INFLUENCE OF MUTATIONS WHICH DETERMINE ACCUMULATIONS IN THE BODY OF 3-HYDROXY KYNURENINE, ON THE BEHAVIOUR AND FUNCTIONS OF THE NERVOUS SYSTEM IN HONEY BEES

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The beginning of the study concerning the role of the kynurenine metabolites of tryptophan on the behaviour and functions of the nervous system in insects was performed into the laboratories of the Institute of Piysiology "I. P. PAVLOV" of the USSR Academy of Sciences in Leningrad. The study is connected to the great role, already known, of these compounds on the genesis of neuro-psychological disorders in humans.

Several authors have shown the importance of kynurenines on the essential activity of insects (LINZEN, 1974) and particularly in bees, in their physiological change for wintering period (ZHEREBKIN, 1979).

In our report the results of the study on the particularities of behaviour (individually obtained or inborn) and neurological ones reflecting the functional activity of the nervous system, of the brick (bk) and chartreuse red (ch^r) mutations in honey bee, that determine the accumulations in the organism of 3-hydroxykynurenine (3-HOK), are presented.

With bk mutations the activity of the enzyme phenoxykynonsintase is low and as a result, the transformation of 3-HOK into ommochromes is blocked, a fact that leads to the accumulation of 3-HOK (10μg/ml) and xanturenic acid into the haemolymph — both products of tryptophan metabolism, but that normally, cannot be found in wild bees (or they are present only as traces). With respect to the tryptophan and kynurenine contents, the bk mutants can not be distinguished from wild bees. The eyes of these mutants are red in colour, because, due to the incomplete blocking of the ommochromes synthesis, their eyes contain a small amount of screening pigments (only 10% as compared to the normal level in wild bees) (DUSTMANN, 1975).

The last step into the ommochrome formation chain — transformation of 3-HOK into ommochromes — is also interrupted by the allelic mutations of the locus (chartreuse) (DUSTMANN, 1975). Apart from bk mutants, with ch mutants, the accumulation of 3-HOK occurs only into the pigmentary cells of the eye (33 mg/head). The 3-HOK content in the haemolymph, as well as the tryptophan and kynurenine contents are not different from the normal levels.

The colour of the eyes in mutant individuals is determined by 3-HOK, that is not found free in the eye, but associated with protein. Side by side with granulated 3-HOK, in the ch/ch eyes may appear also normal ommochromic granules, that favour a large variability of the eye colour. In the homozygote ch^r individuals the eyes get a brown-reddish colour.

In honeybees of 5 genotypes: +/+, bk/+, bk/bk, ch^r/+, ch^r/ch^r, we studied the velocity of formation and transformation of the conditioned reflexes of food acquirement, depending on the colour, smell and tactile excitants. We recorded simultaneously the rhythm of dance (the number of signal movements in 15 seconds), the percent of dancing individuals, the number of stimulating feedings necessary for the appearance of the first signal movements, the rhythm of visits to the source of food (50% sugar syrup feeders). In immobilized individuals we studied the elaboration of the food, conditioned reflexes determined by the food smell.

In honeybees of the same genotypes several neurological characteristics were studied: the excitability of the muscle-epithelial sack, in 3 days old larvae and imagos, the time of narcose falling using ether (that indirectly characterizes the functional state of the synaptic transfer to the locomotory organs) and the natural bioelectric activity (AFB) of the locomotion segmentary center (the second thoracic segment), the frequency — impulses/sec.

These methods were presented in detail (LOPATINA et al., 1976). Due to the fact that the 3-HOK content is changing into the imago ontogenesis, in bk mutants, we studied in relation to the age, the dynamics of the conditioned reflexes activity (CRA). It has been proved that in immobilized mutants, 3,7 and 10 days old, the velocity of food conditioned reflexes formation (smell dependent) is higher than in 20 days old individuals (Table 1).

