to swim straight. Swimming has become a common measure of motor abilities in rodents because it requires the integration of trunk and limbs (Marshall & Berrios, 1979). Neurological test batteries which emphasize reflex and neuromotor ontogeny, such as the Cincinnati Behavioral Teratogenicity Test Battery and the Barlow and Sullivan Screening Battery, include swimming ontogeny as one of their measures (Adams, 1986; Vorhees, 1985; 1986). In fact swimming is listed as one of the most commonly used measures of motor coordination in teratological evaluations (Buelke-Sam & Kimmel, 1979).

As swimming is a measure that can be used to test rodents very early in the postnatal period it has been used frequently to examine a variety of environmental and genetic factors on motor behavior. The literature reviewed below provides a background of the different measures researchers have developed to examine swimming behavior in rodents. As swimming is the

assay used in the present research a full review of the previous methodologies is necessary to enable evaluation of the different types of methodologies.

Detailed Form and Body Position Coding Schemes

Coding schemes have been developed that provide information about the form of the swim, including the position of the body in relation to the water surface and/or the relative participation of all four limbs in the swim. For instance, Schapiro et al. (1970) developed a scale ranging from zero to three which is based on the position of the animal's nose with respect to the water surface. The higher the nose was kept out of the water the higher the score, a score of three indicating the most mature nose position, a score of zero indicating the least mature position. They also designed a second scale to include the relative activity (scale 0-4) of the four limbs during the swim, as they observed that older rats tended to inhibit the use of the front limbs during the swim, whereas younger animals used all four limbs.

Schapiro et al. (1970) reported that Sprague-Dawley rats less than six days of age were not able to keep their noses out of the water and often floated motionless with arched backs. Limb movements were not coordinated, with the animals displaying hyperextensive reflex activity of the extremities and toes. By day six postpartum, although able to maintain their equilibrium, pups showed poorly coordinated flexor and extensor movements and these movements were not sufficient to keep them swimming in any one direction. This resulted in circling or random directionless movements. At day seven the beginning of flexion and contralateral extension of the limbs was evident, accompanied by apparent attempts to get out of the tank. By

postnatal day eight the pups had started turn-around movements when swimming by using the front limbs. It was not until days 10-12 postpartum that pups displayed well-developed patterns of sequential flexion and extensions of the front limbs. Also by day twelve postpartum the animals were able to keep part of the head including the face and nose out of the water during swimming. Coordination of all four limbs was not evident until day 15 postpartum. After that time the use of the front limbs changes in the swim and by day 22 the forelimbs are held in extension while the hindlimbs provide the main source of thrust of the swim.

To examine the effects of circulating hormones, on central nervous system maturation, Schapiro et al. (1970) examined the swimming patterns of thyroxine and cortisol injected rat pups. In terms of the maturation of body posture while swimming, thyroxine accelerated the maturation process, whereas cortisol retarded it. Similar effects were evident on the progressive cessation of forelimb activity during development. They concluded that the ontogeny of the complex neuromuscular adaptive mechanisms involved in swimming can be modified by the hormonal environment present during early neuronal development.

Using the Schapiro et al. (1970) criteria Anderson and Schanberg (1975) found that maturation of the adult swimming posture could be advanced approximately two days in young rats by thyroxine and decreased by cortisol. Salas (1972) used the Schapiro et al. (1970) coding scheme to examine the effects of early malnutrition on swimming development in the rat. Rat pups were disallowed from nursing for 12 hours per day from postnatal days 4-13. Differences in swimming ability were not evident before postnatal day four as neither control nor malnourished animals could swim well. However after that time the malnourished rats exhibited a two to three day developmental lag in

maturation of swimming ability, in both body position and forelimb use. Salas also reported that disappearance of circling to straight line swimming was delayed in these malnourished animals.

The Schapiro et al. (1970) scheme is perhaps the mostly widely used coding scheme for the study of swimming ontogeny, although it is frequently modified slightly. For instance, Preache and Gibson (1976) adopted the scheme for use in the study of cyclophosphamide treatment on Swiss Webster mice. Cyclophosphamide is an immunosuppressive agent given to transplant patients and also is given as an antineoplastic agent for some forms of cancer. They tested swimming ability from 5-21 days postpartum and evaluated nose position (0 - animal failed to surface, 1 - top of head but not nose was above the surface, 2 - the top of the nose was above the surface, 3 - nose mostly out of the water but ears submerged, and 4 - nose and ears both out of the water) and front paw movement (0 - no paw movement, 1 - all four paws paddling, 2 - one forelimb extended intermittently but paddling is still predominant, 3 - paddling occasionally but extension is the predominant response and 4 - both forelimbs extended leaving paddling to hindlimbs). Cyclophosphamide treatment delayed swimming ability and this effect was dose dependent. There were significant differences between treated and control groups between days six and eighteen postpartum in terms of nose position. In the case of forepaw movement, differences did not emerge until postnatal day nine and were dose dependent. Their findings indicate that this drug can functionally impair behavioral development.

Using a slightly modified Schapiro et al. (1970) scale, Murphy and Nagy (1976) examined the effect of thyroxine stimulation on swimming behavior in mice. Their modification involved four point scales for both the head (0 - nose and forepart of head below surface, 1 - nose touching water surface, but

below it, 2 - nose just above surface, 3 - nose maintained above surface) and the forepaws (0-uncoordinated paddling, often in circles, 1 - coordinated paddling with paws pushed straight forward and down, 2 - some forepaw inhibition but some spontaneous paddling of at least one forepaw, 3 - hindpaws doing the paddling, forepaws inhibited). Both head position and forepaw paddling scores provided evidence that thyroxine treatment results in accelerated swimming development. Using the same modification of the basic Schapiro et al. (1970) scale Nagy, Porada and Anderson (1977) examined the effects of litter size, and in consequence malnutrition, on swimming behavior in the mouse. Adult-like swimming patterns were delayed two to three days in pups from litters of size sixteen versus pups from litters of size six thus indicating that litter size, if not malnutrition, resulted in delays of this motor behavior.

Fride and Weinstock (1984) used the basic Schapiro et al. (1970) method when examining the effects of prenatal noise (electric bell) and light (incandescent bulb) stressors on swimming development (style: where 1 - floating, 2 - circling and 3 - straight line swimming, and nose: where 0 - nose below surface, 1 - nose just above surface, 2 - eyes at water surface and 3 - ears held at water surface). They reported a delay in swimming development but only when the stress was unpredictable. In fact when stress was induced on a daily basis it was found that swimming development was accelerated.

Modifications of the Schapiro et al. (1970) scoring method have been used to determine the effects of neonatal exposure to paint thinner on rat swimming behavior as measured by head position and forepaw paddling (Lorenzana-Jimenez & Salas, 1980). Head position was categorized using a three point scale (1 - nose and forepart of head below the surface of the water, 2 - nose just above the surface of the water, 3 - nose maintained well above the

surface) and forepaw paddling was categorized using a four point scale (0 - front paws in hyperextension, inhibition of forepaws, 1 - inhibition of forepaws with interspersed spontaneous paddling of one forepaw, 2 - well coordinated movements of forepaws, 3 - uncoordinated paddling, no inhibition). Exposure to paint thinner resulted in developmental delays on both measures. Rats exposed to toluene neonatally exhibited a developmental delay in swimming behavior using both the head position and limb paddling measures (Lorenzana-Jimenez & Salas, 1983). Also, there was a delay in the attainment of straight line swimming, persistence in circling, and time to escape from water, in the toluene exposed group.

Najam and Panksepp (1989) adapted the Schapiro et al. (1970) scale to examine swimming behavior, using head position (0 - pup unable to keep nose out of the water to 3 - nose out of water, head tilted at an angle) and leg activity (0 - uncoordinated use of both font legs to 3 - forepaws inhibited), in rat pups that had been given morphine and naloxone neonatally. It was unfortunate that the researchers did not fully define all the values in the scale, but neither did Schapiro et al. (1970), in their original publication. Najam and Panksepp (1989) found that morphine-treated pups displayed a two to three day lag in the development of mature swimming patterns relative to the naloxone-treated ones.

In an expansion of the Schapiro et al. (1970) criteria Vorhees, Butcher, Brunner and Sobotka (1979) included three aspects of swimming development as part of the developmental test battery for determining neurobehavioral toxicity (Cincinnati Psychoteratogenicity Screening Test Battery). The three dimensions of their swimming ability scale were direction (0 - sank, 1 - floated, 2 - swan in circle or arc and 3 - swam in a straight line or nearly straight line), angle in water or head position (0 - submerged, 1 - nose

at surface, 2 - nose and top of head at or above surface, and 3 - same as 2 except waterline at midear level) and use of limbs (0 - no paddling, 1 - paddling with all four limbs, and 2 - paddling with hindlimbs only, forelimbs stationary).

Vorhees, Brunner and Butcher (1979) examined the effects of prenatal exposure to three psychotropic drugs (prochlorperazine, propoxyphene and fenfluramine) on Sprague-Dawley rat pups' swimming ability using the same basic three dimensional coding scheme. However, they added one level to the angle in water or head position code, (4 - nose and top of head above water surface, with waterline at the bottom of the ears). Fenfluramine treated pups were developmentally delayed on the swimming direction and head position scales. Propoxyphane treated pups were developmentally delayed in terms of head position, although prochlorperazine did not have an effect when measured on any of the swimming scales.

Since the scale's development in 1979 Vorhees and colleagues have used their swimming scale to examine a number of toxicity scenarios. For instance, Vorhees, Butcher, Brunner and Sobotka (1981) have reported that chronic exposure (both prenatally and postnataliy) to butylated hydroxytoluene (an antioxidant food additive) led to delays in forelimb inhibition (holding forelimbs still). Prenatal exposure to naloxone resulted in an accelerated swimming direction development in rat pups (Vorhees, 1981). Prenatal exposure to diphenylhydantoin delayed all three aspects of swimming development, whereas prenatal exposure to phenobarbitol only resulted in a delay on the development of mature swimming angle and then only on postnatal day 12 (Vorhees, 1983). However, short-term prenatal exposure to alcohol did not have an effect on swimming ontogeny (Vorhees & Fernandez, 1986).

Using the Vorhees et al. (1979) procedure Poppe, Stuckhardt and Szczech (1983) found that pups born to ochratoxin A (a toxin produced by storage fungi) treated dams had lower swimming scores than those born to vehicle control dams. Peruzzi, Lombardelli, Abbracchio, Coen and Cattabeni (1985) found that perinatal caffeine treatment had a significant effect on rat pup swimming (tested on postnatal day six), with the treated animals receiving lower scores on the angle in the water (or head position) dimension of the Vorhees et al. (1979) scale. Administration of a soy lecithin preparation during the perinatal period resulted in developmental delays in swimming behavior (using a composite score from the Vorhees et al. (1979) scales), although much of this effect was due to body angle and less so to limb paddling and forward movement (Bell & Lundberg, 1985).

Using a combination of Schapiro et al. (1970) and Vorhees et al. (1979) scales Ryan and Pappas (1985) examined the effect of neonatal 6-hydroxydopamine lesion of spinal norepinephrine terminals on swimming ontogeny. When direction (0 - sank, 1 - floated, 2 - swam in circle, 3 - swam in straight line), angle of nose (0 - nose submerged, 1 - nose at surface, 2 - nose and top of head at or above surface, 3 - water surface at mid-ear or below) and limb usage (0 - no paddling, 1 - front limb paddling only, 2 - all four limbs paddling, 3 - paddling with hindlimbs only) were combined into a composite score there was no effect on swimming behavior from this type of lesion.

When examining the effects of thyroxine on swimming ontogeny Davenport and Gonzalez (1973) used a five point scale which evaluates body position as follows: 0-entire body under the surface with nose pointing downward, 1 - nose parallel to surface of the water and head and ears barely under the surface, 2 - the nose just above the surface of the water (just broke the surface) , 3 - the nose maintained above the surface of the water, and 4 -

the nose and ears both above the surface of the water. They found that the thryoxine group showed more advanced swimming behavior than the control group. Using the same coding scale Davenport, Hagquist and Hennies (1975) found even more acceleration of swimming behavior when rats were given triiodothyronine (a thyroid hormone).

St. Omer and Mohammad (1987) used only the angle of head criterion to evaluate the effects of prenatal exposure to a mixture of 2,4-dichlorophenoxyacetic and 2,4,5- trichlorophenoxyacetic acids (a combination used in Agent Orange) on swimming behavior. Swimming scores were significantly lower for the treatment animals than control animals on postnatal day seven although they were not statistically different for the rest of the period studied (postnatal days 9-21). Prenatal or postnatal exposure to secalonic acid D mycotoxin (a fungus toxin) also resulted in lower swimming scores as measured by the angle dimension (St. Omer & Bolon, 1990). Montella and Reddy (1991) reported, using the angle of head criteria that postnatal secalonic acid D mycotoxin exposure resulted in lower swimming scores throughout the second postnatal week in mice. Again using only the head angle dimension to evaluate swimming, St. Omer et al. (1991) concluded that prenatal methylenedioxymethamphetamine (a mild hallucinogen) exposure had no effect on the ontogeny of swimming performance in rat pups.

Adams (1982) examined the effects of perinatal exposure to the mild tranquilizer chlordiazepoxide on swimming behavior in rats (postnatal days 3-30) using a two-dimensional scale including direction (3 - straight, 2 - circling, 1 - floating) and head angle (4 - ears above the surface, 3 - ears half out of the water, 2 - nose and top of head above the surface, 1 - not able to hold head up). Rats exposed to chlordiazepoxide perinatally showed impaired swimming, as measured by the direction criterion (i.e., more circling), although

not in terms of the head angle criterion. Adding a third dimension (limb movement, 1 - all four limbs used, 2 - hindlimbs only) to the Adams (1982) scales Pantaleoni et al. (1988) examined swimming behavior of rat pups exposed perinatally to polychlorinatedbiphenyls (PCBs). Perinatal PCB exposure did not have an effect on limb movement, however, it did cause delays in direction and head angle aspects of swimming development.

To determine the effects of experimentally induced maternal phenylketonuria on the behavior of rat pups, Hanulak and Hull (1987) combined parts of the Schapiro et al. (1970) and Adams (1982) swim coding schemes into a new 3-dimensional scheme. Using head angle (0 - head below surface, 1 - top of head at surface but nose below it, 2 - nose above, 3 - ears half out, and 4 - ears out of water), direction (0 - sinking, 1 - floating, 2 - circling and 3 - swimming straight) and front paw movement suppression (0 - no movement to 4 - vigorous movement), they found that pups born to alpha-methylphenylalnine and phenylalanine treated mothers (experimentally induced maternal PKU) were deficient relative to the control pups in terms of head position in the water (days 10 and 12), direction of swimming (day 18) and front-paw movement suppression (day 20).

In contrast, Rice and Millan (1986) developed a detailed eleven stage scale (0 - pup sinks with either no movement or uncoordinated limb movement, 1 - first sinks then orients to a vertical position, slow uncoordinated limb movements, nose bobs above surface, 2 - body orientation at a 30-45° angle under the surface with nostrils and head below surface, all extremities involved in slow uncoordinated movements and swim may be straight or alternated with semi-circular motions, 3 - horizontal body orientation, nostril position alternates between above and below surface, head and ears below surface, more consistent and continuous limb movements, straight line swim, 4

- horizontal body crientation, nostril above surface, ears submerged, forward movement, staggering effect due to uncoordinated movement of all limbs, 5 - horizontal body orientation below water surface, nostrils above surface, ears submerged, all four limbs used except periodically forelimbs held still under mandible, semi-circular or straight movement dependent on limb use, 6 - body horizontal with the dorsal side of the rump above the surface, semi-coordinated limb movements, forelimbs immobile, circular movements due to more effective movements of one of the hindlimbs, 7 - body below water surface, head moves up and down but nostrils above surface, surface of water alternates between top and midline of ears, forelimbs immobile, mostly straight line swimming but some circling due to one hindlimb being more effective at swimming movements than the other, 8 - body crientation changes from horizontal below the surface of the water to horizontal with all but mid to upper back of the dorsal side above the surface, head position alternates from midlevel to bottom of the ears above the surface, hindlimbs coordinated, forelimbs immobile, straight line swimming pattern, 9 - body orientation alternates as in stage eight, head and ears above the surface, coordinated hindlimb paddling, immobile forelimbs and straight line swimming movement, 10 - same as stage eight except now the entire dorsal side of the body is above the surface) to test Swiss Webster mice perinatally treated with methimazole (an antithyroid agent). Methimazole exposed mice exhibited developmental delays in swimming ontogeny, not reaching stage ten until postnatal day twenty, whereas the controls reached stage ten by day eighteen.

Although the scales listed above provide a semi-quantitative picture of swimming behavior, they lack both sophistication and resolution. The combination of several qualitative measures such as head angle, swim

direction, and limb usage, does not reflect or represent a true quantitative scale. These types of ordinal scales can be used more effectively when the differences between groups are massive. However, when distinctions between groups are subtle, the published scales are often not fine-grained enough to enable the detection of essential differences. Simply increasing the number of categories in the scale is obviously not sufficient to provide a systematic quantitative assessment of behavior. Thus, even though Rice and Millan (1986) used 11 categories in their scale, the result is semi-quantitative at best. Therefore, it is critical that more refined quantitative measures are used in combination with these types of ordinal scales.

An additional problem with many of the above studies is that researchers frequently record in real time without objective recordings when using these scales. In fact, in only a few of the studies reviewed above is there a mention of video recording of the swimming behavior, and often there is no reference to the number of people coding the behavior, an exception being Ryan and Pappas (1985). It may be argued that recording in real time is not problematic as the scales are basically ordinal in nature. However, without a video tape record one can never be sure of the role of experimental error, especially when only one person codes the data, under those conditions. The situation is compounded when the scale choices are subjective and have numerous categories.

Finally, although the criteria in many of the studies are similar (head position/angle, swim direction, limb usage) there is a serious lack of uniformity in the scales used to measure swimming. Many researchers develop their own scales using slightly different levels within a criterion and somewhat different interpretations of same or similar scales. As many of the scales are not described by their developers in enough detail to allow others to adopt the

same original version, many adapt or modify the original scales. In this way the interpretation of swimming behavior, which is coded as numerical values, is very dependent on the specific categories used to evaluate it by different researchers. This makes replication and cross study interpretation of results very difficult. With more quantitative measures, for instance the time it takes for the animal to move the forelimb one swim stroke or the speed of the movement is less vulnerable to subjective interpretation. These sorts of quantitative measures, made possible with videotape recordings provide a more objective set of swimming measures than the more qualitative ones used in the majority of the above studies.

Interlimb Coordination Schemes

As is evident in the research described in the previous section, body position, style of movement and paddling criteria have proven useful for detecting the effects of a variety of factors, including dietary regimes, drugs, and environmental teratogens on swimming ontogeny. However, these measures do not examine detailed movement properties of the swim. For instance, that a rodent may be able to hold its head above the water surface and paddle using only the hindlimbs does not convey specific kinematic information about the type of movements they are making, nor does it measure the coordination between the limbs in use. One can easily tell, from the literature reviewed, that the effects from a variety of agents are often similar, as measured using these crude scales, especially in the case of developmental retardation of forelimb immobility. It becomes difficult, if not impossible, to ascertain specific effects that may apply to only one factor or category of factors. Movement coordination and timing measures are more

sensitive, and hence may pick up detailed information about very subtle differences in movement organization.

It has been well documented that the number and effectiveness of the limbs involved in swimming change with age (e.g., Schapiro, et al., 1970). Fentress (1972) provided the first detailed descriptions of mouse limb coupling during swimming. During the first postnatal week C57 mice tend to use all four limbs, although the coupling between legs tends to be very loose. Often one of the hindlimbs remains motionless while the other three carry out swimming movements. It is evident that the use of the hindlimbs not only becomes more prevalent with age but that the frequency of these movements changes over time, starting with one hindlimb movement every two-thirds second on postnatal day one and steadily advancing to one every one-seventh second on day twenty-two (adult level). Limb coupling also increases over the first ten postnatal days, after which time the forelimbs display the characteristic immobility evident in the more mature swimming patterns. As summarized by Fentress (1972), these changes in the limb coordination and participation during swimming illustrate "... both increased coupling of individual movements and increased differentiation of movements during ontogeny" (p. 122).

Over the past twenty years only a few researchers have produced detailed interlimb coordination and/or kinematic analyses of rodent swimming development. Bekoff and Trainer (1979) examined swimming ontogeny in rat pups, developing a coding system by which interlimb coordination could be calculated. They calculated stroke-cycle durations and latencies (the time of onset of a stroke-cycle in one limb to the onset of the subsequent stroke-cycle in a second limb), and then based on the duration and latency measures they calculated phases for each of the limb pairs. In this way they would ascertain if any particular pair of limbs was moving in phase or out of phase. Their

findings indicate that interlimb coordination begins early in the postnatal period, with limbs on the same side of the body (ipsilateral), the forelimb pair (front bilateral) and the hindlimb pair (hind bilateral) moving out of phase (limbs alternate, phase value = 0.5) in relation to each other. Bekoff and Trainer suggest that this early development indicates that the underlying neural circuitry develops prenatally. They also report a gradual decrease in stroke-cycle duration over the first two postnatal weeks, which may reflect maturations in sensory input. However, as will be discussed in more detail in later sections of this thesis, it is possible that the coordination results, as indicated in their reported phase values, are flawed due to an error in mathematical logic.

In a comparative analysis of limb phase calculations and EMG recordings Cazalets, Menard, Cremieux and Clarc (1990) examined the development of swimming in rat pups. Measuring swim behavior on postnatal days three, eight and fifteen they demonstrated phase values of 0.5 for forelimbs, right forelimb and hindlimb, and hindlimbs, indicating that for each of these pairs the two limbs involved are moving as would be expected in the movement of a normal quadruped. They were also able to establish the same pattern of alternating activity in the EMGs of related muscle groups. In agreement with Bekoff and Trainer (1979) they did not see any improvement in forelimb coupling with age. However, in contrast to the findings of Bekoff and Trainer (1979) they did discover an increase in the degree of linkage in the hindlimbs.

Walton, Lieberman, Llinas, Begin and Llinas (1992) recently used free-style swimming in combination with terrestrial locomotion to establish critical periods in motor development. They manipulated the motor environment of the animals by suspending them by the tail so that the hindlimbs could not make physical contact with the ground, (i.e. could not bear any weight). This

measure of hindlimb unloading was a non-invasive means of examining the effects of motor environment manipulation. Through this method they established a critical period in motor development between postnatal days 8-13, in which suspended rats had poor to nonexistent swimming ability as measured by hindlimb stroke duration. Either before or after that time suspension had little effect.

Recently, using a combination of Bekoff and Trainer's (1979) duration, latency and phase system and the Schapiro et al. (1970) limb paddling coding scheme Bolivar, Manley and Fentress (1996) have provided the first multiple measure study of swimming ontogeny in the neurological mutant weaver mouse. Weaver mutants displayed a lag in the development of limb use relative to control animals and a generalized slowness of limb movement (as measured by the duration of the stroke-cycle during the swim) which correlated with the onset of the use of a particular limb in the swim. However, despite early postnatal differences (some as early as day three), as ataxia became evident in these mice, during terrestrial locomotion, swimming ability did not continue to degrade. This indicates that the swimming assay is tapping into a slightly different set of motor skills than are measured by terrestrial locomotion.

Research Objectives

It is evident from the studies reviewed above that neurological mutant mice have been used to elucidate the relationships between genetics, biochemistry, neuroanatomy and behavior, although this is possible only through the implementation of detailed behavioral analyses. Unfortunately, most researchers who have behaviorally examined neurological mutant mice have

used measures which, while providing information on the ability to perform motor actions, rarely provide a measure of how motor actions are actually performed. For instance, measuring the latency until falling from a rotorod, wooden beam or rotating grid does not detail specific actions necessary to maintain balance (Lalonde, Bensoula, & Filali, 1995; Lalonde, Filali, Bensoula, & Lestienne, 1996). Therefore, studies are needed which examine a particular behavior using several complementary measures. This in turn will provide a more comprehensive explanation of the behavior, which will enable richer connections to neuroanatomical structure.

A second issue seldom addressed by researchers examining behavior in neurological mutants is that of developmental timetables. Most of the existing research has been conducted on adult mice. By adulthood the symptoms of the disorder are often quite obvious, but their origins and progressions remain elusive. Researchers in biochemistry and neuroanatomy have been able to trace the developmental progression of the neurological anomalies of these animals. It is imperative for those wanting to connect behavior to neurological mutations to trace the development of the behavioral effects. As one starts to investigate the developmental progression, of either the assembly or disassembly of a behavior, detailed analyses of the behavior become even more critical to capture subtle developmental changes (Bolivar, Danilchuk & Fentress, 1996; Bolivar, Manley & Fentress, 1996).

This thesis research was designed to: (i) provide qualitative and quantitative assessments of motor development in the neurological mutant jimpy mouse in an attempt to document the assembly of movement patterns during ontogeny and (ii) describe any alterations of these patterns as a function of developmentally based central nervous system myelin deficiency.

With these goals in mind, it was necessary to devise techniques for the quantitative examination of the ontogeny of motor coordination during a rhythmic movement (i.e., rodent swimming) using three complementary levels of analyses, which are: (i) documenting the development of limb use - swim style, (ii) determining limb stroke-cycle profiles for individual limbs - duration, missed strokes and velocity, and iii) determining interlimb coordination - latencies and phases. There are two main categories of objectives and hypothesis in this research: i) those directly related to developmental issues independent of genotype , and ii) those directly related to genotype.

General Developmental Hypotheses

The first objective was to examine the development of limb usage during swimming. In our research with the weaver mouse, the emergence of different swimming styles became evident across early development (Bolivar, Manley & Fentress, 1996). It was hypothesized that the same progression of swimming styles - forelimb to four limbs to hindlimb - would be evident in all mice in this study.

The second objective was to determine the developmental patterns of stroke-cycle durations, strokes missed by limbs and limb velocities. It was hypothesized that different developmental patterns would emerge for forelimbs and hindlimbs and these patterns would be influenced by transitions between swim styles.

The third objective was to investigate the effects of age on interlimb coordination. It was hypothesized that mice would become more proficient at coordinating the movements of their limbs (measured by phase relations) as a function of age.

The fourth objective was to determine the degree of consistency of individual animals' scores across the 10 stroke-cycle swimming session and if this consistency varied with age. As Cazalets et al. (1990) found an improvement in hindlimb cycle stability with age in rat pups, it is possible that the same type of developmental trend may exist in this strain of mouse.

The fifth objective was more general in scope. As this study makes use of multiple measures of swimming behavior, it was expected that these measures, when taken together, would provide a more complete and integrated description of age related changes in swimming behavior. It was hypothesized that different measures of swimming behavior would produce different types of developmental curves, and that these curves would in some ways be interrelated and other ways be independent.

Genotype Related Hypotheses

The first objective was to examine the developmental differences between central nervous system myelin deficient jimpy mice and littermate controls in the utilization of the four limbs during swimming. The development of limb use in normal and jimpy mice was compared during the first twenty one days of life. Since jimpy mice display dysmyelination of the entire central nervous system, and develop tremours in the hindquarters starting around postnatal days 11-13, then one would expect that neural transmission, along the length of the spinal cord, would be affected especially after the onset of tremours. As a result difficulties in achieving more advanced swimming styles, involving the hindlimbs, were predicted. It was hypothesized that jimpy mice would show a developmental delay in the initiation of the increasingly more advanced swimming styles relative to the controls.

The second objective was to examine stroke-cycle durations, strokes missed by limbs and the velocity at which limbs moved during the stroke-cycle, over development. It was hypothesized that jimpy mice would show longer stroke-cycle durations for the hindlimbs after the onset of tremours. The number of strokes missed by the hindlimbs of jimpy mice was expected to be higher than control littermates after the onset of tremours. The limb velocities of jimpy mice, measured only in the right fore- and hindlimbs, were expected to be less in the hindlimb only, after the onset of tremours, thus mirroring the hypothesized duration results. The distinction between mutant and control animals was expected to increase with age primarily because the normal development of increased communication within and between central nervous system circuits would be disordered in the mutant animals.

The third objective was to document the development of interlimb coordination (e.g., latencies and phases between limb strokes) during swimming for both jimpy and littermate control mice. It was hypothesized that jimpy mice would show less coordination between pairs of limbs than their normal littermates, in particular the ipsilateral (right forelimb and right hindlimb), contralateral (right forelimb and left hindlimb) and bilateral hindlimb pairs. It was not expected that coordination in the bilateral forelimb pair would be affected by the jimpy mutation, since the forelimbs drop out of use before the onset of tremours.

The fourth objective was to examine the degree of consistency within individual animal's scores for both jimpy and littermate control groups. It was expected that jimpy mutants would display a tendency for less consistency in duration and phase scores throughout the 10 stroke-cycle swimming session than would littermate controls.

METHOD

Subjects

Originally 32 *jp*/Y and 61 control mouse pups from 25 litters were tested. These pups were the offspring of B6CBACa-$A^{w\text{-}J}$/A-*Ta jp* females and B6CBACa-$A^{w\text{-}J}$/*A* males obtained from Jackson Laboratories (Bar Harbor, Maine). As the jimpy mutation is maintained on a hybrid background, there is a degree of heterogeneity within offspring of any particular litter as evident in slight differences in coat color. Each litter and its parents were housed in a clear polypropylene cage (27.5 x 15.5 x 13.0 cm) with a stainless steel wire lid. Purina Rodent Laboratory Chow and tap water were provided *ad libitum* and the colony maintained on a 12:12 h light: dark cycle (lights on at 12:00 p.m.) in a single housing room controlled for temperature (21° C ± 2) and humidity (50% ± 5).

Due to the sex-linked nature of the mutation, only male pups were used in the study. Consequently at postnatal day two (day of birth = day 0) litters were culled to include male pups only, in order to maximize the chances of survival of the mutant animals. Litter size varied from three to six male pups (two litters of six, four litters of five, seven litters of four and 12 litters of three). Mutant animals were later identified by the presence of hindlimb tremours (onset 11-13 days of age) and tonic-clonic seizures (onset 20-24 days of age). However, one litter contained only *jp*/Y pups and four litters contained only control pups. These litters were excluded from the statistical analyses. This resulted in 20 litters (two litters of six, three litters of five pups, six litters of four pups and nine litters of three pups) being used for initial data analyses.

All male mice within a litter were tested. It was necessary to test all the mice because prior to the onset of full phenotypic expression of the mutant proteolipid protein gene, one could not be certain of whether or not a mouse was a jimpy. However, for data analyses only one control and one mutant were randomly selected from any one litter (a total of 40 mice). This measure was taken to ensure that no one litter was over represented in the results. It has long been argued in both prenatal and postnatal research that taking multiple animals from the same litter may have detrimental effects on any conclusions drawn from the data. This is due to a reduction in within-group variance. The argument is that animals within a litter are more alike than animals from different litters which violates the homogeneity of variance assumption inherent in many statistical procedures. Litter effects have been reported for behavioral variables such as odor-footshock conditioning, orienting to an auditory stimulus and trials to criterion in a water maze task, as well as physiological variables such as body weight and baseline body temperature (McKinzie, Roepe & Spear, 1993; Myers, 1991). Abbey and Howard (1973) have argued that any time multiple animals from the same litter are used in an analysis one must take into account that a correlation may be present among the measurements of animals in a given litter and modify the statistical calculations accordingly. Other non-statistical ways to address the litter issue include using only one animal from a given litter in each of the treatment groups (as used in this study) or by using the litter as the unit of analysis (i.e., collapsing all the animals from a litter into one score).

Apparatus

A color video camera (Panasonic Digital 5100) fitted with a high resolution lens (Panasonic WVLZ1415 15X zoom lens) with the shutter speed set at 1/2000 second was used for videotaping. The video camera was connected to a VCR (Panasonic AG7400 S-VHS) through a time-code generator (SMPTE). The time code generator sequentially records unique frame numbers, on the audio track of the tape, for each frame of recorded video. The electronically recorded tag is used by the PEAK system to identify and capture any frame of video that is selected by the user. In addition the frame number is recorded on the tapes so that it can be visualized during playback. Video recordings (30 fps) were made on Super-VHS (S-VHS) tapes. A VCR (Panasonic AG7300 S-VHS) with full shuttle/jog control over tape playback (in both forward and reverse) was used during frame-by-frame analysis of the tapes. Videotaping was done on Super-VHS (S-VHS) tape to provide high resolution images for analysis of movement, with the PEAK 2D Video/Computer Motion Measurement System from PEAK Performance Technologies, Inc., (Englewood, Colorado). This movement system has been used in non-human studies to examine the development of locomotion in postnatal rats (Walton et al., 1992), locomotion in cockroaches (Full & Tu, 1990), chick embryo movements (Chambers, Bradley & Orosz., 1995) and reaching and grasping movements in rats (Whishaw & Gorny, 1994; Whishaw et al., 1994; Whishaw, Pellis & Gorny, 1992a, 1992b).

The Peak system digitally reconstructed each individual frame's two component pictures (resulting in 60 pictures per second). The system captured analog images from the videotape (playback on a Panasonic AG7300 S-VHS VCR) and imported them into a frame grabber board where

the two pictures in each frame were split and digitally reconstructed. The digital picture was displayed on a video monitor (Sony Trinitron, Model # PVM-1341) along with a cross-hair cursor which was controlled by a computer mouse. Due to an inability to place markers on limb points of mice submerged in water, each picture had to be manually digitized (approximately 75,000 points in total). The points digitized included the end points of limbs (i.e., paw) facing the camera (i.e., right side of the body). Using the computer mouse, the points were selected and stored as "X-Y" coordinates in a data file. The data were scaled and then filtered using a fourth order, zero-lag Butterworth digital filter. An optimal cutoff value, determined by the program, was used for the filtering process, based on the frequency of particular limb movements. The software was then used to calculate velocities for any given point.

Behavioral Testing

Mice were videotaped every second day from postnatal days 3 to 21. To begin the testing procedure, a pup was removed from the home cage and weighed. During testing individual mice were suspended mid-thoracically by a 1 cm wide rubber band, which was kept taut by having one end of the band pulled through a 10 cm straw with a string. The mice were lowered into a partitioned aquarium with a swimming channel 40 cm in length, 6 cm in width and 25 cm in depth. They were allowed to swim for a maximum of 30 seconds. The young mice were thereby prevented from dropping beneath the water surface and at the same time given the freedom to make swimming movements. In addition, they maintained a relatively fixed position with respect to the camera which facilitated videotaping and later kinematic analysis. The presence of the rubber band around the mouse's midsection

should not interfere with swimming performance as previous research did not reveal any changes in swimming patterns when young mice were suspended by a harness (Cazalets et al., 1990). Water temperature was maintained at 38 ± 2° C by a 50 watt aquarium heater (Hagen). At the end of the session, the pup was marked on the tail with non-toxic markers of various colors for identification and returned to its home cage. Pups were tested in random order on each day.

Swimming Style Coding Analysis

It has been well documented that throughout the course of early postnatal development rodents such as mice and rats display differential patterns of limb use during swimming (see the introduction for a review). Involvement of the limbs proceeds in a rostral-caudal direction. Based, in part, on the coding schemes of a variety of rodent studies (e.g., Fentress, 1972; Schapiro et al., 1970; Vorhees et al., 1979) swimming style categorizations were developed based on limb paddling. During a swimming session the mouse's swim style was categorized as follows:

0 = no swim - the mouse did not make limb swimming movements at all but hung suspended from the elastic band in the water

1 = forelimb swim - the mouse used only the forelimbs during the swim session, the hindlimbs remaining limp or sticking out straight from the body; although on occasion one of the hindlimbs would move in swimming like movements, there was not complete participation of the hindlimbs

2 = four limb swim - all four limbs made swimming like movements

3 = hindlimb swim - only the hindlimbs made swimming like movements, the two forelimbs being held virtually motionless under the animal's chin.

This coding scheme is identical to that used by Bolivar, Manley and Fentress (1996) in an examination of the ontogeny of swimming behavior in weaver mutant mice.

After randomly selecting one jimpy and one control littermate from each litter, videotapes of the mice were observed at slow speed for the entire swimming period (a total of 400 swim sessions). The most advanced swimming style displayed by the animal during 10 complete stroke-cycles was used for coding purposes. It was observed that within a given swimming session individual animals often vacillated between more and less developmentally advanced styles of swimming. The most advanced swimming style was recorded to obtain a measure of maximum potential.

Measures of Individual Limb Movement

Duration

For quantitative assessment, videotapes were analyzed frame-by-frame (30 frames per second, a 10 stroke-cycle session ranging from two to five seconds). The 10 stroke-cycle swimming sessions that had been used to code swimming style were used to record interlimb coordination. Onset of the stroke-cycle was defined as the frame at which the limb is at its maximum anterior extension (Bekoff & Trainer, 1979). This is also the frame before the limb begins its downward stroke. This frame was called frame zero and the onset of the stroke in the lead or reference limb occurred at this time.

The stroke-cycle duration was defined as the time between the onset of one stroke-cycle until the onset of the next stroke-cycle (i.e., the time it takes for the limb to go from maximum anterior extension thorough the swim stroke

and back up to maximum anterior extension again). Stroke-cycle durations (approximately 3000 in total) for all of the limbs used in the swim were calculated for each of the 40 mice on each of the 10 days (postnatal days 3-21) tested in the manner originally developed by Bekoff and Trainer (1979). The frame number of the onset of the first stroke-cycle was subtracted from the frame number representing the onset of the next stroke-cycle. These differences were calculated for each pairing of the 10 stroke-cycles giving a total of 9 duration measures per limb for each day of testing. The average of the nine durations for each limb was then taken as the measure for each mouse per day. In this way a data base consisting of 36 durations could be collapsed into four average durations (one for each limb) per mouse per testing day. Finally, averages were converted from frame numbers to time, in milliseconds (msec.), by multiplying each average by 33.333 (the number of milliseconds between frames; 1 frame = 1/30 second). Initially limb durations were analyzed by collapsing together the data for the front limb pair, and the hind limb pair, so that the bilateral limb pairs were being considered as a unit. Additionally, to ensure that the limbs within the bilateral pairs were not moving differently, mean durations were calculated separately for each limb.

Measurement of Velocity

The PEAK System software calculates linear velocity, using a fourth order central difference for all pictures except the first two and last two. All manual digitizations were done so that the points of interest would not be either the first or last two pictures. The formula used to calculate a velocity for each picture is:

$$V(i) = (D(i-2)-8*D(i-1)+8*D(i+1)-D(i+2))/(12*Timediff)$$

where V = velocity

i = picture number

D = smoothed data value

Timediff = the time difference between pictures in seconds

Mean velocities for the whole 10 stroke-cycle swim session were calculated by the software. Mean maximum velocities were determined by extracting the value for the highest velocity attained, during the initial thrust phase of each of the 10 stroke-cycles, and then averaging to arrive at a mean maximum velocity for each mouse in each swim session.

Missed Strokes

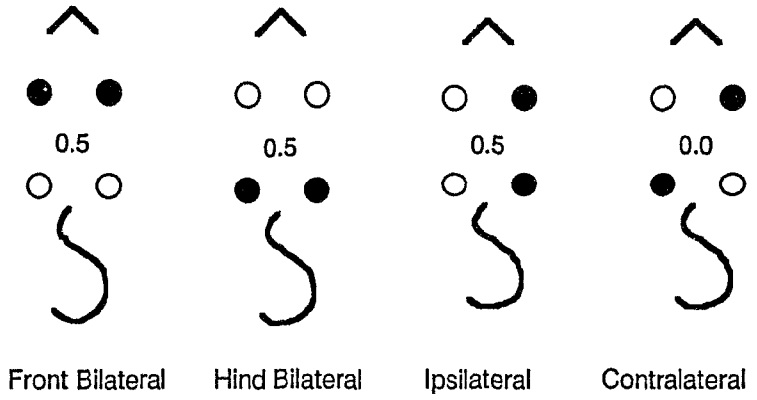
On occasion, control and *jp*/Y mice would miss one or more stroke-cycles for one of the limbs. This happens when during the time that the other limb(s) make a complete stroke a particular limb has not completed its stroke. In this way the number of missing strokes from a 10 stroke-cycle was counted for each limb separately. As in the duration analysis, the bilateral limb pairs were initially considered as a unit, and then examined as individual limbs.

