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Research report

The development of skilled walking in the rat

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ABSTRACT

The rat is an altricial species and consequently undergoes considerable postnatal development. Careful analysis of the emergence and disappearance of motor behaviours is essential to gain insight into the temporal pattern of maturation of motor system structures. This study presents a qualitative analysis of the developmental progression of skilled movement in the rat by using a skilled walking task. A new rung bridge task was used to expose rat pups to a novel environment in order to reveal their potential capabilities. Ten rat pups were filmed daily from postnatal day 7 through postnatal day 30 as they explored the rung bridge task. Discrete changes in skilled and non-skilled walking in fore- and hind-limbs were evaluated by scoring seven categories and 24 subcategories of motor behaviour, including limb flexion and extension, coordination, posture, sensorimotor responses, distal control, and tail use in rat pups. Frame-by-frame analysis of ambulatory movement revealed six distinct stages of locomotor development. The most significant transformation to mature gait patterns was found between postnatal days 15 and 19, and maturation of all motor behaviour was completed by postnatal day 27. The findings are discussed in relation to the maturation of underlying structures and their relevance to studies of brain damage.

1. Introduction

The Norway rat (*Rattus norvegicus*) is an altricial species and consequently undergoes considerable postnatal development. Previous studies have discovered a number of particular motor behaviours that occur within certain time frames of rat pup development. For example, Altman and Sudarshan [1] observed pivoting and crawling behaviour, and Eilam [2] provided a time course of gait development. Furthermore, Westerga and Gramsbergen [19] noted significant time windows of musculoskeletal development that are paralleled by integration of descending and ascending systems [3–5]. These identifiably distinct stages of rat pup development are accompanied by corresponding behavioural modifications.

Most previous studies suggested that postnatal development of the rat central nervous system and associated behavioural changes follow two temporal gradients: a rostral–caudal and a ventral–dorsal gradient [6]. These gradients are mainly displayed as forelimb control preceding hind limb control [7]. However, there are exceptions to the rule of rostral–caudal/ventral–dorsal gradients in that forelimb elevation precedes head elevation [31]. Furthermore, appreciable differences exist between motor columns within a segment that deviate from the original notion of the existence of rostral–caudal/ventral–dorsal gradients in development [5]. It is possible that such exceptions to the absolute chronology of motor skill acquisition in rat ontogeny also exist for skilled movement.

Though extensive research has examined neural and anatomical motor system development, there is a dearth of research providing a time frame as to when these systems are functional at the behavioural level. The goal of the present study was to catenate previous observations into easily identifiable stages through qualitative analysis of skilled movement from postnatal day 7 to postnatal day 30 (P7–P30). Furthermore, the aim was to establish a time course as to when typical and atypical behaviours emerge and disappear. In order to assess the potential capabilities of rat pups it was important to place them in a novel environment to stimulate spontaneous behaviour and exploration. A novel apparatus, the rung bridge stepping task, was developed based on the ladder rung walking task (see [8,9]). The ladder rung walking task is commonly used to study skilled walking and recovery of function after brain injury in adult rats. The new rung bridge stepping task seemed an ideal apparatus for assessing ambulation and discrete fore- and hind-limb movements due to the complexity of skilled movement required to successfully traverse the apparatus. Based on early studies by Nornes and Das [6], we anticipated that qualitative behavioural analysis would reflect a temporal rostral–caudal gradient as well as a ventral–dorsal gradient during development of the central nervous system.

