Appendix 8 - 6B

CONFIDENTIAL

STUDY REPORT

VERSION: 1.0

STUDY OF THE EFFECTS OF ING 911 HYDROLYSATE GIVEN AT A 50 MG/KG/DAY DOSE THROUGHOUT PREGNANCY ON THE MATERNAL BEHAVIOUR AND THE PHYSICAL, NEUROMOTOR AND BEHAVIOURAL DEVELOPMENT OF YOUNGS BETWEEN BIRTH AND ADULTHOOD IN WISTAR RAT

PROTOCOL CODE

INGREDIA No.18/1100/ING 911-Behavioural Toxicology

SPONSOR

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ETAP - Applied Ethology

STUDY IN CONFORMITY WITH THE GOOD ANIMAL EXPERIMENT PRACTICES

NOVEMBER 2001

EXPERIMENT PERIOD: January - July 2001

Contents

QUALITY ASSURANCE

Study: INGREDIA No.18/1100/ING 911-Behavioural Toxicology
Study title: Study of the effects of "ING 911" hydrolysate, given at a 150 mg/kg daily dose throughout pregnancy, on the maternal behaviour and the physical, neuromotor and behavioural development of youngs between birth and adulthood in Wistar rat.

I, undersigned, declare that the work described in this report accurately corresponds to the results obtained.
Moreover, I declare that the present study was carried out in accordance with the Good Laboratory Practices.

November 22nd, 2001

Pr. D. DESOR, Scientific Consultant and Quality Assurance
Ethopharmacology - Biostatistics
Ministerial Authorisation of Animal Experiment No. 04140/1991.

AUTHORS

I, undersigned, declare that the work described in this report was completed under my responsibility and that this report accurately corresponds to the results obtained.
Moreover, I declare that the present study was carried out in accordance with the Standard Work Protocols of ETAP Company and in accordance with the Good Laboratory Practices, including the original document filing.

M. MESSAOUDI, Scientific Manager November 22nd, 2001
Behavioural Biology - Ethopharmacology
Ministerial Authorisation of Animal Experiment No. 04535/1991

We, undersigned, responsible for carrying out these study experiments, declare that the study was carried out in accordance with the procedures described in this report and that data presented hereafter strictly correspond to the experimental results.

A. NEJDI, Study Manager November 22nd, 2001
Behavioural Biology - Human Nutrition
Authorisation of Animal Experiment

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Ministerial Authorisation No. B-54805.

1 – SUMMARY

Treatment effects on the treated females

The pregnancy times of females of the various treatment groups are equivalent statistically.

The number of youngs and the sex-ratio at birth of litters born from females treated with ING 911 hydrolysate are statistically comparable to those of litters from control females. The treated females have also maternal and care-to-young behaviour comparable to those of control females treated with powdered milk.

Treatment effects on the physical development of the youngs

The weight evolution, age of incisor cutting and age of eye opening of youngs born from treated females are comparable to those born from control females.

Treatment effects on the neuromotor development of the youngs

Between birth and weaning, on the basis of various measurement tests of neuromotor development, the performances of youngs born from treated females are statistically equivalent to those of the youngs born from control females.

Treatment effects on the behavioural and cognitive development of the youngs

In the open-field test, the locomotor and exploratory activity and the emotional state of the youngs born from treated females are statistically equivalent to those of the youngs born from control females.

In the Morris water maze as in the aversive light stimulus avoidance test, the youngs born from treated females show short and long term memory and training performances statistically equivalent, even better, compared to those of the youngs born from control females.

On the basis of the study experimental results, the daily treatment of females with the oral dose of 150 mg/kg of ING 911 hydrolysate during the first, second, third week of pregnancy, or throughout pregnancy, does not modify their pregnancy time, their maternal and care-to-young behaviour, and the quality of their litters compared to the placebo.

Similarly, the treatment of females with ING 911 hydrolysate does not induce physical, neuromotor, behavioural and cognitive development disorders in young males and females compared to youngs born from control females.

2 - INTRODUCTION

At the INGREDIA's request, ETAP-Applied Ethology has evaluated the "ING 911" hydrolysate effects on the maternal behaviour and the physical, neuromotor and behavioural development of the youngs. The hydrolysate was given orally at the dose of 150 mg/kg to fertilised females either during the first, second or third week of pregnancy, or throughout the three weeks of pregnancy.

Forty litters, divided into five treatment groups (n = 8), were studied. After recording of their size, the litters were reduced to 8 youngs (in general 4 males and 4 females), in order to study the development of youngs from comparable-sized litters.

The treatment effects were evaluated regarding quality of maternal behaviour (litter size, sex-ratio and test of nest construction) and the youngs through a testing battery of variables relating to physical (weight evolution, incisor cutting and eye opening), neuromotor (eversion tests, grip reflex, righting reflex, hanging and locomotor coordination) and behavioural (locomotor, exploratory activity and emotional state in open-field, spatial training in the Morris water maze and operative conditioning in the aversive light stimulus avoidance test) developments between birth and adulthood.

3 - MATERIALS AND METHODS

3.1 - Animals

Sixty adult female rats from the Wistar strain (Breeding centre of Charles River/Iffa-Credo, 69-St-Germain sur l'Arbresle, France), were used at the beginning of experiment. After crossing with adult male Wistar rats (Breeding centre of Charles River/Iffa-Credo, 69-St-Germain sur l'Arbresle, France), the fertilised females were randomly divided into five treatment groups: a control group (S0) treated with an oral dose of 150 mg/kg of powdered milk throughout pregnancy and four groups treated with an oral dose of 150 mg/kg of ING 911 hydrolysate either every day during the first week (S1), the second week (S2) or the third week of pregnancy (S3), or every day during all the pregnancy time (S123). The doses of powdered milk and hydrolysate were diluted in a 5-ml volume of a 0.5% aqueous methylcellulose solution. Out of treatment periods with the hydrolysate, the females of the S1, S2 and S3 groups were receiving every day 5 ml/kg of vehicle. At birth, the litters were coded and installed in type-F polycarbonate cages (48 x 27 x 20 cm, U.A.R., 91 - Epinay-Sur-Orge, France). After recording of their size, the litters were reduced to 8 youngs (in general 4 males and 4 females), in order to study comparable-sized groups. In the cases where litters contained less than 8 youngs, they were completed with youngs from litters with the same treatment. The youngs of every litter were subcutaneously marked, according to a given code, with a vital dye (alcyan blue) to be individualised. Following hair growth, a definitive marking, with picric acid, was applied to the hairs according to a specific code. The females and their youngs were housed in a air-conditioned animal care facility, at a temperature of 22-24°C. The females had food (dry food M25, Ets Piètrement, 77- Provins, France) and drink *ad libitum* and were subjected to a light-dark cycle of 12 hours (light from 8 pm to 8 am).
All the females of the various groups were treated similarly and under the same conditions

3.2 - Experimental procedures

3.2.1 - Treatment effects on females

3.2.1.1 - Treatment effects on the litter quality and sex-ratio at birth

The litter size allows to evaluate the treatment impact on the quality of the litters (litter size, sex-ratio) at birth throughout different pregnancy periods.

3.2.1.2 - Treatment effects on the quality of maternal behaviour and care to youngs

The test of nest construction (nest structure and quantity of material used for its construction) in the nursing female allows to detect possible disturbances of maternal behaviour inherent in various toxic treatments (test carried out between D4 and D7 after birth).
Four levels were assigned to the nest construction quality (from 0 to 4):
- level 0: total absence of nest;
- level 1: beginning of construction without precise structure;
- level 2: beginning of structure in a semicircle;

- level 3: complete round structure without nest covering;
- level 4: complete round structure with nest covering.

3.2.2 - Treatment effects on youngs

3.2.2.1 - Treatment effects on the physical development of youngs

The weight evolution of the youngs (weighed on D2, D5, D9, D12, D15 and D18), the dates of incisor cutting (checking between D8 and D11) and eye opening (checking between D12 and D16) are good indicators of the physical development of the young rat.

3.2.2.2 - Treatment effects on the neuromotor development of youngs

Between birth and weaning, every young was evaluated using a test series involving different degrees of neuromotor maturation.

- Eversion test (Day 3)

When it is laid down face upwards, the young rat seeks to return to its natural dorso-ventral position. The measured variable is the time necessary for the young to reverse (neuro-muscular maturation measurement).

- Grip test (Day 4)

When it is placed on a wire plate, the young rat must grip not to fall when the plate is rotated. The measured variable is the angle obtained compared to the horizontal line when the young "lets go" and fall (grip reflex measurement).

- Righting reflex test (Day 9)

When it is placed "upside down" on a 20° inclined plane, the young rat turns over to find itself "head upwards". The measured variable is the time necessary for the animal to carry out a complete half-turn of 180° (measurement of equilibration, maturation of the cerebellum and semicircular canals of the inner ear).

- Wire-hanging test (Day 12)

The forepaws of the young rat are put in contact with a tight wire suspended 40 cm above the ground. This stimulation triggers a grip reflex and the animal hangs on to the wire. The measured variable is the hanging time (muscular force and "fatigability" measurement).

