Theses of PhD Dissertation

COMPUTER-ASSISTED ANALYSIS OF THE DEVELOPING BRAIN MOTOR SYSTEM AND COORDINATED LOCOMOTION IN THE FOAL

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HISTORICAL BACKGROUND AND INTRODUCTION

The distinguished position of the horse among domestic mammals dates back to the origins of Eurasian civilizations. Ever since the horse has been domesticated, its usefulness in locomotion, transport and warfare was realized. Particularly people living on plain areas could make use of the power, speed, and endurance of this animal. Without the horse the migration of people, the encounter of cultures and civilizations, the development of trading could not have happened as they did. Hence all sophistication in animal breeding and husbandry, all experience and skills accumulated in dealing with animals, all efforts to ensure animal health and welfare were concentrated on this species.

The body of knowledge collected developed into a separate field of animal sciences called hippology. Hippology had its climax in the 18-19th centuries particularly when horse racing was institutionally introduced in a number of countries as a means to further improve the efficiency of horse breeding and to produce horses bred with the purpose to satisfy best the most special needs of economy as well as of imperial armies.

Hippology, quite understandably, focused on horse gait. Its ultimate goal was to analyze the function of equine locomotor apparatus to an extent which would allow to select the best horses for a given purpose on one hand, and to obtain a full and perfect control over the animal’s behaviour on the other.

Hippology, however, could never develop beyond the confines of mere observation. Around the middle of the 19th century pioneer efforts were taken in cinematography which had, among other things, a dynamical impact on the development of motion analysis. Several attempts have started in different technical directions. The first scientific descriptions and notations of the equine gait were published from the 1870’s. The very first correct step succession at walk have been illustrated in hoof diagrams by Wilhelm Baumeister in 1870 (Baumeister, 1870)

Three years later Etienne-Jules Marey introduced his pneumatic device previously used in human gait experiments. As a result detailed hoof diagrams with accurate phase duration signs of each limb were generated. The principle of this device is still used in recent accelerometers. Marey, however, earned his fame from the own constructed photographic gun. In his pioneering studies all images appeared overlapping each other giving thereby a montage effect but later he could separate them into series suitable for the purposes of analysis (Marey, 1873).

Parallel to Marey's investigations Eadweard Muybridge's "movies" appeared with his still sequences taken of a running horse. This observation aimed to clarify whether there is a moment of suspension when no hooves are touching the ground. His films are renowned as artistically valuable works and became world-wide known.

It is noteworthy that the Hungarian historical painter, Bertalan Székely (1835-1910) was one of the pioneers in preparing so-called zoetrope bands which were recently recovered (Szőke and Beke, 1992) correspondence with Marey has been explored and exhibited with his other works in Budapest in 1992.

Nevertheless, the period of application of revolver-equipped snapshot gun and other tricky equipment was just a short prelude to the movie camera which became for the next decades the exclusive device of recording.

Films made with the conventional movie camera having the speed of 24 pictures per second could reproduce horse gait in a quality that appeared perfect for the human eye. The slow motion analysis of these recordings proved to be particularly useful in learning more about the details of different gait forms. In our times methods reached an even higher sophistication using various video cameras, including the extra high-speed CCD-cameras. The appearance of high-speed photography enabled application of high speed cameras in motion analysis (Kobluk et al., 1989; Martinez del Campo et al., 1991). After the 1970's when electronic devices have been introduced in the everyday life, special shutter systems, highly accurate synchronization of moving mechanical parts were developed for cameras. Advances in photochemical techniques enabled highly sensitive films to be produced industrially. All these improvements allowed to take pictures in the fractions of milliseconds or movies recording over 10,000 images per second.