Table 1

THE STUDY OF THE BK MUTANTS DURING ONTOGENESIS

Age of honey bees	Number of experiments	‰ of conditioned reflexes					
		fragments of the conditioned reflexes stereotype					
		(1+s)×5	(g+s)×5	(1+s)×8	(g+a)×10	(1+s)×10	(g+a)×5
20	5	3,60	5.60	8.25	6.00	7.60	0
10	4	33.0*	310.00	33.13	23.00	24.50	0
7	5	40.86*	39.94*	34.92*	25.06*	23.64**	8.66**
3	6	44.77**	42.00*	33.18	20.88**	18.05	0

NOTE : Between the brackets
1+s — sugar syrup mixed with lavender flavour
g+s — sugar syrup mixed with carnation scent
a — water ; outside the brackets the number of repetitions is given
* $p<0.05$, ** $p<0.01$

On the contrary, in wild bees, as in many other animal species (including mammals) the educational capacity increases with age. The comparative study of the CRA in bk mutants and wild Carniolan bees in different stages of ontogenesis proved also the existence of some significant differences, with respect to the educational capacity in 10 days old individuals and 15—20 days old ones. At the age of 10 days, the bk mutants have a 1.5—2 times higher velocity of conditioned reflexes formation than in wild honeybees, but they are inferiour to the latter, with respect to the velocity of transformation — of the temporary connections under changing the signal value of the excitants. On the contrary, in 15—20 days old bk mutants, comparing to the normal individuals, the process of food searching conditioned reflexes formation is decreasing (1.7—2.5 times) under the action of colour (27.5 and 11.6 combinations, respectively, $p<0.01$ and of smell (17.4 and 10.8 combinations, $p<0.05$) while studying the velocity of changing the attitude towards excitants, under changing their signal value, the bk mutants show no difference from wild honeybees. In this case, the latent periods of the conditioned reflexes changed as follows : they were shorter in bk mutants comparing to normal honeybees, during the first half of ontogenesis (2.0 s, and 2.5 s, $p<0.001$) and longer during the second half of ontogenesis (2.9 sec and 2.2 sec., respectively, $p<0.05$).

The effect of bk mutation on the velocity of elaboration and transformation of the conditioned reflexes against smell, allows us to suppose a dependancy of the latter on the biochemical changings produced by mutations.

The role of the possible visual defect determined by the ommochromes defficiency is probably limited to the diminution of the capacity of spatial orientation and of conditioned reflexes formation to visual excitants.

In ch^r homozygotes comparing to the heterozygote forms of the same mutation, 15 days in age, the velocity of food searching conditioned reflexes formation is diminishing, (i.e. similarly to both visual and tactile excitants).

The inborn forms of behaviour in food searching and signalization and the neurological characteristics were studied in bk and ch^r mutants only during the second half of ontogenesis. The data obtained (KUZMINA et al., 1979, 1981) prove the inhibition of behaviour functions and of the functional activity of the nervous system in mutants comparing to wild honeybees. These agree to the data obtained for the group of older bk mutants studied with respect to the condioned reflexes formation activity.

Fig. 1 shows the decrease of the dancing rhythm in mutants, comparing to normal individuals. The rhythm of visists paid to the source of food and the percent of dancing individuals among mutants was also diminished comparing to wild honeybees. On the contrary, in order to determine the mutant individuals ready to begin the dance after visiting the food source, 2—3 times more food was necessary than in normal insects.

The effects of mutations on the neurological characteristics were the following: bk mutants fall into narcosis with ether, with 1.4 minutes earlier than normal bees ($p<0.005$). In both mutants, comparing to the wild bees, the excitability of the peripheric nervous system proved to be 1.3—1.8 times lower ($p<0.01$).

