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FRONTAL DECORTICATION AND ADAPTIVE CHANGES IN STRIATAL CHOLINERGIC NEURONS: NEUROPHARMACOLOGICAL AND BEHAVIORAL IMPLICATIONS

Silvana Consolo
Istituto di Ricerche Farmacologiche "Mario Negri"
Via Eritrea 62
20157 Milan, Italy

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EOARD
223/231 Old Marylebone Rd
London NW1 5TH UK

20157 MILANO, Italy - Via Eritrea, 62 - Tel. (02) 39014.1 - Telefax (02) 3546277
Cable address: NEGRINST MILANO - Telex 331268 NEGRI I

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Molecular Mechanism studies: autoradiographic and saturation binding experiments showed a marked reduction of (^{3}H)hemicholinium-3 [(^{3}H)HCh-3] binding to sodium-dependent high-affinity choline uptake (SDHACU) sites in striatum two weeks after frontal decortication. Oxiracetam (100 mg/kg i.p.), a no tropic drug, did not affect the distribution of (^{3}H)HCh-3 binding sites in sham-operated rats but completely overcame the reduction in the Bmax of decorticated striatum. This result is consistent with the increase in SDHACU in decorticated striatum after oxiracetam treatment. Frontal decortication did not change the percentage of the mAChR subtypes M1 and M3 present in the striatum and did not affect Gpp(NH)p modulation of binding affinity for the muscarinic cholinergic agonist oxotremorine (OTMN) in the striatum. Frontal decortication did not affect the activation of phosphoinositide turnover (PI) induced by carbachol but it enhanced the stimulation of PI turnover induced by ibotenic acid, a glutamatergic agonist. Behavioral studies: in the radial maze test decorticated animals' performance was severely impaired in spatial learning and maze running strategies (MRS) were altered similarly to fornix- or hippocampus- damaged rats. Ibotenic and quisqualic acid

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quisqualic and ibotenic lesions of nucleus basalis magnocellularis, learning and memory processes.

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chemical lesions of the nucleus basalis magnocellularis (NBM) did not affect learning performance. Only ibotenic lesions modified MRS but in the opposite direction from the decorticated group suggesting that the striato-cortical connections rather than the projections from NBM to frontal cortex are involved in the control of spatial learning and in organization of MRS.

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List of abbreviations: ACh, acetylcholine; ChAT, choline-o-acetyl/transferase; OTMN, oxotremorine; OXI, oxiracetam; SDHACU, sodium-dependent high affinity choline uptake; DC, decorticated; HCh-3, hemicolinium; PZ, pirenzepine; QNB, l-quinuclidinyl benzilate; NMS, N-methyl scopolamine; mAChR, muscarinic acetylcholine receptor; PI, phosphoinositide; CARB, carbachol; InsPs, inositol-phosphates; IBO, ibotenic acid; QUIS, quisqualic acid; NBM, nucleus basalis magnocellularis.

1. Molecular Mechanisms in Decorticated Rats

i.[^{3}H]Hemicholinium binding autoradiography: restoration studies

The interruption of the corticostriatal pathway by undercutting the frontal cortex resulted, after two weeks, in a 40% reduction of basic cholinergic processes such as ACh release *in vivo* and sodium-dependent high-affinity choline uptake (SDHACU) (see previous Reports). The depression of cholinergic function in deafferented striatum has been demonstrated also by the evaluation of [^{3}H]HCh-3 binding to SDHACU sites, determined biochemically in tissue homogenates, or by autoradiography in brain sections. We have also reported that using appropriate drugs such as choline, the ACh precursor, or oxiracetam, a typical nootropic compound, it was possible to promote the recovery of both basic cholinergic function and pharmacological responses to different agonists. Thus, we have tested the ability of oxiracetam (OXI) to restore, in a neuroanatomical context by autoradiography, the normal density of [^{3}H]HCh-3 binding sites in deafferented striatum.

The autoradiographic distribution of [^{3}H]HCh-3 binding sites has been evaluated according to Lowenstein et al (1987). The rats were decapitated and the whole brain was removed and rapidly frozen in N-pentane at -20-25 °C . Coronal and sagittal sections were cut at -20°C in a cryostat and thaw-mounted onto acid-cleaned gelatin coated slides. The slides were incubated with 10 nM [^{3}H]HCh-3 for 30 min at room temperature in 50 mM glycylglycine buffer pH 7.8 containing 200 mM NaCl. Non-specific binding was determined in adjacent sections processed in the same manner except that 10 μM unlabelled HCh-3 was added to the incubation medium. After dessication, the slides were exposed to tritium-sensitive film "Hyperfilm" (Amersham, UK) for four weeks and developed using a standard technique; tritiated microscales (Amersham) were coexposed. Quantitative autoradiographic analysis was done with a RAS 3000 Image Analysis System (Loats System, U.S.A.).

Digitized images of the [^{3}H]HCh-3 binding distribution in coronal sections of bilateral deafferented rats treated with OXI are Illustrated in Fig 1. A substantial decrease of autoradiographic signal of [^{3}H]HCh-3 binding was seen in striata of bilaterally deafferented rats compared to the sham-operated rats; no changes were noted in other structures. In sham-operated rats, OXI did not alter the distribution of [^{3}H]HCh-3 binding sites in either striatum or accumbens, but in deafferented rats the treatment with OXI appeared to restore the normal distribution of [^{3}H]HCh-3 binding sites.

The autoradiographic distribution of [^{3}H]HCh-3 binding sites in sham-operated or decorticated rats treated with OXI was quantified by image analyser (Table 1). Brain coronal sections, which included the anteromedial part of the caudate-putamen and the accumbens-tubercle olfactorium region, were analyzed. As previously shown, bilateral deafferentation produced a decrease (22%) in the [^{3}H]HCh-3 binding sites in the anteromedial portion of the striatum. No changes were detected in the posterior part adjacent to the globus pallidus or in the accumbens-olfactory tubercle region. In sham-operated rats OXI did not significantly alter the density of [^{3}H]HCh-3 binding sites but there was a slight increase in the signal in the limbic area. In deafferented rats, the nootropic drug overcame the effect of the lesion and even produced a significant increase -15%- in striatal [^{3}H]HCh-3 binding sites compared to sham-operated animals. Similar trend was seen also considering only the laterodorsal portion of striatum, where the density of [^{3}H]HCh-3 binding sites was higher. This result is consistent with the increase in SDHACU in deafferented striatum after OXI treatment.

In addition, it appears from the results that OXI only acts this way in animals with impaired cholinergic function. This is in agreement with previous biochemical experiments showing that OXI restores ACh release *in vivo* in striata of decorticated rats and prevents the electroshock- or scopolamine-induced decrease in brain ACh but has no effect in control animals.

The findings of this study point to the possibility that OXI normalizes basic cholinergic processes by increasing the availability of choline for ACh synthesis. The finding that in certain conditions OXI could improve the synthesis of phospholipids, particularly the phosphatidylcholine pool, proposed as a "reservoir" for generating choline for the synthesis of ACh speaks in favor of this hypothesis However, OXI may possibly restore the tone of cholinergic neurons in deafferented striatum by activation of NMDA receptors, as recently suggested in the hippocampus. Indeed the corticostriatal pathway uses glutamate as putative neurotransmitter which has been shown to increase ACh release from striatal slices *in vitro* through NMDA-type receptors possibly located on the cholinergic cell. Therefore, the direct or indirect activation of NMDA receptors by OXI could normalize the activity of cholinergic interneurons in deafferented striata.

ii. Effect of frontal decortication on muscarinic receptor subtypes in striatum

The impairment of ACh release in vivo as a result of the loss of the excitatory corticostriatal pathway could cause understimulation of cholinergic receptors in the striatum, and as an adaptation to this, an up regulation of muscarinic acetylcholine receptors (mAChRs). It was thus firstly examined whether frontal decortication caused changes in the number (B_{max}) or affinity (K_d) of muscarinic receptors, using as radioligand N-methylscopolamine

[(^{3}H)NMS], a quaternary muscarinic antagonist that does not discriminate between the muscarinic receptor subtypes. Saturation curves of (^{3}H)NMS were determined as described by Giraldo et al., 1987. It was found that both the B_{max} and the K_d do not change 14 days after decortication (Table 2).

In general muscarinic receptors are classified as neuronal M1 [high affinity for pirenzepine (PZ)], cardiac M2 (low affinity for PZ / high affinity for AF-DX 116) and glandular M3 (low affinity for both PZ and AF-DX 116). It has been shown that rat striatum contains about 30% of the M1 subtype and about 70% of the M3 subtype and it seems to be devoid of the M2 type (Giraldo at al., 1987). In order to examine whether frontal decortication caused changes in the percentages of the muscarinic receptor subtypes known to be present in the striatum, competition experiments against (^{3}H)NMS were done using the selective antagonists PZ and AF-DX 116. Binding curves were derived indirectly from competition experiments against 0.35nM (^{3}H)NMS and 0.5 (^{3}H) PZ as already described by Giraldo at al., 1987.

