Abstract. Pups from the strains 129SvEv/Bkl and C57BL/6/Bkl and F1 hybrids of these were subjected to an analysis of the development of swimming pattern (from the day of birth until 21 days old) to study the neuromuscular development of neonatal and juvenile mice of common laboratory strains in gene modification programmes. Swimming as a parameter of scrutiny was chosen because it requires total coordination of the body’s muscles, and we found that the gradual change from a neonatal to an adult swimming pattern can be scored objectively. Five different parameters were scored: the position of the head in the water, the use of front legs, the use of hind legs, the use of the tail and whether or not the animals could maintain a straight course in the water or not. Each parameter could be objectively scored as 0 (neonatal), 1 (juvenile) or 2 (adult) level of development. Both the F1 hybrids C57BL/6/Bkl♂ x 129SvEv/Bkl♀ and 129SvEv/Bkl♀ x C57BL/6/Bkl♂ were fully developed swimmers after only 13 to 14 days, respectively, while it took until 19 or 20 days for the mice of the pure strains to develop an adult swimming pattern. The study also revealed some major differences in swimming pattern between the two strains. The 129SvEv/Bkl♂ maintained a horizontal position in the water and were strong swimmers, whereas the C57BL/6/Bkl♀ mice consistently exhibited a startle reaction when first introduced to the water, maintained a more vertical position in the water and tired easily.

Swimming requires the smooth integrated organization of a co-ordinated series of reflex responses including the righting reflex, vestibular reflexes and extensor reflexes. This makes swimming suitable for assessing functional and behavioural development of the sensory-muscular system in neonatal and juvenile individuals. In young rats, the development of the adult mode of swimming is accomplished by Day 20, at which time the juvenile rat has a fully developed adult swimming pattern. It is quite easy to objectively characterise the normal development of the adult pattern from Day 7 to Day 21 by assessing four parameters: head position in water, use of front limbs, use of tail, and line of swimming (1-4). Rat pups born to stressed mothers have been reported to be of lower birth weight (5), to gain weight more slowly (6) and exhibit poorer locomotor control than pups born to non-stressed mothers (5). Pavlovska-Teglia and co-workers (2) and Young and co-workers (3) showed that oral administration of even relatively low levels of corticosterone had a significant negative influence on the maturation and learning ability of the neonatal rat pup assessed by achievements on swim testing. Kerton and Hau (4) confirmed these findings and found that prolactin deprived corticosterone-treated pups showed an even greater retardation in normal development of the swim pattern.

In mice, life expectancy of many gene-modified strains is shorter than that of the parent strains, but it can be difficult to detect phenotypic changes from normal in terms of normal development and performance in various behavioural tests. Phenotypic characterisation of gene modified mice calls for motor function analyses, perhaps in particular for mutants developed to study ataxias and other neuromuscular diseases. We hypothesized that the pattern of how juvenile mice develop an adult mode of swimming is not only very stable within a strain, but also very susceptible to changes in neuromuscular function and development. Consequently, a characterisation of these normal developmental changes may be a useful tool in mouse phenomics. In the production of mutant mice via microinjection of gene-targeted embryonic stem (ES) cells, C57BL/6/Bkl mice are often used as blastocyst donors and 129SvEv/Bkl mice are routinely used for ES cells (7).
The aim of the present study was to characterise these two strains as well as their two possible F1 hybrid strains with regard to neuromuscular development analysed by the gradual development of an adult swim pattern.