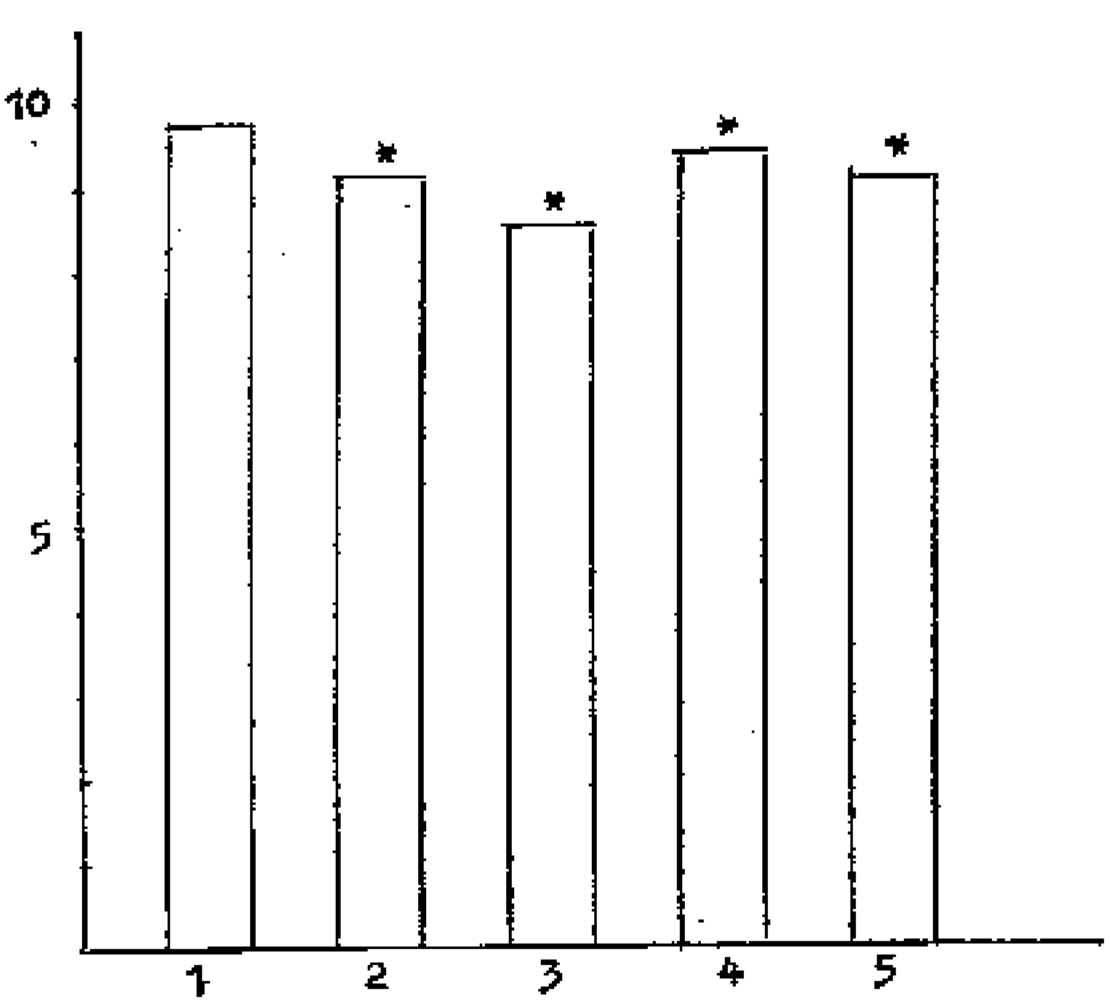

Fig. 1 — The rhythm of dancing in honeybees (the number of cycles/15 sec.) 1) wild honeybees (+/+) ; 2) heterozygotes for brick mutation ; 3) homozygotes for brick mutation ; 4) heterozygotes for chartreuse red mutation : 5) homozygotes for chartreuse red mutation
** The difference from the wild type is significant, $p<0.01$*

In bk homozygote mutants the natural bioelectric activity was lower (fig. 2).

