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Ontogeny of Psychomotor and Sensory Functions in the Rat: Effects of Sexual Dimorphism

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Abstract

Background: We studied the development of eight (8) different psychomotor and sensory functions in male and female rats, from postnatal day 10 to 45, with the aim of determining whether the ontogenesis of these functions was subject to sexual dimorphism. **Methods:** Wistar rats bred according to standard conditions in our laboratories were put into reproduction. Ten days after whelping, male and female pups were identified and subjected to a battery of behavioral tests on postnatal days 10, 15, 20, 25, 30 and 45, to assess the development of the following psychomotor and sensory functions: Exploratory activity, locomotor activity, emotional defecation, hind paws lifting reflex latency, wire-grasping time, Latencies of execution of crawling along the wire and of leap onto the ground, nociception (tail flick) and body weight. **Results:** Only complex brain functions generated by cerebral cortex activities, *i.e.* exploratory activity and leap execution latency, do not undergo differential development sex-dependent. However, voluntary motor functions initiated in the motor cortex, and requiring high peripheral muscle performance such as crawling execution latency and wire-grasping time developed more rapidly in males than in females. Correlatively, body weight *i.e.* muscle mass index increased more speedily in males than in females. On the other hand, studies of automatic motor functions such as locomotor activity, and reflex motor functions *i.e.* hind paws lifting reflex latency and tail flick latency showed earlier development in females than in males. In addition, the study of emotional response, an emanation of limbic structures, showed prodigious development in females compared to males. **Conclusion:** Our studies have shown that there is a developmental sexual dimorphism of the central nervous system in the rat. Indeed, studies of automatic and reflex motor functions, whose activities are essentially linked to the spinal cord and brainstem, indicated that hindbrain areas develop more speedily in females

than in males. Likewise, study of the emotional response emanating from diencephalic limbic structures, in particular the hypothalamus, showed a prodigious and early development in females compared to males. Taken together, our studies indicate that the vast majority of brain structures and functions reached maturation earlier in females than in males. Estrogen is the trigger hormone for early maturation of the female brain.

Keywords

Developing Rats, Brain Ontogeny, Psychomotor Functions, Sexual Dimorphism

1. Introduction

The literature has extensively described variations in anatomical, physiological, biochemical and neurobiological parameters during the development of the central nervous system (CNS) [1]. Marginally, the concept of sexual dimorphism has gradually emerged in comparative studies between male and female brain development. This concept would enable an individual to adopt male or female sexual behavior from puberty onwards. Indeed, several brain nuclei and structures are likely to undergo numerous morphological changes throughout life and gonadal steroids are involved in the sexually dimorphic development of the brain, behaviors and physiological functions [2]. For example, the limbic system plays a very important role in many emotional behaviors such as aggression, affectivity, fear, learning and memory [3]. The limbic system contains a number of nuclei and structures whose anatomy and function are often influenced by sexual dimorphism.

The hippocampus, one of the main nuclei of the limbic system, is a telencephalic brain structure primarily involved in learning and memory [4] [5]. Its structure is divided into cytoarchitectonically different subfields, notably the cornu ammonis (CA) 1-4, and the dentate gyrus [6]. One of the most consistent sex differences in the developing hippocampus comes from studies looking at cell proliferation. As early as postnatal days P1 and P4, male rats show increased cell proliferation in the CA1, CA3 and dentate gyrus compared to females [7] [8], leading to a greater neuron number in these subregions at P7 [9] and in CA1 and CA2/3 at P21 [10]; all of which may contribute to a larger volume size for male hippocampus [11]. Conversely, at P21, female rats have a greater number of dendritic segments on dentate gyrus granule cells than males [12]. There is also a greater density of synaptic spines and boutons in the CA1 of female mice compared with males at P15 [13]. This suggests the presence of sex differences in hippocampal morphology during early development [14].

Moreover, microglia are involved in the formation, elimination, and maturation of synapses [15] [16] [17]; they play a key role in circuit maturation and sexual differentiation of the developing brain [18] [19]. Microglial phagocytosis

of progenitor cells can reduce overall cell proliferation [20]. Accordingly, differences in microglial phagocytosis may contribute to sex differences in neonatal hippocampal cell proliferation. Indeed, female rats display a greater proportion of phagocytic microglia compared to males [21]. This increased number of phagocytic microglia in P2-3 females can be decreased to male levels through neonatal estradiol treatment, but not dihydrotestosterone [21].

The amygdala is a key structure involved in emotional learning [22] [23]. In early life, it undergoes rapid and dynamic changes in morphology, volume, cell proliferation and physiological properties; some of which follow sex-specific patterns [14]. The medial amygdala (MeA) is particularly implicated in sexually divergent behaviors [24] and matures earlier than other amygdala regions. Thus, the MeA attains adult-typical volumes earlier than other subnuclei in both sexes, but with females reaching their adult-typical volume before males. Indeed, the MeA volume increases during the first postnatal days, with female rats reaching adult-like MeA volume by P5 [25], whereas in males the MeA continues to gradually increase in size, reaching a volume larger than that of females by P21, which is maintained into adulthood [25].

The hypothalamus also shows functional differences between male and female brains. Several hypothalamic nuclei undergo dimorphic sexual development. For example, the central part of the medial preoptic nucleus (MPNc) and the ventromedian hypothalamus (VMH) volumes were greater in males than in females, while the paraventricular nucleus (PVN) volume showed no gender differences [26].

