phine addicts[16,17] could be mediated by the release of noradrenaline accumulated during morphine abuse. The fact that conjunctival naloxone had no effect on the pupil size of healthy volunteers not treated with morphine[16] or treated with a single clinical dose of morphine[6] does not exclude the possibility that the iris opiate receptors take part in the physiological control of the pupillary size.

A miosis, reversible by naloxone eye-drops, has in fact been observed in man after strenuous physical exercise[18]. This fact confirms the opinion that the activity of the natural opioid system, 'silent' in its basic condition, is operant in stressful conditions capable of affecting homeostatic balance.

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Arginine has a morphine-like action in insects

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Summary. Injections of arginine and morphine increased the voltage of electric shocks necessary to induce a defensive reaction in the Praying Mantis. The effect of both was inhibited by naloxone.

Recently it has been shown that morphine has an antinoxious action in 2 arthropods: the mantis shrimp[2] and the honey bee[3]. Morphine increases the threshold for behavioral responses to electric shocks, i.e. the violent flexure of the body of the shrimp and the extrusion of the sting in the bee; and naloxone, if it is injected together with morphine, inhibits this action. It is also known that morphine and endorphines affect memory formation in vertebrates[4], but no report is known for arthropods. In the praying mantis *(Stagmatoptera biocellata)* it has been shown that the levels of arginine in the cerebral ganglia increased in those insects which learned not to attack a moving star or not to display a defensive reaction (the deimatic reaction[5]). This increase in the level of arginine was not due to the training activity in itself but appeared only in those animals which later retained the learned task[6]. Injections of arginine before training induced in this insect consolidation of long term memory in training situations in which otherwise formation of long term memory never have occurred[7]. Therefore, in this work we compare the antinoxious effect of arginine to that of morphine in the praying mantis, in order to prepare for future investigation of the relation between morphine and memory.

Praying mantises display a defensive response, i.e. a deimatic reaction[5] when they are touched, presented to a bird, or submitted to electric shocks on their legs, abdomen, thorax or head. Electric shocks of increasing voltage were given until the mantises displayed a full deimatic reaction[8]. For this purpose, 96 female mantises (12 for each dose tested), were fixed on a mantis holder, and a teflon cannula was implanted chronically into the thoracic cavity in order to make the injections[7]. The injected volume was always 50 µl. Two stainless steel electrodes were implanted chronically into the epicranial sklerite to the extent just necessary to perforate the cuticle. Through them, square bimodal electric pulses (1 msec duration and 100 Hz) of increasing voltage were applied until a full deimatic reaction was observed[8]. 1 min after measuring this first threshold, the insects were injected with the drug to be tested, and the voltage threshold was again measured at 1, 2 and 4 h after the injection of the drug. This new voltage could be either 0, 25, 75 or 100% higher than the voltage threshold measured before the injection. If no response was elicited with a 100% voltage in-

Percentage of stimulus threshold increase necessary to produce a full deimatic reaction after various types of injections

Amount injected (mg/g of insect)		Stimulus threshold (%) after injection Median (range) n = 12 after 1 h	2 h	4 h
Arginine	+Naloxone			
0	0	0 (0– 25)[a]	0 (0-25)[a]	0 (0–25)[a]
3.5	0	25 (25– 75)[b]	25 (0– 50)[b]	0 (0–25)[a]
5.0	0	100 (0–100)[c]	50 (25–100)[c]	25 (0–50)[b]
6.5	0	100 (100–100)[c]	87 (75–100)[d]	50 (0–75)[b]
5.0	0.032	37 (25– 75)[b]	25 (0– 50)[a,b]	0 (0–25)[a]
Morphine	+Naloxone			
0.35	0	100 (0–100)[c]	50 (0–100)[c]	0 (0–75)[b]
0.35	0.032	25 (0–100)[b]	0 (0–100)[a,b]	12 (0–25)[a]
0	0.032	12 (0– 75)[a,b]	25 (0– 50)[a,b]	25 (0–25)[a]
Analysis of variance H		49.80	37.12	25.56
(Kruskal-Wallis) p		< 0.001	< 0.001	< 0.001

[a,b,c] and [d] indicate statistically different medians for each column given by the Mann-Whitney U test, $\alpha = 0.05$.

crease, this was also computed as 100%, as no higher voltages were tested to avoid damaging the insects.

The effect of injections of arginine, morphine, naloxone and mixtures of them on the voltage threshold was studied (table). We confirmed our earlier results[8] showing that the dose of morphine which produced an increase of 50% in the stimulus threshold 2 h after injection (ED_{50}) was 0.35 mg/g of insect. Arginine also inhibited the deimatic reaction in a dose dependent manner, increasing the stimulus threshold. The same concentration of naloxone antagonised the effect of morphine and arginine at their ED_{50}. Naloxone injected alone had no significant effect on the stimulus threshold until concentrations of 64 µg/g or more were injected[8].

