

## The Laboratory Rat: Relating Its Age with Human's

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### ABSTRACT

By late 18<sup>th</sup> or early 19<sup>th</sup> century, albino rats became the most commonly used experimental animals in numerous biomedical researches, as they have been recognized as the preeminent model mammalian system. But, the precise correlation between age of laboratory rats and human is still a subject of debate. A number of studies have tried to detect these correlations in various ways, But, have not successfully provided any proper association. Thus, the current review attempts to compare rat and human age at different phases of their life. The overall findings indicate that rats grow rapidly during their childhood and become sexually mature at about the sixth week, but attain social maturity 5-6 months later. In adulthood, every day of the animal is approximately equivalent to 34.8 human days (i.e., one rat month is comparable to three human years). Numerous researchers performed experimental investigations in albino rats and estimated, in general, while considering their entire life span, that a human month resembles every-day life of a laboratory rat. These differences signify the variations in their anatomy, physiology and developmental processes, which must be taken into consideration while analyzing the results or selecting the dose of any research in rats when age is a crucial factor.

**Keywords:** Adult, human age, laboratory rat, physiology, puberty, rat age

### INTRODUCTION

The laboratory rat is an inevitable part of today's biomedical research. They are recognized as the preeminent model in numerous fields, including neurobehavioral studies, cancer and toxicology.<sup>[1]</sup> It is difficult to evaluate the number of animals employed in scientific experiments every year. An estimation suggests some dozens of millions per year, being 15 million in the United States, 11 million in Europe, five million in Japan, two million in Canada and less than one million in Australia. Almost 80% of the experimental animals are rodents that include mice, rats, guinea pigs and others (10% are fish, amphibians, reptiles and birds). A third group includes rabbits, goats, bulls and in smaller amounts, dogs, cats and some species of primates.<sup>[2]</sup> They substitute the human being as an experimental object in scientific researches. Among the rodents, rats are the mostly used animals for experimental purposes (accounting for approximately 20% of the total number

of mammals used for scientific purposes), followed by mouse, rabbit, dog, pig and primate, especially for *in vivo* studies. About 85% of the articles in Medline, and 70.5% of the articles in Lilacs, employed rats and mice.<sup>[3]</sup> All over the past 80 years, rats have been utilized in investigations in almost every aspect of biomedical and behavioral research. A recent publication dealing with biomedical research applications lists the following areas of biomedical investigation as ones in which the rat is widely used and is particularly useful in: Toxicology, teratology, experimental oncology, experimental gerontology, cardiovascular research, immunology, dental research immunogenetics and experimental parasitology.<sup>[4]</sup> The rat is also the most widely used laboratory mammal in behavioral studies, for which, incidentally, the mouse is not well suited. Rats have traditionally been the animal of choice in much nutritional research, although it should be noted that their natural habit of coprophagy may limit their suitability for certain of these studies.

Their use in scientific research started in the 16<sup>th</sup> century, although the development of the laboratory rat as an experimental model really began in 1906 when the Wistar Institute developed the Wistar rat model (*Rattus norvegicus*).<sup>[5]</sup> They are thought to have originated in some parts of Asia. *Rattus rattus* was well established in Europe by 1100 A.D., with *Rattus norvegicus* commonly found in Europe in the 1700s. By the 1800s, these animals were used for neuroanatomy studies in the United States and in Europe. It was in the late 1800s and early 1900s that individual stocks and strains had their beginnings. Today, there are 51 known species of the *Rattus* of both albino and pigmented types that are available. There are recognized differences between wild and laboratory rodents. For example, laboratory rats have smaller adrenals and preputial glands, earlier sexual maturity, no reproductive cycle seasonability, better fecundity and a shorter lifespan than their free-ranging wild counterparts.<sup>[6]</sup> Currently, Wistar rats and Sprague-Dawley rats are gradually becoming the most used laboratory animals worldwide.

## AGE DETERMINATION OF A LABORATORY RAT: COMMON METHODS

Numerous methods have been investigated in several studies to correlate the ages of small mammals with that of a human, i.e. using the weight

of the eye lens,<sup>[7]</sup> growth of molar teeth,<sup>[8]</sup> counting of endosteal layers in the tibia,<sup>[9]</sup> musculoskeletal growth along with the closure and thickening of the epiphyses,<sup>[10]</sup> etc., but, all of the techniques are relative methods and do not exactly define the absolute age; thus, researchers generally employ more than one method at a time to have a proper idea about the age of the experimental animal.