The spinal nucleus of the bulbocavernosus (SNB) within the lumbosacral spinal cord is a sexually dimorphic pool of motoneurons that innervate the perineal musculature. The sexual dimorphism in the SNB develops neonatally. Male rodents have many more motoneurons in the spinal nucleus of the bulbocavernosus (SNB) than do females. This sex difference is caused by hormone-regulated death of SNB motoneurons and their target muscles [27]. Androgens have a masculinizing effect on the male rat SNB by preventing motoneuron cell death neonatally [28]. This prevention of cell death allows the increase in motoneuron number seen in male rat compared to female rats.

Together, these observations show that almost all studies have compared the maturation of CNS nuclei and structures between males and females. Few studies have compared the maturation of CNS functions between males and females. We therefore set out to compare 8 different psychomotor and sensory functions generated by CNS activity between male and female rats.

2. Materials and Methods

2.1. Animals

Nulliparous Wistar female rats bred in our colony, 12-old weeks and weighing 190 - 200 g, were used in our experiments. They were maintained under standard laboratory conditions in ambient temperature (30˚C ± 2˚C) at 12 h

light/dark cycles and relative humidity (85% ± 3%). Rats were housed in numbers of 3, in polypropylene cages (27 × 37 × 18 cm) with floors covered in wood shavings. They were fed a pellet-type diet and accessed water ad libitum. One week before tests starting, they were acclimatized to experimental conditions. All experiments were carried out in accordance with the National Institutes of Health "Guide to the Care and Use of Laboratory Animals".

For mating, females were grouped three per cage in which a Wistar male was introduced at 18:00 h daily. The presence of a vaginal plug indicates the first day of gestation. Approximately one week before parturition, the dams were housed individually in plastic cages and checked daily for the presence of pups. The date of parturition was designated as postnatal day 1 (P1). Litter size was adjusted within 24 h of birth so that each mother was nursing 8 - 10 pups randomly assigned to each mother.

After birth, the pups were left undisturbed until they were 10 days old. Sex identification was performed on postnatal day 10. At weaning (day 21), the pups were then housed in some sex group of 3 per cage. Testing was carried out at 10, 15, 20, 25, 30 and 45 days of age. The same pups were assessed from postnatal days 10 to 45. Similarly, pup body weight was measured from postnatal days 10 to 45. Mothers remained with the pups at all times until weaning, except during test sessions. All offspring had ad lib access to laboratory food in pellet form and plain water.

2.2. Behavioral Tests

Neural development was assessed by a battery of behavioral tests (described previously in Bâ and Seri [1], Bâ [29] which examine the development of psychomotor and sensory functions in the rat pups. Eight neurodevelopmental abilities were tested in the offspring, from the 10th to the 45th postnatal day: Exploratory activity, locomotor activity, emotional reaction, hind paws lifting reflex, wire-grasping time, crawling and leap execution latencies, Nociception.

2.2.1. Hole-Board Test

The apparatus was a Plexiglas board with 16 equidistant holes (4 × 4 holes). Electric photocells, directly incorporated in the inner side of each hole, provided automated measurement of the number of head-dip responses by a microcomputer.

The hole-board test measures exploratory activity, locomotor activity and emotional responses in pups during a 5-minute trial.

- Exploratory activity

Each rat was placed singly in the center of the hole-board and the number of head-dip responses was recorded. Only one 5 min trial was performed at every age.

- Locomotor activity

The space of the hole-board was crossed by two perpendicular luminous rays allowing to cipher the displacement of the animal each time their trajectories

were interrupted. The number of crossed rays was recorded during a 5 rain trial by an automatic apparatus. Only one 5 min trial was performed at every age.

- ***Emotional reaction***

The new situation evoked by the experimental context of the hole-board generates anxiety in the animal [22]. The number of emitted defecations was counted during a 5 min trial of exposure at every age. After each trial, the floor of the apparatus was wiped with dilute acetic acid and dried to remove traces of the previous path.

2.2.2. String Test

The testing apparatus consisted of a piece of iron wire, 0.7 mm in diameter and 37 cm long, tied tightly between two vertical bars and suspended 35 cm over the ground.

The testing apparatus was used to measure hind paws lifting reflex, wire grasping time, crawling execution latency along the wire and leap execution latency onto the ground.

- ***Hind paws lifting reflex***

The animal was left gripped by its fore paws at the middle of the wire. The time spent by the animal to retrieve its balance by bringing its hind paws upon the wire, was measured.

- ***Wire-grasping time***

The rat is compelled to get the grip in the middle of the wire by its fore paws and the observer counts time until the fall of the animal.

- ***Crawling and leap execution latencies***

The rat was compelled to get the grip on the middle of the wire. The time spent to reach one of the two vertical bars by crawling execution, or to leap onto the ground was timed.

2.3. Tail-Flick Test

The tail-flick was evoked using a feedback-controlled projector lamp (24 V - 100 W, 60˚C) focused on the dorsal skin of the tail, at the half length. To test pain responsiveness, a latency timer and the radiant heat were co-activated at the beginning of a trial. The heat source and timer automatically stopped as soon as a flick of the tail out of the path of the emitted heat was obtained [30].

2.4. Statistical Analyses

The two-way ANOVA was used to assess effects of age (4 - 6 factors) × sex (2 factors) on neurobehavioral measures in developing offspring. Scheffé's post hoc F-test was used to compare means of neurobehavioral measures between males and females.