2. Materials and methods

2.1. Subjects

Two litters of Long-Evans Hooded rats (*R. norvegicus*), consisting of 16 and 19 pups, respectively, were used. Postnatal day 1 (P1) is considered the first calendar

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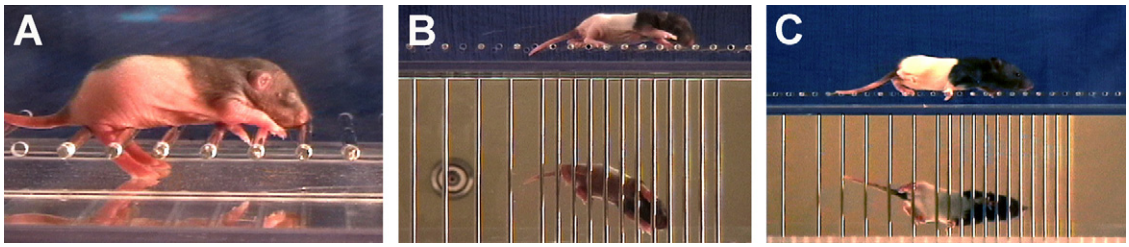

Fig. 1. Photographs of rat pups performing the rung bridge stepping task. (A) A P7 animal illustrating forelimb use while hind limbs remain mainly passive. (B) A P10 animal showing fore- and hind-limb use and onset of occasionally weight bearing steps. (C) A P17 animal showing weight bearing and coordinated stepping patterns.

day of birth. Pups were housed in the Lethbridge vivarium with dam and littermates until weaned at 22 days. They were then split into two groups with littermates. Housing consisted of clear Plexiglas hanging cages (45.4 cm × 24 cm × 19.5 cm high) with 1/8″ Laboratory Animal Bedding (Bed-o'cobs, Andersons, Maumee, OH, USA). Food and water were available *ab libitum*. The housing room was maintained at 20–21 °C with a 12 h light-and-dark-cycle (lights on at 07:00). Animals were behaviourally tested in the morning hours from P7 to P30.

2.2. Behavioural testing

Five randomly chosen pups of either sex from each of the two litters ($n = 10$) were filmed for one trial daily at the same time of day from P7 to P30 on the rung bridge stepping task. Dams were kept in the testing room during testing until pups were weaned at 21 days.

2.3. Rung bridge stepping task apparatus

The rung bridge stepping task consisted of two Plexiglas walls connected by a floor of metal rungs, modified from the original ladder rung walking task as described by Metz and Whishaw [8], Metz and Whishaw [10]. Dimensions of the apparatus were 1 m in length, 20 cm in height, and 8 cm wide (see Fig. 1). The rungs were 3 mm in diameter and were arranged in sections at distances ranging from 1 to 3 cm. The placement of fore- and hind-limbs on the rungs was recorded. The rung bridge stepping task apparatus was placed on a table surface so that pups could place their limbs on the surface if they missed a rung. The rungs were elevated 1 cm above the table surface.

Each pup was filmed for 3 min or until it crossed the entire length of the apparatus. During later stages of development, rat pups traversed a distance of 50 cm (half the length of the apparatus) per trial while video recorded.

2.4. Video recording

Pups were filmed using either a Canon NTSC ZR50 or ZR50 MC Digital Video Camcorder (Canon USA Inc., Lake Success, NY). Video recordings were made on mini Digital Video Cassettes with a shutter speed of 1/500. The video recordings were analyzed frame-by-frame at 30 f/s.

2.5. Skilled walking rating score

Qualitative analysis of fore- and hind-limb ambulatory movements was performed using a novel rating scale (Table 1) comprising seven categories and 24 subcategories associated with skilled movement. The scoring system was developed based on movements made by adult rats. Each component was rated on a 3-point scale with a score of 0 for absent movement, a score of 1 for atypical movement, and a score of 2 for typical (adult-like) movement. Scores were normalized for the number of steps and mean scores were taken for each sequence. The evaluation of each of the movements listed in Table 1 is described in the following sections. Movements were analyzed across six major developmental stages: *asynchronous activity* (P1–P6); *pivoting stage* (P7–P10); *crawling stage* (P11–P14); *integration stage* (P15–P18); *immature ambulation* (P19–P26); *mature ambulation* (P27–30). Furthermore, spontaneous rearing and grooming behaviour was documented as well as the day of eye opening and ear unfolding.

2.5.1. Orientation

Orientation of head, trunk and limb were scored using three main categories.

