129P A COMPARISON OF THE ABILITIES OF CHEMOKINE LIGANDS TO COMPETE FOR [I^{125}] MIP-1α AND [I^{125}]RANTES BINDING TO THE CHEMOKINE RECEPTOR CCR1

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Chemokines are chemotactic cytokines involved in recruitment of leukocytes into inflamed tissue. Chemokines such as RANTES and MIP-1α are agonists at the CC chemokine receptor CCR1 (Saunders & Tarby, 1999). Proudfoot and co-workers (1995) have previously described radioligand-dependent differences in agonist binding profiles at CCR1. The aim of the present work was to characterise the binding of [I^{125}]MIP-1α and [I^{125}]RANTES to CCR1 transfected CHO cells and to compare the binding profiles of chemokine agonists and recently described non-peptide antagonists using these radioligands.

Radioligand binding was measured using scintillation proximity assay (SPA). CHO cell membranes stably expressing CCR1 (0.3 μg) were incubated for 6h with SPA beads (0.5 mg) and either increasing concentrations of [I^{125}]MIP-1α or [I^{125}]RANTES (0.001- 1 nM; saturation binding), or 50 pM of [I^{125}]MIP-1α or [I^{125}]RANTES in the presence of cold ligand (0.001 nM-100μM; competition binding). Non-specific binding (NSB) was determined using MIP-1α (1 μM). Binding parameters were estimated by non-linear regression on total binding data and expressed as mean ± s.e.mean of 3-5 experiments.

In saturation binding experiments, [I^{125}]MIP-1α and [I^{125}]RANTES bound to CCR1 with pK_D values of 9.6 ± 0.2 and 9.1 ± 0.1 and Hill slope coefficients of 0.8 ± 0.04 and 1.1 ± 0.2 respectively. However, pK_D values could not be determined to a high degree of accuracy due to the relatively low maximum concentrations of radioligands that could be achieved. The use of SPA technology precluded the calculation of B_{max} values.

In the presence of [I^{125}]MIP-1α, MIP-1α displaced ligand binding monophasically, whereas RANTES displaced binding biphasically (Table). Both agonists displaced binding to NSB levels. Compound 1 (Formamide,N-[5-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl] 2,2dipheny pentyl]-,monohydrochloride; Kato *et al.*, 1997) displaced binding biphasically to NSB levels. In contrast, Compound 2 (1,3,8-Triazaspiro[4.5]decan-4-one,8-[3-(10H-phenothiazin-10-yl)propyl]-1phenyl; Bright *et al.*, 1998) displaced binding monophasically with partial inhibition of ligand binding (Table).

Using [I^{125}]RANTES, MIP-1α and Compounds 1 and 2 displaced binding in a monophasic manner to NSB levels. Low concentrations (< 10 nM) of RANTES displaced binding (by 111 ± 2 % at 1 nM), but quantitative analysis was precluded because higher concentrations augmented binding non-specifically, as previously reported (Proudfoot *et al.*, 1995).

These experiments suggest that there may be two binding sites within the binding domain of CCR1. The data is consistent with MIP-1α having a similar affinity for both sites whereas RANTES discriminates between them. The low concentration of RANTES used in competition studies would only label its high affinity site. We hypothesise that Compound 1 binds to both sites with differing affinities, whereas Compound 2 only binds to the high affinity RANTES site.

	[I^{125}]MIP-1α			[I^{125}]RANTES		
Compound	pIC_{50}	Hill Slope	% Inhibition	pIC_{50}	Hill Slope	% Inhibition
MIP-1α	8.15 ± 0.09	0.84 ± 0.05	99.7 ± 0.3	8.80 ± 0.10	0.74 ± 0.09	96.5 ± 1.22
RANTES	9.67 ± 0.05 7.11 ± 0.14	0.40 ± 0.06*	109.8 ± 9.4	ND	ND	ND
Compound 1	7.41 ± 0.30 5.04 ± 0.17	0.57 ±0.08*	78.2 ± 14.9	6.47 ± 0.10	0.91 ± 0.13	88.0 ± 10.1
Compound 2	5.48 ± 0.11	1.33 ± 0.18	48.6 ± 4.6*	5.71 ± 0.06	1.04 ± 0.12	98.0 ± 16.1

Table. Mean pIC_{50}, Hill slope co-efficient and % inhibition values at CCR1. Values are mean ± s.e.m; n=3-5. Where Hill slope coefficients were significantly different from 1 (t-test), the data was fitted to a 2 site model. % Inhibition is defined as maximum inhibition of radioligand binding as a % of specific binding. *indicates $p<0.05$ (t-test) and ND indicates not determined.

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130P EFFECT OF mGluR ACTIVATION ON THE RELEASE OF ENDOGENOUS GLUTAMATE FROM CULTURED C6 GLIOMA CELLS

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Glial cells respond to many neurotransmitters by generating waves of rising intracellular calcium, which may trigger the release of glutamate. We have recently shown that 5-HT stimulates the release of glutamate from C6 glioma cells in a calcium-dependent manner, an effect mediated by the $5\text{-}HT_{2A}$ receptor (Meller et al., 1997). C6 glioma cells also express metabotropic glutamate receptors (mGluR) (Albasanz et al., 1997). Here we report the effect of the mGluR agonists on the release of glutamate from these cells.

C6 glioma cells were grown in 24 well plates at a density of ~20,000 cells/ well for 4 days in DMEM plus 10 % FCS and then 1 % dialysed FCS and di-butyryl cAMP (1 mM) for a further 3 days. Cells were washed 3 times with 1 ml Krebs gassed with 95 % O_2/ 5% CO_2 and then incubated at 37 °C with 0.5 ml Krebs (±drugs) in a shaking water bath for 10 min. Following incubation, 200 μl supernatant samples were removed and assayed for glutamate content using a HPLC-EC method (Meller et al., 1997). Briefly, 50 μl samples were reacted with 5 μl of a fresh OPA/ sulphite reagent for 20 min at room temp. Derivatised glutamate was separated on a 4.6 x 250 mm C_{18} Microsorb HPLC column, with a mobile phase comprising 0.1 M Na_2HPO_4, 1 mM EDTA, 10 % methanol (v/v) at pH 5.8., and detected using a glassy carbon electrode (+0.85 V vs. Ag/AgCl). Data are expressed as mean ± s.e.mean (n) and analysed statistically using Students unpaired t-test.

The mGluR agonist (±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (tACPD) (0.01 μM-1.0 mM) produced a concentration dependent increase in the release of glutamate from C6 glioma cells (max. effect = 316±16 (10) % of basal levels; EC_{50} = 88 μM). tACPD had no effect on aspartate or serine levels. Kainic acid (0.1 μM-1.0 mM) caused a small increase in glutamate release (max effect= 152±7 (4) % of basal levels; EC_{50} = 105 μM). However, the group III mGluR agonist L-2-amino-4-phosophonobutyric acid (LAP4) (0.1 μM-0.1 mM) had no effect. Other mGluR agonists were tested but interfered with the chromatography, which precluded their further use. The effect of tACPD (1 mM) was inhibited by the group I mGluR antagonist DL-2-amino-3-phosphonopropionic acid (DL-AP3) (IC_{50} = 0.5 mM; $p<0.05$), but not the $mGluR_1$ antagonist (selective vs. $mGluR_5$ (Moroni et al., 1997)) RS-1-aminoindan-1,5-dicarboxylic acid, at concentrations up to 1 mM.

The glutamate response to tACPD (1 mM) was not inhibited in low calcium medium (1 mM EGTA/ 5 mM $MgCl_2$) (285±16 (8) % vs. 294±22 (10) % of control). The glutamate response to 0.1 mM tACPD was inhibited following 10 min preincubation of the C6 glioma cells with either BAPTA-AM (10 μM) (154±6 (4) % vs. 109±12 (4) % of control; $p<0.05$) or thapsigargin (1.0 μM) (156±7 (4) % vs. 128±9 (4) % of control; $p<0.05$). The effect of tACPD (1 mM) was also inhibited by caffeine (20 mM) (322±20 (4) % vs. 220±16 (4) % of control; $p<0.05$). Finally, the effect of tACPD (0.1 mM) was not blocked by the glutamate uptake blocker 2,4 trans-pyrollidine dicarboxylic acid (50 μM) or the anion transport inhibitor frusemide (5 mM).

These data suggest that activation of the group I mGluR (possibly $mGluR_5$) on C6 glioma cells, stimulates the release of glutamate. The release of glutamate is unlikely to be due to osmotic swelling or reversal of the glutamate uptake, but may be associated with the release of calcium from intracellular stores. However, unlike 5-HT, the glutamate release triggered by mGluR activation is not dependent on external calcium.

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131P PHARMACOLOGICAL CHARACTERISATION OF HUMAN A_1 AND A_{2A} RECEPTORS CO-EXPRESSED WITH $G_{\alpha_{16}}$ IN CHO-K1 CELLS USING A FLUORIMETRIC IMAGING PLATE READER (FLIPR)

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The human adenosine A_1 and A_{2A} receptors have been stably expressed in CHO-K1 cells. The A_1 and A_{2A} receptors are endogenously negatively and positively coupled to adenylate cyclase respectively. We have co-expressed the promiscuous G-protein $G_{\alpha_{16}}$ that couples both receptors to phospholipase C (Milligan et al., 1996), with the goal of developing a rapid high throughput functional screening assay using a fluorometric imaging plate reader (FLIPR).

Cells were preloaded with the calcium sensitive dye Fluo-3-AM for approx. 90 min. Following removal of unincorporated dye, no fluorescence signal to agonists was obtained from transfected cells that did not contain $G_{\alpha_{16}}$. In cells that co-expressed $G_{\alpha_{16}}$, agonist addition caused a rapid rise in intracellular calcium.

Table 1: Agonist characterisation of human A_1 and A_{2A} receptors co-expressing $G_{\alpha_{16}}$

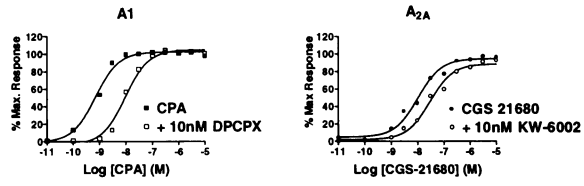
Compound	A_1 pEC_{50}	A_{2A} pEC_{50}
CPA	9.0	6.5
CHA	8.8	6.7
NECA	8.5	7.7
CGS-21680	5.8	7.4
Adenosine	8.3	7.1
R-PIA	9.2	7.5
S-PIA	7.9	6.5

Abbreviations used are N^6-cyclopentyladenosine (CPA), N^6-cyclohexyladenosine (CHA), and 5'-N-ethylcarboxamidoadenosine (NECA), N^6-(2-phenylisopropyl)adenosine (PIA).

These results agree with the published literature (Jarvis et al., 1989; Klotz et al., 1998).

Antagonist potency was calculated according to the following equation $pA_2 = -Log[A] - Log (DR-1)$ where A is the concentration of antagonist used and DR represents the dose ratio of the calculated EC_{50} values for the agonists in the absence and presence of the antagonist. Due to low expression levels of the A_{2A} receptors in CHO-K1 cells, 10mM sodium butyrate was used to increase receptor expression levels.

Figure 1 Antagonism of A_1 and A_{2A} mediated responses



The cells were pre-incubated with a fixed concentration of antagonist for 10 minutes prior to agonist addition.

The selective A_1 antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) had a pA_2 of 9.2 and 7.4 at A_1 and A_{2A} receptors confirming its selectivity for the A_1 receptor. The selective A_{2A} antagonists KW-6002 had a pA_2 of 6.5 and 8.3 at A_1 and A_{2A} receptors respectively (figure 1).

In conclusion, these results confirm the role of $G_{\alpha_{16}}$ as a useful tool for linking G-protein coupled receptors to a calcium signal, and the utility of FLIPR as a universal high throughput-screening tool for G-protein coupled receptors.

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132P CHARACTERIZATION OF A NEWLY SYNTHESIZED PYRIMIDO[4,5-*b*]INDOLE (PPPIA) AS AN ADENOSINE A1 RECEPTOR AGONIST

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Adenosine is an endogenous nucleoside that is formed under pathophysiological conditions, including hypoxia. The depression of neuronal activity by inhibiting neurotransmitter release from presynaptic nerve terminals and the change of the potassium conductance of postsynaptic nerve cells appear to be mediated by adenosine A1 receptors. The aim of the present study was to characterize a newly synthesized pyrimidoindole, (*R*)-9-(1-phenylethyl)-2-(4'-pyridyl)-9*H*-pyrimido[4,5-*b*]indol-4-amine (PPPIA), which showed a high affinity to the A1 receptor in binding studies (K_i=46nM), as an adenosine A1 receptor agonist or antagonist. Therefore, in a first series of experiments we investigated PPPIA on the electrically evoked noradrenaline (NA) release from rat hippocampal slices. In additional experiments the effects of PPPIA were tested on membrane potential and input resistance observed during hypoxia in rat cortical pyramidal neurons.

Brain slices (300µm thick) of male Wistar rats (own breed: 150-200g) containing the primary somatosensory region of the parietal cortex or the hippocampus were prepared. Hippocampal slices loaded with ^{3}H-NA (0.1µM) were subjected to electrical field stimulation (360 pulses; 3Hz) during superfusion in the presence of (+)-oxaprotiline (1µM), an inhibitor of NA reuptake and rauwolscine (0.1µM), an α_2-adrenoceptor antagonist. ^{3}H-NA release was measured in 4 min fractions. In cortical slices changes of membrane potential and input resistance of pyramidal cells in layers II-III were investigated by means of the intracellular recording technique. Hypoxia (5 min duration) was evoked by superfusing the slices with medium in which 95%O_2-5%CO_2 was replaced by 95%N_2-5%CO_2.