3. Results

Behavioral developmental parameters were measured separately in males and

females from postnatal day 10 to 45, in order to determine whether sexual dimorphism determines the development of the central nervous system in rats.

3.1. Ontogeny of Novelty-Induced Functions in Male and Female Rat Pups

In our studies, novelty-induced functions occur in animal placed in new surroundings which triggers behavioral responses including exploratory activity and emotional reaction materialized by defecation.

- Exploratory activity

Analysis of exploratory activity using a 2-way ANOVA indicates a significant development of this function in the pups of both sexes from postnatal day 10 to 45 [$F(5, 131) = 46.471$, $p < 0.0001$] and no reliable "age × sex" interaction [$F(5, 131) = 1.910$, $p = 0.0968$]. Between-sex comparison shows that males do not differ significantly from females [$F(1, 131) = 0.145$, $p = 0.7036$]. Analysis of this function shows no sexual dimorphism during development (**Figure 1**).

- Emotional reaction

A 2-way ANOVA on emotional defecation shows an important development of this function in both sexes across age [$F(5, 131) = 120.929$, $p < 0.0001$], with a strong "age x sex" interaction [$F(5, 131) = 18.855$, $p < 0.0001$]. Females exhibit prodigious development of emotional reaction compared to males [$F(1, 131) = 36.749$, $p < 0.0001$] (**Figure 2**). This function undergoes a highly significant dimorphic development according to sex.

3.2. Ontogeny of Reflex Motor Functions in Male and Female Rat Pups

- Hind paws lifting reflex

The average latency of hind paws lifting reflex undergoes an ontogenic reduction with age [$F(3, 88) = 50.816$, $p < 0.0001$], and a noticeable "age × sex" interaction [$F(3, 88) = 11.866$, $p < 0.0001$] (**Figure 3**). The ontogenic reduction of average latency is more rapid in females in than males [$F(1, 88) = 27.334$, $p < 0.0001$]. This function undergoes a solid dimorphic development according to sex (**Figure 3**).

- Wire-grasping time

The use of a 2-way ANOVA on grasping times (**Figure 4**) indicates an exponential development of this function [$F(3, 88) = 174.210$, $p < 0.0001$], with an efficient "age × sex" interaction [$F(3, 88) = 11.301$, $p < 0.0001$]. The mean wire-grasping time increases more significantly in males than in females [$F(1, 88) = 6.136$, $p = 0.0152$]. This function displays a tangible sexually dimorphic development.

3.3. Ontogeny of Automatic Motor Function in Male and Female Rats

- Locomotor activity

A 2-way ANOVA shows that locomotor activity develops substantially as a function of age [F (5, 131) = 55.757, p < 0.0001], with a strong "age × sex" interaction [F (5, 131) = 7.591, p < 0.0001] (Figure 5). Females compared to males exhibit a more remarkable development of locomotor activity [F (1, 131) = 36.568, p < 0.0001]. The development of this function is highly dimorphic according to sex.

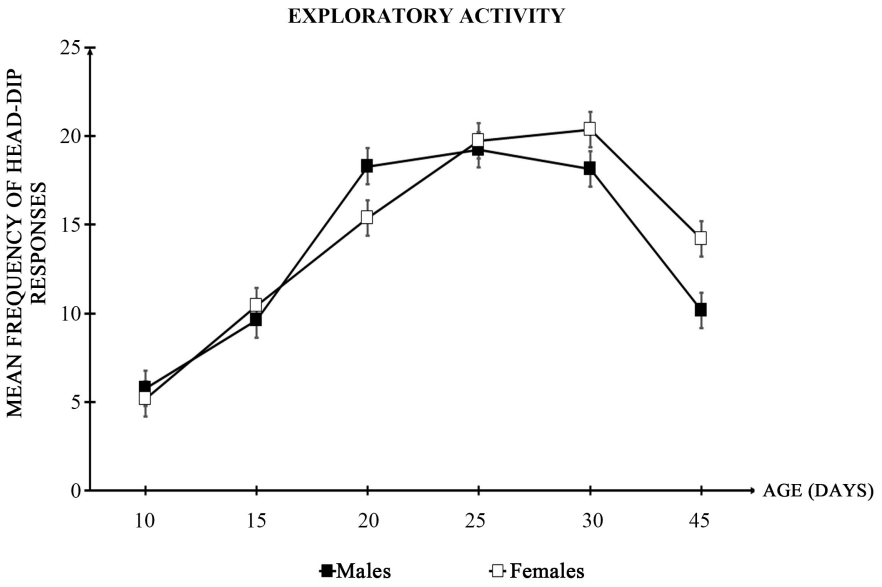

Figure 1. Development of exploratory activity in male and female rats. Exploratory activity expressed as a mean frequency of head-dip responses is shown as a function of age. N = 12 male pups; N = 12 female pups.