- Locomotor coordination test (Day 19)

The rat is put in a water-filled cylindrical container equipped with a threaded rod topped by a platform. The task, based on a good locomotor coordination, consists for the rat in swimming towards the threaded rod and climbing onto it so as to push itself up onto the platform 50 cm above the water surface.

A satisfactory nervous and muscular maturation is required to pass this test.
The studied variables are the latency time to grip the rod, the total time used to push itself up onto the platform and the number of attempts to carry out the complete locomotor coordination test.

3.2.2.3 - Treatment effects on the behavioural and cognitive development of youngs
After weaning, two males and two females of every litter were randomly selected for the behavioural and cognitive tests.

- Locomotor, exploratory activity and emotional state in open-field (Day 45)
The device consists of an arena (Ø 50 cm) divided into nine compartments, a power station and eight peripherals.
This test allows to evaluate, on 5-minute test sessions, the animal disorders regarding locomotor activity, exploratory behaviour and emotional state.
The studied variables are the number of crossed compartments and uprightings (locomotor and exploratory activity), the number of droppings and micturitions, and the number of passages through the central compartment of the device (anxiety criteria).

- Morris water maze: spatial memory (Days 50 and 51)
When it is placed in a water-filled round basin (Ø150 cm), the rat swims and seeks to escape from the aversive aquatic environment. The first test session (Day 50) comprises 5 trials, during which the animal learns how to locate the site of a platform immersed 2 mm below the water surface and to take refuge there. During the first two trials (test familiarisation trials), the platform is placed against the basin wall. For the other trials, it is placed 10 cm distant from the wall. The second test session (Day 51) comprises two trials, during which the platform is placed 10 cm distant from the basin wall as from the beginning. The rat is left on the platform for a 30-second rest period between two trials so that it can take bearings within the device.
This test allows to evaluate the locomotor behavioural quality during swimming, the possible disturbances in the progressive training acquisition and the short and long term spatial memorisation.
The studied variables are the swimming behavioural quality and the latency time to take refuge onto the platform.

- Discriminating training: TESLA (Days 60 and 61)
The device of the aversive light stimulus avoidance test (TESLA ®) is composed of a brightly lit cage, provided with an active lever and an inactive one. For every 20-minute training session, any pressing on the active lever, during the light phase, gives place to a 30-second darkness period as positive reinforcement.
This test allows to evaluate possible disorders regarding manipulatory activity of the levers and training acquisition through the discrimination between active and inactive levers. A retest, 24 hours afterwards, allows to estimate the long-term training memorisation.

The studied variables are the number of pressings on the active lever and the number of pressings on the inactive one.

3.3 - Statistical analyses

The Kruskal-Wallis test was used to show any heterogeneity in the results of the various tests involving the animals of the 5 treatment groups. In the cases of significant heterogeneity, the Mann-Whitney test was used to compare the scores of every treatment group with those of the reference group S0. The Friedman test was used to show any heterogeneity in the repeated measurements in the Morris test and the Wilcoxon test was used to compare in twos the paired variables in both Morris and TESLA tests.

The statistical analyses were carried out using the StatView 5 softwares (SAS, Abacus Concept., Inc). The results were expressed as average ± average standard error (E.S.M.).

4 - RESULTS

Out of the 50 pregnant females, 42 were fertilised and gave birth: 9 "S0", 7 "S1", 8 "S2", 9 "S3" and 9 "S123".

4.1 - Treatment effects on females

4.1.1. - Treatment effects on pregnancy period, litter size and sex-ratio at birth

Kruskal-Wallis tests do not show heterogeneity in pregnancy periods, litter sizes and sex-ratio in various treated female groups (Tab. 1).

Table 1

Treatment effects on pregnancy period, litter size and sex-ratio at birth

Treatment groups	S0 (n = 9)	S1 (n = 7)	S2 (n = 8)	S3 (n = 9)	S123 (n = 9)
Pregnancy time (d) KW: H (ddl=4) = 2.16; p = 0.71	21.67 ± 0.17	21.71 ± 0.29	21.75 ± 0.16	21.78 ± 0.17	22.00 ± 0.17
Litter size KW: H (ddl=4) = 3.93; p = 0.42	9.00 ± 1.16	7.86 ± 1.06	10.88 ± 0.74	9.89 ± 1.20	9.22 ± 1.04
Sex-ratio (% of males) KW: H(ddl=4) = 5.95; p = 0.20	58.57 ± 3.26	47.93 ± 7.90	48.28 ± 3.72	63.24 ± 8.17	45.08 ± 4.53

KW: Kruskal-Wallis test.

4.1.2 - Treatment effects on nest construction quality

The Kruskal-Wallis test does not show heterogeneity in the scores of various treated female groups (Tab. 2) regarding the nest construction.

Table 2

Treatment effects on nest construction quality

Treatment groups	S0 (n = 9)	S1 (n = 7)	S2 (n = 8)	S3 (n = 9)	S123 (n = 9)
KW H (ddl=4) = 5.60; p = 0.23	3.22 ± 0.51	3.29 ± 0.42	3.50 ± 0.50	2.78 ± 0.49	1.79 ± 0.70

KW: Kruskal-Wallis test.

4.1.3 - Treatment effects on the quantity of material used in nest construction

The Kruskal-Wallis test does not show heterogeneity in the quantities of material used in the nest construction of various treated female groups (Tab. 3).

Table 3

Treatment effects on the quantity of material used in nest construction

Treatment groups	S0 (n = 9)	S1 (n = 7)	S2 (n = 8)	S3 (n = 9)	S123 (n = 9)
KW H (ddl=4) = 6.32; p = 0.18	7.80 ± 1.46	8.86 ± 1.14	8.75 ± 1.25	7.63 ± 1.32	4.44 ± 1.76

KW: Kruskal-Wallis test

4.2 - Mother treatment effects on youngs

A litter of the treatment group "S3" is made up only of males. Consequently the results relating to young females of the group S3 only involve 8 litters.

4.2.1 - Treatment effects on the physical development of youngs

4.2.1.1 - Weight evolution (D2, D5, D9, D12, D15 and D18)

4.2.1.1.1 - Weight evolution in males

On days 2, 5, 9, 12, 15 and 18, the Kruskal-Wallis test does not show heterogeneity in the weights of the various young male rat groups (Tab. 4).

Table 4

Weight evolution in males (g)

Treatment groups	S0 (n = 9)	S1 (n = 7)	S2 (n = 8)	S3 (n = 9)	S123 (n = 9)
D2 KW: H (ddl=4) = 2.92; p = 0.57	7.42 ± 0.26	7.48 ± 0.36	7.41 ± 0.3	6.91 ± 0.31	7.67 ± 0.23
D5 KW: H (ddl=4) = 3.3; p = 0.49	11.56 ± 0.45	11.84 ± 0.63	11.9 ± 0.33	10.93 ± 0.44	11.85 ± 0.39
D9 KW: H (ddl=4) = 6.38; p = 0.17	19.59 ± 0.98	20.05 ± 1.23	19.81 ± 0.43	18.29 ± 0.46	19.92 ± 0.58
D12 KW: H (ddl=4) = 5.02;p = 0.29	26.41 ± 1.21	26.82 ± 1.50	26.71 ± 0.45	24.91 ± 0.51	25.99 ± 0.46
D15 KW: H (ddl=4) = 3.90; p = 0.42	33.54 ± 1.57	32.74 ± 1.90	32.61 ± 0.63	31.13 ± 0.47	32.57 ± 0.66
D18 KW: H (ddl=4) = 2.58; p = 0.63	39.94 ± 1.65	38.35 ± 2.13	38.70 ± 1.07	37.40 ± 0.49	38.82 ± 0.91

KW: Kruskal-Wallis test.

4.2.1.1.2 - Weight evolution in females

On days 2, 5, 9, 12, 15 and 18, the Kruskal-Wallis test does not show heterogeneity in the weights of the various young female groups (Tab. 5).

Table 5

Weight evolution in young females (g)

Treatment groups	S0 (n = 9)	S1 (n = 7)	S2 (n = 8)	S3 (n = 8)	S123 (n = 9)
D2 KW: H (ddl=4) = 4.61; p = 0.33	7.02 ± 0.3	7.30 ± 0.36	7.20 ± 0.22	6.63 ± 0.24	7.25 ± 0.25
D5 KW: H (ddl=4) = 6.87; p = 0.14	11.08 ± 0.52	11.81 ± 0.53	11.74 ± 0.16	10.67 ± 0.41	11.16 ± 0.34
D9 KW: H (ddl=4) = 8.25; p = 0.08	18.74 ± 1.15	19.87 ± 1.07	19.64 ± 0.25	17.94 ± 0.58	19.00 ± 0.55
D12 KW: H (ddl=4) = 6.22; p = 0.18	25.54 ± 1.35	26.68 ± 1.47	26.42 ± 0.37	24.45 ± 0.67	25.05 ± 0.57
D15 KW: H (ddl=4) = 1.90; p = 0.75	32.39 ± 1.71	32.66 ± 1.84	32.20 ± 0.62	30.67 ± 0.73	31.61 ± 0.83
D18 KW: H (ddl=4) = 2.20; p = 0 70	37.96 ± 1.93	38.45 ± 2.02	37.91 ± 0.98	36.56 ± 0.69	37.59 ± 0.89

KW: Kruskal-Wallis test.

