A rat model of neurobehavioral development

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Summary. The neurobehavioral evolution of the normally growing rat has been investigated by means of a series of reflex, motor and sensory tests from birth up to weaning. A sequential development of behavioral reponses has been assessed over this 21-days period, the 2nd week following birth representing an important step in the neurobehavioral maturation of the rat. This rat model may be considered as an useful reference to evaluate changes that may be induced by pharmacological and toxicological agents in the developing exposed rat.

The aim of this study was to assess a rat model of neurobehavioral development during early postnatal life, with the view to establish normative data in the untreated animal[2]. Although postnatal behavioral features have been reported in some rodents (Altman and Sudarshan[3], Altman[4], Fox[5], Wahlsten[6]), our normative investigation appeared useful in order to identify more precisely the sequential phenomena involved in the progressive acquisition and improvement of reflex activities, motor and sensory abilities, as a function of age in the rat.

The assessment of an adequate and well-stated model of behavioral activities in the normal young rat appears to be justified with the view to be able to evaluate the changes induced in the timing and/or the quality of several behavioral responses following fetal exposure to maternal drugs or postnatal administration to the pups.

Material and methods. 10 female Sprague-Dawley rats were mated by the pairing system. They delivered 10 healthy litters, each of them being reduced at birth to 8 pups (4 females, 4 males). Procedures for testing behavioral responses were derived and adapted from those reported by Altman[4], Fox[5] and Wahlsten[6]. Each of the 80 pups was submitted to a test battery according to a similar schedule and at the same daily time. The evaluation of the responses was performed by the same investigator using a 9-scale grading system, number 9 being the maximal score[7].

The figure illustrates the handling procedure introduced by us in order to maintain each pup of a litter under minimal intralitter variations. For further details, refer to the legend of the figure.

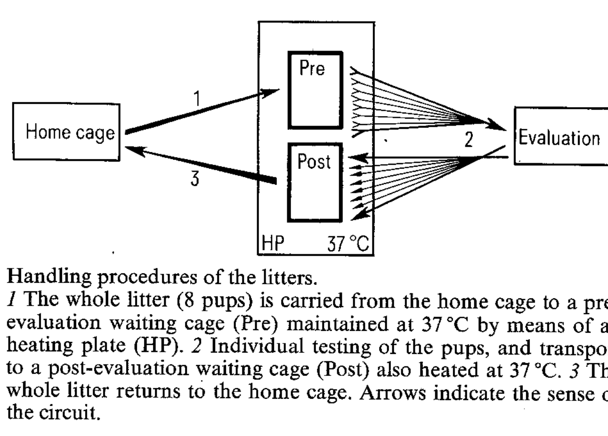
Results and discussion. The mean scores obtained from the behavioral tests applied to the pup rat from the 1st day after birth up to weaning time (21 postnatal days) are summarized in the table. The analysis of the sequential development of behavioral responses evidences that the 1st 10 days are marked by the gradual disappearance of primitive reflexes, namely: crossed extensor, flexor dominance and rooting reflex. This is paired with the progressive emergence of other activities which are essential to the feeding and the survival of the pup: surface righting reflex, limb placing reactions, grasping reflex, cliff drop aversion, suckling ability and negative geotaxis. Most of these activities are facilitated by postural extension. Thereafter, more complex motor skills develop as evidenced by the improvement of the performance to specific tests: air righting, sensory responses, vibrissae and visual placing responses, screen tests, chimney, bar-holding and rotarod. Parallely, sense organs gradually mature (eye opening, ear unfolding and opening); this anatomic maturation coincides with the acquisition of adult-like sensory reactions (auditory and visual responses, tactile and auditory startles, visual and auditive orientations, ear twitch, and cornea reflex). At the end of the 2nd week, a normal postural tone distribution is attained. Tooth eruption was completed during the 2nd week and fur development during the 3rd week.