In mutant individuals, the natural bioelectric activity was nearly similar to that of the ivory-umber (i^u) mutants, in which haemolymph kynurenine is accumulated, and that of the wild honeybees after appli-

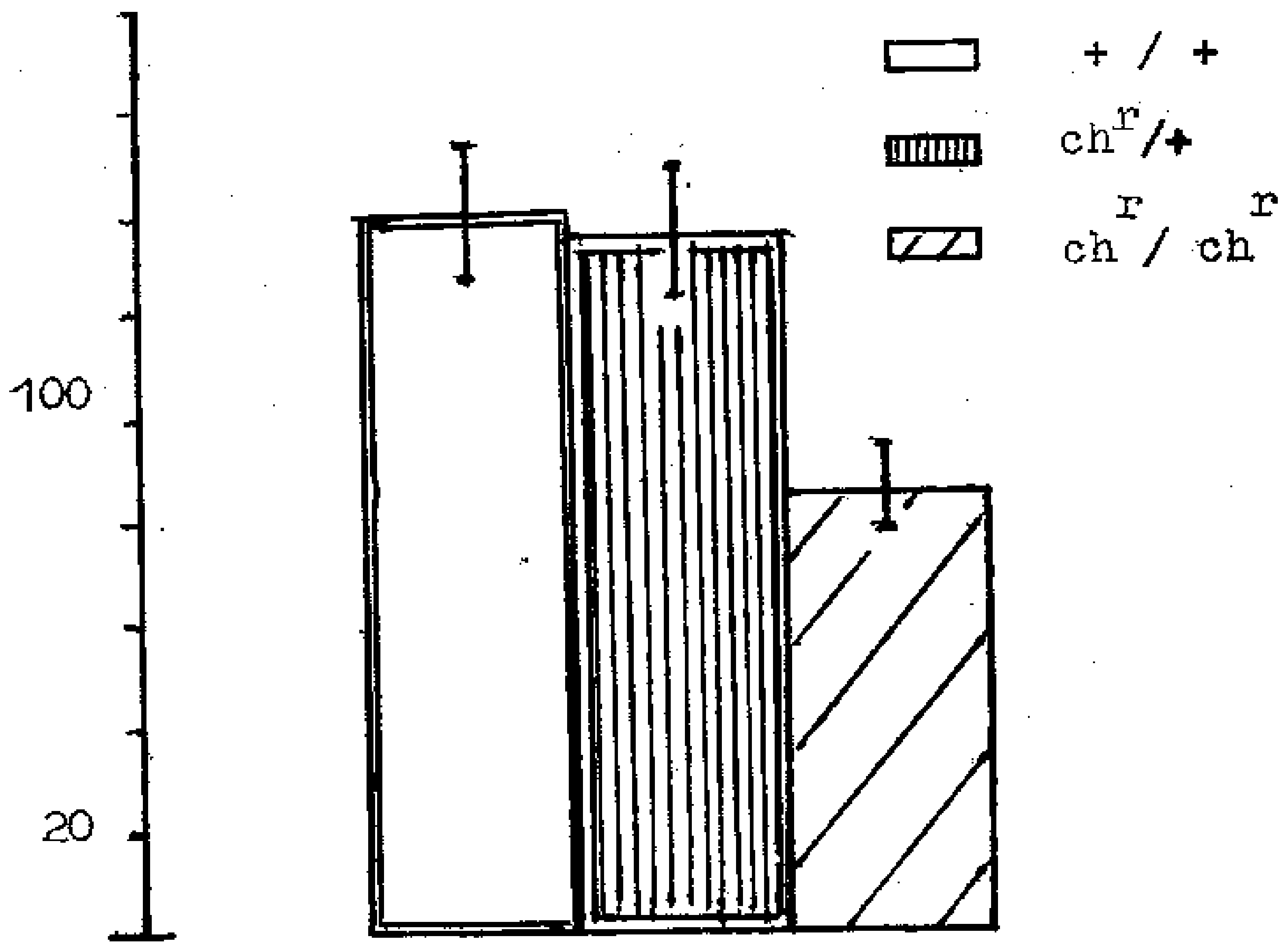

Fig. 2 — The normal bioelectric activity of the second thoracic ganglion of the wild honeybee and of the chr mutants (chr/+chr) on the abscissa — the genotype ; ordinate — the frequency (impulses/sec.)