4.2.1.2 – Age of incisor cutting

The Kruskal-Wallis test does not show heterogeneity in the ages of incisor cutting in the various litters of the 5 treatment groups (Tab. 6).

Table 6

Age of incisor cutting

Treatment groups	S0 (n = 9)	S1 (n = 7)	S2 (n = 8)	S3 (n = 9)	S123 (n = 9)
KW H (ddl=4) = 1.36; p = 0.85	9.44 ± 0.34	9.29 ± 0.29	9.13 ± 0.30	9.44 ± 0.18	9.11 ± 0.26

KW: Kruskal-Wallis test.

4.2.1.3 - Age of eye opening

The Kruskal-Wallis test does not show heterogeneity in the ages of eye opening in the various litters of the 5 treatment groups (Tab. 7).

Table 7

Age of eye opening

Treatment groups	S0 (n = 9)	S1 (n = 7)	S2 (n = 8)	S3 (n = 9)	S123 (n = 9)
KW H (ddl=4) = 2.25; p = 0.69	14.78 ± 0.32	14.29 ± 0.42	14.88 ± 0.30	15.00 ± 0.29	14.67 ± 0.37

KW: Kruskal-Wallis test.

4.2.2 - Treatment effects on the neuromotor development of youngs

4.2.2.1 - Eversion test (Day 3)

4.2.2.1.1 - Eversion test in young males

The Kruskal-Wallis test does not show heterogeneity in the eversion latency times of male rats of the 5 treatment groups (Tab. 8).

Table 8

Eversion latency time of young males (s)

Treatment groups	S0 (n = 9)	S1 (n = 7)	S2 (n = 8)	S3 (n = 9)	S123 (n = 9)
KW H (ddl=4) = 0.97; p = 0.91	12.67 ± 3.62	12.14 ± 4.21	12.13 ± 4.28	10.33 ± 2.68	14 ± 4.67

KW: Kruskal-Wallis test.

4.2.2.1.2 - Eversion test in young females

The Kruskal-Wallis test does not show heterogeneity in the eversion latency times of young females of the 5 treatment groups (Tab. 9).

Table 9

Eversion latency time of young females (s)

Treatment groups	S0 (n = 9)	S1 (n = 7)	S2 (n = 8)	S3 (n = 8)	S123 (n = 9)
KW H (ddl=4) = 1.49; p = 0.83	8.89 ± 1.37	11.29 ± 2.67	10.75 ± 2.90	15.00 ± 6.2	14.11 ± 3.55

KW: Kruskal-Wallis test.

4.2.2.2 - Grip test (Day 4)

4.2.2.2.1 - Grip test in young males

The Kruskal-Wallis test does not show heterogeneity in the angles of fall of the male rats of the 5 treatment groups in the grip test (Tab. 10).

Table 10

Angle of fall of young males in the grip test (°)

Treatment groups	S0 (n = 9)	S1 (n = 7)	S2 (n = 8)	S3 (n = 9)	S123 (n = 9)
KW H (ddl=4) = 2.91; p = 0.57	97.56 ± 3.82	93.57 ± 7.81	92.75 ± 4.90	102.67 ± 4.28	96.67 ± 5.62

KW: Kruskal-Wallis test.

4.2.2.2.2 - Grip test in young females

The Kruskal-Wallis test does not show heterogeneity in the angles of fall of the young females of the 5 treatment groups in the grip test (Tab. 11).

Table 11

Angle of fall of young females in the grip test (°)

Treatment groups	S0 (n = 9)	S1 (n = 7)	S2 (n = 8)	S3 (n = 8)	S123 (n = 9)
KW H (ddl=4) = 1.57; p = 0.81	97.44 ± 5.07	102.57 ± 3.85	99.50 ± 5.24	103.75 ± 7.28	95.78 ± 5.43

KW: Kruskal-Wallis test.

4.2.2.3 - Righting reflex test (Day 9)

4.2.2.3.1 - Righting reflex test in young males

The Kruskal-Wallis test does not show heterogeneity in the righting reflex times of the male rats of the 5 treatment groups in the righting reflex test (Tab.12).

Table 12

Righting reflex time of young males (s)

Treatment groups	S0 (n = 9)	S1 (n = 7)	S2 (n = 8)	S3 (n = 9)	S123 (n = 9)
KW H (ddl=4) = 1.71; p = 0.91	15.78 ± 1.75	18.29 ± 5.66	16.13 ± 3.48	14.00 ± 2.19	13.78 ± 2.40

KW: Kruskal-Wallis test.

4.2.2.3.2 - Righting reflex test in young females

The Kruskal-Wallis test does not show heterogeneity in the righting reflex times of the young females of the 5 treatment groups in the righting reflex test (Tab. 13).

Table 13

Righting reflex time of young females (s)

Treatment groups	S0 (n = 9)	S1 (n = 7)	S2 (n = 8)	S3 (n = 8)	S123 (n = 9)
KW H (ddl=4) = 5.25; p = 0.26	17.00 ± 2.73	16.86 ± 2.41	14.00 ± 2.30	15.00 ± 2.93	18.22 ± 1.87

KW. Kruskal-Wallis test.

4.2.2.4 - Wire-hanging test (Day 12)

4.2.2.4.1 - Wire-hanging test in young males

The Kruskal-Wallis test does not show heterogeneity in the hanging times of the male rats of the 5 treatment groups in the wire-hanging test (Tab. 14).

Table 14

Hanging time of young males (s)

Treatment groups	S0 (n = 9)	S1 (n = 7)	S2 (n = 8)	S3 (n = 9)	S123 (n = 9)
KW H (ddl=4) = 2.91; p = 0.57	31.11 ± 12.23	29.29 ± 11.94	28.38 ± 10.82	27.22 ± 10.79	30.44 ± 11.00

KW: Kruskal-Wallis test.

4.2.2.4.2 - Wire-hanging test in young females

The Kruskal-Wallis test does not show heterogeneity in fall latencies of the young females of the 5 treatment groups in the wire-hanging test (Tab. 15).

Table 15

Hanging time of young females (s)

Treatment groups	S0 (n = 9)	S1 (n = 7)	S2 (n = 8)	S3 (n = 8)	S123 (n = 9)
KW H (ddl=4) = 1.16; p = 0.88	29.22 ± 5.82	32.86 ± 5.66	30.63 ± 2.63	28.75 ± 4.24	33.89 ± 4.67

KW: Kruskal-Wallis test.

4.2.2.5 - Locomotor coordination test (day 19)

4.2.2.5.1 - Locomotor coordination test in young males

The Kruskal-Wallis test does not show heterogeneity in the latency times to grab the rod, the times to push itself up onto the platform and the number of attempts to successfully complete the locomotor coordination test in the male rats of the 5 treatment groups (Tab. 16).

Table 16

Locomotor coordination test in young males

Treatment groups	S0 (n = 9)	S1 (n = 7)	S2 (n = 8)	S3 (n = 9)	S123 (n = 9)
Latency time to grab the rod (s) KW: H (ddl=4) = 1.71; p = 0.79	15.56 ± 3.96	24.14 ± 6.53	20.00 ± 6.06	20.56 ± 6.30	19.22 ± 4.99
Time for pushing up onto the platform (s) KW: H (ddl=4) = 0.86; p = 0.93	36.11 ± 3.66	46.86 ± 7.13	38.25 ± 3.49	39.22 ± 4.45	40.78 ± 3.39
Number of attempts to successfully complete the test KW: H (ddl=4) = 1.48; p = 0.83	1.17 ± 0.11	1.07 ± 0.05	1.17 ± 0.08	1.09 ± 0.05	1.06 ± 0.04

KW: Kruskal-Wallis test.

4.2.2.5.2 - Locomotor coordination test in young females

The Kruskal-Wallis test does not show heterogeneity in the latency times to grab the rod, the times to push itself up onto the platform and the number of attempts to successfully complete the locomotor coordination test in the young females of the 5 treatment groups (Tab. 17).

Table 17

Locomotor coordination test in young females

Treatment groups	S0 (n = 9)	S1 (n = 7)	S2 (n = 8)	S3 (n = 8)	S123 (n = 9)
Latency time to grab the rod (s) KW: H (ddl=4) = 4.01; p = 0.40	42.89 ± 8.28	49.00 ± .20	38.25 ± 4.82	38.25 ± 3.49	48.56 ± 5.32
Time for pushing up onto the platform (s) KW: H (ddl=4) = 0.65; p = 0.96	21.22 ± 5.95	14.43 ± 1.70	21.25 ± 6.10	17.00 ± 5.17	18.67 ± 3.08
Number of attempts to successfully complete the test KW: H (ddl=4) = 6.33; p = 0.18	1.28 ± 0.16	1.00 ± 0	1.30 ± 0.12	1.36 ± 0.16	1.15 ± 0.09

KW: Kruskal-Wallis test.