The adenosine A1 receptor agonist N^6-cyclopentyladenosine (CPA, 100µM) and the pyrimidoindole PPPIA (1 and 10µM) inhibited electrically evoked ^{3}H-NA release from hippocampal slices in a dose-dependent manner. PPPIA was more potent than CPA. The effect of CPA and PPPIA on ^{3}H-NA release was partially antagonized by the A1 receptor antagonist 8-cyclopentyl-2,3-dipropylxanthine (DPCPX, 0.1µM), which caused a slight increase in overflow per se. Hypoxia induced a membrane depolarization and decrease of input resistance in cortical pyramidal cells. The hypoxic depolarization was inhibited by CPA (100µM). DPCPX (0.1µM) had no effect on the hypoxic depolarization per se but antagonized the inhibitory effect of CPA. Like CPA, PPPIA (1 and 10µM) diminished the hypoxic depolarization. In the presence of PPPIA (1µM), CPA had no additional inhibitory effect on the hypoxic depolarization. When the slices were superfused with DPCPX (0.1µM), PPPIA at 1µM and 10µM failed to inhibit the hypoxic depolarization. The inhibitory effects induced by both, CPA and PPPIA were reversed by washout. CPA and PPPIA did not influence the hypoxic changes of the input resistance.

In summary, our results indicate that the newly synthesized pyrimidoindol PPPIA might be an adenosine receptor agonist, which decreases the electrically evoked release of NA and inhibits the hypoxic depolarization by acting on A1 receptors.

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133P KINETICS OF CO LIGATION WITH A MUTANT LACKING THE CALMODULIN BINDING DOMAIN OF ENDOTHELIAL NITRIC OXIDE SYNTHASE BY FLASH PHOTOLYSIS AND STOPPED-FLOW SPECTROPHOTOMETRY

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Nitric oxide synthase (NOS) catalyzes conversion of L-arginine to nitric oxide, which subsequently stimulates a host of physiological processes (Knowles, 1989). The calmodulin binding domain comprises 20 aminoacids and links the flavin-domain with the hemedomain. It was previously believed that its deletion should not change the haemepocket but could influence dimerization and access to the haemepocket by positioning the flavin domain closer to the haemedomain.

The kinetics of CO and NO ligation with a Mutant lacking the Calmodulin Binding Domain NOS were determined in the presence and absence of tetrahydrobiopterin and Arg to allow comparison with wildtype NOS.

Geminate recombination in the nanosecond time domain is followed by bimolecular association in the millisecond time domain. Complex association kinetics imply considerable heterogeneity but can be approximated with two forms, one fast 1-9 10^6 and another slow 1-5x 10^3 $M^{-1}s^{-1}$ both 4-10 times slower than in wildtype neuronal NOS with the slow component predominating in the presence of arginine. Previously, we had not found any significant difference in the kinetic data between wt-nNOS and wt-eNOS. The relative proportions of the two forms varied dependent on the presence of arginine like in wildtype but tetrahydrobiopterin did not seem to affect association data indicating that the calmodulin domain contributes to the tetrahydrobiopterin binding. Geminate recombination is substantial and varying from 10% to 45% with its amplitude being positively correlated with the fast component as in wildtype.

The results seem to indicate that the calmodulin binding domain contributes to tetrahydrobiopterin binding and the haeme pocket, consistent with the recently published crystall structure.

Table 1
kinetic rate constants for binding CO to calmodulin domain deletion mutant of ferrous eNOS

System	agem/ΔA(0)	kgem	af/(af+as)	ka,f	ka,s	kd,f	kd,s
		s-1		[M-1s-1]	[M-1s-1]	s-1	s-1
delta cal	0.15±0.03	1.4 e7	0.94±0.02	5.9 e5	1.0 e3	< 15	3
delta cal+Arg	0.05±0.03	1.2 e7	0.20±0.07	1.2 e5	2.7 e3	< 5	0.7
delta cal+BH4	0.22±0.03	1.5 e7	0.86±0.04	9.0 e5	3.9 e3	< 50	0.8
delta cal+Arg+Bh4	0.03±0.02	1.2 e7	-	-	4.9 e3	–	1.4

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134P KINETICS OF CO LIGATION WITH A CYS331Ala MUTANT OF NEURONAL NITRIC OXIDE SYNTHASE BY FLASH PHOTOLYSIS AND STOPPED-FLOW SPECTROPHOTOMETRY

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Nitric oxide synthase (NOS) catalyzes conversion of L-arginine to nitric oxide, which subsequently stimulates a host of physiological processes.

The kinetics of CO ligation with the Cys331Ala mutant of neuronal NOS were determined in the presence and absence of tetrahydrobiopterin and Arg to allow comparison with wildtype neuronal NOS.

Flash photolysis was used to determine association and dissociation constants. In addition stopped- flow mixing was used to varify dissociation constants. As in wildtype neuronal NOS geminate recombination in the nanosecond time domain is followed by bimolecular association in the millisecond time domain. Complex association kinetics imply considerable heterogeneity but can be approximated with two forms, one fast 2-3x and another slow 2-4x 10^4 $M^{-1}s^{-1}$ like in wildtype neuronal NOS but the fast component predominating. The relative proportions of the two forms varied less with tetrahydrobiopterin and arginine than in wildtype indicating that tetrahydrobiopterin binding was abrogated by the Cys331Ala point mutation. Geminate recombination is substantial and varying from 40% to 60% with its amplitude being positively correlated with the fast component as in wildtype (Table 1). Overnight incubation with L- Arginine seems to generate kinetic properties of wt- protein and allow normal BH_4- binding as indicated by incubation with increasing concentrations of L-Arg. (Table 2).

Table 1
Kinetic rate constants for binding CO to a Cys331Ala mutant of ferrous nNOS

System	agem/ΔA(0)	Kgem	af/(af+as)	ka,f	ka,s	kd,f	kd,s
		s-1	[M-1s-1]	[M-1s-1]	s-1	s-1	
HoLo	0.50±0.05	1.2e7	0.96±0.02	4.3e6	11e4	<50	<10
HoLo+Arg	0.46±0.03	1.0e7	0.60±0.08	1.7e6	12e4	<50	<2
HoLo+BH4	0.61±0.05	1.4e7	0.93±0.04	3.2e6	8e4	<100	<2
HoLo+Arg+BH4	0.50±0.06	1.4e7	0.75±0.1	4e6	4e4	<100	<1

Table 2
100% CO Fe2+ Cys 331 [Arg] dependence

System	Amp(fast)/Amp(slow)	ka,f	ka,s
0Arg	4.51	4.1x e6	7.5x e5
0.1mM Arg	1.09	3.1x e6	2.1x e4
1.0mM Arg	0.24	4.3x e6	2.1x e4
10mM Arg	0.77	4.3x e6	2.1x e4
0mM Arg+ 250 µMBH4	0.51	5.5x e6	2.0x e4
0.1mM Arg+250 µMBh4	0.2	4.2x e6	2.0x e4
1.0mM Arg+250 µMBH4	0	n.a.	2.1x e4
10mM Arg+ 250 µMBH4	0	n.a.	2.1x e4

The data are consistent with other data indicating the collaps of the hame pocket preventing tetrahydrobiopterin binding and weakening the proximal base-haeme bond. Incubation with at least 1mM L-Arginine seems to restore the structural integrity of the haeme pocket indicated by rate constants similar to wt- Protein.

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135P KINETICS OF CO LIGATION WITH NITRIC OXIDE SYNTHASE BY FLASH PHOTOLYSIS AND STOPPED-FLOW SPECTROPHOTOMETRY

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Interaction of CO with haemeproteins has physiological importance. This is especially true for nitric-oxide sythases (NOS), haeme/flavoenzymes that produce NO and citrulline from L-arginine (Arg) and are inhibited by CO in vitro (Knowles, 1989; Palmer, 1989).

The kinetics of CO ligation with both neuronal NOS and its heme domain module were determined in the presence and absence of tetrahydrobiopterin and Arg to allow comparison with other hemeproteins and to determine the equilibrium constant of CO- binding.

Rat nNOS-Holoenzyme and the amino-terminal haeme-binding domain (residues 1-714) of rat nNOS and were expressed in E. Coli purified and reconstituted as described previously (McMillan and Masters, 1995; Roman, 1995). Flash photolysis used an improved version of an instrument described previously (8). Photolysis laser pulses were 4 ns in duration at 545 nm with up to 4 mJ of energy over an area of 0.1cm^2. The probe had an 8-nm bandwidth selected from a stable tungsten lamp, except that measurements of geminate recombination required a pulsed xenon flash. The photomultiplier was wired with both a large standing current and large interdynode capacitors to optimize linearity. The digitizer had been upgraded to a lecroy Model 9361 digitizing oscilloscope.

Stopped-flow measurements were carried out at 20^{0}C using a Durrum instrument following protocols described earlier (Sharma, 1983). The CO dissociation rates were determined both by the microperoxidase method (Sharma, 1976) and by replacing CO with NO (Olson, 1981).

Geminate recombiation in the nanosecond time domain is followed by bimolecular association in millisecond time domain. Complex association kinetics imply considerable heterogeneity but can be approximated with two forms, one fast (2-3 x 10^6 M^{-1} s^{-1}) and another slow 2-4 x10^4 M^{-1} s^{-1}). The relative proportions of the two forms vary with conditions. The relative proportions of the forms vary with conditions (Table 1). For the haemedomain, fast forms dominate except when both reagents are absent.

Geminate recombination is substantial, 50%, only when fast forms predominate. Stopped flow mixing found dissociation constants near 0.3s^{-1} (Table 1).

Table 1
Kinetic rate constants for binding CO to nNOS heme domain and nNOS holoenzyme
Rates listed here do not always represent single exponential decays

System[a]	$a_{gem}/\Delta A(0)$	k_{gem}	$a_f/(a_f+a_s)$	$k_{a,f}$	$k_{a,s}$	k_d
Dom	0.35±0.10	2.0±0.5x10^7	0.95±0.03	2.1±0.2x10^6	3.5±0.5 x 10^4	0.15
Dom + Arg	0.40±0.10	1.6±0.5x10^7	0.09±0.04	3.5±0.2x10^6	2.2±1.5 x 10^4	0.32
Dom + BH_4	0.55±0.10	1.8±0.3x10^7	0.95±0.22	3.0±0.4x10^6	3.0±0.3 x 10^4	0.16
Dom + Arg + BH_4	0.08±0.03	1.8±0.5x10^7	0.30±0.05	1.5±0.1x10^6	2.1±0.1 x 10^4	0.34
Holo	0.45±0.05	1.3±0.5x10^7	0.94±0.02	2.8±0.2x10^6	4.2±0.5 x 10^4	
Holo + Arg	0.09±0.03	1.2±0.4x10^7	0.10±0.03	2.0±0.2x10^6	1.8±0.2 x 10^4	
Holo + BH_4	0.10±0.03	1.8±0.5x10^7	0.14±0.03	1.9±0.2x10^6	2.2±0.2 x 10^4	0.16 and 15
Holo + Arg + BH_4	<0.03		0.03±0.01	~ 2-3x10^6	2.0±0.1 x 10^4	0.33

[a]Dom, domain; Holo, Holoenzyme

These data imply an equilibrium constant such that very little CO should bind at physiological conditions unless large CO concentrations are present locally.

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136P APICAL UPTAKE OF L-DOPA BY L-TYPE AMINO ACID TRANSPORTER IN PORCINE KIDNEY LLC-PK$_1$ CELLS

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The urinary excretion of dopamine, an intrarenal natriuretic hormone, parallels the urinary excretion of sodium during both low and high sodium intakes, suggesting that amine formation may depend on sodium renal delivery (Goldstein et *al.*, 1989). The aim of present study was to examine the result of manoeuvres that affect cellular sodium gradients on the apical inward transfer of L-DOPA, the immediate precursor of dopamine, in LLC-PK$_1$ cells (Soares-da-Silva et *al.*, 1998). In an additional set of experiments, 2-aminobicyclo(2,2,1)-heptane-2-carboxylic acid (BHC) and N-(methylamino)-isobutyric acid (MeAIB) were used to define the type of amino acid transporter involved in the apical inward transfer of L-DOPA. LLC-PK$_1$ cells (ATCC CRL 1392; passages 210-215) were grown in Medium 199 supplemented with 100 U ml^{-1} penicillin G, 0.25 µg ml^{-1} amphotericin B, 100 µg ml^{-1} streptomycin, 3% foetal bovine serum and 25 mM HEPES. After 6 days, the cells formed a monolayer and each 2 cm^2 culture well contained about 80 µg of cell protein; 24 h before the experiments the cell culture medium was changed to a serum free medium. In uptake studies, cells were preincubated (30 min) with Hanks' medium with added tolcapone (1 µM) and benserazide (1 µM). Cellular L-DOPA was assayed by h.p.l.c. with electrochemical detection. L-DOPA was applied from the apical cell side at non-saturating (2.5 µM) and saturating (up to 1000 µM) concentrations for 6 min. Results are arithmetic means with s.e.mean, n=4-5. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Newman-Keuls test for multiple comparisons. A P value less than 0.05 was assumed to denote a significant difference. Non-linear analysis of the saturation curve for L-DOPA revealed a K_m value (in µM) of 47.4±8.3 and a V_{max} value (in pmol mg protein^{-1} 6 min^{-1}) of 3069±224. Reducing extracellular sodium (from 140 mM to 70, 35 and 0 mM) did not affect the accumulation of L-DOPA (2.5 µM). Moreover, in the absence of extracellular sodium (replaced by an equimolar concentration of choline), K_m (38.1±7.8) and V_{max} (2608±190) values for L-DOPA were similar to those observed in the presence of sodium. Manoeuvres which affect transepithelial flux of sodium, such as acidification of the extracellular milieu (from pH=7.4 to pH=6.9 or pH 6.4) and the addition of amphotericin B (2.5 µg ml^{-1}), amiloride (100 µM) or ouabain (500 µM) failed to affect the accumulation of L-DOPA. N-(methylamino)-isobutyric acid (MeAIB; 1 mM) failed to affect the uptake of L-DOPA, whereas 2-aminobicyclo(2,2,1)-heptane-2-carboxylic acid (BHC) produced a concentration-dependent inhibition of L-DOPA uptake (IC_{50}=251±26 µM). The inhibitory effect of 1 mM BHC on the accumulation of L-DOPA was of the competitive type, as evidenced by the increase in K_m (192±21) but not V_{max} (4959±289) values for L-DOPA uptake. It is concluded that LLC-PK$_1$ cells are endowed with the L-type amino acid transporter through which L-DOPA can be taken up.

