in hospitalized subjects[6] would probably reduce this variability.

Large statistically significant practice effects were seen, as subjects invariably performed much better on their 2nd and 3rd mazes than on their first. The effect could have been greatly reduced by presenting the subjects with a practice maze before the initiation of the experiment itself. Also maze T proved to be significantly harder for the subjects to learn than the other 2 mazes.

In summary, we have found evidence to suggest that a low oral dose of scopolamine which did not interfere with storage of a long list of digits interfered with some factors in a spatial learning task. The fact that the overall rate of learning was not impaired makes it unlikely that the impairments observed were due to disruption of attentiveness. The types of impairment produced by scopolamine included entering more blind alleys and the insertion of extra turns in drawings of a 'mental map' of the maze. The large amount of variability in the scopolamine condition indicates the need for a more reliable route of administration.

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The postnatal evolution of muscular twitches in the developing rat

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Summary. The reported study concerns the evolution of muscular twitches during the 21 postnatal days of the normal rat pups. The data indicate an age-dependent progression of these twitches in different body regions.

Previous studies on the neurobehavioral evolution of the rat during the postnatal development (Lapointe[2], Lapointe and Nosal[3]) had evidenced a particular developmental feature. During the 21 days of observation, the rat pups exhibited brief and unexpected muscular twitches that were of variable intensity. Such saccadic movements have been previously reported in growing animals: in the rat by Bolles ad Woods[4], Gramsbergen[5], Jouvet-Mounier and Astic[6] and in the mouse by Fox[7]. In addition, Jouvet-Mounier et al.[8] also observed similar movements in rats, mice and guinea-pigs.

However, no systematic study on the body distribution of muscular twitches during the postnatal growth of the rat has been described until now. As we were interested to include this developmental characteristic in our rat model of neurobehavioral evolution (Lapointe, Lin et Nosal[9,10]) we have undertaken a selective investigation on the onset, progression, body distribution and variations of the muscular twitches during the first 3 weeks following birth.

In addition, this behavioral criterion may be useful to appreciate changes in the wake-sleep state of growing rats during the preweaning period of life.

Material and methods. 10 fertile female Sprague-Dawley rats were mated with 10 males of the same strain. They delivered healthy litters which were reduced at birth to 8 pups (4 females and 4 males). A total of 80 pups were used for this study. Controls were performed daily at the time corresponding to the birth hour (±1 h). This daily time has been selected in order to avoid age-related differences between the litters. According to several criteria (morphological and behavioral), which were previously investigated, we found that a few h difference within the first 48 h could be also significant on the neurological state of the neonatal rats. In the case of older pups, identical daily timed controls were applied to all animals with the view to minimize individual variations occurring in the 24-h biological rhythms.

For this specific testing, the pups were placed individually in a plastic cage for a 1-min duration. The body was arbitrarily subdivided into 6 separate zones: the head, the trunk, the dorsoventral region, the tail, the limbs and finally the entire body. The frequency of muscular twitches was recorded by means of a manual counter solely when the animal was at the rest state. However, the same pups were submitted each day to the entire test battery (Lapointe and Nosal[3]) following muscular twitches evaluation. The total time of observation for each pup was of 5–6 min per day.

Experimental results were expressed in twitches/min/animal with the respective SEM for each age from day 1 to day 17. We have chosen the experimental procedure of 1 daily testing on more numerous animals, rather than several twitch tests on a restricted number of animals.

Results. In the postnatally growing rat, muscular twitches are typically characterized by: a) an intermittent occurrence; b) an unexpected sequence of appearance in different regions and c) brief movements of varying magnitude.