4.2.3 - Treatment effects on the behavioural and cognitive development of youngs

4.2.3.1 - Locomotor, exploratory activity and emotional state in open-field (Day 45)

4.2.3.1.1 - Locomotor, exploratory activity and emotional state of young males

The Kruskal-Wallis test does not show heterogeneity in the numbers of crossed compartments, the numbers of uprightings, the numbers of passages through the central compartment, the numbers of droppings and micturitions in the male rats of the 5 treatment groups in open-field (Tab. 18).

Table 18

Locomotor, exploratory activity and emotional state of young males in open-field

Treatment groups	S0 (n = 8)	S1 (n = 8)	S2 (n = 8)	S3 (n = 8)	S123 (n = 8)
Number of crossed compartments KW: H (ddl=4) = 2.38; p = 0.66	52.13 ± 9.05	57.00 ± 4.74	61.25 ± 5.30	49.13 ± 5.34	56.63 ± 7.88
Number of uprightings KW: H (ddl=4) = 5.30; p = 0.26	14.38 ± 2.63	21.5 ± 1.55	18.63 ± 2.17	18.5 ± 2.89	19.25 ± 3.47
Number of passages through the central compartment KW: H (ddl=4) = 2.15; p = 0.71	2.63 ± 0.80	2.88 ± 0.92	3.63 ± 0.91	3.75 ± 0.70	4.25 ± 1.18
Number of droppings KW: H (ddl=4) = 4.45; p = 0.35	2.75 ± 1.33	4.5 ± 1.02	3 ± 0.71	2.63 ± 1.07	4.38 ± 0.73
Number of micturitions KW: H (ddl=4) = 3.64; p = 0.46	0.25 ± 0.16	0.50 ± 0.19	0.50 ± 0.19	0.13 ± 0.13	0.38 ± 0.18

KW: Kruskal-Wallis test.

4.2.3.1.2 - Locomotor, exploratory activity and emotional state of young females

The Kruskal-Wallis test does not show heterogeneity in the numbers of uprightings, the numbers of droppings and micturitions in females of the five treatment groups in open-field.

The Kruskal-Wallis test shows any heterogeneity in the numbers of crossed compartments and the numbers of passages through the central compartment of the females of the five treatment groups in open-field. The Mann-Whitney test shows that the rats of groups S1, S3 and S123 cross significantly more compartments than the rats of the group S0. The rats of groups S1 and S123 cross the central compartment significantly more than those of the group S0 (Tab. 19).

Table 19

Locomotor, exploratory activity and emotional state of young females in open-field

Treatment groups	S0 (n = 8)	S1 (n = 8)	S2 (n = 8)	S3 (n = 8)	S123 (n = 8)
Number of crossed compartments KW: H (ddl=4) = 14.42; p = 0.006	74.75 ± 4.53	94 00 ± 2.91 U = 6.5 p = 0.007	83.13 ± 4.47 U = 20 p = 0.21	108.75 ± 8.76 U = 8 p = 0.01	88.88 ± 3.49 U = 11.5 p = 0.03
Number of uprightings KW: H (ddl=4) = 2.02; p = 0.73	25.88 ± 1.60	30.75 ± 2.50	27.75 ± 1.88	29.88 ± 4.01	28.75 ± 1.71
Number of passages through the central compartment KW: H (ddl=4) = 8.83; p = 0.066	4.13 ± 0.52	5 75 ± 0.53 U = 12.5 p = 0.03	4.38 ± 0.84 U = 31 p = 0.91	5.63 ± 0.94 U = 21 p = 0.21	6.75 ± 0.59 U = 7.5 p = 0.009
Number of droppings KW: H (ddl=4) = 4.73; p = 0.32	0.88 ± 0.44	1.25 ±0 41	1.50 ± 0.93	2.00 ± 0.89	0.50 ± 0.50
Number of micturitions KW: H (ddl=4) = 5.20; p = 0.27	0.00 ± 0.00	0.25 ± 0.16	0.50 ± 0.19	0.25 ± 0.16	0.25 ± 0.16

KW: Kruskal-Wallis test
U: Mann-Whitney test (vs. S0).

4.2.3.2 - Morris water maze: spatial memory (Days 50 and 51)

4.2.3.2.1 - Morris water maze: spatial memory of young males (Tab. 20)

On days 50 and 51, the Kruskal-Wallis test does not show heterogeneity in the average latency times to take refuge on the platform of the male rats of the five treatment groups in the Morris water maze test.

The Friedman test shows any heterogeneity in the latency times to take refuge on the platform in trials 3, 4 and 5 of the young males of the five treatment groups. The Wilcoxon test shows that on D50 the males of all the treatment groups have significantly improved their performances between trial 3 and trial 5.

In addition, the Wilcoxon test shows that on D51 the males of all the treatment groups have significantly improved their average latency times to take refuge on the platform compared to D50 (average of trials 1 and 2 vs. average of trials 3, 4 and 5).

Table 20

Latency time to take refuge on the platform of young males (s)

Treatment groups	S0 (n = 17)	S1 (n = 13)	S2 (n = 16)	S3 (n = 18)	S123 (n = 18)
Average of trials 3-4-5 (D50) KW: H(ddl=4) = 2.72; p = 0.61	32.65 ± 5.06	29.69 ± 5.44	27.02 ± 4.40	35.13 ± 5.78	35.20 ± 4.03
Average of trials 1-2 (D51) KW: H(ddl=4) = 2.82; p = 0.59	19.18 ± 2.52	16.08 ± 2.40	16.63 ± 1.86	15.14 ± 1.75	20.81 ± 2.91
Friedman test [Chi2(ddl=2)] (Tests 3-4-5)	Chi2 = 12.12 p = 0.002	Chi2 = 6.54 p = 0.04	Chi2 = 9.13 p = 0.01	Chi2 = 15.20 p = 0.001	Chi2 = 8.33 p = 0.02
Wilcoxon test (T3 vs. T5)	Z = 3.10 p = 0.002	Z = 2.83 p = 0.005	Z = 2.54 p = 0.01	Z = 3.07 p = 0.002	Z = 2.85 p = 0.004
Wilcoxon test (Average of trials 3-4-5 vs. average of trials 1-2)	Z = 2.07 p = 0.04	Z = 2.20 p = 0.03	Z = 2.77 p = 0.006	Z = 2.83 p = 0.005	Z = 3.03 p = 0.003

KW: Kruskal-Wallis test.

4.2.3.2.1 - Morris water maze: spatial memory of young females (Tab. 21)

On days 50 and 51, The Kruskal-Wallis test does not show heterogeneity in the average latency times to take refuge on the platform of the young females of the five treatment groups in the Morris water maze test. The Friedman test shows any heterogeneity in the latency times to take refuge on the platform of trials 3, 4 and 5 of the young females of the five treatment groups. The Wilcoxon test shows that on D50 the females of all the treatment groups have significantly improved their performances between trial 3 and trial 5. In addition, the Wilcoxon test shows that on D51 the females of all the groups have significantly improved their average latency times to take refuge on the platform compared to D50 (average of trials 1 and 2 vs. average of trials 3, 4 and 5).

Table 21

Latency time to take refuge on the platform of young females (s)

Treatment groups	S0 (n = 15)	S1 (n = 15)	S2 (n = 16)	S3 (n = 17) #	S123 (n = 12) #
Average of trials 3-4-5 (D50) KW: H(ddl=4) = 6.94; p = 0.14	28.71 ± 3.67	38.91 ± 5.80	40.56 ± 5.39	24.49 ± 2.49	32.75 ± 4.28
Average of trials 1-2 (D51) KW: H(ddl=4) = 1.42; p = 0.84	17.93 ± 3.24	18.70 ± 3.44	20.56 ± 3.74	15.50 ± 1.57	18.42 ± 1.77
Friedman test [Chi2(ddl=2)] (Trials 3-4-5)	Chi2 = 6.53 p = 0.04	Chi2 = 6.81 p = 0.03	Chi2 = 14.63 p = 0.001	Chi2 = 6.71 p = 0.04	Chi2 = 6.50 p = 0.04
Wilcoxon test (T3 vs. T5)	Z = 2.67 p = 0.008	Z = 2.78 p = 0.005	Z = 3.18 p = 0.002	Z = 2.39 p = 0.02	Z = 2.20 p = 0.03
Wilcoxon test (Average of trials 3-4-5 vs. average of trials 1-2)	Z = 2.61 p = 0.009	Z = 2.78 p = 0.005	Z = 2.78 p = 0.005	Z = 2.69 p = 0.007	Z = 2.75 p = 0.006

KW: Kruskal-Wallis test.

#: two females (S3 and S123) were withdrawn due to immobility in the device throughout the D50 trials.

4.2.3.3 – Aversive light stimulus avoidance test (TESLA): discriminating training (Days 60 and 61)

4.2.3.3.1 - Overall number of lever pressings from young males

On days 60 and 61, the Kruskal-Wallis test does not show heterogeneity in the total pressings on both levers from the young males of the five treatment groups in the TESLA (Tab. 22 and 23).