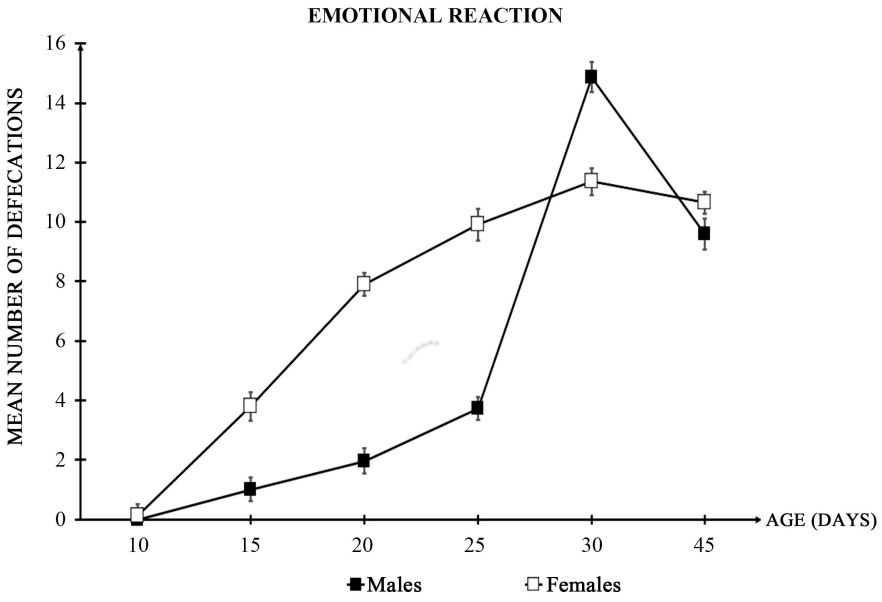

Figure 2. Development of emotional reaction in male and female rats. The mean number of emitted defecations during a 5 min trial of exposure is expressed in terms of age. N = 12 male pups; N = 12 female pups.

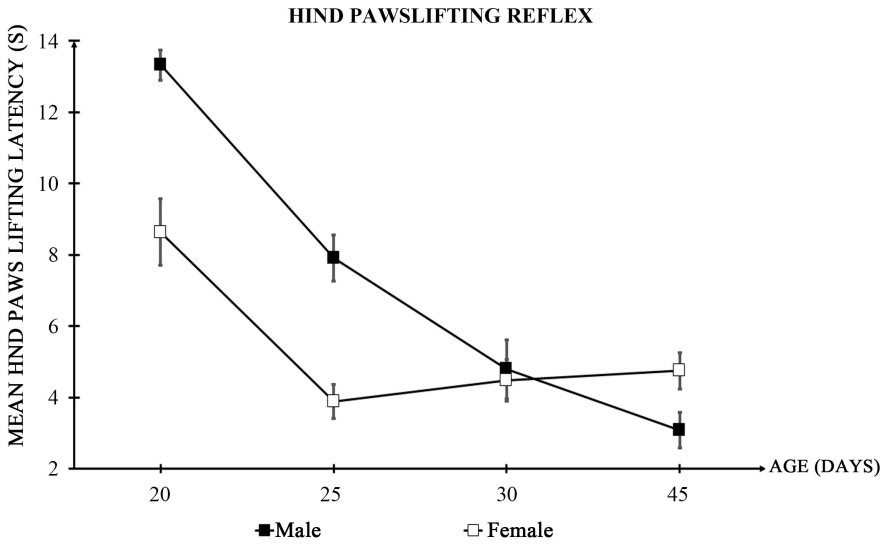

Figure 3. Development of hind paws lifting reflex in male and female rats. The mean latency of hind paws lifting reflex is shown as a function of age. N = 12 male pups; N = 12 female pups.

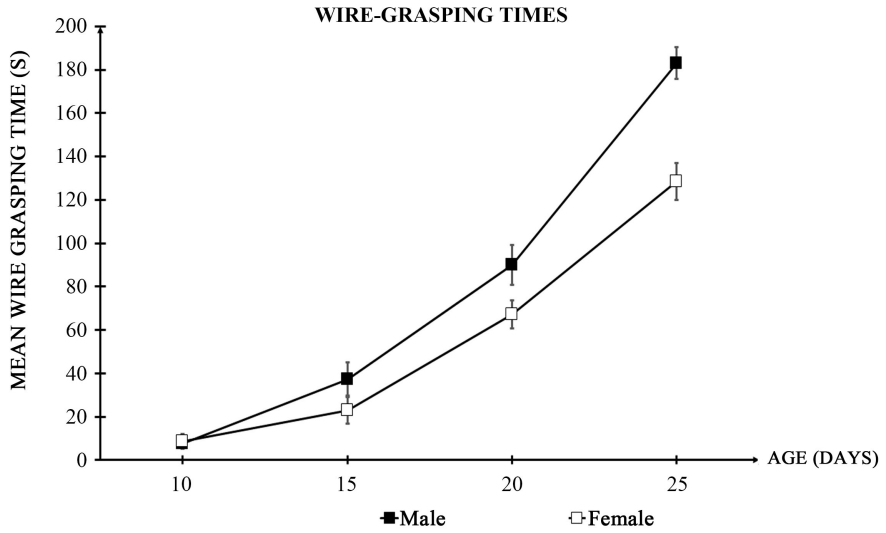

Figure 4. Development of wire-grasping times in male and female rats. The mean wire-grasping time is shown as a function of age. N = 12 male pups; N = 12 female pups.

3.4. Ontogeny of Voluntary Motor Functions in Male and Female Rats

- Crawling along the wire

A 2-way ANOVA on the measures of crawling execution latencies shows a significant reduction in these latencies from postnatal day 10 to 45 in male and female rat pups [$F(3, 88) = 84.644$, $p < 0.0001$], but with no credible "age × sex" interaction [$F(3, 88) = 3.035$, $p < 0.1139$]. Post hoc comparison of means ($p \leq 0.05$), using Scheffé's F test, shows fastest ontogenic reductions of latencies in males than females [$F(1, 88) = 32.291$, $p < 0.0001$]. A sexually dimorphic development of this function is manifest (**Figure 6**).