### Weight of eye lens: A useful measure

Several studies have been performed using the weight of the eye lens in an attempt to use the development and growth of the lens throughout the mammalian life as an indicator that could help correlate the ages of different species.<sup>[7]</sup> The weight of an eye lens increases along an asymptotic curve throughout the normal life span of many mammals.<sup>[11]</sup> In laboratory rats, this increase in lens weight has been found to be largely independent of the nutritional status of the animals. This technique was taken as a useful measure in the late 1980s to correlate the ages of different mammalian species at different stages of life. However, this method proved a useful indicator only up to 3-4 months; beyond that point, the technique is not precise enough to determine the exact age of the rat.<sup>[12]</sup>

### Teeth: A test of age

Some researchers have developed methods to determine the ages of smaller mammals by using the growth of molar teeth, mostly by the *molar ageing method* or the *crown method*.<sup>[8]</sup> In rodents, the first upper molars clearly show age-dependent changes. The molar of young animals consists solely of a specialized prismatic part (i.e. crown), which is compensated by the growth of the roots that starts approximately at the age of 2.5 months, and is continuous, pushing the crown upwards. In aged animals, most of the crown wears away and the roots are long. On account of differences in diet and also in primary molar hardness, molar wear may differ geographically; therefore, the molar ageing methods are perhaps not directly applicable outside the area in which they are developed. If accurate assessment of dental development is possible, this method should be given more emphasis in age estimates. In the absence of dental information, assessments of skeletal maturation (including long bone lengths and maturation of other skeletal elements) can be used.<sup>[13]</sup>

### Counting endosteal layers in tibia

Although many age determination techniques have been developed, the most widely used method in vertebrates involves a technique of counting endosteal layers in the tibia that allows to accurately determine the age of the experimental animals. It has been reported in several studies that, in younger animals, more lamellae were found than their age in years.<sup>[9,14]</sup>

### Musculoskeletal examination: Epiphyseal closure

Because dental development is minimal in fetal animals, most estimates of age rely on bone formation, especially long bone lengths, and also other bones such as the assessment of the development of the ilium and the petrous portion of the temporal. If accurate measurements can be taken, formulae exist to allow the calculation of body length and subsequently, the age. Mostly, bones of the upper and lower limbs and hip joint are used to determine the age of the experimental animal. In the childhood of the animal, closure of metopic suture and appearance of ossific centers are used.<sup>[10]</sup> During the first 6 years of human life, appearance of ossific centers are observed mainly in the bones of the humerus, femur, tibia, radius, patella and ulna. In addition, closures of epiphyseal plates are an indicator of the adolescent period. Closures of epiphysis in the bones of the upper limbs (wrist, shoulder joint, humerus, ulna, radius, metacarpals and phalanges) are found during the age of 14-18 years of human life, while epiphyseal closure lower limbs (femur and tibia) are found during 18-25 years of age. During early adulthood, bone remodeling and maintenance is the prime indicator, while in late adulthood bone wears and tears help in the determination of the animal age; two pubic rami of the hip are found at the age of 6 years, suture at the acetabulum at 15 years, ischial tuberosity with the ischium at 21 years and iliac crest with the ilium at 23 years. In addition to skeletal measurements and dental evaluation, the extent of formation and union of epiphyses is important. Epiphyseal evaluation involves gross examination in skeletal remains and radiological assessment in fleshed material.<sup>[15]</sup>

### Rat age and human age: Revealing the relation

Biomedical researchers who use rats as an experimental model often face numerous questions

like “*what is the relationship between age of the rat and human?*,” “*when are these animals considered adults or aged?*” or “*how old is a rat in people years?*.” Only a few research works have attempted to answer these questions. These questions could be answered in various ways. Most of the researchers used to relate human and rat age by simply correlating their life span, which is not acceptable, because, for a specific research work, one uses a particular developmental phase of rat-life. Thus, one should consider different phases of their life to have an accurate correlation.