The inhibition curves generated by PZ in displacing (^{3}H)NMS from specific muscarinic receptors sites in the striatum of sham and DC rats were shallow with an nH significantly less than one, and the data points fitted best to a two binding site model, indicating the presence of two major populations of receptors. One receptor population (M1 sites), amounting to 35% of total receptors, bound PZ with high affinity (K_d, 35 nM) whereas second and major (65%) population of sites bound with low affinity (K_d, 386 nM) both in sham-operated and in DC rats (Table 3). From the binding experiments with PZ alone, the nature of second receptor population cannot be more precisely defined since PZ does not discriminate between the M2 and M3 low affinity binding sites. Therefore, we did competition experiments with the novel antimuscarinic

PZ analog, AF-DX 116 in sham and DC rats. AF-DX 116/ (^{3}H)NMS competition experiments in the striatal membrane preparation of both groups generated a steep curve with nH not significantly different from unity suggesting that AF-DX 116 bound to a uniform population of sites with low affinity (M3 sites). The affinity constants, 1.1 and 1.2 μM for sham and DC rats respectively, fell within a range similar to the K_d value, 2.9 μM, shown for the glandular M3 (Table 4). Based on these findings we can conclude that the percentages of the mAChR subtypes, M1 and M3, present in the striatum were similar in sham operated and DC rats.

iii. Effect of frontal decortication on the agonist binding to muscarinic receptors in striatum

It is well accepted that in brain and in heart the high affinity states of the mAChR result from interaction with G proteins (Nathanson, 1987). Indeed, the guanyl nucleotide GTP and the phosphohydrolase resistant analogue, Gpp(NH)p,which uncouple G proteins from the receptors have been demonstrated to modulate the binding affinity for the muscarinic cholinergic agonists in the heart and cortex (Rosenberger at al 1979). It was thus examined whether frontal decortication caused changes in the Gpp(NH)p modulation of the binding affinity for the muscarinic cholinergic agonist, oxotremorine (OTMN) in the striatum (Table 5).

OTMN /(^{3}H)1-quinuclidinyl benzilate (QNB) competition curves from striatal membrane preparations of sham operated and DC rats were obtained in the absence and presence of 100 μM Gpp(NH)p by using concentrations of the agonist from 1nM to 300 μM. In the absence of Gpp(NH)p, the data points differed significantly from the theoretical curve in both groups with nH being less than unity: 0.67 ± 0.08 nM $p<0.01$ (sham-operated group) and 0.61 ± 0.05 nM $p<0.01$ (DC group). Computer analysis of the curves from sham and DC striata showed the existence of two receptor affinity states : in sham striata OTMN dispaced 16.2 ± 0.51% with high affinity [IC_{50}= 14 ±1.2 nM] and 81±1.1% with low affinity [IC_{50} = 5.6 ± 0.5 μM] in sham striata whereas in DC striata it bound 14.3 ± 3.3% of receptors with high affinity [IC_{50} 13.0 ±1.0] and 85.9 ± 4.2% with low affinity [1.3 ± 0.1 μM] were found in DC striata. The addition of 0.2 mM Gpp(NH)p and 10 mM Mg^{++} to the incubation medium resulted in a almost complete conversion of the mAChRs that OTMN recognized with high affinity to a low affinity state: 96.2 ± 2.9% with a IC_{50} of 3.3 ± 0.28 μM in sham and 94.6 ± 0.37% with IC_{50} 3.1 ± 0.01 μM in DC striata. In both cases the nH was 0.93 ±0.02.

These data demonstrate that the GTP regulatory function at muscarinic receptors in rat striatum does not change after decortication.

iv. Effect of frontal decortication on signal transduction at postsynaptic cell sites: measurement of (^{3}H) inositol-phosphate accumulation

Receptor binding studies can detect changes in the characteristics (B_{max} and K_d) of the mAChRs, but they cannot yield any information on receptor function, i.e. whether the reaction of the agonist with the receptor leads to a normal or altered biochemical response. It is well accepted that ACh or the cholinomimetic drugs, acting at muscarinic receptors in the striatum, inhibit adenylate cyclase, stimulate cGMP synthesis and elicit phosphoinositide (PI) breakdown. Thus, we have investigated whether frontal decortication affected the transduction mechanism coupled to muscarinic receptors in the striatum at the postsynaptic level, i.e the activation of phosphoinositide turnover (PI) induced by the muscarinic agonist carbachol (CARB). In addition, since glutamate is the putative neurotransmitter in the corticostriatal pathway, we have also investigated the lesion effects on the activation of PI turnover by the glutamatergic agonist, ibotenic acid (IBO).

Effect of frontal decortication on phospoinositide turnover stimulated by CAR in striatum: The accumulation of (^{3}H)-inositol-phosphates [(^{3}H)InsPs] was determined according to the method of Brown et al 1984, in tissue miniprisms from striata of DC and sham-operated rats. Fig. 2 shows the dose-response effect of CARB on (^{3}H)InsPs accumulation in miniprisms from lesioned and sham-operated animals. In sham-striata CARB-stimulated PI turnover in a concentration-dependent manner (1μM -1mM). Formation of (^{3}H)InsPs was about 200 % above control values at 1μM CARB and the maximal effect, about 600% was achieved at 1 mM when the plateau was reached. The EC_{50} is $1.3 \times 10^{-4} \pm 43$. As shown in the figure, we did not find any change in the response of DC rats at any of the doses studied.

Effect of frontal decortication on phosphoinositide turnover stimulatea by IBO: The effect of DC lesion on PI turnover stimulated by IBO, an excitatory amino acid which mimics the effect of glutamate, was examined (Fig. 3). In miniprisms from striata of sham-operated rats the stimulation of (^{3}H)InsPs accumulation induced by IBO was 80% and 90% at 100 μM and 1mM, respectively, while in DC miniprisms the effect was already evident (25%) at 10 μM of the agonist and the maximal effect, about 350%,was achieved at 1mM. The enhancement of IBO stimulation in DC striata is very likely caused by glutamate receptor supersensitivity induced by the removal of the glutamatergic input, as previously observed (Nicoletti at al., 1987). Further experiments are in progress to analyze the effects of decortication on other signal transduction mechanisms coupled to mAChRs in the striatum.

2. Behavioral Effects of Frontal Decortication

In previous experiments it was found that the neurochemical alterations observed after disconnecting the basal ganglia from the cortex were accompanied by marked deficits in active avoidance conditioning and Lashley maze learning. However, since this lesion also induces a general pattern of behavioral disinhibition (decrease of emotionality, increased reactivity to novelty), it was difficult to estabilish whether DC rats have specific memory deficits.

Effect of frontal decortication on spatial learning

In order to investigate to what extent lesioned animals are impaired in processing and storing information when submitted to learning problems, we examined their acquisition rate in the radial maze, a complex special learning task (preliminary data were given in Report of April, 1990).

Two groups of rats (bilaterally operated and sham-operated) were tested in a radial maze consisting of eight identical paths (width 12 cm; length 60 cm) radiating from an octogonal platform. For three consecutive days, each rat was placed on the central platform and allowed to explore the maze in which a large amount of food pellets had been placed. On the 4th day, the actual procedure started. For ten consecutive daily trials, each rat was placed on the central platform and allowed to make eight runs, with all the paths previously baited.

Three dependent variables were recorded: (1) the mean number of unrepeated path choices out of eight, (2) the rank of occurrence of the first error, (3) the degree of divergence of the sequence of runs. The results were statistically evaluated by one-way ANOVA for repeated measures. There was an increase of performance in sham-operated as well as in lesioned rats as learning proceeded (Fig. 4), but the number of correct path choices were less frequent [significant lesion effect, $F(1,24)= 21.70$ $p>0.001$] in lesioned than in sham-operated animals (Fig.4 A) and the first error occurred earlier [$F(1,24)=15.57$, $p> 0.0006$] (Fig.4 B). Differences were also observed in the maze running strategies (MRS) which were significantly more divergent [significant lesion effect, $F(1,24)=11.6$, $p>0.002$] in lesioned than in sham-operated rats (Fig.4 C).

As in simpler tasks, disconnecting the cortex from the basal ganglia significantly impaired performance in the radial maze. Interestingly, the lesioned rats not only made a larger number of errors in this task but also displayed very different MRS. Control rats tended to visit adjacent paths consecutively, whereas lesioned rats alternated their choices in an apparently arbitrary manner. It can thus be assumed that the mapping operations resulting from their different exploratory strategies will lead to different representations of their experimental environment, making for poor spatial memory.

However, the higher degree of divergence displayed by lesioned rats in running the maze is similar to that observed in animals with damage to the septo-hippocampal pathway, the main cholinergic input to the hippocampus. Important behavioral impairment has also been observed in rats lesioned in the nucleus basalis magnocellularis, source of cholinergic innervation to the cerebral cortex (Olton and Wenk 1987).