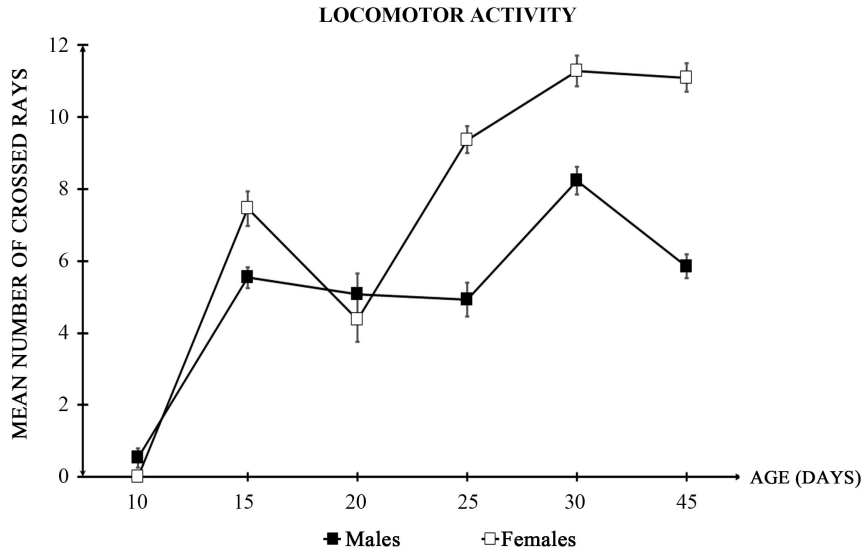

Figure 5. Development of locomotor activity in male and female rats. The mean number of crossed rays, expression of locomotor activity, is presented as a function of age. N = 12 male pups; N = 12 female pups.

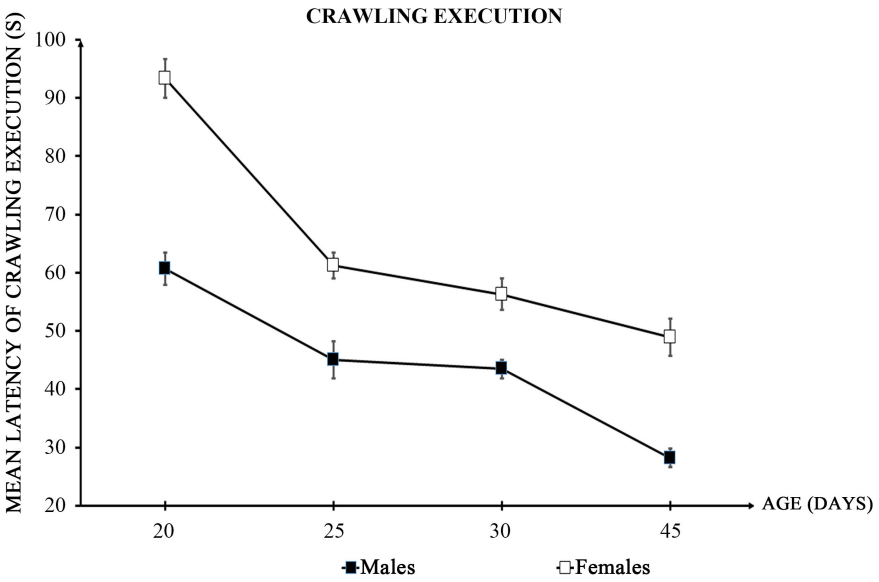

Figure 6. Development of crawling execution latency in male and female rats. The mean latency of crawling execution is presented as a function of age. N = 12 male pups; N = 12 female pups.

- Leap on the ground

A two-way ANOVA analyzing leap execution latencies showed their highly significant reduction in male and female rats pups as a function of age [F (3, 88) = 19.2474, $p < 0.0001$]. However, there was no significant "age × sex" interaction [F (3, 88) = 0.8826, $p < 0.46$]. Post hoc comparison of mean latencies ($p \leq 0.05$), using Scheffé's F test shows no significant difference between males and females [F (1, 88) = 2.481, $p < 0.1434$). The development of this function is not dimorphic according to sex (Figure 7).

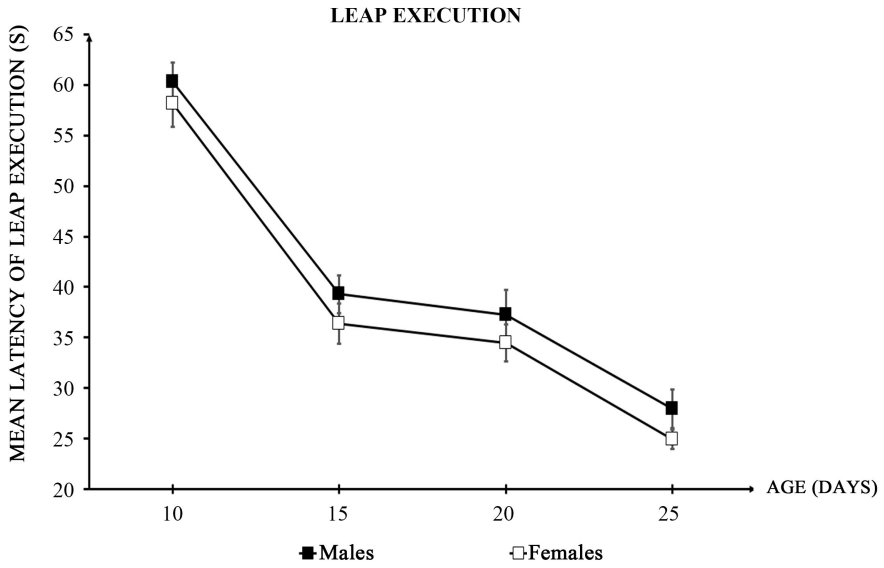

Figure 7. Development of leap execution latency in male and female rats. The mean latency of leap execution is presented as a function of age. N = 12 male pups; N = 12 female pups.