Table 22

Overall number of pressings on the active (AL) and inactive (IL) levers from young males (D60)

Treatment groups	S0 (n = 17)	S1 (n = 12) #	S2 (n = 16)	S3 (n = 18)	S123 (n = 18)
Overall AL + IL pressings KW: H (ddl=4) = 3.77; p = 0.44	25.29 ± 6.57	34.92 ± 5.72	27.81 ± 5.40	32.78 ± 6.11	26.61 ± 5.71

KW: Kruskal-Wallis test.
#: a rat of the group S1 was withdrawn: it spent its time to nibble the levers (false pressings)

Table 23

Overall number of pressings on the active (AL) and inactive (IL) levers from young males (D61)

Treatment groups	S0 (n = 17)	S1 (n = 12)	S2 (n = 15) #	S3 (n = 18)	S123 (n = 18)
Overall pressings AL + IL KW: H (ddl=4) = 0.39; p = 0.98	29.35 ± 6.49	40.69 ± 9.86	30.69 ± 7.86	28.67 ± 6.03	30.22 ± 6.90

KW: Kruskal-Wallis test.
#: a rat of the group S2 was withdrawn. it spent its time to nibble the levers (false pressings).

4.2.3.3.2 - Discrimination between both levers by young males (24 and 25)

On day 60, the Wilcoxon test shows that the young males of the group S1 press significantly more on the active lever than on the inactive one. The rats of the other groups do not discriminate significantly between both levers.

On day 61, the Wilcoxon test shows that the young males of groups S1, S2, S3 and S123 press significantly more on the active lever than on the inactive one. The rats of the group S0 tend to press more on the active lever than on the inactive one.

Table 24

Discrimination between both levers by young males during test 1

Treatment groups	S0 (n = 16) #	S1 (n = 12)	S2 (n = 15) #	S3 (n = 18)	S123 (n = 16) #
Test 1 (D60)					
Active lever pressings	14.44 ± 4.02	20.75 ± 3.78	17.00 ± 3.71	17.94 ± 3.32	16.19 ± 3.57
Inactive lever pressings	12.25 ± 3.21	14.17 ± 2.07	12.60 ± 2.26	14.83 ± 3.04	13.63 ± 3.00
Wilcoxon test AL vs. IL	Z = 0.57 p = 0.68	Z = 2.28 p = 0.03	Z = 1.31 p = 0.19	Z = 1.49 p = 0.14	Z = 0.77 p = 0.44

#: the rats which did not press on the active lever and/or the inactive one during the test 1 were withdrawn from the statistical analyses.

Table 25

Discrimination between both levers by young males during test 2

Treatment groups	S0 (n = 16) #	S1 (n = 12)	S2 (n = 14)#¤	S3 (n = 18)	S123 (n = 16) #
Test 2 (D61)					
Active lever pressings	18.25 ± 3.98	20.58 ± 5.86	17.14 ± 4.62	17.78 ± 3.94	17.69 ± 4.39
Inactive lever pressings	12.50 ± 2.74	12.92 ± 2.84	13.79 ± 3.27	10.39 ± 2.10	11.50 ± 2.75
Wilcoxon test AL vs. IL	Z = 1.79 p = 0.07	Z = 2.13 p = 0.03	Z = 2.10 p = 0.04	Z = 2.51 p = 0.01	Z = 2.50 p = 0.01

#: the rats which did not press on the active lever and/or the inactive one during test 1 were withdrawn from the statistical analyses.
¤: a rat of the group S2 was withdrawn: it spent its time to nibble the levers (false pressings).

4.2.3.3.3 - Overall number of lever pressings of young females

On days 60 and 61, the Kruskal-Wallis test does not show heterogeneity in the total pressings on both levers of the young females of the five treatment groups in the TESLA (26).

Table 26

Overall number of pressings on both levers of young females

Treatment groups	S0 (n = 15)	S1 (n = 15)	S2 (n = 16)	S3 (n = 18)	S123 (n = 13)
AL and IL pressings (D60) KW: H (ddl=4) = 3.19; p = 0.53	16.00 ± 4.35	17.60 ± 3.88	28.63 ± 6.04	17.22 ± 4.03	20.31 ± 4.88
AL and IL pressings (D61) KW: H (ddl=4) = 6.20; p = 0.18	12.13 ± 5.83	15.40 ± 4.25	32.00 ± 7.85	16.39 ± 3.41	19.23 ± 5.67

KW: Kruskal-Wallis test.

4.2.3.3.4 - Discrimination between both levers by young females (27 and 28)

On day 60, the Wilcoxon test shows that the young females of the group S1 press significantly more on the active lever than on the inactive one. The rats of the other groups do not discriminate significantly between both levers.

On day 61, the Wilcoxon test shows that the young females of groups S1, S2, S3 and S123 press significantly more on the active lever than on the inactive one. The rats of the group S0 tend to press more on the active lever than on the inactive one.

Table 27

Discrimination between both levers by young females during test 1

Treatment groups	S0 (n = 13) #	S1 (n = 14) #	S2 (n = 15) #	S3 (n = 16) #	S123 (n = 12) #
Test 1 (D 60)					
Active lever pressings	10.39 ± 3.10	11.50 ± 2.88	16.40 ± 3.05	10.69 ± 2.38	13.25 ± 3 16
Inactive lever pressings	7.23 ± 1.80	7.29 ± 1.49	14.07 ± 3.25	8.44 ± 2.03	8.67 ± 1.98
Wilcoxon test AL vs. IL	Z = 2.25 p = 0.02	Z = 1.89 p = 0.06	Z = 1.77 p = 0.08	Z = 1.97 p = 0.05	Z = 2.36 p = 0.02

#: the rats which did not press on the active lever and/or the inactive one during test 1 were withdrawn from the statistical analyses.

Table 28

Discrimination between both levers by young females during test 2

Treatment groups	S0 (n = 13) #	S1 (n = 14) #	S2 (n = 15) #	S3 (n = 16) #	S123 (n = 12) #
Test 2 (D 61)					
Active lever pressings	8.46 ± 4.04	10.14 ± 2.83	18.93 ± 4.85	10.50 ± 2.09	12.50 ± 3.59
Inactive lever pressings	5.15 ± 2.72	5.71 ± 1.83	13.93 ± 3.70	7.56 ± 1.71	8.08 ± 2.52
Wilcoxon test AL vs. IL	Z = 2.70 p = 0.007	Z = 2.67 p = 0.008	Z = 2.24 p = 0.03	Z = 2.13 p = 0.03	Z = 2.57 p = 0.01

#. the rats which did not press on the active lever and/or the inactive lever during test 1 were withdrawn from the statistical analyses.

5 - CONCLUSION

Treatment effects on the treated females

The pregnancy times of the females of the various treatment groups are statistically equivalent.
The number of youngs and the sex-ratio at birth of the litters born from females treated with ING 911 hydrolysate are statistically comparable to those of the litters of control females. The maternal and care-to-young behaviour of treated females are also comparable to those of control females treated with powdered milk.

Treatment effects on the physical development of youngs

The youngs born from treated females show a weight evolution, an age of incisor cutting and an age of eye opening comparable to those born from control females.

Treatment effects on the neuromotor development of youngs

Between birth and weaning, on the basis of various measurement test of the neuromotor development, the youngs born from treated females show performances statistically equivalent to those of youngs born from control females.

Treatment effects on the behavioural and cognitive development of youngs

In the open-field test, the locomotor and exploratory activity and the emotional state of the youngs born from treated females are statistically equivalent to those of the youngs born from control females.
In the Morris water maze as in the aversive light stimulus avoidance test, the youngs born from treated females show short and long term memory and training performances, statistically equivalent, even better, compared to those of the youngs born from control females.

On the basis of the study experimental results, the daily treatment of females with the oral dose of 150 mg/kg of ING 911 hydrolysate during the first, second, third week of pregnancy, or throughout pregnancy, does not modify their pregnancy time, their maternal and care-to-young behaviour, and the quality of their litters compared to the placebo.
Similarly, the treatment of females with ING 911 hydrolysate does not induce physical, neuromotor, behavioural and cognitive development disorders in young males and females compared to youngs born from control females.