**What is the relation between their “life periods”?**

Laboratory rats live about 2-3.5 years (average 3 years),<sup>[16]</sup> while the worldwide life expectancy of humans is 80 years, with variations in countries in accordance with their socioeconomic conditions.<sup>[17]</sup>

Therefore, taking their life span together, it can be calculated as:

$$(80 \times 365) \div (3 \times 365) = 26.7 \text{ human days} = 1 \text{ rat day; and}$$

$$365 \div 26.7 = 13.8 \text{ rat days} = 1 \text{ human year.}$$

Thus, one human year almost equals two rat weeks (13.8 rat days) while correlating their entire life span.<sup>[18,19]</sup>

However, while considering the different phases of rat life, including weaning to aged phase, it could be easily noticed that rats have a brief and accelerated childhood in respect of humans. Rats develop rapidly during infancy and become sexually mature at about 6 weeks of age. Humans, on the other hand, develop slowly and do not hit puberty until about the age of 11-12 years. Social maturity is obtained in 5-6 months of age.<sup>[19,20]</sup>

**When do the baby rats weaned?**

The unique bond between mammalian mothers and their infants, whom they create and maintain by nursing, is irrevocably broken during weaning. In a strict sense, the weaning process involves a developmental reorganization of ingestive behavior. Infant altricial mammals subsist entirely on mother's milk; as adults, they independently select and ingest solid foods. Weaning is the transition between these two forms of subsistence, and constitutes an essential element in the progression to adult function in all mammals. In a general sense, weaning also represents a milestone in the achievement of more global forms of independence, a prominent and universal discontinuity in mammalian development that marks a significant change in life pattern.<sup>[10,11]</sup>

Weaning (or nursing) is the first phase of rat

life, which is a developmental process unique to all mammalian young. It is the process of gradually introducing a mammalian infant to an adult diet (solid food) and withdrawing the supply of its mother's milk [Table 1]. In *Rattus norvegicus*, a species important in laboratory studies of ingestive behavior, the young begin to reliably ingest solid food on about Day 18 (P18). Time spent suckling begins to decline around P20, while time spent ingesting solid food increases. By about P34, the young no longer suckle and weaning is essentially complete. The average weaning age for humans is approximately 6 months (180 days),<sup>[21]</sup> while it is 3 weeks for laboratory rats (~P21).<sup>[22]</sup>

Thus,

$$180 \div 21 = 8.6 \text{ human days} = 1 \text{ rat day and}$$

**Table 1:** General physiology and reproductive data of *Rattus norvegicus*

Common physiological data	
Body temperature	37°C
Respiratory rate	75-115 breaths/min
Heart rate	260-400 beats/min
Daily water consumption	10-12 ml/100 g body weight
Daily food consumption	10 g/100 g body weight
Litter size	6-12
Birth weight	5 g
Weaning age	21 days
Sexual maturity	7 weeks (~P49)
Breeding duration	12-16 months
Male adult weight	450-550 g
Female adult weight	250-300 g
Life span	2.5-3.5 years
Reproduction parameters	
Male rats	
Age at pairing (mating)	8-10 weeks
Weight at pairing	250-300 g
Female rats	
Age at pairing (mating)	8-10 weeks
Weight at pairing	180-225 g
Length of oestrous cycle	4-5 days
Duration of oestrus	10-20 h
Time of ovulation	8-11 h after onset of estrous
Menopause	15-18 months
Gestation	
Time of copulation	Near midpoint of previous dark cycle
Time sperm is detected in vagina	Day 1
Time of implantation	Late day 5
Length of gestation	21-23 days

$$365 \div 8.6 = 42.4 \text{ rat days} = 1 \text{ human year.}$$

Therefore, in this developmental phase, one human year equals 42.4 rat days.<sup>[18,19]</sup>

#### **Is my rat going through "puberty"?**

The second phase of rat-life is its puberty, when reproduction first becomes possible, i.e., when germ cells are released. Research on the reproductive physiology using pubertal and adult rats as experimental animal began in the 1930s. Since then, the species has been more thoroughly characterized in these research fields than any other laboratory animal model. This biomedical field basically employs pubertal or adult rats.<sup>[23-25]</sup> Long and Evans<sup>[26]</sup> found that rats reached puberty at an average age of 50 days after birth (P50). Humans, on the other hand, develop slowly and do not reach puberty until about the average age of 11.5 years ( $11.5 \times 365 = 4198$  days). Rats, on the other hand, become sexually mature at 6 weeks (P42).<sup>[27]</sup> However, it has been reported in several studies that have compared birth weight of rat and human that, rats are not "born" until Day 12 after birth (P12). This means that rats reach sexual maturity at approximately 38 days (i.e. 12 days less than their actual pubertal age at P50).