Measures of Interlimb Coordination

Before outlining the interlimb coordination measures it is important to clarify the terminology used throughout the thesis. Occasionally terms for specific limb pairs vary in the literature. The term ipsilateral is used to identify comparisons of pairs of limbs on the same side of the body, whereas contralateral is used to identify a forelimb and hindlimb pairing from opposite

sides of the body. Finally, although the term homologous has been used to identify the forelimb and hindlimb pairs (Bekoff & Trainer, 1979), in this thesis these pairs will be called bilateral, with specific reference to whether they involve the forelimbs or hindlimbs (e.g., the bilateral forelimb pair).

To assist in interpreting the latency and phase figures a schematic diagram of a mouse is included at the bottom of each figure indicating the limbs which are being compared. In the case of the phase graphs the diagram includes the ideal phase value for the particular limb pair. These diagrams are presented below for reference.



Latency

Stroke latency was defined as the time between the onset of the stroke-cycle of the lead, or reference limb, and the onset of the stroke-cycle of a second limb. Once again this is the basic formula used by Bekoff and Trainer (1979) in their research on limb movement during swimming in rats. In all swim types other than hindlimb only, the lead or reference limb was the right forelimb; in the case of the hindlimb only swim it was the right hindlimb. Latencies were calculated for the forelimb pair, hindlimb pair, one contralateral

pair (the right forelimb and left hindlimb) and one ipsilateral pair (right forelimb and right hindlimb).

Phase

Phase relations have been calculated in two distinct ways in this thesis. First, they are calculated using the method described by Bekoff and Trainer (1979). Phase was calculated by dividing the latency of a specific limb, relative to the lead limb, by the duration of the lead limb in the corresponding stroke-cycle in the manner of Bekoff and Trainer (1979). Values were calculated for the same pairs as described for the latency measure. In all of the pairings, except that of the contralateral, perfect coordination between the two limbs during the swim is represented by a phase of 0.5 as these two limbs should alternate during the swim. In the case of the contralateral forelimb-hindlimb pair the phase value should approach 0.0 as these two limbs should begin strokes cycles at the same time.

This method allows phase values to range from 0.0 to 1.0, with out-of-phase equaling 0.5 and in-phase equaling either 0.0 or 1.0. This coding system is based on the assumption that one limb must always serve as the lead limb with the other limb always following the first. However, there is a serious limitation with this assumption, if indeed the limbs are switching during the swim session and the original lead limb becomes the follower. For instance if the lead limb begins a stroke-cycle and the latency before the second limb begins its stroke-cycle is short there will likely be a low phase relation between the two limbs; i.e., the two limbs will have a phase close to 0.0 and be in-phase. If instead the second limb does not start its stroke-cycle until about halfway through the stroke-cycle of the first limb then the phase

value will approach 0.5 and be classified as out-of-phase. However, if the second limb does not begin its stroke-cycle until almost the point at which the first limb begins a new stroke-cycle then according to the Bekoff and Trainer system the limbs will be considered in-phase and be assigned a phase value close to 1.0. In this way there are two types of situations that can lead to in-phase relations and one that can lead to out-of-phase, with the other values falling somewhere between the extremes.

If there are several phase values for a particular animal that are averaged together to give a mean phase for the swim session a potential problem arises. An animal may have some phase values close to 1.0 and some values close to 0.0, and would subsequently be considered to have the limbs moving in-phase, but when averaged together using this formula the opposite would result. The average may be closer to 0.5 implying that the limbs were out-of-phase. In fact, if the phase values were totally random on a scale from 0.0 to 1.0, the average would be 0.5 indicating that chance alone could provide a perfect out-of-phase relation between two limbs.

Alternatively a corrected method of phase calculation was developed based on the recognition that the Bekoff and Trainer (1979) method is based on a flaw in mathematical logic. Bekoff and Trainer's (1979) individual phase values were corrected by subtracting all of those phase values over 0.5 from 1.0 to provide a range from 0.0 to 0.5. Rather than being symmetrical around 0.5 the corrected formula yields a range from 0.0 to 0.5 thus avoiding errors that infer a phase of 0.5 when in fact that is not the case. In this method the phase value as a degree of relatedness is independent of which limb is serving as the lead limb and differs in that fundamental way from the calculations of Bekoff and Trainer (1979).

Measures of Consistency

Each of the measures listed above is based on a mean score for each animal collapsed over the 10 stroke-cycle session on any given testing day. To ascertain the degree of consistency for individual animals over the 10 stroke-cycle session the degree of variation of the within session scores was calculated for each animal for each testing day. Average degree of variation ratings were then calculated for both jimpy and littermate control groups.

In the case of stroke-cycle durations the coefficient of variation was calculated for the 10 stroke-cycles of each animal (cf. Sokal and Rohlf, 1981) To determine the amount of variability across the 10 stroke-cycle session for phase relations (corrected method only) the standard deviation was calculated for each 10 stroke-cycle session. Average standard deviations for each of the two groups were then calculated for each testing day.

Statistical Analyses

Jp/Y and control groups were compared in terms of general swimming style and body weight using a 2 X 10 within subjects ANOVA with genotype (*jp*/Y or control) and age (postnatal days 3-21) as the within-subjects variables. All 20 litters were used in the analyses. Tukey's HSD post-hoc tests were used to analyze significant ANOVA results further.

Stroke-cycle durations for all four limbs, number of missed strokes for all four limbs, mean velocity and mean maximum for the ipsilateral limb pairs, stroke latencies of bilateral pairs of limbs (forelimb and hindlimb pairs), phase values of bilateral limb pairs, and consistency measures for the stroke-cycle durations and bilateral limb pair phases were analyzed via within-subjects ANOVAs using reduced sample sizes. Examining the data it became evident that on specific days one set of limbs (forelimbs or hindlimbs) was not used by

many of the mice, thereby creating days with missing data. Although an 2 X 10 within-subjects ANOVA would be the preferred statistical method to include all the postnatal days tested, this was not possible due to the days with missing data. Therefore, a reduced ANOVA was used, the levels of which depended on the measure. For example on measures involving the forelimbs only, data were included from postnatal days 3-11 in the ANOVA. In the case of hindlimb only swim the data included postnatal days 9 -21.

To allow use of any type of ANOVA it was necessary first to eliminate five litters from the analyses based on limb usage during the early postnatal period. Pups who did not use their forelimbs during postnatal days three, five and seven were eliminated from the duration, latency, phase, missed strokes and velocity analyses. Each time a mutant or control animal was eliminated its littermate was also removed from the other group, to prevent uneven representation from a litter and to allow pairing for within-subjects ANOVAs. This resulted in a sample size of 15 *jp*/Y and 15 littermate controls for these ANOVAs. Tukey's HSD post-hoc tests were used to further analyze significant ANOVA results.

There were, however, still instances in which a set of limbs was not in use for the entire developmental period. For example, the hindlimbs were not used by many of the animals until day seven or nine, whereas the forelimbs stopped being used by day 13 in many cases. Therefore Mann-Whitney tests were used to compare *jp*/Y and control animals on those days in which a within-subjects ANOVA was not possible. The Mann-Whitney test was employed due to the reduced sample size resulting from the large number of instances in which individual animals did not utilize a particular limb during the swim on a given postnatal day. It was employed in lieu of a paired measure as it was often the case that one animal (either *jp*/Y or control) from a litter

might not make use of the limb although the littermate would use it, hence making littermate pairing impossible. In some cases, and in particular days three and 13, reducing the data set to include only littermate pairs would reduce the sample size to the point of making even a Mann-Whitney test questionable. Therefore, all the available data were utilized with the non-paired procedure.

In the case of stroke latency, phase values and phase consistency of the ipsilateral and contralateral pairs there were only two days (day 9 and 11) in which both forelimbs and hindlimbs were used during swimming by all the mice in the sample, thus making an ANOVA possible only on those two days, and seriously limiting the information from the statistic. Therefore, Mann-Whitney tests were used to compare *jp*/Y and control mice on each day tested. Due to repeated testing, a conservative stance was taken in these cases and only those *p* values of .01 or less were considered significant, to reduce the chances of Type 1 error.

RESULTS

Weight

As can be seen in Figure 1, there were significant main effects in weight for both genotype and age, i.e., *jp*/Y mice weighed significantly less than littermate controls, $F(1,19)=47.759$, $p<.01$, and overall, weight increased with age, $F(9,171)=436.026$, $p<.01$. There was also a significant genotype by age interaction, $F(9,171)=37.668$, $p<.01$, with the jimpy mice actually losing weight after postnatal day 17. Although there was no significant difference between the mutant and control groups on postnatal days three and five, after that time *jp*/Y pups weighed significantly less than controls. Using Tukey's post-hoc tests weight differences between the two groups first became statistically significant ($p<.01$) on postnatal day seven and continued throughout the rest of the period studied. These data present a progressive and smooth divergence between *jp*/Y and control groups across development.

Swimming Style

The progression of swimming styles for *jp*/Y and littermate control mice across development can be found in Figure 2. A change in the number of limbs involved during swimming behavior was evident for both *jp*/Y (see Figure 2A) and control mice (see Figure 2B) across the developmental period studied. Even at postnatal day three, most mice showed swimming movements, with only two *jp*/Y and four control animals failing to swim on that day. Thirteen mutants and 10 control mice used the forelimb swim style, the rest (*jp*/Y=5, control=6) used a four limb style. By postnatal day five all of the

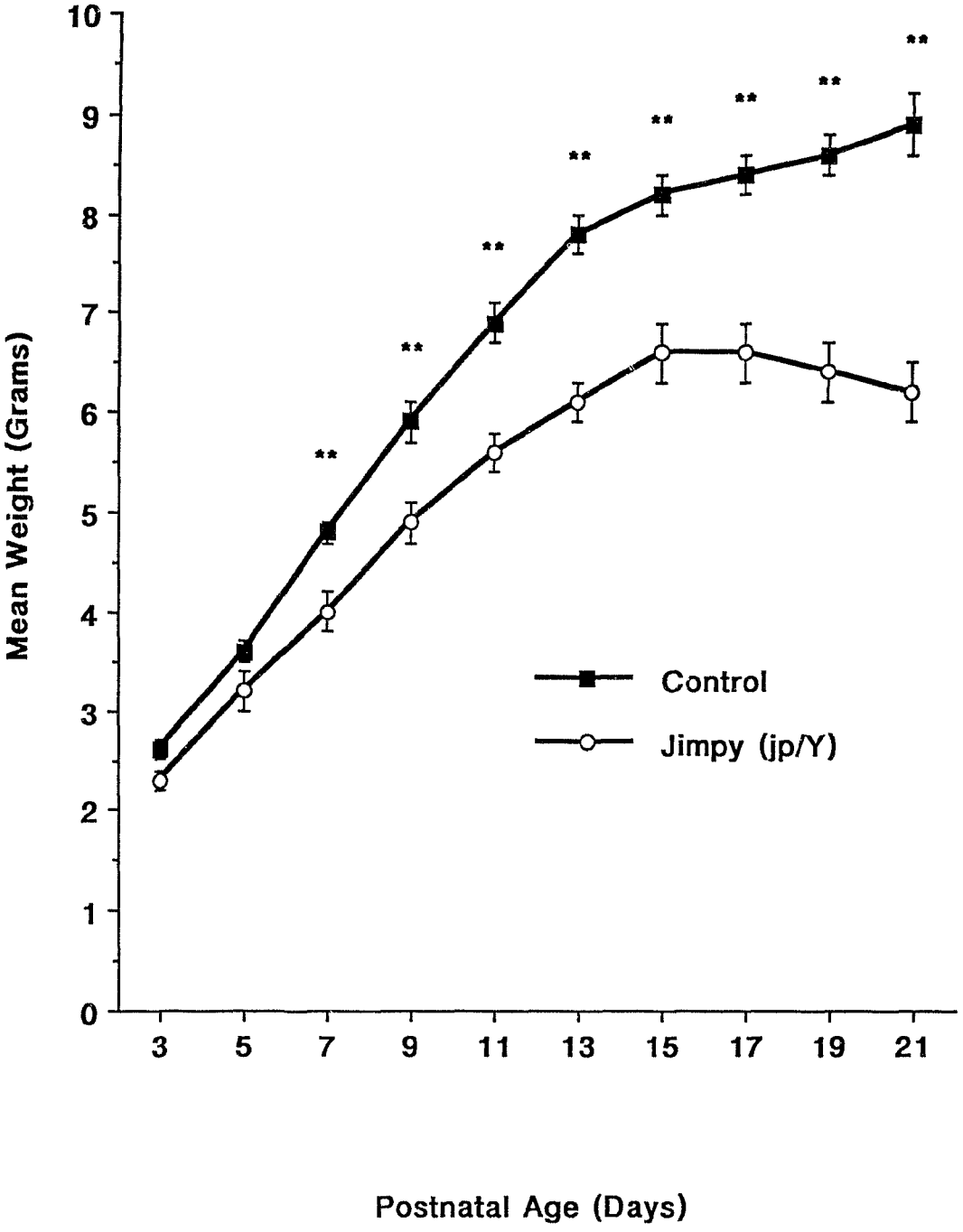


Figure 1. Mean weight (± SEM) for control and jimpy (jp/Y) mice from 3 to 21 days of age. **p≤ .01.

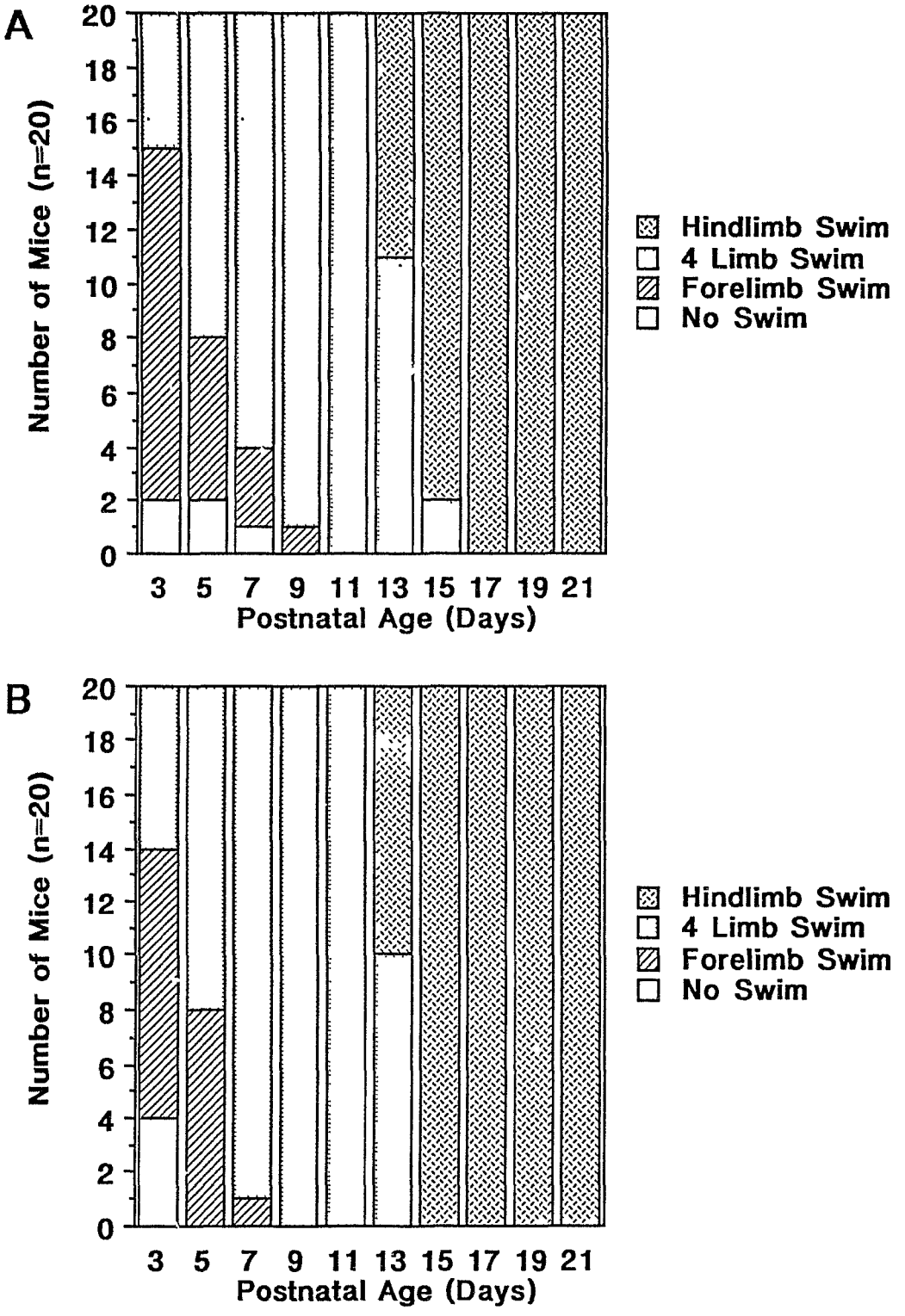


Figure 2. The progression of swimming styles of (A) jimpy (jp/Y) and (B) control mice from 3 to 21 days of age.

control and all but two of the mutant animals were swimming. Eight of the control mice and six of the mutants used the forelimb swim style, with the rest (*jp*/Y=12, control=12) using a four limb style. By the third day of testing, postnatal day seven, 19 of the control mice were using the four limb swim, whereas one animal was using the forelimb swim style. However, only 16 *jp*/Y mice were using the four limb swim, and of the remaining mice, one did not perform swimming movements and the other three used the forelimb swim style. At postnatal day nine all controls and 19 mutants were using the four limb swim style, the remaining mutant using the forelimb swim. By postnatal day 11 there was no difference between mutant and control groups, as all animals were using the four limb style. At day 13 the change to the hindlimb swim became evident, with 10 controls and 11 mutants using the four limb swim style, whereas 10 controls and 9 mutants were using the more advanced hindlimb swim style. On postnatal day 15 two mutants still used the four limb swim style, but all other mutants, as well as all the control mice, used the hindlimb style. From postnatal days 17-21 all animals of both groups used the hindlimb swim style.

If swimming styles (no swim through hindlimb swim) are ranked for relative maturity (no swim=0, forelimb swim=1, four limb swim=2 and hindlimb swim=3) the development of swimming styles in both groups becomes evident. As can be seen in Figure 3, with age the maturity of swimming style increases, although not in a linear fashion. Instead, developmental transition points emerge at specific ages as the mice go from one type of swim to the next. Early in the postnatal period (postnatal days 3-7) there is an almost linear increase in swimming maturity scores, as the mice move from either a no swim style or a forelimb style to a four limb style. However, from days 7-11 of swimming style (four limb swim) at this point in development. There is

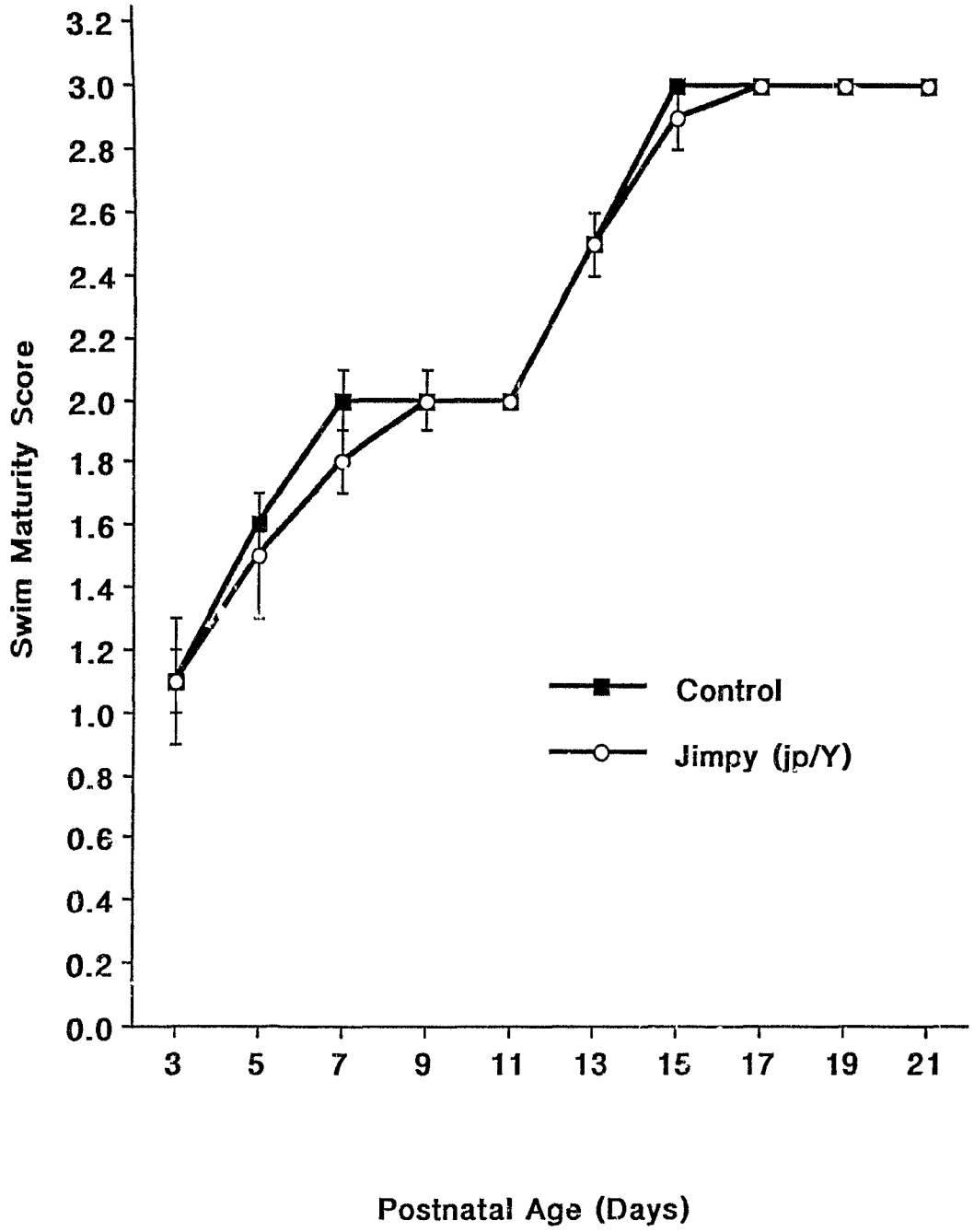


Figure 3. Mean swim maturity scores (± SEM) for control and jimpy (jp/Y) mice from 3 to 21 days of age.

another period of increased swimming scores from day 11 to day 15, again indicating a period of change in which animals are changing from one type of swim to the next (i.e., four limb to hindlimb style). Finally from postnatal day 15 until the end of testing (day 21) the scores remained similar indicating that the animals have now reached the most mature swimming style (hindlimb swim) and continue to perform at this level. An ANOVA confirmed this change in swimming maturity score as a function of age ($F(9,171)=150.040$, $p<.01$). As can also be seen in Figure 3 there was no significant main effect on swimming maturity as a function of genotype (*jp*/Y versus control), nor was there an interaction between genotype and age. Both groups of mice showed the same general pattern of transition from one type of swimming style to the next, at about the same time in postnatal development. Postnatal days 7-11 and 15-21 were marked by stable patterns of swim maturity scores, indicating a discontinuity in developmental trends. These developmental trends are similar for each genotype and clearly distinct from the developmental pattern of weight (see Figure 1).

Forelimb Stroke-Cycle Analyses

Duration

Forelimb durations were analyzed by 2 X 5 ANOVAs with genotype (*jp*/Y or control) and age (days 3 - 11) as the two within-subjects variables. Collapsing the stroke-cycle duration data from the two front limbs, an overall difference between *jp*/Y and littermate control mice became evident ($F(1,14)=6.219$, $p<.05$). Throughout the period when these limbs were in use (postnatal days 3-13) *jp*/Y mice displayed longer stroke-cycle durations for the forelimbs than did littermate controls (see Figure 4). Using Tukey's

tests there were no significant differences between the groups on individual postnatal days. However, as noted by Weinberg and Goldberg (1990) it is possible to have a significant overall *F* and not be able to obtain significant differences between individual pairs of means with this conservative test. There was no significant difference between the two groups on postnatal day 13 using the Mann-Whitney test. There was no significant interaction between genotype and age. Descriptively, the *jp*/Y group displayed longer stroke-cycle durations than the littermate controls throughout the period studied.

Also evident in Figure 4, there was a general decrease in stroke-cycle duration as a function of age ($F(4,56)=25.095$, $p < .01$), although after postnatal day eleven duration tends to increase slightly. This is the period that immediately precedes the time in which the forelimbs are dropping out of use (see Figure 2). The essentially continuous nature of stroke-cycle duration changes is in marked contrast to the discontinuous changes observed in overall swim style (see Figure 3).

Having compared duration for the front limbs as a unit, each limb was then examined separately to determine any asymmetries in stroke-cycle durations between the two limbs. Although there was a tendency for the *jp*/Y mice to display longer right forelimb stroke-cycle durations than controls, there was no significant difference between the two groups ($F(1,14)=4.117$, $p < .10$) (see Figure 5). There was also no significant difference between the two groups for postnatal day 13 using the Mann-Whitney test. There was an overall significant main effect of age, as both groups of mice showed a decrease in stroke-cycle duration as a function of age ($F(4,56)=25.847$, $p < .01$). Again, stroke-cycle duration tended to decrease with age for both groups of mice until postnatal day 13 when it showed the same type of increase as with the collapsed forelimb data.

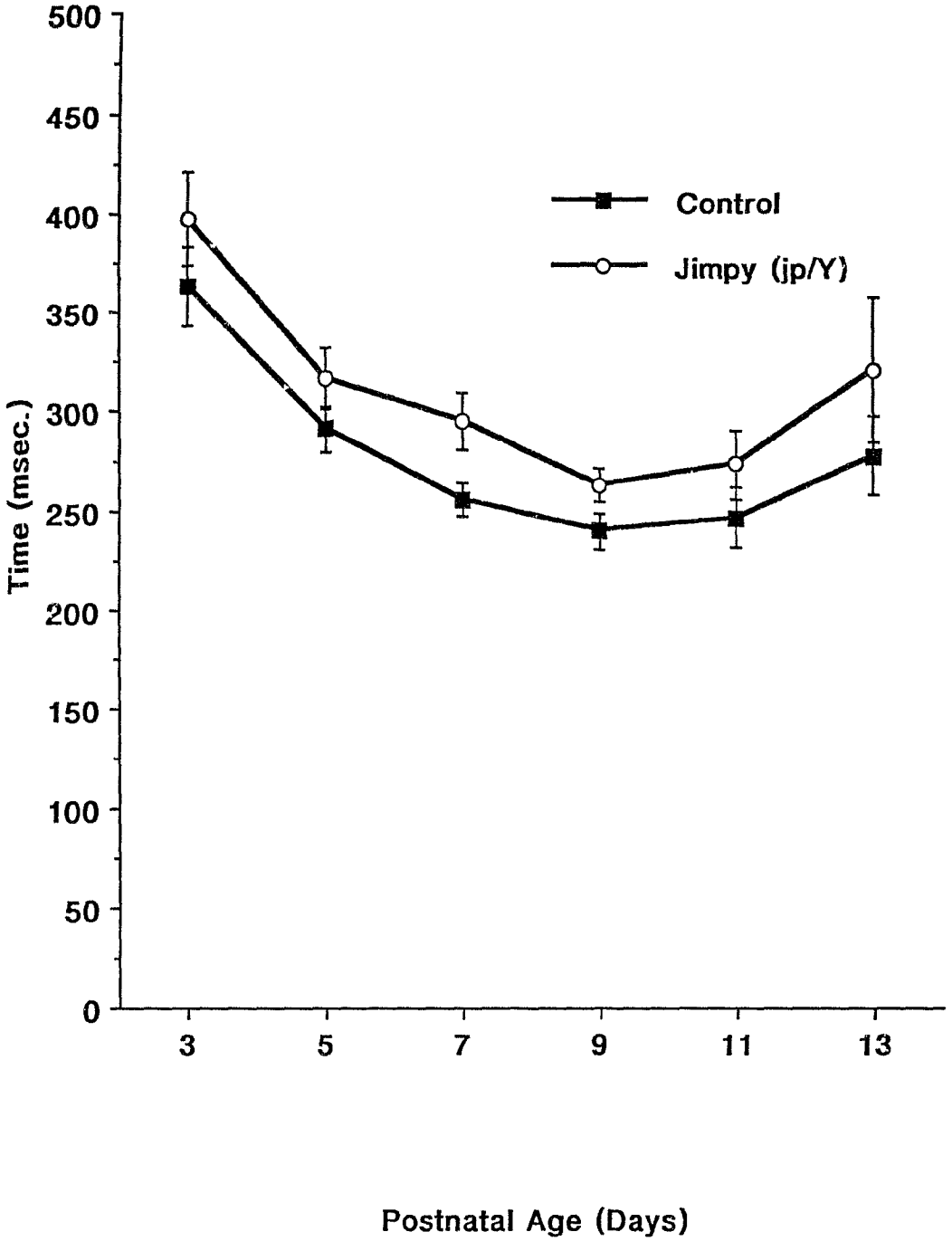


Figure 4. Mean stroke-cycle duration (± SEM) of the forelimbs for control and jimpy (jp/Y) mice from 3 to 13 days of age.

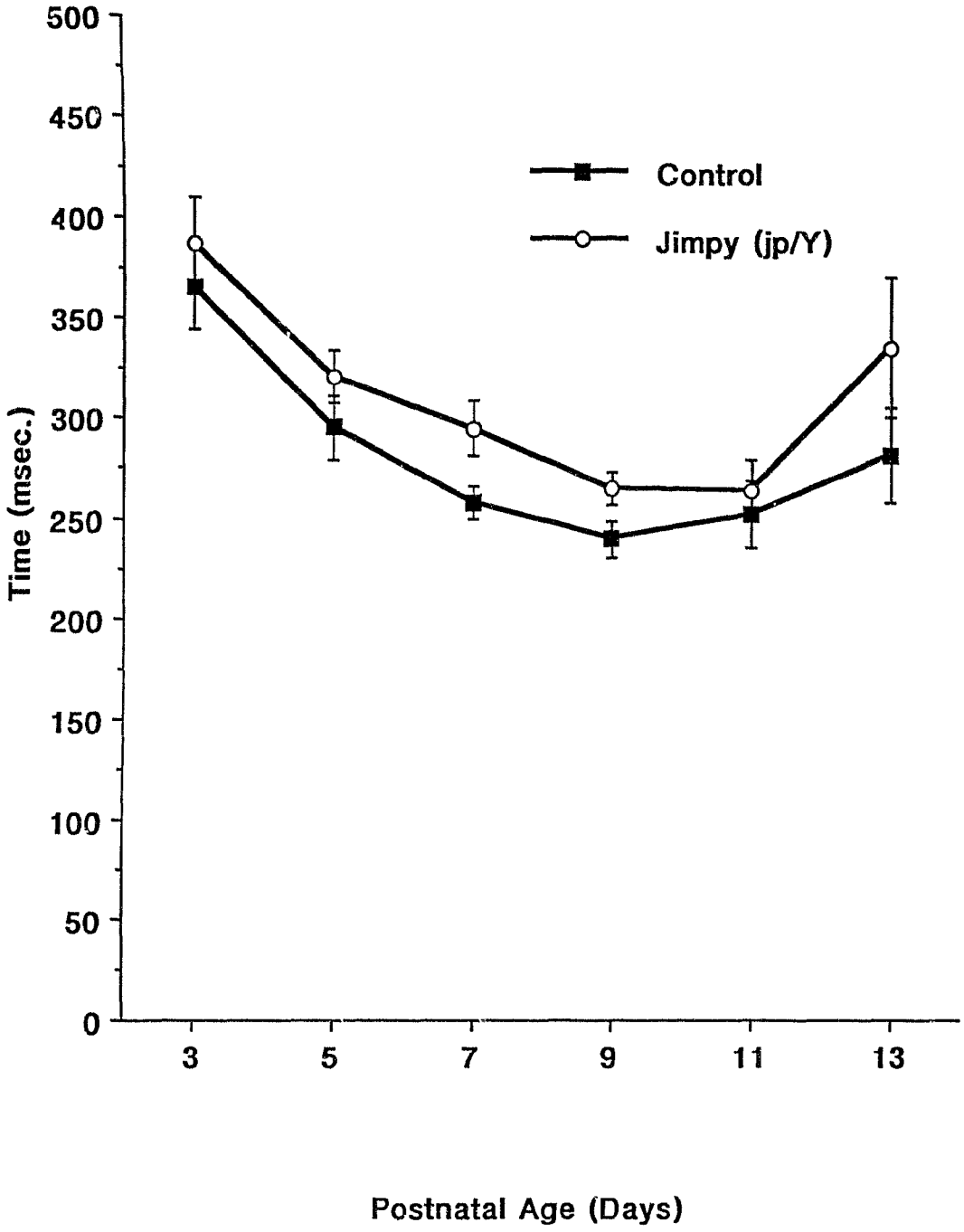


Figure 5. Mean stroke-cycle duration (± SEM) of the right forelimb for control and jimpy (jp/Y) mice from 3 to 13 days of age.

In the case of the left forelimb there is a significant difference between the two groups with *jp*/Y mice displaying longer stroke-cycle durations than controls ($F(1,14)=9.317$, $p<.01$) (see Figure 6). However, using Tukey's post-hoc tests there were no significant differences between *jp*/Y and control groups on any of the individual postnatal days. Nor was there a significant difference between the two groups on postnatal day 13 using the Mann-Whitney test. Stroke-cycle duration decreased as a function of age ($F(4,56)=17.981$, $p<.01$), and as with the right forelimb durations tended to increase as the forelimbs were dropping out of use. There was no interaction between genotype and age, as in general the *jp*/Y group had longer durations than did the littermate control group. It is noteworthy that the data for forelimb duration for the two limbs separately (Figures 5 and 6) are indistinguishable for the control mice with a slight asymmetry for the *jp*/Y group.

Right Forelimb Mean Velocity

A 2 X 5 ANOVA, with genotype (*jp*/Y or control) and age (postnatal days 3-11) as the within-subjects variables, was used to analyze mean velocity for the right forelimb. There was no significant difference in velocity of right forelimb movement between *jp*/Y and control mice (see Figure 7). There was no difference in velocity between *jp*/Y and control mice on day 13 via a Mann-Whitney test. There was a significant main effect due to age ($F(4,56)=36.214$, $p<.01$), as velocity tends to increase with age up to postnatal day 9 for the control and 11 for the *jp*/Y mice, after which it appeared to stabilize until postnatal day 13 when it decreased slightly. Furthermore, there was no

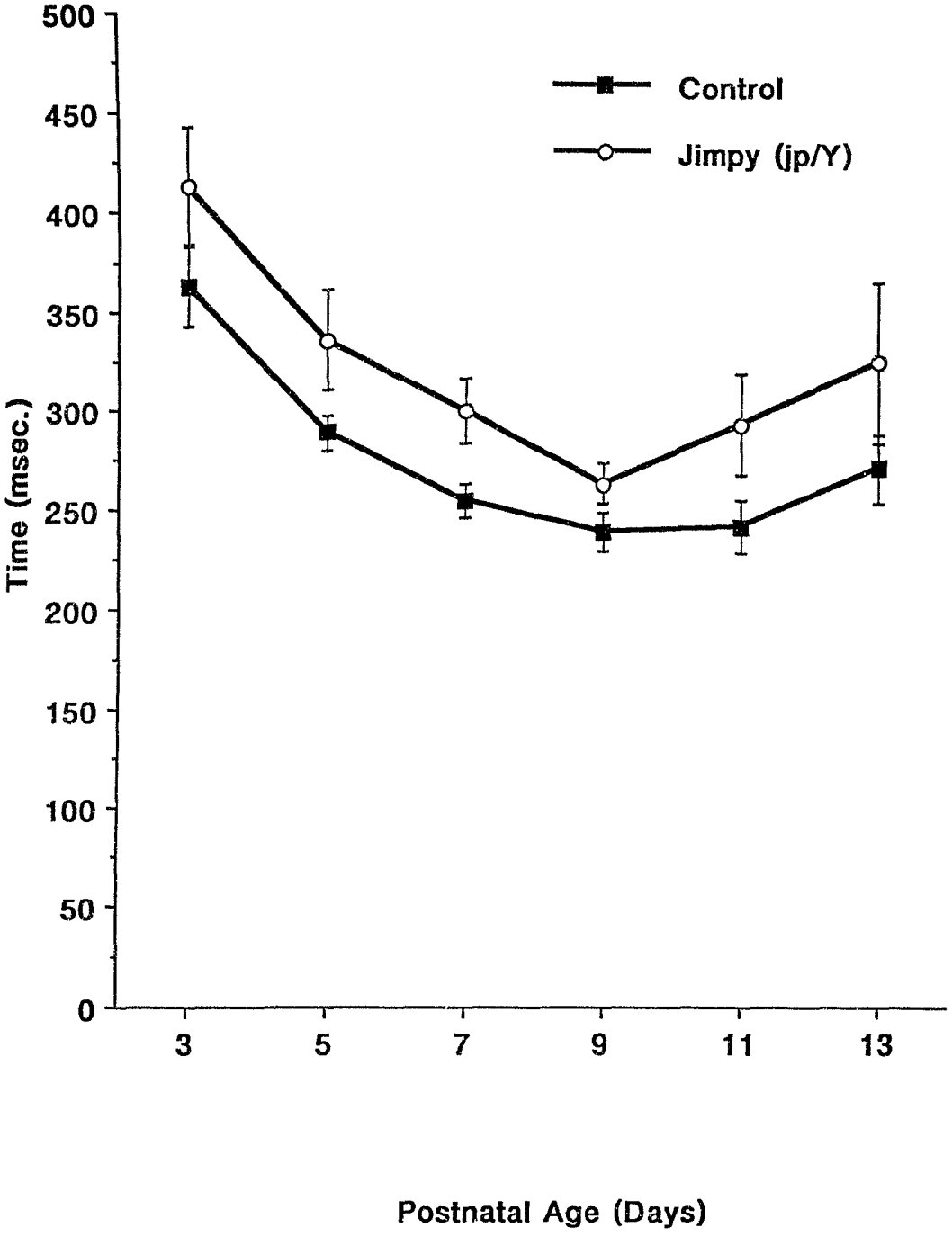


Figure 6. Mean stroke-cycle duration (± SEM) of the left forelimb for control and jimpy (jp/Y) mice from 3 to 13 days of age.

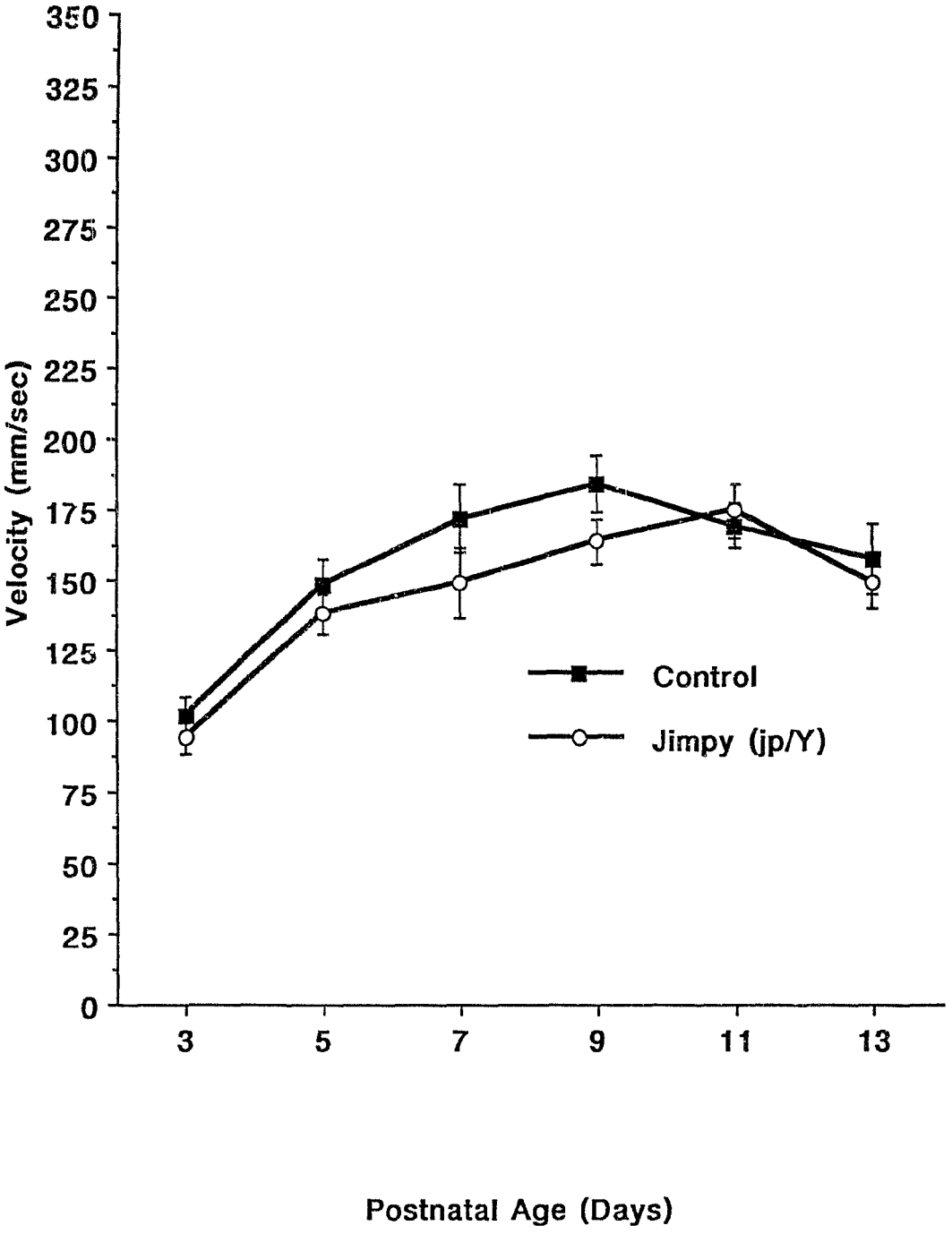


Figure 7. Mean velocity (± SEM) of the right forelimb, during the ten stroke swim session, for control and jimpy (jp/Y) mice from 3 to 13 days of age.

significant genotype by age interaction, as the age trend was very similar for both groups. Consistent with the decrease in right forelimb stroke-cycle duration (see Figure 5) mice show increased velocity with age (see Figure 7).