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7 - INDIVIDUAL RESULTS

7.1 - Maternal behaviour

Litter No.	Treatment	Pregnancy time (d)	Quality of nest construction	Quantity of material used for the nest (g)
3	S0	21	4	10
6	S1	21	3	10
7	S2	21	4	10
9	S3	22	4	10
10	S3	21	0	0
11	S123	22	0	0
13	S0	22	4	10
14	S0	21	1	0.2
16	S1	22	3	10
17	S1	23	4	10
18	S1	21	4	10
21	S2	22	4	10
23	S3	22	4	10
24	S3	21	4	10
25	S3	22	2	10
26	S3	22	4	10
28	S123	21	4	10
29	S123	22	0	0
32	S0	22	4	10
33	S1	21	4	10
35	S2	22	4	10
38	S123	23	0	0
39	S123	22	4	10
40	S0	22	4	10
42	S2	22	0	0
43	S123	22	4	10
44	S123	22	0	0
45	S2	22	4	10
46	S2	22	4	10
47	S2	22	4	10
48	S0	21	0	0
49	S123	22	0	0
50	S0	22	4	10
51	S3	22	3	6.7
52	S3	22	3	10
53	S0	22	4	10
54	S1	22	4	10
55	S3	22	1	2
56	S0	22	4	10
58	S1	22	1	2
59	S2	21	4	10
60	S123	22	4	10

7.2 – Litter size, sex-ratio, age of incisor cutting and age of eye opening

Litter No.	Treatment	Litter size	Number of males	Number of females	Age of incisor cutting (d)	Age of eye opening (d)
3	S0	10	7	3	10	15
6	S1	4	1	3	10	14
7	S2	8	3	5	10	15
9	S3	12	9	3	9	15
10	S3	7	4	3	10	15
11	S123	10	5	5	9	15
13	S0	7	4	3	9	15
14	S0	7	4	3	9	16
16	S1	7	4	3	9	13
17	S1	5	2	3	8	13
18	S1	8	2	6	9	16
21	S2	9	5	4	9	14
23	S3	12	10	2	9	15
24	S3	14	11	3	9	16
25	S3	9	3	6	9	13
26	S3	4	4	0	9	15
28	S123	11	5	6	9	15
29	S123	8	2	6	9	13
32	S0	6	4	2	8	14
33	S1	10	8	2	9	15
35	S2	10	4	6	8	14
38	S123	10	5	5	8	13
39	S123	5	3	2	8	14
40	S0	3	2	1	8	13
42	S2	13	8	5	8	14
43	S123	4	1	3	9	15
44	S123	11	6	5	10	16
45	S2	9	3	6	9	15
46	S2	13	6	7	9	16
47	S2	12	7	5	10	16
48	S0	11	6	5	11	15
49	S123	10	6	4	10	16
50	S0	11	5	6	10	16
51	S3	11	8	3	10	16
52	S3	14	5	9	10	15
53	S0	14	6	8	10	15
54	S1	12	5	7	10	15
55	S3	6	2	4	10	14
56	S0	12	8	4	10	14
58	S1	9	6	3	10	14
59	S2	13	7	6	10	15
60	S123	14	5	9	10	15

7.3 - Average weights of young males of every litter (g)

Litter No.	Treatment	Day 2	Day 5	Day 9	Day 12	Day 15	Day 18
3	S0	7.3	11.9	18.9	25.7	31.9	37.1
6	S1	6.9	11.2	18.4	23.5	28.2	31.8
7	S2	6.0	10.0	17.5	25.0	31.1	37.5
9	S3	6.7	10.6	18.2	25.3	30.3	37.0
10	S3	6.5	11.1	19.4	26.3	33.4	40.0
11	S123	7.9	12.3	20.1	27.2	33.1	37.7
13	S0	8.3	12.8	20.3	26.0	33.2	40.4
14	S0	7.0	10.7	17.3	23.2	28.9	35.6
16	S1	8.5	13.6	20.4	27.1	33.3	39.8
17	S1	9.1	14.0	25.8	33.1	39.8	44.9
18	S1	6.4	10.1	17.2	23.2	28.9	34.7
21	S2	8.1	12.6	18.6	25.2	29.9	34.1
23	S3	5.4	9.3	16.7	23.6	30.1	37.5
24	S3	5.9	8.6	17.0	24.1	30.7	37.4
25	S3	7.4	12.0	19.5	25.7	31.1	35.8
26	S3	7.9	12.3	19.3	25.1	30.7	36.0
28	S123	6.8	11.3	19.0	25.4	31.5	37.8
29	S123	8.2	13.0	19.9	27.0	33.7	40.4
32	S0	7.9	11.3	18.5	25.1	31.9	38.7
33	S1	6.9	10.0	16.5	22.9	27.1	31.9
35	S2	7.6	12.0	20.6	27.9	33.7	40.4
38	S123	8.0	12.0	21.3	26.1	33.4	38.5
39	S123	7.8	11.7	22.0	26.7	35.2	43.6
40	S0	8.9	14.4	26.9	35.7	45.6	52.5
42	S2	7.4	11.9	19.9	25.8	30.9	35.2
43	S123	8.9	13.7	22.1	27.6	34.2	40.8
44	S123	7.1	11.1	17.5	23.3	28.7	33.9
45	S2	8.5	12.7	20.7	27.3	33.7	40.3
46	S2	7.3	12.0	20.5	27.1	34.1	41.1
47	S2	8.0	12.7	21.1	28.5	34.8	42.9
48	S0	6.3	9.7	16.9	24.3	33.0	38.9
49	S123	7.5	11.8	19.8	26.0	32.7	39.6
50	S0	7.0	11.5	19.7	26.3	33.3	38.0
51	S3	7.4	12.0	19.5	26.5	32.9	38.8
52	S3	6.9	10.6	16.0	21.7	28.9	35.8
53	S0	6.9	10.9	18.7	25.9	31.9	39.9
54	S1	7.4	13.0	22.6	30.6	38.6	45.0
55	S3	8.2	12.0	19.1	25.7	31.9	38.4
56	S0	7.3	10.9	19.1	25.4	32.2	38.4
58	S1	7.1	11.0	19.4	27.3	33.3	40.4
59	S2	6.4	11.2	19.6	27.0	32.6	38.0
60	S123	6.7	9.7	17.5	24.6	30.7	37.1

7.4 – Average weights of young females of every litter (g)

Litter No.	Treatment	Day 2	Day 5	Day 9	Day 12	Day 15	Day 18
3	S0	6.7	11.1	17.9	24.9	34.0	36.9
6	S1	6.9	11.7	18.4	23.7	28.6	31.4
7	S2	6.7	11.5	19.0	26.6	32.9	39.1
9	S3	6.1	9.4	15.5	22.0	26.9	33.3
10	S3	6.4	11.2	19.4	26.7	33.5	40.0
11	S123	6.5	10.3	17.0	22.7	28.6	34.6
13	S0	7.7	11.4	16.8	23.5	28.4	33.0
14	S0	6.5	10.2	16.8	22.7	27.9	31.3
16	S1	7.8	12.6	19.2	25.8	32.0	38.2
17	S1	9.2	14.3	25.1	33.5	40.0	45.4
18	S1	6.2	10.8	18.4	24.1	29.8	36.8
21	S2	7.8	12.0	18.9	25.2	29.4	33.5
23	S3	7.1	11.5	18.8	25.0	30.4	35.2
24	S3	5.6	8.6	16.8	23.8	30.4	36.9
25	S3	7.2	11.9	20.0	26.5	32.5	37.5
26	S3	*the litter does not comprise females*					
28	S123	6.2	10.3	17.8	23.9	29.4	35.5
29	S123	8.4	13.0	20.2	27.4	34.8	39.1
32	S0	7.6	11.0	18.6	24.8	31.5	38.2
33	S1	6.7	10.2	16.8	22.6	26.6	32.6
35	S2	7.2	12.0	20.8	27.9	34.1	39.8
38	S123	7.7	12.1	21.4	26.2	32.3	38.9
39	S123	7.4	11.1	21.0	25.3	33.7	41.1
40	S0	8.9	14.9	27.6	36.1	45.2	51.9
42	S2	7.3	11.9	19.0	24.9	29.8	34.1
43	S123	8.2	11.9	19.6	27.1	33.8	40.3
44	S123	6.9	10.7	17.5	22.8	28.1	33.3
45	S2	7.7	11.9	19.8	26.1	32.1	38.1
46	S2	6.9	11.6	19.7	26.6	33.5	40.9
47	S2	7.8	12.2	20.6	27.5	33.5	40.5
48	S0	6.1	9.5	16.4	23.1	31.1	36.5
49	S123	7.0	11.4	19.1	25.3	33.0	38.5
50	S0	6.7	10.8	18.6	25.1	31.6	37.4
51	S3	6.6	10.9	18.1	24.3	30.8	36.7
52	S3	6 3	10.3	16.0	21.6	29.0	35.6
53	S0	6.2	10.1	17.6	24.8	30.1	37.5
54	S1	7.2	12.3	22.2	30.1	37.7	44.0
55	S3	7.7	11.6	18.8	25.6	31.8	37.3
56	S0	6.7	10.5	18.4	24.9	31.9	38.9
58	S1	7.1	10.7	19.1	26.8	33.9	40.8
59	S2	6.1	10.8	19.4	26.5	32.4	37.4
60	S123	6.9	9.7	17.6	24.8	30.8	36.9

7.5 - Average performances of young males from every litter in the neuromotor development tests