These results show that arginine exerts an action in the praying mantis similar to that of morphine. The fact that arginine also affects memory consolidation suggests some similarity to the effect that opiates have in vertebrates[4], indicating that this amino acid could have a neural activity in insects like that of endorphines and other opiates in vertebrates, although the effect of opioids on memory in insects is not yet known. The concentration of arginine that facilitates memory consolidation in the praying mantis[7] is the same as the ED_{50} found here. The actual amount reaching the nervous tissue is unknown, since the substances were injected into the thoracic cavity of the insects. This fact could explain the need for high doses of these drugs in our experiments and in those with shrimps and bees, although other explanations are also possible[2,3,8]. These findings taken together suggest a neuro-modulator role for arginine which was formerly unexpected.

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Hydrogen bond catalysis of mononucleotide ethylation supports non-random DNA alkylations by N-ethyl, N-nitrosourea

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Summary. The axial (ax.) and equatorial (eq.) diastereomeric forms of phosphate triesters resulting from reactions of N-ethyl, N-nitrosourea with 3 cyclic mononucleotides were analyzed by column liquid chromatography (CLC). Evidence is presented that the 2'OH group of 3', 5'cAMP essentially contributes to the stereoselective eq. alkyl substitution, most probably by hydrogen bonding catalysis. The neighboring group direction of ethylation gives substantial support to non-random DNA alkylations by NEU.

Recently it has been shown that when intact cells are exposed to 2 polycyclic hydrocarbons[2,3] and N-acetoxy-acetylaminofluorence (AAAF)[4], these compounds bind preferentially to sequences of chromosomal DNA involved in various functions of genetic control. This is in keeping with the molecular model of tumorigenesis[5] suggesting that mutagenic agents reacting with promotor and/or supressor sequences could turn on transforming genes. Also, a number of authors agree on the fact[6-11] although not on the type[6-10] of non-random DNA alkylations by N-nitroso compounds. In contrast to the genotopic selectivity of bulky substituents[2-4], for which steric accessibility to chromatin components[12,13] was assumed to be an essential factor, no chemical reason has been forwarded so far to explain how certain DNA regions could be hyperreactive to methylating and ethylating N-nitroso compounds. We have investigated stereoselective phosphate alkylation of 3', 5' cAMP, 3', 5' c(2'0-ethyl)AMP, 3', 5' c(2'-deoxy) AMP and adenylyl (3' → 5') adenosine (ApA) by N-ethyl, N-nitrosourea (NEU). In this report we present evidence that the 2'OH group catalyzes the ethylation reaction of NEU. Such neighboring group catalysis may have implications for the mechanisms of non-random DNA alkylation by N-nitroso compounds.

It was shown 30 years ago[14] that phosphate groups of DNA could be esterified by alkylating agents and that the triester so formed alkylated nucleobases in a second step reaction[15]. After Loveless[16] had described O^6-alkylated products from in vitro reactions of deoxyguanosine with NEU and 2 other mutagens, it was gradually recognized in the 1970s, and finally become well established, that over 80% of NEU modification of nucleic acids is on oxygens[17].

When we reacted 0.2 M 3', 5' cAMP (^{14}C uniformly labelled, approximately 80 µCi/mmol^{-1}) with 1 M NEU in 200 µl ethanol containing 20% 1 M triethylammonium hydrogencarbonate buffer pH 7.2 for 5 h under gentle shaking at 20 °C we obtained an approximate yield of 25% of alkylated nucleotide and more than 90% of the NEU was decomposed. This was monitored by thin layer chromatography (stationary phases: cellulose (TLC_A) and silica gel (TLC_B); mobile phases: iso-propanol −1% $(NH_4)_2SO_4$ = 2/1, v/v (TLC_A) and chloroform-methanol = 17/3, v/v (TLC_B)) and high efficiency column liquid chromatography (CLC). 92% of total alkylated 3', 5' cAMP was substituted in the phosphate (with the consequence of a hetero-tetracoordinated phosphorus), 8% in the ribose (2'-OH) and no detectable amount in the purine base moiety. This was in keeping with our previous results from adenine nucleotide reactions with NEU[18].

When we synthesized the neutral P-O-ethyl ester of 3', 5' cAMP according to Preobrazhenskaya et al.[19] by activation with di-phenyl-phosphorochloridate and subsequent alcoholysis with ethanol we found an approximately 1:1 ratio of both