Right Forelimb Maximum Velocity

To evaluate maximum velocity, a 2 X 5 ANOVA with genotype (*jp*/Y or control) and age (postnatal days 3-11) as the two within-subjects variables was used. The maximum velocity data are consistently higher than mean velocities, indicating the variations in velocity that occur during a swim session. A Mann-Whitney test was used to evaluate the difference between *jp*/Y and control mice for postnatal day 13. In general *jp*/Y mice had lower maximum velocities than did controls ($F(1,14)=7.886$, $p<.05$). However, there were no differences between *jp*/Y and control groups at individual days via the Tukey's test, nor at day 13 via the Mann-Whitney test. As is evident in Figure 8, generally *jp*/Y mice tend to have lower maximum velocities than control mice, especially after postnatal day seven. There was a significant main effect of age ($F(4,56)=81.754$, $p<.01$), as for both groups maximum velocity increased with age up to postnatal day 9 when it started to decrease, with the onset of the hindlimb swim style. There was no significant interaction between genotype and age. As with the mean velocity (see Figure 7) there is an increase in maximum velocity with age until postnatal day 9, at which time the right forelimb appears to reach its highest velocity level.

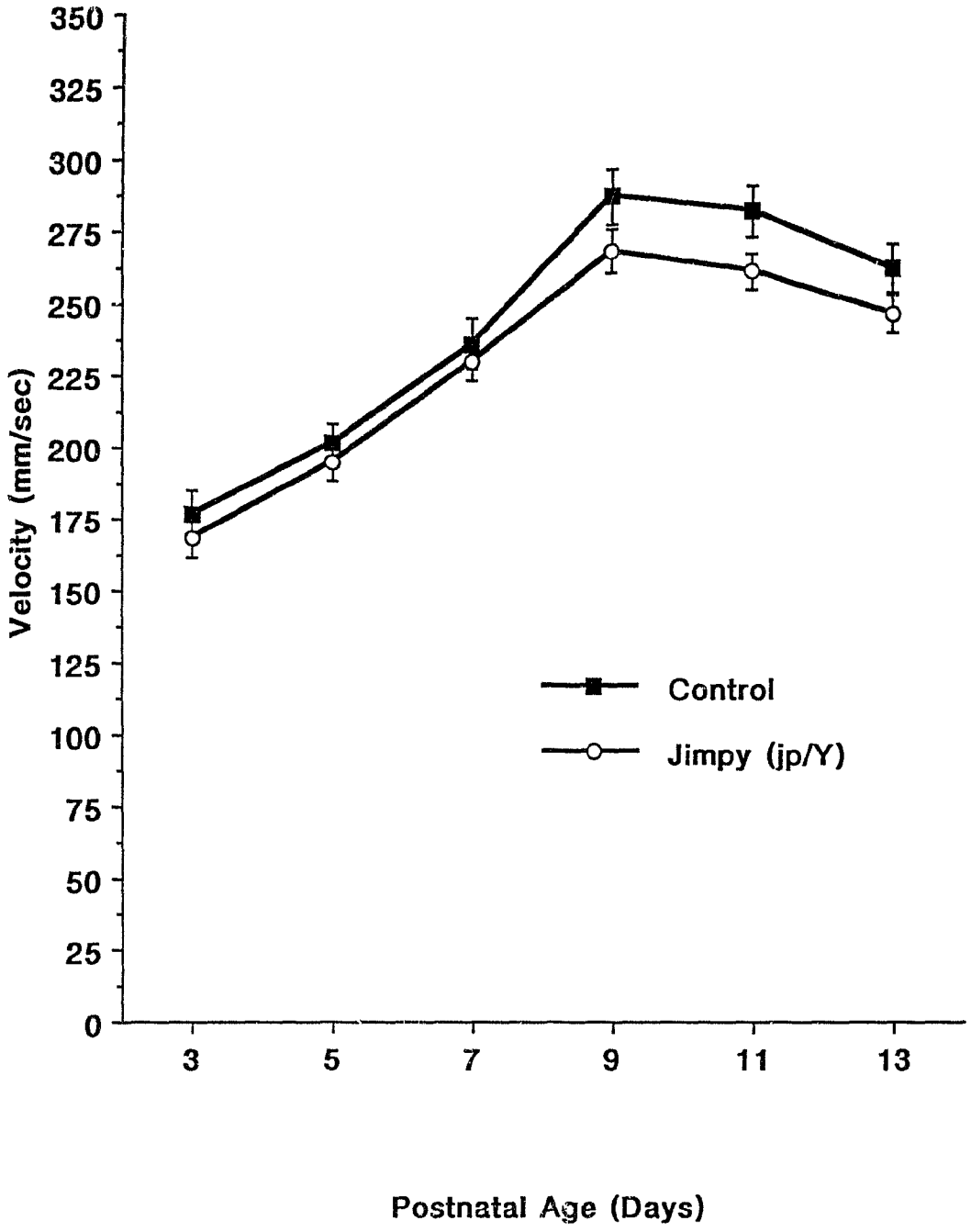


Figure 8. Mean maximum velocity (± SEM) of the right forelimb, during the onset of the swim stroke, for control and jimpy (jp/Y) mice from 3 to 13 days of age.

Hindlimb Stroke-Cycle Analyses

Duration

Hindlimb durations were analyzed by 2 X 7 ANOVAs with genotype (*jp*/Y or control) and age (days 9 - 21) as the two within-subjects variables. Collapsing the stroke-cycle duration data from the two hindlimbs there was a significant main effect due to genotype, with *jp*/Y mice displaying longer durations than littermate controls ($F(1,14)=8.464$, $p < .05$) (see Figure 9). Using Tukey's tests to compare the two groups on individual days there was a significant difference between *jp*/Y and littermate controls on postnatal day 19. There were no significant differences between the two groups on the postnatal days not included in the ANOVA (days 3-7) using Mann-Whitney tests.

There was a significant main effect due to age on hindlimb stroke-cycle duration ($F(6,84)=28.731$, $p < .01$) (see Figure 9). In general stroke-cycle duration became shorter as a function of age. There was no significant interaction between genotype and age. In general, after postnatal day 11, *jp*/Y mice tended to have longer hindlimb stroke-cycle durations than littermate controls, although both groups showed the same basic developmental pattern of decreasing durations. There is, in both groups, evidence of a plateau from postnatal day 15 onward, as the hindlimbs appear to be moving as fast as possible. As with the collapsed durations of the forelimbs (see Figure 4) mice tend to show decreased stroke-cycle duration with age. In the case of the forelimbs this trend disappears when the limbs drop out of use, whereas for the hindlimbs the decrease tends to reach a plateau at postnatal day 15. Note that hindlimb durations are maintained in

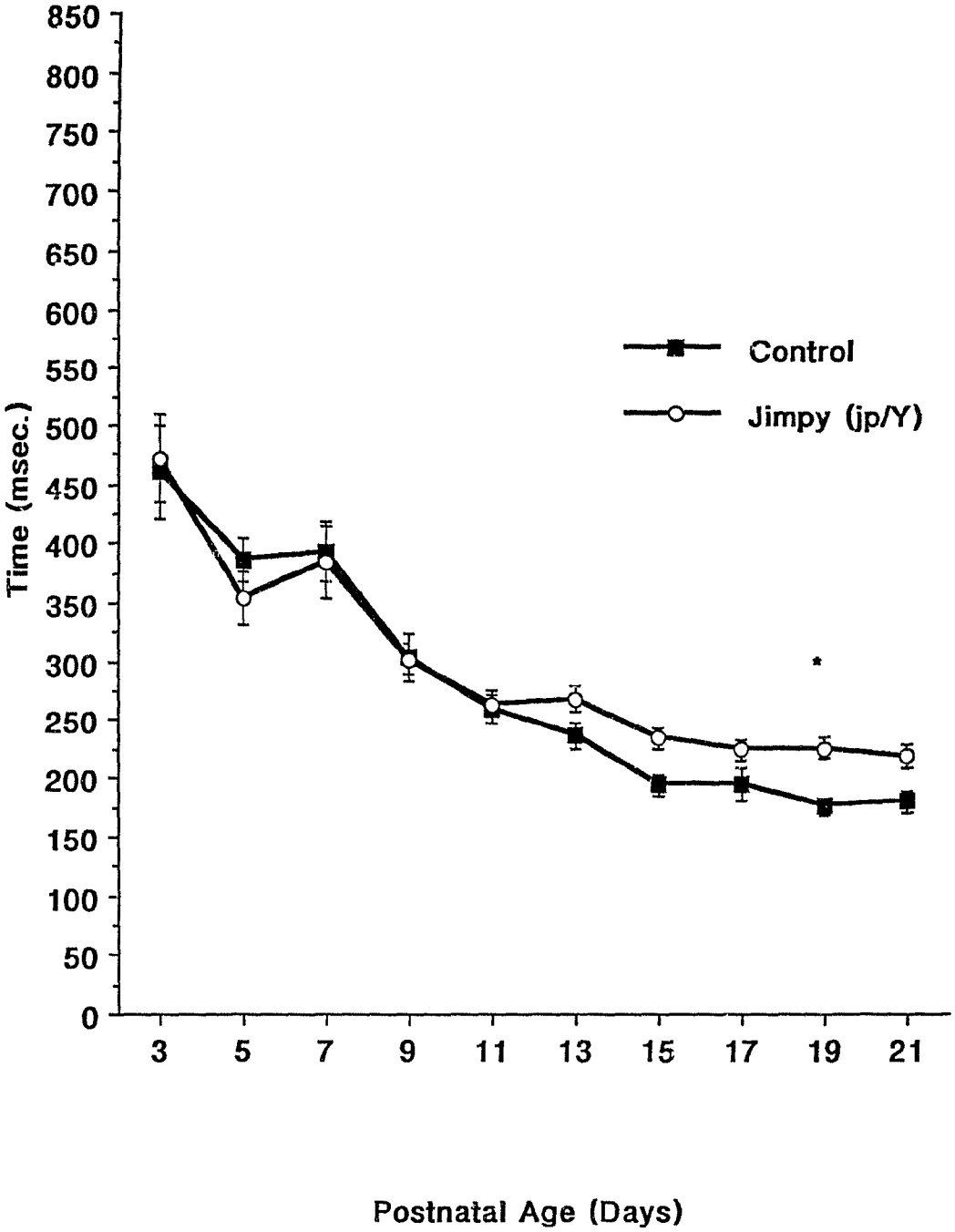


Figure 9. Mean stroke-cycle duration (± SEM) of the hindlimbs for control and jimpy (jp/Y) mice from 3-21 days of age.

jp/Y mice even during their weight loss (see Figure 1), therefore making it unlikely that differences in muscular strength can account for the results.

Having established both genotype and age effects with the collapsed data the hindlimbs were now analyzed separately to detect asymmetries. In the case of the right hindlimb, *jp*/Y mice had significantly longer stroke-cycle durations than did controls ($F(1,14)=17.009$, $p<.01$) (see Figure 10). Using Tukey's test on individual postnatal days there were significant differences between *jp*/Y and control groups on day 13 ($p<.05$) and day 19 ($p<.05$). There were no significant differences between the two groups prior to the days included in the ANOVA, using Mann-Whitney tests, on postnatal days 3-7. Stroke-cycle duration decreased with age, although at day 15 it tended to stabilize ($F(6,84)=18.780$, $p<.01$). However, there was no significant interaction between genotype and age.

The results for the stroke-cycle duration of the left hindlimb were similar to those for the right hindlimb. There was a significant difference between *jp*/Y and control mice, with the *jp*/Y mice displaying longer stroke-cycle durations than the controls ($F(1,14)=7.931$, $p<.05$) (see Figure 11). There was also a significant decrease in stroke-cycle duration up to postnatal day 15, when it tended to stabilize ($F(6,84)=28.328$, $p<.01$). There was a significant genotype by age interaction ($F(6,84)=2.353$, $p<.05$). Initially (days 9-13 for the ANOVA) there is little difference between the two groups, and it is only after postnatal day 13 that the *jp*/Y animals begin to lag behind the control animals. However, using Tukey's test only on postnatal day 19 ($p<.05$) there was a significant difference between the two groups. There was also no difference between the two groups for days not included in the ANOVA (days 3-7) using Mann-Whitney tests. Comparisons between Figure 10 and Figure 11 show slightly greater bilateral distinctions for *jp*/Y than for control mice,

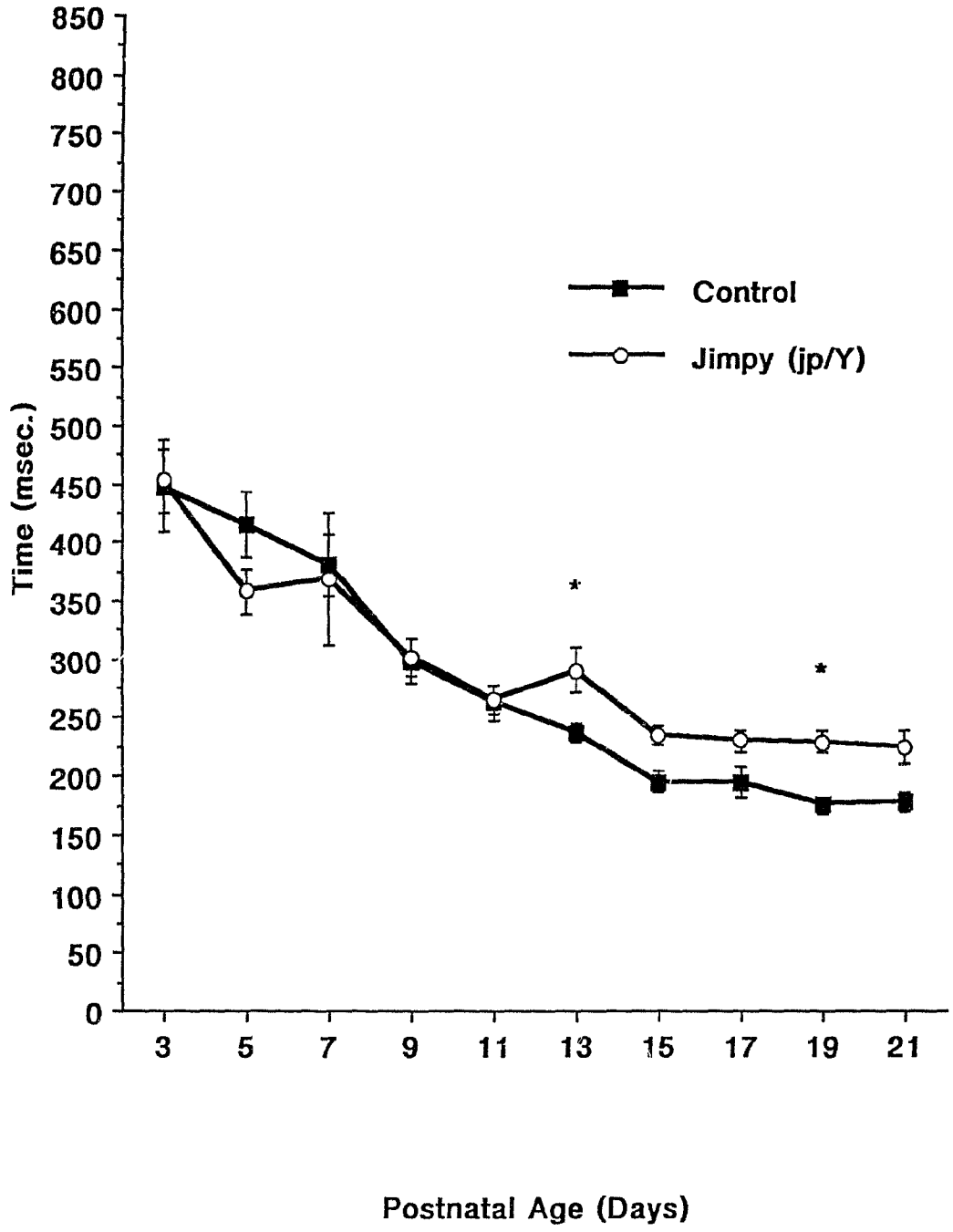


Figure 10. Mean stroke-cycle duration (± SEM) of the right hindlimb for control and jimpy (jp/Y) mice from 3 to 21 days of age. *p≤ .05.

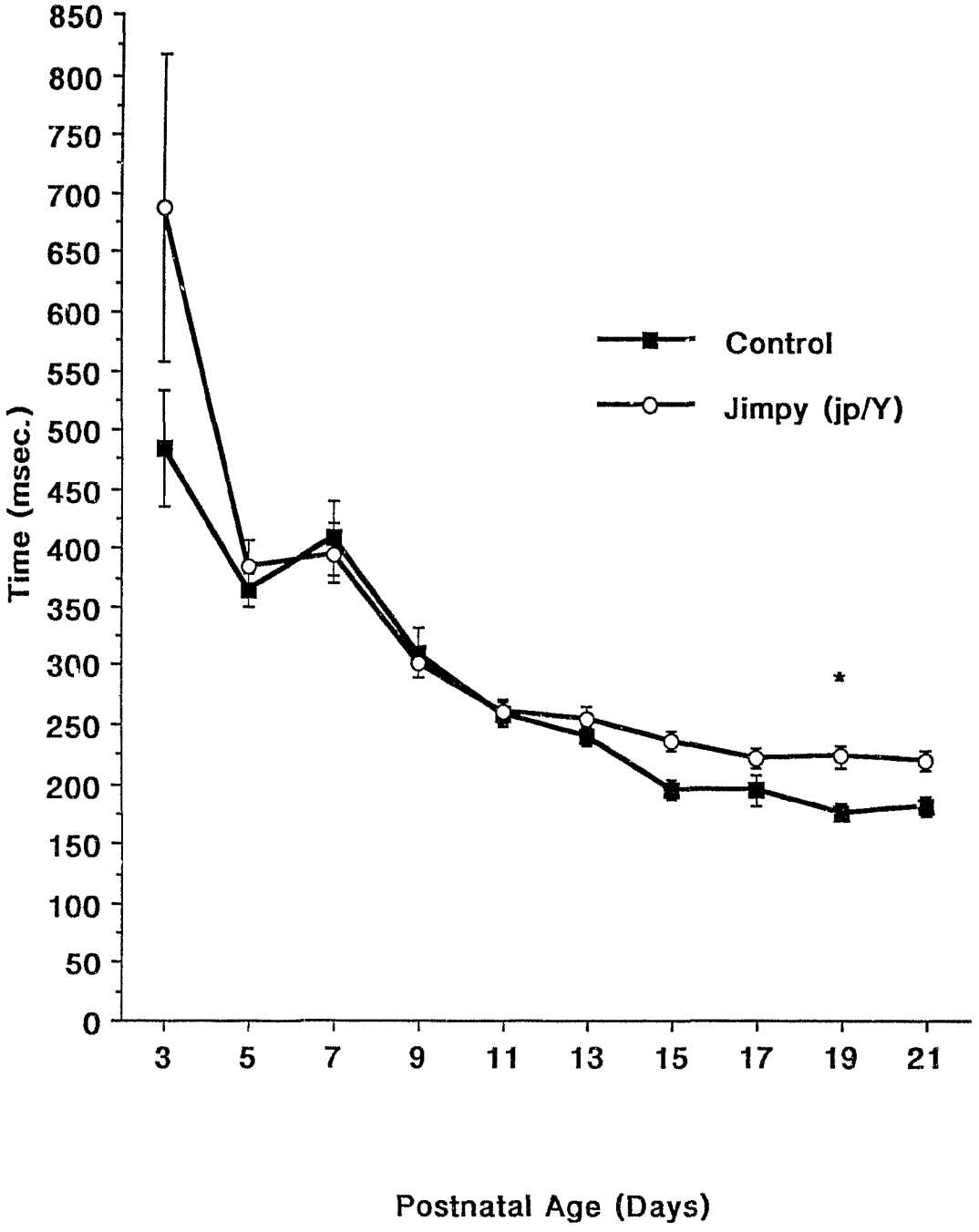


Figure 11. Mean stroke-cycle duration (± SEM) of the left hindlimb for control and jimpy (jp/Y) mice from 3 to 21 days of age. *p≤ .05.

which may account for the statistical significance (using Tukey's posthoc tests) on postnatal day 13 for the right hindlimb but not the left hindlimb. This modest asymmetry is similar to that observed in the forelimbs (see Figures 5 & 6), suggesting less precision in the coupling of bilateral limb pairs in the *jp*/Y mice.

Right Hindlimb Mean Velocity

A 2 X 7 within-subjects ANOVA, with genotype (*jp*/Y or control) and age (days 9-21) as the two variables, was used to evaluate the right hindlimb mean velocities of *jp*/Y and control mice (Figure 12). There was a significant main effect of genotype: *jp*/Y mice had lower velocities than control mice ($F(1,14)=68.596$, $p<.01$). This difference first became significant at postnatal day 13 ($p<.01$), and continued through days 15 ($p<.05$), 17 ($p<.05$), 19 ($p<.01$) and 21 ($p<.05$) using Tukey's tests. However, prior to day 13 there were no differences between the two groups either by Tukey's tests (days 9-11) or by Mann-Whitney tests (days 3-7). There was a significant main effect due to age, as generally for both groups mean velocity increased with age ($F(6,84)=59.835$, $p<.01$). In addition, there was a significant genotype by age interaction ($F(6,84)=4.984$, $p<.01$), the result of the divergence in the *jp*/Y and control groups after postnatal day 11. This developmental pattern is particularly interesting in light of the fact that even when right hindlimb stroke-cycle duration is basically flat, after postnatal day 13 (see Figure 10), the velocity continues to increase. Figure 12 has more significant differences at individual days between *jp*/Y and control groups, which may in part be determined by increased divergence in weight (see Figure 1).

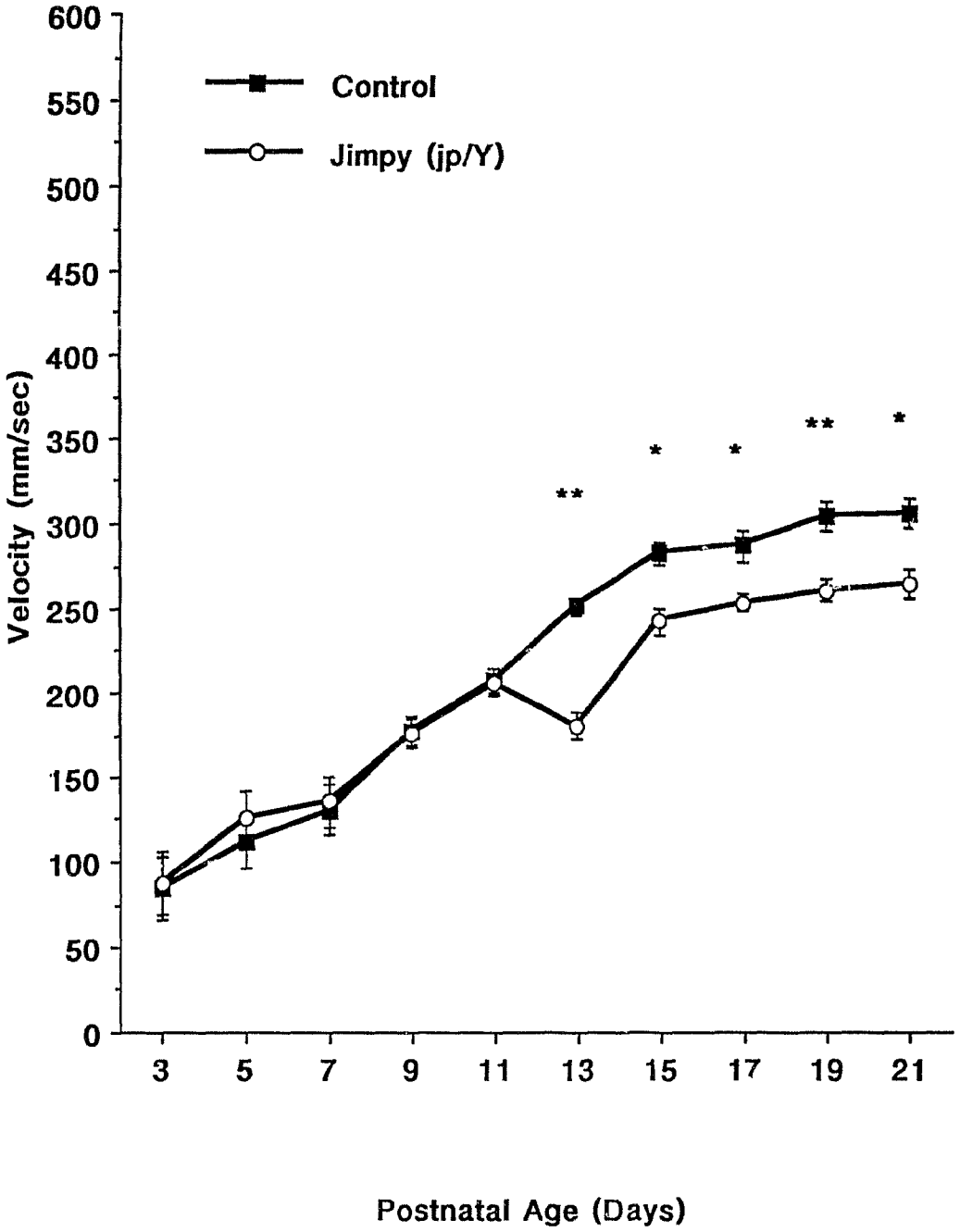


Figure 12. Mean velocity (± SEM) of the right hindlimb, during the ten stroke swim session, for control and jimpy (jp/Y) mice from 3 to 21 days of age. *p≤ .05. **p≤ .01.

Right Hindlimb Maximum Velocity

To evaluate the maximum velocity of the right hindlimb a 2 X 7 ANOVA was used with genotype (*jp*/Y or control) and age (days 9-21) as the within-subject factors. There was a significant difference in maximum velocity for the *jp*/Y and control groups ($F(1,14)=60.710$, $p<.01$), a significant main effect due to age ($F(6,84)=56.587$, $p<.01$). and a significant genotype by age interaction ($F(6,84)=3.438$, $p<.01$). There were no significant differences between *jp*/Y and control groups on postnatal days 3-7 via Mann-Whitney tests. As is evident in Figure 13, although the two groups are virtually identical and increasing with age at the same rate until postnatal day 9, from that point on a divergence becomes evident. From postnatal day 11 onward the *jp*/Y group lags significantly behind the control group (day 11($p<.05$), day 13 ($p<.01$), day 15 ($p<.05$), day 17 ($p<.01$), day 19 ($p<.01$), and day 21 ($p<.01$)).

Missed Strokes

Forelimbs

Missed strokes were analyzed using 2 X 5 ANOVAs with genotype (*jp*/Y or control) and age (days 3-11) as the within-subjects variables and Mann-Whitney tests for postnatal day 13. Although there was no significant difference between the two groups when the missed stroke data were collapsed over both forelimbs (giving a total number of missed strokes) there was a nearly significant tendency for the *jp*/Y group to have a higher number of missed strokes than the control group ($F(1,14)=4.053$, $p<.10$) (see Figure 14). *Jp*/Y and control groups did not differ on postnatal day 13 using the Mann-Whitney test. There was no significant main effect due to age or interaction between age and genotype. However, it is worth noting that on

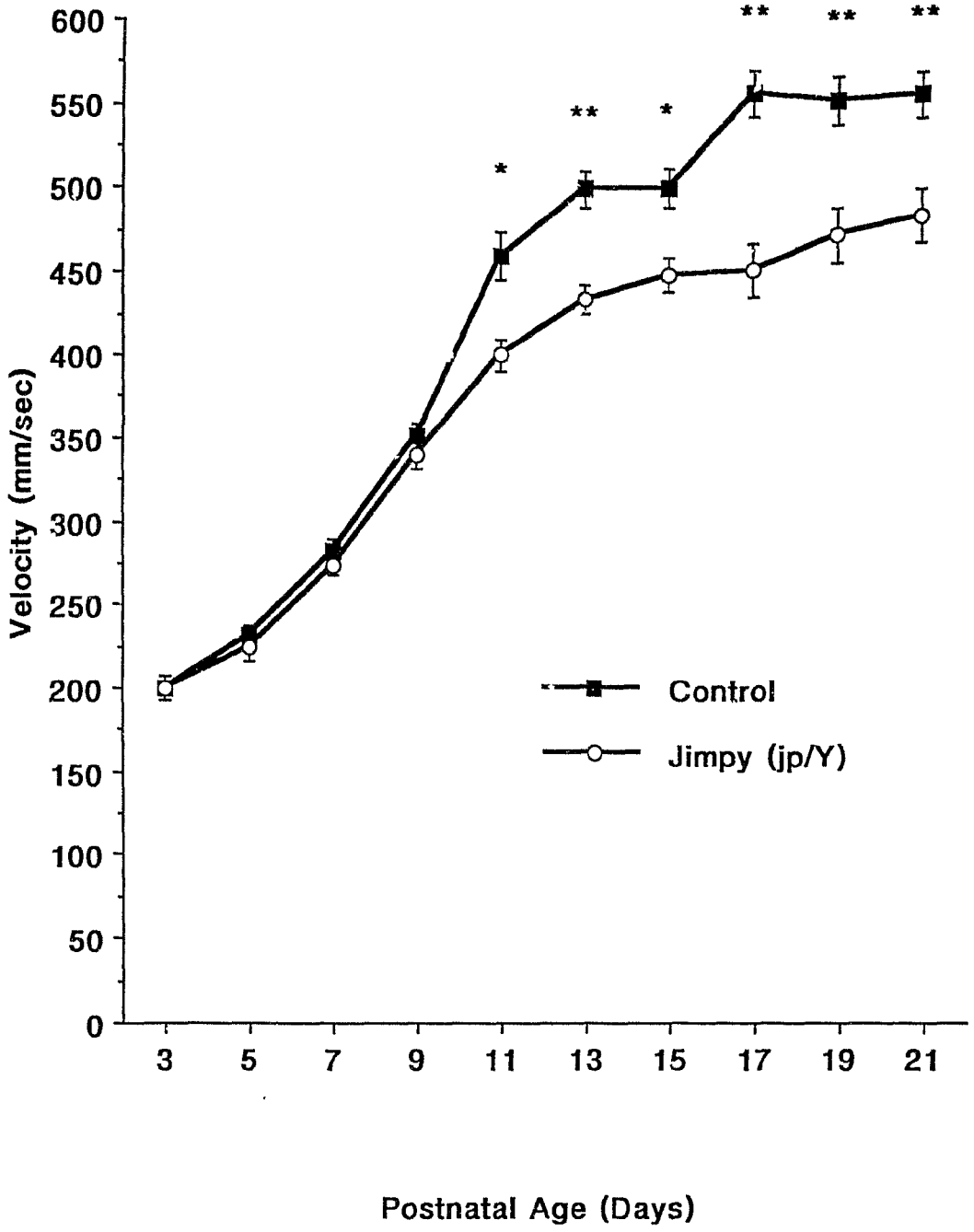


Figure 13. Mean maximum velocity (± SEM) of the right hindlimb, during the onset of the swim stroke, for control and jimpy (jp/Y) mice from 3 to 21 days of age. *p≤ .05. **p≤ .01.

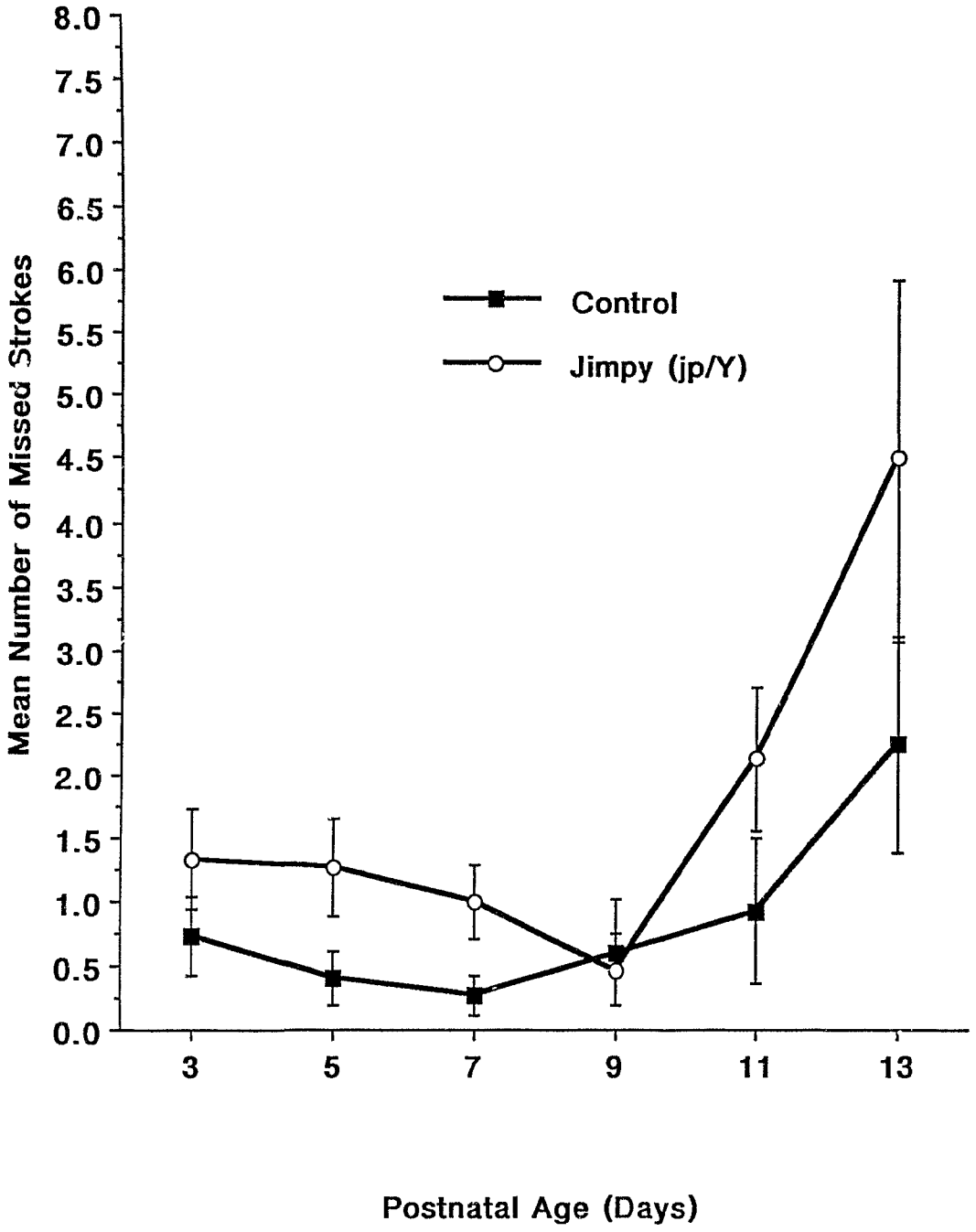


Figure 14. Mean number of strokes missed (± SEM) by the forelimbs, in a ten stroke session, for control and jimpy (jp/Y) mice from 3 to 13 days of age.

postnatal day 13, when forelimb duration tends to increase slightly for both groups of mice (see Figure 4) that the number of missed strokes also increases, thus coinciding with the forelimbs dropping out of use and the transition to hindlimb swim style (see Figures 2 & 3). As the *jp*/Y and littermate controls groups both have the same developmental trend in terms of transition from one style of swim to the next, these data suggest that within the transition period the *jp*/Y group is more likely to miss strokes.

To examine individual forelimb trends, each limb was analyzed separately. There was no significant difference in the number of missed strokes in the right forelimb between the *jp*/Y and control groups either in the ANOVA or the Mann-Whitney tests (see Figure 15). There was no main effect of age and no interaction between genotype and age. There was a low number of missed strokes for this limb over postnatal days 3 through 9 for both groups of mice. After postnatal day 9 there was an increased number of missed strokes.

Overall, *jp*/Y mice showed more missed strokes of the left forelimb than did controls ($F(1,14)=5.762$, $p<.05$) (see Figure 16). Individual Tukey's tests did not reveal significant differences between the groups and in addition, there was no significant difference between the two groups on postnatal day 13 using the Mann-Whitney test. There was no main effect of age and no interaction between genotype and age. In general, missed strokes were less for control than *jp* 'Y mice and until postnatal day 13 (when the animals are switching to a hindlimb swim style) there is little change in the number of missed strokes for either group as a function of age.

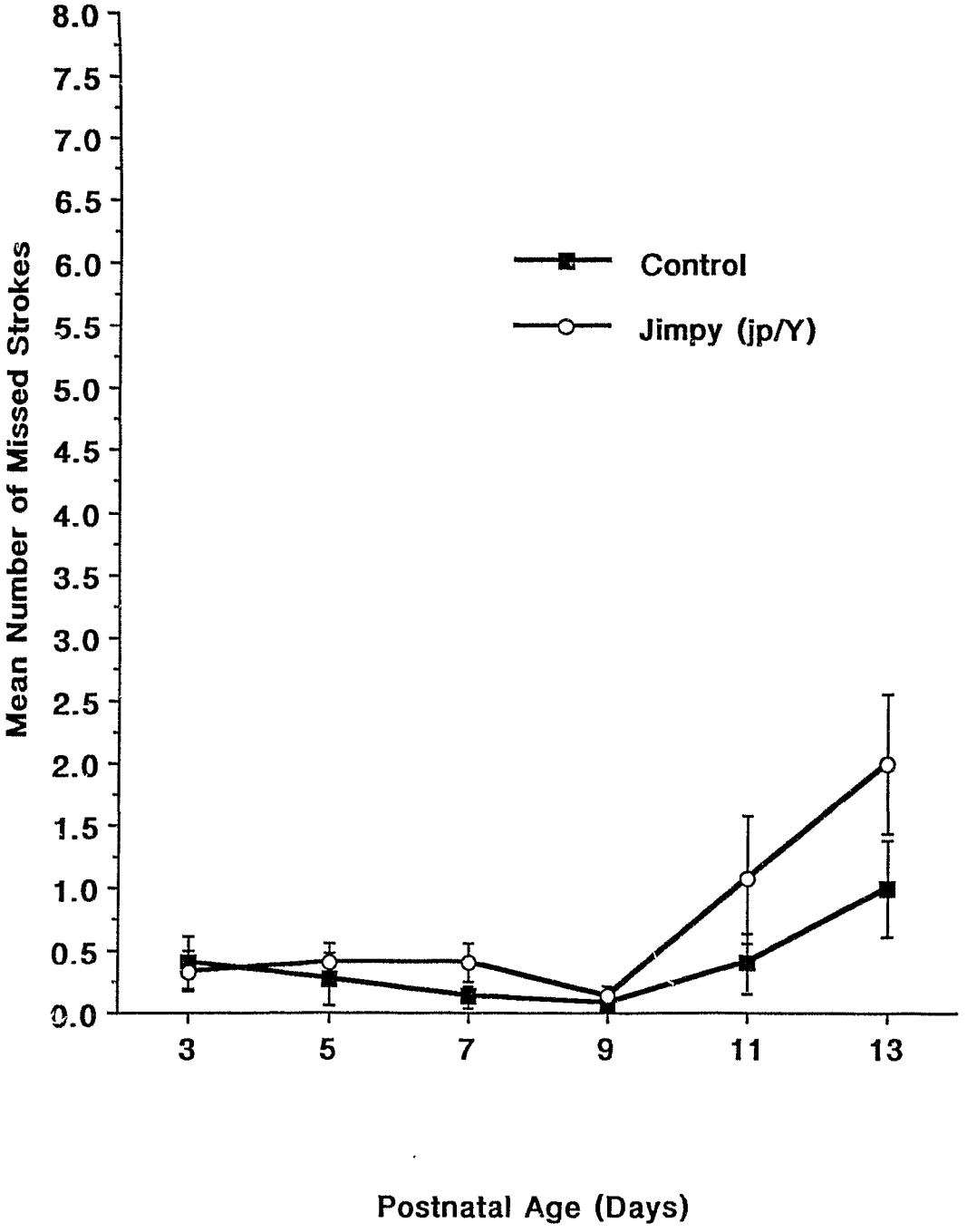


Figure 15. Mean number of strokes missed (± SEM) by the right forelimb, in a ten stroke session, for control and jimpy (jp/Y) mice from 3 to 13 days of age.