Our results generally are in good agreement with those reported by Altman[3] in the rat, and Fox[4] and Wahlsten[6] in the mouse, as regards the nature of the progressive responsiveness. Nevertheless, our data show a marked timing difference as regards the acquisition of the surface righting reflex. Those authors have found a maximal value several days before us. This discrepancy may be attributed to a more severe scoring system used by us in the evaluation of behavioral responses.

According to our present data, impressive evolutionary phenomena will take place in the course of the 1st 2 weeks following birth. The early neonatal period is characterized by the predominance of behavioral responses that will ensure adequate survival of the rat pups. Thereafter new responses develop so that the neurological state of the developing rat will vary considerably during the 1st 2 postnatal weeks. Around the 12th day of age, which represents an important developmental step, the pups will acquire progressively the basic organization that is required for their functional autonomy. In this phase of behavioral evolution, more complex activities, such as sense organs and locomotor responses, establish themselves and progress in replacement of the primitive responsiveness of the neonates. In the 16–18-day-old rats, the neurobehavioral development is grossly achieved except for sexual maturation and adult-like socialization that will be continued still after weaning.

Besides this model of behavioral evolution[8,9], morphologic investigations on the developing cerebellum have lead us to the assessment of a rat model of neuronogenesis, i.e. of the neuronal maturation in the cerebellar Purkinje cell (Nosal and Radouco-Thomas[10]). Structural and ultrastructural studies performed in the growing rat and mouse from birth up to weaning (21 days of age) evidenced several developmental stages in this cerebellar neuron that were identified as the morphologically 'undifferentiated cell' (initial state), the early, intermediate and late neuroblasts and finally the young neuron (Nosal et al.[11,12]).

The sequential events occurring in the developing Purkinje cell of the rat cerebellar cortex may be summarized as follows: the nuclear maturation appears to be attained as



Handling procedures of the litters.
1 The whole litter (8 pups) is carried from the home cage to a pre-evaluation waiting cage (Pre) maintained at 37 °C by means of an heating plate (HP). *2* Individual testing of the pups, and transport to a post-evaluation waiting cage (Post) also heated at 37 °C. *3* The whole litter returns to the home cage. Arrows indicate the sense of the circuit.