3. Effect of Neurotoxin-induced Lesions of Cortical Afferents

Since the mechanical disconnection used in this study also damages the cholinergic projections from the nucleus basalis to the frontal cortex, it seemed necessary to check to what extent the behavioral alterations observed depended on the interruption of this particular pathway, or were due to the more complete disconnection of the basal forebrain from the cortex. We compared the behavioral and biochemical effects of specific chemical lesions of the nucleus basalis magnocellularis (NBM) with the frontal decortication.

i. Effect of ibotenic and quisqualic lesions on spatial learning

Ibotenic and quisqualic lesions were made. Equitensin (1% pentobarbital, 4% chloral hydrate) anesthetized rats were bilaterallly lesioned in the NBM by stereotaxic microinjections (4 x 0.75 ml for each hemisphere) of ibotenic (IBO: 35 mM) or quisqualic (QUIS: 50 mM) acid (coordinates: 1.3 mm posterior to bregma, 2.5 mm lateral to midline, 6.5 and 7.2 mm below dura). In sham-operated animals, the skull was opened, but no lesion was made. The experiments were done 7 and 14 days after IBO or QUIS lesion, respectively.

Two groups of rats bearing these lesions and their respective controls were tested in a radial maze as previously described, for ten consecutive daily trials. Rats from the IBO and QUIS groups did not differ from SHAM in the quantitative aspects of performance (Fig. 5). The number of correct choices and the rank of occurrence of the first error were similar in the three groups. However, differences in the organization of MRS were observed for lesioned and control groups. MRS were less divergent in IBO and QUIS than in SHAM

[F(2,45) = 6.25, p> 0.004], the main modification being in the IBO group which differs significantly from QUIS and SHAM (Newman-Keuls pairwise comparisons, p> 0.01). No significant difference was found between these two latter groups.

In conclusion, the behavior of DC rats differs from that of rats of the QUIS and IBO groups. Decortication drastically impairs spatial learning and strongly modifies MRS strategies which mimic those observed in fornix or hippocampus damaged rats. IBO and QUIS lesions of the nucleus basalis modify MRS but in the opposite direction from the DC group. This suggests that striato-cortical connections other than the projections from nucleus basalis to frontal cortex participate in the control of spatial learning and organization of MRS.

ii. Effect of ibotenic and quisqualic lesions on ACh release from frontal cortices in vivo and choline acetyl transferase activity

Application of IBO to degenerate the baso-cortical cholinergic system moderately reduced ChAt activity - a marker of cholinergic neuron integrity in the cortical regions - but it also caused clear behavioral impairment. The NBM lesion with QUIS, a glutamate receptor subtype agonist, caused a large drop in ChAt activity in the frontal cortex but less severe behavioral impairment.

We originally assessed cortical cholinergic functions in both conditions and in DC rats by measuring ACh *output* by *microdialysis*. We also measured frontal cortices ChAt activity in all three groups of lesioned rats (DC, IBO and QUIS). Extracellular ACh content was measured in frontal cortices of 14-day bilaterally decorticated and sham-operated rats, using the microdialysis technique. The results are shown in Fig. 6. ACh levels in the 20 min perfusate samples are uncorrected for the recovery which was 50% for a probe 8.0 mm long. The

ACh output was constant over at least 200 min in the sham-operated and DC rats. The average ACh content in the perfusate was 6.0 ± 0.1 pmol/20 min in the sham-operated and 2.5 ± 0.1 pmol/20 min in DC with a significant ($p<0.01$) decrease of about 60%. Fig. 7 shows the effect on ACh release from frontal cortices of bilateral NBM lesioned rats induced by ibotenic IBO or QUIS compared to sham-operated rats. ACh output was constant over at least 200 min in the three groups but while the average ACh content in the perfusate of IBO (5.4 ± 0.1 pmol/20 min) was similar to that of sham-operated rats (5.7 ± 0.1 pmol/20 min), in the QUIS the cortical ACh output was significantly ($p<0.01$) lowered, to 3.2 ± 0.1, a decrease of about 40%. Our results indicate that there is a loss of cortical ACh release in vivo in the DC and in the QUIS rats, but not in the IBO ones.

To investigate this point further, we measured ChAt activity in the frontal cortex and in hippocampus of the lesioned and sham-operated rats (Table 6). Neither decortication nor NBM lesions affected hippocampal ChAt activity, underlining the specific involvement of cholinergic cortical function. NBM lesions induced by IBO or QUIS reduced cortical ChAt activity by about 30% and 40%, respectively, while decortication had no effect. The different decreases in cortical ChAt activity induced by IBO or QUIS correlates well with their different effects on cortical ACh release in vivo, while the absence of modification of cortical ChAt activity observed in DC rats and the marked impairment of cortical ACh release needs to be clarified.

In summary, our findings show that the decrease of cortical ACh release in vivo observed in the DC and QUIS groups is correlated with a reduction of cortical ChAt activity only in the QUIS rats. The IBO lesion did not alter ACh output from frontal cortices and reduced cortical ChAt activity less than the lesion induced by QUIS.

The differences in ACh release in the IBO and QUIS groups do not correlate with any behavioral alterations since (1) IBO, QUIS and SHAM radial maze performances were similar and (2) MRS differed for IBO and SHAM in spite of their similar rate of ACh release. It seems, therefore, that cholinergic depletion in the frontal cortex does not by itself represent a specific marker either for spatial learning deficits or for MRS alterations. Probably, a reduction of ACh release in the frontal cortex associated with particular alterations of other neurotransmitter systems would correlate better with specific patterns of behavioral deficits.

Data analysis

The binding data were computer analyzed with a non linear least squares regression program for single or multiple independent binding site models. The sum of squares errors from data fitting was then statistically compared between models to determine best fit. The hill coefficient (nH) was calculated by linear regression analysis and assessed for statistically significant deviation from unity (Student's t-test).

The EC_{50} was determined by an ALLFIT program, using four parameter logistic functions.

The results of behavioral experiments were statistically evaluated by a one way ANOVA for repeated measures

Table 1. <u>EFFECT OF OXIRACETAM ON THE [^{3}H]HCh-3 BINDING DISTRIBUTION IN FRONTAL DEAFFERENTED RAT BRAIN DETERMINED BY AUTORADIOGRAPHY.</u>

Brain regions	[3H]HCh-3 binding (fmol/mgP)				F int.
	Sham-oper.	Deafferented	Oxiracetam	Deaffer.+ OXI	
Striatum anteromedial	361.4 ± 14.9	283.8 ± 15.4*	359.6 ± 12.5	411.7 ± 19.1* P<0.001	17.8
Striatum laterodorsal	476.9 ± 20.5	396.0 ± 23.4°	472.7 ± 23.0	545.0 ± 23.1* P<0.001	11.1
Accumbens Olfact. Tub.	307.2 ±17.2	274.0 ± 18.4	365.1 ± 29.2	361.3 ± 20.0*	n. s.

The data are the means ± S.E.M. of 4-5 rats (3 - 5 sections per brain).*P< 0.01, °P<0.05 vs respective sham-operated group, ANOVA (2 x 2) test and Tukey's test.The rats were killed two weeks after frontal bilateral deafferentation and 2 h after oxiracetam (OXI) treatment (100 mg/kg). One concentration (10nM) of [^{3}H]HCh-3 was used.

Table 2. BINDING ESTIMATES OF [3H]–NMS IN STRIATUM OF SHAM OPERATED AND FRONTALLY DECORTICATED RATS

	Bmax fmoles/mg protein)	KD (nM)
SHAM	1067±123	0.19±0.04
DECORTICATED	1061±163	0.18±0.03

The data are the means ± S.E.M. ($n = 4$)
The experiment was performed 14 days after lesion

Table 3. **BINDING ESTIMATES OF PIRENZEPINE IN STRIATUM OF SHAM OPERATED AND FRONTALLY DECORTICATED RATS**

	SHAM		DECORTICATED	
KD1 (nM)	14±8	35%±12	17±5	35%±8
KD2 (nM)	386±145	65%±12	356±45	63%±8
nH	0.67±0.01*		0.70±0.01*	

The data are the means ± S.E.M. ($n = 3$)
The experiment was performed 14 days after lesion
* significantly different from unity ($p < 0.05$)

Table 4. BINDING ESTIMATES OF AF-DX 116 IN STRIATUM OF SHAM OPERATED AND FRONTALLY DECORTICATED RATS

	KD (nM)	nH
SHAM	1125 ± 17	0.87
DECORTICATED	1179 ± 35	0.87

The data are the means $\pm$ S.E.M. ($n = 3$)
The experiment was performed 14 days after lesion

Table 5. INFLUENCE OF Gpp(NH)p ON OTMN DISPLACEMENT OF [^{3}H]-L-QNB IN STRIATUM OF SHAM-OPERATED AND FRONTALLY DECORTICATED RATS

	R_1 (%)	R_2 (%)	Ic_{50} 1 (nM)	Ic_{50} 2 (μM)	nH
-Gpp(NH)p					
SHAM	16.2 ± 0.5	81.1 ± 1.1	14.0 ± 1.2	5.6 ± 0.5	0.7 ± 0.1
DC	14.3 ± 3.3	85.9 ± 4.2	13.0 ± 1.0	1.3 ± 0.1	0.6 ± 0.1
+Gpp(NH)p					
SHAM	-	96.1 ± 2.9	-	3.3 ± 0.3	0.9 ± 0.1
DC	-	94.7 ± 0.4	-	3.0 ± 0.1	0.9 ±0.1

The data are the means ± S.E.M. of 3 experiments performed in triplicate. Gpp(NH)p was added at the dose of 100 μM in the presence of 10 mM $MgCl_2$. The data were evaluated by non linear least-squares regression analysis for simple or multiple independent binding site models (Sacchi et al., 1983).