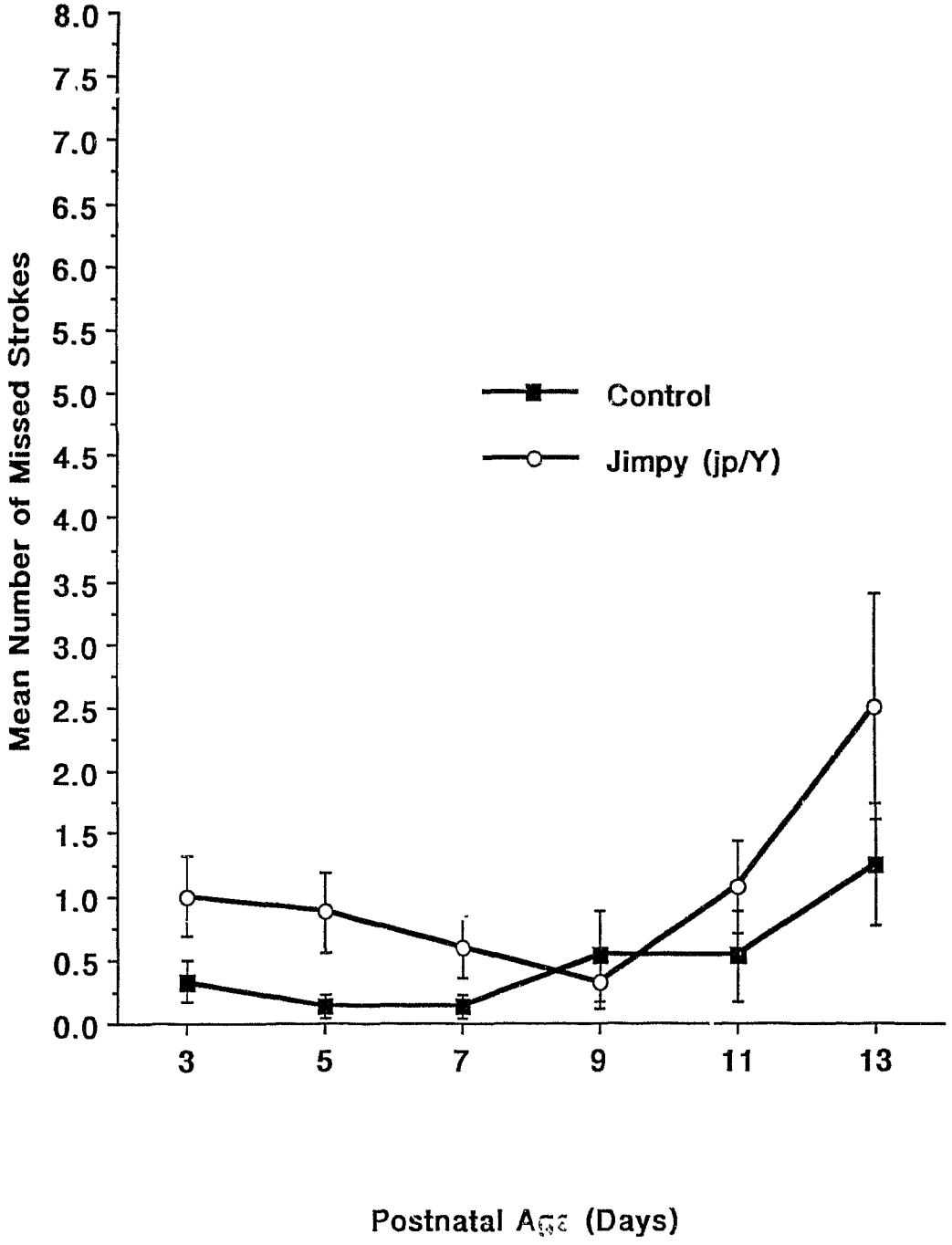


Figure 16. Mean number of strokes missed (± SEM) by the left forelimb, in a ten stroke session, for control and jimpy (jp/Y) mice from 3 to 13 days of age.

Hindlimbs

To analyze the missed strokes data for the hindlimbs 2 X 7 ANOVAs with genotype (*jp*/Y or control) and age (days 9-21) as the within-subjects variables was used. Mann-Whitney tests were used to compare the *jp*/Y and control groups on earlier days (postnatal days 3-7). After collapsing the missing strokes data from both hindlimbs to obtain a total number of missed strokes for the hindlimbs, there was no main effect of genotype, although there was an age effect ($F(6,84)=18.905$, $p<.01$) and a genotype by age interaction ($F(6,84)=2.415$, $p<.05$). With age both groups of mice show a decrease in the number of missed strokes, which is consistent with what would be expected as the role of the hindlimbs becomes more critical for the swim (see Figure 17). Although both *jp*/Y and littermate control groups follow similar developmental patterns there are differences between the two groups, with the *jp*/Y group displaying a tendency to have more missed strokes after postnatal day 11, which coincides with the transition to a hindlimb swim style (see Figures 2 & 3). After the littermate control animals begin using the hindlimb swim style their hindlimbs rarely missed strokes. In contrast, from postnatal day 7 onward there is an increase in missed strokes for the forelimbs of controls, even after they have been using the limbs for several days and in some cases the same swimming style for several days (see Figure 14). With the total number of missed strokes (see Figure 17), as with hindlimb duration (see Figure 9) as age increases both tend to decrease, up to postnatal day 15, after which they remain stable indicating that the animals have reached maximum swimming potential.

To determine the presence of limb asymmetries the number of missed strokes of each limb was examined separately. Overall there was no

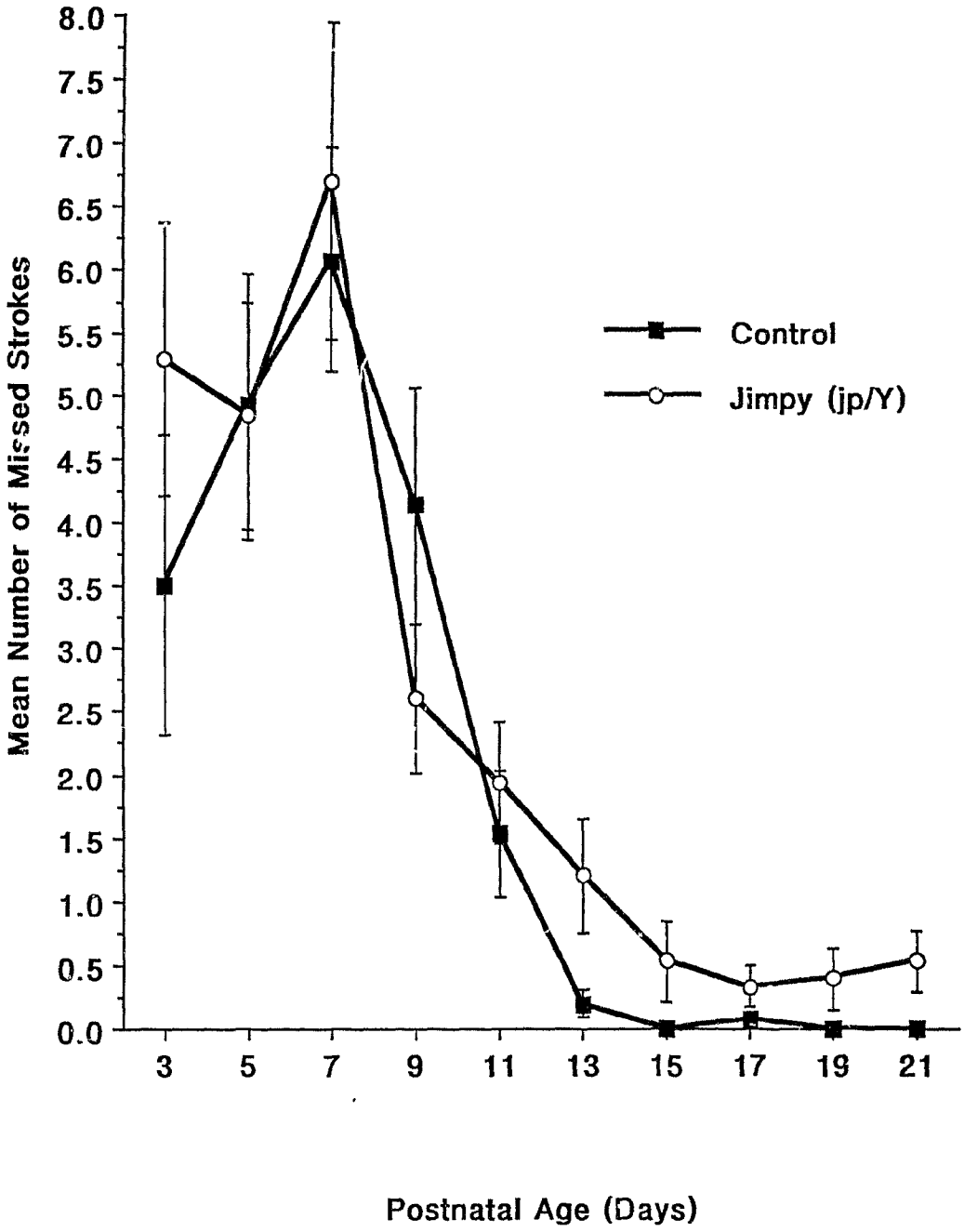


Figure 17. Mean number of strokes missed (± SEM) by the hindlimbs, in a ten stroke session, for control and jimpy (jp/Y) mice from 3 to 21 days of age.

significant difference between the *jp*/Y and littermate control groups for the right hindlimb (see Figure 18). Using Mann-Whitney tests, there were no significant differences between the two groups on postnatal days 3-7. There was a significant main effect of age ($F(6,84)=12.764$, $p<.01$). Both *jp*/Y and control mice exhibited a relatively high number of missed strokes during the early development of the four limb swim. The tendency for missed strokes declined consistently from postnatal day 7, and from postnatal day 15 missed strokes were very rare for the control group, although they were still present for the *jp*/Y group. By postnatal day 11 the *jp*/Y group showed more missed strokes than the control group, and although they continued to show more missed strokes for the rest of the period studied, the difference between the two groups was greatest on postnatal days 11 and 13. These differences are represented by a significant genotype by age interaction ($F(6,84)=2.268$, $p<.05$). However, once again the Tukey's tests did not reveal significant differences at individual days. It appears, especially for the right hindlimb, that the difference between *jp*/Y and control groups is greatest at the transition between four limb and hindlimb swim styles.

For the left hindlimb, there were no significant differences in the number of missed strokes between *jp*/Y and control groups, either in the ANOVA or in the Mann-Whitney tests (see Figure 19). Both groups showed a decrease in the number of missed strokes as a function of age ($F(6,84)=19.946$, $p<.01$) although this tended to stabilize from postnatal day 13 onward. There was no significant interaction between genotype and age, as both groups showed the same decrease in missed strokes with age. In the case of the left hindlimb there less difference between *jp*/Y and littermate control groups, indicating a modest asymmetry between the two hindlimbs in terms of number of strokes missed during the swim session. This modest asymmetry is not surprising as

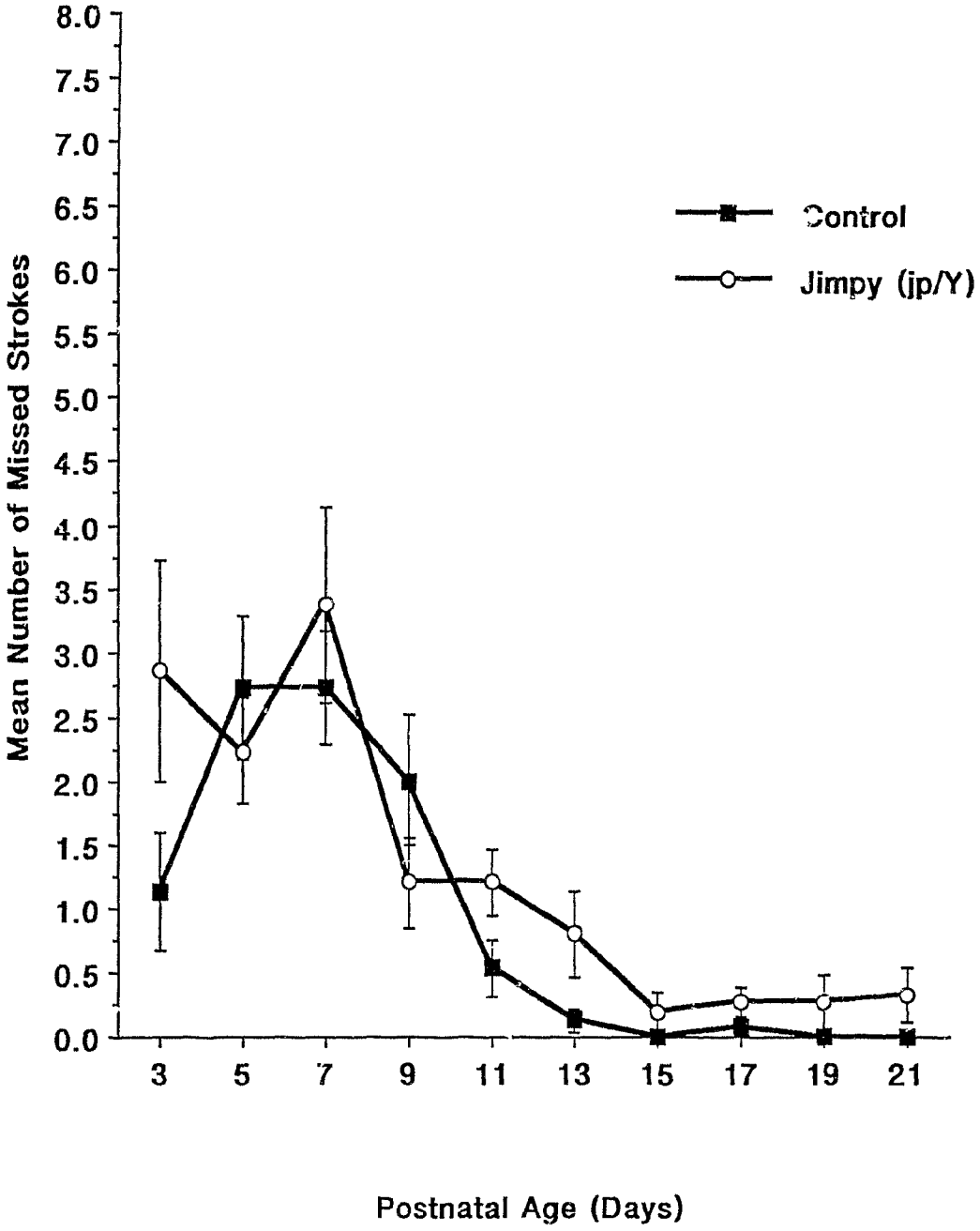


Figure 18. Mean number of strokes missed (± SEM) by the right hindlimb, in a ten stroke session, for control and jimpy (jp/Y) mice from 3 to 21 days of age.

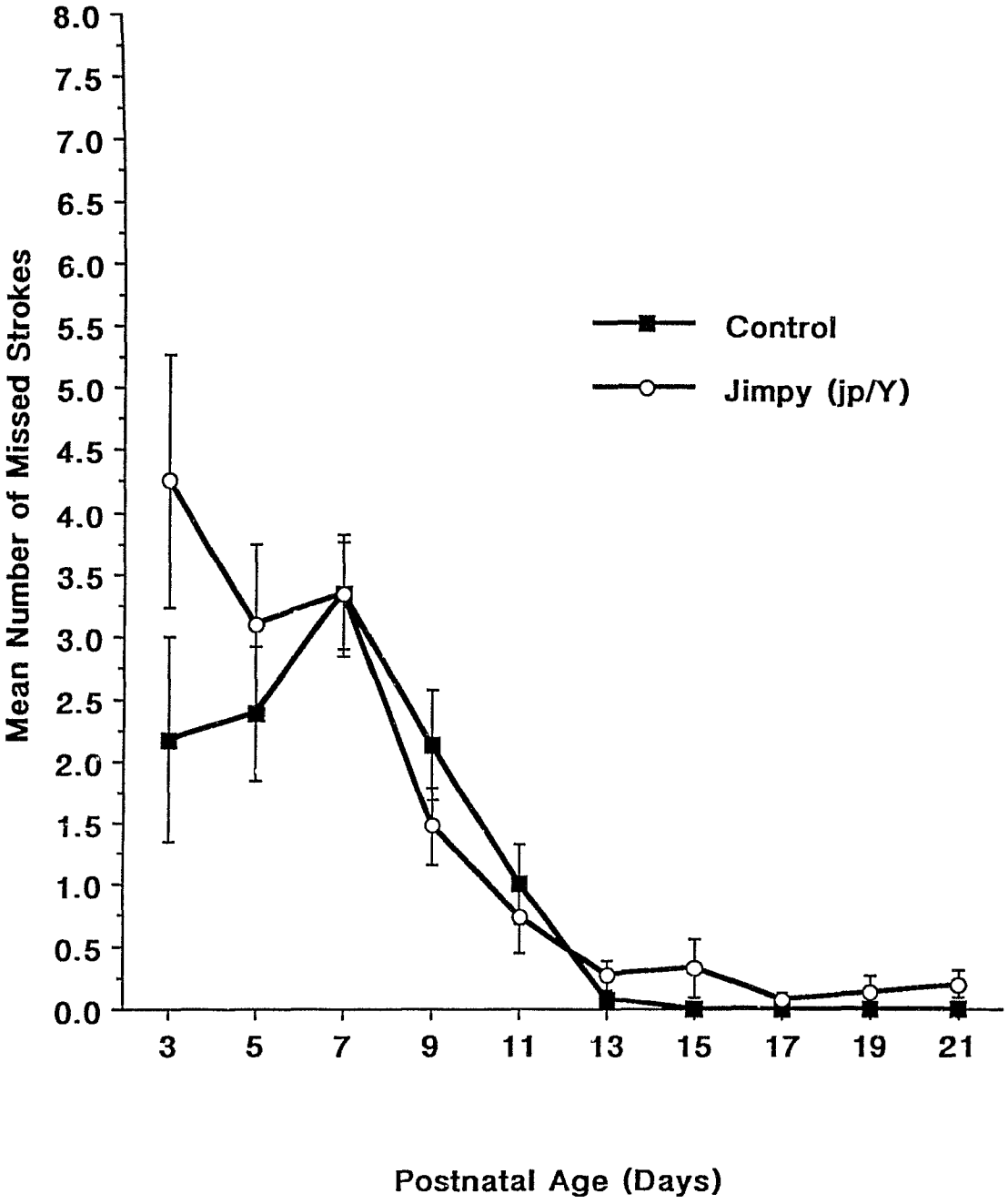


Figure 19. Mean number of strokes missed (± SEM) by the left hindlimb, in a ten stroke session, for control and jimpy (jp/Y) mice from 3 to 21 days of age.

a similar one existed between the stroke-cycle durations for the two hindlimbs (see Figures 10 & 11).

Interlimb Coordination Measures

Bilateral Forelimb Pair Latency

The latency of the forelimb pair was analyzed by a 2 X 5 ANOVA with genotype (*jp*/Y or control) and age (days 3 - 11) as the two within-subjects variables. There was no significant difference between *jp*/Y and control mice in terms of latency in stroke cycle onset between the right and left forelimb pair at any of the days tested (see Figure 20). On day 13 there was no difference between *jp*/Y and control groups via the Mann-Whitney test. However, a main effect due to age was evident, as with increased age latencies decreased for both groups of mice, up to day 9 when they tended to stabilize ($F(4,56)=15.594$, $p < .01$). There was no significant interaction between genotype and age indicating that both groups follow the same basic trend. Although the *jp*/Y mice display somewhat longer forelimb stroke-cycle durations, relative to controls (see Figures 4-6), these are not reflected in the latency relationships for the two forelimbs.

Bilateral Hindlimb Pair Latency.

The stroke latency between the hindlimb pair was analyzed by a 2 X 7 ANOVA with genotype (*jp*/Y or control) and age (days 9 - 21) as the two within-subjects variables. When comparing the latency between onset of stroke-cycles in this bilateral pair there was no significant effect of genotype either in the days covered by the ANOVA or in those examined by the Mann-

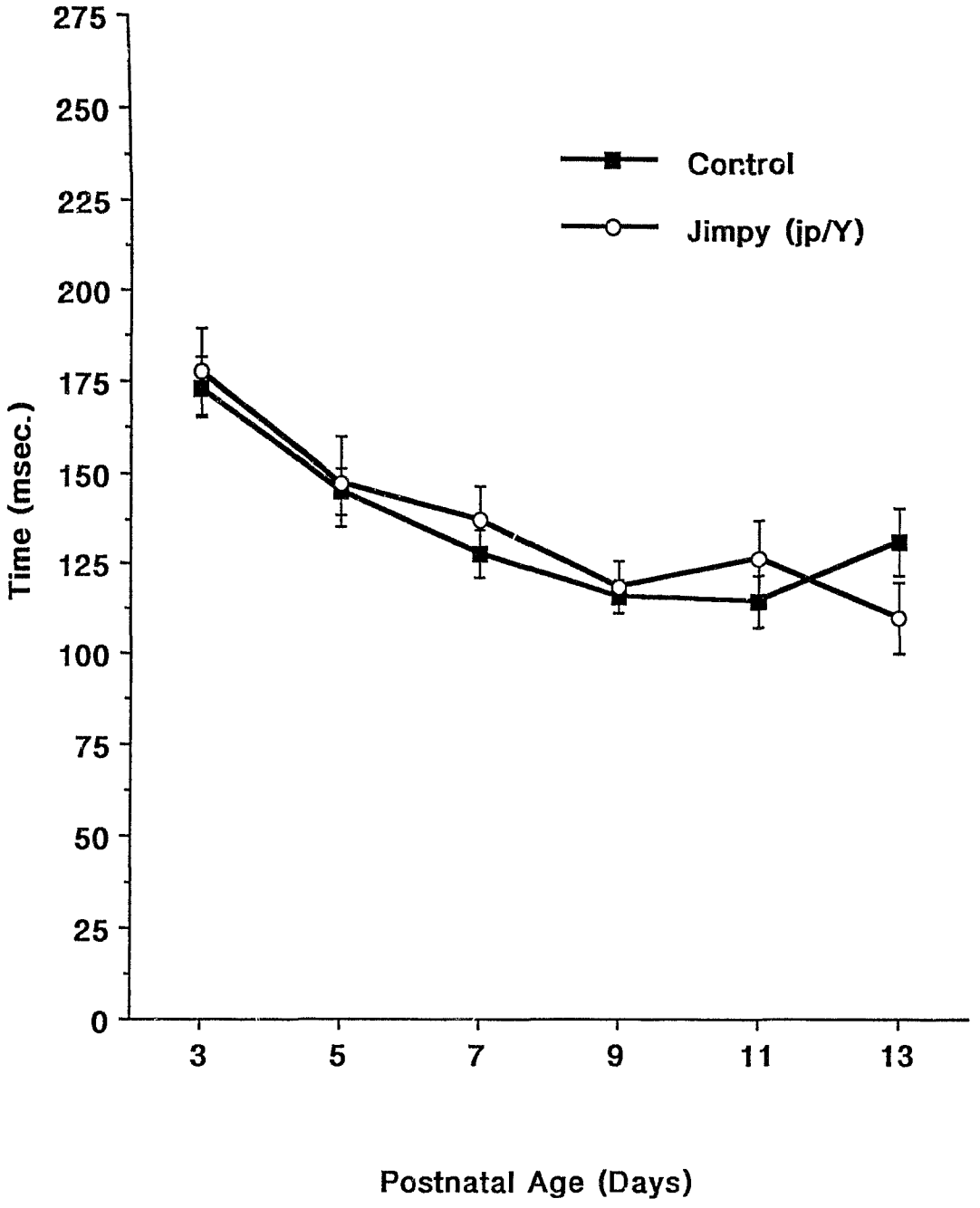


Figure 20. Mean stroke latency (± SEM) of the left forelimb relative to the right forelimb for control and jimpy (jp/Y) mice from 3 to13 days of age.

Whitney test (postnatal days 3-7). There was a main effect due to age, in general as age increased latency decreased ($F(6,84)=12.906$, $p<.01$) (see Figure 21). There was also a significant interaction between genotype and age ($F(6,84)=3.596$, $p<.01$), with the *jp*/Y pups actually showing reduced latencies relative to control pups from postnatal days 7-13, which is the period in which these animals should be using a four limb swimming style and alternating these two limbs. However, when both groups begin to use the hindlimb swim style a crossover occurs and the *jp*/Y group show longer latencies than the control group. Using the Tukey's test there were no significant differences between *jp*/Y and control mice for any of the individual days. Once again although there is a general pattern of slowed durations for the *jp*/Y relative to the littermate control group (see Figures 9-11) this can not in itself account for the timing between onset of stroke-cycles in these two bilateral limbs.

Ipsilateral and Contralateral Limb Pair Latencies

In the case of the right ipsilateral limb pair, there were no significant differences between *jp*/Y and control groups (see Figure 22). An ANOVA was not practical for this data set because there were only two days (day 9 and 11) in which both forelimbs and hindlimbs were used during swimming by all the mice. Therefore, Mann-Whitney tests for each testing day were used, and there were no significant differences between the *jp*/Y and control groups at any of the days tested. Only on postnatal day 11 did the difference between *jp*/Y and control groups approach significance ($U(n=15,15)=67.5$ and 157.5, $p<.10$). As can be seen in Figure 22, on postnatal day 11, mutant animals actually have shorter latencies between these two limbs, than do

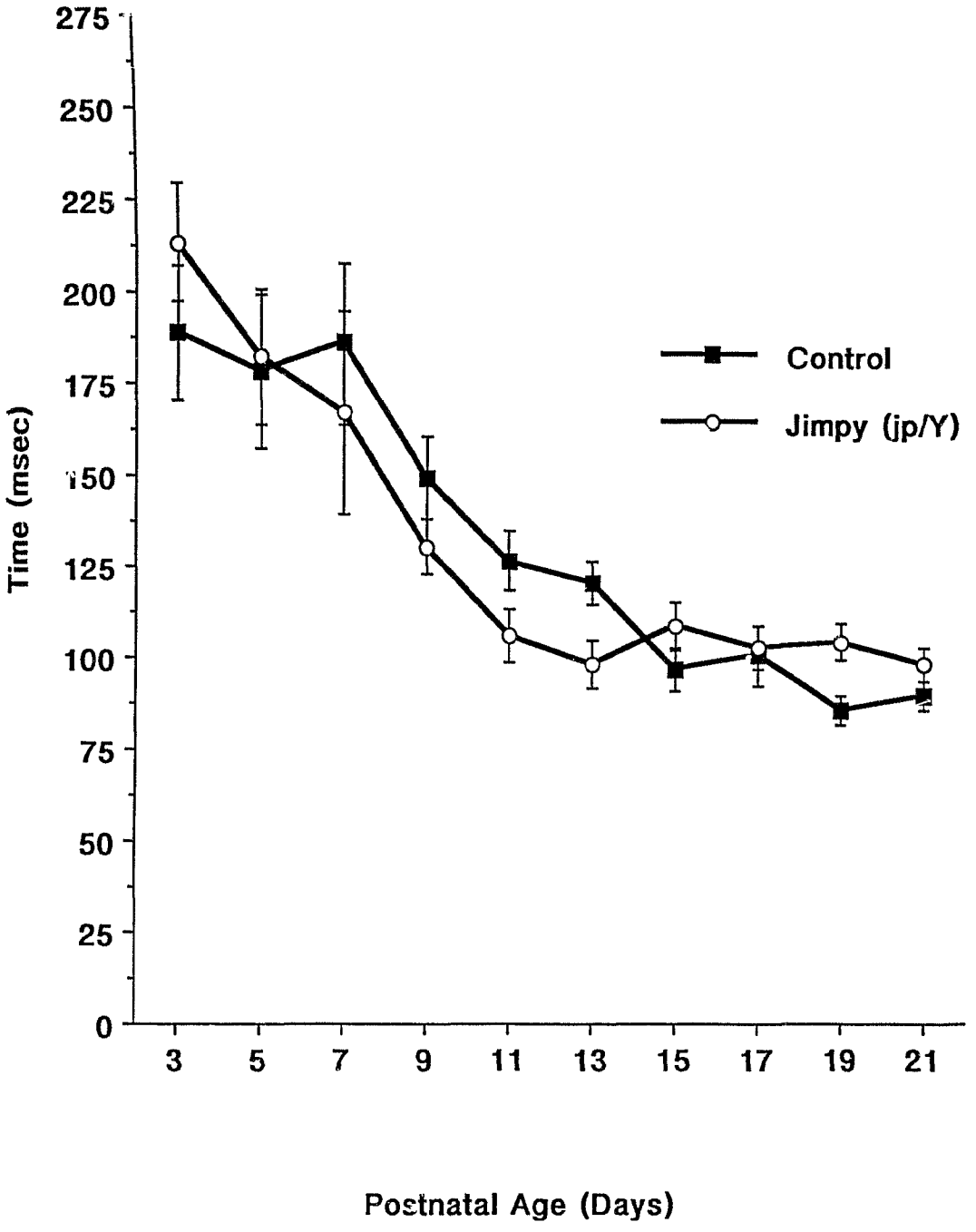


Figure 21. Mean stroke latency (± SEM) of the left hindlimb relative to the right hindlimb for control and jimpy (jp/Y) mice from 3 to 21 days of age.

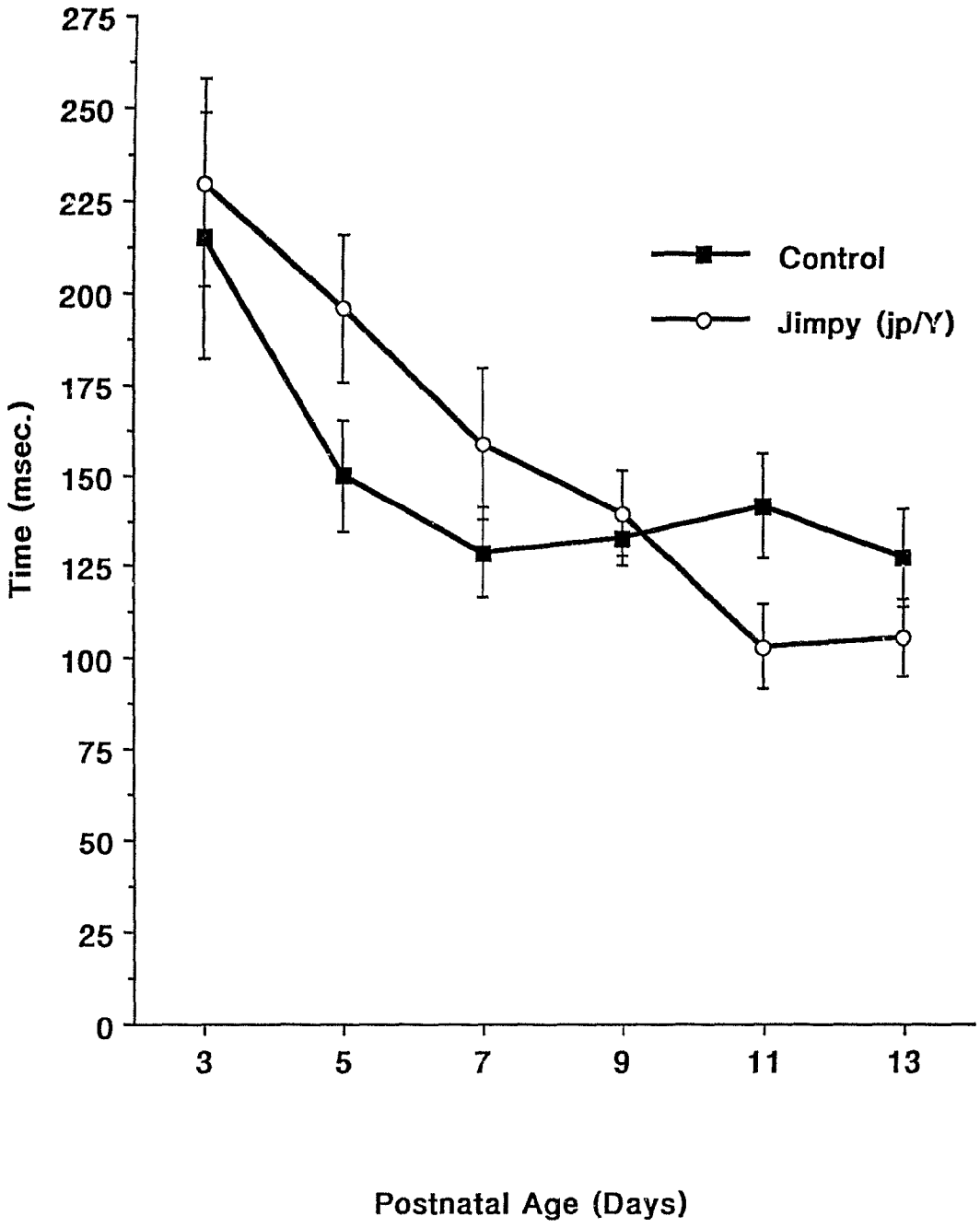


Figure 22. Mean stroke latency (± SEM) of the right hindlimb relative to the right forelimb for control and jimpy (jp/Y) mice from 3 to 13 days of age.

control animals, indicating they are moving those two limbs closer together in time than are controls.

The situation was similar for the contralateral pair data set, as only on postnatal days 9 and 11 did all the mice use both sets of limbs, making the information from an ANOVA of very limited relevance to the developmental period studied. As is evident in Figure 23, there were no consistent differences between *jp*/Y and control animals across age. On postnatal day 11 *jp*/Y mice showed significantly longer latencies than control mice, indicating that although the control animals are moving these two limbs fairly close together in time (the classic quadruped gait), the *jp*/Y mice have a longer time period between onset of the left hindlimb after the onset of the right forelimb ($U(n=15.15) = 46.5$ and 178.5, $p<.01$). Although postnatal day 13 also shows the increased latency for the *jp*/Y group there is no significant difference using the Mann-Whitney test ($U(n=7,10)=17$ and 53, $p<.10$). On postnatal days 5 and 7 differences between *jp*/Y and control groups also approached significance ($U(n=13,11) = 35$ and 106, $p<.05$) for day 5 and ($U(n=15,12) = 54$ and 126, $p<.10$) for day 7), although at these days the *jp*/Y animals had shorter latencies than did controls. Developmental trends become evident in light of the transition from one swimming style to another. Control mice continue to reduce the latency between the stroke onset of the two contralateral limbs (with the exception of postnatal day 3) until the forelimbs begin to drop out of the swim at postnatal day 13. *Jp*/Y mice, in contrast, show a decrease only up to postnatal day 9, and increase thereafter.

The normal distinction between ipsilateral and contralateral latency seen in control mice by postnatal day 11 (see Figures 22 & 23) is not present in *jp*/Y mice. Comparing the mean latencies between the ipsilateral and contralateral pairs of limbs, both in relation to the right forelimb, it is evident that after having

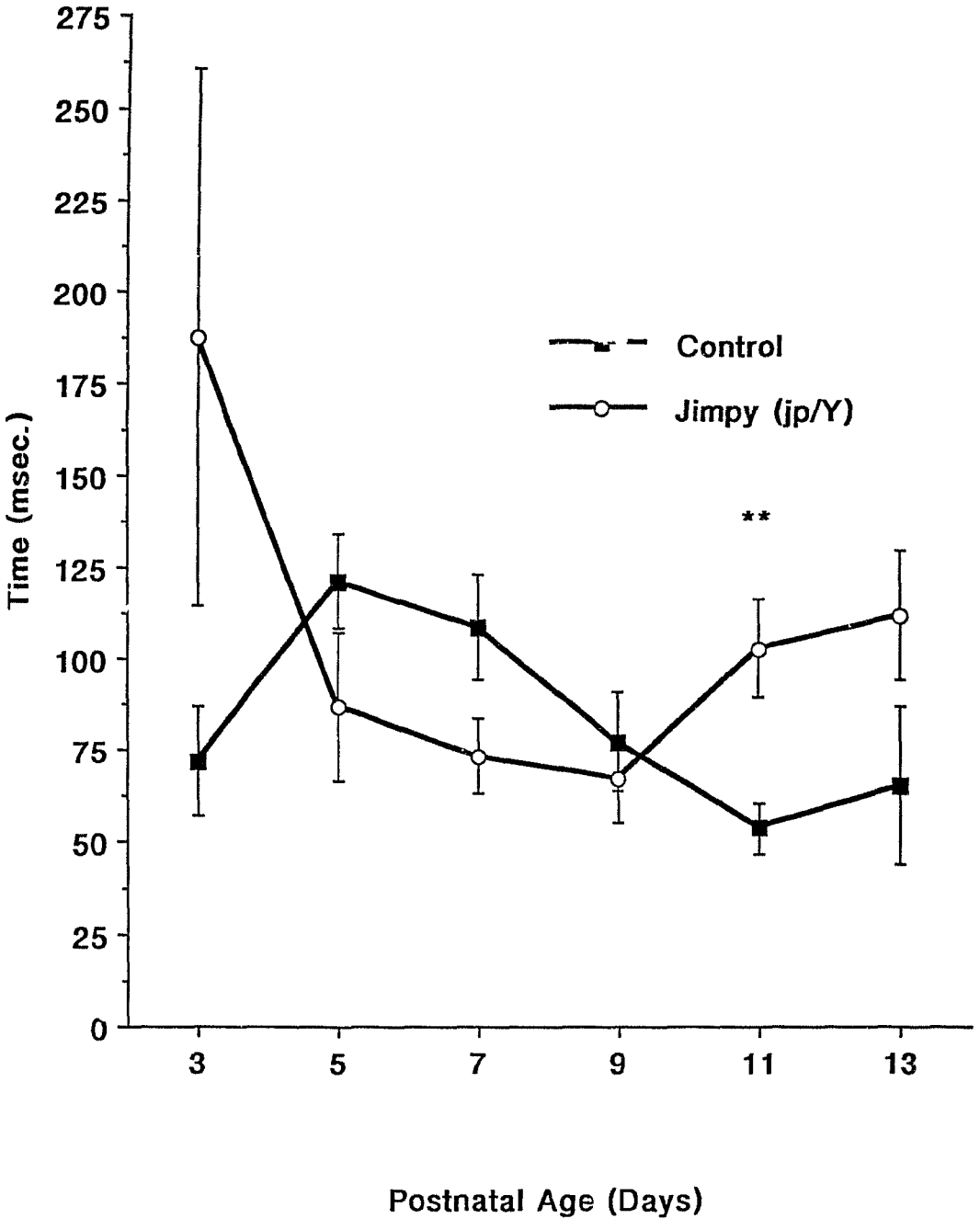


Figure 23. Mean stroke latency (± SEM) of the left hindlimb relative to the right forelimb for control and jimpy (jp/Y) mice from 3 to 13 days of age. **p≤.01.

initiated a stroke-cycle in the right forelimb, it is more quickly followed in time by the initiation of a stroke-cycle in the ipsilateral limb than in the contralateral limb for control mice (ipsilateral latency=141.31 msec. and contralateral latency=53.67 msec.). However, in the case of the *jp*/Y mice the latencies between onset of either hindlimb is the same (ipsilateral latency=103.09 msec. and contralateral latency=102.76 msec.). This represents a distinction between *jp*/Y and littermate controls in terms of limb coupling between forelimbs and hindlimbs.