Mean scores of the neurobehavioral tests

Age (days)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Surface righting reflex	1.95	3.60	5.75	5.69	6.00	6.72	7.93	8.7	8.75	8.8	9	9	9	9	9	9	9	9	9	9	9
Postural flexion	4.4	2.8	0.06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Postural extension	0.15	1.06	5.5	5.3	5.9	5.2	5.2	4.19	3.06	0.05	0.9	0	0	0	0	0	0	0	0	0	0
Normal posture	0	0	0	0	0	0.22	1.8	2.23	3.31	5.31	6.9	6.55	8	9	9	9	9	9	9	9	9
Forelimb placing	2.7	4.15	8.15	8.5	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Hindlimb placing	0	0.06	1.74	3.56	4.56	5.32	5.76	7	8.5	8.75	9	9	9	9	9	9	9	9	9	9	9
Visual placing	0	0	0	0	0	0	0	0	0	0	0	0	0.03	0.75	6	9	9	9	9	9	9
Vibrissae placing	0.18	0.16	2.92	2.87	4.19	4.93	6.7	7	7.42	7.74	8.87	9	9	9	9	9	9	9	9	9	9
Forelimb grasp reflex	0.72	1.17	2.3	2.5	2.9	3.25	4.2	4.5	6.5	8.5	9	9	9	9	9	9	9	9	9	9	9
Hindlimb grasp reflex	0	0	0	0	0	0	0	0.4	0.58	0.7	1.33	1.38	2.89	3.25	4.26	5.89	6	6	6.8	7	7
Visual orientation	0	0	0	0	0	0	0	0	0	0	0	1.6	3.04	5.98	8.23	8.9	9	9	9	9	9
Auditory orientation	0	0	0	0	0	0	0	0	0	0	0	0.46	3.45	5.17	7.10	7.47	7	8.97	9	9	9
Crossed extensor	2.37	4.8	6.67	7.43	3.12	0.18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rooting reflex	8.62	8.75	8.56	9	8.25	6.78	3.84	4.67	0.1	0	0	0	0	0	0	0	0	0	0	0	0
Geotaxis (25° incline)	1	4.39	3.17	4.21	4.64	6.59	6.76	8.31	9	8.95	9	9	9	9	9	9	9	9	9	9	9
Cliff drop aversion	0.57	0.6	1.57	1.96	3.16	4.8	6.2	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Air righting	0	0	0	0	0	0	0	0	0	0.16	0.93	2.63	4.51	6.04	8.05	9	9	9	9	9	9
Eye opening	0	0	0	0	0	0	0	0	0	0	0	0.02	0.35	2.68	7.9	9	9	9	9	9	9
Ear opening	0	0	0	0	0	0.25	0.25	0.25	0.25	0.58	0.92	1.5	4.4	6.8			8.2	8.78	9	9	9
Ear unfolding	0	1.48	2.72	3.55	3.56	3.54	4	4.92	4.67	4.92	5.75	6.58	7.50	8.0	7.25	8.17	8.6	8.9	9	9	9
Fur development	0	0	0.77	1.9	2.16	2.87	3	4.42	5.27	6	6.55	7.3	7.92	8.08	8.55	8.42	9	9	9	9	9
Tooth eruption sup. max.	0	0	0	0	0	0	18%	50%	50%	100%											
Tooth eruption inf. max.	0	0	0	0	0	0	0	0	1%	1.9%	80%	92%	100%								
Auditory startle reaction	0	0	0	0	0	0	0	0	0	0.06	0.51	2.4	4.64	5.56	5.74	7.25	8.1	9	9	9	9
Tactile startle reaction	2.25	2.17	2.89	2.75	3	3.19	2.88	2.33	3.06	3.30	3.15	4.28	5.68	5.84	7.1	7.75	8.6	9	9	9	9
Ear twitch response	0	0	0	0	0.6	0.6	0.3	1.36	1.54	1.8	2.42	2.47	2.63	4.08	5.75	7	8.75	9	9	9	9
Cornea reflex	1	1	1	1	1	1	0.4	1	1	1.8	1	1	1.16	2.62	6.76	9	9	9	9	9	9
Chimney	0	0.25	0.25	0	0.6	0.58	0.25	1.46	3.10	3.50	6.33	7.11	7.2	6.93	6.43	6.1	7.4	8.4	8.45	7.04	8.87
Bar holding	0	0	0	0	0	0.28	0.6	3.12	3.48	4.82	5.7	6.59	7.59	8.57	9	9	9	9	9	9	9
Rotarod	0	0	0	0	0	0	0	0	0	0	0	0.17	0.19	0.67	1.46	3.65	3.2	4.9	6.58	8.2	8.3
Level screen test	0	0	0.7	1	2.1	3.09	3.22	4	5.12	6.08	6.33	6.92	7.17	8.5	9	9	9	9	9	9	9
Vertical screen test	0	0	0	0.1	1.47	2.39	2.37	2.82	3.78	5.54	6.88	7.21	8.18	8.62	9	9	9	9	9	9	9
Screen climb test	0	0	0	0.3	0.8	1.64	1.58	3.66	4.6	6.55	8.41	8.75	9	9	9	9	9	9	9	9	9
Suckling	8.7	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9

early as the 4–5 days after birth; the cytoplasmic differentiation and growth proceeds more slowly and is continued until the adult-like stage (rats aged 3–4 weeks). These ultrastructural features mainly concern the progressive evolution of the morphologic and functional intraneuronal apparatus designated by us as the 'nucleus-ribosome system'. The NRS is essentially involved in the production of m-RNA and r-RNA and in the biosynthesis of neuronal proteins during the maturational period, as well as during the functioning of adult Purkinje cells.
Besides the cell body evolution, the dendritic arborization develops extensively, starting from the 2nd postnatal week. Intracerebellar connections are gradually established as the synaptogenesis proceeds and the neuronal prolongements become myelinized.
A comparative evaluation of maturational processes, implied in the behavioral development and in certain brain structures development, indicates that the 2nd week following birth constitutes a crucial phase in the neurobehavioral maturation of the rat. This assertion is further supported by the neurochemical and neurophysiological changes that have been reported by several investigators to occur at this time in the developing rat brain.
To conclude, the described neurobehavioral model, which has been assessed in the course of the rat postnatal development, may be considered in an attempt to integrate structural data with some functional aspects in the maturing brain. This model in normally growing rats may be used as a reference point to evaluate the modifications possibly induced by pharmacological and toxic agents on the prenatal and postnatal neurobehavioral development of the rat.