Head orientation: Animals received a score of 0 in this category when their upper torso and/or head were opposite to the direction of movement. Animals received a score of 1 when the head was lateral to the direction of the movement. If the head was facing forward or towards the direction of the movement, a score of 2 was given.

Trunk orientation: Animals received a score of 0 when the caudal region of the trunk was in an orientation opposite to the direction of movement. A score of 1 was given when the trunk was lateral to the direction of movement. A score of 2 was given if the trunk aligned with the direction of movement.

Limb orientation: Animals received a score of 0 when the limb was at a 90–45° angle relative to the trunk. A score of 1 was given when the limb was positioned at a 45–30° angle, and a score of 2 was given when the limb was positioned at an angle greater than 30°.

2.5.2. Flexor/extensor activity of paw and digits

Paw and digit flexion and extension were measured by three categories, modified from Metz and Whishaw [8].

Flexion: Animals received a score of 0 when no flexion of the wrist was observed. A score of 1 was given when the wrist exhibited partial flexion, and a score of 2 was given when the wrist exhibited full flexion.

Extension: An animal received a score of 0 when it did not extend its paw and digits after a lift (see Fig. 3A). A score of 1 was given when the animal opened its digits without an extension parallel to the rungs. A score of 2 was given when the paw and digits were extended parallel to the rung before placement.

Close digits with lift: An animal received a score of 0 when the paw remained open and digits extended with a lift from the surface or rung. A score of 1 was given when the digits came together with a partial flexion. A score of 2 was given when the digits were closed upon lifting with full flexion.

2.5.3. Gait development

Limb placement, abduction and adduction were scored separately for fore- or hind-limbs.

Placement on rung: Animals received a score of 0 when no apparent attempt to place the limb on a rung was made. A score of 1 was given when the pup initiated an attempt to place the limb on a rung but was unable to reach the rung or place the limb. A score of 2 was given when the animal made a successful attempt to place the limb on a rung, regardless of the quality of the placement.

Abduction: An animal received a score of 0 when lateral articulation of the limb was observed. If the animal exhibited partial limb abduction it was given a score of 1 and if it demonstrated forward stepping without lateral diversion it was given a score of 2. The hind limb was also scored for abduction using the same criteria for 0 and 1 though a slight abduction of the hind limb was permitted for a score of 2 as this reflects the adult movement.

Adduction: An animal received a score of 0 when the limb showed articulation towards the body axis. A score of 1 was given when the limb exhibited partial limb adduction, and a score of 2 was given when forward stepping occurred without any adduction.

Coordinated stepping pattern: Stepping coordination was scored according to the description of lateral walk by Eilam [2]. An animal received a score of 0 when the forelimb and hind limb did not alternate in succession. A score of 1 was given when there was more than a 50% overlap of the swing phase of two successive steps and a score of 2 was given when the hind limb and forelimb step were in succession with no more than a 50% overlap of the swing phase of two successive steps.

2.5.4. Postural control

Postural control was measured as indicated by weight bearing, base of support and limb placement.

Weight bearing: An animal received a score of 0 when it did not elevate the trunk from the surface/rungs. A score of 1 was given when the trunk was elevated with partial support. A score of 2 was given when the animal lifted its shoulders and hindquarter off the surface.

Base of support: A score of 0 was given when the hind limbs were oriented at an angle of greater than 30° away from the body. A score of 1 was given when the hind limbs were oriented laterally from the body, but less than 30° away. A score of 2 was given when the hind limbs were tucked underneath the torso.

Limb placement on rung: A score of 0 was given when the limb was placed on the rung with an ulnar deviation between 45° and 90°. An ulnar deviation of 30–45° was scored as 1 point and a deviation of <30° was given a score of 2 points.

2.5.5. Sensorimotor responses

Five separate behaviours were scored to assess sensorimotor responses. Scores were based on an earlier error rating scale [8,9]. Fore- and hind-limbs were scored separately.

Placing response on rung: A score of 0 was given when the pup attempted to place a limb on a rung but slipped off and did not bear weight. A score of 1 was given when a limb was placed on a rung but the animal used other rungs for orientation