Phase

Bekoff and Trainer (1979) Method

Although there are difficulties inherent in the interpretation of phase data with the Bekoff and Trainer (1979) method, analyses using this method were made to allow direct comparisons of the present results using their calculation and using the corrected calculation. In the corrected method phase relations were restricted to the 0.0 to 0.5 range, so that a more accurate picture of phase relations could be attained.

Bilateral Forelimb Pair (Bekoff & Trainer Method)

The phase of the forelimb bilateral pair was analyzed by a 2 X 5 ANOVA with genotype (*jp*/Y or control) and age (days 3-11) as the two within-subjects variables. There was no significant difference between *jp*/Y and control pups, although there was a tendency for the control group to remain closer to the 0.5 phase value than the *jp*/Y group ($F(1,14)=3.981$, $p < .10$) (see Figure 24). There was no significant difference between *jp*/Y and control groups on

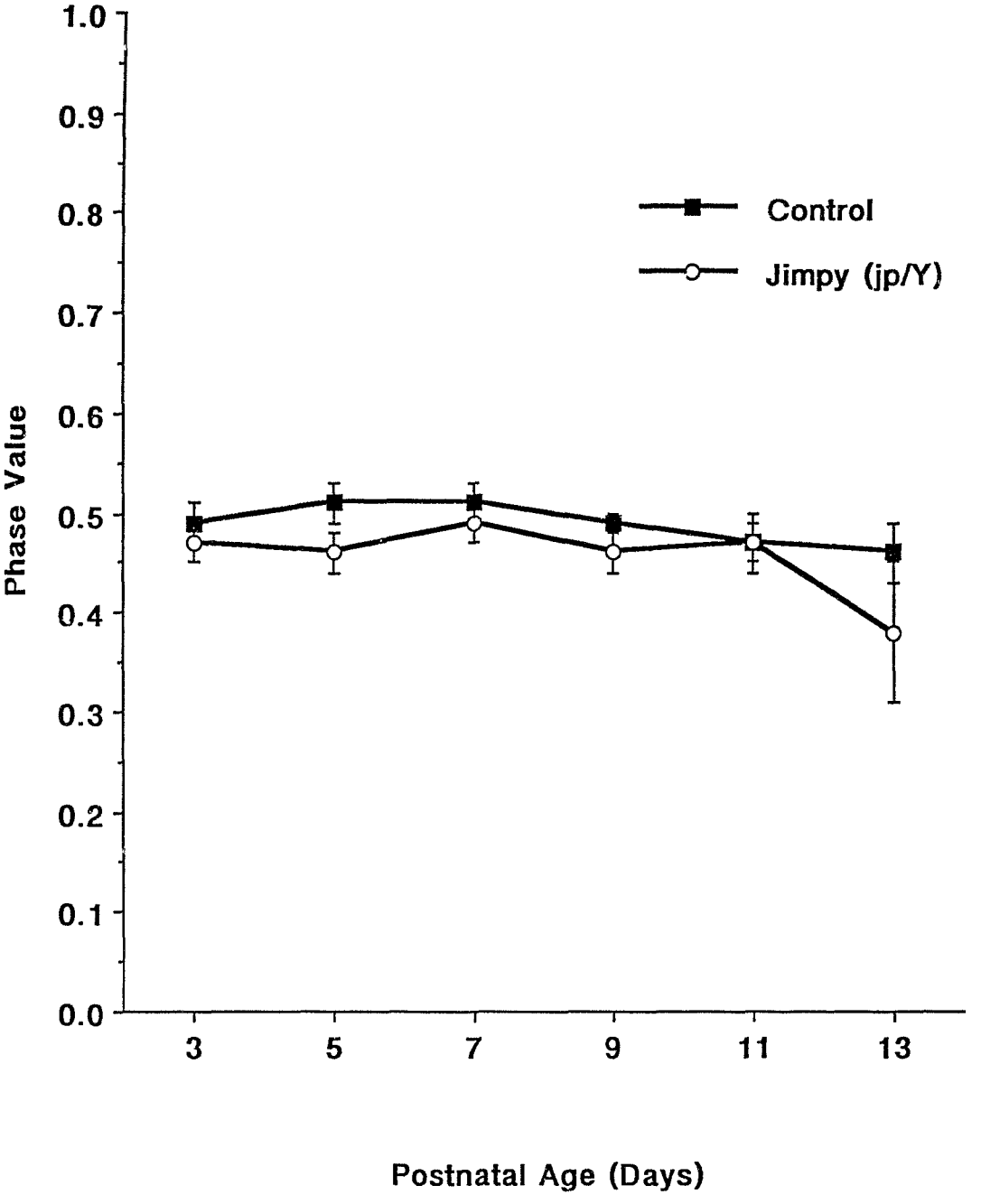
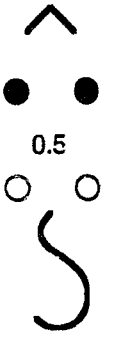


Figure 24. Mean phase value (± SEM) of the left forelimb relative to the right forelimb for control and jimpy (jp/Y) mice from 3 to 13 days of age (Bekoff & Trainer Method).



postnatal day 13 using the Mann-Whitney test. In general both groups obtained phase values close to 0.5, suggesting that the movement of one member of the pair of limbs was exactly out of phase with the movement of the other. There was no age main effect or interaction between genotype and age. These data are very similar to the results published on rats by Bekoff and Trainer (1979). However, this apparent precise 0.5 phase relations is in part an artifact of the method.

Bilateral Forelimb Pair (Corrected Method)

Phase of the forelimb bilateral pair was analyzed by a 2 X 5 ANOVA with genotype (*jp*/Y or control) and age (days 3-11) as the two within-subjects variables. There was no significant difference between *jp*/Y and littermate control groups across the ages included in the ANOVA and also no difference at postnatal day 13 using the Mann Whitney test. As with the calculation using the Bekoff and Trainer (1979) method *jp*/Y mice do not show a deficit in interlimb coordination with the forelimb pair. In fact the patterns displayed in Figure 24 and Figure 25 are similar in form, although the actual phase values are higher using the Bekoff and Trainer method of calculation. Figure 25 also looks very similar to the latency graph for the bilateral forelimb pair (see Figure 20), which also showed no differences between the two groups. Although there were some duration deficits in the individual forelimbs of the *jp*/Y mice (see Figure 4-6) these do not translate into interlimb coordination deficits.

As was the case with the phase values using the Bekoff and Trainer (1979) method (see Figure 24) there was no main effect of age and no genotype by age interaction. Both *jp*/Y and control groups display the same basic pattern, no phase differences as a function of age (see Figure 25). The

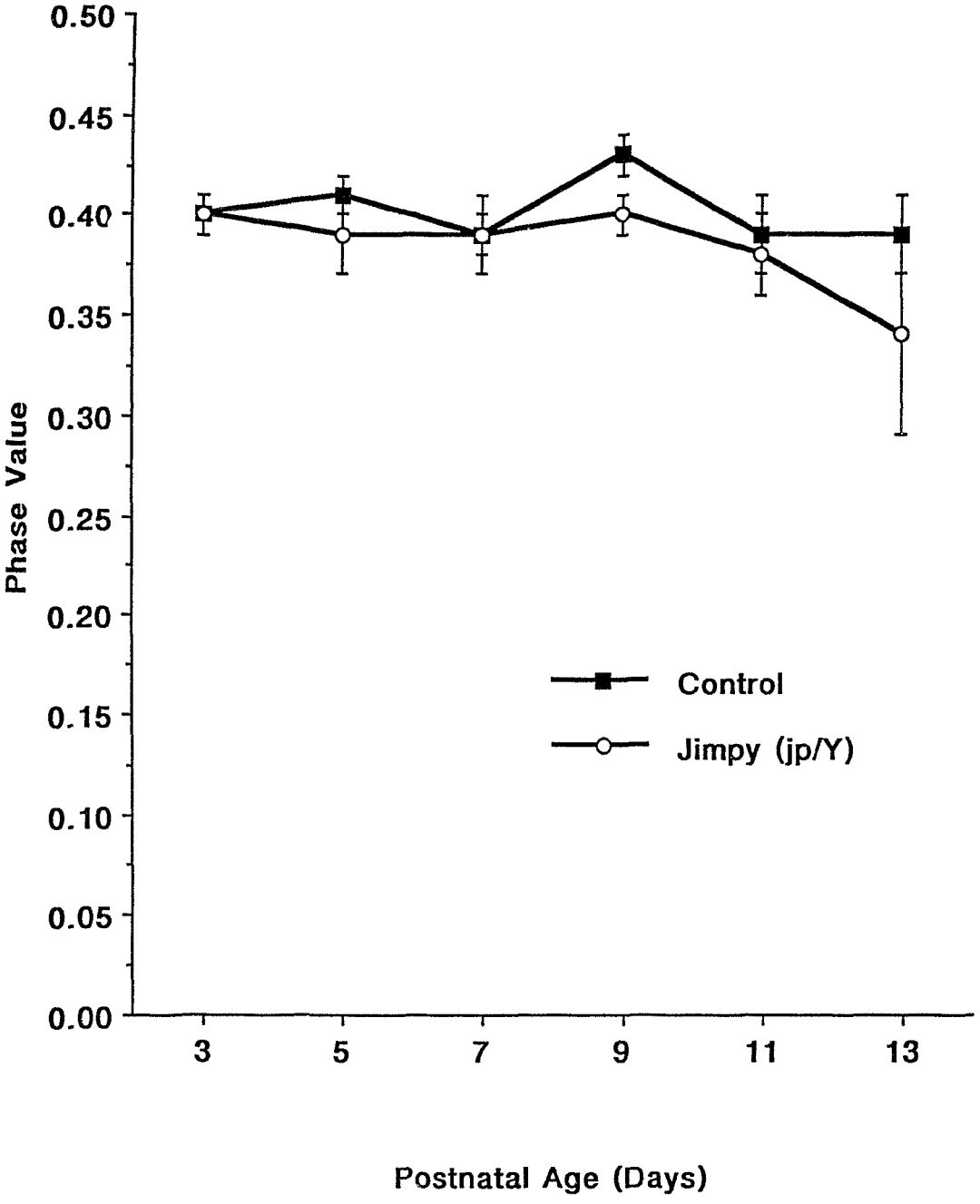
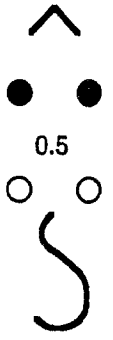


Figure 25. Mean phase value (± SEM) of the left forelimb relative to the right forelimb for control and jimpy (jp/Y) mice from 3 to 13 days of age (Corrected Method).



major difference between the two phase graphs (Figures 24 & 25) is in the mean phase values. The Bekoff and Trainer method (Figure 24) produces phases closer to 0.5, whereas the other method (Figure 25) produces somewhat lower than 0.5 (perfect out-of-phase) phase relations. However in both cases the phase value for these two limbs approaches 0.5.

Bilateral Hindlimb Pair (Bekoff & Trainer Method)

The phase of the bilateral hindlimb pair was analyzed by a 2 X 7 within-subjects ANOVA with genotype (*jp*/Y or control) and age (days 9-21) as the two within-subjects variables. There was a significant difference between *jp*/Y and control groups for the phase values of this limb pair ($F(1,14)=19.439$, $p<.01$), with *jp*/Y mice moving the two hindlimbs slightly more in phase (less than 0.5) than the control mice (see Figure 26). This difference became significant on postnatal day 13 ($p<.05$), using Tukey's test. Prior to postnatal day 9 there were no significant differences between *jp*/Y and control groups, using Mann-Whitney tests. There was no main effect due to age, or an interaction between genotype and age, as both groups of mice tended to maintain fairly constant scores close to 0.5 throughout the entire developmental period studied. There is more tendency for genotype differences in this bilateral limb pair than in the forelimbs (see Figure 24), although the differences are generally occurring after the forelimbs have dropped out of use. As all the measures used to study the hindlimbs tended to show *jp*/Y deficits, includin duration (Figures 14-16), missed strokes (Figures 17-19), velocity (Figu 12-13), and latency (Figure 21), these phase differences after postnatal day 11 are not unexpected. Again, these

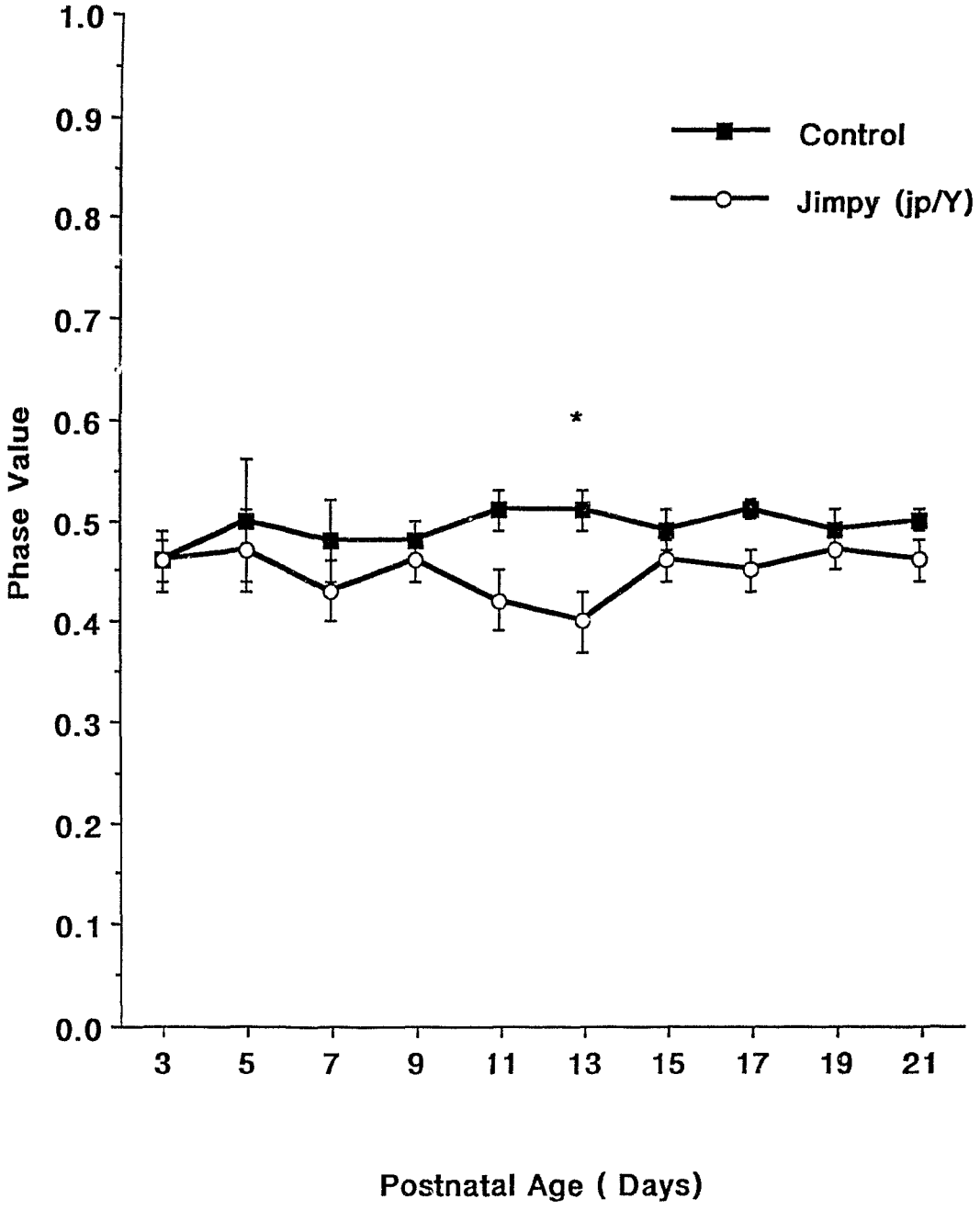
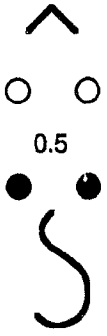


Figure 26. Mean phase value (± SEM) of the left hindlimb relative to the right hindlimb for control and jimpy (jp/Y) mice from 3 to 21 days of age (Bekoff & Trainer Method). *p≤ .05.



data are comparable with Bekoff & Trainer's (1979) findings, but are limited by the method employed.

Bilateral Hindlimb Pair (Corrected Method)

Phase of the hindlimb pair was analyzed by a 2 X 7 within-subjects ANOVA with genotype (*jp*/Y or control) and age (days 9-21) as the two within-subjects variables. There was a significant main effect due to genotype ($F(1,14)=12.400$, $p<.01$), with *jp*/Y mice moving the two hindlimbs slightly more in-phase (closer to 0.0) than the control mice (see Figure 27). This difference was significant on postnatal day 13 ($p<.05$), using Tukey's test. Prior to postnatal day 9 there were no significant differences between *jp*/Y and control groups, using Mann-Whitney tests. There was no main effect due to age, or an interaction between genotype and age. Once again, in comparison with Bekoff and Trainer (see Figure 26), while the overall pattern of the graphs are similar the corrected method of phase calculation provides more genotype differences, especially after postnatal day 15. As was the case with the phases calculated in the Bekoff and Trainer methodology, there were more genotype differences in this bilateral limb pair than in the forelimb pair (see Figure 24), although the differences generally occurred after the forelimbs had dropped out of use. As all the measures used to study the hindlimbs tended to show *jp*/Y deficits, including duration (Figures 14-16), missed strokes (Figures 17-19), velocity (Figures 12-13), and latency (Figure 21), these phase differences are consistent with the pattern of deficits after postnatal day 11.

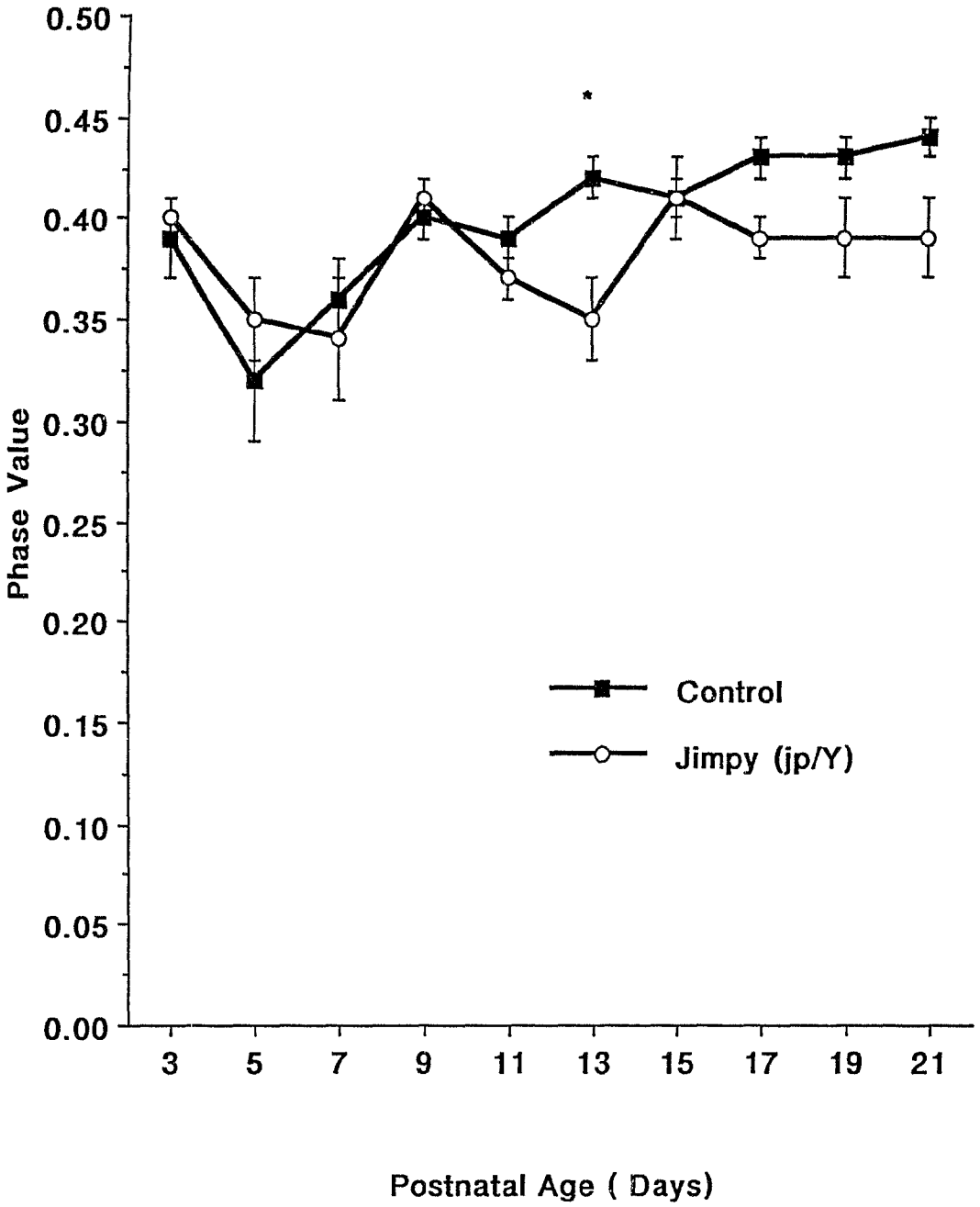
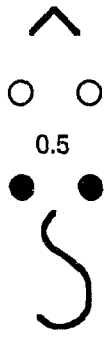


Figure 27. Mean phase value (± SEM) of the left hindlimb relative to the right hindlimb for control and jimpy (jp/Y) mice from 3 to 21 days of age (Corrected Method). *p≤ .05



Ipsilateral Limb Pair Phase (Bekoff & Trainer Method)

In the case of the ipsilateral pair, the phase relationship approached 0.5 for both groups, with the Bekoff and Trainer (1979) method again suggesting that the two limbs of the pair were alternating. Due to the fact that the front and hind limbs were used in combination only on postnatal days 9 and 11, the usefulness of the ANOVA was limited. Only on postnatal day 11 did the difference between *jp*/Y and control animals approach significance ($U(n=15,15) = 69$ and 156, $p<.10$), using the Mann-Whitney test, indicating that the mutant animals might be moving these two limbs more in phase than the controls (see Figure 28). There was no obvious change in phase as a function of age, as across the developmental period studied the phase values remained close to 0.5.

Ipsilateral Limb Pair Phase (Corrected Method)

In contrast, the ipsilateral phase relationship calculated using the corrected method (see Figure 29) displays phases considerably lower than 0.5 for both groups. This indicates that the ipsilateral pair of limbs were moving not as exactly alternating as the Bekoff and Trainer calculation would suggest. Instead the phase values were closer to 0.3 than the ideal 0.5 demonstrated using the Bekoff and Trainer calculation.

Usefulness of the ANOVA was again limited due to the combination of forelimb and hindlimb movements. In contrast to Figure 28, a significant difference between *jp*/Y and control mice emerged at postnatal day 5 ($U(n=11,13) = 26$ and 117, $p<.01$), using the Mann-Whitney test, indicating that the mutant animals might be moving these two limbs more out of phase than the controls on that particular day (see Figure 29). This difference was

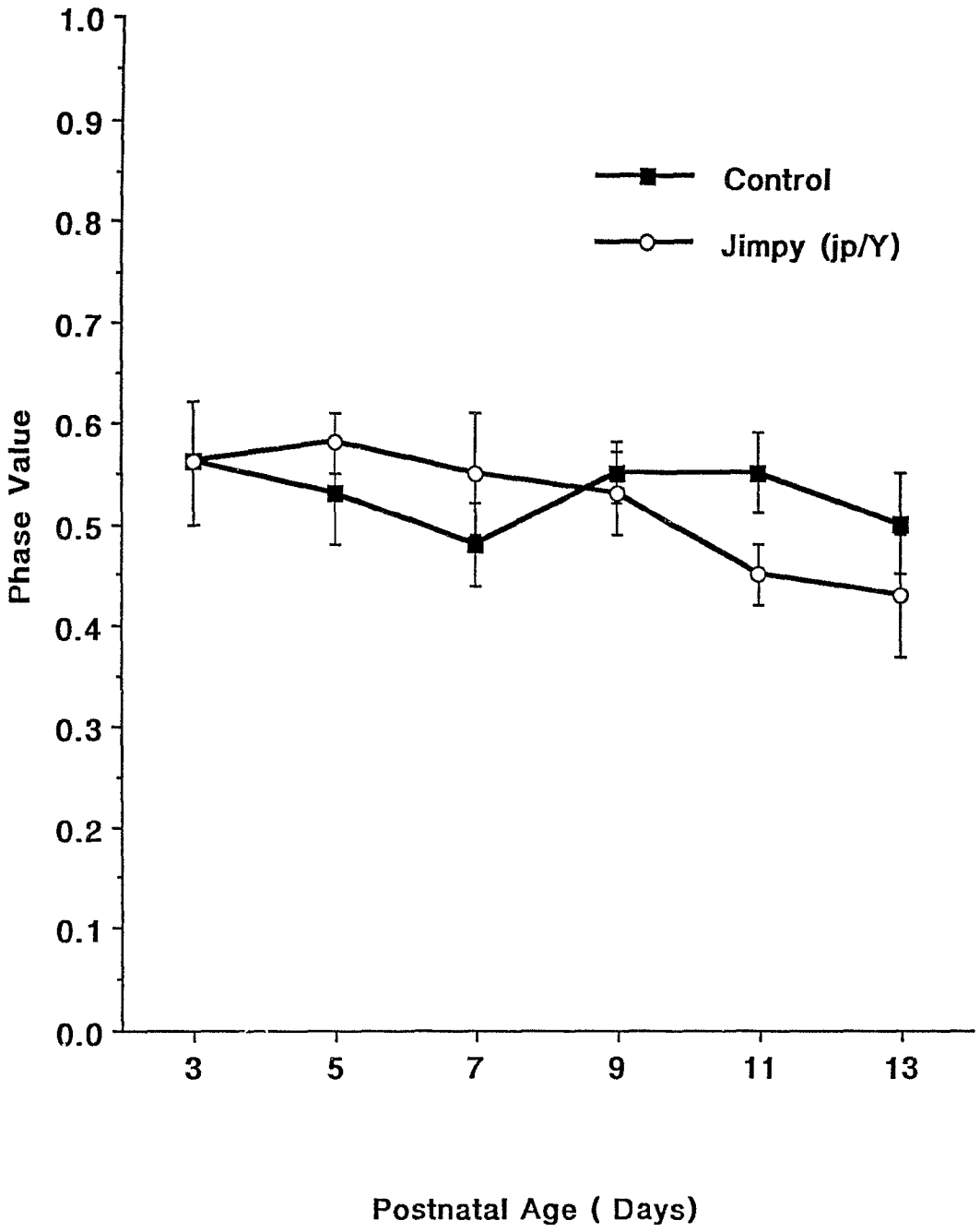
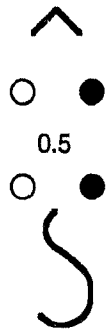


Figure 28. Mean phase value (± SEM) of the right hindlimb relative to the right forelimb for control and jimpy (jp/Y) mice from 3 to 13 days of age (Bekoff & Trainer Method).



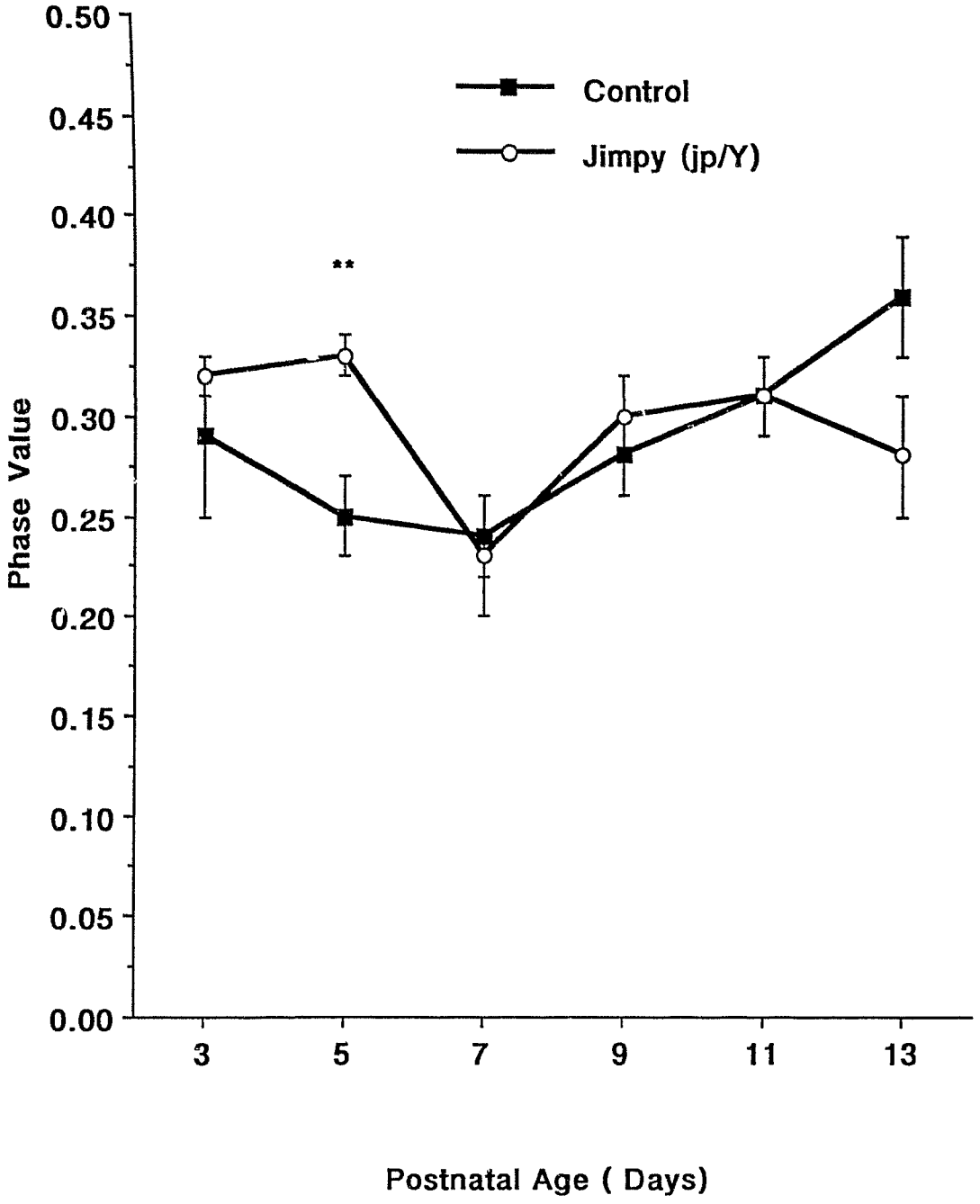
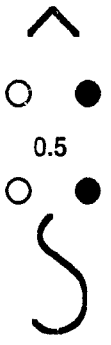


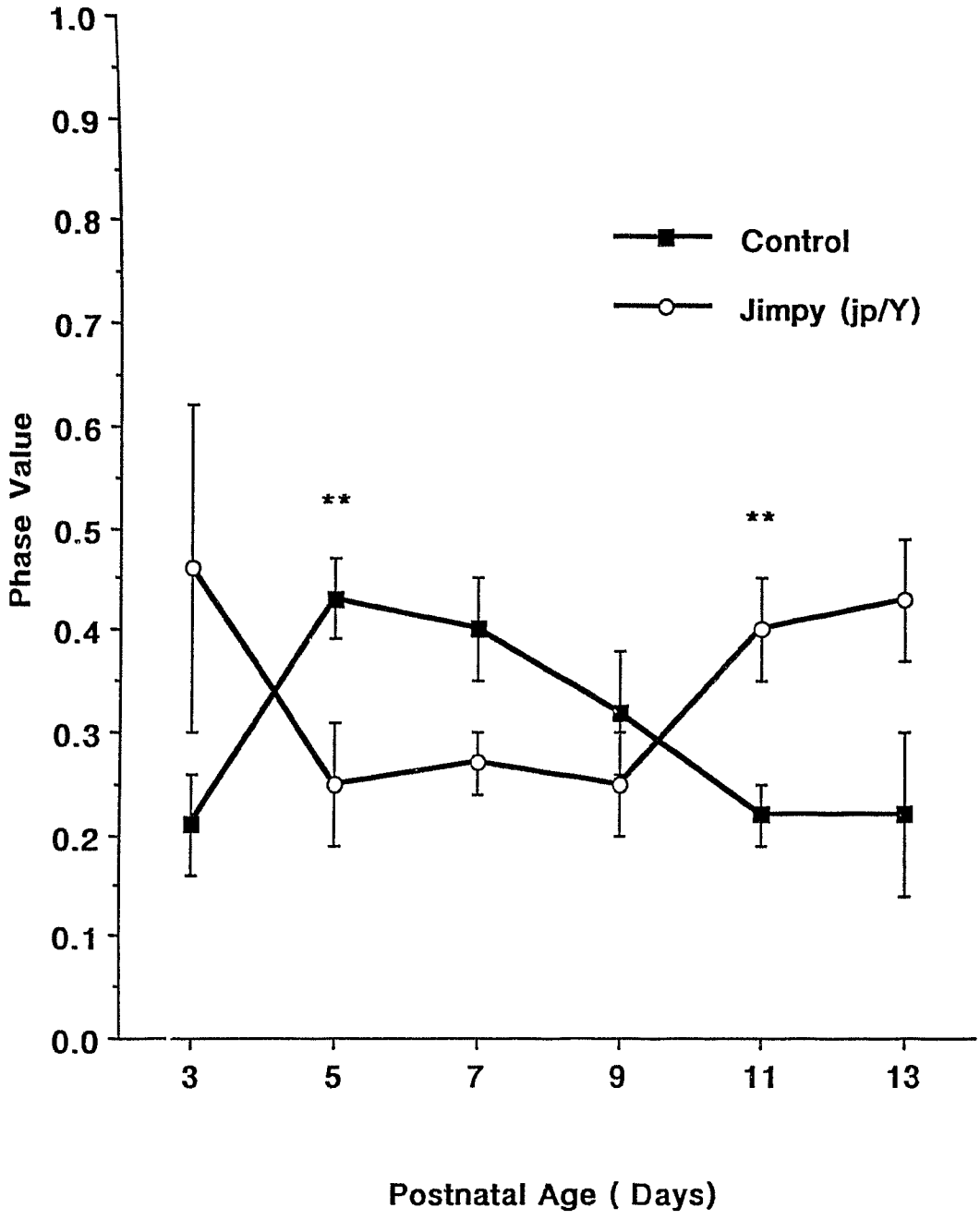
Figure 29. Mean phase value (± SEM) of the right hindlimb relative to the right forelimb for control and jimpy (jp/Y) mice from 3 to 13 days of age (Corrected Method). **p≤ .01.



more pronounced than that produced by the Bekoff and Trainer calculation of phase (see Figure 28). There was also a tendency for the *jp*/Y group to be more in-phase than the control group at postnatal day 13 (U(n=8,9)= 15.5 and 56.5, $p<.05$), indicating that the mutant animals were more likely to move the ipsilateral limbs together than the littermate control mice were. This difference was not as obvious using the Bekoff and Trainer calculation of phase and in fact the postnatal day 11 distinction between the groups presented in Figure 28 does not emerge when the phase is calculated in the corrected way (see Figure 29). There was a tendency for the littermate control group to show higher phase values with age indicating that as they age mice become better at producing out of phase coordination in this limb pair. However, for the *jp*/Y group this was not the case and in fact after postnatal day 11 they actually show a decrease in ability to produce out of phase coordination.

Contralateral Limb Pair Phase (Bekoff & Trainer Method)

With the phase of the contralateral pair (right forelimb and left hindlimb) an ANOVA was not practical as only two days could be included in the analysis. Therefore, Mann-Whitney tests for individual days were used. *Jp*/Y mice displayed lower phase values (closer to the ideal 0.0) than control mice on postnatal day 5 (*U* (n=13,11)=24 and 119, $p<.01$), indicating that they tended to move the contralateral pair of limbs closer together in time than did the control mice (see Figure 30). However, the reverse is the case by postnatal day 11 (*U* (n=15,15)=51 and 171, $p<.01$) and to a lesser extent day 13 (*U* (n=8,9)=17 and 55, $p<.10$), at which time the *jp*/Y mice appear to



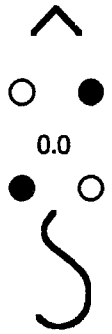


Figure 30. Mean phase value (± SEM) of the left hindlimb relative to the right forelimb for control and jimpy (jp/Y) mice from 3 to 13 days of age (Bekoff & Trainer Method). **p≤ .01.

be alternating the contralateral limbs instead of moving them together, in contrast to the control animals (see Figure 30).

A comparison of ipsilateral (Figure 28) and contralateral (Figure 30) mean phase values illustrates how the *jp*/Y and control groups differ in terms of the relationship between the right forelimb and the two hindlimbs. For control mice the phase relationships on postnatal day 11 are quite different (ipsilateral phase=.55 and contralateral phase=.22). However, in the case of the *jp*/Y mice the phase values are virtually the same (ipsilateral phase=.45 and contralateral phase=.40). As was the case with the comparison of ipsilateral and contralateral latencies (Figures 22 & 23) this represents a distinction between *jp*/Y and littermate controls in terms of limb coupling between the right forelimb and the hindlimbs.

Contralateral Limb Pair Phase (Corrected Method)

The contralateral limb pair (right forelimb and left hindlimb) phase relationship using the corrected method (see Figure 31) was again somewhat different than that obtained using the Bekoff and Trainer method of phase calculation (see Figure 30). Once again an ANOVA was not practical in this situation as only on two days did all mice use both forelimbs and hindlimbs. Mann-Whitney tests for individual days were again used as an alternative to the ANOVA. *Jp*/Y mice displayed lower phase values (closer to the ideal 0.0) than control mice on postnatal day 5 (U (n=13,11)=15.5 and 127.5, $p<.01$) indicating that they tended to move the contralateral pair of limbs closer together in time than did the control mice (see Figure 31). This is the same type of genotype difference seen in the contralateral phases as calculated by Bekoff and Trainer (see Figure 30). The reverse was the case after postnatal

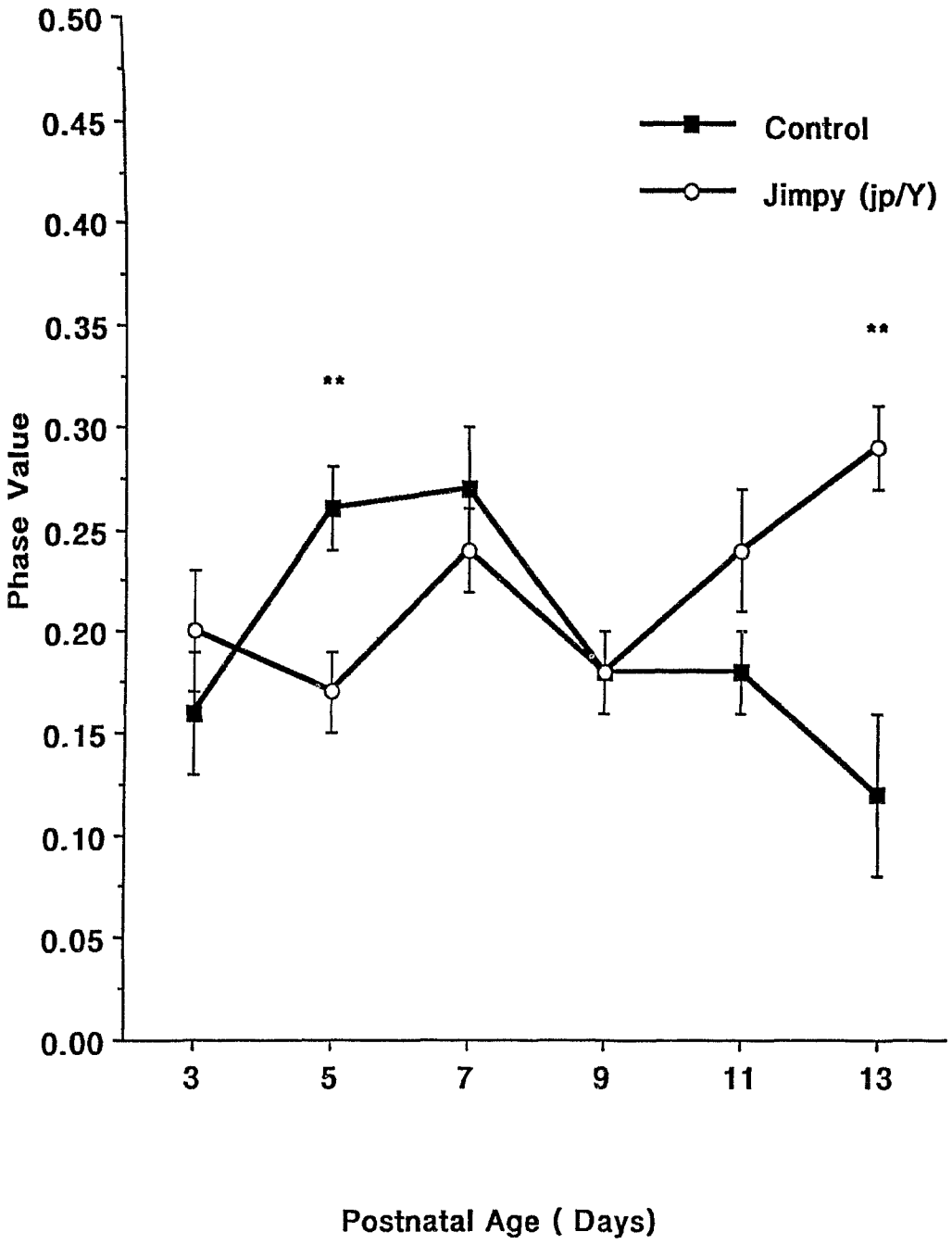


Figure 31. Mean phase value (± SEM) of the left hindlimb relative to the right forelimb for control and jimpy (jp/Y) mice from 3 to 13 days of age (Corrected Method). **p≤ .01.

day 9, with a tendency on postnatal day 11 (U (n=15,15)=64 and 159, $p<.05$) and a significant difference on postnatal day 13 (U (n=8,9)=7.5 and 64.5, $p<.01$) for *jp*/Y mice to display more out of phase movement involving the contralateral pair of limbs than littermate controls. At this time in development the *jp*/Y mice appear to be alternating the contralateral limbs instead of moving them together. in contrast to littermate control animals. This phase difference was also evident in the Bekoff and Trainer method of calculation (see Figure 30).