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137P Ca^{2+}/CALMODULIN-MEDIATED PATHWAYS AND THE CONTROL OF APICAL UPTAKE OF L-DOPA IN PORCINE KIDNEY LLC-PK_1 CELLS

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The formation of renal dopamine, an intrarenal natriuretic hormone, depends on the uptake of filtered or circulating L-DOPA and its decarboxylation in tubular epithelial cells, but the mechanism and cellular regulation of L-DOPA epithelial inward transfer is poorly understood (Soares-da-Silva et *al.*, 1998). The present study examined the result of manoeuvres that affect molecular mechanisms, namely those concerning protein kinase A (PKA), protein kinase C (PKC), protein kinase G (PKG), protein tyrosine kinase (PTK) and Ca^{2+}/calmodulin mediated pathways, on the uptake of L-DOPA. LLC-PK_1 cells (ATCC CRL 1392; passages 210-215) were grown in Medium 199 supplemented with 100 U ml^{-1} penicillin G, 0.25 µg ml^{-1} amphotericin B, 100 µg ml^{-1} streptomycin, 3% foetal bovine serum and 25 mM HEPES. After 6 days, the cells formed a monolayer and each 2 cm^2 culture well contained about 80 µg of cell protein; 24 h before the experiments the cell culture medium was changed to a serum free medium. In uptake studies, cells were preincubated (30 min) with Hanks' medium with added tolcapone (1 µM) and benserazide (1 µM). L-DOPA was assayed by h.p.l.c. with electrochemical detection. L-DOPA was applied from the apical cell side at non-saturating (2.5 µM) and saturating (up to 1000 µM) concentrations for 6 min. Results are arithmetic means with s.e.mean, n=4-5. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Newman-Keuls test for multiple comparisons. A P value less than 0.05 was assumed to denote a significant difference. Cyclic AMP (0.5 mM), forskolin (50 µM), isobutylmethylxanthine (1 mM) and cholera toxin (5 µg ml^{-1}) failed to affect the accumulation of a non-saturating (2.5 µM) concentration of L-DOPA. Similarly, cyclic GMP (1 mM), zaprinast (30 µM), LY 83583 (30 µM) and sodium nitroprusside (100 µM) failed to affect the accumulation of L-DOPA (2.5 µM). The PKC activator phorbol 12,13-dibutyrate (PDBu, 1 µM), the inactive phorbol ester 4α-phorbol 12,13-didecanoate (PDDC, 1 µM) and the PKC inhibitor chelerythrine (50 µM) also failed to affect the accumulation of L-DOPA (2.5 µM). The PTK inhibitors genistein and tyrphostin 25 were found to increase the accumulation of L-DOPA (2.5 µM), whereas their negative controls genistin and tyrphostin 1 were devoid of effect. At the highest concentrations (300 µM), genistein and tyrphostin 25 significantly ($P<0.05$) increased L-DOPA accumulation by 112±23% and 37±4%, respectively. The Ca^{2+}/calmodulin inhibitors calmidazolium and trifluoperazine produced concentration-dependent inhibition of L-DOPA (2.5 µM) uptake with IC_{50}'s of 71.5±1.2 µM and 54.7±1.0 µM, respectively. The inhibitory effect of 30 µM calmidazolium on the accumulation of L-DOPA was of the non-competitive type, as evidenced by the decrease in V_{max} (3522±327 vs. 1739±21 pmol mg $protein^{-1}$ 6 min^{-1})) but not K_m (55±15 vs. 61±21 µM) values for L-DOPA uptake. It is concluded that L-DOPA uptake in LLC-PK_1 cells is a carrier-mediated system which appears to be under the control of Ca^{2+}/calmodulin mediated pathways. Protein tyrosine kinases may also affect the intracellular accumulation of L-DOPA.

Soares-da-Silva et *al.* (1998). Am. J. Physiol., 274, F243-F251.

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138P INFLUENCE OF BRADYKININ POTENTIATING $PEPTIDE_{9A}$ (BPP_{9A}) ON BRADYKININ (BK)-EVOKED EFFECTS IN DIFFERENT TARGETS

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It is now generally accepted that bradykinin potentiating peptides and ACE inhibitors can potentiate BK effects beyond blocking its enzymatic inactivation. To investigate the mechanism this potentiation is based on we examined the influence of $BPP_{9\alpha}$ (20 nM) on BK evoked contractions in isolated guinea pig ileum and on the binding of [^{3}H]BK to guinea pig ileum membranes. Further investigations concerning binding and signal transduction were made on the well characterized BK B_2 receptor of human epidermoid carcinoma cells A431.

Ilea of guinea pigs (300-500 g, both sex) were placed into organ baths with Tyrode solution (37°C) gassed with carbogen. Isotonic contractions were recorded under a resting tension of 500 mg. Radioligand binding studies in guinea pig ileum membranes were performed in a buffer containing 25 mM TES, 1 mM 1,10-phenanthroline, 140 µg/ml bacitracin, 1 mM dithiothreitol, and 0.1 % BSA. Membranes were incubated with [^{3}H]BK (1-2 nM) and increasing concentrations of unlabelled BK for 30 min at 4°C. The reaction was stopped by filtration through glassfibre filters. The [^{3}H]BK binding to A431 cells was performed in a buffer containing 25 mM TES, 300 mM sucrose, 1 mM 1,10-phenanthroline, 10 µM phosphoramidon, and 0.1 % BSA. Cells were incubated with [^{3}H]BK (1-2 nM) and increasing concentrations of unlabeled BK for 30 min at 37°C. After washing with cold PBS the cells were solubilized with 5 % SDS. $[Ca^{2+}]_i$ was measured in A431 cells by incubating the cells with FURA-2/AM (1µM) for 30 min at 37°C. Fluorescence was monitored with excitation wavelengths of 340 and 380 nm and emission wavelength of 500 nm. Maximum and minimum fluorescence ratio signals were measured using Triton X-100 and EGTA (0.1 % [v/v], 10 mM, respectively). Formation of inositol phosphates was determined by prelabelling A431 cells with 4 µCi/ml [^{3}H]inositol for 24 h. After pretreatment with 10 mM LiCl cells were stimulated for 10 min at 37°C with BK. The reaction was stopped with 10 % trichloroacetic acid and the extracts were placed on AG 1-X8 columns. The inositol phosphate fraction was eluted with 1 M ammonium formate and 0.1 M formic acid.

$BPP_{9\alpha}$ increased significantly the pEC_{50} values (mean ± s.e.m.) of BK induced contractions of isolated guinea pig ilea from 7.19 ± 0.25 to 7.45 ± 0.23 (n = 9, $P<0.01$, paired t-test), whereas binding parameters of the B_2 receptor in guinea pig ileum membranes (K_i = 0.43 ± 0.12 nM, n = 5) were not changed in the presence of $BPP_{9\alpha}$ (K_i = 0.25 ± 0.08 nM, n = 5). Whether or not $BPP_{9\alpha}$ had any effect on receptor internalisation could not be determined, but this study is currently being extended using cultured myocytes of ileum. At human A431 cells competition binding studies showed no significant change of the K_i value (1.24 ± 0.45 nM, n = 6) in the presence of $BPP_{9\alpha}$. Likewise, there could be found no influence of $BPP_{9\alpha}$ on the BK induced Ca^{2+} flux (BK: EC_{50} = 8.15 ± 3.56 nM, with $BPP_{9\alpha}$: EC_{50} = 5.10 ± 1.41 nM, n = 5) and on inositol phosphate formation (BK: EC_{50} = 3.74 ± 1.74 nM, with $BPP_{9\alpha}$: EC_{50} = 8.15 ± 4.02 nM, n = 3). The results could support the hypothesis of Minshall *et al.* (1997) that the expression of ACE is necessary for the potentiation of BK B_2 receptor evoked responses, because the content of ACE in A431 cells is very low (G. Vietinghoff, personal communication) in comparison to e.g. endothelium. Future work will include investigations into the involvement of G proteins and a potential crosstalk between ACE and the BK B_2 receptor.

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139P CONSTITUTIVE ACTIVITY OF ENDOGENOUSLY EXPRESSED β_2-ADRENOCEPTORS IN BC3H1 CELLS

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β_2-Adrenoceptors can exist in a constitutively active state following site-directed mutagenesis (Lefkowitz et al., 1993) or overexpression of wild-type β_2-adrenoceptors in transfected cell lines (Chidiac et al., 1994). In both these systems, ICI 118551 can act as an inverse agonist and reduce the elevated basal accumulation of cyclic AMP resulting from constitutive receptor activity. Here we show that β_2-adrenoceptors endogenously expressed in BC3H1 cells also exhibit constitutive receptor activity.

BC3H1 cells were cultured at 37°C in DMEM supplemented with 2mM glutamine and 20% FCS under an atmosphere of 10% CO_2 in humidified air. ^{3}H-cyclic AMP accumulation was measured in ^{3}H-adenine-labelled cells as described previously (McDonnell et al., 1998). β_2-Adrenoceptor number was determined using ^{125}I-iodocyanopindolol (McDonnell et al., 1998).

Isoprenaline -stimulated (log EC_{50} = -6.93 ± 0.11, n=16) ^{3}H-cyclic AMP accumulation in BC3H1 cells was antagonised with high affinity by the β_2-adrenoceptor selective antagonist ICI 118551 (30nM, apparent PA_2 = -8.98 ± 0.33, n=4). A similar value was obtained (PA_2 = -9.30 ± 0.20, n=4) when the β_2-selective agonist salbutamol was used. In contrast, the β_1-selective antagonist atenolol was much weaker (PA_2 = -5.81 ± 0.15, n=4 [isoprenaline] & -5.22 ± 0.11, n=4 [salbutamol]).

ICI 118551 produced a significant ($p < 0.001$, two-way ANOVA) and concentration-dependent (log IC_{50} -8.15 ± 0.12) reduction (28.0 ± 1.6 % at 300nM; n=7) of basal ^{3}H-cAMP accumulation in BC3H1 cells. In contrast, propranolol (n=8) and atenolol (n=6) produced no significant effect on basal cyclic AMP levels at concentrations up to 3μM and 100μM respectively. In addition, alprenolol (100nM) was able to attenuate the inhibitory effect of ICI 118551 on basal ^{3}H-cAMP accumulation (from 29.8±2.6% to 9.0±2.6% inhibition of basal; n=3). Binding experiments with [^{125}I]-iodocyanopindolol confirmed that BC3H1 cells have a relatively low expression of endogenous β_2-adrenoceptors (77.9 ± 18.5 fmol/mg protein; log K_D -11.1 ± 0.2pM; n=4). These data suggest endogenously expressed β_2-adrenoceptors in BC3H1 cells are constitutively active and sensitive to inverse agonists such as ICI 118,551.

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140P CB$_1$ CANNABINOID RECEPTOR-MEDIATED ACTIVATION OF K$^+$ CURRENT IN DDT$_1$MF-2 CELLS IS ENTIRELY DEPENDENT ON THE PRESENCE OF EXTRACELLULAR Ca^{2+}

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To date, three cannabinoid receptors, CB_1, CB_{1A} and CB_2, have been described in a variety of tissues. Stimulation of CB receptors can lead to inhibition of adenylyl cyclase, activation of inwardly rectifying K^+ channels, and inhibition of voltage dependent Ca^{2+} channels. In addition, the herbal cannabinoid receptor agonist, Δ^9-THC, induces increases in $[Ca^{2+}]_i$ in populations of DDT$_1$MF-2 smooth muscle cells (Filipeanu *et al.*, 1997). We have shown previously that increases in $[Ca^{2+}]_i$ upon H_1-histaminergic and P_2-purinergic receptor stimulation in DDT$_1$MF-2 cells are reflected as Ca^{2+} dependent K^+ currents (Molleman *et al.*, 1991a). The purpose of the present study is to characterise the changes in membrane conductances evoked by CB receptor stimulation in single DDT$_1$MF-2 cells.

The DDT$_1$MF-2 cells, derived from Syrian hamster vas deferens, where cultured as described previously (Molleman *et al.*, 1991b). The whole cell version of the patch clamp technique was applied for recording the K^+ currents, using a holding potential of -30mV. The cells where continuously superfused at a rate of 4ml.min^{-1}.The extracellular solution contained (in mM): NaCl 125, KCl 6, $MgCl_2$ 2.5, NaH_2PO_4 1.2, HEPES 10, glucose 11, sucrose 67, $CaCl_2$ 1.2, pH 7.35. The intracellular solution contained (in mM): NaCl 5, KCl 142, $MgCl_2$ 1.2, HEPES 20, glucose 11, K-ATP 5, Na-GTP 0.1, pH 7.2. All drugs were dissolved in ethanol, and shielded from light. The maximal bath concentration of ethanol was 0.1%. Statistical analysis was by unpaired Student's t-test.

The CB receptor agonist, CP55,940, evoked a dose dependent outward current (peak of 558 ±47pA at 10μM, which occurred after 124 ±6seconds, n = 6). The reversal potential for this current (established using a ramp protocol) was -84 ±5mV (n = 6) which is close to the calculated K^+ equilibrium potential of -80mV. The ramp protocol also revealed that the current showed no rectification between membrane potentials of -130 and -30mV. Removal of extracellular Ca^{2+} (n = 8), or the addition of Cd^{2+} (0.1mM, n = 3) to the extracellular solution completely abolished the outward current. The outward current was also abolished when intracellular K^+ was substituted by Cs^+ (n = 4). The CB_1 antagonist, SR141716 (1μM) delayed the onset of the outward current evoked by 10μM CP55,940 (peak occurred at 332 ±9seconds, a significant increase of 268%, $P<0.001$, n = 6), and reduced the current to 133 ±21pA (a significant inhibition of 77%, $P<0.001$, n = 6), whereas the CB_2 antagonist, SR144528 (1μM) had no effect on the outward current (514 ±25pA, n = 5).