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Dopamine-β-hydroxylase, adrenaline, noradrenaline and dopamine in the venous blood of adrenal gland of man: A comparison with levels in the periphery of the circulation[1]

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Summary. In 10 human subjects plasma dopamine-β-hydroxylase activity was found in the adrenal vein blood to be as high as in the periphery of the circulation. Adrenaline concentration in the adrenal vein blood was in the mean 170 times, noradrenaline concentration 11 times and dopamine concentration little higher than levels in the periphery.

Adrenaline (A), noradrenaline (NA) and dopamine (DA) as well as the enzyme dopamine-β-hydroxylase (DBH), which are all circulating in human blood, originate from the sympatho-adrenal system[2]. During activity of sympathetic nerves, NA, DA and DBH are released from the nerve endings by an exocytotic process into the extraneuronal space, then they reach the bloodstream secondarily. Adrenaline is a product of the adrenal medulla only; it is secreted directly into the adrenal blood. Venous blood of the adrenal gland leaves the organ by way of the suprarenal vein to enter the common circulation. The enzyme DBH, which catalizes the synthesis of NA from DA, is stored in the adrenal medulla as well as in the sympathetic nerves. It is not known in man whether DBH is released from the adrenal medulla into the blood. Animal experiments in vivo suggest that circulating DBH mainly originates from the sympathetic nerves rather than from the adrenal medulla[3]. In order to ascertain in man whether DBH, which is stored in the adrenal medulla, is released into adrenal blood together with the adrenal catecholamines, DBH

Comparison of catecholamine concentrations and dopamine-β-hydroxylase (DBH) activity in the plasma of iliac vein and plasma of suprarenal vein. Study in 10 human subjects: No. 1–5 have normal blood pressure, No. 6–9 are patients with essential hypertension, and subject No. 10 is a woman with terminal renal insufficiency and primary nephrosclerosis (f, female; m, male)

Subject No.	Age (years)	Sex	Blood pressure (mm Hg)	Plasma DBH activity (units/ml) Iliac vein	Suprarenal vein	Plasma catecholamine concentration (ng/ml) Iliac vein A	NA	DA	Suprarenal vein A	NA	DA
1	19	m	110/ 70	181	191 (106%)	0.10	0.38	0.19	18.83	2.67	0.30
2	35	m	130/ 80	193	196 (102%)	0.08	0.19	0.14	9.28	1.61	0.23
3	42	f	140/ 80	70	67 (96%)	0.13	0.34	0.21	11.80	7.01	0.26
4	28	m	140/ 90	293	291 (99%)	0.07	0.12	0.12	13.42	1.52	0.16
5	43	m	140/ 90	78	79 (101%)	0.04	0.13	0.20	8.15	0.91	0.23
6	54	m	150/100	68	74 (109%)	0.04	0.26	0.13	5.53	0.43	0.15
7	52	m	170/110	224	230 (103%)	0.07	0.30	0.20	4.25	1.17	0.23
8	56	m	200/130	190	181 (95%)	0.07	0.10	0.18	17.85	3.65	0.15
9	47	m	250/160	88	82 (93%)	0.10	0.07	0.21	13.90	2.30	0.30
10	40	f	200/120	38	37 (97%)	0.14	0.26	0.40	33.26	4.08	0.70
		n=10	$\bar{X}\pm SD$	142±85	143±85	0.08±0.03	0.22±0.11	0.20±0.08	13.63±8.41	2.54±1.96	0.27±0.16