A comparison of ipsilateral (Figure 29) and contralateral (Figure 31) mean phase values on postnatal day 13 provides an interesting illustration of how *jp*/Y and littermate control groups differ in terms of the relationships between the right forelimb and one of the hindlimbs. The phase values on that day are quite different for littermate control mice (ipsilateral phase=.36 and contralateral phase=.12), but in the case of the *jp*/Y mice they are virtually identical (ipsilateral phase=.28 and contralateral phase=.29). As was the case with the comparison of ipsilateral and contralateral latencies (Figures 22 & 23), and phase calculated by the Bekoff and Trainer method (Figures 28 & 30) this represents a distinction between *jp*/Y and littermate controls in terms of limb coupling between the right forelimb and the hindlimbs.

Summary of Phase Comparisons

The Bekoff and Trainer (1979) method greatly inflated the out of phase relations (0.5), giving a misleading picture of swimming gaits. This is in large part due to averaging of points both above and below the 0.5 value. In the corrected analysis only the extent of deviations in a single direction contributed

to the scores. This avoids the confounds of distance and direction of data point deviations from 0.5.

Additionally, while the overall shape of many graphs was similar for the two methods, several important distinctions were observed. Overall the 0.5 phase relationship obtained for both bilateral limb pairs and the ipsilateral pair using the Bekoff and Trainer method was not obtained using my corrected method of phase calculations. Phase values tended to be slightly lower than 0.5 in each of these three cases. In the case of the contralateral pair, which in a normal quadruped gait would be expected to have a phase value of 0.0, the corrected method obtained values closer to 0.0 than did the Bekoff and Trainer method. Finally, by collapsing the data around the 0.5 phase relationships important distinctions and trends in the data were often obscured with the Bekoff and Trainer (1979) method. These include the continued tendency for differences between *jp*/Y and control groups after postnatal day 15 for the bilateral hindlimb pair, the significant difference between *jp*/y and control groups on postnatal days 5 and 13 for the ipsilateral pair and the significant difference between the two groups on postnatal day 13 in terms of the contralateral pair.

Consistency

Forelimb Durations

Coefficients of variation for forelimb stroke-cycle durations were analyzed by 2 X 5 ANOVAs with genotype (*jp*/Y or control) and age (days 3-11) as the two within-subjects variables. For the degree of consistency of right forelimb stroke-cycle durations there was no significant difference between *jp*/Y and

littermate control groups, nor was there a difference between the two genotypes on postnatal day 13 using the Mann-Whitney test (see Figure 32). Also evident in Figure 32, there was no significant main effect due to age and no interaction between genotype and age. In the case of the consistency of the stroke-cycle durations of the left forelimb a similar pattern emerged (see Figure 33). There were no main effects of genotype or age and no interaction. Nor was there a difference between the two genotypes on postnatal day 13 using the Mann-Whitney test. *Jp*/Y mice did not display any more inconsistency in stroke-cycle durations during the 10 stroke-cycle session for either forelimb than did littermate controls. Additionally, young mice were not significantly more inconsistent in their individual stroke durations than were older individuals. These findings are in contrast to the developmental decline in stroke-cycle duration (see Figure 5 & 6).

Hindlimb Durations

Coefficients of variation for the hindlimb stroke-cycle durations were analyzed by 2 X 7 ANOVAs with genotype (*jp*/Y or control) and age (days 9-21) as the two within-subjects variables. There was no significant difference in terms of consistency during the 10 stroke-cycle session between the *jp*/Y and littermate control groups (see Figure 34). There was also no significant difference between the two genotypes on days analyzed by Mann-Whitney tests (days 3-7). *Jp*/Y mice did not display more inconsistency during the 10 stroke-cycle session that did littermate controls. There was no significant difference in consistency during the 10 stroke-cycle session as a function age, although there was a slight decline with age in the period prior to the days included in the ANOVA (see Figure 34). There was, no interaction between

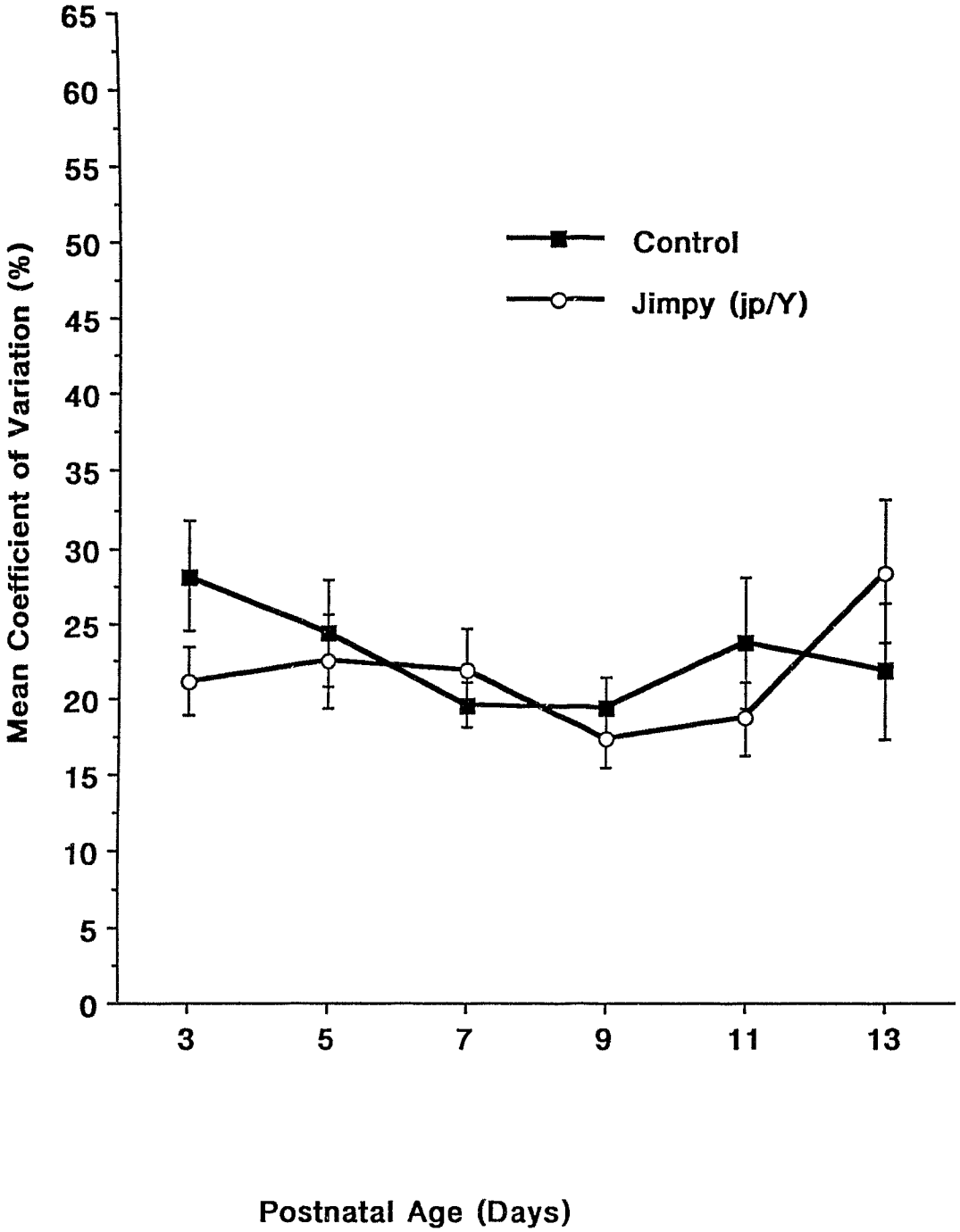


Figure 32. Mean coefficient of variation (±SEM) of the right forelimb durations for control and jimpy (jp/Y) mice from 3 to 13 days of age.

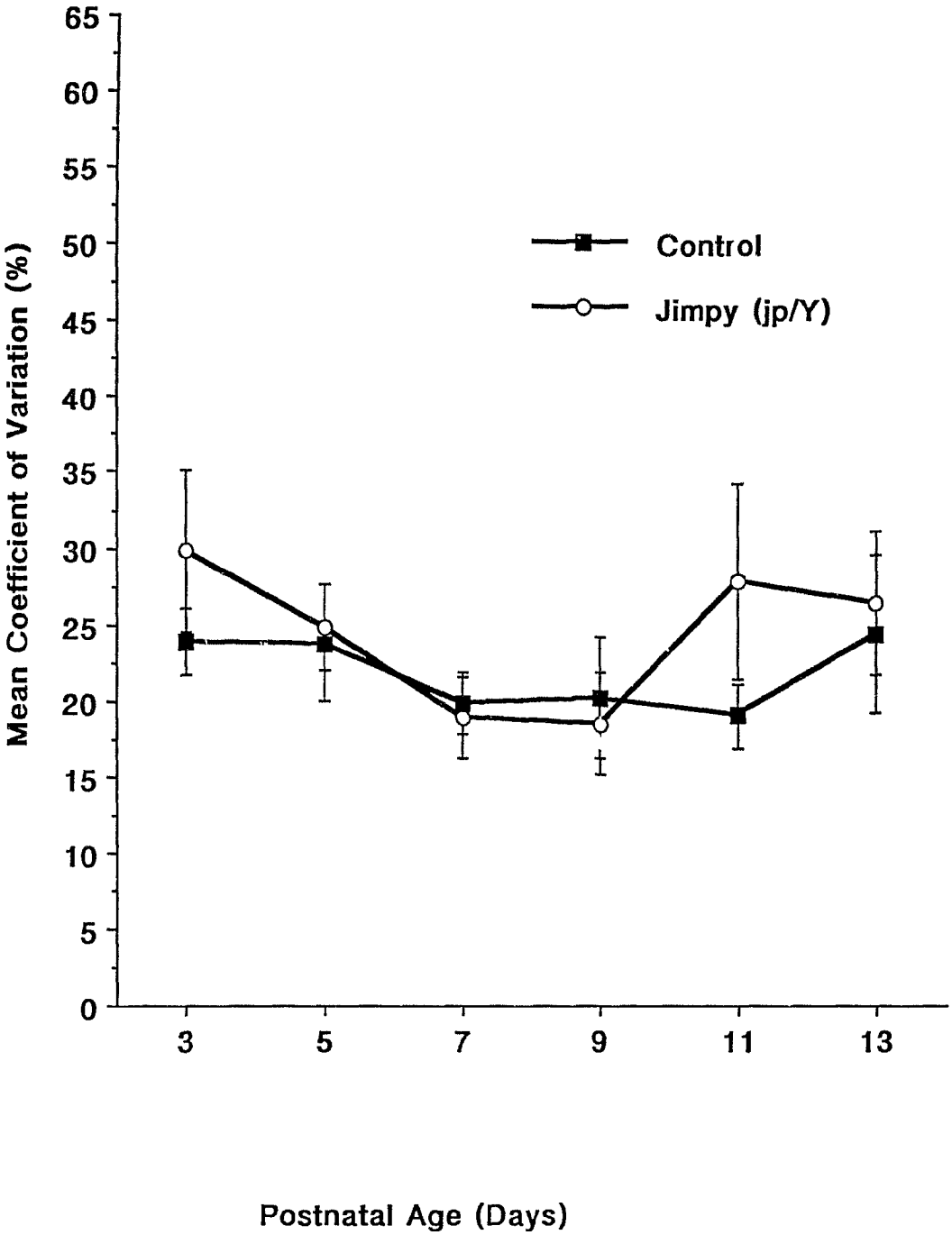


Figure 33. Mean coefficient of variation (±SEM) of the left forelimb durations for control and jimpy (jp/Y) mice from 3 to 13 days of age.

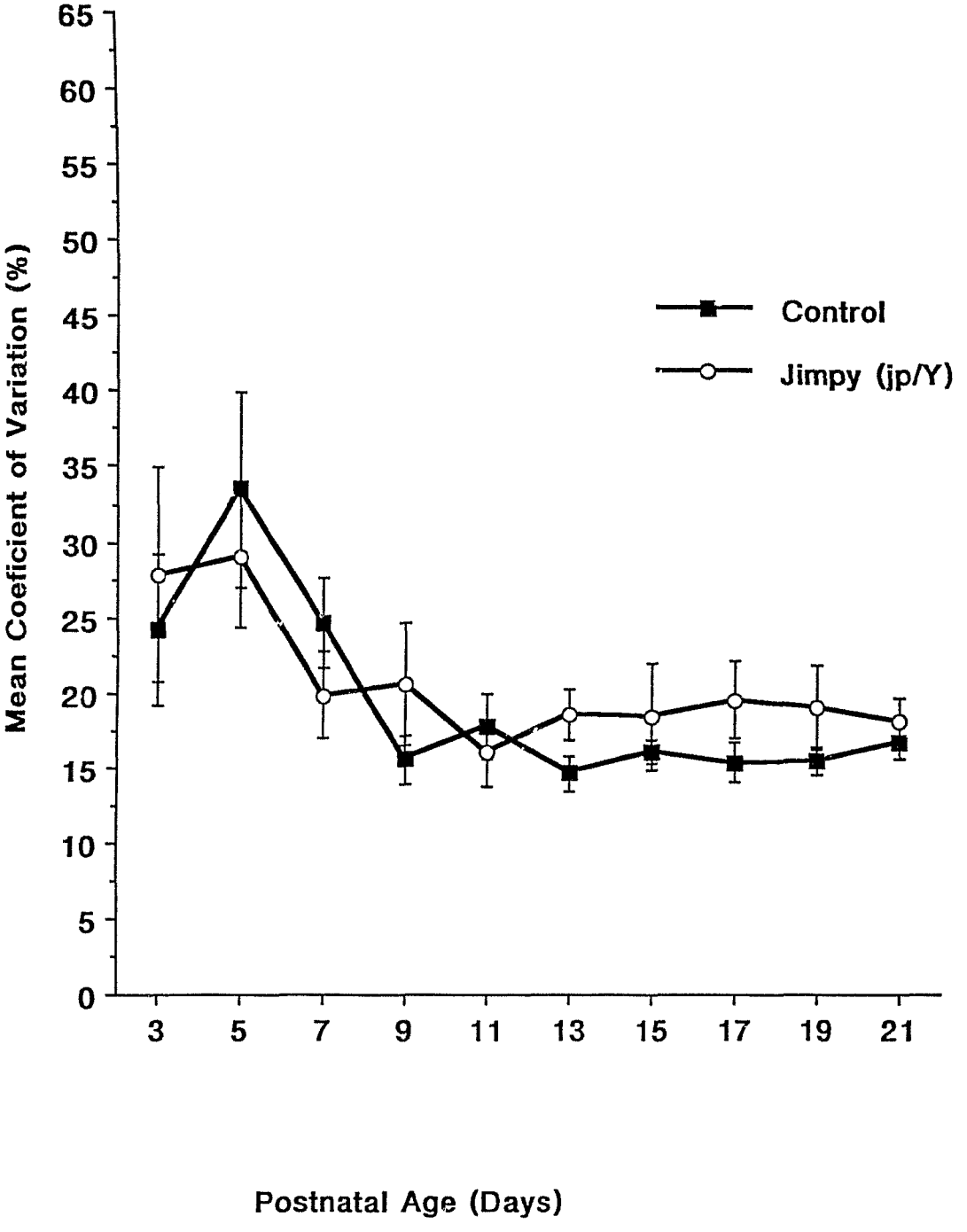


Figure 34. Mean coefficient of variation (±SEM) of the right hindlimb durations for control and jimpy (jp/Y) mice from 3 to 21 days of age.

genotype and age. The findings for the left hindlimb were similar to those of the right hindlimb. There were no genotype differences, no significant age effects and no interaction (see Figure 35). However, once again, in the postnatal period not included by the ANOVA, there was a slight decline in variation for both *jp*/Y and littermate control groups. Although the age trends for the hindlimbs were not significant, they showed similar developmental trends as that displayed in the mean stroke-cycle duration graphs (see Figures 10 & 11).

Bilateral Forelimb Phase

The consistency of the phase values across the 10 stroke-cycle session were analyzed in a 2 X 5 ANOVA with genotype (*jp*/Y or control) and age (days 3 - 11) as the two within-subjects variables. There was no significant difference in phase variability over the 10 stroke-cycle session between the *jp*/Y and control groups (see Figure 36). There was also no significant difference between the two groups on postnatal day 13 via the Mann-Whitney test. There was no main effect due to age or any interaction between genotype and age (see Figure 36). There does not appear to be an increase in consistency within the swim session as a function of age for either group.

Bilateral Hindlimb Phase

The variability of the phase values across the 10 stroke-cycle session were analyzed in a 2 X 7 ANOVA with genotype (*jp*/Y or control) and age (days 9 - 21) as the two within-subjects variables. There was no significant difference in phase variability over the 10 stroke-cycle session between the *jp*/Y and control groups, although there was some hint that after postnatal day 11 *jp*/Y

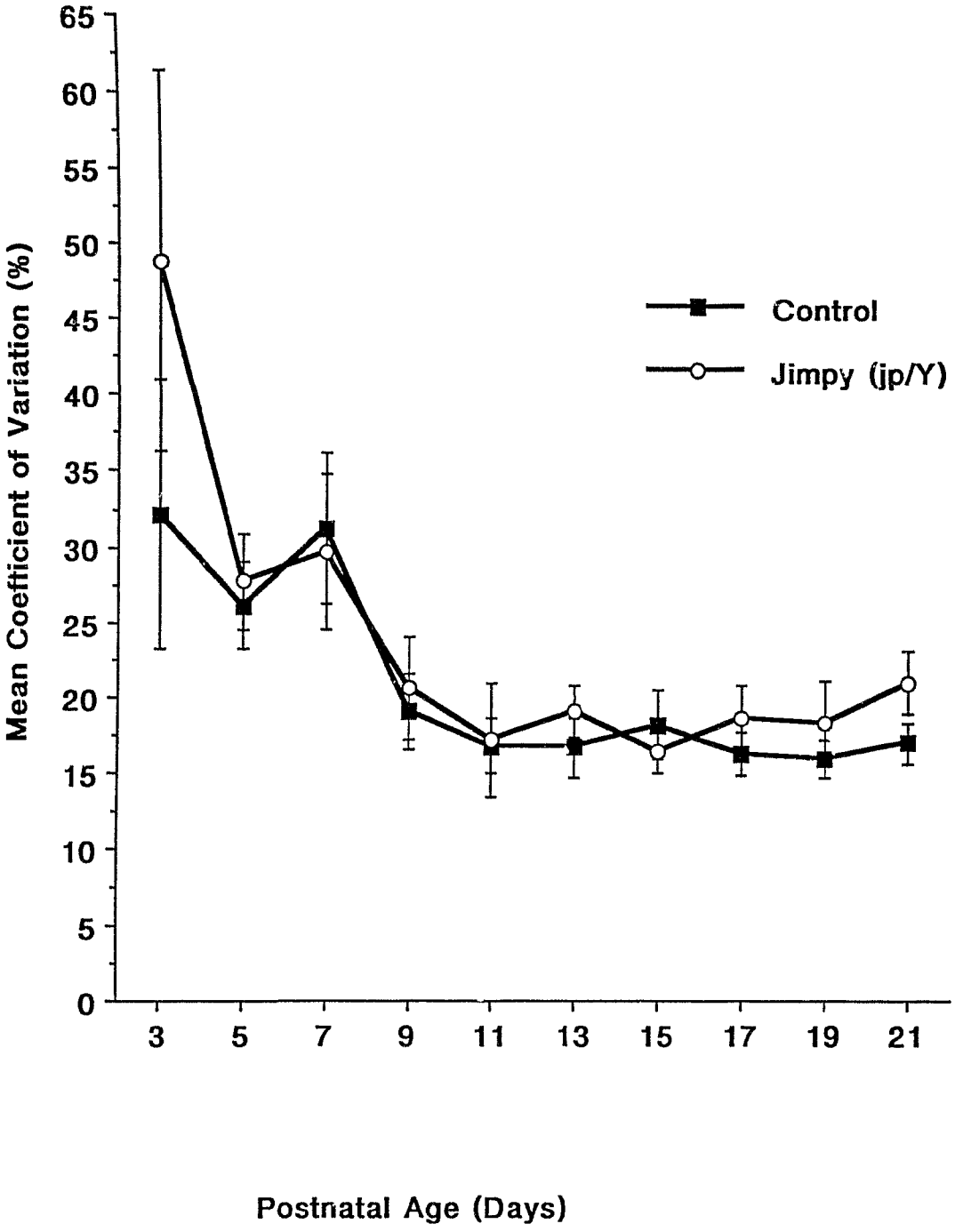


Figure 35. Mean coefficient of variation (±SEM) of the left hindlimb durations for control and jimpy (jp/Y) mice from 3 to 21 days of age.

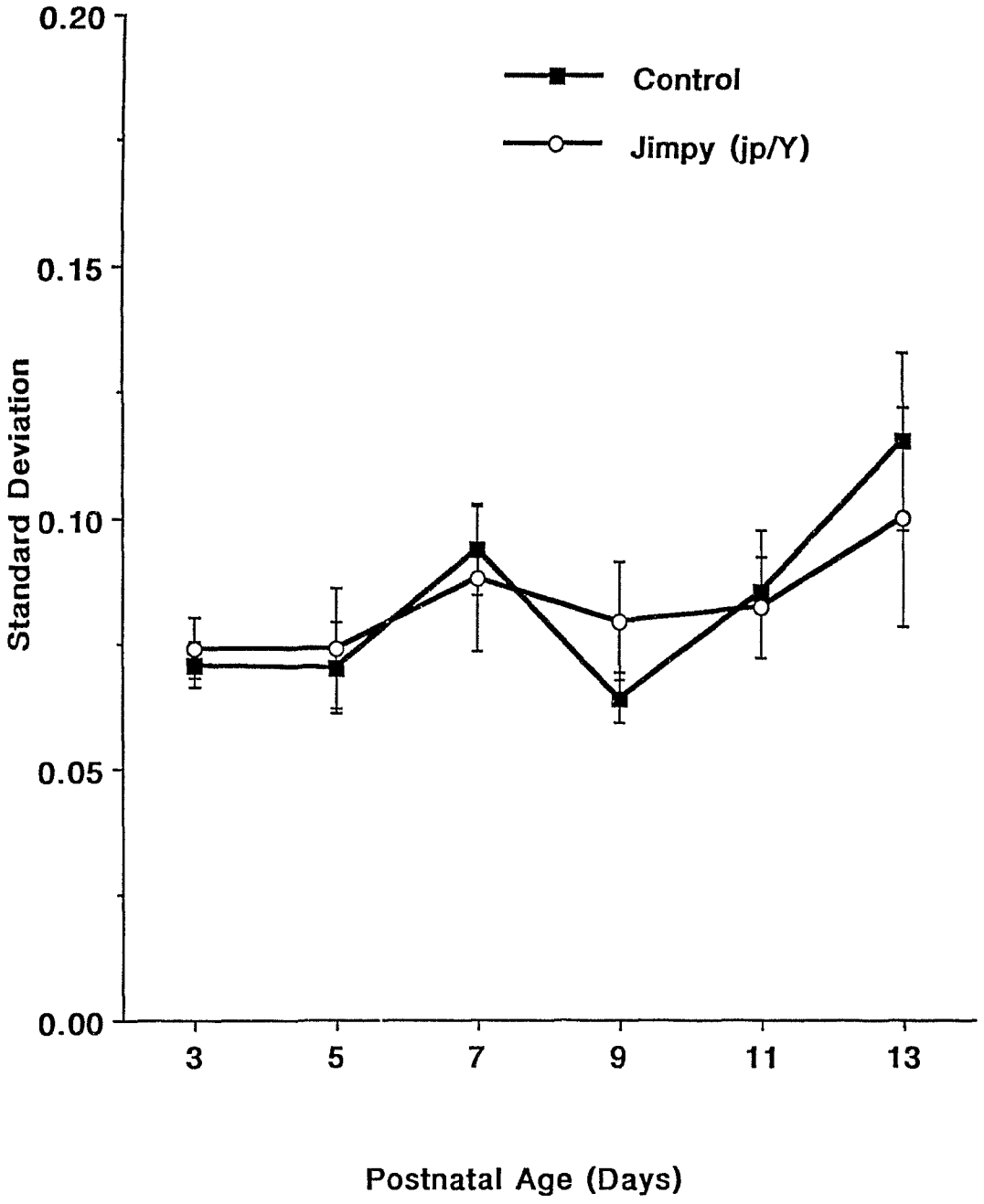
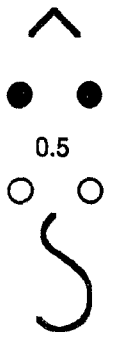


Figure 36. Mean standard deviation (±SEM) of the forelimb bilateral phase values for control and jimpy (jp/Y) mice from 3 to 13 days of age.



displayed more variability than did littermate controls (see Figure 37). There was no significant differences between the two groups on postnatal days 3-7 via Mann-Whitney tests. As can be seen in Figure 37, the variability did not change significantly with age and there was no interaction between genotype and age.

Ipsilateral Limb Phase

In the case of the ipsilateral pair there was no significant difference between *jp*/Y and control groups at any of the days tested via the Mann-Whitney test (see Figure 38). Only on postnatal days 7 and 11 did the difference between *jp*/Y and control groups approach significance. On day seven the littermate control group had a tendency to display a higher variability than the *jp*/Y group ($U\ (n=15,12)=51$ and 129, $p<.10$), whereas on day 13 the reverse was the case ($U\ (n=8,9)=17$ and 55, $p<.10$). Of note is the fact that the phase value variation for either bilateral limb pair (Figures 36-37) was less than that of the ipsilateral pair.

Contralateral Limb Phase

There was no significant difference between the *jp*/Y and littermate control groups on any of the days analyzed by Mann-Whitney tests (see Figure 39). Only on postnatal day 13 did the difference between the two groups approach significance ($U\ (n=8,9)=18$ and 54, $p<.10$), with the *jp*/Y groups showing higher levels of inconsistency than littermate controls. The variation of the phase values of the contralateral limb pair was greater than that of either bilateral limb pair (Figures 36-37).

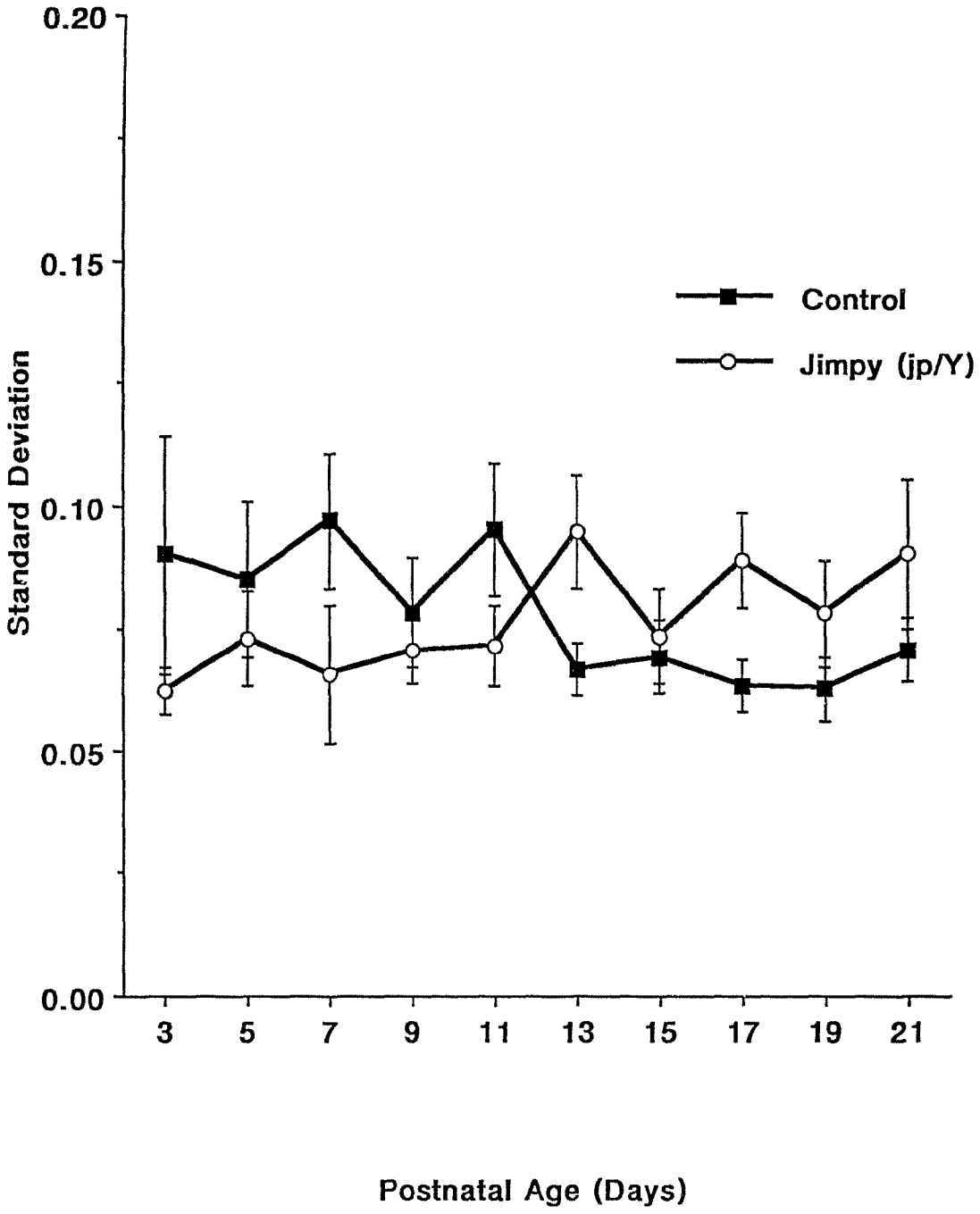
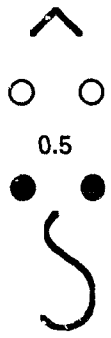
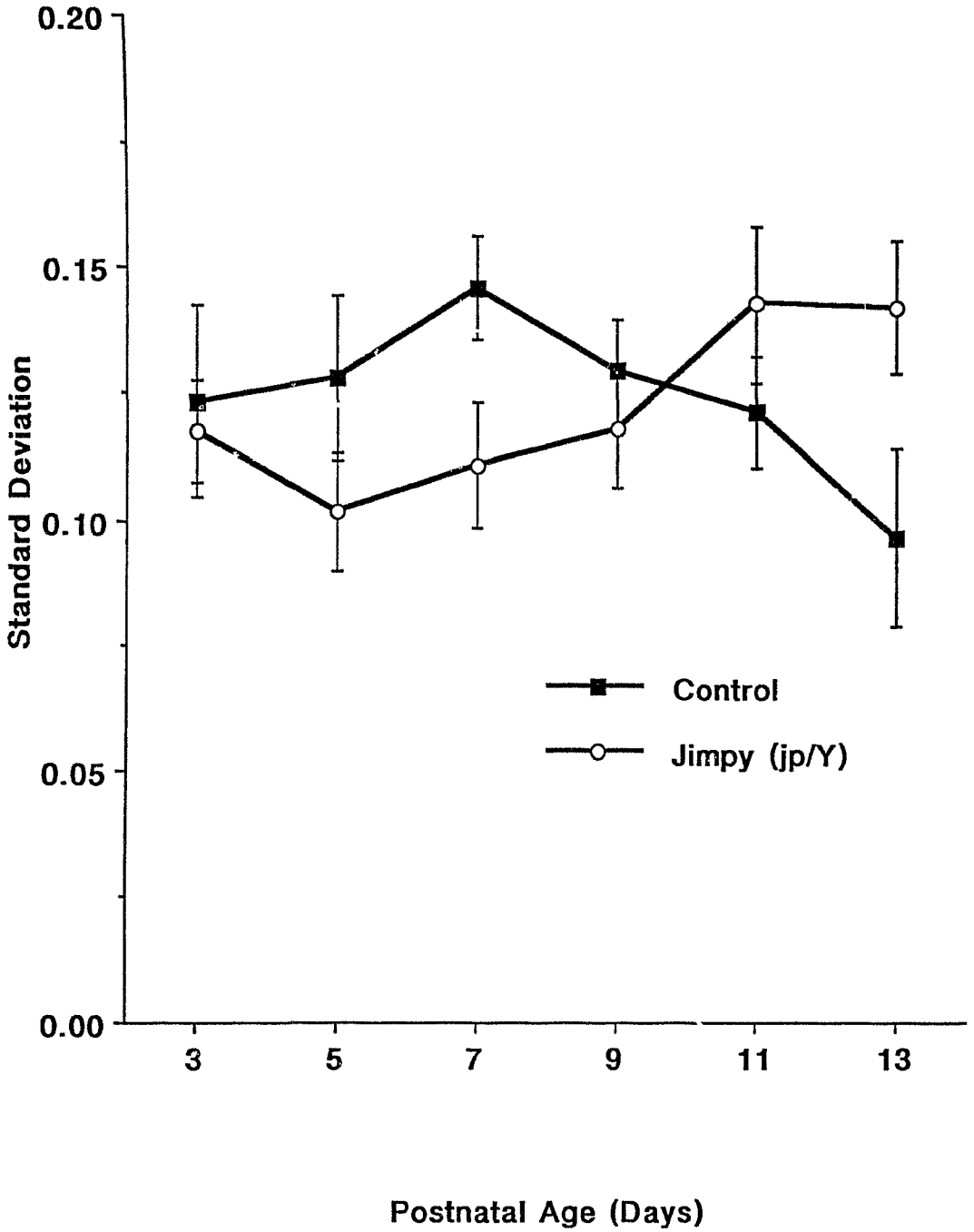


Figure 37. Mean standard deviation (±SEM) of the hindlimb bilateral phase values for control and jimpy (jp/Y) mice from 3 to 21 days of age.





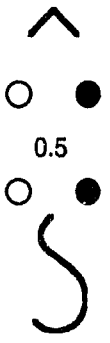


Figure 38. Mean standard deviation (±SEM) of the ipsilateral limb phase values for control and jimpy (jp/Y) mice from 3 to 13 days of age.

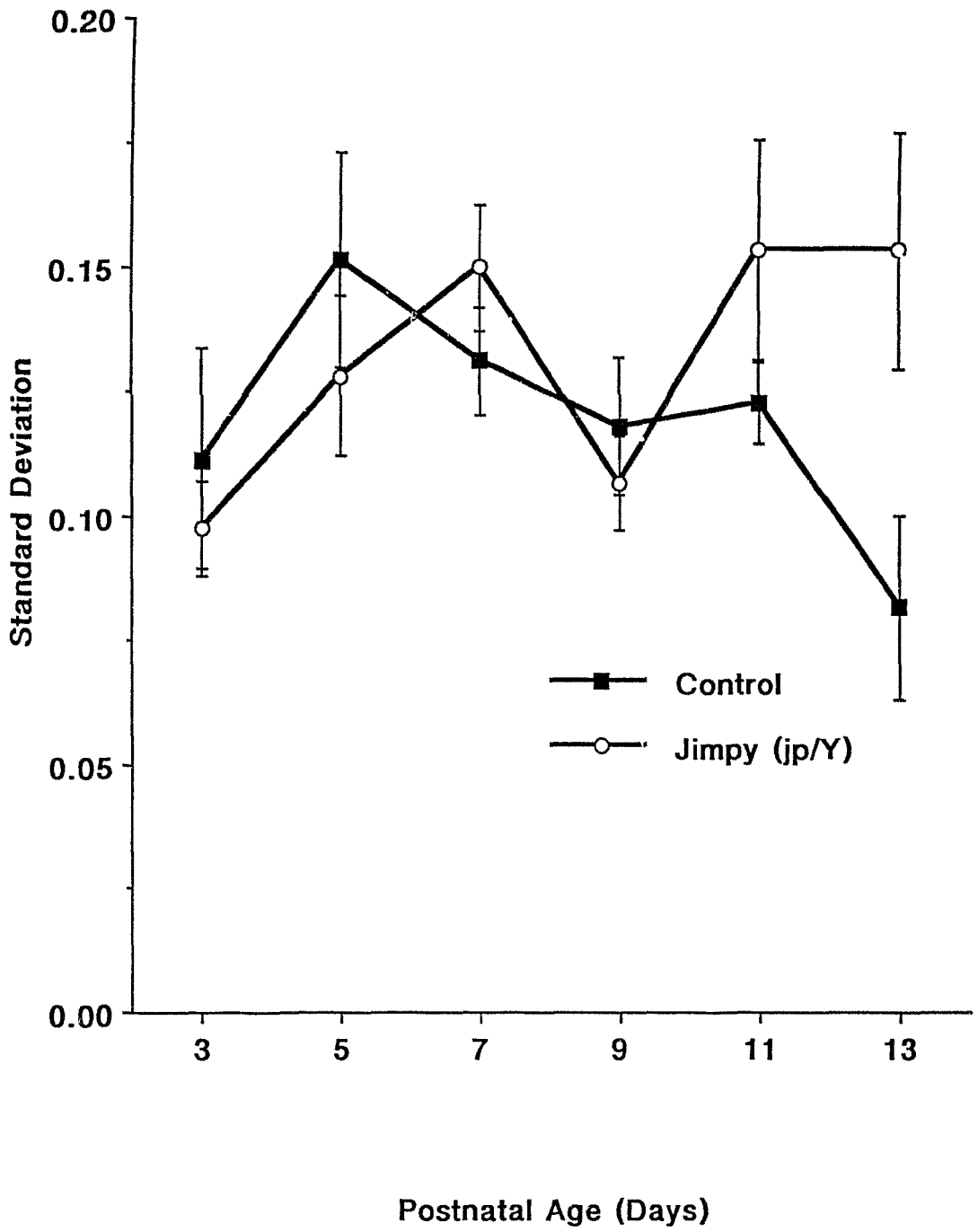
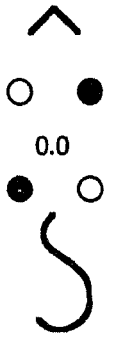


Figure 39. Mean standard deviation (±SEM) of the contralateral limb phase values for control and jimpy (jp/Y) mice from 3 to 13 days of age.