Table 6. Effect of neurotoxin-induced lesions in NBM and of deafferentation of frontal cortex on ChAT activity in rat cortex and hippocampus

	FRONTAL CORTEX	HIPPOCAMPUS
	ChAt (µmoli/h/gP)	
SHAM	27.3 ± 1.3	24.1 ± 4.8
LES-QUIS	16.4 ± 2.8*	27.7 ± 1.8
SHAM	33.8 ± 5.2	22.1 ± 1.0
LES-IBO	23.1 ± 6.7**	24.9 ± 3.6
SHAM	18.9 ± 1.8	20.9 ± 1.4
DECORTICATED	21.0 ± 3.7	19.7 ± 3.6

The data are the means ± S.E. of two experiments. *$p<0.01$, **$p<0.05$ vs Sham group; Student's t test.

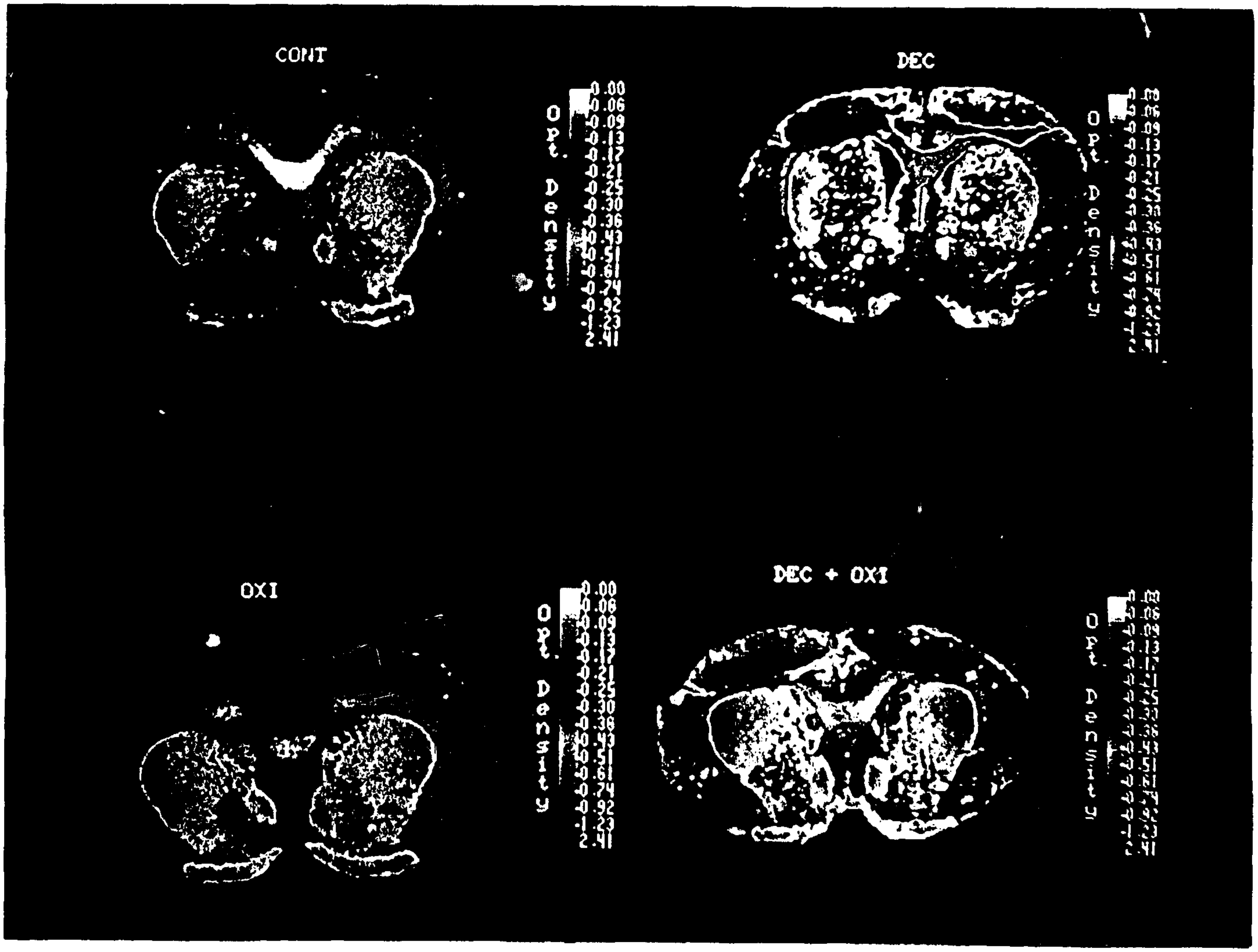

CONT
DEC
OXI
DEC + OXI
Opt. Density
0.00
-0.06
-0.09
-0.13
-0.17
-0.21
-0.25
-0.30
-0.36
-0.43
-0.51
-0.61
-0.74
-0.92
-1.23
-2.41

Fig. 1

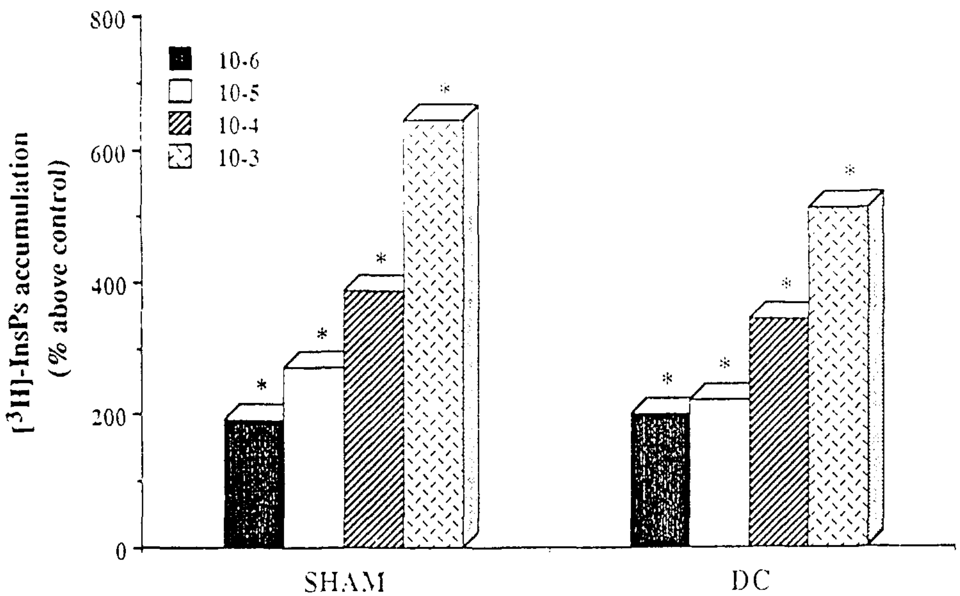


Fig.2 Dose-dependent stimulation of [^{3}H]-InsPs accumulation induced by carbachol (CARB) in miniprisms from striata of sham-operated (SHAM) and decorticated (DC) rats. Control values were 857±31 cpm /29700±1700 cpm in lipids (SHAM) and 1080±40 cpm /30000±3500 cpm in lipids (DC). Results are expressed as a percentage above control response. Each bar represents the mean ± S.E. of three experiments performed in triplicate. *$p<0.01$ vs respective control values. ANOVA(2X2) and Tukey's test for unconfounded means.