3.5. Ontogeny of Sensory Function in Male and Female Rats

- Nociception

A 2-way ANOVA used to analyze tail-flick latencies indicates a highly significant decrease in nociceptive thresholds from postnatal day 10 to 45 in male and female rat pups [$F(5, 131) = 49.345$, $p < 0.0001$] but with no significant "age × sex" interaction [$F(5, 131) = 0.851$, $p < 0.683$]. Post hoc comparison of means ($p \leq 0.05$), using Scheffé's F test, shows a significant late ontogenic reduction in nociceptive thresholds in male compared with female pups [$F(1, 131) = 22.856$, $p < 0.0001$], (**Figure 8**). The ontogeny of nociception is determined by a noticeable dimorphism sexuel.

3.6. Ponderal Growth in Male and Female Rats

A 2-way ANOVA on weight gain in growing pups (**Figure 6**) indicates an exponential development of this function as a function of age [$F(5, 131) = 249.687$, $p < 0.0001$], with a reliable "age × sex" interaction [$F(5, 131) = 2.345$, $p < 0.0447$]. Between-sex comparison shows that males differ significantly from females [$F(1, 131) = 18.716$, $p < 0.0001$]. Ponderal growth in rat pups is driven by a credible sexual dymorphism (**Figure 9**).

4. Discussion

Our results describe the ontogenic development in rats of a wide range of motor and sensory behaviors generated by CNS activity in male and female pups.

The study of the development of locomotor activity reflects the maturation of the spinal circuit, as step length and gait coordination are under the control of spinal automatism [1]. Our studies show that the development of locomotor ac-

tivity is more vigorous in females than in males, suggesting that the spinal cord develops more rapidly in females than in males. According to McCallum-Loudéac *et al.* [31], overall gene expression changes as the spinal cord matures, with an over-representation of genes playing an important role in myelination and signal transduction. In addition, neonatal rodent brains showed more oligodendrocytes in female compared to male [32] and 17β-estradiol (E2) treatment increased oligodendrocyte progenitor number and myelin formation [32], which may contribute to gender-related specific differences in spinal tracts conduction velocity.

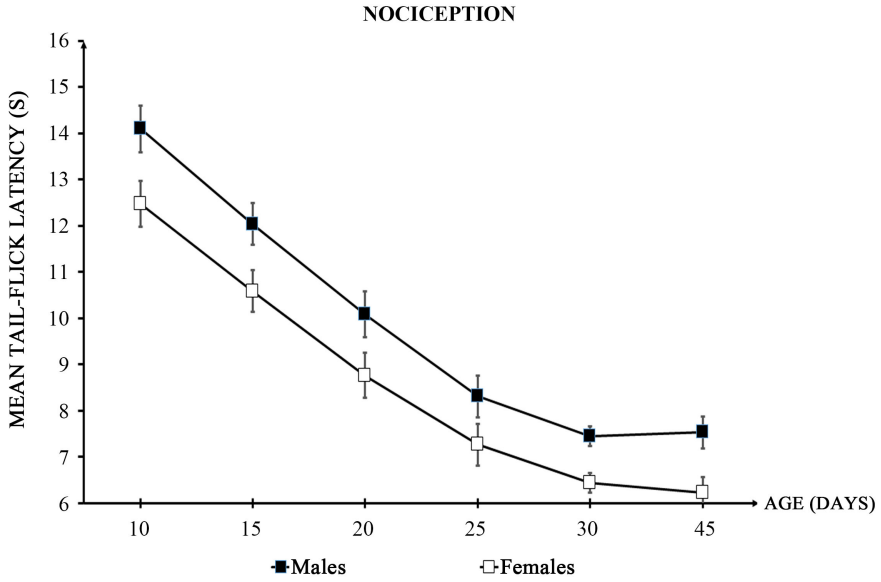

Figure 8. Development of nociceptive thresholds in male and female rats. The mean tail-flick latency is shown as a function of age. N = 12 male pups; N = 12 female pups.