DISCUSSION

It is informative to compare the various graphs provided in this thesis. A fundamental question is to what extent one set of developmental profiles corresponds with another. When profiles are similar, shared causal processes may be operating. However, divergence among profiles indicates separable developmental events.

Weight Profiles and Their Relation to Basic Movements

The weight change profiles (Figure 1) express continuous weight changes with age, with a progressive divergence between *jp*/Y and control mice. Indeed, the continuous weight gain in control animals can be contrasted with a descriptive weight decline in *jp*/Y mice from postnatal day 17. As seen in Figure 2, however, the *jp*/Y and control mice have each achieved 100% hindlimb swimming by postnatal day 17, and maintain their mature swimming patterns through postnatal day 21. While there is some suggestion that *jp*/Y mice are developmentally retarded, this does not represent a significant difference, nor can it be accounted for by patterns of weight change.

One of the key arguments that might have otherwise been raised relative to behavioral differences between jimpy and littermate controls, or for that matter differences between neurological mutant mice and their littermates more generally, is whether the mutation is the direct cause of the differences or if physiological variables such as weight or weakness play an intermediate role. Bolivar and Brown (1994) also addressed this issue in their description of early behavioral effects of the jimpy mutation. As we suggest, it is possible that weight differences may account for some of the behavioral abnormalities in

the jimpy mouse, such as grooming and locomotion deficits. However, other behavioral differences we observed (i.e., slight movement and ultrasonic vocalization production) precede weight deficits, which do not become evident until postnatal day six. Weight differences in this thesis become statistically significant on postnatal day seven and complement those found by Bolivar and Brown (1994), although the mice were weighed on alternate postnatal days in the two studies.

Swimming abnormalities cannot simply be explained as resulting from weight differences. Although many of the differences between jimpy and littermate controls emerge after the weight differences have become significant, other differences such as the phase differences occurring on postnatal day five precede them. Nor do the swimming deficits observed later in postnatal development increase relative to the increased difference in weight between mutant and control animals. If weight were the sole cause of the swimming abnormalities, one would expect that as the weight of the two groups continue to diverge, so too should the differences in swimming ability. However, this is not the case. Often postnatal days 11 and 13 show larger differences between the two groups than later in development, when the weight difference is at its greatest.

Additionally, other neurological mutants with similar weight deficits as the jimpy mouse show very different swimming behavior profiles. For instance, the weaver mutant shows slower limb movements during the early part of the postnatal period (days three and five) at the same time as they show a general delay in swimming style; however, they do not show any phase abnormalities between any pair of limbs (bilateral, ipsilateral or contralateral) (Bolivar, Manley & Fentress, 1996). The weight differences between homozygous weaver mice and littermate controls look very similar to that of

jimpy mice and their littermate controls (Bolivar, Manley & Fentress, 1996), showing no significant differences until postnatal day seven. In each case the difference between mutant and control groups increases with age. The weaver and jimpy control groups are similar in weight, as would be expected as both mutations are on the same CBA background. If weight were the sole cause of the behavioral differences, then one would expect similar abnormalities in swimming behavior for both types of neurological mutants, which is not the case.

Swim Styles in Relation to Individual Limb Parameters

Figure 3, on swim styles, confirms the close relationship between *jp*/Y and control mice. Note that this graph suggests major discontinuities in swim style over development that are basically superimposable for *jp*/Y and control mice. This discontinuous profile is markedly different from the continuous weight gain profiles of Figure 1. What is also apparent is that Figure 3 reveals no discernible differences in swimming style transitions between *jp*/Y and control mice. A major argument of this thesis is that broad categorization of behavioral style, and changes in this style during development, may be insufficient to document more subtle changes in motor performance produced by mutant genes.

Although not intuitively obvious, continuous changes in specific parameters of motor performance can contribute to overall discontinuities in higher level behavioral categories such as swimming style. For example stroke-cycle duration and velocity for individual limbs (Figures 4-13) have been shown to decrease and increase respectively in a more or less continuous fashion for both groups of mice. This means that changes in stroke-cycle duration and

velocity are alone insufficient explanations for the observed step-wise changes in swimming style (Figure 3). Dynamic systems and related mathematical models substantiate the possibility that combinations of continuous change can lead to step-functions at higher levels of organization (Kelso, 1995).

The data on limb stroke-cycle durations and velocities also indicate the importance of quantifying individual limb parameters for the swimming task. In terms of swimming style, the forelimbs precede the hindlimbs in use, with a forelimb to four limb to hindlimb progression in all mice. Forelimb stroke-cycle durations decrease and increase in a parallel fashion for both *jp*/Y and control mice (Figures 4-6), a fact also reflected in systematic and continuous changes in limb velocity (Figure 7). Measures of maximum forelimb velocity (Figure 8) also provide evidence of similar continuous changes in *jp*/Y and control mice. These maximum velocity increases deviate markedly from mean velocity, indicating that timing generator mechanisms are not rigidly fixed.

Hindlimb stroke-cycle durations (Figures 9-11) and velocity (Figure 12) were also continuously variable over development, but have profiles clearly diffe nt from forelimb profiles. One reason for this is that hindlimb use during swimming extends developmentally beyond the use of the forelimbs. In addition, early hindlimb use has larger stroke-cycle times and smaller velocities than forelimbs, although by postnatal day 21 the hindlimbs are moving more rapidly than ever observed in forelimbs. It is thus obvious that unitary conclusions about limb use parameters during ontogeny must be separated for different limbs.

This latter point is reinforced by the fact tnat significant differences in hindlimb stroke-cycle times and velocities are observed between *jp*/Y and control mice (Figures 9-13) even though the differences between groups by

forelimb measures (Figures 4-8) are less pronounced. For the hindlimb, jimpys move with consistently lower velocity than do controls from postnatal day 13 for mean velocity and postnatal day 11 for maximum velocity (Figures 12 & 13). It is therefore clear that progressive developmental divergence can be seen in hindlimb stroke-cycle duration and velocity measures that are not evident from measurements of the forelimbs. Again, note that these changes are basically continuous over development, in contrast to the more or less step functions observed in transitions between swimming styles.

At this point it is again useful to bring the data into a dynamic systems perspective that is increasingly dominant in the motor behavior literature (e.g., Kelso, 1995; Thelen & Smith, 1994). The basic idea is that motor performance details are best understood by taking together a unified constellation of factors rather than trying to isolate single unitary variables. As noted, one consequence of these models is that combined continuous changes along simple variables can lead to sudden discontinuities among the total ensemble of these variables. To the extent this turns out to be true, it provides a caution in trying to understand motor performance and/or developmental processes by concentrating solely upon single variables. As illustration, abstractions involving "central pattern generators" in motor control (Grillner, 1990) must be interpreted within the broader context of factors normally viewed as extrinsic to these pattern generators (Fentress, 1990). To be more specific, spinal pattern generators in basic movements such as swimming can be influenced importantly by sensory feedback, supraspinal processes and muscular strength (Shepherd, 1994). These are important considerations for any attempts to provide formal models of motor control, development and the effects of specific mutations upon movement parameters.

One valuable measure of central pattern robustness is the appearance of missed strokes within swimming cycles (Figures 14-19). For both forelimbs (Figures 14-16) and hindlimbs (Figures 17-19) it is clear that individual limbs may "miss a beat" from time to time, and that this tendency varies with age. Interestingly, the forelimbs for both *jp*/Y and control mice increase the proportion of missed strokes from postnatal days 9-13 (the time at which four limb swimming is occurring), while the hindlimbs, for each genotype, show a progressive decreases in missed strokes from postnatal day seven. This clear distinction between forelimb and hindlimb profiles illustrates the value of quantitative application of swimming tests to clarify separable patterns of behavioral ontogeny.

Interlimb Coordination: Latencies

A further value of the swimming test is that it allows one to examine patterns of interlimb coordination during development. Evaluations of forelimb and hindlimb bilateral pairs (Figures 20 & 21) indicate a smooth developmental decline in latencies, with the hindlimbs continuing to show a continuous decline after the forelimbs are no longer in use (postnatal day 13). Jimpy and control mice show similar trends.

Latencies of the ipsilateral pair (right hindlimb-right forelimb, Figure 22) also show a continuous developmental decline in each of the two genotypes, although more pronounced in the jimpy. Importantly the latencies of these ipsilateral stroke pairs are similar in their profiles to both forelimb and hindlimb latencies. On the presumption that swimming involves a "classical" quadruped gait (Bekoff & Trainer, 1979; Cazalets et al., 1990) , one would expect similar latency patterns between each bilateral limb pair and ipsilateral limb pairs.

In contrast, the contralateral limb pair (e.g., right forelimb-left hindlimb, Figure 23) would be expected to exhibit shorter latencies. This is because body-defined limb supports should move more or less together. While this general proposition has been confirmed by my results, the developmental time profiles of contralateral limb pairs are quite distinct from both bilateral and ipsilateral limb pairs. Further, the coordination patterns for *jp*/Y and control mice now appear distinct. For example, at postnatal day 11 *jp*/Y mice exhibited a significantly larger contralateral limb pair latency than did controls. This means that for jimpy mice the contralateral limbs are moved less simultaneously than that observed in littermate controls. An interesting hypothesis is that the reason *jp*/Y and control mice differ more on contralateral limb pair latency than either bilateral or ipsilateral limb pairs is that the normal phase locking of swimming movements is delayed due to reduced transmission times in long-distance spinal circuits. There is now considerable evidence that interlimb coordination is mediated by these spinal level circuits, although obviously one has to be careful to acknowledge supraspinal and other influences (Grillner, 1990). Upon the assumption that deficiencies in myelin can both increase neural transmission time and degrade signal integrity, these data provide suggestive evidence for the role of myelin in a basic spinally mediated movement, swimming.

Interlimb Coordination: Phase Relationships

Phase relationships provide a valuable assay of interlimb coordination. At two extremes, limb pairs may be either perfectly in phase or exactly out of phase (alternating). Quadrupeds can show a variety of limb phase relationships during locomotion and other activities. For example, a horse can

move with a walking gait, a trot, or a gallop (see Grillner, 1990; Kelso, 1995 for extended discussion). Swimming behavior in rodents is generally viewed as a trot-like gait, where both bilateral and ipsilateral limb pairs move more or less in an alternating phase, whereas contralateral limb pairs move synchronously (Bekoff & Trainer, 1979).

A previous study on rat swimming ontogeny by Bekoff and Trainer (1979) has indeed provided quantitative support for the early alternating phase between bilateral (forelimb-forelimb; hindlimb-hindlimb) and ipsilateral (forelimb-hindlimb on same side of body) limb pairs in rats. Further, these authors argue that the rules of limb coupling are basically invariant across development. This led them to the potentially important conclusion that neural circuits involved in interlimb coupling are established early and remain fixed throughout early postnatal ontogeny. Their data are of major interest since they link hypotheses of "central pattern generators" for single limbs with connections between limbs.

A subsequent electromyographic study of rat swimming ontogeny by Cazalets et al. (1990) provided data that "the ontogeny of the central pattern generators sustaining swimming activity is much more complex than was previously thought" (p. 216). These authors picked three developmental time frames for analyses: i) 3 days (forelimb swim, ii) 8 days (four limb swim) and iii) 15 days (hindlimb swim). Each rat was tested once. Period, phase and latency measures were quantified for various muscle groups.

They found that during early ontogeny, forelimb swimming sequences were regular but could be interrupted by missed strokes. Muscle bursts in the forelimbs, however, occasionally overlapped, and variations in the percentage of phase calculations for the forelimbs during ontogeny remained variable. These measures of coupling variability were essentially constant for the

forelimbs, but progressively decreased in the hindlimbs over the three time periods studied. Thus, as in my findings, quantitative ontogenetic patterns for forelimb and hindlimb coordination follow distinct patterns.

Both Bekoff and Trainer (1979) and Cazalets et al. (1990) examined ipsilateral, but unfortunately not contralateral, phase relations between forelimbs and hindlimbs. Re-evaluation of the phase calculations of Bekoff and Trainer (1979) revealed a fundamental error in interpretation. If 0.5 is taken to represent perfect alternation between the limbs and 0.0 is taken to represent simultaneous movement between the limbs, then phase relations that are in-between alternation and simultaneous should range between 0.0 and 0.5. If the deviations between alternating and simultaneity are measured for different lead limbs, such as in a galloping motion, then in principle a given limb could closely precede limb movements of the other limb which in turn would exhibit a latency in excess in what would be found in perfect alternation. Therefore, it might appear that numbers between 0.5 and 1.0 (again simultaneous movements) should be included in the calculation. This is indeed what Bekoff and Trainer (1979) did. The difficulty with this method is that random variations around 0.5 on a 0.0 to 1.0 scale will artificially drive mean values to 0.5. Thus, limbs can appear to be more precisely alternating in their phase relationships than is actually the case. To correct for this error values between 0.5 and 1.0 were converted to values between 0.0 and 0.5. Since systematic asymmetries between defined lead and following limbs would not be expected across animals (e.g., right versus left handed mice) this simple correction avoided artificial migration toward 0.5 that occur in the original Bekoff and Trainer (1979) method.

In this thesis I have explicitly compared my data on phase coupling using the method of Bekoff and Trainer (1979) and a corrected calculation in which

all deviations from 0.5 are scaled in a single direction. As predicted from the logic of this transition, the phase values I attained for both bilateral and ipsilateral limb pairs were lower by the corrected method than by the Bekoff and Trainer (1979) method. The contralateral limb pair was illogically near 0.5 with the Bekoff and Trainer (1979) formula, but dropped toward simultaneous movements with the corrected formula. The distinction between bilateral/ipsilateral (predicted at 0.5 by Bekoff & Trainer, 1979) and contralateral (logically predicted but not tested by Bekoff & Trainer (1979) at 0.0) was lessened by the corrected analysis. This indicates that the swimming gait of mice cannot be summarized accurately as a simple trot (simultaneously contralateral limbs).

In support of the conclusions of Bekoff and Trainer (1979) and Cazalets et al (1990), the phase relations of the two forelimbs are relatively stable developmentally at around 0.4 for both *jp*/Y and control mice (Figures 24 & 25). There are no significant genetic or developmental distinctions. There is perhaps a slight suggestion that jimpy mice deviate further from limb alternation and show greater variability (error bars) as the forelimbs stop being used, but basically the profiles for each genotype are both similar and stable over time.

As for forelimb pairs, phase values of hindlimb pairs (Figures 26 & 27) hover around 0.4 for both genotypes and over time [versus 0.5 for the Bekoff and Trainer (1979) method]. Interestingly, at postnatal day 13 the time at which animals are switching from four limb to hindlimb swimming, the jimpy mice express hindlimb phase relationships that deviate significantly more from 0.5 than do the controls. There is also a descriptive tendency for lower phase values in *jp*/y mice on postnatal days 17, 19 and 21. Finally, the variability in phase values for *jp*/Y mice is considerably higher than for the control mice

during these periods of maximum deviation. Upon the assumption that bilateral phase values near 0.5 reflect the most effective swimming coordination, there is thus a suggestion that, at least for certain developmental periods, *jp*/Y mice swim in a less effective way than do littermate controls. These distinctions are more marked for the hindlimbs than for the forelimbs.

Graphs for the ipsilateral pairing (Figures 28 & 29) again deviate from 0.5 in the corrected calculations. Further, at postnatal day five the corrected calculations reveal a significant difference between *jp*/Y and control animals with the *jp*/Y mice showing a closer correspondence to the hypothetical "appropriate" 0.5 gait than do control animals. Further, the controls, descriptively increase their approach to 0.5 from postnatal day seven while the *jp*/Y mice first approach 0.5 (postnatal day 11) and then deviate from 0.5 (postnatal day 13).

Assuming the validity of these statistical results, it is clear that a complex developmental pattern of hindlimb phase relationships may distinguish *jp*/Y and control animals. The pattern of results could be accounted for on the presumption that early connections with supraspinal mechanisms are both less complete at postnatal day five in *jp*/Y mice than control mice, and that these early supraspinal connections modulate hindlimb phase relationships otherwise controlled at the spinal level. There is indeed much suggestive literature that during development early connections across central nervous system levels can disrupt previously existing patterns (Michel & Moore, 1995; Thelen & Smith, 1994). The distinctions between control and *jp*/Y mice on postnatal day 13 may similarly reflect imperfect signal transmission in the jimpy mice.

The corrected contralateral limb phase relationship also is farther from 0.5 than the picture attained by the Bekoff and Trainer (1979) calculation, as

would be expected if these limbs tend toward simultaneous movements (Figures 30 & 31). As with the ipsilateral limb pairs, *jp*/Y mice deviate significantly from control mice on postnatal day 5 and additionally on postnatal day 13. While without electrophysiological data it is only possible to infer circuit transmission distinctions, these data are also compatible with the dual hypothesis that a) day five distinctions reflect early interference of basic spinal patterns by supraspinal or sensory influences in control mice, and b) spinal regulation in *jp*/Y mice that involves intersegmental and transverse cord communication operates differently in these mutant mice and their control littermates. Indeed, if *jp*/Y mice have spinal cord transmission delays these may show up primarily in long distance cord coordination. This possibility opens up a host of potential future electrophysiological experiments.

It is important to note that myelination in mammals develops in a pattern that basically ascends spinal cord, brain stem, midbrain and higher central nervous system regions (e.g., Jacobson, 1963; Michel & Moore, 1995). While it is obvious that there is much overlap in the myelination of these regions, and that neural function can both precede and potentially contribute to myelination patterns, there is good reason to suppose that basic spinal and brain stem mechanisms in actions such as swimming mature early. As noted by Michel and Moore (1995) quantitative measures at complementary levels are critical to any evaluation of the role of myelination in intact organism behavior.

Consistency

Examining the degree of consistency across the 10 stroke-cycle swimming session allowed examination of cycle stability and how this stability was affected by age and genotype. In contrast to Cazalets et al. (1990), who

found that cycle stability increased with age in rat pups, the degree of consistency did not change systematically with age or genotype for either stroke-cycle durations or interlimb phase values (see Figures 32-39). From these data one can conclude that central pattern generators do not operate in too rigid a way and this fact is consistent across the data set. Although some variations may be due to neurobehavioral circuit noise, it would thus be an error to assume that these animals do not adjust output or modulate the rhythm of swimming behavior. Even though swimming is a very basic rhythmic behavior the variations observed warn against central motor program concepts that are rigidly formulated.

Interestingly, although limb stroke-cycle durations generally decreased with age (see Figures 4-6 & 9-11) there was no comparable decrease in variability of these scores across the 10 stroke-cycle swimming session as a function of age (Figures 32-35). Additionally, although the jimpy mice tended to display longer stroke-cycle durations than littermate controls (see Figures 4-6 & 9-11), there was no comparable effect on variability within the 10 stroke-cycle session.

In the case of the variation of the phase values over the 10 stroke-cycle swimming session there were no systematic differences as a function of age or genotype. However, there was both within and between animal variability (Figures 36-39). Further, the variability in the phases values of the ipsilateral and contralateral pairs tended to be higher than that of the bilateral pairs. The linkage between the central pattern generators appears to be looser up and down the spinal cord than across the cord. These data suggest that forelimb and hindlimb profiles are modified somewhat independently of each other. Further, there is also a hint that phase values of ipsilateral and contralateral limb pairs in littermate control mice become tighter with age. This

developmental trend does not appear to be present in the jimpy group and this may be a result of myelin deficiency.

It is interesting to note that Figures 32-39 also provide a measure of individual variation within the two groups of mice, as measured by the error bars. Especially in measures involving the hindlimbs, there appears to be more variation among individuals in the jimpy group than among individuals within the littermate control group. One might speculate that this higher degree of individual variation might be the result of the mutation affecting individuals differently, due to overall differences in genetic background. In fact, these measures may provide a way to examine the degree of expressiveness of the mutation across individual mutant mice. Given the fact that this mutation is expressed on a hybrid background the degree of expression of the behavioral effects of the mutation might be affected by the combination of other genes. This in turn, may lead to higher inter-animal variation in the jimpy group in terms of swimming behavior. In future research with the jimpy and other mutant strains it is important to consider the possibility of this type of individual variation when examining behavioral profiles.

Overall the consistency measures also provide support for the multiple measure approach to understanding a particular behavior. It is evident from this research that one cannot predict from one measure to another how age or genotype will affect the behavior. Although duration and phase relations differ between the genotypes and across age these differences were not complemented by differences in consistency within the 10 stroke-cycle swimming session. Making global assumptions about how various aspects of a behavior will be affected by the effects on one measure would be incomplete at best or completely incorrect, as is the case with consistency measures.

The Importance of Multiple Behavioral Measures

The patterns of limb coupling seen with swimming behavior in mice are likely to contribute importantly to more complicated sequential behaviors such as grooming. Therefore, the timing mechanisms evident in this thesis research can be generalized to studies involving more complicated behavioral patterns. It is particularly valuable to consider the way in which this set of measures enables the dissection of age and swimming style effects on other aspects of swimming behavior.

One more general issue addressed in this research is that of the importance of using complementary measures when measuring a behavior (Fentress, 1992: Fentress & Bolivar, 1996). The issue of one measure providing incomplete information has been illustrated with the results from the swimming styles analysis when compared to limb usage of the jimpy mouse. This generalized coding scheme based on limb usage has been the sole measure of researchers examining the effects of various types of lesions on swimming ability (e.g., Kolb, 1987; Kolb, Holmes & Whishaw, 1987, Kolb & Whishaw, 1981a, 1981b, 1983). When using swimming style measurements alone there were no significant differences between jimpy and littermate controls. The only prior reference to the swimming ability of this mutant by Sidman et al. (1964), did not report any swimming abnormalities in jimpy mice. If the only measure of swimming ability was swimming style (i.e., limb usage), then the present findings would be in agreement with that study. This raises some concerns about the way in which swimming has been used to measure motor ability in many studies of toxicology, lesion, drug, and nervous system assembly and disassembly research (Adams, 1986; Weiner & Lang, 1989). If the findings vary from measure to measure it becomes critical to evaluate the measure(s) one proposes to use. In this study, although differences were

evident between jimpy and control groups on several of the measures used, there were no significant differences when examining limb usage.

So, the question arises: is limb usage a sensitive measure of factors affecting swimming ability? Generalized coding schemes, which examine body position and limb usage, have been developed and used by many researchers (e.g., Marshall & Berrios, 1979; Petrosini, Molinari & Gremoli, 1990; Schapiro et al., 1970; Syapin, 1982; Vorhees, Butcher et al., 1979; Whishaw et al., 1981a). Usually the general swimming style codings include several separate measures such as limb usage and body position, and direction of swim, although again fine-grained detail on kinematics and coordination is for the most part missing. In this study of the jimpy mutant it is the limb kinematics and coordination measures (phase relationship between limbs) that provide the most interesting details of the abnormalities during swimming. This is not to say that limb usage is not a good measure of swimming ability; it is simply not enough on which to build a complete picture of motor capability. In fact, limb usage has proven useful in evaluations of swimming behavior in the neurological mutant weaver mouse over the first three postnatal weeks; however, the addition of other measures provided a much richer description of the behavior (Bolivar, Manley & Fentress, 1996).

In contrast to the weaver mutant, the abnormalities in jimpy mouse swimming can be found only by detailed analyses of the limb movements. Although the weaver mutants show generalized slowness in limb movements, they do not provide any evidence of phase relation deficits. In contrast the jimpy mouse, in measures involving the length of the spinal cord, show interlimb coordination deficits.

The early postnatal period has previously proven to be a very good time for detecting abnormalities in other measures of jimpy mouse behavior

patterns. Bolivar and Brown (1994) have shown differences in jimpy pups as early as postnatal day two, in ultrasonic vocalization production, and the amount of time engaged in slight movement. Other behavioral differences do not become evident until later in development, locomotion at postnatal day 12 and grooming at postnatal day 18. The findings of the present study complement the early developmental differences described by Bolivar and Brown (1994). Differences in limb movements and coordination become evident earlier, using the swimming assay, than the locomotion assay used in the Bolivar and Brown (1994) study. This is not surprising as research has shown that in many cases young rodents are capable of making coordinated movements given the appropriate environmental conditions. For instance Smotherman and Robinson (1989) have shown that face wiping can be elicited much earlier than would normally occur by placing the animals in a substrate-free environment. Swimming coordination can be reliably measured in early postnatal rodent development (Bekoff & Trainer, 1979; Bolivar, Manley & Fentress, 1996) and has also been produced in rat fetuses (Bekoff & Lau, 1980). Therefore it is very useful measure of early coordination, enabling one to examine limb coordination earlier than terrestrial locomotion tests.

Independent of the findings related to the jimpy mouse motor abnormalities, is the confirmation of the general trend of swimming development in mice. As indicated by many researchers, rodent swimming appears to follow a specific rostral-caudal pattern of development, with the forelimbs used primarily early in development, then all four limbs and finally the hindlimbs used as the main propulsive thrust of the swim (Fentress, 1972, Kolb and Whishaw, 1981a, Schapiro et al., 1970). In fact using the same methodology we have found that the neurological mutant weaver mouse and its littermates follow this same pattern, although a bit delayed in the case of the

homozygous weaver pups. This rostral-caudal progression is similar to that seen in the ontogeny of rodent grooming (Richmond & Sachs, 1980) and terrestrial locomotion (Altman & Sudarshan, 1975; Eilam & Golani, 1988).

In the case of both jimpy and control mice there are clear transitions from one style of swim to the next. Additionally, a general decrease in swim stroke-cycle duration as a function of age is evident for both the hindlimbs and forelimbs of the mice in this study, and is in agreement with the developmental trend previously reported with rat pups (Bekoff and Trainer, 1979) and weaver mice (Bolivar, Manley & Fentress, 1996). As they mature and perfect the swim style rats and mice appear to increase the speed of the limb movements; however, when they switch to a more mature swim style, limbs may in fact slow down for a period of time or cease to be used at all depending on the particul swim style. Once again, these illustrate how important multiple measures are when quantifying behavior and that often these measures provide results that are both inter-related and complimentary.

Three generalizations outlined by Goodall and Guastavino (1986) in the evaluation of mutant mice are noteworthy. First, the behavior observed may be directly related to the known physiological consequences of the gene's action (e.g., the ataxia produced by granule cell deficit in the cerebellum of the weaver mouse). Second, in other cases the effects are secondary and arise because of deficits in some subcomponent of a particular behavior (e.g., mounting behavior in male staggerer mice may be affected directly by cerebellar abnormalities and have repercussions for more complicated reproductive behavior). Third, the behavioral effects observed appear to be, as yet, unrelated to the known anatomical and physiological deficits (e.g., jimpy mice display abnormal immune system function (Carr et al., 1996)). Even if the weight deficits were sufficient to explain many of the behaviors that

does not eliminate the importance of connecting genetic mutation to behavior. Perhaps, as suggested by Bolivar and Brown (1994) malnourishment of the jimpy mutant does play a role in the behavioral abnormalities. But, it is the mutation, either directly or indirectly, that results in the proposed malnutrition. Perhaps the mutants are less able to nurse and feed on the pellets provided as solid food (although the pellets were placed on the floor of the cage for easy access). If so, then the mutation is, to use Goodall and Guastavino's (1986) terms, indirectly responsible, and therefore the behavior is a consequence of the genetic mutation. To establish the effects of malnutrition on jimpy mouse behavior in future it might be useful to compare jimpy animals with enriched diets to littermate controls, so that one can eliminate many of the weight differences evident between the two groups and determine the role, if any, played by this variable.

Future Research

There are several future avenues for this research. First, a number of variations on the current experimental design could address a wide range of issues. For instance, a cross-sectional design could be used to evaluate swimming behavior. As is the case in all longitudinal designs it is possible that learning played a role in the improved performance over age seen in both jimpy and littermate control mice. In future a cross-sectional design, in which mice are tested only once with the swimming assay, would eliminate this possibility. However, as swimming behavior is a basic rhythmic activity, not a higher level cognitive one, it is assumed that the effects of repeated testing of the same animals would be minimal.

The effects of water temperature on swimming performance of jimpy and littermate control mice could be investigated. In this study the water

temperature was kept constant at a warm temperature (38 ± 2° C) since young mice do not thermoregulate. However, it is possible that different temperatures could produce new or more pronounced difference in swimming behavior between the two genotypes. Additionally, it has been shown that multiple sclerosis (a demyelinating disorder) symptoms can be exacerbated by placing the affected individual in a hot water bath (see McDonald & Silberberg, 1986). In future studies using higher and lower water temperatures may produce exaggerated and reduced swimming abnormalities, respectively, in the dysmyelinating jimpy mouse group.

Second, a combination of this behavioral assay with other measures may be useful. For instance, electrophysiological information about the conduction speed of neural impulses could shed light on the interlimb and intralimb deficits seen in the jimpy mouse. This future research could combine electrophysiological techniques with behavioral ones.

Alternatively, the swimming assay could be combined with other behavioral assays to establish more firmly the general kinds of deficits resulting from dysmyelination of the central nervous system. For instance more detailed movement examinations of the behavioral assays used by Bolivar and Brown (1994) may reveal further differences in the sequencing and coordination of grooming and locomotion. We currently have research underway to examine the sequential order of grooming components in jimpy mice and littermate controls in the manner in which Bolivar, Danilchuk and Fentress (1996) examined the weaver mouse. In the case of locomotion, detailed information about limb coordination, stride length and limb velocity could be measured.

Electromyographic recordings of swimming jimpy mutants could be correlated with behavioral observations in a manner similar to that developed by Bekoff and colleagues (Bekoff, 1976; Bekoff, Stein & Hamburger, 1975;

Bekoff, Kauer, Fulstone & Summers, 1987, Bekoff, Nusbaum, Sabichi & Clifford, 1987; Watson & Bekoff, 1990) when examining hatching and walking movements in chick embryos. They found that the combination of EMG and kinematic analyses of movements could provide a more complete picture of underlying similarities and differences in these movement patterns.

Third, one could determine whether or not there were abnormalities in the swimming behavior of other dysmyelinating mutants such as quaking and shiverer, which show myelin deficits in both peripheral and central nervous systems. As each mutation appears to follow a slightly different time course in its development, it would be interesting to trace the development of swimming behavior, and attempt to directly correlate the behavioral changes with the cellular and biochemical changes. One could correlate behavioral development with assays of myelin deficits in various portions of the central nervous system. This would enable more accurate structure-function relations and one might be able to assess the developmental timetable more accurately for myelin deficits and how these deficits relate to behavioral abnormalities. In the same vein, this could be combined with conduction speed analyses to correlate the behavior with speed of neural transmission.

Conclusions

In this study I have shown that by using a combination of fine-grained movement analyses, the motor development of the dysmyelinating jimpy mutant mouse is complex, with velocity and coordination deficits that are age-specific. The various measures employed to examine swimming have allowed the tracing of multiple dimensions in early postnatal changes of motor behavior. Thereby I have clarified the cont tion of the jimpy mutation to one

type of behavior more comprehensively than previously reported. This clarification of early postnatal distinctions in the behavior of jimpy and control mice, along with the developmental progression of these distinctions, allows the formation of a more complete link between behavior and the cellular/biochemical literature on jimpy mice than previously available. Together with the findings of Bolivar and Brown (1994) the results of this study provide a much richer description of the behavior of the jimpy mouse, thereby creating a behavioral profile that is far broader than originally established, i.e., hindquarter tremours and tonic-clonic seizures.

My data have confirmed a number of initial hypotheses as outlined in the thesis introduction. The initial set of hypotheses concern age-related processes that might be shared among jimpy and control mice. First, the same basic progression of swimming styles forelimb to four limb to hindlimb-was found in both jimpy and control mice. Second, distinctly different developmental patterns emerge for forelimbs and hindlimbs and the details of these patterns were also reflected in overall transitions in swimming style. Third, on the model that swimming behavior should reflect a basic quadruped gait where both bilateral and ipsilateral limb pairs move alternately, my data confirmed an overall improvement for each of the genotypes with age. Fourth, I confirmed that individual measures of swimming development can both correspond and be dissociated. This is important because of the fact that coordinated behavior depends essentially on both the differentiation of specific operations and their integration into higher order patterns (Fentress & Bolivar, 1996).

The second set of hypotheses involve expected distinctions between jimpy and littermate control animals. While I confirmed that detailed analyses of intralimb and interlimb motor parameters distinguished jimpy from control mice,

an unexpected result was that overall the transitions in swimming style were indistinguishable between the two genotypes. This pair of findings is important in that they emphasize how hierarchical levels of motor coordination may proceed by quite distinct developmental rules. Furthermore, specific genetic alterations in development, such as the myelin deficiency studied here, may have a major expression at one level and not another. This possibility was hinted at in an early paper by Fentress (1972), but up to the present time there were no definitive data that could be used to isolate level specific motor disorders in a mutant mouse.

Other hypotheses partly supported by my data included the prediction of a greater number of missed strokes restricted to the hindlimbs of jimpy mice. Importantly, the simple hypothesis that jimpy would show less coordination between pairs of limbs than normal littermates was only partly confirmed. While there were clear duration, velocity, latency, and phase relationship distinctions, these were not obviously accompanied by marked disorders in fine motor control. This is of particular interest since jimpy mice develop tremours, a phenomenon that reflects a variety of central and peripheral nervous system disorders (Weiner & Lang, 1989).

These results are compatible with the idea of distributed movement control systems with somewhat independently organized subroutines (Kelso, 1995). Certain views of simple unitary central pattern generating mechanisms are insufficient to provide a full dissection of movement control pathways. Careful use of mutant mice can indeed provide a valuable method for dissecting isolable subsystems in movement pattern generation.

REFERENCES

Abbey, H., & Howard, E. (1973). Statistical procedure in developmental studies on species with multiple offspring. *Developmental Psychobiology, 6,* 329-335.

Adams, J. (1986). Methods in behavioral teratology. In E. P. Riley & C. V. Vorhees (Eds.), *Handbook of Behavioral Teratology* (pp. 67-97). New York: Plenum Press.

Adams, P. (1982). Effects of perinatal chlordiazepoxide exposure on rat preweaning and postweaning behavior. *Neurobehavioral Toxicology and Teratology, 4,* 279-282.

Allin, J. T., & Banks, E. M. (1972). Functional aspects of ultrasound production by infant albino rats (*Rattus norvegicus*). *Animal Behaviour, 20,* 175-185.

Altman, J., & Sudarshan, K. (1975). Postnatal development of locomotion in the laboratory rat. *Animal Behaviour, 23,* 896-920.

Anderson, T. R., & Schanberg, S. M. (1975). Effect of thyroxine and cortisol on brain ornithine decarboxylase activity and swimming behavior in developing rat. *Biochemical Pharmacology, 24,* 495-501.

Bartlett, W. P., & Skoff, R. P. (1989). Expression of the jimpy gene in the spinal cords of heterozygous female mice. 2. Oligodendroglial and endothelial cell hyperplasia. *Developmental Brain Research, 47,* 1-11.

Baudoin, C., Feron, C., & Magnusson, M. S. (1991). Male-female interactions in staggerer and non-mutant mice: Impairment to react to novelty as a possible explanation of staggerer male social behavior. *Behavioural Processes, 24,* 49-58.

Bekoff, A. (1976). Ontogeny of leg motor output in the chick embryo: A neural analysis. *Brain Research, 106,* 271-291.

Bekoff, A., Kauer, J. A., Fulstone, A., & Summers, T. R. (1989). Neural control of limb coordination. II. Hatching and walking motor output patterns in the absence of input from the brain. *Experimental Brain Research, 74,* 609-617.

Bekoff, A., & Lau, B. (1980). Interlimb coordination in 20-day-old rat fetuses. *Journal of Experimental Zoology, 214,* 173-175.

Bekoff, A., Nusbaum, M. P., Sabichi, A. L., & Clifford, M. (1987). Neural control of limb coordination. I. Comparison of hatching and walking motor output patterns in normal and deafferented chicks. *Journal of Neuroscience, 7,* 2320-2330.

Bekoff, A., Stein, P. S. G., & Hamburger, V. (1975). Coordinated motor output in the hindlimb of the 7-day chick embryo. *Proceedings of the National Academy of Sciences (U.S.A.), 72,* 1245-1248.

Bekoff, A., & Trainer, W. (1979). The development of interlimb co-ordination during swimming in postnatal rats. *Journal of Experimental Biology, 83,* 1-11.

Bell, J. M., & Lundberg, P. K. (1985). Effects of a commercial soy lecithin preparation on development of sensorimotor behavior and brain biochemistry in the rat. *Developmental Psychobiology, 18,* 59-66.

Bensoula, A. N., Guastavino, J. -M., Lalonde, R., Portet, R., Bertin, R., & Krafft, B. (1995). Spatial navigation of staggerer and normal mice during juvenile and adult stages. *Physiology and Behavior, 58,* 823-825.

Berridge, K. C. (1994). The development of action patterns. In J. A. Hogan & J. J. Bolhuis (Eds.), *Causal mechanisms of behavioral development* (pp. 147-180). Cambridge: Cambridge University Press.

Berridge, K. C., & Fentress, J. C. (1986). Contextual control of trigeminal sensorimotor function. *Journal of Neuroscience, 6,* 325-330.

Berridge, K. C., Fentress, J. C., & Parr, H. (1987). Natural syntax rules control action sequence of rats. *Behavioural Brain Research, 23,* 59-68.

Billings-Gagliardi, S., Karthigasan, J., Kirschner, D. A., & Wolf, M. K. (1990). Quaking*jimpy double mutant mice: Additional evidence for independence of primary deficits in jimpy. *Molecular Brain Research, 7,* 189-198.

Blatt, G. J., & Eisenman, L. M. (1985a). A qualitative and quantitative light microscopic study of the inferior olivary complex in the adult staggerer mutant mouse. *Journal of Neurogenetics, 2,* 51-66.

Blatt, G. J., & Eisenman, L. M. (1985b). A qualitative and quantitative light microscopic study of the inferior olivary complex of normal, reeler, and weaver mutant mice. *Journal of Comparative Neurology, 232,* 117-128.

Bloedel, J. R. (1992). Functional heterogeneity with structural homogeneity: How does the cerebellum operate? *Behavioral and Brain Sciences, 15,* 666-678.

Bolivar, V. J., & Brown, R. E. (1994). The ontogeny of ultrasonic vocalizations and other behaviors in male jimpy (*jp*/Y) mice and their normal male littermates. *Developmental Psychobiology, 27,* 101-110.

Bolivar, V. J., & Brown, R. E. (1995). Selective Retrieval of Jimpy Mutant Pups Over Normal Male Littermates by Lactating Female B6CBACa-A^{W-J}/A-*Ta jp* Mice. *Behavior Genetics, 25,* 75-80.

Bolivar, V. J., Danilchuk, W., & Fentress, J. C. (1996). Separation of activation and pattern in grooming development of weaver mice. *Behavioural Brain Research, 75,* 49-58.

Bolivar, V. J., Manley, K., & Fentress, J. C. (1996). Early detection of movement consequences of mutation in the swimming behavior of weaver mice. *Developmental Psychobiology, 29,* 123-137.

Bolles, R. C., & Woods, P. J. (1964). The ontogeny of behavior in the albino rat. *Animal Behaviour, 12,* 427-441.

Boufares, S., Guastavino, J. -M., Larsson, K. (1993). Restoration of staggerer mouse maternal behavior following long-term breeding selection. *Physiology and Behavior, 53,* 1151-1155.

Buelke-Sam, J., & Kimmel, C. A. (1979). Development and standardization of screening methods for behavioral teratology. *Teratology, 20,* 17-30.

Bulloch, K., Hamburger, R. N., & Loy, R. (1982). Nest-building behavior in two cerebellar mutant mice: Staggerer and weaver. *Behavioral and Neural Biology, 36,* 94-97.

Carr, R. I., Bolivar, V. J., Sadi, D., Wong, F. K., Bitter-Suermann, B., Walsh, N., Fentress, J. C., & Brown, R. E. (1996). *Abnormal immune system function in 4 week old jimpy mice, a dysmyelinating CBA mutant.* In preparation.

Caston, J., Delhaye-Bouchaud, N., & Mariani, J. (1996). Motor behavior of heterozygous staggerer mutant (+/*sg*) versus normal (+/+) mice during aging. *Behavioural Brain Research, 72,* 97-102.

Cazalets, J. R., Menard, I., Cremieux, J., & Clarc, F. (1990). Variability as a characteristic of immature motor systems: An electromyographic study of swimming in the newborn rat. *Behavioural Brain Research, 40,* 215-225.