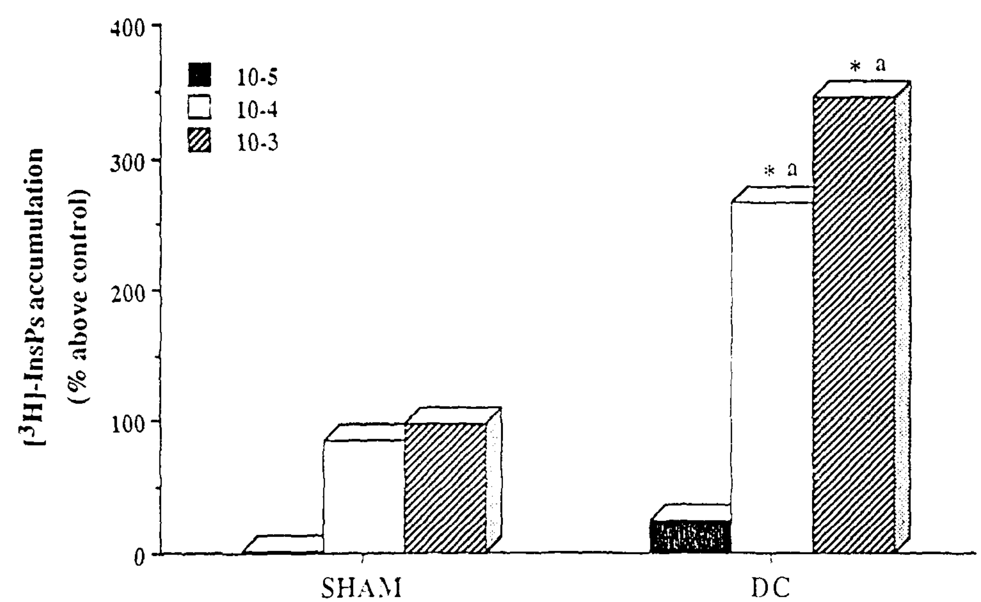
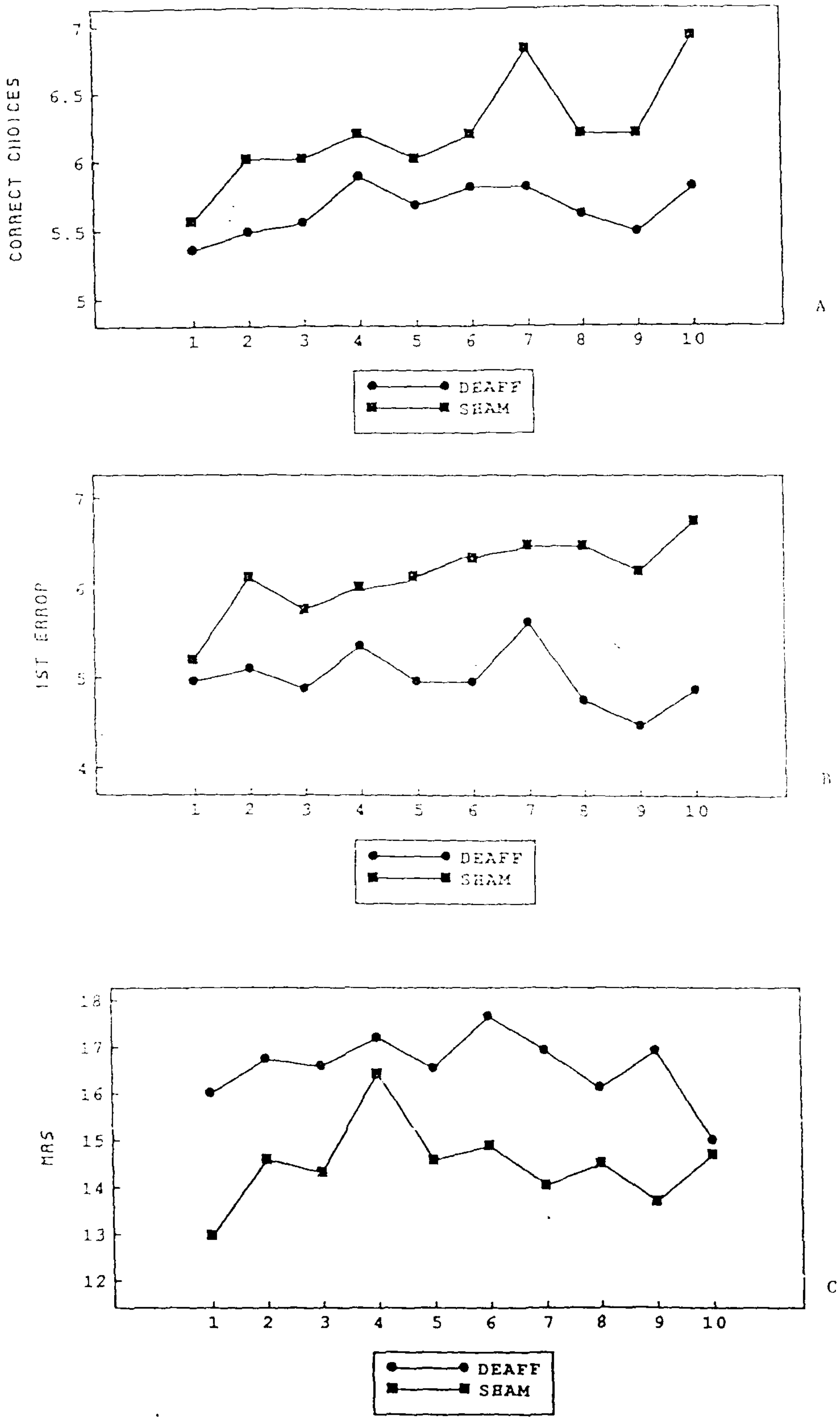


Fig.3 Dose-dependent stimulation of [^{3}H]-InsPs accumulation induced by ibotenic acid (IBO) in miniprisms from striata of sham-operated (SHAM) and decorticated (DC) rats. Control values were 1350±130 cpm /38000±2600 cpm in lipids (SHAM) and *1800±190 cpm /43700±4100 cpm in lipids (DC). Results are expressed as a* percentage above control response. Each bar represents the mean ± S.E. of three experiments *performed in triplicate.* *p<0.01 vs *respective SHAM group.* $F(3/24)=3.83$, ap<0.01. ANOVA(2X2) and Tukey's test for unconfounded means.



CORRECT CHOICES
7
6.5
6
5.5
5
1 2 3 4 5 6 7 8 9 10
A
DEAFF
SHAM
1ST ERROR
7
6
5
4
1 2 3 4 5 6 7 8 9 10
B
DEAFF
SHAM
MRS
18
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16
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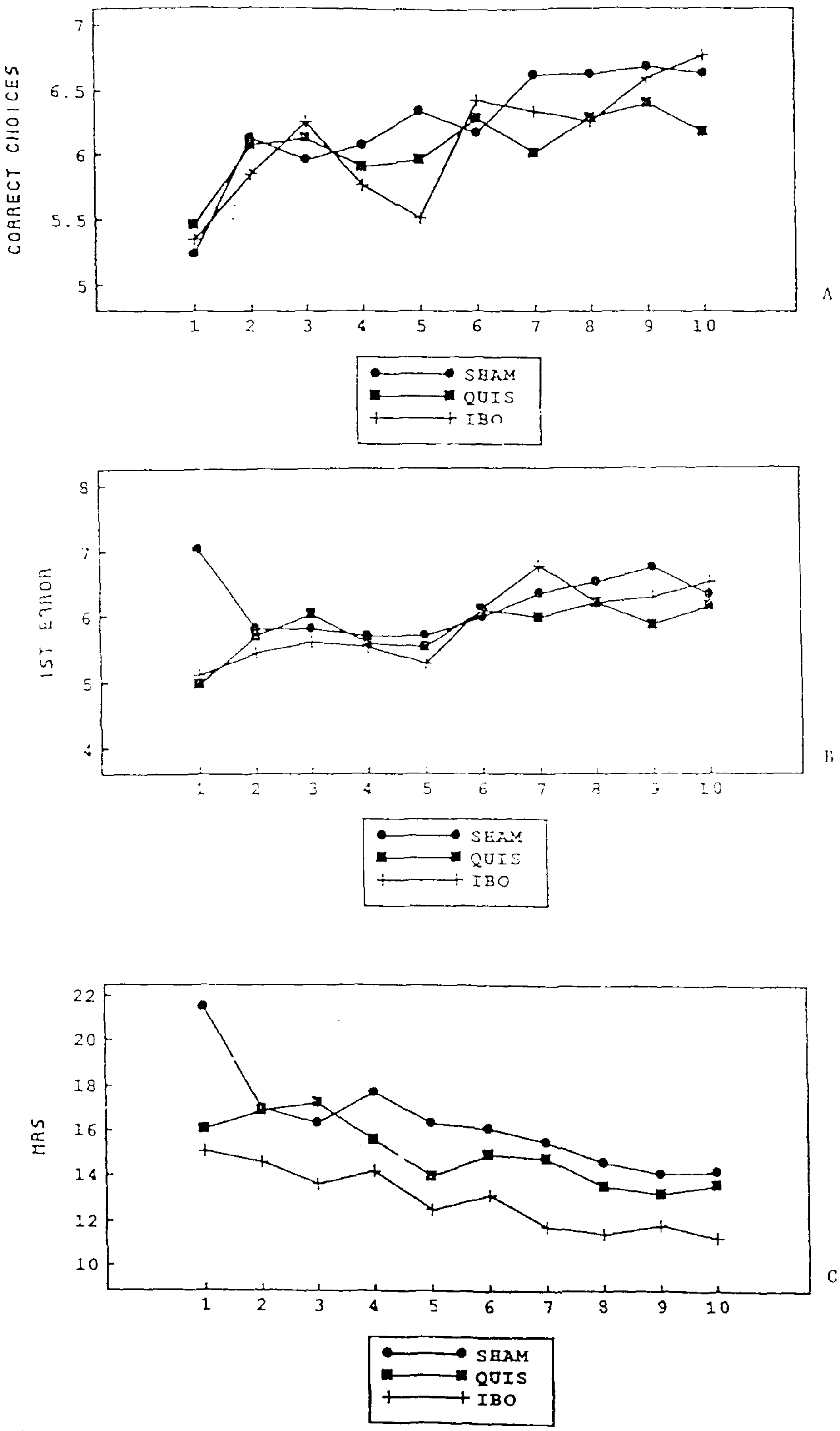
14
13
12
1 2 3 4 5 6 7 8 9 10
C
DEAFF
SHAM