The results show that CB_1 receptor activation in DDT$_1$MF-2 cells evokes a Ca^{2+} dependent K^+ current which is entirely dependent on the presence of extracellular Ca^{2+}. This suggests that the observed current therefore reflects Ca^{2+} release from a plasma membrane bound store or direct Ca^{2+} influx. The increase in $[Ca^{2+}]_i$ evoked by Δ^9-THC in the absence of extracellular Ca^{2+} (Filipeanu *et al.*, 1997) was not reflected as a membrane conductance change in our experiments. This could be because i. the increase in $[Ca^{2+}]_i$ is localised away from the plasma membrane, ii. an extra factor such as Ins(1,3,4,5)P_4 is required for ion channel activation (Molleman *et al.*, 1991a), or iii. Δ^9-THC has additional effects that are not mediated by the CB_1 receptor.

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Molleman, A. *et al.*(1991a) *J. Biol. Chem.* **266**, No.9, 5658-5663.
Molleman, A. *et al.*(1991b) *Eur. J. Physiol.* **417**, 479-485.

141P EFFECT OF A POINT MUTATION IN THE HUMAN D_{2SHORT} DOPAMINE RECEPTOR ON AGONISM AND INVERSE AGONISM

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Many G-protein coupled receptors (GPCRs) can demonstrate activity in the absence of an agonist. These constitutively active receptors have several predicted properties, including increased agonist affinity, basal activity and agonist potency in functional assays. The Extended Ternary Complex Model (Samama *et al.*, 1993) proposed that constitutively active receptors have an increased tendency to adopt an active (R*) conformation, which can couple to G-proteins to form the active R*G complex. Mutagenesis studies have indicated that substitution of amino acids in the C-terminal portion of the third intracellular loop of many GPCRs can increase constitutive activity, demonstrated by agonist-independent functional responses.

In the present study, a threonine residue in the third intracellular loop (residue 343) of the human D_{2short} dopamine (D_{2S}) receptor was mutated to an arginine. In order to determine if this mutated (T343R D_{2S}) receptor demonstrated characteristics of constitutive activity, inhibition or stimulation of adenosine 3',5'-cyclic monophosphate (cAMP) accumulation was monitored following addition of the agonist, dopamine or the inverse agonist, butaclamol respectively. Measurements of adenylyl cyclase activity were performed on whole Chinese hamster ovary cells stably expressing the wild-type (WT) and T343R D_{2S} receptors as described previously (Hall & Strange, 1997). Following addition of forskolin (10μM) to the cells, the effects of the ligands on cAMP accumulation were investigated.

The agonist dopamine inhibited forskolin-stimulated cAMP accumulation to an equal extent in both WT and T343R D_{2S} receptors (Table 1). However, dopamine displayed an 8-fold higher potency in T343R transfected-cells compared with WT. The addition of butaclamol to forskolin-stimulated WT receptors did not result in any further stimulation over basal levels of cAMP accumulation. In contrast, there was a 278% increase in forskolin-stimulated cAMP levels with the T343R D_{2S} receptors. Butaclamol displayed an EC_{50} of 3.7nM for this effect (Table 1). The ability of butaclamol to act as an inverse agonist at the T343R mutated receptors but not at the WT suggests that the mutants have an increased constitutive activation of signalling systems.

Table 1. Potency and efficacy of dopamine and butaclamol

Receptor:	Ligand	pEC_{50} (EC_{50}, nM)	% relative inhibition (dop) or stimulation (but)
WT:	Dopamine	7.54 ± 0.15 (28)	84.3 ± 3.1
T343R:	Dopamine	8.46 ± 0.28 (3.47) *	83.3 ± 4.8
WT:	Butaclamol	No stimulation	No stimulation
T343R:	Butaclamol	8.43 ± 0.47 (3.72)	278.1 ± 2.5

Data are mean ± s.e.mean of 3-6 experiments.
*, $p < 0.05$ compared with corresponding WT potency (Student's *t* test).

Previous work has shown that substitution of a threonine residue (T343) with an arginine in the human D_{2S} receptor resulted in an increased affinity for two dopamine agonists (dopamine and NPA) in both the absence and presence of GTP (Wilson *et al.*, 1999), suggesting that this mutated receptor may have an increased tendency to adopt the R* conformation. In agreement with this theory, the present results demonstrate that these mutated receptors also exhibit an increased potency for dopamine and an ability for the inverse agonist, butaclamol to suppress basal activity. Therefore, these receptors can be used to investigate the mechanisms of inverse agonism.

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142P IDENTIFICATION OF A TRYPTOPHAN RESIDUE AS A DETERMINANT OF AGONIST BINDING IN THE 5-HT_3 RECEPTOR

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The nicotinic acetylcholine (nACh) receptor family of neurotransmitter receptors (which includes GABA, glycine and 5-HT_3 receptors) possess a region of sequence close to the cys-cys loop that has been implicated as a major determinant of ligand specificity. We have used site-directed mutagenesis to change the tryptophan (W) residue in this region of sequence (W183) to a conserved (tyrosine, Y) and non conserved (serine, S) amino acid residue in the 5-HT_3R_{Ab} subunit, and have examined the properties of the mutant homomeric receptors using radioligand binding, functional and immunological assays.

Mutations were performed on the full length 5-HT_3R_{Ab} subunit DNA inserted into the expression vector pRc/CMV, which was then transiently transfected into HEK 293 cells. Expressed 5-HT_3 receptors were examined using 5-HT_3-specific antisera, radioligand binding, and whole cell voltage clamp as previously described (Hargreaves *et al.*, 1996; Spier *et al.*, 1999).

Table 1. Functional properties of W183 mutants

	5-HT		mCPBG	
Receptor	EC_{50} (μM)	n_H	EC_{50} (μM)	n_H
Wild-type	2.10 ± 0.40	2.22 ± 0.20	0.81 ± 0.09	1.97 ± 0.20
W183Y	194 ± 19.2*	1.92 ± 0.26	19.6 ± 2.8	2.05 ± 0.18
W183S	not detected		not detected	

(Values = s.e.mean, n = 5, *significantly different, $p < 0.05$)

5-HT_3-specific antisera show that both mutant receptors can be expressed on the surface of HEK293 cells, suggesting correct assembly and targeting. Radioligand binding studies, however, revealed no specific binding for either mutant receptor, using both [^{3}H]granisetron, a 5-HT_3 receptor antagonist at up to 10nM, and [^{3}H]m-chlorophenylbiguanide (mCPBG), a 5-HT_3 receptor agonist, at up to 50nM. Whole cell patch clamp electrophysiology, on the other hand, whilst revealing no agonist-induced responses for the S-containing mutant, did show that the Y-containing mutant was functional, although the EC_{50}s for 5-HT and mCPBG were increased 92 and 24 fold respectively compared to wild-type (Table 1).

Therefore mutation of W183 to Y caused a large increase in agonist EC_{50}, while the W183S mutant had no apparent ligand binding or function, but was expressed at the plasma membrane. The cooperative nature of ligand-binding was unaffected by the W183Y mutation, suggesting that a structural change in the receptor is unlikely, and we propose that residue W183 is an important determinant of neuro-transmitter binding in the 5-HT_3 receptor.

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143P CATECHOLAMINE-STIMULATED [^{35}S]GTPγ-S BINDING IN RAT HEART

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Agonist stimulated ^{35}S-GTP γ-S binding to cell and tissue membranes is a widely used method for the characterisation of G protein-coupled receptor function. A recent modification of the technique involves the use of tissue sections, in an autoradiographic protocol, to preserve anatomical detail (Sim et al.,1995). We have applied the method, for the first time, to sections of rat heart and we report marked stimulations of ^{35}S-GTP γ-S binding due to catecholamines that are not, apparently, adrenoceptor-mediated.

Hearts from male Wistar rats (200-300g) were rapidly frozen and cut longitudinally into 15μm sections. Thawed sections were incubated with agonists and, when appropriate, antagonists in the presence of ^{35}S-GTP γ-S (0.05nM) and 50μM GDP for 90 mins. Sections were apposed to ^{35}SβMax film (Amersham) for 1-2 days at -70°C. After development, densiometric analysis of the autoradiograms was performed and results expressed as disintegrations per minute /1000 per mm^2 (Kdpm/mm^2) by reference to co-exposed radioactive standards. In some experiments, heart or blood vessel membranes were substituted for tissue sections (Traynor and Nahorski, 1995).

^{35}S-GTP γ-S binding was somewhat greater in the atria than the ventricles but data shown refer to the ventricles alone. Basal labelling (26.4±4.1, n=66 sections) was substantially increased by noradrenaline, adrenaline, dopamine and octopamine. Stimulations were concentration-related and no maximum responses were achieved over the concentration ranges used (3-300μM). At 300μM, binding was increased by 8.9±2.5, 5.4±0.3, 5.4±1.0 and 4.8±0.4 fold basal in the presence of adrenaline, noradrenaline, dopamine and octopamine respectively. Only small responses to isoprenaline (1.8±0.2) and carbachol (1.3±0.1 fold basal) were observed. The responses to adrenaline (100μM) were not significantly inhibited by propranolol, prazosin or rawolscine (all 1μM; $p>0.05$, Students t test, n=24-32 sections) and the responses to dopamine were unaffected by SCH 23390 or sulpiride (1μM, n=6). There were no differences in the responses to the (-) isomer of adrenaline compared with the racemic (±) mixture. Qualitatively similar responses to adrenaline and dopamine were also observed in rat heart membrane preparations.

In isolated hearts from animals treated with pertussis toxin (10μg/kg, i.p. for 3 days) the profound bradycardia resulting from exposure to the cholinoceptor agonist carbachol was abolished. However, the adrenaline-enhanced ^{35}S-GTP γ-S binding in sections from the same hearts was still evident.

In membrane preparations from the rat mesenteric vasculature noradrenaline also enhanced ^{35}S-GTP γ-S binding (204± 16 fold basal at 10μM, n=3) and this was unaffected by the presence of the adrenoceptor antagonists prazosin, yohimbine or propranolol (all 1μM).

In rat heart and mesenteric vasculature, catecholamines profoundly enhance ^{35}S-GTP γ-S binding. Although this phenomenon is normally assumed to represent receptor agonist-mediated changes in the conformation of associated G proteins we have no evidence for the involvement of α or β-adrenoceptors or of a pertussis toxin-sensitive G protein. It remains to be determined whether the responses reflect a receptor-independent process or the involvement of a non-adrenoceptor mechanism.

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144P INHIBITION AND ENHANCEMENT OF APOPTOSIS BY α_1-ADRENOCEPTOR STIMULATION

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We have recently reported that human α_1-adrenoceptors can promote or inhibit cellular proliferation depending on whether activation of extracellular signal-regulated kinase (ERK) or p38 mitogen-activated protein kinase (p38) dominates (Keffel et al. 1999). We have now investigated the effect of α_1-adrenoceptor stimulation on apoptosis.

Human α_{1A}-adrenoceptors were stably expressed in Rat-1 fibroblasts at ≈ 1 pmol/mg protein (Alexandrov et al. 1998). Confluent cells were cultured in 12 well plates containing collagen-coated cover slips in the absence of serum (unless otherwise indicated) for 16 h in the absence or presence of the indicated agents. Apoptosis was measured by counting the percentage of cells (based on at least 1000 cells in at least 8 viewing fields) with condensed or fragmented chromatin, as indicated by staining with Hoe 33342 (2'-[4-ethoxyphenyl]-5-[4-methyl-1-piperazinyl]-2,5'-bi-1H-benzimidazole; 5 μg ml^{-1}), by a blinded investigator. Data are expressed as mean ± s.e. mean of % of apoptotic cells in 4-6 experiments. Statistical significance of the effects of the α_1-adrenoceptor agonist, phenylephrine (PE; 100 μM), was determined by paired t-tests with $P < 0.05$ considered significant.

Only a small fraction of cells cultured in the presence of 10% fetal calf serum exhibited signs of apoptosis (1.5 ± 0.4%), and this was not significantly affected by PE treatment (2.5 ± 0.4%). In serum-starved cells 9.3 ± 1.5% of cells were apoptotic, but PE treatment reduced this to 2.4 ± 0.3% ($P < 0.01$). In the presence of PD 98,059 (2-(2-amino-3-methoxyphenyl)-4H-1-benzopyran-4-one; 50 μM), an inhibitor of ERK activation, a large fraction of cells was apoptotic (12.1 ± 1.3%), and the protective effect of PE was mitigated and no longer statistically significant (8.1 ± 1.4%). In the presence of the p38 inhibitor SB 203,580 (4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1H-imidazole; 10 μM) 7.5 ± 1.2% of cells were apoptotic, and PE reduced this to 2.3 ± 0.2% ($P < 0.01$).

In the presence of the protein kinase C inhibitor, bisindolylmaleimide I (3 μM), 17.6 ± 4.1% of cells were apoptotic; PE did not reduce but rather enhanced this to 30.7 ± 4.6% ($P < 0.01$). In the presence of serum the percentage of apoptotic cells in the bisindolylmaleimide I-treated group was only 1.4 ± 0.2%, but was also enhanced by PE treatment (3.8 ± 0.4%; $P < 0.001$).

We conclude that α_1-adrenoceptor stimulation can counteract the pro-apoptotic effect of serum withdrawal, and this may involve the ERK rather than the p38 family of mitogen-activated protein kinases. This is surprising since α_1-adrenoceptor stimulation in our cells activates p38 but inhibits ERK (Alexandrov et al. 1998). In contrast α_1-adrenoceptor stimulation can enhance the pro-apoptotic effect of bisindolylmaleimide I independent of the presence of serum.

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Keffel, S. et al. (1999) *Naunyn-Schmiedeberg's Arch. Pharmacol.* 359 Suppl.: R26.

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145P PHARMACOLOGY OF HUMAN DOPAMINE D2 RECEPTOR COUPLING TO CHANGES IN CYTOPLASMIC Ca^{++} THROUGH THE CHIMERIC G-PROTEIN, Gqi 5, DETERMINED BY FLIPR FLUORIMETRY

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The fluorescent imaging plate reader (FLIPR) is an optical screening tool for cell-based fluorescent assays (Schroeder & Nagle, 1996), particularly measurement of cytoplasmic calcium using the calcium sensitive dye, Fluo-3AM. Gi-coupled receptors are not readily amenable to whole cell calcium fluorescence studies. Conklin *et. al.* (1993) showed that chimeric G-proteins were able to couple receptors which normally inhibit adenylyl cyclase to changes in cytoplasmic calcium concentration, but undertook little pharmacological characterisation of the response. We therefore used the human dopamine D2 receptor as a model to further characterise responses mediated by the chimeric G-protein Gqi5, and compared the results with data obtained in another functional assay.