A further major development was the introduction of the treadmill. While filming free-moving animals, unpredictable elements cannot be excluded. On the treadmill, however, animals can be kept in one place even during the fastest gait, the strategic points of limbs can be labeled with conspicuous tags to facilitate further analysis and measurements. Of course animals must be trained to perform in the treadmill (Althouse and Auer, 1987; Fredericson et al, 1983) so that results obtained have to be critically compared to those derived from the natural free motion or motion during other tasks (Ingen Schenau, van 1980; Corley and Goodship, 1993). Nevertheless, the monitoring of the horse gait in a treadmill and the subsequent analysis of recordings, provided valuable information as to the mechanics of joints and proved to be extremely useful in the diagnosis of lameness (Stashak, 1987).

An important objective, however, of normal gait analysis was to allow a prediction of future performance in young animals (Clayton, 1989; Grant, 1989, 1992; Back, 1994b). This goal, however, has never been achieved. Unfortunately, what looks even worse, up to now there appeared to be no coherent conclusion whatsoever that could have emerged from the wealth of data collected during the past 150 years. These data did not inspire any new training methods and were of no help in predictions. It has to be stated that, this is still not the case. The reasons may lie in the insufficient knowledge of locomotion control by the central nervous system. While experimental neurosciences have for long clarified the pathways of skeletal muscle innervation both in the central and peripheral nervous systems in several species, the equine central nervous system is practically unexplored. Lacking detailed information on the topography and development of the motor pathways of the horse there is no biological basis of assessment, consequently no objective methods of improvement or selection can be introduced. Veterinary textbooks rely on descriptions derived from other species, usually small domestic mammals. The brains of the cat, dog, sheep, etc. are small enough to be fixed and embedded as a whole and cut with a conventional microtome. The method of tract-tracing is based on series of sections through the entire brain from where the course of specially stained fibres can be reconstructed. In addition to inconveniences caused by the huge body mass, the high costs of keeping and the fact that the horse is much too valuable to be sacrificed for experimental purposes, all experience suggests that the sampling of horse brains of given ages is a long and cumbersome procedure. Moreover, the size of the equine brain matches with that of the young human brain giving no chance for serial sectioning either in a conventional microtome or a cryostat.

Tract-tracing is based on the demonstration of bundles of myelinated fibres. A number of staining technologies have been developed to visualize the myelin sheath (see Pannese, 1994). On the other hand, formation of the myelin sheath around an axon is a process taking place in the late embryonic and early postnatal period (Río Hortega, 1930). There are, however, wide interspecies variations in the timing of myelination. Some animals are born with an almost unmyelinated central nervous system, while others in differently advanced stages of myelinization. Some pathways are notorious by their late myelination being protracted into early adulthood. This applies particularly to the motor system. It is also important to note that the acquisition of the myelin sheath also means the functional maturation of an axon since it becomes only then capable of a full velocity (saltatory) impulse conduction (Lillie, 1925; Huxley and Stämpfli, 1949). This is clearly reflected by the pattern of movements of the newborn. Species with more advanced myelination of their motor pathways at birth develop earlier their independent locomotion than the ones with neonatally poorly myelinated motor tracts (Szalay et al., 2000).

This parallelism between the degree of neonatal locomotion and the advance of myelination of the motor pathways encouraged us to take myelination as a major morphological reference to compare motion patterns with.

Recent advances in microtome technology – initiated primarily by the demand to cut series of sections from whole adult human brains – led to the construction of a special high-performance microtome on which large-size sections can be readily cut. This was completed with the manufacturing of large-size slide glasses and special coverslips, as well as with the modification of the specimen stage of the microscope such that it can carry large area preparations. This equipment, particularly when joined through a video camera to a computer was thought to be suitable to map the adult and young horse brains.

Our objective was therefore to describe the motor system of the adult horse brain and to correlate it with brains from various developmental periods of horse embryos and foals.

AIMS AND SCOPE

Our primary aim was to provide a morphometric analysis of the adult and developing horse brains. By this we hoped to obtain some basic reference data for further functional morphological studies. To this end, we attempted

(i) to monitor the cytoarchitectonic maturation of motor centres, particularly the motor areas of the neocortex, striatum, brainstem and the cerebellar cortex

(ii) to follow the course of myelination of motor centres and pathways in areas of the brain which may be instrumental in the innervation of the skeletal musculature and in the control of motor coordination.