FIG. 4

FIG. 5



CORRECT CHOICES
7
6.5
6
5.5
5
1 2 3 4 5 6 7 8 9 10
A
SHAM
QUIS
IBO
8
7
6
5
4
1 2 3 4 5 6 7 8 9 10
B
SHAM
QUIS
IBO
MRS
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1 2 3 4 5 6 7 8 9 10
C
SHAM
QUIS
IBO

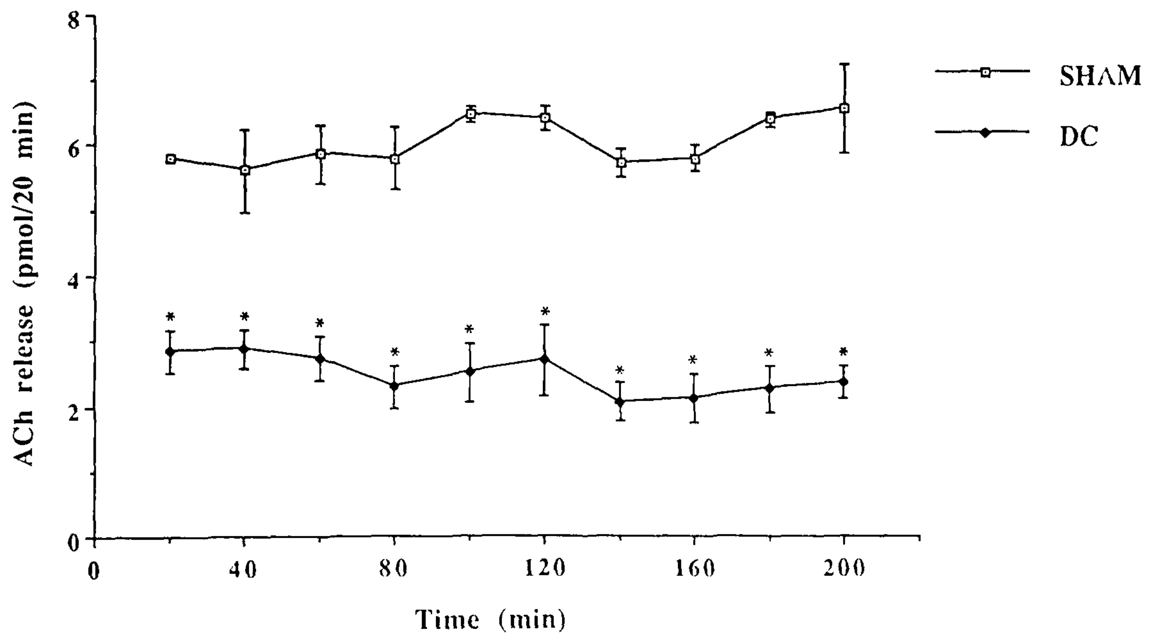

Fig.6 Effect of bilateral frontal decortication (DC) on ACh release <u>in vivo</u> from frontal cortices. The data [mean and S.E. (vertical bars) values from five rats] represent the ACh content in each 20 min fraction and are expressed as pmol/20 min. Data were evaluated statistically by Dunnett's test. *$p<0.01$ vs SHAM group.

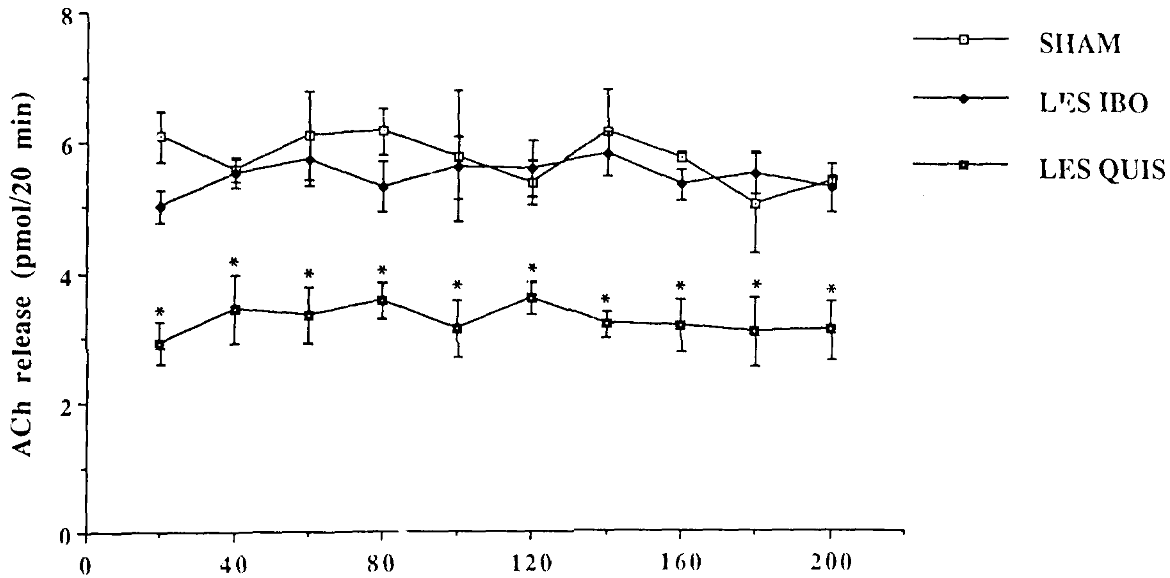

Fig.7 Effect of ibotenic (IBO) and quisqualic (QUIS) acid lesions of the nucleus basalis magnocellularis on the ACh release in vivo from frontal cortices.The data [mean and S.E. (vertical bars) values from five rats] represent the ACh content in each 20 min fraction and are expressed as pmol/20 min. Data were evaluated statistically by Dunnett's test. *p<0.01 vs SHAM group.

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Decrease in [^{3}H]hemicholinium binding to high-affinity choline uptake sites in deafferented striatum: restoration by oxiracetam

GianLuigi Forloni, Nadia Angeretti, Daniela Amoroso, Antonio Addis and Silvana Consolo

Istituto di Ricerche Farmacologiche 'Mario Negri' Milan (Italy)

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Key words: Choline uptake; Corticostriatal pathway; Autoradiography

Frontal cortical deafferentation of the rat striatum reduces the tone of striatal cholinergic neurons. We used biochemical and autoradiographic techniques to investigate whether the [^{3}H]hemicholinium-3 ([^{3}H]HCh-3) binding to sodium-dependent high-affinity choline uptake sites was influenced by this lesion. Frontal deafferentation produced a reduction of about 30% in the number of [^{3}H]HCh-3 binding sites (B_{max}) in striatum, with no significant changes in the binding affinity (K_d). Autoradiography showed a significant reduction of [^{3}H]HCh-3 binding sites in the anteromedial portion of the striatum, but not in the posterior part of frontal deafferented rats. Oxiracetam (100 mg/kg), a nootropic drug, did not affect the distribution of [^{3}H]HCh-3 binding sites in sham-operated rats but completely overcame the reduction in the number of [^{3}H]HCh-3 binding sites in deafferented striatum.