CHO cells expressing the human D2 receptor (Coldwell *et. al.*, 1999) were transfected with the LEC-Gqi5 vector (Molecular Devices) using LipofectAMINE (Gibco). After 24 h, cells were seeded at 25000/100 µl/well in microplates. 24 h later, cells were loaded with Fluo-3AM (4 µM) for 90 min before washing with Tyrode's buffer. Antagonist or buffer were preincubated for 30 min at 37°C. Cell plates were transferred to FLIPR and 84 images taken at 1 or 5 s intervals. Basal fluorescence was 6000-10000 relative fluorescence units. Agonists were added after 20 s. Peak increase in fluorescence over basal was determined and non-cumulative concentration-response curves fitted using a four parameter logistic fit (Bowen & Jerman, 1995).

Quinpirole caused large (>120%) increases in fluorescence intensity in cells 48 h after transfection with Gqi5, but not in sham transfected controls. Responses approximately halved each 24 h thereafter. Responses to quinpirole were inhibited by pertussis toxin

Table1: Radioligand binding inhibition constants and functional potencies of agonists at human dopamine D2 receptors

Compound	radioligand binding* pK_i	micro-physiometer* pEC_{50}	FLIPR pEC_{50}
Dopamine	6.12	6.85±0.16	8.04±0.06
Pergolide	6.09	7.93±0.23	8.15±0.08
Pramipexole	5.94	8.00±0.06	8.34±0.10
Quinpirole	5.98	8.10±0.09	8.09±0.05
Quinelorane	7.58	8.50±0.21	8.65±0.09

Values are mean±s.e.mean. n = 3-14. *Coldwell *et. al.* (1999).

(100 ng/ml; 64±7%; n=4) and iodosulpride (pKb 9.26±0.08; n=4) or clozapine (pKb 7.26±0.12; n=4). Functional potencies of most agonists were higher than radioligand binding affinities, but similar to the potencies in another functional assay (Table 1). The exception was dopamine, which was more potent in FLIPR assays possibly due to the rapidity of the calcium response, as oxidation may lead to apparently low dopamine potency in microphysiometer assays (Coldwell *et. al.*, 1999).

These results suggest that the pharmacology of the Gi-coupled dopamine D2 receptor is not altered by interaction with Gqi5 and signaling through changes in cytoplasmic concentration, although further work with other standard compounds is required.

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146P THE EFFECT OF NF-κB INHIBITORS ON IRF-1 EXPRESSION IN RAW 264.7 MACROPHAGES

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Interferon regulatory factor-1 (IRF-1) is a 48 kDa protein transcription factor believed to play an essential role in the regulation of nitric oxide induction in response to gamma interferon (IFN-γ) and lipopolysaccharide (LPS) (Kamijo *et al.*, 1994). However, little is known about the mechanisms by which INF-γ and LPS regulate IRF-1 expression and activity. A number of transcription factor consensus binding sites have been identified within the IRF-1 promoter including INF-γ -sensitive gamma activation factor (GAF)/gamma activation site (GAS) complex and NF-κB binding sites (Harada, *et al.*, 1994). In this study we examined the ability to LPS to regulate IRF-1 induction and the role of the NF-κB signalling pathway.

IRF-1 protein expression in RAW 264.7 macrophages was determined by Western Blotting. The immunoblots were quantified by optical scanning densitometry. IRF-1, GAF/GAS and NF-κB - DNA binding activities were detected by Electrophoretic Mobility Shift Assay (EMSA). All values represent the mean ± s.e.m. of at least 4 experiments. Statistical analysis employed a student t-test.

In RAW 267, INF-γ (30iu/ml) stimulated a large increase in cellular IRF-1 protein expression (control=0.149±0.001, IFN-γ (4h)=1.035±0.079) and IRF-1 DNA-binding activity. LPS (1µg ml^{-1}) gave a smaller, 2-3 fold, increase in IRF-1 expression (control=0.1483±0.0097, LPS(4h)=0.33±0.024) and failed to significantly increase IRF-1 DNA-binding. However, LPS also reduced the onset of INF-γ-stimulated induction of IRF-1 expression.

Pyrrolidine dithiocarbamate (PDTC), an inhibitor of NF-κB (Brennan & O'Neill 1996), significantly inhibited IRF-1 expression induced by LPS (1µg ml^{-1}) in a concentration dependent (control=0.002 ± 0.0003. control + PDTC = 0.002 ± 0.0001, LPS = 0.35 ± 0.039, LPS + PDTC = 0.1 ± 0.01). However, PDTC did not effect either NF-κB DNA-binding activity nor GAF/GAS DNA binding activity stimulated by LPS. Pre-incubation of RAW 264.7 macrophages with 10µM N-α tosyl-$_L$-lysine chloromethyl ketone (TLCK), a serine protease inhibitor (Kim *et al.*, 1995), significantly reduced IRF-1 expression stimulated by LPS or IFN-γ (control=0.035 ± 0.003, control + TLCK = 0.034 ± 0.005; LPS = 0.35 ± 0.08, LPS + TLCK = 0.09 ± 0.01; IFN-γ = 0.67 ± 0.10, IFN-γ + TLCK = 0.227 ± 0.01). However, a concentration of 100µM TLCK was required to abolish NF-κB and GAS/GAF DNA-binding activities stimulated by LPS and partially inhibit GAF/GAS binding activity stimulated by INF-γ (control = 0.0125 ± 0.002, control + TLCK= 0.006 ± 0.0013, INF-γ = 0.59 ± 0.05, INF-γ + TLCK = 0.25 ± 0.003).

These studies suggest that relative to IFN-γ, LPS stimulates expression of IRF-1 to a minor extent in RAW26.7 macrophages and that NF-κB is likely to play a role in the process. However, the NF-κB inhibitors used in this study may have non-specific effects unrelated to their established actions.

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147P "OUTSIDE-IN" SIGNALLING MECHANISMS UNDERLYING CD11b/CD18-MEDIATED NADPH OXIDASE ACTIVATION IN HUMAN ADHERENT BLOOD EOSINOPHILS

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Recent findings have implicated the β_2 integrin, CD11b/CD18, as a prominent mediator of eosinophil adhesion and the subsequent activation of the NADPH oxidase. To date, few studies have examined the cellular signalling pathway which underlies these responses, which is complicated by the need to differentiate between the pathways that increase receptor-ligand binding ("inside-out" signalling) and those that mediate receptor-ligand induced responses ("outside-in" signalling) such as cell spreading and NADPH oxidase activation. To investigate the latter pathway, we have adopted a pharmacological approach to examine the mechanism of superoxide anion generation by the NADPH oxidase during the adhesion of human.

Human eosinophils were purified from the peripheral blood according to the method of Hansel *et al.*, (1989). Adherence and superoxide anion generation were estimated fluorimetrically, in Calcein-AM-treated eosinophils, and by lucigenin-enhanced chemiluminescence respectively.

Incubation of human eosinophils on tissue culture-treated plates resulted in time-dependent adhesion and attendant activation of the NADPH oxidase. These responses reached maxima at approximately 20 and 30 min respectively and were attenuated (by > 85%) by blocking antibodies to both CD18 (6.5E) and CD11b (KIM 249). SB 203580 (10 pM – 10 μM), an inhibitor of the α and β isoforms of p38 mitogen-activated protein (MAP) kinase, inhibited NADPH oxidase activity in a concentration-dependent manner (pIC_{50} = -6.57 ± 0.14, n = 5) but had no effect upon adhesion. In contrast, an inhibitor of MAP kinase kinase-1 (MKK-1), PD 098059 (0.3 nM – 10 μM), had no affect on either eosinophil adhesion or respiratory burst. The selective inhibitors of PKC, Ro-318220 (broad spectrum; 30 nM – 10 μM), GF 109203X (novel and conventional isoforms; 30 nM – 100 μM) and Gö6976 (conventional isoforms; 1 nM – 10 μM) suppressed CD11b/CD18-dependent oxidative burst in a concentration-dependent manner (pIC_{50}s = -6.61 ± 0.11, -6.05 ± 0.20 and -4.89 ± 0.56 respectively, n = 5) but did not affect adhesion. PP1 (30 nM – 100 μM), a selective inhibitor of src-related tyrosine kinases, abolished both adhesion and superoxide anion generation with similar potencies (pEC_{50} = -5.53 ± 0.11 and – 5.99 ± 0.11, respectively, n = 5) whilst piceatannol (1 nM – 10 μM), a syk tyrosine kinase inhibitor, failed to attenuate either response. Wortmannin (10 pM – 1 μM) , an inhibitor of phosphatidylinositol 3-kinase (PtdIns 3-kinase) abolished superoxide anion production (pIC_{50} = -9.06 ± 0.23, n = 5) and partially inhibited (50.4 ± 0.4% at 1 μM) adhesion with a similar potency (pEC_{50} = -9.27 ± 0.29, n = 5).

The observation that inhibitors of PKC and p38 MAP kinase can attenuate respiratory burst in the absence of an affect upon adhesion, suggest that these enzymes selectively mediate CD11b/CD18-mediated NADPH oxidase activation in adherent human eosinophils ("outside-in" signalling). In addition, a possible role for Src-kinase and Ptd 3-kinase, but not MKK-1 or syk-kinase, is suggested although the actions of the former maybe secondary to the inhibition of adhesion.

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148P PHARMACOLOGICAL PROFILE OF THE HUMAN CCR2b RECEPTOR EXPRESSED IN RAT BASOPHILIC LEUKAEMIA CELLS: EVIDENCE FOR PARTIAL AGONIST ACTIVITY OF MCP-3 AND MCP-4

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Binding studies in monocytes have shown that MCP-4 and MCP-3 are very potent in displacing high affinity binding of ^{125}I-MCP-1 (IC_{50} for MCP-4, MCP-3 and unlabeled MCP-1 of 2.1±1.4, 0.85-1.6 and 0.7±0.2 nM respectively), suggesting that all three chemokines interact with the CCR2b (MCP-1) receptor (Berkhout *et. al.*).

We have investigated the signalling of MCP-3 and MCP-4 in a functional assay, calcium mobilisation, using the Fluorescence Imaging Plate Reader (FLIPR; Molecular Devices U.K.). RBL cells transfected with the human CCR2b receptor were grown in suspension, plated out in serum containing medium on to black wall clear bottomed 96-well plates, at a density of 20,000 cells per well. Twenty-four hours later cells were incubated in medium at 37°C for 90 min in 5% CO_2/95% O_2 with the cytoplasmic Ca^{2+} indicator, FLUO-3AM (4 μM; Molecular Probes) and 2.5 mM probenecid to reduce dye leakage. The cells were then washed four times with 125μl of Tyrodes buffer, (145 mM NaCl, 2.5 mM KCl, 10 mM Hepes, 10 mM Glucose, 1.2 mM $MgCl_2$ and 1.5 mM $CaCl_2$ pH7.4) then after 30 min incubation in the presence or absence of antagonists at 37°C in 5% CO_2/95% O_2, the cells were transferred into FLIPR to monitor cell fluorescence reflecting intracellular calcium.

Wild type RBL cells responded to ATP, acting through an endogenous purinergic receptor, (typically 250% increase over basal at 100 μM) pEC_{50} 6.64 ± 0.11 (n = 6) but showed no response to MCP-1. RBL cells transfected with the human CCR2b receptor responded to MCP-1 (typically 169% increase over basal at 1 μM) with a pEC_{50} 8.32 ± 0.11 (n=4) and ATP, (typically 269% increase over basal at 100 μM) pEC_{50} 6.35 ± 0.10 (n = 9) and a very small effect (typically 8% increase over basal at 100 nM) of MCP-3 and MCP-4 was observed. When preincubated for 30 min with MCP-1, MCP-3 and MCP-4, then challenged with 30nM MCP-1 the response to MCP-1 was diminished (pIC_{50} 8.41 ± 0.1, 7.93 ± 0.19 and 7.24 ± 0.18 respectively, n=3), but no effect of MCP-2, MIP-1α or RANTES was observed at the CCR2b receptor. (Data are mean ± s.e.m.)

These results show that FLIPR can be used to measure chemokine receptor activation seen as increases in intracellular calcium. Rapid successive exposure to their ligands is known to desensitize the signalling capacity of chemokine receptors. In these experiments pre-exposure to MCP-1 blocked the response of 30nM MCP-1 and pre-exposure to MCP-3 or MCP-4 reduced the response of 30nM MCP-1. These and previous results from Berkhout et. al. suggest that MCP-3 and MCP-4 may modulate MCP-1 signalling through the human CCR2b receptor.

Berkhout *et. al.*, (1997) J. Biol. Chem. **272**, 16404-16413

149P ENDOGENOUS 5-HT AND THE EFFECT OF GROWTH MEDIA ON EXPRESSION AND FUNCTION OF HUMAN RECOMBINANT 5-HT_4 AND 5-HT_7 RECEPTORS IN HEK-293 CELLS

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Serum used to supplement cell culture growth media contains significant amounts of serotonin (5-HT), a fact evidenced by the name of this neurotransmitter but often neglected. In this light, it is unfortunate that the effects of prolonged 5-HT exposure on recombinant 5-HT receptors expressed in cultured cells have not been systematically studied. In the present study we have used a commercial ELISA assay (KMI Diagnostics) to measure the concentrations of 5-HT in various growth media. In addition, by using radioligand binding and functional methods, we have examined the expression and functional activity of human recombinant 5-HT_4 and 5-HT_7 receptors (5-HT_{4B} and 5-HT_{7b} isoforms) following cell culture using media containing various concentrations of 5-HT.