Litter No.	Treatment	Eversion test	Grip test	Righting reflex test	Wire-hanging test	Locomotor coordination test		
		Eversion latency time (s)	Angle of fall (°)	Righting reflex time (s)	Hanging duration (s)	Latency time to grab the rod (s)	Time for pushing up onto the platform (s)	Number of attempts to successfully complete the test
3	S0	11	101	22	34	17	6	1.00
6	S1	10	80	2	12	36	8	1.00
7	S2	29	105	12	11	33	53	1.67
9	S3	6	100	10	17	27	12	1.33
10	S3	8	101	19	22	27	66	1.25
11	S123	4	84	10	17	35	10	1.00
13	S0	7	114	18	30	36	15	1.00
14	S0	8	112	12	17	40	44	1.25
16	S1	2	75	10	43	47	42	1.25
17	S1	5	100	45	38	82	13	1.00
18	S1	10	132	15	21	37	10	1.00
21	S2	5	111	19	20	39	12	1.00
23	S3	3	105	10	14	64	13	1.00
24	S3	5	134	10	17	25	26	1.00
25	S3	28	103	16	26	54	31	1.00
26	S3	15	102	13	29	34	10	1.00
28	S123	5	124	14	20	37	15	1.00
29	S123	2	115	16	18	52	20	1.00
32	S0	8	95	14	19	57	16	1.00
33	S1	13	72	31	20	43	47	1.00
35	S2	4	100	39	31	47	14	1.00
38	S123	3	102	31	39	22	7	1.00
39	S123	16	83	13	28	44	48	1.00
40	S0	4	80	23	30	40	8	1.00
42	S2	6	93	13	35	46	8	1.00
43	S123	39	85	5	44	50	7	1.00
44	S123	18	72	10	28	43	13	1.25
45	S2	7	80	12	23	20	7	1.00
46	S2	8	82	8	43	36	13	1.25
47	S2	4	71	16	24	34	41	1.25
48	S0	40	85	16	24	32	10	2.00
49	S123	5	101	14	46	32	12	1.25
50	S0	13	98	18	39	38	23	1.25
51	S3	8	92	10	41	37	7	1.20
52	S3	16	89	9	43	47	8	1.00
53	S0	16	103	6	29	39	9	1.00
54	S1	36	100	18	30	24	38	1.25
55	S3	4	98	29	36	38	12	1.00
56	S0	7	90	13	58	26	9	1.00
58	S1	9	96	7	41	59	11	1.00
59	S2	34	100	10	40	51	12	1.20
60	S123	34	104	11	34	52	41	1.00

7.6 - Average performances of young females from every litter in the tests of neuromotor development

Litter No.	Treatment	Eversion test	Grip test	Righting reflex test	Wire-hanging test	Locomotor coordination test		
		Eversion latency time (s)	Angle of fall (°)	Righting reflex time (s)	Hanging duration (s)	Latency time to grab the rod (s)	Time for pushing up onto the platform (s)	Number of attempt to successfully complete the test
3	S0	12	102	12	28	15	10	1.00
6	S1	9	100	11	15	23	9	1.00
7	S2	10	93	16	25	33	14	1.00
9	S3	3	127	11	32	46	15	1.00
10	S3	8	83	35	28	53	52	2.33
11	S123	3	92	14	27	36	33	1.00
13	S0	6	127	24	12	32	33	1.00
14	S0	3	107	17	12	49	53	1.00
16	S1	3	113	12	52	48	16	1.00
17	S1	6	102	16	41	86	22	1.00
18	S1	12	118	19	13	38	15	1.00
21	S2	4	100	11	22	47	44	1.50
23	S3	3	93	11	20	43	16	1.00
24	S3	3	117	9	11	24	17	1.33
25	S3	3	138	15	18	39	11	1.60
26	S3	no female in the litter						
28	S123	10	133	22	23	32	31	1.17
29	S123	4	98	20	38	67	22	1.00
32	S0	14	80	10	20	93	12	1.00
33	S1	25	90	25	26	52	16	1.00
35	S2	9	124	14	32	64	18	1.00
38	S123	27	96	31	32	50	9	1.00
39	S123	4	95	15	20	24	8	1.00
40	S0	7	80	14	19	18	9	1.00
42	S2	4	116	29	27	19	8	1.00
43	S123	13	73	16	34	73	15	1.67
44	S123	13	82	18	29	47	24	1.50
45	S2	30	85	9	42	29	9	1.00
46	S2	10	80	9	24	33	12	1.76
47	S2	10	103	12	40	45	53	1.50
48	S0	12	82	30	18	41	8	2.25
49	S123	33	98	15	68	54	13	1.00
50	S0	14	98	27	55	66	10	1.25
51	S3	53	88	12	34	25	10	1.33
52	S3	11	90	13	40	39	7	1.00
53	S0	6	100	13	45	25	46	2.00
54	S1	14	103	10	40	47	9	1.00
55	S3	36	94	14	47	37	8	1.25
56	S0	6	101	6	54	47	10	1.00
58	S1	10	92	25	43	49	14	1.00
59	S2	9	95	12	33	36	12	1.67
60	S123	20	95	13	34	54	13	1.00

7.7 - Performances of young males in the open-field test

Rat No.	Treatment	Number of crossed compartments	Number of uprightings	Number of passages through the central compartment	Number of droppings	Number of micturitions
1	S0	23	4	0	0	0
2	S0	25	3	2	1	1
3	S0	50	11	3	1	1
4	S0	58	21	4	0	0
5	S1	34	23	0	4	1
6	S1	55	16	5	3	1
7	S1	45	17	1	2	0
8	S1	52	22	3	4	1
9	S2	59	15	3	3	1
10	S2	49	23	6	4	1
11	S2	65	25	7	0	0
12	S2	68	17	2	5	1
13	S3	62	25	5	0	1
14	S3	66	35	4	7	0
15	S3	48	14	6	0	0
16	S3	48	15	5	0	0
17	S123	78	24	10	5	1
18	S123	60	21	4	4	0
19	S123	54	4	1	1	1
20	S123	11	4	0	4	0
21	S1	60	18	1	2	0
22	S1	65	22	8	3	1
23	S1	74	29	2	9	0
24	S1	71	25	3	9	0
25	S123	58	22	6	4	0
26	S123	42	23	2	8	1
27	S123	71	30	4	6	0
28	S123	79	26	7	3	0
29	S2	78	25	6	6	0
30	S2	76	21	1	2	0
31	S2	32	7	0	1	0
32	S2	63	16	4	3	1
33	S0	25	20	0	3	0
34	S0	77	21	3	7	0
35	S0	87	18	7	10	0
36	S0	72	17	2	0	0
37	S3	24	10	2	0	0
38	S3	66	15	3	5	0
39	S3	35	13	0	3	0
40	S3	44	21	5	6	0

7.8 - Performances of young females in the open-field test

Rat No.	Treatment	Number of crossed compartments	Number of uprightings	Number of passages through the central compartment	Number of droppings	Number of micturitions
1	S0	85	32	5	0	0
2	S0	72	25	3	2	0
3	S0	55	28	3	2	0
4	S0	78	22	4	0	0
5	S1	91	35	5	0	0
6	S1	99	36	5	1	0
7	S1	90	28	5	1	0
8	S1	88	22	5	3	1
9	S2	85	24	3	0	0
10	S2	63	32	3	4	1
11	S2	82	27	8	7	0
12	S2	100	21	3	1	1
13	S3	109	18	7	4	1
14	S3	93	24	5	7	0
15	S3	123	40	3	3	0
16	S3	125	45	10	1	0
17	S123	103	36	9	0	1
18	S123	92	24	8	4	0
19	S123	89	26	8	0	0
20	S123	79	31	5	0	0
21	S1	91	26	9	1	0
22	S1	97	24	5	3	1
23	S1	85	43	5	0	0
24	S1	111	32	7	1	0
25	S123	73	21	7	0	0
26	S123	93	32	4	0	1
27	S123	98	29	7	0	0
28	S123	84	31	6	0	0
29	S2	75	27	7	0	1
30	S2	94	37	4	0	1
31	S2	72	23	1	0	0
32	S2	94	31	6	0	0
33	S0	98	29	3	0	0
34	S0	74	21	3	0	0
35	S0	69	30	5	0	0
36	S0	67	20	7	3	0
37	S3	107	43	6	1	0
38	S3	57	16	2	0	1
39	S3	137	25	8	0	0
40	S3	119	28	4	0	0

7.9 - Performances of young males in the Morris water maze

Rat No.	Treatment	Day 50			Day 51	
		Trial 3 (s)	Trial 4 (s)	Trial 5 (s)	Trial 1 (s)	Trial 2 (s)
1	S0	25	10	4	20	6
2	S0	7	19	8	16	17
3	S0	20	12	9	13	15
4	S0	49	54	8	6	8
5	S1	11	30	10	14	13
6	S1	43	11	37	6	6
7	S1	59	11	18	22	17
8	S1	19	22	18	43	26
9	S2	8	9	7	8	9
10	S2	7	24	9	15	4
11	S2	8	9	45	13	13
12	S2	41	36	16	40	8
13	S2	34	16	10	12	6
14	S2	88	32	5	6	31
15	S2	23	13	16	17	15
16	S2	81	13	10	62	8
17	S3	55	22	7	20	23
18	S3	87	8	5	13	11
19	S3	12	60	24	9	6
20	S3	50	20	29	6	20
21	S3	21	7	8	8	7
22	S3	9	7	6	7	18
23	S3	41	56	13	35	12
24	S3	37	16	4	35	22
25	S0	130	39	24	74	13
26	S0	17	11	9	8	17
27	S0	94	10	6	9	9
28	S0	18	7	13	71	10
29	S1	10	12	12	7	7
30	S1	29	30	18	17	15
31	S1	84	27	19	11	14
32	S1	180	23	30	29	21
33	S123	19	16	61	19	17
34	S123	35	8	21	5	17
35	S123	13	16	17	11	37
36	S123	22	11	24	17	6
37	S123	25	5	6	13	9
38	S123	8	9	21	10	9
39	S123	128	12	13	20	8
40	S123	41	14	23	39	9