Materials and Methods

Two litters from each of the strains of 129SvEv/Bkl and C57BL/6/Bkl (B&K International, Sollentuna, Sweden) and F1 hybrids of these were studied. The parents' genotypes were either 129SvEv/Bklf x 129SvEv/Bklm, C57BL/6/Bklf x C57BL/6/Bklm, 129SvEv/Bklf x C57BL/6/Bklm or C57BL/6/Bklf x 129SvEv/Bklm. The abbreviation "f" added to the strain designates female and "m" designates male, and the female is always mentioned before the male in a mating. Thus 129SvEv/Bklf x C57BL/6/Bklm is the designation used for 129SvEv/Bkl female mated with C57BL/6/Bkl male. The mice were maintained in a conventional animal facility. On arrival the parents (7 weeks old nullipareous) were caged in Polycarbonate type III cages (Scanbur, Koge, Denmark), 3 females together with one male. A total of 8 cages were used, 2 for each mating system. The rats were kept in standard animal rooms and subjected to standard animal house conditions: the light regime was 12/12 hour dark/artificial light cycle, temperature was maintained at 21±1°C, the relative humidity was between 30-60%, and the cages were cleaned twice a week. Wooden chips (Finn Tapvei, Finland) were used as bedding. Water and standard pelleted diet (R36, Laktamin, Stockholm, Sweden) were available ad libitum.

Swim tests were carried out daily, in the animal holding room, from Day 1 to 20 (Day 1 being the day when the pups had been born during the night) at 10 p.m. in an aquarium approximately 40 x 40 x 50 cm, containing water at a depth of 10 cm maintained at 30–34°C. Mice younger than 7 days were left in the water for approximately 10 seconds and older mice for 20 seconds. Swimming ability was evaluated by five rating systems (Table I). The first two systems, head rating and front leg rating, were used as described for rats by Schapiro and co-workers (1). The scoring of line of swimming and tail movements were modified from those published for rats by Lorenzana-Jimenez and Salas (8). In addition, the hind leg movements were rated as described by Pavlovska-Teglia et al. (2).

During the swimming analysis, the general behaviour in water and the overall swimming capability were also assessed. This included how the mice reacted when put into the water (i.e. startle reactions, swimming or immobile) and how the complete adult swimming behaviour differed between the strains (i.e. body angle in the water, fatigue, overall behaviour). After swimming, the mice were dried before being returned to their cages.

Ethics committee approval. The project was approved by the regional ethics committee in Tierp, Sweden.

Results

Head position. The C57BL/6/Bklf x 129SvEv/Bklm F1 hybrids started to raise their heads on Day 3 and all had reached the adult pattern on Day 12. The F1 hybrid 129SvEv/Bklf x C57BL/6/Bklm first showed score 1 signs on Day 5 and were somewhat less mature, but caught up and reached the adult pattern on Day 11. The first pups of the pure strain 129SvEv/Bklf x 129SvEv/Bklm raised the head to water level on Day 6 and by Day 13 all were displaying the adult pattern. The C57BL/6/Bklf x C57BL/6/Bklm pups did not begin to raise their heads above water level until Day 9, but then they caught up quickly and were all displaying the adult swimming pattern on Day 13 (Figure 1).

Front leg movement. During swimming tests partial, and then total, cessation of the use of forelimbs as in the adult mode of swimming developed as follows. The C57BL/6/Bklf x 129SvEv/Bklm F1 hybrids began to show score 1 signs of a mixed front leg swimming pattern on Day 3 and all displayed the adult swimming pattern by Day 13. The F1 hybrid 129SvEv/Bklf x C57BL/6/Bklm first showed score 1 signs on Day 10 but all had reached score 2 on Day 14. The 129SvEv/Bklf x 129SvEv/Bklm pups began to show a development on Day 11 but did not achieve the adult swimming pattern until Day 19. The C57BL/6/Bklf x C57BL/6/Bklm hybrids began to show score signs of a mixed hind leg swimming pattern on Day 3 and all had achieved the adult swimming pattern by Day 12. The F1 hybrids 129SvEv/Bklf x C57BL/6/Bklm first showed score signs on Day 10, but all quickly reached the adult pattern by Day 11. The 129SvEv/Bklf x 129SvEv/Bklm pups began development on Day 11, but did not achieve the adult pattern until Day 14. The pure strain of C57BL/6/Bklf x C57BL/6/Bklm did not start development until Day 11 and had completed development by Day 20 (Figure 2).