HEK-293 cells expressing human recombinant 5-HT_4 and 5-HT_7 receptors were cultured in MEM containing 10% v/v qualified fetal bovine serum (qFBS), 10% v/v dialysed fetal bovine serum (dFBS), or in serum-free medium (SFM; IS-293, Irvine Scientific). Membrane radioligand binding assays used either [^{3}H]-GR113808 (0.01-5 nM, non-specific binding defined by 1μM unlabelled GR113808) or [^{3}H]-5-CT (0.01-6 nM, non-specific binding defined by 10μM 5-HT) for 5-HT_4 and 5-HT_7 receptors respectively. Tubes were incubated at 20°C for 40 min or 37°C for 1 h, respectively, before filtration over GF-B filters and washing (2 x 4 s). The amount of bound [^{3}H]-ligand was measured by liquid scintillation spectroscopy. In functional studies (accumulation of [^{3}H]-cyclicAMP in [^{3}H]-adenine loaded cells) the methodology was as described by Alvarez and Daniels (1992).

As determined by the ELISA assay, the final concentrations of 5-HT in the growth media used were 2μM, 10nM and below assay detection limit (0.3nM) for media containing qFBS, dFBS and SFM respectively. Heat treatment of qFBS did not have a marked effect on 5-HT content. Table 1 shows the results obtained from binding and functional studies.

In 5-HT_4-expressing cells, both 5-HT and 5-methoxytryptamine (5-MeOT) were considerably more potent in stimulating [^{3}H]-cyclicAMP accumulation when cells were grown in SFM ($P<0.05$).

Table1

	5-HT_4 cells			5-HT_7 cells		
	qFBS	dFBS	SFM	qFBS	dFBS	SFM
B_m (pmol/mg.)[a]	2.3	4.6	3.5	6.9 *	3.6	1.9
pK_d [a]	9.9	9.7	9.9	9.0	9.3	9.5
5-CT[b]	-	-	-	8.1	8.3	8.6
5-HT[b]	8.3	8.6	9.4	7.2	7.4	7.4
5-MeOT[b]	7.6	8.2	8.8	-	-	-
SB-204070[b]	8.5	8.3	8.8	-	-	-
Spiperone[c]	-	-	-	6.9	6.9	7.1

[a]Radioligand binding, [b]pEC_{50} cyclicAMP accumulation, [c]pK_B cyclicAMP accumulation. Data are means, s.e.mean ≤0.2 (*s.e.mean=1.4)., n=3-5.

In 5-HT_4 cells cultured in qFBS medium, SB-204070 was a partial agonist (56±3%) with respect to 5-HT: in cells cultured in dFBS and SFM, the compound showed increasing efficacy (74±8% and 108±6% respectively) but no change in pEC_{50}. In 5-HT_7 cells grown in qFBS medium, basal (unstimulated) [^{3}H]-cyclicAMP accumulation was inhibited by approximately 50% in a concentration-dependent manner by spiperone (IC_{50} ~ 106nM). This apparent "negative efficacy", sometimes described as "inverse agonism", was not observed in cells grown in SFM.

The results of the present study demonstrate the importance of taking into consideration the possible effects of endogenous agonist(s) contained in cell culture medium when studying recombinant receptor systems.

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150P EVIDENCE FOR AN INDIRECT MODE OF ACTION OF THAPSIGARGIN ON INTESTINAL EPITHELIAL CELLS

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Increases in intracellular calcium induce a direct secretory response from intestinal epithelial cells. This effect, manifest as an increase in short-circuit current (Isc) was obtained from cultured monolayers of the T_{84} human colonic intestinal cell line (Barrett, 1993). Similarly thapsigargin, which elevates intracellular calcium by inhibiting Ca^{2+} ATPase, also initiated increases in Isc of T_{84} cells (Kachintorn *et al.*, 1993). It would appear that thapsigargin could provide means of obtaining direct, calcium mediated, secretory responses from intact preparations of rat colonic mucosa. Such responses were required for an investigation of the effects of potassium channel inhibition on calcium and cAMP dependent secretagogues. Muscle stripped preparations of rat (Wistar,male, 200-300 g) colon and monolayers of T_{84} cells were set up in Ussing chambers for recording of Isc. Drug applications were to basolateral domain unless otherwise stated. Initially thapsigargin (dissolved in DMSO) was given apically, the intention being to avoid possible indirect secretory responses arising from stimulation of basolateral secretomotor neurones.

Apically applied thapsigargin (1-3 μM) had no significant effect on basal Isc of rat colonic preparations (0.3±0.1 $\mu A.cm^{-2}$, n=7, P>0.05). However basolaterally applied thapsigargin (1μM) caused significant increases in basal Isc (28±4 $\mu A.cm^{-2}$, n=7, P<0.05) when compared to DMSO vehicle (10 μl, 0.6 ±0.3 $\mu A.cm^{-2}$, n=4). In the presence of tetrodotoxin (1 μM) responses to thapsigargin (1 μM) were greatly reduced (4 ± 1.9 $\mu A.cm^{-2}$, n=4, P<0.05). Evidence for predominantly indirect effect of thapsigargin on intestinal epithelial cells was supported by the relative insensitivity shown by T_{84} monolayers to the compound. Thapsigargin (1-3 μM) had no significant effect on monolayer Isc (2.5±0.5 $\mu A.cm^{-2}$, n=7, P>0.05) when compared to DMSO (10 - 30 μl, 1.1± 0.8 $\mu A.cm^{-2}$, n=4). In contrast to thapsigargin, acetylcholine (10 μM) significantly increased Isc in T_{84} monolayers (12.7 ± 1.9 $\mu A.cm^{-2}$, n=6, P<0.05). In conclusion thapsigargin appears to have a mainly indirect secretory effect on colonic epithelial cells. In the rat colon its actions were mainly neuronal in origin, while in T_{84} cells it had relatively little effect compared to acetylcholine. Although Kachintorn *et al.* (1993) showed that T_{84} cells responded to thapsigargin, another study (Devor *et al*, 1997) demonstrated a similar lack of sensitivity as described above. Intraneuronal calcium mobilisation, required for neurotransmitter exocytosis, occurs after propagation of a nerve action potential into the nerve terminal. As tetrodotoxin blocks sodium channels required for the latter we suggest that thapsigargin may be acting on interneurones within the submucosal plexus.

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Kachintorn *et al.* (1993) *Br.J.Pharmacol.* 109, 510-517.
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151P ADENOSINE A_1 RECEPTOR-MEDIATED ACTIVATION OF THE MAP KINASE SIGNALLING PATHWAY IN DDT_1MF-2 CELLS

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The mitogen-activated protein kinase (MAPK) signalling pathway can be activated by a variety of G_i/G_o protein coupled receptors (for review see Sudgen & Clerk, 1997). Our previous studies have shown that the G_i/G_o-protein coupled human adenosine A_1 receptor activates the MAPK pathway in transfected Chinese hamster ovary (CHO) cells (Dickenson *et al.*, 1998). In this study we have investigated whether the endogenous adenosine A_1 receptor in hamster vas deferens DDT_1MF-2 smooth muscle cells activates the MAPK signalling pathway.

DDT_1MF-2 cells were grown in 6-well plate cluster dishes and serum starved for 16 h, in DMEM containing 1% bovine serum albumin, prior to determining MAPK activation. After agonist stimulation cells were solubilised in lysis buffer as described previously (Dickenson & Hill, 1998). Protein samples (25 µg) were then separated using 10% SDS-PAGE and transferred to nitrocellulose membranes. MAPK activation was determined by immunoblotting with an antibody (anti-phospho-MAPK) that detects only activated p42-kDa and p44-kDa MAPK.

Stimulation of DDT_1MF-2 cells with the selective adenosine A_1 receptor agonist N^6-cyclopentyladenosine (CPA) produced a rapid and transient activation of MAPK with dominant activation of p42-kDa MAPK. The MAPK response to CPA was concentration-dependent ($p[EC_{50}] = 8.98 \pm 0.24$; n=6) and time-dependent (peak activation occurring at 5 min). Responses to CPA (100 nM) were antagonised by the selective adenosine A_1 receptor antagonist, 1,3, dipropylcyclopentylxanthine (DPCPX) yielding an apparent K_D value of 1.2 ± 0.1 nM (n=3). Pertussis toxin pre-treatment (100 ng ml^{-1} for 16 h) completely inhibited the response to 1 µM CPA, demonstrating that G_i/G_o protein(s) couple the adenosine A_1 receptor to the MAPK pathway in DDT_1MF-2 cells. Responses to 1 µM CPA were reduced by pre-treatment (30 min) with the MAP kinase kinase 1 (MEK1) inhibitor, PD 98059 (50 µM; 79 ± 11% inhibition; n=4). Our previous results have shown that the adenosine A_1 receptor induces MAPK activation in transfected CHO cells by tyrosine kinase and phosphatidylinositol 3-kinase (PI-3K) dependent mechanisms (Dickenson *et al.*, 1998). In this study, pre-treatment of DDT_1MF-2 cells with the tyrosine kinase inhibitor, genistein (100 µM; 30 min) had no significant effect on the activation of MAPK by CPA (1 µM; 94 ± 12% of control response; n=4). In marked contrast, genistein (100 µM; 30 min) inhibited the MAPK responses mediated by insulin (100 nM; 96 ± 2% inhibition; n=3) and histamine (100 µM; 87 ± 8% inhibition; n=4). Treatment (30 min) of DDT_1MF-2 cells with the selective PI-3K inhibitor, wortmannin (100 nM), inhibited the MAPK response to 1 µM CPA by 56 ± 10% (n=5).

In summary, we have shown that stimulation of the endogenous adenosine A_1 receptor in DDT_1MF-2 cells activates MAPK via signalling pathway(s) involving PI-3K and MEK1. Interestingly, adenosine A_1 receptor-induced MAPK activation in DDT_1MF-2 cells is unaffected by the tyrosine kinase inhibitor, genistein. Therefore adenosine A_1 receptor-induced MAPK activation in DDT_1MF-2 cells may involve genistein-insensitive tyrosine kinases or be independent of tyrosine kinase activation altogether.

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152P AUTORADIOGRAPHIC ANALYSIS OF THE BINDING OF THE ANTAGONIST RADIOLIGAND [^{3}H]-ZM241385 TO A_{2A} ADENOSINE RECEPTORS IN RAT STRIATUM

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Autoradiographic analysis of A_{2A} adenosine receptors in the central nervous system has typically been conducted using agonist radioligands such as [^{3}H]-NECA (Alexander & Reddington, 1989) or [^{3}H]-CGS21680 (Jarvis *et al.*, 1989). However, these have proved problematic due to binding of radioligand to sites distinct from A_{2A} receptors (e.g. Cunha *et al.*, 1996). The antagonist ZM241385 has proved useful as a means of identifying A_{2A} adenosine receptor-mediated responses both *in vivo* and *in vitro* (Keddie *et al.*, 1996; Poucher *et al.* 1995). Binding of a tritiated version has recently been examined in particulate preparations from rat brain (Alexander, 1998). Here we report binding of [^{3}H]-ZM241385 to sections of rat frozen brain.

20 µm parasagittal sections of rat (Wistar, 150-300 g, either sex) frozen brain were cut using a cryostat and mounted on gelatin-subbed slides. After drying, sections were exposed to [^{3}H]-ZM241385 over 90 minutes at room temperature, in buffer (50 mM Tris, 1 mM EDTA, pH 7.4) containing adenosine deaminase (1 $U.mL^{-1}$). After rapid washing and drying, sections were apposed to photographic film (Hyperfilm, LKB) for 8-15 weeks. [^{3}H]-labelled standards exposed alongside the tissue allowed conversion of grain density to $fmol.mm^{-2}$. Saturation analysis was conducted over the nominal radioligand concentration range of 0.25 - 8 nM, while competition curves were conducted at 0.25 nM. Competition for binding was examined in the presence of antagonist at 100 nM and agonists at 1 µM. Non-specific binding was defined by 1 mM theophylline. Data reported are means ± SEM from three separate experiments using 3-8 tissue sections per animal. Statistical significance of displacement of [^{3}H]-ZM241385 was assessed using a repeated measure ANOVA, with Dunnett's multiple comparison test.

Specific binding was observed in the caudate putamen, nucleus accumbens and olfactory tubercle. Analysis of saturation isotherms in the caudate putamen showed the radioligand to have a K_d of 0.43 ± 0.06 nM, with a B_{max} of 293 ± 22 $fmol.mm^{-2}$.

A rank order of agonist competition for [^{3}H]-ZM241385 binding in the caudate putamen was: NECA (24 ± 6 % control, $P<0.01$) ≥ CGS21680 (34 ± 5 %, $P<0.01$) > CCPA (109 ± 8 %). Antagonists displayed the rank order of competition: SCH58261 (0 ± 1 %, $P<0.01$) > XAC (95 ± 11 %) = DPCPX (98 ± 6 %).

The rank orders of agonist and antagonist competition, together with the high affinity of [^{3}H]-ZM241385 are consistent with the radioligand binding to A_{2A} adenosine receptors in the rat striatum. Future studies should examine its binding in other brain regions to sites other than A_{2A} adenosine receptors (cf. Cunha *et al.* 1996).

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153P P2Y- AND P2X-RECEPTOR-MEDIATED MITOGENIC EFFECTS ON ASTROCYTES *IN VIVO*

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ATP is released during tissue injury and is known to exert morphogenic and mitogenic effects on astrocytes via P2-purinoceptors *in vitro* (Neary et al., 1996). Cultured astrocytes were described to possess P2X- (Walz et al., 1994), P2Y- and P2U-receptors (King et al., 1996). In previous studies we have shown that the 2-methylthio-ATP (mixed P2X/P2Y-receptor agonist)-induced astrogliosis in the nucleus accumbens (NAc) of rats was inhibited by pyridoxal-phosphate-6-azophenyl-2,4-disulphonic acid (PPADS) a P2-receptor antagonist. When given alone, PPADS depressed astrogliosis caused by injection of the solvent only (involvement of endogenous ATP). This supported the hypothesis that purine nucleotides are involved in astroglial proliferation also *in vivo* (Franke et al., 1999). To characterize these P2-receptors in the present study the effects of more specific P2X (α,β methylene ATP)- and P2Y (adenosine 5'-O-(2-thiodiphosphate), ADP-β-S)- receptor agonists were investigated.
The substances were infused (1 µl at a rate of 12 µl/h) in the NAc of rats (anaesthesia: ketamine hydrochloride (90mg/kg)/ xylazine hydrochloride (15mg/kg) i.p.). Male Wistar rats (280-320g) received bromodeoxyuridine (BrdU, a proliferation marker, 100 µM) or PPADS (30 µM) and BrdU at first; 15 min later a second injection containing the respective agonists (100 µM each) or PPADS and the agonists. All compounds were dissolved in artificial cerebrospinal fluid (aCSF). After a postinjection time of 4 days the rats were transcardially perfused with paraformaldehyde. For quantification of proliferating astrocytes double immunocytochemical staining with antibodies against BrdU and glial fibrillary acidic protein (GFAP) was performed. All GFAP-positive cells and all GFAP-/BrdU-double stained cells were counted in identical areas of the NAc (see Franke et al., 1999).
After infusion of the agonists an up-regulation of GFAP-immunoreactivity, hypertrophy of astrocytes and an increase in the number of counted cells in comparison to the aCSF treated control side were observed. PPADS inhibited both the P2X- and the P2Y-agonist induced astrogliosis.