Hemicholinium is a potent, reversible inhibitor of sodium-dependent high-affinity choline uptake[23] (SDHACU), a rate-limiting step in acetylcholine (ACh) synthesis[21]. The availability of [^{3}H]hemicholinium ([^{3}H]-HCh-3) has permitted the development of a ligand-binding method to label the SDHACU sites[19,28]. Previous studies have documented a close correlation between the regional distribution of [^{3}H]HCh-3 binding to crude membrane preparation and the choline acetyltransferase (ChAT) activity[28]. Changes in [^{3}H]HCh-3 binding site density have been observed as a consequence of pharmacological manipulation in vivo[13] and in vitro[18], consistent with the alteration of cholinergic neuron activity. The association of [^{3}H]HCh-3 binding sites with the cholinergic terminals has been further proved by the autoradiographic technique. In this context the distribution of [^{3}H]HCh-3 binding sites in central nervous system correlates significantly with the immunocytochemical distribution of ChAT and the histochemical distribution of acetylcholinesterase, the two classical presynaptic markers of the cholinergic system[7,14,27].

Removal of excitatory corticostriatal afferents by cortical ablation or undercut resulted in a reduction of the basic cholinergic activity in the striatum as reflected by the decrease of ACh turnover[10,29], ACh release in vivo[5] and SDHACU[20]. However, even when their activity is depressed, the striatal cholinergic interneurons remain potentially functional and capable of responding to certain stimuli[6]. Choline, a precursor of ACh, or oxiracetam (OXI), a second generation nootropic drug in the 2-pyrrolidinenone class whose therapeutic value is currently being investigated[16], were reported to restore cholinergic biochemical activity in decorticated rats. The deafferented striatum constituted an interesting model for testing whether the [^{3}H]HCh-3 binding can reflect changes in cholinergic function induced by altered physiological equilibrium of intact cholinergic neurons rather than dramatic changes of cholinergic activity induced by drugs or lesions[13,26].

In this study, we used biochemical and autoradiographic techniques to investigate [^{3}H]HCh-3 binding to SDHACU sites in rat striatum after monolateral or bilateral chronic deafferentation. We also investigated whether OXI altered the autoradiographic distribution of [^{3}H]HCh-3 binding sites in sham-operated and frontal deafferented rats.

Female Sprague–Dawley rats (CD/COBS, Charles River, Italy) weighing 200–250 g were used. Monolateral and bilateral frontal deafferentation was done under ether anesthesia by undercutting the cortex with a thin glass knife fashioned from a cover slip, as described by Consolo et al.[6]. In sham-operated animals, the skull was opened but no lesion was made. The experiments were done 14 days after the lesion. The extent of damage to the corticostriatal pathway induced by the undercut was assessed in randomly selected groups of 4 animals per experiment, by measuring the uptake of [^{3}H]glutamate in crude homogenate preparations[8]. When [^{3}H]glutamate

Correspondence: G.-L. Forloni, Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea 62, 20157 Milano, Italy

TABLE I

Effect of frontal deafferentation on [³H]HCh-3 binding to the sodium-dependent high-affinity choline uptake (SDHACU) sites in striatal membranes

The data are the means ± S.E.M. of 4–6 determinations. *$P < 0.01$, Student's t-test. Rats were killed two weeks after bilateral deafferentation. Parameters derived from 'non-linear fitting' analysis of the saturation curves (1–24 nM).

	Sham-operated	*Deafferented*
B_{max} (fmol/mg P)	170 ± 20	120 ± 10*
K_d (nM)	7.2 ± 1.7	7.3 ± 1.1

uptake was approximately halved the lesion was effective.

[^{3}H]HCh-3 binding assay. The rats were killed by decapitation, striata and hippocampi were dissected and crude membranes obtained by sonication were centrifuged at 20,000 g for 15 min at 4 °C. The resulting pellets were washed twice then resuspended in glycylglycine buffer pH 7.8 to yield a final concentration between 200 and 800 μg/ml, used in the binding assay. The binding of [^{3}H]HCh-3 (NEN, U.S.A. 147 Ci/mmol; 1–24 nM) was performed as described by Sandberg and Coyle[19], with minor modifications. Washed crude membrane preparations were incubated at 25 °C for 30 min. Incubations were terminated by vacuum filtration with a Brandel Cell Harvester over glass fiber filters (Schleicher and Schuell No. 32) previously soaked in a 0.3% (v/v) aqueous solution of polyethyleneimine to reduce the binding of [^{3}H]HCh-3 to filters. Non-specific binding was defined as binding in the presence of 1 μM unlabelled HCh-3.

Autoradiography. The rats were decapitated and the whole brain was removed and rapidly frozen in N-pentane at −20 to −25 °C. Coronal and sagittal sections were cut at −20 °C in a cryostat and thaw-mounted onto acid-cleaned gelatin-coated slides. The slides were incubated with 10 nM [^{3}H]HCh-3 for 30 min at room temperature in 50 mM glycylglycine buffer pH 7.8 containing 200 mM NaCl. Non-specific binding was determined in adjacent sections processed in the same manner except that 10 μM unlabelled HCh-3 was added to the incubation medium. After desiccation, the slides were exposed to tritium-sensitive film 'Hyperfilm' (Amersham, U.K.) for 4 weeks and developed using the standard technique; tritiated microscales (Amersham) were coexposed. Quantitative autoradiographic analysis was done with a RAS 3000 Image Analysis System (Loats System, U.S.A.).

Table I shows the effect of frontal deafferentation on [^{3}H]HCh-3 binding parameters. The lesion reduced the [^{3}H]HCh-3 binding sites (B_{max}) in striatum of about 30%, with no change in the affinity (K_d). This effect is consistent with the general depression of cholinergic striatal activity observed after interruption of the corticostriatal pathway[6]. Neither binding parameter of deafferented rats was modified in the hippocampal region (data not shown), indicating the region specificity of the phenomenon.

The effect of frontal deafferentation on [^{3}H]HCh-3 binding sites was investigated in a neuroanatomical

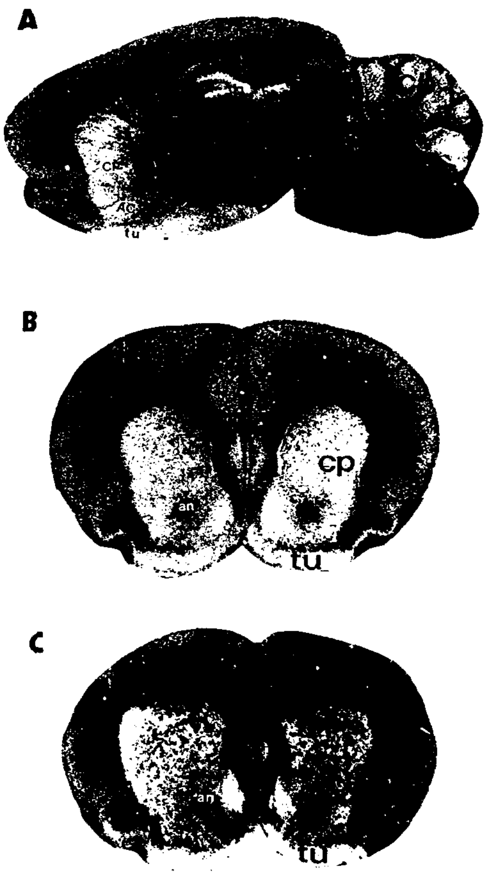

Fig. 1. Autoradiograms with [^{3}H]HCh-3 binding site distribution in sagittal (A) and coronal (B) sections of sham-operated and coronal section of deafferented rat (C). The signal (white) was high in caudate-putamen (cp) with a mediolateral gradient, olfactory tubercle (tu) accumbens (ac) and dentate gyrus (dg), medium in the superior layers of cortex (cx), the pyramidal layer of hippocampus (h) and the habenula (ha). Note the absence of signal (black) in the anterior commensure (an). In cerebellum (cb) the high signal appears largely due to a non-specific binding as previously demonstrated[1]. In C note the reduction of autoradiographic signal in deafferented striata (cp); in contrast the signal in olfactory tubercle (tu) appears unchanged.

TABLE II

Effect of frontal deafferentation on the [³H]HCh-3 binding in striatum and accumbens–olfactory tubercle determined by autoradiography

The data are the means ± S.E.M. of 4–5 rats (3–4 sections per brain). *$P < 0.01$, Student's t-test. Rats were killed two weeks after frontal bilateral deafferentation. One concentration (10 nM) of [³H]HCh-3 was used.

	Binding [³H]HCh-3/fmol/mg P	
	Sham-operated	*Deafferented*
Striatum		
Anteromedial part	425.2 ± 20.2	293.6 ± 12.0*
Posterior part	317.6 ± 16.4	325.4 ± 20.1
Accumbens–olfactory tubercle	197.0 ± 8.7	170.3 ± 23.9

context by autoradiography technique. Fig. 1 illustrates the autoradiograms of [^{3}H]HCh-3 binding in sagittal (A) and coronal (B) sections. The autoradiographic signal was high in the striatum, accumbens and dentate gyrus, intermediate in hippocampus and cerebral cortex and low in thalamus and hypothalamus. This further indicates that the distribution of [^{3}H]HCh-3 binding sites in rat brain fits well with the localization of cholinergic terminals[3], as previously shown by other investigators[1,14].