Chambers, S. H., Bradley, N. S., & Orosz, M. D. (1995). Kinematic analysis of wing and leg movements for type I motility in E9 chick embryos. *Experimental Brain Research, 103,* 218-226.

Churchland, P. S., & Sejnowski, T. J. (1992). *The computational brain.* Cambridge: MIT Press.

Collins, J. J., & Stewart, I. N. (1993). Coupled nonlinear oscillators and the symmetries of animal gaits. *Journal of Nonlinear Science, 3,* 349-392.

Cordo, P., & Harnad, S. (Eds.) (1994). *Movement control*. Cambridge: Cambridge University Press.

Coscia, E. M., & Fentress, J. C. (1993). Neurological dysfunction expressed in the grooming behavior of developing weaver mutant mice. *Behavior Genetics, 23,* 533-541.

Crepel, F., Delhaye-Bouchaud, N., Guastavino, J. -M., & Sampaio, J. (1980). Multiple innervation of cerebellar Purkinje cells by climbing fibers in *staggerer* mutant mouse. *Nature, 283,* 483-484.

Dautigny, A., Mattei, M. -G., Morello, D., Alliel, P. M., Pham-Dinh, D., Amar, L., Arnaud, D., Simon, D., Mattei, J. -F., Guenet, J. -L., Jolles, P., & Avner, P. (1986). The structural gene coding for myelin-associated proteolipid protein is mutated in *jimpy* mice. *Nature, 321,* 867-869.

Davenport, J. W., & Gonzalez, L. M. (1973). Neonatal thyroxine stimulation in rats: Accelerated behavioral maturation and subsequent learning deficit. *Journal of Comparative and Physiological Psychology, 85,* 397-408.

Davenport, J. W., Hagquist, W. W., & Hennies, R. S. (1975). Neonatal hyperthyrodism: Maturational acceleration and learning deficit in triiodothyronine-stimulated rats. *Physiological Psychology, 3,* 231-236.

Davis, M., Jacobs, B. L., & Schoenfeld, R. I. (Eds.). (1989). *Annuals of the New York Academy of Sciences, Vol. 563. Modulation of defined vertebrate neural circuits*. New York: New York Academy of Sciences.

Deshmukh, D. S., Inoue, T., & Pieringer, R. A. (1971). The association of the galactosyl diglycerides of brain with myelination. II. The ability of the myelin-deficient mutant, jimpy mouse, to synthesize galactosyl diglycerides effectively. *Journal of Biological Chemistry, 246,* 5695-5699.

Duncan, I. D., Hammang, J. P., Goda, S., & Quarles, R. H. (1989). Myelination in the jimpy mouse in the absence of proteolipid protein. *Glia, 2,* 148-154.

Eilam, D., & Golani, I. (1988). The ontogeny of exploratory behavior in the house rat (*Rattus rattus*): The mobility gradient. *Developmental Psychobiology, 21,* 679-710.

Eto, Y., & Suzuki, K. (1973). Cholesterol ester metabolism in rat brain. A cholesterol ester hydrolase specifically localized in the myelin sheath. *Journal of Biological Chemistry, 248,* 1986-1991.

Fannon, A.M., Mastronardi, F.G., & Moscarello, M.A. (1994). Isolation and identification of proteolipid proteins in *jimpy* mouse brain. *Neurochemical Research, 19,* 1005-1012.

Fentress, J. C. (1972). Development and patterning of movement sequences in inbred mice. In J. A. Kiger, Jr. (Ed.), *The Biology of Behavior* (pp. 83-131). Corvallis: Oregon State University Press.

Fentress, J. C. (1973). Development of grooming in mice with amputated forelimbs. *Science, 179,* 704-705.

Fentress, J. C. (1990). Organizational patterns in action: Local and global issues in action pattern formation. In G. M. Edelman, W. E. Gall & W. M. Cowan (Eds.), *Signal and sense. Local and global order in perceptual maps* (pp. 357-382). New York: Wiley-Liss.

Fentress, J. C. (1991). Analytical ethology and synthetic neuroscience. In P. Bateson (Ed.), *The development and integration of behavior. Essays in honour of Robert Hinde.* (pp. 77-120). Cambridge: Cambridge University Press.

Fentress, J. C. (1992). Emergence of pattern in the development of mammalian movement sequences. *Journal of Neurobiology, 23,* 1529-1556.

Fentress, J. C., & Bolivar, V. J. (1996). Developmental aspects of movement sequences in mammals. In K. -P. Ossenkopp, M. Kavaliers & P. R. Sanberg (Eds.), *Measuring movement and locomotion: From invertebrates to humans* . Austin: R. G. Landes Company.

Fentress, J. C., & Stilwell, F. P. (1973). Grammar of a movement sequence in inbred mice. *Nature, 244,* 52-53.

Feron, C., & Baudoin, C. (1993). Sexual experience and preferences for odors of estrous females in staggerer mutant male mice. *Behavioral and Neural Biology, 60,* 280-281.

Feron, C., & Baudoin, C. (1995). Social isolation partially restores reproduction of male staggerer mutant mice. *Physiology and Behavior, 58,* 107-110.

Feron, C., Baudoin, C., & Magnusson, M. S. (1991). Effect of post-weaning social experience with normal females on the behavior of adult male staggerer mice interacting with normal females. *Behavioural Processes, 23,* 145-152.

Fox, M. W. (1965a). Neuro-ontogeny of neuromuscular mutant mice. *Journal of Heredity, 56,* 55-60.

Fox, M. W. (1965b). Reflex-ontogeny and behavioral development of the mouse *Animal Behaviour, 13,* 234-241.

Fride, E., & Weinstock, M. (1984). The effects of prenatal exposure to predictable or unpredictable stress on early development in the rat. *Developmental Psychobiology, 17,* 651-660.

Full, R. J., & Tu, M. S. (19[illegible]). Mechanics of six-legged runners. *Journal of Experimental Biology, 148*, 129-146.

Galli, C., & Galli, D. R. C. (1968). Cerebroside and sulphatide deficiency in the brain of "jimpy mice", a mutant strain of mice exhibiting neurological symptoms. *Nature, 220,* 165-166.

Gallistel, C. R. (1980). *The new organization of action: A new synthesis*. Hillsdale, New Jersey: Lawrence Erlbaum Associates.

Golani, I. (1992). A mobility gradient in the organization of vertebrate movement: The perception of movement through symbolic language. *Behavioral and Brain Sciences, 15,* 249-308.

Golani, I., Bronchti, G., Moualem, D., & Teitelbaum, P. (1981). "Warm-up" along dimensions of movement in the ontogeny of exploration in rats and other infant mammals. *Proceedings of the National Academy of Sciences (U.S.A.), 78,* 7226-7229.

Golani, I., & Fentress, J. C. (1985). Early ontogeny of face grooming in mice. *Developmental Psychobiology, 18,* 529-544.

Goldowitz, D., & Eisenman, L. M. (1992). Genetic mutations affecting murine cerebellar structure and function. In P. Driscoll (Ed.), *Genetically defined animal models of neurobehavioral dysfunctions* (pp. 66-88). Boston: Birkhauser.

Goldowitz, D., & Koch, J. (1986). Performance of normal and neurological mutant mice on radial arm maze and active avoidance tasks. *Behavioral and Neural Biology, 46,* 216-226.

Goldowitz, D., Wahlsten, D., & Wimer, R. E. (Eds.). (1992). *Techniques for the genetic analysis of brain and behavior: Focus on the mouse*. New York: Elsevier.

Goodall, G., & Gheusi, G. (1987). Abnormal patterns of maze patrolling in the mutant mouse staggerer. *Behavioral and Neural Biology, 47,* 307-320.

Goodall, G., & Guastavino, J. -M. (1986). The neurological mutant: Scope and limitations as a tool for the genetic analysis of behavior. In J. Medioni & G. Vaysse (Eds.), *Genetic Approaches to Behavior* (pp. 59-68). Toulouse: Privat.

Goodall, G., Guastavino, J. -M., & Gheusi, G. (1986). The swimming activity of the staggerer mutant mouse. *Behavioural Processes, 13,* 287-299.

Goodlett, C. R., Hamre, K. M., & West, J. R. (1992). Dissociation of spatial navigation and visual guidance performance in Purkinje cell degeneration (*pcd*) mutant mice. *Behavioural Brain Research, 47,* 129-141.

Gow, A., Friedrich, V. L., & Lazzarini, R. A. (1994). Many naturally occurring mutations of myelin proteolipid protein impair its intracellular transport. *Journal of Neuroscience Research, 37,* 574-583.

Gramsbergen, A., & Westerga, J. (1992). Locomotor development in undernourished rats. *Behavioural Brain Research, 48,* 57-64.

Grillner, S. (1990). Neurobiology of vertebrate motor behavior- From flexion reflexes and locomotion to manipulative movements. In G. M. Edelman, W. E. Gall & W. M. Cowan (Eds.), *Signal and sense. Local and global order in perceptual maps* (pp. 187-208). New York: Wiley-Liss.

Guastavino, J. -M. (1982). Sexual experience and successful mating in *staggerer* mutant mice. *Behavioural Processes, 7,* 183-188.

Guastavino, J. -M. (1983). Constraint of the mother with pups restores some aspects of the maternal behavior of mutant *staggerer* mice. *Physiology and Behavior, 30,* 771-774.

Guastavino, J. -M. (1984a). A tilted rotational stimulation improves the gait of a cerebellar mutant mouse: The *staggerer*. *Behavioural Processes, 9,* 79-84.

Guastavino, J. -M. (1984b). Environmental features determining successful rearing in the mutant mouse *staggerer*. *Physiology and Behavior, 32,* 225-228.

Guastavino, J. -M., & Goodall, G. (1985). Permanency of gait improvement induced by vestibular stimulation in the mutant mouse staggerer. *Journal of Neurogenetics, 2,* 273-283.

Guastavino, J. -M., & Larsson, K. (1992). The staggerer gene curtails the reproductive life span of females. *Behavior Genetics, 22,* 101-112.

Guastavino, J. -M., Larsson, K., Allain, C., & Jaisson, P. (1993). Neonatal vestibular stimulation and mating in cerebellar mutants. *Behavior Genetics, 23,* 265-269.

Guastavino, J. -M., Larsson, K., & Jaisson, P. (1992). Neurological murine mutants as models for single-gene effects on behavior. In D. Goldowitz, D. Wahlsten & R. E. Wimer (Eds.), *Techniques for the genetic analysis of brain and behavior: Focus on the mouse* (pp. 375-390). New York: Elsevier.

Gupta, M., Felten, D. L., & Ghetti B. (1987). Selective loss of monoaminergic neurons in weaver mutant mice- an immunocytochemical study. *Brain Research, 402,* 379-382.

Hanulak, A. T., & Hull, E. M. (1987). Behavioral deficits in a rat model of maternal PKU. *Psychobiology, 15,* 75-78.

Herrup, K. (1983). Role of staggerer gene in determining cell number in cerebellar cortex. I. Granule cell death is an indirect consequence of staggerer gene action. *Developmental Brain Research, 11,* 267-274.

Herrup, K., & Mullen, R. J. (1979). Regional variation and absence of large neurons in the cerebellum of the staggerer mouse. *Brain Research, 172,* 1-12.

Herrup, K., & Trenkner, E. (1987). Regional differences in cytoarchitecture of the weaver cerebellum suggest a new model for weaver gene action. *Neuroscience, 23,* 871-885.

Hogan, E. L., Joseph, K. C., & Schmidt, G. (1970). Composition of cerebral lipids in murine sudanophilic leucodystrophy: The jimpy mutant. *Journal of Neurochemistry, 17,* 75-83.

Hudson, L. D., Berndt, J., Puckett, C., Kozak, C. A., & Lazzarini, R. A. (1987). Aberrant splicing of proteolipid protein mRNA in the dysmyelinating jimpy mutant mouse. *Proceedings of the National Academy of Sciences (U.S.A.), 84,* 1454-1458.

Hudson, L. D., Friedrich, V. L., Behar, T., Dubois-Dalcq, M., & Lazzarini, R. A. (1989). The initial events in myelin synthesis: Orientation of proteolipid protein in the plasma membrane of cultured oligodendrocytes. *Journal of Cell Biology, 109,* 717-727.

Inagawa, K., Watanabe, S., Tsukada, Y., & Mikoshiba, K. (1988). The role of myelin in learning performance observed in two strains of myelin-deficient mutant mice (shiverer and mld). *Behavioral and Neural Biology, 50,* 184-192.

Jacobson, S. (1963). Sequence of myelinization in the brain of the albino rat. A. Cerebral cortex, thalamus and related structures. *Journal of Comparative Neurology, 121,* 5-29.

Joseph, K. C., & Hogan, E. L. (1971). Fatty acid composition of cerebrosides, sulphatides and ceramides in murine sudanophilic leucodystrophy: The jimpy mutant. *Journal of Neurochemistry, 18,* 1639-1645.

Kelso, J. A. S. (1995). *Dynamic patterns. The self-organization of brain and behavior*. Cambridge: MIT Press.

Knapp, P. E., Benjamins, J. A., & Skoff, R. P. (1996). Epigenetic factors up-regulate expression of myelin proteins in the dysmyelinating jimpy mutant mouse. *Journal of Neurobiology, 29,* 138-150.

Knapp, P. E., Booth, C. S., & Skoff, R. P. (1993). The pH of jimpy glia is increased: Intracellular measurements using fluorescent laser cytometry. *International Journal of Developmental Neuroscience, 11,* 215-226.

Knapp, P. E., & Skoff, R. P. (1987). A defect in the cell cycle of neuroglia in the myelin deficient jimpy mouse. *Developmental Brain Research, 35,* 301-306.

Knapp, P. E., & Skoff, R. P. (1993). Jimpy mutation affects astrocytes: Lengthening of the cell cycle in vitro. *Developmental Neuroscience, 15,* 31-36.

Knapp, P. E., Skoff, R. P., & Redstone, D. W. (1986). Oligodendroglial cell death in jimpy mice: An explanation for the myelin deficit. *Journal of Neuroscience, 6,* 2813-2822.

Kolb, B. (1987). Recovery from early cortical damage in rats. I. Differential behavioral and anatomical effects of frontal lesions at different ages of neural maturation. *Behavioural Brain Research, 25,* 205-220.

Kolb, B., Holmes, C., & Whishaw, I. Q. (1987). Recovery from early cortical lesions in rats. III. Neonatal removal of posterior parietal cortex has greater behavioral and anatomical effects than similar removals in adulthood. *Behavioural Brain Research, 26,* 119-137.

Kolb, B., & Whishaw, I. Q. (1981a). Decortication of rats in infancy or adulthood produced comparable functional losses on learned and species-typical behaviors. *Journal of Comparative and Physiological Psychology, 95,* 468-483.

Kolb, B., & Whishaw, I. Q. (1981b). Neonatal frontal lesions in the rat: Sparing of learned but not species-typical behavior in the presence of reduced brain weight and cortical thickness. *Journal of Comparative and Physiological Psychology, 95,* 863-879.

Kolb, B., & Whishaw, I. Q. (1983). Dissociation of the contributions of the prefrontal, motor, and parietal cortex to the control of movement in the rat: An experimental review. *Canadian Journal of Psychology, 37,* 211-232.

Kurihara, T., Nussbaum, J. L., & Mandel, P. (1969). 2'-3'-cyclic nucleotide 3'-phosphohydrolase in the brain of the "jimpy" mouse, a mutant with deficient myelination. *Brain research, 13,* 401-403.

Lachapelle, F., Gumpel, M., & Baumann, N. (1994). Contribution of transplantations to the understanding of the role of the PLP gene. *Neurochemical Research, 19,* 1083-1090.

Lachapelle, F., Lapie, P., & Gumpel, M. (1992). Oligodendrocytes from jimpy and normal mature tissue can be 'activated' when transplanted in a newborn environment. *Developmental Neuroscience , 14,* 105-113.

Lalonde, R. (1986a). Acquired immobility response in weaver mutant mice. *Experimental Neurology, 94,* 808-811.

Lalonde, R. (1986b). Delayed spontaneous alternation in weaver mutant mice. *Brain Research, 398,* 178-180.

Lalonde, R. (1987a). Exploration and spatial learning in staggerer mutant mice. *Journal of Neurogenetics, 4,* 285-291.

Lalonde, R. (1987b). Motor abnormalities in staggerer mutant mice. *Experimental Brain Research, 68,* 417-420.

Lalonde, R. (1987c). Motor abnormalities in weaver mutant mice. *Experimental Brain Research, 65,* 479-481.

Lalonde, R., Bensoula, A. N., & Filali, M. (1995). Rotorod sensorimotor learning in cerebellar mutant mice. *Neuroscience Research, 22,* 423-426.

Lalonde, R., & Botez, M. I. (1986). Navigational deficits in weaver mutant mice. *Brain Research, 398,* 175-177.

Lalonde, R., Botez, M. I., & Boivin, D. (1986). Spontaneous alternation and habituation in a t-maze in nervous mutant mice. *Behavioral Neuroscience, 100,* 350-352.

Lalonde, R., Botez, M. I., & Boivin, D. (1987). Object exploration in staggerer mutant mice. *Physiology and Behavior, 41,* 115-117.

Lalonde, R., Botez, M. I., Joyal, C. C., & Caumartin, M. (1992). Motor abnormalities in lurcher mutant mice. *Physiology and Behavior, 51,* 523-525.

Lalonde, R., Filali, M., Bensoula, A. N., & Lestienne, F. (1996). Sensorimotor learning in three cerebellar mutant mice. *Neurobiology of Learning and Memory, 65,* 113-120.

Lalonde, R., Filali, M., Bensoula, A. N., Monnier, C., & Guastavino, J. -M. (1996). Spatial learning in a Z-maze by cerebellar mutant mice. *Physiology and Behavior, 59,* 83-86.

Lalonde, R., Joyal, C. C., & Cote, C. (1993). Swimming activity in dystonia musculorum mutant mice. *Physiology and Behavior, 54,* 119-120.

Lalonde, R., Manseau, M., & Botez, M. I. (1988a). Spontaneous alternation and exploration in staggerer mutant mice. *Behavioural Brain Research, 27,* 273-276.

Lalonde, R., Manseau, M., & Botez, M. I. (1988b). Spontaneous alternation and exploration in weaver mutant mice. *Behavioural Brain Research, 31,* 111-114.

Larsson, K., Guastavino, J. -M., & Ly, A. K. (1986). Altered reproductive behavior of the neurological mutant mouse staggerer. In J. Medioni & G. Vaysse (Eds.), *Genetic approaches to behavior* (pp. 69-79). Toulouse: Privat.

Lashley, K. S. (1951). The problem of serial order in behavior. In L. A. Jeffress (Ed.), *Cerebral mechanisms in behavior: The Hixon symposium* (pp. 112-136). New York: John Wiley & Sons, Inc.

Lorenz, K. Z. (1981). *The foundations of ethology.* New York: Springer-Verlag.

Lorenzana-Jimenez, M., & Salas, M. (1980). Effects of neonatal exposure to paint thinner on the development of swimming in rats. *Neurobehavioral Toxicology, 2,* 1-5.

Lorenzana-Jimenez, M., & Salas, M. (1983). Neonatal effects of toluene on the locomotor behavioral development of the rat. *Neurobehavioral Toxicology and Teratology, 5,* 295-299.

Lyon, M. F., & Searle, A. G. (Eds.). (1989). *Genetic variants and strains of the laboratory mouse* (2nd ed.). New York: Oxford University Press.

Macklin, W. B., Gardinier, M. V., King, K. D., & Kampf, K. (1987). An AG->GG transition at a splice site in the myelin proteolipid protein gene in jimpy mice results in the removal of an exon. *FEBS Letters, 223,* 417-421.

Mann, C. C. (1994). Behavioral genetics in transition. *Science, 264,* 1686-1689.

Mariani, J., & Changeux, J. -P. (1980). Multiple innervation of Purkinje cells by climbing fibers in the cerebellum of the adult staggerer mutant mouse. *Journal of Neurobiology, 11,* 41-50.

Marshall, J. F., & Berrios, N. (1979). Movement disorders of aged rats: Reversal by dopamine receptor stimulation. *Science, 206,* 477-479.

Matthieu, J. -M., Quarles, R. H., Webster, H., Hogan, E. L., & Brady, R. O. (1974). Characterization of the fraction obtained from the CNS of jimpy mice by a procedure for myelin isolation. *Journal of Neurochemistry, 23,* 517-523.

Matthieu, J. -M., Widmer, S., & Herschkowitz, N. (1973). Jimpy, an anomaly of myelin maturation. Biochemical study of myelination phases. *Brain Research, 55,* 403-412.

McDonald, W. I. (1986). The pathophysiology of multiple sclerosis. In W. I. McDonald & D. H. Silberberg (Eds.), *Multiple sclerosis*. London: Butterworths.

McKinzie, D. L., Roepe, S. B., & Spear, N. E. (1993, November). *Prevalence of "litter effects" as assessed through multiple measures: Implications for ontogenetic studies*. Paper presented at the annual meeting of the International Society for Developmental Psychobiology, Alexandria, VA.

Michel, G. F., & Moore, C. L. (1995). *Developmental Psychobiology. An interdisciplinary science* . Cambridge: MIT Press.

Miller, S. L. (1992). Nongenetic animal models of myelin disorders. In A. A. Boulton (Series Ed.), G. B. Baker (Series Ed.) & R. F. Butterworth (Vol. Ed.), *Neuromethods: Vol. 21. Animal models of neurological disease, I. Neurodegenerative diseases* (pp. 205-273). Totowa, New Jersey: Humana Press.

Misslin, R., Cigrang, M., & Guastavino, J. -M. (1986). Responses to novelty in staggerer mutant mice. *Behavioural Processes, 12,* 51-56.

Montella, P. G., & Reddy, C. S. (1991). Neurotoxic effects of secalonic acid D in mice during subchronic postnatal exposure. *Pharmacology, Biochemistry and Behavior, 40,* 241-247.

Morello, D., Dautigny, A., Pham-Dinh, D., & Jolles, P. (1986). Myelin proteolipid protein (PLP and DM-20) transcripts are deleted in *jimpy* mutant mice. *EMBO Journal, 5,* 3489-3493.

Moriguchi, A., Ikenaka, K., Furuichi, T., Okano, H., Iwasaki, Y., & Mikoshiba, K. (1987). The fifth exon of the myelin proteolipid protein-coding gene is not utilized in the brain of *jimpy* mutant mice. *Gene, 55,* 333-337.

Murphy, J. M., & Nagy, Z. M. (1976). Neonatal thyroxine stimulation accelerates the maturation of both locomotor and memory processes in mice. *Journal of Comparative and Physiological Psychology, 90,* 1082-1091.

Myers, M. M. (1991). Identifying relationships between early life experiences and adult traits. In H. N. Shair, G. A. Barr & M. A. Hofer (Eds.), *Developmental Psychobiology: New methods and changing concepts* (pp. 5-18). New York: Oxford University Press.

Nadon, N. L., Arnheiter, H., & Hudson, L. D. (1994). A combination of PLP and DM20 transgenes promotes partial myelination in the jimpy mouse. *Journal of Neurochemistry, 63,* 822-833.

Nagy, Z. M., Porada, K. J., & Anderson, J. A. (1977). Undernutrition by rearing in large litters delays the development of reflexive, locomotor, and memory processes in mice. *Journal of Comparative and Physiological Psychology, 91,* 682-696.

Najam, N., & Panksepp, J. (1989). Effect of chronic neonatal morphine and naloxone on sensorimotor and social development of young rats. *Pharmacology, Biochemistry and Behavior, 33,* 539-544.

Nave, K. -A., Bloom, F. E., & Milner, R. J. (1987). A single nucleotide difference in the gene for myelin proteolipid protein defines the *jimpy* mutation in mouse. *Journal of Neurochemistry, 49,* 1873-1877.

Nave, K. -A., Lai, C., Bloom, F. E., & Milner, R. J. (1986). Jimpy mutant mouse: A 74-base deletion in the mRNA for myelin proteolipid protein and evidence for a primary defect in RNA splicing. *Proceedings of the National Academy of Sciences (U.S.A.), 83,* 9264-9268.

Nussbaum, J. L., Neskovic, N., & Mandel, P. (1969). A study of lipid components in brain of the 'jimpy' mouse, a mutant with myelin deficiency. *Journal of Neurochemistry, 16,* 927-934.

Oliverio, A., & Messeri, P. (1973). An analysis of single-gene effects on avoidance, maze, wheel running, and exploratory behavior in the mouse. *Behavioral Biology, 8,* 771-783.

Olmstead, C. E. (1987). Neurological and neurobehavioral development of the mutant 'twitcher' mouse. *Behavioural Brain Research, 25,* 143-153.

Pantaleoni, G., Fanini, D., Sponta, A. M., Palumbo, G., Giorgi, R., & Adams, P. M. (1988). Effects of maternal exposure to polychlorobiphenyls (PCBs) on F1 generation behavior in the rat. *Fundamental and Applied Toxicology, 11,* 440-449.

Patil, N., Cox, D., Bhat, D., Fahan, M., Myers, R., & Peterson, A. (1995). A potassium channel mutation in weaver mice implicates membrane excitability in granule cell differentiation. *Nature Genetics, 11,* 126-129.

Pearson, K. G. (1987). Central pattern generation: A concept und scrutiny. In H. McLennan, J. R. Ledsome, C. H. S. McIntosh, & D. R. Jones (Eds.), *Advances in physiological research* (pp. 167-185). New York: Plenum.

Peruzzi, G., Lombardelli, G., Abbracchio, M.P., Coen, E., & Cattabeni, F. (1985). Perinatal caffeine treatment: Behavioral and biochemical effects in rats before weaning. *Neurobehavioral Toxicology and Teratology, 7,* 453-460.

Peters, A., & Proskauer, C. C. (1969). The ratio between myelin segments and oligodendrocytes in the optic nerve of the adult rat. *Anatomical Record, 163,* 243.

Petrosini, L., Molinari, M., & Gremoli, T. (1990). Hemicerebellectomy and motor behavior in rats. I. Development of motor function after neonatal lesion. *Experimental Brain Research, 82,* 472-482.

Phillips, R. J. S. (1954). *Jimpy*, a new totally sex-linked gene in the house mouse. *Zeitschrift fur indukt. Abstammungs- und Vererbungslehre, 86,* 322-326.

Poppe, S. M., Stuckhardt, J. L., & Szczech, G. M. (1983). Postnatal behavioral effects of ochratoxin A in offspring of treated mice. *Teratology, 27,* 293-300.

Posner, M. I., & Raichle, M. E. (1994). *Images of mind.* New York: Scientific American Library.

Preache, M. M., & Gibson, J. E. (1976). Effects of cyclophosphamide treatment of newborn mice on the development of swimming and reflex behavior and on adult behavioral performance. *Developmental Psychobiology, 9,* 555-567.

Privat, A., Valat, J., Lachapelle, F., Baumann, N., & Fulcrand, J. (1982). Radioautographic evidence for the protracted proliferation of glial cells in the central nervous system of jimpy mice. *Developmental Brain Research, 2,* 411-416.

Rakic, P., & Sidman, R. L. (1973a). Sequence of developmental abnormalities leading to granule cell deficit in cerebellar cortex of weaver mutant mice. *Journal of Comparative Neurology, 152,* 103-132.

Rakic, P., & Sidman R. L. (1973b). Weaver mutant mouse cerebellum: Defective neuronal migration secondary to abnormality of Bergmann glia. *Proceedings of the National Academy of Sciences (USA), 70,* 240-244.

Rezai, Z., & Yoon, C. H. (1972). Abnormal rate of granule cell migration in the cerebellum of "weaver" mutant mice. *Developmental Biology, 29,* 17-26.

Rice, S. A., & Millan, D. P. (1986). Validation of a developmental swimming test using Swiss Webster mice perinatally treated with methimazole. *Neurobehavioral Toxicology and Teratology, 8,* 69-75.

Richmond, G., & Sachs, B. D. (1980). Grooming in Norway Rats: The development and adult expression of a complex motor pattern. *Behavior, 75,* 82-96.

Richter, J. A., Stotz, E. H., Ghetti, B., & Simon, J. R. (1992). Comparison of alterations in tyrosine hydroxylase, dopamine levels, and dopamine uptake in the striatum of the weaver mutant mouse. *Neurochemical Research, 17,* 437-441.

Robinson, S. R., & Smotherman, W. P. (1991). The amniotic sac as scaffolding: Prenatal ontogeny of an action pattern. *Developmental Psychobiology, 24,* 463-485.

Robinson, S. R., & Smotherman, W. P. (1992a). Motor competition in the prenatal ontogeny of species-typical behavior. *Animal Behaviour, 44,* 89-99.

Robinson, S. R., & Smotherman, W. P. (1992b). Organization of the stretch response to milk in the rat fetus. *Developmental Psychobiology, 25,* 33-49.

Roffler-Tarlov, S., & Graybiel, A. M. (1984). Weaver mutation has differential effects on the dopamine-containing innervation of the limbic and nonlimbic striatum. *Nature, 307,* 62-66.

Roffler-Tarlov, S., & Graybiel, A. M. (1987). The postnatal development of the dopamine-containing innervation of dorsal and ventral striatum: Effects of the weaver gene. *Journal of Neuroscience, 7,* 2364-2372.

Rosenfeld, J., & Friedrich, V. L. (1986). Oligodendrocyte production and myelin recovery in heterozygous jimpy mice: An autoradiographic study. International *Journal of Developmental Neuroscience, 4,* 179-187.

Roussel, G., Neskovic, N. M., Trifilieff, E., Artault, J. -C., & Nussbaum, J. -L. (1987). Arrest of proteolipid transport through the Golgi apparatus in jimpy brain. *Journal of Neurocytology, 16,* 195-204.

Ryan, C., & Pappas, B. A. (1985). Neonatal 6-hydroxydopamine lesion of spinal norepinephrine terminals fails to affect the ontogeny of swimming behavior. *Developmental Brain Research, 17,* 258-262.

Salas, M. (1972). Effects of early malnutrition on the development of swimming ability in the rat. *Physiology and Behavior, 8,* 119-122.

Sarlieve, L. L., Farooqui, A. A., Rebel, G., & Mandel, P. (1976). Arylsulphatase A and 2'-3'-cyclic nucleotide 3'-phosphohydrolase activities in the brains of myelin deficient mutant mice. *Neuroscience, 1,* 519-522.

Schapiro, S., Salas, M., & Vukovich, K. (1970). Hormonal effects on ontogeny of swimming ability in the rat: Assessment of central nervous system development. *Science, 168,* 147-151.

Schmidt, M. J., Sawyer, B. D., Perry, K. W., Fuller, R. W., Foreman, M. M., & Ghetti, B. (1982). Dopamine deficiency in the weaver mutant mouse. *Journal of Neuroscience, 2,* 376-380.

Schneider, A., Griffiths, I. R., Readhead, C., & Nave, K. -A. (1995). Dominant-negative action of the jimpy mutation in mice complemented with an autosomal transgene for myelin proteolipid protein. *Proceedings of the National Academy of Sciences (U.S.A.), 92,* 4447-4451.

Shepherd, G. M. (1994). *Neurobiology* (3rd ed.). New York: Oxford University Press.

Shojaeian, H., Delhaye-Bouchaud, N., & Mariani, J. (1985). Decreased number of cells in the inferior olivary nucleus of the developing staggerer mouse. *Developmental Brain Research, 21,* 141-146.

Shojaeian Zanjani, H., Herrup, K., Guastavino, J. -M., Delhaye-Bouchaud, N., & Mariani, J. (1994). Developmental studies of the inferior olivary nucleus in *staggerer* mutant mice. *Developmental Brain Research, 82,* 18-28.

Shojaeian Zanjani, H., Mariani, J., & Herrup, K. (1990). Cell loss in the inferior olive of the *staggerer* mutant mouse is an indirect effect of the gene. *Journal of Neurogenetics, 6,* 229-241.

Sidman, R. L. (1968). Development of interneuronal connections in brains of mutant mice. In F. D. Carlson (Ed.), *Physiological and biochemical aspects of nervous integration* (pp. 163-193). Englewood Cliffs, New Jersey: Prentice Hall.

Sidman, R. L. (1983). Experimental neurogenetics. In S. S. Kety, L. P. Rowland, R. L. Sidman & S. W. Matthysse (Eds.), *Genetics of neurological and psychiatric disorders* (pp. 19-46). New York: Raven Press.

Sidman, R. L., Dickie, M. M., & Appel, S. H. (1964). Mutant mice (quaking and jimpy) with deficient myelination in the central nervous system. *Science, 144,* 309-311.

Sidman, R. L., Lane, P. W., & Dickie, M. M. (1962). Staggerer, a new mutation in the mouse affecting the cerebellum. *Science, 137,* 610-612.

Simon, J. R., & Ghetti, B. (1992). Topographic distribution of dopamine uptake, choline uptake, choline acetyltransferase, and GABA uptake in the striata of weaver mutant mice. *Neurochemical Research, 17*, 431-436.

Simon, J. R., Richter, J. A., & Ghetti, B. (1994). Age-dependent alterations in dopamine content, tyrosine hydroxylase activity, and dopamine uptake in the striatum of the weaver mutant mouse. *Journal of Neurochemistry, 62,* 543-548.

Skoff, R. P. (1982). Increased proliferation of oligodendrocytes in the hypomyelinated mouse mutant-jimpy. *Brain Research, 248,* 19-31.

Skoff, R. P., & Ghandour, M. S. (1995). Oligodendrocytes in female carriers of the jimpy gene make more myelin than normal oligodendrocytes. *Journal of Comparative Neurology, 355,* 124-133.

Skoff, R. P., & Knapp, P. E. (1990). Expression of the jimpy phenotype in relation to proteolipid protein appearance. *Annals of the New York Academy of Sciences, 605,* 122-134.

Smeyne, R. J., & Goldowitz, D. (1989). Development and death of external granular layer cells in the weaver mutant cerebellum: A quantitative study. *Journal of Neuroscience, 9,* 1608-1620.

Smeyne, R. J., & Goldowitz, D. (1990). Purkinje cell loss is due to a direct action of the weaver gene in Purkinje cells: Evidence from chimeric mice. *Developmental Brain Research, 52,* 211-218.

Smotherman, W. P., & Robinson, S. R. (1986). Environmental determinants of behavior in the rat fetus. *Animal Behaviour, 34,* 1859-1873.

Smotherman, W. P., & Robinson, S. R. (1987). Prenatal expression of species-typical action patterns in the rat fetus (*Rattus norvegicus*). *Journal of Comparative Psychology, 101,* 190-196.

Smotherman, W. P., & Robinson, S. R. (1989). Cryptopsychobiology: The appearance, disappearance, and reappearance of a species-typical action pattern during early development. *Behavioral Neuroscience, 103,* 246-253.

Sokal, R. R. & Rohlf, F. J. (1981). *Biometry. The principles and practice of statistics in biological research* (2nd ed.). San Francisco: W. H. Freeman and Company.

Sotelo, C. (1975). Anatomical, physiological and biochemical studies of the cerebellum from mutant mice. II. Morphological study of cerebellar cortical neurons and circuits in the weaver mouse. *Brain Research, 94,* 19-44.

St. Omer, V. E. V., Ali, S. F., Holson, R. R., Duhart, H. M., Scalzo, F. M., & Slikker, W. (1991). Behavioral and neurochemical effects of prenatal methylenedioxymethamphetamine (MDMA) exposure in rats. *Neurotoxicology and Teratology, 13,* 13-20.

St. Omer, V. E. V., & Bolon, B. (1990). Secalonic acid D mycotoxin affects ontogeny of brain catecholamines and swimming in mice. *Neurotoxicology and Teratology, 12,* 85-90.

St. Omer, V. E. V., & Mohammad, F. K. (1987). Ontogeny of swimming behavior and brain catecholamine turnover in rats prenatally exposed to a mixture of 2,4-dichlorophenoxyacetic and 2,4,5-trichlorophenoxyacetic acids. *Neuropharmacology, 26,* 1351-1358.

Syapin, P. J. (1982). Effects of the tottering mutation in the mouse: Multiple neurologic changes. *Experimental Neurology, 76,* 566-573.

Takahashi, J. S., Pinto, L. H., & Vitaterna, M. H. (1994). Forward and reverse genetic approaches to behavior in the mouse. *Science, 264,* 1724-1733.

Thelen, E., & Smith, L. B. (1994). *A dynamic systems approach to the development of cognition and action*. Cambridge: MIT Press.

Triarhou, L. C., Norton, J., & Ghetti, B. (1988). Mesencephalic dopamine cell deficit involves areas A8, A9 and A10 in weaver mutant mice. *Experimental Brain Research, 70,* 256-265.

Triarhou, L. C., Norton, J., & Hingtgen, J. N. (1995). Amelioration of the behavioral phenotype in weaver mutant mice through bilateral intrastriatal grafting of fetal dopamine cells. *Experimental Brain Research, 104,* 191-198.

Vorhees, C. V. (1981). Effects of prenatal naloxone exposure on postnatal behavioral development of rats. *Neurobehavioral Toxicology and Teratology, 3,* 295-301.

Vorhees, C. V. (1983). Fetal anticonvulsant syndrome in rats: Dose- and period- response relationships of prenatal diphenylhydantoin, trimethadoine and phenobarbital exposure on the structural and functional development of the offspring. *Journal of Pharmacology and Experimental Therapeutics, 227,* 274-287.

Vorhees, C. V. (1985). Comparison of the Collaborative Behavioral Teratology Study and Cincinnati behavioral teratology test batteries. *Neurobehavioral Toxicology and Teratology, 7,* 625-633.

Vorhees, C. V. (1986). Methods for assessing the adverse effects of foods and other chemicals on animal behavior. *Nutrition Reviews, 44,* 185-193.

Vorhees, C. V., Brunner, R. L., & Butcher, R. E. (1979). Psychotropic drugs as behavioral teratogens. *Science, 205,* 1220-1225.

Vorhees, C. V., Butcher, F [illegible] Brunner, R. L., & Sobotka, T. J. (1979). A developmental test b [illegible] for neurobehavioral toxicity in rats: A preliminary analysis usi[illegible] nosodium glutamate calcium carrageenan, and hydroxyurea. *Toxicology and Applied Pharmacology, 50,* 267-282.

Vorhees, C. V., Butcher, R. E., Brunner, R. L., & Sobotka, T. J. (1981). Developmental neurobehavioral toxicity of butylated hydroxytoluene in rats. *Food and Cosmetics Toxicology, 19,* 153-162.

Vorhees, C. V., & Fernandez, K. (1986). Effects of short-term prenatal alcohol exposure on maze, activity, and olfactory orientation performance in rats. *Neurobehavioral Toxicology and Teratology, 8,* 23-28.

Walton, K. D., Lieberman, D., Llinas, A., Begin, M., & Llinas, R. R. (1992). Identification of a critical period for motor development in neonatal rats. *Neuroscience, 51,* 763-767.

Watson, S. J., & Bekoff, A. (1990). A kinematic analysis of hindlimb motility in 9- and 10-day-old chick embryos. *Journal of Neurobiology, 21,* 651-660.

Weinberg, S. L., & Goldberg, K. P. (1990). *Statistics for the behavioral sciences* . Cambridge: Cambridge University Press.

Weiner, W. J., & Lang, A. E. (1989). *Movement disorders. A comprehensive survey*. Mount Kisco, New York: Futura Publishing Company.

Westerga, J., & Gramsbergen, A. (1990). The development of locomotion in the rat. *Developmental Brain Research, 57,* 163-174.

Whishaw, I. Q., & Gorny, B. (1994). Arpeggio and fractionated digit movements used in prehension by rats. *Behavioural Brain Research, 60*, 15-24.

Whishaw, I. Q., Gorny, B., Tran-Nguyen, Ly T. L., Castaneda, E., Miklyaeva, E. I., & Pellis, S. M. (1994). Making two movements at once: Impairments of movement, posture, and their integration underlie the adult skilled reaching deficit of neonatally dopamine-depleted rats. *Behavioural Brain Research, 61,* 65-77.

Whishaw, I. Q., Pellis, S. M., & Gorny, B. P. (1992a). Medial frontal cortex lesions impair the aiming component of rat reaching. *Behavioural Brain Research* , *50*, 93-104.

Whishaw, I. Q., Pellis, S. M., & Gorny, B. P (1992b). Skilled reaching in rats and humans: Evidence for parallel development or homology. *Behavioural Brain Research* , *47*, 59-70.

Yanagisawa, K., & Quarles, R. H. (1986). Jimpy mice: Quantitation of myelin-associated glycoprotein and other proteins. *Journal of Neurochemistry, 47,* 322-325.