Table 2. Mutagenic effect of the product of spermidine-nitrite interaction

Sample	His+ revertants/plate							
	TA 1535		TA 1537		TA 100		TA 98	
	−S-9	+S-9	−S-9	+S-9	−S-9	+S-9	−S-9	+S-9
None	14	11	10	11	88	88	21	29
100 mM $NaNO_2$	155	171	15	9	130	111	30	39
100 mM spermidine	16	17	-	-	111	87	-	-
100 mM spermidine + 120 mM $NaNO_2$	112	91	19	20	396	108	65	33

A 200-µl aliquot of the sample was preincubated with bacterial cells in 0.1 M phosphate buffer, pH 7.4 (−S-9) or in S-9 mix (+S-9) for 20 min at 37 °C and then deposited in a soft agar overlay on minimal medium according to the procedure of Yahagi et al.[9]. Each value represents the mean of the numbers of revertant colonies on 2 plates after 48 h of incubation at 37 °C. −, Not determined.

prepared from PCB-pretreated rats (S-9), positive results were obtained with strains TA1535, TA100 and TA98 (table 2). In the presence of S-9, positive results were obtained with strain TA1535. Similar results were recently reported by Kokatnur et al.[10]. Using TA 1535 and TA100, N-nitrosospermidine was assayed in a range from 7.5 to 30 µmoles per plate. No significant mutagenicity was observed. The weak direct-acting mutagenicity found in the reaction mixture might be attributed to the reaction product(s) other than N-nitrosospermidine.

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Increase in in vivo (^{3}H) spiperone binding in the rat hippocampal formation and striatum after repeated treatment with haloperidol

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Summary. An increase in in vivo (^{3}H) spiperone binding was observed in rat hippocampal formation and striatum after repeated treatment with haloperidol. This suggests that in hippocampus as well as in striatum prolonged blockade of dopaminergic transmission by a neuroleptic agent results in the development of a supersensitivity of the dopamine receptors.

Recent biochemical[2-5] and electrophysiological[6] studies have provided evidence for a dopaminergic innervation of the mammalian hippocampal formation originating from the substantia nigra and ventral tegmental area[7,8]. Moreover, the recent demonstration of specific (^{3}H) spiperone hippocampal binding sites selectively inhibited by dopamine (DA) agonists and antagonists[3,4] suggests the existence of a discrete population of DA receptors in the rat hippocampal formation. This view was supported by the increase in hippocampal dihydroxyphenylacetic acid (DOPAC) levels induced by acute administration of neuroleptics[2,3,9]. Repeated treatment with neuroleptic agents is known to result in an increase in the number of striatal DA receptors in rat[10] and mouse[11], probably as a compensatory response to the sustained blockade of dopaminergic transmission. It was therefore of interest to study whether a similar phenomenon may also occur in the hippocampal formation. For this purpose, we have investigated the effects of repeated treatment with haloperidol on in vivo (^{3}H) spiperone binding in rat striatum and hippocampal formation.

Methods. Male rats [Tif:RAI f (SPF) weighing 120–130 g at the beginning of the experiment] were injected s.c. daily for 10 or 21 days with haloperidol (1 mg/kg). Control animals were similarly treated with saline. In vivo radioreceptor assay was performed 4–12 days following the last injection of the drug. 12 µCi (^{3}H) spiperone (sp.act. 39 Ci/mmole, NEN) was administered i.v. and the animals were killed 2 h later. Specific (^{3}H) spiperone binding was measured as previously described[4].

Results. As shown in the table repeated treatment with haloperidol for 10 days followed by a 4-day washout period produced an increase of (^{3}H) spiperone binding in both hippocampus and striatum; however, this effect was statistically significant only in the former region. In vivo (^{3}H) spiperone binding was increased significantly by more than

20% in both regions 4 days after the end of a repeated treatment for 21 days with haloperidol but returned to control level after 8 or 12 days of haloperidol withdrawal.