Hind leg movement. The C57BL/6/Bklf x 129SvEv/Bklm F1 hybrids began to show score signs of mixed hind leg swimming pattern on Day 3 and all had achieved the adult swimming pattern by Day 12. The F1 hybrids 129SvEv/Bklf x C57BL/6/Bklm first showed score signs on Day 10, but all quickly reached the adult pattern by Day 11. The 129SvEv/Bklf x 129SvEv/Bklm pups began development on Day 11, but did not achieve the adult pattern until Day 14. The pure strain of C57BL/6/Bklf x C57BL/6/Bklm also did not start development until Day 11 and had finished developing by Day 14 (Figure 3).

Tail movement. In the beginning the pups did not use their tails at all, after which they began to move their tails like a propeller with circular movements in the water. They then

| Table I. Score assessment scale for scoring swimming ability in mice. |
|-----------------|-----------------|-----------------|
| Score           | Neonatal: 0     | Juvenile: 1     | Adult: 2        |
| Head position   | Nose under water| Nose at water level| Nose above water level |
| Front leg       | Dog swim        | Mix             | Immobile        |
| Hind leg        | Dog swim        | Mix             | Coordinated movement |
| Tail movement   | Immobile        | Propeller       | Rudder          |
| Line of swim    | Circle          | Unpurposeful line of swimming | Purposeful line of swimming |
Figure 1-5. Cumulative score (Table I) for 10 pups of the four genotypes tested.

Figure 6. Total sums of the five rating systems (Table I) for 10 pups of the four genotypes tested.
progressed to lateral tail movements as in the juvenile and adult swimming patterns. The C57BL/6/Bklf x 129SvEv/Bklm F1 hybrids started to show score signs on propeller tail movement on Day 3 and all displayed the adult tail movement pattern on Day 12. The F1 hybrid 129SvEv/Bklf x C57BL/6/Bklm first showed score signs on Day 7, but all quickly reached score 2 on Day 11. The 129SvEv/Bklf x 129SvEv/Bklm pups began development on Day 6 or Day 8 and achieved the adult movement pattern by Day 12. The C57BL/6/Bklf x C57BL/6/Bklm began development already on Day 6, but did not exhibit the adult pattern until Day 14 (Figure 4).

**Line of movement.** Orientation in space, posture and coordination of the locomotor activities are the components that determine the line of swimming. In the beginning, the pups swam in small circles. The diameter of the circles progressively grew larger as they began to maintain the nose above the water and with their strength developing. By the time the animals attempted to swim in straight lines, they sometimes changed direction and began to swim in circles in the opposite direction. The C57BL/6/Bklf x 129SvEv/Bklm F1 hybrids began to show score signs on breaking the circular swimming on Day 3 and all had an adult straight swimming pattern on Day 13. The F1 hybrid 129SvEv/Bklf x C57BL/6/Bklm first showed development on Day 4 but all quickly finished development by Day 11. Both the strains 129SvEv/Bklf x 129SvEv/Bklm and C57BL/6/Bklf x C57BL/6/Bklm began development on Day 6 but did not achieve the adult swimming pattern until Day 14 (Figure 5).

**Total locomotor development.** When the total sums of the five rating systems were added, the C57BL/6/Bklf x 129SvEv/Bklm F1 hybrids began to show score signs of a juvenile swimming pattern on Day 3 and all had achieved the adult swimming pattern by Day 13. The F1 hybrids 129SvEv/Bklf x C57BL/6/Bklm first showed score signs on Day 4, but all quickly reached score 2 by Day 14. The 129SvEv/Bklf x 129SvEv/Bklm began development on Day 6, but all did not achieve the adult pattern until Day 18. The pups of C57BL/6/Bklf x C57BL/6/Bklm also did not start development until Day 6 and did not finish development until Day 20 (Figure 6).