The autoradiogram of the [^{3}H]HCh-3 binding distribution in coronal section of bilateral deafferented rat is illustrated in Fig. 1C. A substantial decrease of [^{3}H]HCh-3 binding sites was seen in striata of bilaterally deafferented rats compared to the sham-operated rats; no changes were noted in other structures. A similar reduction was found in striata of unilaterally lesioned rats (data not shown). This picture is consistent with the determination of other cholinergic parameters[6] and confirms that collateral fibers, described in frontal cortex[15], did not influence the functional depression of striatal cholinergic neurons induced by deafferentation.

Table II summarizes the results of quantitative image analysis. The absolute values of [^{3}H]HCh-3 binding site density obtained by autoradiography appeared different from the B_{max} calculated from the saturation curve in homogenate. The slight discrepancy in the binding site numbers is justified by the different conditions of evaluation[1,13]. Bilateral deafferentation produced a decrease (32%) in the [^{3}H]HCh-3 binding sites in the anteromedial portion of the striatum, equivalent to that observed in equilibrium binding experiments. No changes were detected in the posterior part adjacent to the globus pallidus or in the accumbens–olfactory tubercle region. Excitatory inputs have been described from the frontal cortex to these parts[4,9], but apparently they do not directly modulate the activity of cholinergic neurons.

The autoradiographic distribution of [^{3}H]HCh-3 binding sites was quantified in frontal deafferented and sham-operated rats after intraperitoneal OXI at the optimal dose of 100 mg/kg on the basis of previous studies[5] and our preliminary investigations (data not shown). Brain coronal sections, which included the anteromedial part of the caudate-putamen and the accumbens–tubercle olfactorium region, were analyzed. In sham-operated rats OXI did not significantly alter [^{3}H]HCh-3 binding in either striatum or accumbens, but there was a slight increase of the signal in the limbic area. In deafferented rats, the nootropic drug overcame the effect of the lesion and even produced a significant increase — 15% — in striatal [^{3}H]HCh-3 binding sites compared to sham-operated animals. A similar trend was seen also considering only the laterodorsal portion of striatum, where the density of [^{3}H]HCh-3 binding sites was higher. This result is consistent with the increase in SDHACU in deafferented striatum after OXI treatment[7], and provides strong support for the concept that [^{3}H]HCh-3 binding is a reliable index of SDHACU activity.

In addition, it appears from the results that OXI acts only in animals with impaired cholinergic function. This is in agreement with previous biochemical experiments

TABLE III

Effect of oxiracetam on the [³H]HCh-3 binding distribution in frontal deafferented rat brain determined by autoradiography

The data are the means ± S.E.M. of 4–5 rats (3–5 sections per brain). *$P < 0.01$, **$P < 0.05$ vs respective sham-operated group, ANOVA (2 × 2) test and Tukey's test. The rats were killed two weeks after frontal bilateral deafferentation and 2 h after oxiracetam (OXI) treatment (100 mg/kg). One concentration (10 nM) of [³H]HCh-3 was used.

Brain regions	*[³H]HCh-3 binding (fmol/mg P)*				*F int*
	Sham-operated	*Deafferented*	*Oxiracetam*	*Deafferented + OXI*	
Striatum					
Anteromedial	361.4 ± 14.9	283.8 ± 15.4*	359.6 ± 12.5	411.7 ± 19.1*	17.8 $P < 0.001$
Laterodorsal	476.9 ± 20.5	396.0 ± 23.4**	472.7 ± 23.0	545.0 ± 23.1*	11.1 $P < 0.001$
Accumbens–olfactory tubercle	307.2 ± 17.2	274.0 ± 18.4	365.1 ± 29.2	361.3 ± 20.0*	n.s.

showing that OXI restores ACh release in vivo in striata of decorticated rats[5] and prevents the electroshock- or scopolamine-induced decrease in brain ACh[22] but has no effect in control animals.

The findings of this study, like those of the previous one[5] point to the possibility that OXI normalizes basic cholinergic processes by increasing the availability of choline for ACh synthesis. In accordance with this hypothesis is the finding that in certain conditions OXI could improve the synthesis of phospholipids[25], particularly the phosphatidylcholine pool, proposed as a 'reservoir' for generating choline for the synthesis of ACh[2,11]. It was reported that ACh can be synthetized from choline derived from the breakdown of endogenous phosphatidylcholine, formed de novo by the stepwise methylation of phosphatidylethanolamine[11]. Interestingly, in deafferented striatum, the transmethylation pathway is enhanced, possibly to sustain the striatal cholinergic activity depressed by the lesion[24].

However, OXI may possibly restore the tone of cholinergic neurons in deafferented striatum by modulation of NMDA receptors, as recently suggested in the hippocampus[17]. Indeed the corticostriatal pathway uses glutamate as putative neurotransmitter[8] which has been shown to increase ACh release from striatal slices in vitro through NMDA-type receptors possibly located on the cholinergic cell[12]. Therefore the direct or indirect activation of NMDA receptors by OXI could normalize the activity of cholinergic interneurons in deafferented striata.

In conclusion, according to the depression of cholinergic function, both autoradiographic and saturation binding experiments showed a reduction in the density of [^{3}H]HCh-3 binding sites in deafferented striatum. This reduction was overcome by treatment with OXI. The restoration experiments with OXI indicate the possibility of investigating by autoradiography the functional stage of cholinergic neurons in a neuroanatomical context and identifying the decorticated striatum as a model to test drugs affecting cholinergic activity.

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AUTHORS and Address

M. Ammassari-Teule, D. Amoroso*, G.L. Forloni*, C. Rossi Arnaud and S. Consolo*.

Istituto di Psicobiologia e Psicofarmacologia, Via Reno, 1 Rome 00198, Italy & (*) Istituto Mario Negri, Via Eritrea 62, 20157 Milan, Italy.

Abstract

The present research examines the behavioral and neurochemical alterations resulting from different lesions of the striato cortical pathways. The first lesion was a mechanical disconnection of all striato-cortical projections under the corpus callosum. The two others consisted in more selective damages of the projections from the nucleus basalis to the frontal cortex performed by injecting either ibotenic or quisqualic acid in that nucleus. Three groups of rats bearing those lesions and their respective controls were tested in a radial eight-arm maze for ten consecutive daily trials. On each day, three dependent variables were recorded: the number of unrepeated path choices, the rank of occurrence of the first error and the degree of divergence of the sequences of runs. At the end of the experiment, the rats were sacrificed and basic neurochemical cholinergic parameters were assessed. The results show that the mechanical disconnection of striato-cortical pathways induces severe deficits of acquisition associated with modifications of maze-running strategies (MRS). Ibotenic acid lesions impair acquisition in a less drastic fashion while quisqualic acid lesions do not affect acquisition performance but strongly modify MRS. Neurochemical data indicate modifications of all cholinergic parameters after the three types of lesions. However, the main specific and consistent cholinergic depletion was obtained with quisqualic acid lesions. These results suggest that the hypothesis according to which the cholinergic pathway from the nucleus basalis to the frontal cortex controls spatial learning and memory should be reconsidered. Its role in the organization of exploration suggests a more direct participation to the reactivity to environmental stimuli which, in turn, could influence cognitive functions.

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AUTHOR(S) (in capitals) / Address:

D. AMOROSO, G. L. FORLONI, N. ANGERETTI, A. ADDIS and S. CONSOLO, Lab. of Cholinergic Neuropharmacology , "Mario Negri" Institute,20157 Milano, Italy.

Abstract

Frontal deafferentation of rat striatum reduces the tone of striatal cholinergic interneurons. After two weeks, the lesion resulted in 40% reductions in acetylcholine (ACh) release in vivo, in sodium-dependent high-affinity uptake of choline (SDHACU) and in a 30% decrease in the number of [^{3}H]hemicholinium-3 ([^{3}H]HCh-3) binding (BMAX) to SDHACU sites with no significant change in the binding affinity (KD). Autoradiography showed a significant reduction of [^{3}H]HCh-3 binding sites in the anteromedial portion of the striatum, but not in the posterior part of frontal deafferented rats. Acute treatment with oxiracetam (OXI) 100 mg/kg, i.p., induced a recovery of ACh output from the striata of deafferented rats. At 30 min after drug injection, ACh release was already significantly higher in the OXI-treated group than in saline-treated group, with complete recovery occurring by 80 min. The full effect lasted at least 100 min longer. OXI also normalized SDHACU activity 2 h after its administration. Consistantly with this result, the nootropic drug completely overcame the reduction of [^{3}H]HCh-3 binding sites in the deafferented striatum. Restoration of the basic biochemical cholinergic processes is most likely associated with enhanced ACh formation. The results identify the deafferented striatum as a useful model for studing means to restore the deficit in cholinergic neurotransmission. US AIR FORCE grant AFOSR 87-0399