Operant Behavior of Pre and Post Pubertal Rats Irradiated with I^{131} in the Last Trimester of Gestation

It has been shown in rats made hypothyroid neonatally by surgical thyroidectomy (EAYRS and LISHMAN[1]), or with radioactive iodide and/or thiouracil (EAYRS[2]), suffer behavioral deficits, inversely related to time of thyroid destruction, from 0 to 25 days of age. Such behavioral deficits parallel reduced dendritic branching resulting from hypothyroidism (EAYRS[2,3], HORN[4] and BALL[5]).

Alternatively to the extensive neonatal handling of these behavioral studies, thyroid destruction with I^{131} was done in utero at the beginning of the third trimester of gestation, based on time of fetal colloid formation (SPEERT et al.[6]) and transplacental passage of maternal I^{131} to the fetus (SCHULTZ et al.[7]). Behavioral approaches were restricted to operant conditioning and performance in a Skinnerian, fixed interval, positively water reinforced program, where external stimuli were minimized, the reward being only to reestablish homeostasis after water deprivation, at the critical 25 day age and post pubertally. Through this it is hoped that a simple methodology might be developed to determine whether behavioral deficit from hypothyroidism can be assessed with minimal environmental manipulation.

Materials and methods. Sperm-positive Holtzmann rats were injected i.p. with NaI^{131} 3000 μC/kg on the 14th day of gestation. They were housed in individual cages in a 14 h on, 10 h off, light controlled, closed room and kept there through gestation and their pups' behavioral studies.

At 25 days of age, pairs of experimental and control pups were matched for sex and weight. All were water deprived for 24 h and conditioned to a positively water reinforced, fixed interval (1 sec) schedule. Acquisition of audiovisual association (AVA) to the shaping and self-sustaining lever pressing (SSLP) were recorded in terms of min from the beginning of the training procedure they learned. A maximum of one 40 min training period per day for 4 successive days per animal was allowed. No water was given during this period, except from the reward dipper. Food was given ad libitum, except in the test cage. SSLP rates were also recorded.

One each of a matched pair of experimental and control pups was given a tracer dose of NaI^{125}200 μC/kg i.p. and sacrificed on the 4th day after the beginning of conditioning (1 Wk) 3 to 24 h after injection of tracer dose, alternating experimentals and controls, and tracer iodine levels in whole thyroid gland and serum counted. Thyroids were saved for H+E staining and sera for iodine-labeled protein : inorganic ratios through their separation by a Sephadex-25® starch column. The other of the pair was saved in the light-controlled room until the beginning of the 3rd week (3 Wk) after the beginning of testing, handled as for their first day of conditioning or allowed SSLP for 40 min if they retained their task. Pups and mothers were sacrificed and materials collected as for the 1 Wk animals.

Results and discussion. No significant differences were seen between 31 experimental and 34 control animals in either stage of conditioning or operant lever pressing rates (LPR). On retesting 3 Wk after the initial conditioning, reinforced LPR (RLPR) were significantly lower in 13 experimentals than in 11 controls ($P = 0.05$ by student's t-test; Table I.) Correlation tests (Pearson Product Moment Correlation; –1 to 1) comparing 1 Wk and 3 Wk LPR in 40 min and min to acquire AVA and SSLP with RLPR for 1 Wk and 3 Wk tests revealed different populations of experimental and control animals contributed to RLPR, depending on their performances in the two stages of learning AVA and SSLP, as seen in Table II. 1 Wk and 3 Wk RLPR had no correlation (–0.05), whereas control pups had a 0.62 correlation. Knowing slower learners took more minutes to learn, experimentals who were slow learners contributed much more (0.76) to high lever pressing rates in the 1 Wk than the 3 Wk tests (0.07). Half of this trend was seen in controls (1 Wk 0.51; 3 Wk 0.26). Although experimentals showed no shifts in AVA-RLPR and 3 Wk correlations (0.41 to 0.39), corresponding control data showed a shift from 0.49 to 0.39, showing controls slow in AVA made a greater contribution to RLPR than did experimentals. EAYRS'[1,2] observation of increased, poorly directed, motor activity in early maze learning in his hypothyroid animals is confirmed here, as witnessed by the marked fall in perseverance at the lever task for the experimental animals slow in learning. In addition to this, we find that controls, easily distractable in orienting, improve in their SSLP performances.

Table I. Operant conditioning and lever pressing rates of rat siblings of mothers treated with radioactive iodide in the last trimester of pregnancy

Stages of learning of those who learned		Reinforced lever pressing rates	
Audio-visual association	Self-sustained lever pressing	1 Wk.	3 Wk.
Siblings of I^{131}	Treated mothers	(N = 27)	
34.1 ± 6.1	90.5 ± 9.9	179.7 ± 14.5	
		(N = 13)	
26.2 ± 6.4	83.0 ± 13.7	174.4 ± 19.8	166.1 ± 20.7[a]
Control siblings	(N = 28)		
30.0 ± 4.3	106.5 ± 9.0		
	(N = 11)		
30.0 ± 6.9	95.5 ± 12.2	200.4 ± 22.6	268.3 ± 39.1[a]

[a] Significant (student's t-test; $P = 0.05$).

Table II. Correlation between stages of learning and lever pressing rates[a]

	Reinforced lever pressing rates (40 min)		
	1 Wk		1 Wk
Siblings of I^{131} treated mothers	(N = 13)		
Min to acquire:			
Audio-visual association	0.41		0.39
Self-sustained lever pressing	0.76		0.07
Lever pressing (1 Wk and 3 Wk)		–0.05	
Control siblings (N = 11)			
Min to acquire:			
Audio-visual association	0.49		0.76
Self-sustained lever pressing	0.51		0.26
Lever pressing (1 Wk and 3 Wk)		0.62	

[a] Pearson product moment correlation test.