	GFAP-positive cells	GFAP-/BrdU-double stained cells
aCSF	75.78 ± 3.52	21.73 ± 1.46
α,β meATP	90.07 ± 4.98*	27.96 ± 2.14*
ADP-β-S	104.94 ± 3.40*	35.80 ± 1.28*
α,β meATP/PPADS	82.53 ± 2.73+	21.87 ± 1.27+
ADP-β-S/PPADS	77.54 ± 3.03+	21.49 ± 1.80+

Table1. Effects of P2-agonists and antagonist on the number of counted cells (sum of the different counted areas) in the NAc of rats. Values are expressed as mean ± s.e.m. of 5 animals per group. (*P<0.05, versus aCSF group, +P<0.05 versus agonist group; ANOVA followed by the Bonferroni-test).

Data suggest that P2Y- and P2X-receptor subtypes are present on astrocytes in the NAc *in vivo*. We conclude the dominance of P2Y-receptors in mediating mitogenic changes in astrogliotic process.

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154P DEVELOPMENTAL CHANGES IN NMDA RECEPTOR FUNCTION AND SUBUNIT EXPRESSION IN CULTURED MESENCEPHALIC NEURONES

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Developmental changes in mRNA expression encoding NMDA receptor (NR) subunits and NR function were investigated in primary cultures of mesencephalic (MES) neurones prepared from rats of embryonic day 14. NR subunit expression and free intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) were determined using reverse transcriptase-polymerase chain reaction (RT-PCR) and single-cell fura-2 microfluorimetry, respectively.

Mesencephalic neurones were grown in DMEM F-12 containing 20% foetal calf serum on cover glasses coated with poly-L-lysine. Cultures loaded with 5 µM fura-2/AM (30 min, 37° C) were mounted onto the stage of an inverted microscope and superfused with Mg^{2+}-free HEPES-buffered saline (10 mM, pH 7.4). The fluorescence emission ratio at 510/20 nm was monitored as a measure of $[Ca^{2+}]_i$, subsequent to excitation at 340 and 380 nm. NMDA (plus 10 µM glycine) induced a concentration-dependent increase in $[Ca^{2+}]_i$. In MES kept 8 days in vitro (DIV), 100 µM NMDA enhanced $[Ca^{2+}]_i$ from about 96 nM to 321 nM. The NMDA effect additionally increased with time (up to 14 DIV). Inhibition of the NMDA response by ethanol (100 mM) was 55% and 18% after 8 and 14 DIV, respectively. Accordingly, the inhibitory effect of ifenprodil (1 µM), a preferential non-competitive NR1/2B receptor antagonist, decreased from 75% to 49% during this time interval.
Total/mRNA isolation and RT-PCR were carried out according to standard protocols. Amplification products were analysed by hybridisation with digoxigenin-labelled DNA probes and CSPD chemiluminescent detection. Primer pairs flanking the alternatively spliced regions at the amino and carboxy terminus of NR1 were used, respectively, to estimate the ratios of the corresponding 3' and 5' splice variants. Amplification of the NR subunit 2A, 2B, and 2C cDNA fragments was performed with primer pairs reported by Audinat et al., 1994.
NR1 subunit expression did not reveal significant differences in MES neurones kept 8, 10, or 14 DIV. NR 1-4a was predominantly expressed at each stage. In cultures 8 DIV, the relative amounts of NR subunits 2A, 2B, and 2C corresponded to a ratio of approximately 1:2:1. The NR2C proportion increased with time, whereas both 2A and 2B decreased, accordingly.

In conclusion, the present data reveal developmental changes in NR subunit expression in MES neurones which are associated with an increase in NR function and a decrease in ethanol and ifenprodil sensitivity.

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155P IMMUNOHISTOCHEMICAL LOCALISATION OF THE 5-$HT_{4(A)}$ RECEPTOR IN THE RAT BRAIN

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Recently four splice variants of the 5-HT_4 receptor (5-$HT_{4(a)}$ to 5-$HT_{4(d)}$) whose C-terminal amino acid sequence diverge from L^{358} have been identified and all but the 5-$HT_{4(d)}$ are expressed in the brain (Blondel *et al.*, 1998). All four variants have a similar pharmacological profile, making it impossible to distinguish their cellular distribution with currently available radioligands (Claeysen *et al.*, 1998). To overcome this problem we have raised a polyclonal antibody against a structurally unique portion of the C-terminus of the 5-$HT_{4(a)}$ receptor and report the cellular distribution of this protein in the rat brain.

A polyclonal antiserum raised in sheep against the C-terminal portion of the r5-$HT_{4(a)}$ receptor (Q^{367} to P^{380} + C) by conjugation to Keyhole Limpet haemocyanin was purified by sephadex chromatography and utilised for immunohistochemistry and Western blots. Adult male hooded Lister rats (n = 5) were anaesthetised with sodium pentobarbitone (60 mg kg^{-1} i.p.) to allow sequential intracardiac perfusion with 0.154M sodium chloride and 0.4% w/v paraformaldehyde in 0.1M phosphate buffer (180 ml each). Coronal sections (70 μm) were cut by vibratone and incubated in immunobuffer (NaCl 12, KCl 0.005, Na_2 HPO_4 900, Na_2 HPO_4 1.5, merthiolate 0.1 mM and Triton X-100 0.3% (w/v)) containing 2% (v/v) rabbit serum and 0.4% (w/v) casein to reduce non-specific binding. Following removal of endogenous peroxidase, sections were sequentially incubated with 5-$HT_{4(a)}$ antibody or pre-immune serum (1:40, 48 h), biotinylated rabbit anti-sheep immunoglobulins (1:200, 24 h) and positive cells visualised using an ABC Vectastain elite kit. For Western blots selected rat brain regions were solubilised in 50 mM TRIS (pH 7.4 containing 5% aprotinin and 15 mM PMSF and protein (10 μg) separated by SDS-page, transfered to nitrocellulose and visualised by incubation with 5-$HT_{4(a)}$ antibody (1:20, 12 h), peroxidase conjugated rabbit antisheep antibody (1:200, 1 h) and finally 3,3' diaminobenzidine.

The most abundant (number of cells in 195 μm^2) locations of 5-$HT_{4(a)}$ positive cells were the entorhinal cortex, nucleus of the diagonal band, nucleus accumbens, caudate nucleus (head), ventral pocket of striatum, globus pallidus, intermediate and lateral septal nuclei and the anterior amygdala. Lower levels of positive cells were also present in the frontal cortex, caudate putamen and several septal nuclei. Staining was restricted to the cell membrane and proximal processes of structures (10-20 μm) with a neuronal morphology, was abolished by pre-incubation of the primary antibody with synthetic peptide antigen (100 μM) and absent on incubation with pre-immune serum. In Western blots a single immunopositive band (30 KDa) was detected in striatum, hippocampus, septum and cortex which was prevented by pre-incubation by synthetic antigen (500 μM).

Although a single immunopositive band was identified in Western Blots, the molecular weight was lower than expected (44KDa) possibly due to partial degradation during solubilisation. The cellular distribution of the 5-$HT_{4(a)}$ receptor protein is similar but not identical to that reported for the mRNA (Vilaró *et al.*, 1996) and future studies will examine the phenotype of these neurones.

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156P AUTORADIOGRAPHIC LOCALISATION OF THE 5-HT_6 RECEPTOR IN THE CNS OF THE RAT USING [^{125}I]SB-258585

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An immunolocalisation study of the serotonin 5-HT_6 receptor (Gerard *et al.*, 1997) in the rat brain indicated expression of the receptor in many nuclei throughout the neuraxis. Dendritic and somatic localisation suggested a post-synaptic distribution of the receptor protein.

We have used radiolabelled SB-258585, an analogue of SB-271046 (Bromidge *et al.*, 1999), a potent and selective 5-HT_6 receptor antagonist, in conjunction with selective brain tract lesions to study this receptor distribution further.

[^{125}I]SB-258585 was first characterised in HeLa cells transfected with the human 5-HT_6 receptor, as well as in rat brain membrane homogenates. Binding studies with HeLa cells stably transfected with the human 5-HT_6 receptors indicated a Kd pf 0.8 ± 0.05 nM and Bmax values of 6.1 ± 0.95 pmol/mg protein (Hirst *et al.*, 1999).

Autoradiographic studies in CNS tissues (n=3) of Sprague-Dawley rats showed high densities of binding sites in the cerebral cortex, nucleus accumbens, caudate-putamen and hippocampus (CA1 and dentate gyrus). Moderate densities were found in the thalamus and substantia nigra. Low non-specific binding was seen in the presence of methiothepin or the 5-HT_6 antagonist SB-214111.We were also able to demonstrate a high density of binding in the caudate nucleus and hippocampus (dentate gyrus) of human brain (n=2).

To study whether the distribution of [^{125}I]SB-258585 binding sites was presynaptic or postsynaptic in striatal tissues, we performed unilateral 6-hydroxydopamine (6-OH-DA) lesions of the median forebrain bundle (MFB) of the rat brain (n=3).Two weeks after 6-OH-DA injection, MFB lesioning was confirmed by the observation that apomorphine elicited pronounced contraversive circling and by post-mortem demonstration of complete loss of TH-like immunoreactivity in the striatum and substantia nigra.

Densitometric quantification of autoradiograms resulted in nCi/mg values as follows:control striatum 148.7 ± 11.3; lesioned striatum 141.6 ± 6.1; control substantia nigra 75.6 ± 28.9; lesioned substantia nigra 93.7 ± 14.9. 6-OH-DA lesions had no significant effect on the levels of [^{125}I]SB-258585 binding sites in either the substantia nigra or the corpus striatum.

These results indicate that 5-HT_6 receptors are unlikely to be on dopaminergic cell bodies in the substantia nigra or on dopaminergic terminals in the striatum.Thus, 5-HT_6 receptors may be on cholinergic/GABAergic interneurones in the caudate putamen or on striatal GABAergic projection neurones and their terminals in the substantia nigra. The distribution of [^{125}I]SB-258585 binding sites reported here suggests that this subtype of serotonin receptor may play a role in the regulation of motor function and possibly in the control of memory and/or mood.

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157P CHARACTERISATION OF [^{125}I]SB-258585 BINDING TO 5-HT$_6$ RECEPTORS IN NATIVE TISSUES

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We have recently described binding of the 5-HT$_6$ receptor selective radioligand, [^{125}I]SB-258585 (4-Iodo-N-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]benzene-sulfonamide), to recombinant human 5-HT$_6$ receptors (Hirst *et al.*, 1999). We now report on the pharmacological characterisation of this radioligand binding to native 5-HT$_6$ receptors in rat, pig and human striatal membranes.

Striatal tissue from adult rats (Sprague-Dawley, 200-250g), adult pigs (from a local abattoir) and human caudate putamen tissues (from patients aged 64-76, whose cause of death was non-neurological) were homogenised in 10 volumes of ice cold 50mM Tris-HCl (pH 7.4) and prepared as previously described (Hirst *et al.*, 1999). Binding of 0.1nM [^{125}I]SB-258585 to the striatal membranes was carried out in a buffer containing 50mM Tris-HCl, 10μM pargyline, 5mM $MgCl_2$, 0.1% ascorbic acid and 0.5mM EDTA (pH 7.4), for 45 minutes at 37°C. Non-specific binding was defined with 10μM methiothepin. The assay was terminated by rapid filtration through Whatman GF/B filters, pre-treated with 0.3% polyethyleneimine, and washed with 8 ml of assay buffer. All measurements were performed in at least 3 independent experiments and data shown are mean ± s.e.mean.
In equilibrium binding studies with rat and pig striatal membranes and human caudate putamen membranes, [^{125}I]SB-258585 labelled a single binding site, with high levels of specific binding (59.7 ± 0.9%, 65.5 ± 0.7% and 67.5 ± 1.8%, respectively).

The density (B_{max}) of 5-HT$_6$ receptors in each of the native tissues and the apparent dissociation constants (K_d) for [^{125}I]SB-258585 binding are shown in Table 1 (data from recombinant human 5-HT$_6$ receptors expressed in HeLa cells are included for comparison, Hirst *et al.*, 1999).

Table 1. [^{125}I]SB-258585 binding to striatal membranes and recombinant human 5-HT$_6$ receptors.

	B_{max} (fmol/mg protein)	K_d (nM)
rat striatum	173 ± 22	2.8 ± 0.4
pig striatum	181 ± 25	2.8 ± 0.7
human caudate	215 ± 41	1.3 ± 0.04
HeLa cells	6100 ± 950	0.8 ± 0.05

The pharmacological profile of [^{125}I]SB-258585 binding to human caudate putamen membranes correlated closely with data from recombinant receptors (Kohen *et al.*, 1996, Hirst *et al.*, 1999). The pK_i rank order of potency was SB-271046 > SB-258585 > methiothepin > clozapine > 5-Me-OT > 5-HT > mianserin > ritanserin > 5-CT > mesulergine. Similar values were obtained from the rat and pig striatal membranes.
This is the first demonstration of radioligand binding to 5-HT$_6$ receptors in human brain tissue and confirms the similarity in pharmacology between species and recombinant systems.

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158P THE EFFECT OF STIMULATORS OF DIFFERENTIATION ON 5-HT$_3$ AND MUSCARINIC RECEPTOR EXPRESSION IN NG108-15 CELLS

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The neuroblastoma x glioma hybrid cell line, NG108-15, can be induced to differentiate into a more neurone-like phenotype by a range of stimuli, including low serum and agents which raise cyclic AMP levels in the cells, such as forskolin (Hamprecht, 1977). In this study we have examined the effects of exposing NG108-15 cells to these differentiating stimuli on the expression of muscarinic acetylcholine receptors and 5HT$_3$ receptors.