Previous studies have shown that under in vivo conditions, (^{3}H) spiperone labels mainly DA receptor sites in the rat hippocampal formation[4]. The increase in (^{3}H) spiperone binding observed in the rat hippocampal formation after repeated administration of haloperidol suggests that sustained blockade of dopaminergic transmission results in an increase in DA receptors. This has been found not only in striatum (as previously demonstrated in ex-vivo experiments by Burt et al. and confirmed in vivo in the present study) but also in the hippocampal formation as well as in other limbic areas such as the septum and tuberculum olfactorium. It was not, however, seen in frontal cortex or retina (Bischoff, in preparation). The effect of chronic treatment with haloperidol on in vivo (^{3}H) spiperone binding in hippocampus is time-dependent. A significant effect was first observed after 10 days of treatment, and a further gradual increase was seen up to 28 days (data not shown). With our experimental design it is not possible, however, to decide whether the increase in (^{3}H) spiperone binding reflects a change in the affinity or in the number of DA receptor sites, or both. Although a direct effect of haloperidol on the bioavailability of the radioligand cannot be entirely excluded, it is unlikely in view of the almost total disappearance of haloperidol (as judged by the only weak antagonistic activity against apomorphine-induced stereotyped behaviour in the rat (Delini-Stula, personal communication)) from the brain 4 days after administration. More extensive experiments (dose dependence, chronic treatment with neuroleptics of other types such as phenothiazines) are underway.

In conclusion, the present data suggest that a prolonged blockade of hippocampal dopaminergic transmission by neuroleptics results in the development of a supersensitivity of hippocampal target cells probably related to an increase in DA receptor density and/or affinity. This might account for the occurence of tolerance to the increase in hippocampal DOPAC levels during repeated treatment with haloperidol[9].

Effects of repeated treatment with haloperidol on in vivo (^{3}H) spiperone binding in rat striatum and hippocampus

Duration of treatment (days)	Duration of wash-out period (days)	Specific (^{3}H) spiperone binding Striatum (% of control)	Hippocampus (% of control)
Experiment A			
10	4	116 ± 6(n.s.)	118 ± 5*
Experiment B			
21	4	123 ± 7*	124 ± 3***
21	8	108 ± 5	110 ± 6
21	12	113 ± 8	104 ± 7

In vivo specific (^{3}H) spiperone binding was measured in rat striatum and hippocampus 4–12 days following the withdrawal of a repeated treatment for 10 days (experiment A) or 21 days (experiment B) with haloperidol (1 mg/kg s.c.). Results are means ± SEM of data from 8–12 animals and are expressed as a percentage of control values. *$p < 0.05$; ***$p < 0.001$ vs control (Student's t-test).

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Immunohistochemical localization of pancreatic polypeptide (PP) in the brain of the larval instar of the hoverfly, *Eristalis aenus* (Diptera)[1]

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Summary. 2 pancreatic polypeptide (PP) immunoreactive cells were observed in each half of the protocerebrum of the last (5th) larval instar of the hoverfly, *Eristalis aenus*. No PP immunoreactive nerve fibers could be detected in the brain.

Pancreatic polypeptide (PP) immunoreactive cells have been reported to occur in the brain of the 5th larval instar and adults of the silkworm *(Bombyx mori)*[2] and of the adults of blowfly *(Calliphora erythrocephala)*[3]. As described in a previous report[4], PP immunoreactive cells have been observed in the fused ventral ganglia of the last (5th) larval instar of the hoverfly *(Eristalis aenus)*. In order to test whether or not PP cells are also present in the brain of this larval instar of the hoverfly, the present study has been performed.

The brain of the 5th (last) larval instar of the hoverfly, *E. aenus* (provided by Dr R. Abou-Elella, Department of Entomology, Cairo University, Egypt) was dissected out as described previously[4] and fixed for 24 h in Bouin's fluid, embedded in paraffin and cut serially at 5 µm. For the demonstration of PP cells, the peroxidase-antiperoxidase (PAP) method of Sternberger[5] was applied. The anti-BPP (No. 615-R-110-146-10, donated by Dr R. E. Chance, Lilly Res. Lab., Indianapolis, USA) was used at the dilution of 1:1600. In addition to the controls described previously[4], the first-layer antiserum was pre-incubated with rabbit anti-human Clq complement (Dako, Lot No. 038B) for 24 h at 4 °C.

In the brain of the larval hoverfly 2 PP immunoreactive cells were observed in each protocerebrum hemisphere. One cell was located in the mid-anterior and the other was in a latero-posterior position to the corpora pedunculata. They were small round or polygonal cells (fig.). No PP