Thus, it could be easily calculated that in the pre-pubertal phase:

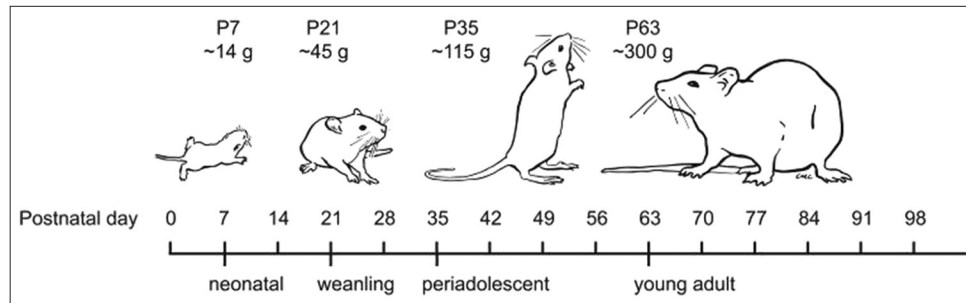
$$4198 \div 38 = 110.5 \text{ human days} = 1 \text{ rat day, and}$$

$$365 \div 110.5 = 3.3 \text{ rat days} = 1 \text{ human year.}$$

Thus, in this phase, one human year equals 3.3 rat days.<sup>[18,19]</sup>

#### **When rats are considered "adult"?**

To determine when an animal is an adult, it is also important to review the developmental stages the animal progresses through to reach adulthood. Both rats and mice show a similar developmental profile [Figure 1]. At P21, rodents are weaned, i.e. separated from their mother. After that, they begin to undergo sexual maturation.<sup>[1,19]</sup> Sexual maturity is generally defined by vaginal opening (females) or balanopreputial separation (males). This point is reached in female rats at approximately P32-P34 but, in males, maturity occurs much later at around P45-P48. However, the age of sexual maturity varies considerably between individuals, ranging from as young as P40 to as old as P76 in male rats.<sup>[28]</sup> It is also important to note that sexual maturity itself does not mark the beginning of adulthood, but rather denotes the beginning of adolescence. Like humans, rats progress through a



**Figure 1:** Correlation of body weight with different phases of postnatal days

period of adolescence characterized by behaviors such as increased risk-taking and social play. These behaviors extend well beyond the pubertal period through the transition to adulthood,<sup>[29]</sup> which begins after the eighth week of post-natal life (~P63). The body weight of an animal is sometimes considered an indicator of its age. However, weight is not an accurate surrogate marker for age. It has been reported that male rats weighing between 250 g and 274 g differed in age by 3 weeks, from P49 (periadolescent) to P70 (young adulthood). In addition, male rats of the same exact age showed up to 100 g variation in body weight [Figure 2]. Weight is, therefore, only an approximate marker of age. Similarly, to identify adulthood by musculoskeletal maturity with rats is problematic as there is no epiphyseal closure in the long bones.<sup>[30,31]</sup> At approximately 7-8 months of age (~210 days), skeletal growth tapers off in male and female Sprague-Dawley rats.<sup>[22]</sup> In humans growth plate closure is rather inconsistent among individuals and among different growth plates within the body. One of the last growth plates to fuse is in the scapula, which closes at about 20 years of age on average ( $365 \times 20 = 7300$  days).<sup>[32]</sup>

Therefore, from this data, it can be calculated that:

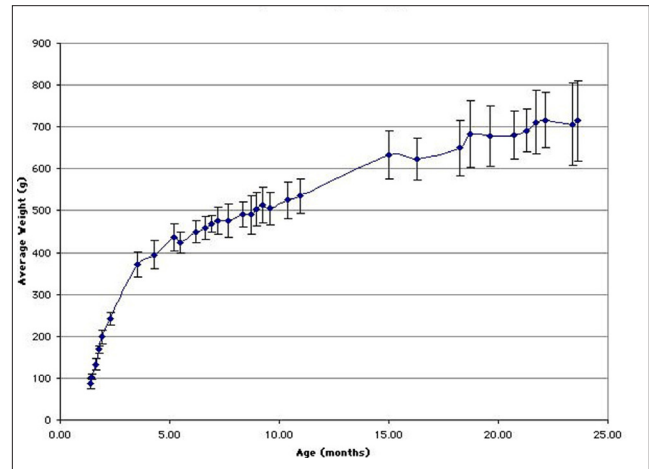
$$7300 \div 210 = 34.8 \text{ human days} = 1 \text{ rat day,}$$

which indicates that  $365 \div 34.8 = 10.5$  rat days = 1 human year.

Thus, during the adolescent phase, 10.5 rat days equals one human year.<sup>[18,19]</sup>

**Reproductive senescence: The rat is no longer sexually active!**

Reproductive senescence in female rats occurs between 15 and 20 months of age. During the fertile period in a female's life in most species, mating usually only occurs when a female is fecund (at the time of ovulation in spontaneous ovulators or when primed to ovulate in reflex ovulators). But, this integration of behavior and physiology can break down during



**Figure 2:** Variations in body weight of male rats throughout the lifespan

aging in female rats. Most aging female rodent exhibits periods of persistent estrus (constant sexual receptivity) that are associated with tonic blood titers of estrogen and low levels of progesterone. Because the tonic estrogen secretion stimulates cornification of the vaginal epithelium, this state is also referred to as persistent vaginal cornification. This is the most common state of acyclicity in laboratory rats.<sup>[19]</sup>

Similarly, the traditional marker of reproductive senescence in women is menopause, characterized by loss of menstrual or fertility cycles at midlife. According to the American Medical Association, the average age of menopause in women is 51 years ( $51 \times 365 = 18615$  days),<sup>[33]</sup> and female rats enter menopause between the ages of 15 and 20 months (600 days).<sup>[34]</sup>

Thus,  $18615 \div 600 = 31.0$  human days = 1 rat day, and

$$365 \div 31 = 11.8 \text{ rat days} = 1 \text{ human year.}$$

Thus, during reproductive senescence, 11.8 rat days equals one human year.<sup>[18,19,34]</sup>

**Post senescence: When the rat is aged!**

If the periods of post-senescence to death are

compared, the following is found: Female rats live an average of 485 days after senescence and female humans live an average of 10,585 days after senescence.

Thus,  $10585 \div 495 = 21.4$  human days = 1 rat day,

meaning  $365 \div 21.4 = 17.1$  rat days = 1 human year.

Thus, in the aged phase, 17.1 rat days equals one human year.<sup>[18,19,34]</sup>

## CONCLUSIONS

Thus, the findings of this review suggest that although rats are indispensable elements of biomedical research,<sup>[35-42]</sup> they are not a miniature form of humans;<sup>[18,19]</sup> differences in anatomy, physiology, development and biological phenomena must be taken into consideration when analyzing the results of any research in rats when age is a crucial factor [Table 2]. Special care should be taken when the intention is to produce correlation with human life. It is important for a researcher to understand that the relative ages are different depending upon the stage of life; therefore, one has to determine the relevant age under investigation and what factors are being analyzed. For this, special attention is needed to verify the phase in

**Table 2:** Rat's age in human years

Correlating human year with rat days with different phases of life		
Entire life span	13.2 rat days	=1 human year
Weaning period	42.4 rat days	
Pre-pubertal period	3.3 rat days	
Adolescent period	10.5 rat days	
Adulthood	11.8 rat days	
Aged phase	17.1 rat days	
Average	16.4 rat days	
Rat age versus human age: Social maturity phase		
Rat age (years)	Human age (years)	
6 months (0.5)	18	
12 months (1.0)	30	
18 months (1.5)	45	
24 months (2.0)	60	
30 months (2.5)	75	
36 months (3.0)	90	
42 months (3.5)	105	
45 months (3.75)	113	
48 months (4.0)	120	

days of the animal and its correlation with age in years of humans.

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