**Differences in swimming patterns.** The study revealed some major differences in swimming pattern between the two original strains. The strain 129SvEv/Bkl had a strong and efficient swimming pattern. When an adult 129SvEv/Bkl was put into the water it floated with the body in a horizontal position and looked purposefully around before starting to swim in a straight line toward a corner of the tank. They were all very strong and calm swimmers. By contrast, the C57BL/6/Bkl adults had difficulties with startle reactions when introduced into the water and showed early exhaustion. They also sank down with their backs deep in the water exhibiting a lordosis position and they consumed a lot of energy paddling at a steep angle with their hind legs. The F1 hybrids 129SvEv/Bklf x C57BL/6/Bklm also showed some of this pattern, although they did not fully display the same steep angle and startle reactions as the pure C57BL/6/Bkl mice and they seemed a little stronger. The C57BL/6/Bklf x 129SvEv/Bklm F1 hybrids exhibited a swim pattern more like that of the pure 129SvEv/Bkl strain.

**Discussion**

This is the first study of how neonatal and juvenile mice gradually develop an adult mode of swimming and how this can be assessed by scoring five different parameters. Considering the taxonomic relationship between mice and rats and our previous studies in the rat (2-5), this was not entirely unexpected. One of the attractions of the present analysis and scoring system is that the performance of the mice seems unaffected by the ambient environment outside the water tank. The method is thus not complicated by the many known and unknown variables, which unfortunately render many behavioural tests difficult to perform and interpret for scientists other than very experienced ethologists.

The study demonstrated that there were clear differences between the four genotypes of mice tested. This is interesting because the genotypes chosen are widely used in the production of genetically modified mice. Considering that many of these are developed with the aim of creating models to study disorders, it seems important to characterise normal locomotor and neuromuscular development in the 129 and C57BL strains as well as crosses between them because many transgenic and knock-out chimeric animals are routinely backcrossed into the C57BL strain.

The pups showing the most rapid development were the F1 hybrids of C57BL/6/Bklf x 129SvEv/Bklm, which were the first to show score signs in all categories and lead the development, although they were not always the first to achieve the adult swim pattern. Second in development of the juvenile pattern and often first to achieve the adult swim pattern was the other F1 hybrid, 129SvEv/Bklf x C57BL/6/Bklm. Pups of both the pure strains 129SvEv/Bklf x 129SvEv/Bklm and C57BL/6/Bklf x C57BL/6/Bklm developed more slowly, indicating a positive heterosis effect in the F1 hybrids.

Head and nose positions during swimming are components of reflexes involved in space orientation, posture, voluntary movements and equilibrium, and they were not fully integrated in pups of the pure mouse strains until Day 13. By contrast, this had already been achieved by
Day 11 and 12 in the F1 hybrids. Shortly after birth, the pups began to use their forelimbs for swimming. They made broad swiping movements with the legs, producing forward movements with little propulsion. They also simultaneously used their hind limbs in the same manner, but this movement was not co-ordinated with the movements of the front limbs. Full co-ordination of the hind limbs was established between Day 11 and Day 12 in the hybrids but not until Day 14 in the pure strains. The establishment of co-ordination of front and hind limb movements is the result of the maturation of the neuromuscular co-ordination, the regulatory centres for which are located in the central nervous system (CNS). Adult mice do not use their forelimbs while swimming, but hold them in hyperextension straightforward. This was achieved by Day 13 to 14 in the hybrids, but not until Day 19 to 20 in the pure strains. Taken together, this suggests that there is a heterosis effect on the neuromuscular development in the CNS in both the F1 hybrids as compared with both the pure strains.

The C57BL/6/Bkl mice seemed to be much poorer swimmers and more afraid to swim than the 129SvEv/Bkl mice. The genes for proficient swimming may come from the male of 129SvEv/Bkl since the F1 offspring of the 129SvEv/Bkl males were much better swimmers than the F1 offspring of the C57BL/6/Bkl males. There was no apparent difference between the development of female and male pups, but this should be scrutinized in larger studies.

In the present study, the mouse pups were subjected to swimming on average every second day. Studies are in progress to determine to what extent development of an adult swimming pattern is stimulated by practice or, in other words, how important learning - cognition - is in the process.

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**References**