(iii) to get an objective measure of brain growth from late fetal to young adult age, including size and volume increase, surface increase and the degree of gyrification.

Secondly, efforts were made to correlate histologically defined maturational states of the motor centres and pathways with the development of motion patterns as distracted from video recordings of animals either performing on a treadmill or moving free.

Finally, we decided to express our findings both histologic and functional, in terms of an objective, automated method, the computer-assisted image analysis which needed a substantial development to be adaptable for these purposes.

The present work is an endeavor to open up new possibilities in the prediction of future sport value of foals. Results may be also relevant to equine neurology.

MATERIALS AND METHODS

Equine brains were removed from the skull in the late prenatal and the postnatal period up to 2 years of age. Postnatal brains were obtained from animals in which infectious and/or neurological diseases were excluded. Lost animals were kept at 4 C° temperature. Sampling was carried out 2-4 hours after death. Brains were cleaned from the meninges, weighed (14 days before birth: 305 g, 7 days before birth: 303 g, newborn: 312 g, 4-day-old: 311 g, 6-week-old: 326 g, 545-day-old: 608 g, 560-day-old: 615 g) and their rostrocaudal extent was measured. Whole brains were fixed in 4-10% formaldehyde buffered at pH 7.4 with phosphate buffer. The duration of fixation was 3-8 months depending on the size of the brain. Following fixation, brains were photographed and immersed for a week into 1% eosin solution. Then they were dehydrated in graded ethanol, each phase lasting 2-5 days, and embedded through chloroform in paraffin. Twenty-micron thick serial sections (full series) were cut in the frontal plane from the entire specimen with a TETRANDER Large-Section Microtome. In 500 µm distances, three subsequent sections were mounted on large-area slide glasses. Sections were processed for Nissl's cresyl violet staining to reveal cytoachtecture, and for Haidenhain's iron-haematoxylin to demonstrate myelin sheath.

Series of histological sections were digitized and evaluated after a segmentation and thresholding procedure (Rosenfeld and Kak, 1976) as 8 bit grey-level images using an NIH Image 1.62 image analysis software.

The contour measurements were carried out in the Nissl-stained material. A rough contour of each section defined at segmentation of low magnification gave the outer perimeter (C_{out}). At higher magnification all the sulci became clearly identifiable and their total perimeter (C_{tot}) could be drawn. The quotient of C_{out} and C_{tot} is the gyrification index (GI; Wosinski et al, 1996). Beside the perimeter, the area of each section has been determined. Principal elements of the brain such as the hemispheres, cerebellum and the brainstem were measured discretely. To determine the ratio of myelinated areas the total area of each section was measured on the Nissl-stained sections, while myelin stained sections were segmented for defining the total territory of myelin by sections.

Within the sections the tracts which may play important role in the execution and coordination of functions of the locomotor system were segmented manually.

The territory of the measured regions and the amount of myelin related to the reference areas were calculated. The two major reference areas were the cerebral cortex as a minimum and the optic nerve as a maximum density of myelination.

The cytoarchitectonics of the primary and the secondary motor cortices were studied. Density of pyramidal cells of layers 3 and 5 was measured by segmentation of their cell bodies and expressed as percentage of total cortical area. In the cerebellum, the perinatal migration of granular cells was investigated from the external granular cell layer through the ganglionic layer to the final (internal) granular layer. The amount of cells was determined with densitometric segmentation in the external granular and molecular layers. The density of Purkinje cells was estimated by calculating the number of cells intersecting a line drawn along the layer of Purkinje cells.