(Continuation)

Rat No.	Treatment	Day 50			Day 51	
		Trial 3 (s)	Trial 4 (s)	Trial 5 (s)	Trial 1 (s)	Trial 2 (s)
41	S0	11	33	20	12	9
42	S1	9	9	6	20	14
43	S123	21	31	13	23	11
44	S123	61	77	18	17	11
45	S3	132	5	6	7	4
46	S3	41	29	32	25	23
47	S3	100	5	5	5	21
48	S3	24	17	46	8	6
49	S1	100	13	10	5	7
50	S1	12	7	12	9	13
51	S1	23	23	17	19	8
52	S1	65	34	55	23	32
53	S2	25	12	8	14	30
54	S2	106	20	6	26	27
55	S2	180	33	23	26	11
56	S2	8	13	26	8	8
57	S3	7	14	6	24	7
58	S3	180	69	36	11	25
59	S3	180	52	11	9	17
60	S123	58	33	25	11	10
61	S123	26	25	16	44	22
62	S123	158	14	10	24	27
63	S123	43	37	19	10	6
64	S0	180	32	56	10	20
65	S0	70	24	8	9	24
66	S0	21	8	38	9	44
67	S0	69	14	27	24	12
68	S2	29	9	8	9	13
69	S2	76	7	8	22	13
70	S2	14	12	13	10	19
71	S2	76	20	5	23	6
72	S3	26	22	16	13	6
73	S3	180	11	15	16	7
74	S3	12	7	7	40	19
75	S123	145	9	6	56	23
76	S123	150	11	10	6	24
77	S123	65	59	30	55	20
78	S123	140	9	14	64	39
79	S0	92	11	9	14	42
80	S0	86	16	9	7	19
81	S0	47	12	15	10	40
82	S0	122	26	6	23	12

7.10 - Performances of young females in the Morris water maze

Rat No.	Treatment	Day 50			Day 51	
		Trial 3 (s)	Trial 4 (s)	Trial 5 (s)	Trial 1 (s)	Trial 2 (s)
1	S0	81	9	6	8	9
2	S0	12	11	9	47	16
3	S0	14	30	20	8	18
4	S0	101	54	27	38	41
5	S1	6	38	8	8	7
6	S1	12	9	4	6	6
7	S1	180	14	6	56	8
8	S1	57	13	17	18	25
9	S1	153	12	7	10	14
10	S1	124	8	8	33	8
11	S1	31	11	9	12	12
12	S1	31	49	46	7	10
13	S1	157	14	16	7	20
14	S1	166	8	41	43	32
15	S1	13	5	18	4	4
16	S2	41	52	8	9	14
17	S2	19	35	12	13	10
18	S2	107	20	56	11	12
19	S2	6	27	4	10	13
20	S2	62	9	6	14	25
21	S2	102	16	5	10	5
22	S2	92	35	24	9	5
23	S2	33	13	56	49	19
24	S3	23	8	5	12	10
25	S3	16	9	8	25	5
26	S3	44	54	29	7	6
27	S3	24	27	5	9	11
28	S3	21	7	27	15	14
29	S3	27	12	29	9	12
30	S3	83	28	6	34	7
31	S3	28	25	4	7	8
32	S3	11	12	8	45	6
33	S3	26	22	6	15	21
34	S3	51	43	20	30	10
35	S3	Female completely motionless in the device			-	-
36	S123	Female completely motionless in the device			-	-
37	S123	28	5	18	13	6
38	S123	31	26	5	10	11
39	S123	72	12	66	35	22
40	S123	20	31	41	9	15

(Continuation)

Rat No.	Treatment	Day 50			Day 51	
		Trial 3	Trial 4	Trial 5	Trial 1	Trial 2
41	S0	61	22	8	15	5
42	S0	44	29	36	5	4
43	S0	25	12	26	9	11
44	S1	9	38	11	8	9
45	S1	69	22	6	85	9
46	S1	70	10	11	64	8
47	S1	165	28	21	16	12
48	S2	43	14	6	7	5
49	S2	27	28	20	19	28
50	S2	180	17	9	68	8
51	S2	180	32	23	57	22
52	S3	12	31	26	9	10
53	S3	17	22	45	40	10
54	S3	32	11	25	23	8
55	S123	30	53	13	11	37
56	S123	96	18	19	8	33
57	S123	20	10	13	9	36
58	S123	36	22	14	14	12
59	S2	61	16	7	22	6
60	S2	130	24	15	11	12
61	S2	7	34	9	58	60
62	S2	180	36	9	6	41
63	S3	29	37	22	10	17
64	S3	66	43	12	43	13
65	S3	33	17	21	21	5
66	S123	145	30	17	25	14
67	S123	73	19	38	31	18
68	S123	47	10	21	8	33
69	S123	19	22	39	17	15
70	S0	26	5	8	32	7
71	S0	13	39	21	5	7
72	S0	43	62	45	80	12
73	S0	49	54	28	30	13
74	S0	50	13	12	24	12
75	S0	25	11	10	10	6
76	S0	67	12	14	39	7
77	S0	21	14	13	10	10

7.11 - Performances of young males in the TESLA

Rat No.	Treatment	Day 60: Test 1		Day 61: Test 2	
		Active lever pressings	Inactive lever pressings	Active lever pressings	Inactive lever pressings
1	S0	7	9	6	6
2	S0	48	39	40	22
3	S0	14	8	34	15
4	S0	27	42	21	26
5	S1	15	6	19	13
6	S1	39	24	30	27
7	S1	36	18	54	17
8	S1	14	15	18	19
9	S2	9	3	6	2
10	S2	44	39	35	32
11	S2	28	12	20	14
12	S2	47	12	51	37
13	S2	13	14	2	12
14	S2	3	8	2	1
15	S2	19	9	22	18
16	S2	9	11	3	5
17	S3	6	7	0	0
18	S3	2	0	0	3
19	S3	21	8	25	10
20	S3	1	7	2	3
21	S3	13	3	1	0
22	S3	28	28	12	9
23	S3	31	12	21	12
24	S3	43	38	33	26
25	S0	11	8	16	2
26	S0	12	19	20	22
27	S0	33	10	49	25
28	S0	2	0	1	1
29	S1	21	17	14	11
30	S1	Withdrawn rat: nibbles the levers		Withdrawn rat: nibbles the levers	
31	S1	25	14	64	33
32	S1	5	7	0	2
33	S123	6	6	1	0
34	S123	7	16	10	8
35	S123	13	8	23	14
36	S123	6	9	3	5
37	S123	47	49	39	35
38	S123	7	6	3	6
39	S123	19	5	8	6
40	S123	8	3	0	1
41	S0	10	10	9	25
42	S1	39	24	25	12

(Continuation)

Rat No.	Treatment	Day 60: Test 1		Day 61: Test 2	
		Active lever pressings	Inactive lever pressings	Active lever pressings	Inactive lever pressings
43	S123	30	10	41	17
44	S123	11	31	38	26
45	S3	43	48	23	22
46	S3	17	12	30	20
47	S3	5	1	14	0
48	S3	25	22	41	20
49	S1	31	22	12	11
50	S1	11	6	3	5
51	S1	2	4	2	1
52	S1	11	13	6	4
53	S2	28	17	21	17
54	S2	11	9	Withdrawn rat: nibbles the levers	
55	S2	2	3	1	1
56	S2	1	0	0	0
57	S3	31	18	57	17
58	S3	18	21	23	6
59	S3	28	18	32	23
60	S123	21	19	11	4
61	S123	7	4	28	26
62	S123	0	1	0	1
63	S123	1	0	4	4
64	S0	4	1	25	10
65	S0	1	8	10	7
66	S0	2	1	1	0
67	S0	3	7	3	1
68	S2	5	11	8	6
69	S2	13	21	18	17
70	S2	22	14	49	30
71	S2	2	6	2	1
72	S3	8	9	2	11
73	S3	1	11	1	1
74	S3	2	4	3	4
75	S123	5	5	1	1
76	S123	50	20	36	29
77	S123	13	13	19	11
78	S123	9	14	43	31
79	S0	49	26	50	20
80	S0	1	3	13	3
81	S0	6	1	1	0
82	S0	3	5	1	5

7.12 - Performances of young females in t

```
ERROR: syntaxerror
OFFENDING COMMAND: h

OPERAND STACK:
```