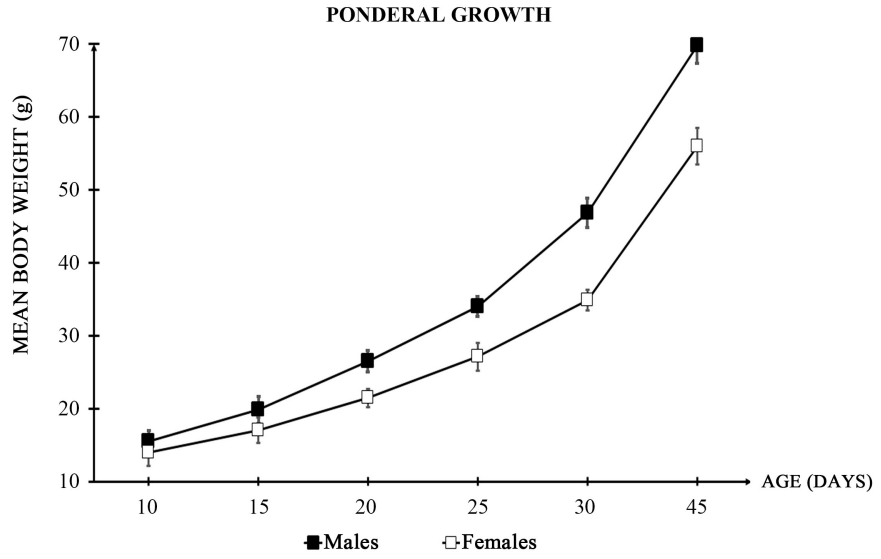

Figure 9. Ponderal growth in male and female rats. The mean body weight is shown as a function of age. N = 12 male pups; N = 12 female pups.

Studying the ontogeny of nociception is another function that shows sexual dimorphism in spinal cord development. Our work has shown that the average latency of tail retraction after its irradiation with a heating light source decreases more rapidly in females than in males. Similar results were found by Guesgen *et al.* [33] reporting that the lambs' latency to respond to a thermal nociceptive stimulus increased with age in males but did not change significantly with age in females. Testosterone is suggested to be anti-nociceptive [34], while estradiol was reported to elicit both pro- and anti-nociceptive effects [35].

There is also sexual dimorphism in the development of motor reflex functions. A study of hind paws lifting reflex shows that latencies decrease significantly from the 10th to the 20th postnatal day, when the most improved values are reached. These latencies decrease more rapidly in females than in males. Hind paw lifting can be classified as postural reflex. Its regulation center should be located in the bulbo-mesencephalic stem [36]. The structures generating this function, such as the bulbo-mesencephalic trunk, appear to develop later in males than in females. Our results support the work of Plandowska *et al.* [37] who showed that girls acquire better postural stability more easily and more quickly than boys. These observations show that the development of posterior brain structures is more rapid in females than in males, thus indicating sexual dimorphism in the development of postural stability.

Exploratory behavior is the most common response of an animal exposed to novelty [38]. The hole-board test provides a relatively reliable measure of stimulus-directed exploratory behavior [39]. Head-dipping behavior reflects the attention paid to the novel objects [39], and would particularly illustrate the animal's aptitude to decode and to integrate sensory stimuli. The structures involved in the decoding and the integration of sensory messages would be the reticular formation, the thalamic relay nuclei and the somatosensory cortex [40]. The ontogenic study of exploratory behavior shows that mesencephalic structures e.g. reticular formations, and diencephalic ones e.g. thalamic relay nuclei with thalamo-cortical projections [1], do not show differential development between the two sexes.

These results are confirmed by our studies carried out on voluntary motor functions. Information on the maturation of the cerebral cortex is provided by measurements of crawling and leap execution latencies during brain ontogeny [1] [29]. Our previous studies have shown that the latency of leap execution onto the ground represents one of the responses of the animal suspended from a wire by its two fore paws, in order to extricate itself from an unstable posture [1] [29], the other response being crawling along the wire to reach one of the two vertical bars.

Our present results show that the measurement of latencies of leap execution onto the ground does not show any significant difference between male and female rat pups. The execution of this function would involve an estimation of spatial dimensions, as the animal carefully orients its future fall towards a certain

location in space. Several studies suggest that the acquisition of spatial information by rats requires the integrity of the hippocampal formation [41] [42]. These observations show that the performance of complex cortical functions does not differ between male and female rats.

On the other hand, our studies have also shown that crawling execution latencies in moving along the wire to reach one of the two vertical bars underwent a more rapid ontogenetic reduction in male than in female pups. This behavior corresponds to the execution of a voluntary motor act initiated in the motor cortical areas and transmitted by the pyramidal pathway to the muscles via the spinal motor centers [1]. As a result, development of the pyramidal pathway is faster in male than in female rats. These observations are confirmed by measurements of wire grasping times, which increase exponentially and more speedy in male than female. According to Hervé *et al.* [43], sex differences in the structure of the corticospinal tract observed during male adolescence may be due to the rising levels of testosterone [44].

These observations suggest that the peripheral skeletal muscles to which these descending pathways terminate must also develop more rapidly in male than female pups. Thus, our studies show that weight growth, witnessed by the increase in peripheral skeletal muscle mass, is faster in males compared to females. According to Gharahdaghi *et al.* [45], several studies show that androgens are involved in increasing muscle mass and strength during development. Thus, exogenous administration of androgens potentiates gains in muscle strength and mass [45]. Furthermore, androgen receptor (AR) antagonists, which prevent endogenous testosterone from binding to ARs, impair muscle growth and strength [46]. Finally, muscle strength and mass are significantly reduced (by up to 20%) in male AR-knockout mice [47].