Series of MRI images were taken from both of the two adult brains. After the removal from the skull a 6 months long period of fixation followed. MRI sequences consisted of 256 coronal sections. A three-dimensional reconstruction of sequences was made in NIH Object Image 1.62 software (Wayne Rasband, National Institutes of Health, USA) in order to assist identification of anatomical features.

Gait analysis studies have been made on the comparison of commercially available VHS video cameras to specially developed CODA-3 (Cartesian Optoelectronic Digital Apparatus, Charnwood Dynamics, Loughborough, UK) motion analysis system (Mitchelson, 1988; Schamhardt et al., 1992; Back et al., 1993) in order to confirm accuracy of the non-professional VHS cinematography. In order to compare results of both systems, parallel recordings were taken. Motion of a 5-year-old Dutch warm blood gelding recorded followed by a 5 minutes warming up exercise on a treadmill. The observed gait types were walk, trot and left lead canter at 1.6, 4 and 7 m/s speed, respectively. For the sake of comparison the same stride of each gait types has been measured in both systems. Fore- and hindlimbs were investigated in different recordings. In the accuracy checkup, moments of each video frame of 13 strides of a trot at 4 m/s have been identified and compared in the dataset of CODA system. Identification of the same event recorded by both systems was assisted by a timer device connected to CODA.

Video recordings on the treadmill have been taken at 1000-1500 lux of illumination from 8 m distance on the left side. Video recordings were played back on a SONY VCR used in freeze-frame mode. Digitization has been performed using a Macintosh 8100/80 AV computer with a built-in digitizing board. For calculations of segmental angles and positions macros were applied coded in a Pascal like macro language of the software developed for this purpose. Joint angles have been calculated as positive and negative values around 0 degree. Scapula rotation was computed as an angle between the axis of the scapular spine and the horizontal plane of the environment. Pelvic rotation was also related to the horizontal plane. The difference between the horizontal plane of the two systems was corrected by mathematical transformation of video data. The difference of averages of angular data was added to video data in averaged strides.

For statistical analyses the Student's t-test and the χ^2-test were applied. For correlations between gait analysis data sets regression tests were used.

RESULTS AND CONCLUSIONS

Our findings obtained by qualitative and quantitative investigations suggest that the overall brain size does not increase between 14 days prior to expected birth to postnatal day 45, meanwhile significant internal changes take place:

- there is an increase in the amount of Nissl-substance, even if viewed at the level of single neurons;
- the amount of myelin increases involving the intensification of myelin staining and the appearance of newly formed myelin. This results in the enlargement of the areas of myelinated motor tracts.

Between postanatal day 45 and adulthood the overall size of the brain, as indicated by external parameters is almost doubled. This is not followed by an equal degree of myelin and Nissl-substance formation showing that the main events of motor pathway maturation occur between prenatal and early postnatal periods, whereas growth in size of the brain follows only after this maturation process.

Concerning locomotion of the horse, a new method of gait analysis was introduced and tested as to accuracy and applicability. The method is based on recordings made by a home video camcorder. It was found that it yields results comparable to those obtained by a specially developed unifunctional device (CODA).

With the help of the home video assisted method of gait analysis we were able to compare the curves of different stages of various gaits relevant to limb joints. Moreover, analytic curves were compared between the young foal and the adult. Comparison allowed to define the characteristics of 'immature' and 'mature' types of curve for each joint studied. This result may serve as a reference in further analyses. As a general feature, the the proximal joints equipped with large groups of muscles yield 'immature' curves for longer postnatal periods than distal joints that in the horse are operated by a reduced musculature.

Temporal data analysis revealed that in foal the duration of phases within a stride is different from that in the adult. In the foal, the characteristcs of stride phases do not change from walk to trot, while in the adult these are conspicuously different.

Gait analysis pointed out in quantitative terms the increase of coordination in limb movements which was shown to be parallel to the maturation of the motor centres and tracts within the brain. It is assumed that there is a causal relationship between the maturation of structural and functional components of the equine locomotion. The verification of this assumption requires further studies.

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