** The difference from the wild type is significant, $p < 0.01$*

cation of a kynurenine solution on the ganglion (CESNOKOVA et al., 1980). We noted also a tendancy of increasing the frequency of low amplitude action potentials development (40—70 microvolts) that represent the basic part of the total activity in wild honeybees, the tendancy of decreasing the frequency of medium amplitude commisures (70—100 microvolts) and a sudden significant increase of the frequency of high amplitude discharges (more than 100 microvolts).

It must be mentioned that in wild honeybees the number of impulses with amplitudes higher than 100 microvolts is very low (0.2—1.3 impulses/second). In this way the simplified AFB patterns, under the action of 3-HOK and kynurenine proved to be very similar.

The study on the behaviour and functions of the nervous system was carried out on homozygote and heterozygote individuals. From graphs 1 and 2, estimating the values of the studied characteristics the heterozygote individuals had an intermediary position between homozygotes and wild honeybees.

Under these conditions the level of the inhibitory effects of bk and ch^r mutations on the velocity of signal movements execution depended on the "dose of the gene". A bk mutant allela diminished the rhythm of dancing with an average of 1.2 cycles/15 seconds, and the ch^r mutant allela with 0.6 cycles/15 sec.

The biphasic action of the bk mutation is clearly expressed by the dynamics of age, the high 3-HOK content in the first stage of the imago ontogenesis and nearly normal in the second half of ontogenesis. Our data agree with the results of the studies of KAMISHEV (1980) carried out on drosophila — carrying the cardinal mutation (cd) similar to the bk mutation in honeybee with respect to the biochemical processes. In 3 days old cd mutants with a rich 3-HOK content in haemolymph, the locomotory activity is significantly higher than that of all the older mutants (7—9 days), as well as that of the flies in the control line — Canton S.

According to the release of the 3-HOK from the organism the locomotory activity of the mutant flies decreases.

The analysis of the data obtained, allows us to suppose that the 3—HOK has a strong stimulating effect on the nervous system of insects, similar to that known in mammals (LAPIN, 1978). The inhibition of the nervous activity in the late stages of development, seems to be related to the result of 3-HOK accumulation in the haemolymph of insects during early ontogenesis, to the more rapid senescence of the mutants as a results of the metabolism amplification in the presence of such a strong stimulating agent (KAMISHEV, 1980). An evidence in this sense, is also the more decreased vitality of the mentioned mutants comparing to that of the wild honeybees.

It is not out of question the noxious effect of the mutations leading to the accumulation of 3—HOK. There are already known the negative effects of the high content of 3—HOK in the organism of insects as for instance the disorders of the normal development and tumoral induction into the excretory system.

Analysing the effects of mutations that determine the accumulation of 3—HOK in insects haemolymph, we must not put out of question the possible participation of serotonine. This fact is proved by KAMISHEV (1981) who demonstrated the increase of serotonine content into the cd mutant organisms comparing to the control flies and its role into the formation of spontaneous motor activity in drosophila. However, the lack of similar evidences for the mutants of honeybees does not allow a suitable analysis.

Are we able to make an analogy between the mechanism of action in bk and ch^r mutants ? To answer this question special researches are still necessary.

INTERNATIONAL FEDERATION
OF BEEKEEPERS' ASSOCIATIONS
A P I M O N D I A

XXIX[th] INTERNATIONAL CONGRESS OF APICULTURE

Budapest, Hungary
August 25—31, 1983

APIMONDIA Publishing House
Bucharest, Romania