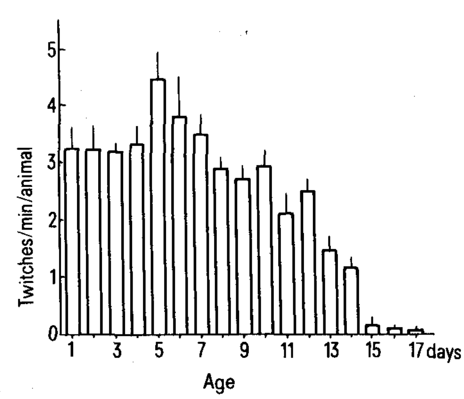
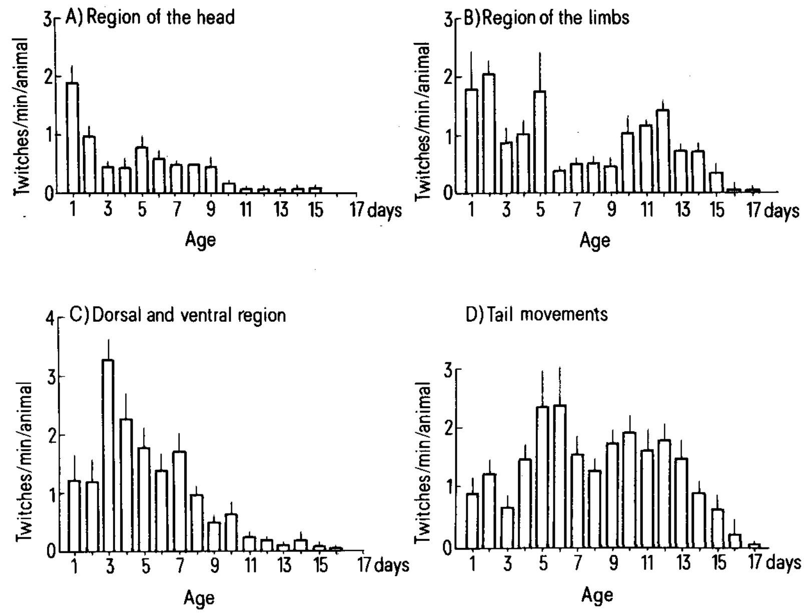


Fig. 1. Evolution of total muscular twitches at different ages (vertical bars represent SEM).

Fig. 2. Evolution of muscular twitches according to different body regions (vertical bars represent SEM).



During the observation period, 3 types of twitches have been identified: 1. localized movements restricted to the right forelimb, for example; 2. regional twitches such as those localized in the anterior region (head and forelimbs) and 3. generalized or gross movements affecting the entire body.

In the course of rat development, the muscular twitches were observed in all mentioned regions of the body. Although abundant in the first 10 days of life, they attained a maximal value on the 5th day. Thereafter they progressively decreased until the 17th day when they almost disappeared (figure 1).

During the day following birth, the head (figure 2,A) and the limbs (figure 2,B) showed numerous twitches. Saccadic movements affected mostly the forelimbs during the first 2 days, whereas in the hindlimbs, maximal twitch activities were found later, on day 12.

The dorsoventral region (figure 2, C) showed its greatest score on the 3rd day and then it subsequently decreased. Only a few twitches were seen in the trunk; this may be due to the bony structure of the thorax which probably hindered the manifestation of such muscular movements.

Finally the tail (figure 2,D) exhibited a large number of twitches all through the developmental period. Moreover we also observed some contractions involving the ears and eyelids but they have not been included in our experimental protocol.

As regards the entire body of the pups (figure 3), it appeared that the optimal twitching attained a plateau from the day 6-8 of life. Thereafter, muscular movements diminished gradually until day 13 and then fell abruptly until day 17. The gross movements varied in magnitude; some of them were so intense that the pups lifted themselves up from the surface within a few mm in a manner that resembled the startle response. In certain young rats, the strength of the movement was great enough to require a righting reflex to recover the initial body position.

Discussion. According to the reported data, the muscular twitches showed a typical sequential development which indicates the initial 'caudo-cranial' evolution of the CNS structures and activities in the infant rat. At birth, the spinal cord and its functions are more mature than the brain. Subsequently, during the postnatal development in non-precocious mammals such as the rat, the CNS evolution

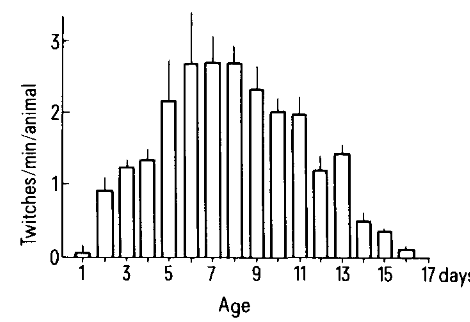


Fig. 3. Evolution of muscular twitches affecting the whole body at different ages (vertical bars represent SEM).

follows a 'cranio-caudal' progression. Thus, at weaning time (21 days after birth), the brain has acquired a maturational state more advanced than the spinal cord.

This differential evolution has been previously demonstrated by the neurobehavioral model already described by Lapointe[2], Lapointe and Nosal[3], and now it appears to be confirmed by the variations of muscular twitches (repartition and magnitude) depending on the age of the rat pup.

According to our results, an inverse relationship seems to exist between this typical primitive muscle activity and the onset and progression of the locomotor abilities. Muscular twitches disappeared at the time when the sense organs (eyes and ears) and the statokinetic system attained their maturity. This disappearance coincides with a progressive increase in more elaborated behavioral activities (e.g. motility and exploration). Such data are suggestive of the gradual changes taking place in the basic sleep-wake relationships during the early post-natal development of the rat. The immature sleep patterns characterized by a high level of paradoxical phases are progressively replaced by a more adult-like alternance of deep versus REM periods of sleep. A similar neurophysiological situation was found in human babies. This evolution in sleep rhythms receives support from animal studies reported by Jouvet-Mounier et al.[7] demonstrating a gradual decrease in the ratio of paradoxical sleep: total sleep during development. These phenomena can be correlated to a shifting from the initial spinal control towards SNC higher centres regulation.