To assess the interactions between diencephalic structures during development, emotional response was evaluated during cerebral ontogeny. The term "emotionality" was first introduced into animal psychobiology by Hall [33], to describe individual differences in urination and defecation, which accompany elevated sympathetic nervous activity. Later, "emotional defecation" was used in various experimental studies where animals, placed in an unfamiliar environment or in situations of tension, displayed a high number of defecations. Emotional reaction is generally under the control of limbic structures, and motivation and emotionality influence, to a high degree, the acquisition and extension of learning [48] [49]. For example, the hypothalamus, one of many limbic structures, is thought to be endowed with emotional properties in rats [50]. In our studies, females show a strong development of emotional response compared to males, suggesting a more rapid development of the diencephalon and subcortical structures in females compared to males [51]. Indeed, in cultures of hypothalamic neurons obtained from male rat fetuses at embryonic day 16, E2-induced hypothalamic axons outgrowth was exerted through a membrane-associated receptor [52]. In rat offspring, Cao and Patisaul [53] used histochemistry to map

the temporal and sexually dimorphic neonatal mRNA expression profiles of ER*α*, ER*β*, in the anterioventral periventricular nucleus (AVPV), medial preoptic area (MPOA), ventromedial nucleus (VMN) and arcuate nucleus (ARC). Females rats had higher levels of ER*α*, in all regions examined, a sex difference that persisted until postnatal day 19 [53]. Indeed, circulating gonadal hormone concentrations do change in a sex-specific manner during the perinatal period, with males exhibiting a peak in testosterone at embryonic day 18 (E18) and shortly after birth [54] [55], whereas testosterone levels remain relatively low in females [14] [56]. Sex differences in circulating perinatal estradiol and androgen concentrations are thus believed to drive, at least partially, sexual differentiation in the rodent brain.

In the diencephalic nuclei and structures of the limbic system, there is a dimorphic difference in maturation processes orchestrated by estradiol and testosterone antagonism. Indeed, hormonal control of cell death (apoptosis) and synaptogenesis are currently the best-established mechanisms for understanding sex differences in the neurobiological maturation processes of diencephalic nuclei and structures. For instance, the nucleus of the stria terminalis (NST) is a heterogeneous and complex structure in the limbic forebrain, playing an important role in the control of autonomic, neuroendocrine and behavioral responses. The volume of the principal nucleus of the stria terminalis (NSTpr) was greater in males than in females without any sex difference. However, more apoptotic nuclei were found in the females' NSTpr than in males, while it's the opposite in NSTl, the lateral division of NST [26]. Conversely, testosterone inhibits apoptosis in the central parts of the stria terminalis and medial preoptic nucleus and controls sexually dimorphic differentiation of these structures [26] [57].

In addition, the sexually dimorphic nucleus of the preoptic area (SDN-POA) in the rat hypothalamus is larger in volume in males than in females due to a larger number of cells in the nucleus. The incidence of apoptosis was determined in part of the SDN-POA, the central division of the medial preoptic nucleus (MPNc) and showed sex difference: Incidence of apoptosis in the MPNc was higher in females than in males [58]. Tsukahara *et al.* [59] concluded that estrogen modulation of Bcl-2 proteins is probably responsible for the sex-dependent different apoptosis induced in SDN-POA postanatally.

Similarly, there is a sex difference in the production of SDN-POA neurons. The neuroblast division that produces SDN-POA neurons may begin earlier and end earlier in females than in males. These differences in neuronal production may also partly explain the sexual dimorphism observed in the volume and number of neurons in the SDN-POA of the adult rat [57].

The role of testosterone (T) in regulating the incidence of apoptosis in the developing MPNc was examined in neonatally castrated males rats following T or vehicle injection. Testosterone had a profound inhibitory effect on the incidence of apoptosis indicating that the regulation of apoptosis by T is one mechanism involved in the sexual differentiation of the SDN-POA. Indeed, perinatal admin-

istration of testosterone propionate in rats increased the volume and number of cells in the MPNc of male pups compared to females, without altering neurogenesis [60], whereas this treatment prevents neuronal loss in the MPNc of female offspring [60].

Consequently, apoptosis regulation may contribute to the development of sex differences. Indeed, the relatively large size of some CNS nuclei in males compared to females may be explained by late synaptic pruning caused by testosterone in males, which may explain the performances recorded in our studies in females compared to males, such as: remarkable development of emotional response, reflex and automatic motor functions and nociception.

On the other hand, microglia participates in the remodeling of synapses in the cortex and hippocampus during postnatal development in mice. The cerebral cortex and hippocampus are vital areas for learning, memory and cognitive functions. Microglia displays sexually dimorphic characteristics during the early postnatal period, which is a critical window for synapse formation and maturation. Measurements of synaptic features revealed sex differences in the density of synaptic spines and knobs during the second postnatal week; and these data were consistent with early development of both microglia and synapses in the female brain [13]. According to Weinhard *et al.* [13], there is a sex-dependent change in microglia volume and phagocytic capacity during the first four postnatal weeks in mice. Interestingly, in the mouse hippocampal CA1, microglial volume and phagocytic capacity peaks at P8 in females, and at P15 in males [13]. This demonstrates an earlier rise and fall in hippocampal microglial volume and phagocytic capacity in females compared to males in early life [14]. Likewise, the developmental switch from $GABA_A$-mediated excitation to inhibition in the hippocampus occurred precociously in female rats compared to males: In female rats, the switch from $GABA_A$-mediated excitation to inhibition in CA1 and CA3 pyramidal neurons occurs between P4 and P7, earlier than in male rats where that transition occurs between P7 and P14, suggesting a longer window of $GABA_A$-mediated excitation compared to females [14].

Taken together, these observations indicate that the vast majority of brain structures and functions reached maturation earlier in females than in males. Estrogen is the trigger hormone for early maturation of the female brain. Consequently, estrogen and testosterone control the sexually dimorphic differentiation of brain structures and functions by independent pathways and mechanisms.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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