ROLE OF KYNURENINES IN THE REGULATION OF THE HONEYBEE'S MEMORY PROCESS

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Our own investigations of the role of kynurenine and kynurenic acid in the regulation of the process of the honeybee are summed up briefly in this article. Data are also presented on the contribution of the specific structural snow gene, which controls a key enzyme of the kynurenine branch of the metabolism of tryptophan (tryptophan oxygenase), to the hereditary determination of the specific characteristics of the dynamics of the formation of the memory trace in the honeybee after a single training session.

We had previously demonstrated that the metabolites of the kynurenine branch of the metabolism of tryptophan (the kynurenines) on the pathway of its conversion into the screening pigments of the eye of insects, possessing marked neurotropic activity [1, 2], exert an influence on the process of the formation of the memory trace in the honeybee [3]. An attempt to elucidate the mechanism of the influence of the kynurenines on the dynamics of the formation of the memory trace demonstrated a facilitatory influence on the memory of the bee of the excitatory amino acids (L-glutamine and L-asparagine) and the possibility of their interaction with kynurenic acid at the level of a common receptor link [5].

Data on the dynamics of the formation of the memory trace in three groups of bees, carrying the snow mutation in the homozygotic and the heterozygotic states and the control (genetic base of introduced mutations), are presented in this report. The snow mutation inactivates the first enzyme of tryptophan metabolism along the kynurenine pathway, tryptophan oxygenase; this leads to the complete absence (s/s) or deficit of kynurenines (s/+). In addition to this, tryptophan and serotonin accumulate in excess in the hemolymph of the mutants. We had previously [3] followed the dynamics of the formation of the memory trace in mutants only in the first 6 min after the ending of a single training session (the combination of a conditional olfactory stimulus with unconditional alimentary reinforcement with 50% sugar syrup). It was demonstrated that they differed to a significant degree from those of control individuals. The difference resided first and foremost in the higher level of conditioned reflex activity in the mutants as compared with the control individuals. At the same time, the homozygotes surpassed the control bees with respect to the number of conditioned reactions over the course of the entire period of observations (after 1, 3, and 6 min), whereas the heterozygotes only in the 3-6 min time interval. One minute after the end of the training procedure, the heterozygotes lagged behind the control individuals to a significant degree with respect to the number of bees responding with the conditioned reaction to the presentation of the conditional signal. The phase of consolidation, for which a sharp reduction in the number of conditioned reflex reactions is characteristic, was also lacking in both the homozygote and heterozygote mutants. A hypothesis of the transience of the stage of consolidation in the mutants was advanced in this context. In continuation of these investigations, the dynamics of the formation of the memory trace in the snow mutants were followed in greater detail, not only in the first 6 min after the end of the training session, but both in earlier periods (30 sec) and more remote periods — 8, 10, 120, and 240 min; the methods were described in [3]. Forty to sixty individuals account for each point of the curve (Fig. 1). The great similarity of the behavior of the bees in the time interval from 1 to 6 min after the end of the training procedure in the present experiment and the one we had done previously [3] can be concluded from the graph. The hypothesis of the earlier periods of the passage by the mutants through the phase of consolidation was not confirmed. To the contrary, the observation of the behavior of the bees in time intervals closer to the ending of the training session, namely 30 sec, revealed the same tendency: the greatest number of conditioned reactions was observed in the snow mutant homozygotes, the least in the heterozygotes, and the control individuals occupied an intermediate position. It is not clear what the reduction
Fig. 1. Dynamics of conditioned reflex responses as a function of the time interval elapsed since the moment of the combination of the conditional signal and the unconditional: a) In control bees; b) in snow mutant homozygotes; c) heterozygotes. Along the abscissa: time interval, min; along the ordinate: CR (conditioned reflexes), %.

as compared with the norm in the conditioned reflex level in the heterozygote mutants in the first minute of testing (30 sec and 1 min) is associated with. Further observations on the course of the formation of the memory trace demonstrated that the phase of consolidation in the mutants commenced later, not at the third minute as in the control individuals, but at the eighth to tenth. It might be thought that prolongation of the short-term phase of memory would entail a disruption of long-term information storage; this was indeed observed in actuality. The number of mutant individuals responding with the conditioned reaction to the presentation of the conditional stimulus 120 and 240 min after the end of the training procedure was only 50-60% of the norm. We had previously shown that the allelic snow mutation snow$^{luna}$ also prolonged the short-term phase of memory, but to a substantially lesser extent than the snow mutation [4]. The phase of consolidation commenced in the s$^{lt}$ homozygotes 1-2 min later as compared with the control individuals. The dynamics of the formation of the memory trace in the s$^{lt}$ heterozygotes in the first 6 min did not differ from those of the control bees. If the locus snow mutants are ranked in accordance with the degree of deviation of the biochemical profile of their hemolymph from the norm, in particular in accordance with the content of kynurenines, the following series is obtained: s/s > s/+ > s$^{lt}$/s$^{lt}$ > s$^{lt}$/+ > +/+ . It is not difficult to appreciate that the locus snow mutants comprise approximately the same series as they do with respect to the time of the appearance in them of phase of consolidation of memory: s/s = s/+ > s$^{lt}$/s$^{lt}$ > s$^{lt}$/+ = +/+ . These data, as well as those obtained earlier [3], make it possible to imagine that it is precisely the kynurenine, their content in the hemolymph, which determine the characteristics of the behavior of the snow mutants. At the same time, the absence or deficiency of kynurenic acid must be reflected to a greater degree in the storage of new information in short-term memory and its transition to long-term memory; whereas the disturbance of long-term storage in memory of newly-developed behavioral patterns may be associated with an absence or deficiency of kynurenine. The aggregate of the experiments carried out have thus made it possible to expand the notions of the boundaries of the neuroactive effect of the kynurenines, having demonstrated for the first time their regulatory role in the formation of the memory trace in the honeybee. This is important not only from the general knowledge point of view, by making it possible to conjecture similar mechanisms in mammals as well, but also for the understanding of the phenomenon of the consolidation in mammals of the neurotic state induced by prolonged stress, in the process of which, as is well known, the powerful activation of the metabolism of tryptophan along the kynurenine pathway takes place. The experiments also suggest that the dynamics of the formation of the memory trace in the honeybee is under the control of the genotype, in particular the snow gene, a structural gene of an enzyme which is key to the metabolism of tryptophan along the kynurenine pathway.