Nevertheless, it has to be underlined that typical twitching activites do not entirely disappear, since they remain as an essential component of paradoxical sleep in adult subjects. Our data involving the early postnatal development of the rat represent a continuity of the studies of Narayanan and Fox[11] during the fetal development of the rat (16-20 days of prenatal life) relating to intrauterine motility. Muscular twitching activities are, therefore, a useful parameter to be included in our neurobehavioral model in the developing rat, both with regards to the total amount of localized and regional muscle activities, as well as the frequency and quantity of gross movements of the entire body.

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Changes in free amino acid level due to physical and chemical agents in ageing *Drosophila*

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Summary. The effects of gamma rays and caffeine on total free amino acids were assessed in unirradiated and irradiated ageing *Drosophila*. As age advances, there is a rise in the quantity of free amino acids in untreated males and females. Gamma rays and caffeine strongly reduce the level of free amino acids in both the sexes.

Insects are known to possess a higher amount of free amino acids. 293-2430 mg/100 ml[1] as compared to mammals which have 42 and 35 mg/100 ml in sheep and humans[2] respectively. This difference makes insects more radio-resistant than mammals. The LD_{50} values range from 10 to 300 kR in adult insects whereas for animals it is between 0.5 and 1.0 kR[3]. The present study was undertaken to study the changes in the level of free amino acids induced by gamma-rays and caffeine in 12-, 24-, 36-, 48-, 60-, 72-, 84- and 96-h-old males and females of adult *Drosophila*. Caffeine (1,3,7-trimethylxanthine) which acts as a radiosensitizer[4,5] of biological damage induced by radiations is gaining importance because of its presence in tea, coffee and coca-cola.

Materials and methods. The flies used in the present studies were the wild type (ORK) of *Drosophila melanogaster*. Adult flies were exposed to 60 kR of gamma irradiation in a 5500 Ci ^{60}Co gamma cell. The dose rate was approximately 60 R/sec. For caffeine feeding, tissue papers were soaked with a solution of 0.2% caffeine and 5% sucrose in water. The adult flies (0-8-h-old) were allowed to feed on this solution. The tissue papers were changed once every 12 h and the entire duration varied from 12 to 96 h depending upon the experiment. Estimation of the free amino acids followed the method of Clark[6]. A standard curve was drawn by using glycine.

Results and discussion. With the increase in age of untreated flies, there is an increase in the quantity of free amino acids

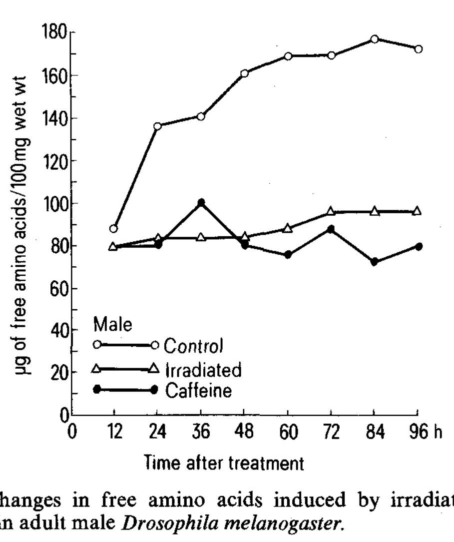

Fig. 1. Changes in free amino acids induced by irradiation and caffeine in adult male *Drosophila melanogaster*.

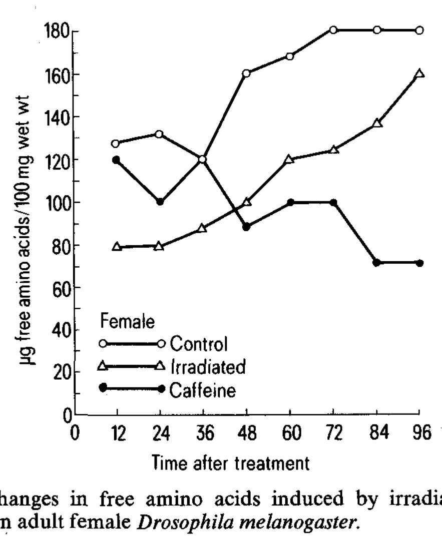

Fig. 2. Changes in free amino acids induced by irradiation and caffeine in adult female *Drosophila melanogaster*.