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Sera collected from a 11 pups and mothers, taken alternately from experimentals and controls, 3–24 h after tracer, showed a progressive decrease with time of whole serum I^{125} with time ($P = 0.005$). Control pup data was not significantly greater than experimental. Protein: inorganic serum I^{125} ratios were lower in experimental (0.54) than in control (0.083) mothers. Pups' sample volumes were insufficient for testing.

In a continuous scale rating, where all animals were assigned thyroid histology ratings of 4 if normal, 1 in extreme destruction and 7 in highest functional potential, no significant differences were seen, except in marked improvement in 'functional potential' from 1 Wk to 3 Wk histology for controls ($P = 0.05$) in Table III. Pooled data for 1 Wk and 3 Wk mothers show significantly less activity in experimental than control mothers ($P = 0.01$), although all fell with time in rating.

Table III. A) Continuous scale rating of thyroids of siblings and their mothers treated with radioactive iodide in the third trimester of gestation

	First week	Third week	First and third week
Siblings of I^{131} treated mothers	3.17 ± 0.37 ($N = 12$)	3.54 ± 0.64 ($N = 13$)	3.36 ± 0.37 ($N = 25$)
Control siblings	2.62 ± 0.49^a ($N = 16$)	4.56 ± 0.75^a ($N = 9$)	3.32 ± 0.37 ($N = 25$)

[a] Student's t-test $P = 0.01$. On the continuous scale rating, 1 = least functional, with frank destruction of follicles, fibrous and leuckocytic invasion; 4 = normal, and 7 = highest functional activity.

B) Differences in histological ratings between mothers and siblings

	First week	Third week	First and third week
I^{131} treated	7.09 ± 0.71^a ($N = 11$)	7.77 ± 0.73 ($N = 13$)	7.46 ± 0.50^a ($N = 24$)
Controls	$4.12 \pm 0.44^{a,b}$ ($N = 16$)	7.44 ± 0.63^b ($N = 9$)	5.32 ± 0.48^b ($N = 25$)

[a] Student's t-test $P = 0.01$. [b] student's t-test, $P = 0.01$. On the continous rating scale rating 1 = least functional compared with mother; 6 = same as mother, and 12 = most active in relation to mother.

Differences between siblings and their mothers' histological ratings were on a 12 unit scale, where 6 equalled mother, 12 highest and 1 lowest from mother (Table III b). Experimentals rated higher than both mothers and controls ($P = 0.01$). 1 Wk to 3 Wk improvement in controls was significant at $P = 0.001$.

Protein bound tracer, without testing T-3 and -4 I^{125} binding cannot reflect amount released to tissues, nor can direct cellular effects of irradiation of brain from our procedure be assessed, but implied by results of NAIR and BAU[8] and SPEERT et al.[6,9].

Conclusion. Irradiation of the rat on the 14th day of gestation induces varying degrees of thyroid destruction in mother and siblings and decreased ratios of iodine labeled protein; inorganic iodine in mothers, compensatory hyperactivity in thyroids of siblings, and increased perseverance of operant behavior, which decreases with maturation. Changes observed in siblings are not fully accountable by either differences in labeled iodide thyroid uptake, serum protein binding or thyroid histology[10].

Résumé. Les petits de rates irradiées au 14e jour de leur gestation, avec 3000 µC/kg de ^{131}I i.p. ont montré une plus grande réduction de leur capacité à presser sur un levier lorsqu'ls l'ont appris avant la puberté. Cet effet fut d'autant plus manifeste chez les animaux qui avaient été conditionnés lentement et qui ont généralement tendance à améliorer leur performance avec l'âge.

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[8] V. NAIR and D. BAU, Brain Res. *16*, 383 (1969).

[9] Kendall's distribution-free correlation test.

[10] Supported by NIMH Grant No. NS-07889.

Hypoprolinemia in Parkinsonism; a Case Report

In recent years various disturbances of amino acid metabolism, including proline, have been reported. SCHÄFER et al.[1] distinguish 2 types of enzyme defects in proline metabolism, both producing hyperprolinemia. Observations on hypoprolinemia have not come to our attention. We therefore wish to report a case of Parkinsonism showing this biochemical abnormality.

This 65-year-old male patient (K.M.) showed a typical Parkinsonian disease which was first observed 1 year before admission. Besides a hypertonus no other pathology could be detected, either clinically or by routine laboratory investigation. The history disclosed that the father of the patient suffered from Parkinson's disease. The patient himself had had no major illness until manifestation of Parkinsonism.

Amino acid analyses were performed in plasma on 4 different occasions within a period of 4 months using the automatic ion exchange method of SPACKMAN et al.[2]. Plasma was deproteinized with picric acid according to STEIN and MOORE[3].

The results (Table I) show the plasma levels of proline in pat. K.M. as about 10% of normal controls. The other amino acids determined were all within normal limits (Table II).

In contrast to hyperprolinemia[4], the hypoprolinemia observed was not accompagnied by a specific pathology. Till the development of hypertonia and Parkinsonism at an advanced age, the patient was a healthy person. Obviously, there is a hereditary factor in the patient's parkinsonism. So far, however, no evidence exists that hypoprolinemia plays a role in Parkinson's disease. As

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