Cells of the NG108-15 line (passage 19-27) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100μM hypoxanthine, 1μM aminopterine and 16μM thymidine (HAT) and 5% foetal calf serum (FCS). In order to induce differentiation, this growth medium was removed on day 4 of culture, when the cells were ca. 30-40% confluent, and replaced with normal growth medium (control) or DMEM + HAT + 1% serum containing either 10μM forskolin (low serum + forskolin) or vehicle (low serum). Cells were maintained in these media for 3 days, then harvested in phosphate buffered saline and stored at -80°C until required. Radioligand binding was carried out on whole cell homogenates using the filtration technique; 3pM-10nM [^{3}H]-N-methylscopolamine ([^{3}H]-NMS) ± 10μM atropine was used to detect muscarinic receptors (Koenig and Edwardson, 1994) and 10pM-30nM [^{3}H]-BRL46470 ([^{3}H]-BRL) was used to detect 5HT$_3$ receptors (Steward et al., 1995). Data were analyzed by non-linear regression analysis to a single site model of binding and compared using *t*-tests, with $P<0.05$ being considered significant.

Inspection of the cells after 72h in low serum or low serum + forskolin suggested that differentiation had occurred; the cells appeared flatter than controls and had longer processes with obvious varicosities. Neither pre-treatment had any significant effect on the K_d values obtained for either ligand, for [^{3}H]-NMS log K_d values were -9.216 ± 0.117, -9.389 ± 0.084, -9.099 ± 0.133 (all values mean ± s.e.mean, n=7) and for [^{3}H]-BRL log K_d values were -8.799 ± 0.123, -8.766 ± 0.088, -8.644 ± 0.120 (all values mean ± s.e.mean, n=5-6), for control, low serum and low serum + forskolin treated cells, respectively.

However, there were effects of the differentiating stimuli on the levels of receptors expressed by the cells. In control cells, the B_{max} for the muscarinic receptor ligand, [^{3}H]-NMS, was 18.4 ± 2.7 fmol.mg protein^{-1}. This value appeared to be decreased very slightly by exposure to low serum, to 16.9 ± 2.6 fmol.mg protein^{-1}. However, exposure to low serum + forskolin produced a marked and significant increase in [^{3}H]-NMS B_{max} to 31.8 ± 5.9 fmol.mg protein^{-1} (all values mean ± s.e.mean, n=7). A somewhat different pattern was obtained with the 5HT3 receptor ligand, [^{3}H]-BRL. In control cells, the B_{max} for [^{3}H]-BRL was 95.7 ± 3.9 fmol.mg protein^{-1}. This value was markedly and significantly decreased by exposure to low serum, to 27.9 ± 6.2 fmol.mg protein^{-1}. However, following exposure to low serum + forskolin, [^{3}H]-BRL B_{max} was not significantly different from control at 67.8 ± 27.0 fmol.mg protein^{-1} (all values mean ± s.e.mean, n=5-6).

Thus it appears that the use of different stimuli for differentiation can produce distinct effects on muscarinic and 5HT$_3$ receptor expression. After 72h, low serum alone decreases 5HT$_3$ receptor number but has little effect on muscarinic receptor density. However, low serum plus forskolin increases expression of both receptors over the levels obtained in low serum alone; for muscarinic receptors this results in a level markedly greater than control, whereas for the 5HT$_3$ receptor the level is still somewhat lower than control. It remains to be determined whether these are true stimulus-dependent effects or whether they reflect different degrees of differentiation.

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159P C-FOS EXPRESSION PATTERNS INDUCED IN THE RAT BRAIN BY CLOZAPINE, HALOPERIDOL AND THE SELECTIVE 5-HT$_6$ RECEPTOR ANTAGONIST SB-271046

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C-Fos expression patterns in the rat brain indicate that Fos-like immunoreactivity (Fos-LI) may be used to characterise antipsychotic compounds (Robertson and Fibinger, 1996). Since 5-HT$_6$ receptors have been implicated in the action of the atypical antipsychotic clozapine (Glatt et al., 1995), we studied the Fos expression induced in the rat brain by the potent and selective 5-HT$_6$ receptor antagonist, SB-271046 (Bromidge et al., 1999) and compared this with that induced by clozapine and haloperidol.

Male Sprague-Dawley rats (n=3) were administered control solution once a day for four days, before being dosed with SB-271046 (10mg/kg p.o.), clozapine (20 mg/kg i.p.), haloperidol (1mg/kg i.p.) or vehicle on the fifth day. Two hours later animals were transcardially perfused under deep anaesthesia with 4% paraformaldehyde in PBS. The brains were then removed and processed for Fos immunocytochemistry (Moorman and Leslie, 1998). Cell counts were made using a light microscope. Resulting means ± S.E.M. are reported (see Table 1). Data were analysed by ANOVA with post-hoc testing using least-squares difference where appropriate.

Haloperidol and clozapine pretreatment resulted in significant Fos-LI throughout the caudate-putamen and nucleus accumbens. In contrast, pre-treatment with SB-271046 did not result in significant Fos-LI in any of the brain areas measured.

The well documented (Robertson and Fibiger, 1996; Arnt and Skarsfeldt, 1998) correlation of brain Fos-LI distribution patterns with 'typical' or 'atypical' antipsychotic compounds was therefore not reproduced SB-271046. This suggests that clozapine does not produce its Fos-LI distribution pattern as a result of its action upon 5-HT$_6$ receptors.

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Table 1. Distribution of Fos immunoreactivity.

Area	Haloperidol	Clozapine	SB-271046	Control p.o.	Control i.p.
Caudate putamen [a]	6.91 ±2.54*	1.3±0.7*#	0	0.18 ±0.18	0
Medial prefrontal cortex[b]	4.33 ±2.56	7.96 ±1.93	2.77 ±1.38	4.22 ±3.22	2.70 ±1.91
Nucleus accumbens [c]	10.92 ±3.35*	3.32 ±1.51*	0.14 ±0.10	0.15 ±0.15	0.04 ±0.04

Figures represent mean number of Fos-positive cells ±SEM within 0.025mm^2 [a] = (F[4,10] = 13.49, P<0.001), [b] = (F[4,10] = 0.52, NS), [c] = (F[4,10] = 15.3, P<0.001) (* = P<0.05 compared to control i.p., # = P<0.05 compared to haloperidol)

160P FOOD RESTRICTION DOWN-REGULATES THE DENSITY OF THE 5-HT$_{1B}$ AND 5-HT$_{2A}$ RECEPTOR IN THE VENTROMEDIAL HYPOTHALAMIC NUCLEUS

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Food restriction is a stressor that produces a marked hyperactivity of the hypothalamic-pituitary-adrenal axis (HPA) (Widdowson et al., 1997). 5-hydroxytrytamine (5-HT, serotonin) is one of multiple neurotransmitters that modulate HPA (Kageyama et al., 1998) and its axon terminals form synapses with corticosterone releasing factor (CRF) containing neurons in the hypothalamus (Fuller et al., 1990). From among the multiple subtypes of serotonergic receptors in the brain, the 5-HT$_1$ and 5-HT$_2$ subtype appear to mediate activation of HPA (Fuller et al., 1990). In this study, we investigated the effects of food restriction on density of 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{2A}$ receptors in the midbrain and hypothalamus with quantitative autoradiography. Male Wistar rats weighing 240-260 g were randomized into controls (n=7) and food restriction (FR) groups (n=7) and were allowed tap water *ad libitum*. While the controls were allowed access to excess amount of lab chow diet, FR rats were only allowed to consume 60 % of their normal daily food requirements. After 10 days, rats were killed by carbon dioxide inhalation. Epididymal and perirenal white adipose tissues were dissected and weighed and brains were removed and frozen in cold isopentane (-35 °C). Quantitative receptor autoradiography was performed on cryostat cut sections (20 μm) using 2nM [^{3}H]8-OH-DPAT (non-specific binding defined with 1μM 5-HT) for 5-HT$_{1A}$ receptors binding in the midbrain, and 2 nM [^{3}H]ketanserin (non-specific binding defined with 1μM cinanserin) for 5-HT$_{2A}$ receptors binding in the hypothalamus. For 5-HT$_{1B}$ receptors binding in the hypothalamus, 150 pM [^{125}I]cyanopindolol (non-specific binding defined with 10μM 5-HT) was used with 30 nM NAN-190 and 30 μM isoproterenol to block 5-HT$_{1A}$ and β-adrenergic receptors, respectively. Over the 10 day period controls gained 32 % in weight, whereas the FR rats showed no increase in body weight. Both epididymal and perirenal fat mass in FR rats were decreased by 50 % (P<0.001, Student's t-test) and 73 % (P<0.001), respectively, compare to controls. Specific [^{3}H]8-OH-DPAT binding to 5-HT$_{1A}$ receptors in the dorsal raphe (controls=12.6 ± 1.4 versus FR= 16.9 ± 1.8 fmol/mg tissue, mean ± S.E.M) and median raphe were not significantly different between the two groups. In contrast, [^{125}I]cyanopindolol binding to 5-HT$_{1B}$ receptors (controls=11.6 ± 1.1 versus FR=8.0 ± 0.8 fmol/mg tissue; p<0.05, ANOVA followed by Bonferonni-corrected t-test) and [^{3}H]ketanserin binding to 5-HT$_{2A}$ receptors (controls=17.5 ± 1.2 versus FR=11.3 ± 1.8 fmol/mg tissue; P<0.05) in the ventromedial hypothalamic nucleus (VMH) showed a significant decrease in FR rats compare to controls. In conclusion, we have demonstrated a decrease in 5-HT$_{1B}$ and 5-HT$_{2A}$ receptor density in the VMH of FR rats, which may be associated with a hyperactivity of the HPA in these rats.

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161P COMPARISON OF DOPAMINE D2 RECEPTOR SITES LABELLED BY [^{3}H]-SPIPERONE IN THE STRIATUM AND NUCLEUS ACCUMBENS WITH REGARD TO MORPHINE PREFERENCE IN THE RAT

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Some individuals are more susceptible to drug dependence than others. It has been suggested this may be due to a greater increase in dopamine (DA) release in the striatum and the nucleus accumbens (NAcc) following drug taking. Some rats previously dependent upon morphine will voluntarily consume morphine when given a choice (Borg & Taylor, 1994). It was determined with microdialysis that high morphine favouring (HMF) rats had a greater morphine induced release of DA in the NAcc (Unpublished). In view of the association of morphine preference with DA release, DA receptor numbers and their sensitivity was investigated. As DA D_2 receptors are believed to be involved in drug preference, this study was undertaken to compare DA D_2 receptor numbers in regard to morphine preference in rats.

Male Glaxo-Wistar rats weighing 220–300g were orally self-administered 0.4 mg/ml morphine in 5 % w/v sucrose solution (no choice) for 3 weeks, followed by a drug free period after which the rats were no longer physically dependent. Rats were then given a choice of 5 % w/v sucrose or 5 % w/v sucrose containing 0.4 mg/ml morphine solutions. Rats were classified as high morphine favouring (HMF; n = 6) when >15 ml/day of morphine containing solution was consumed. Rats were classified low morphine favouring (LMF; n = 6) when consumption was <15 ml/day. Rats were then drug-free for 3 weeks. Rats were then decapitated, their brains removed and the striatum and NAcc were dissected and homogenised in Tris buffer. Tris buffer, haloperidol (300 µM; for non-specific binding), [^{3}H]-Spiperone (1-3000 pM) and 250 µl of membrane homogenate were added to the wells of a Unifilter™ microplate/Optiplate™ incubated for one hour at room temperature and harvested onto a dry pre-soaked Unifilter™ with a FilterMate harvester. Counts were determined using a TopCount Microplate Scintillation Counter. Protein concentrations were determined by the Bradford dye-binding method (Bradford, 1976) using γ-globulin standard. Analysis was performed using unpaired t-tests.

Table 1: Dopamine D_2 binding sites in HMF and LMF rats.

	Striatum [^{3}H]-spiperone		NAcc [^{3}H]-spiperone	
	LMF	HMF	LMF	HMF
B_{max} (fmol/mg protein)	459	2152	1117	23545
K_D (nM)	0.1341	0.3227	0.8549	1.964

Total binding was significantly greater in HMF rats compared to LMF rats (Table 1) in the striatum ($P<0.001$) and the NAcc ($P<0.001$). Furthermore, specific binding was also significantly greater in HMF rats compared to LMF rats in the striatu!m ($P<0.001$) and the NAcc ($P<0.001$).

These findings may indicate that the number of dopamine D_2 receptors in the striatum and the NAcc are greater in high morphine favouring rats. Whether this condition is present before the initial exposure to morphine or occurs subsequent to 'involuntary' and/or 'choice' phases is yet to be determined.

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162P EFFECTS OF DIZOCILPINE ON NORADRENALINE, SEROTONIN AND DOPAMINE EFFLUX AND UPTAKE: AN *IN VITRO* VOLTRAMMETRIC STUDY IN RAT BRAIN SLICES

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Dizocilpine is an N-methyl-D-aspartate (NMDA) blocker used as a neuroprotectant to reduce ischaemic injury *in vivo* (Gill *et al.*, 1991). In the present study, we investigated the possibility that, in addition to its effects on NMDA receptors, dizocilpine might also modulate monoamine efflux and uptake.

Slices (350 µm) of rat nucleus accumbens (NAc), dorsal raphe nucleus (DRN) or locus coeruleus (LC) were taken from male Wistar rats (~150 g) and superfused (60 ml/h) with artificial cerebrospinal fluid (aCSF) at 32°C. Electrical stimulation (20 pulses, 0.1 ms, 10 mA, 100 Hz) was applied every 10 min and monoamine efflux and uptake were recorded by fast cyclic voltammetry (Stamford, 1990). After 3 stable stimulations, dizocilpine (10 µM) was added to the aCSF and its effects on monoamine efflux and uptake half-time (t½) were measured on 3 further stimulations: Dopamine (DA) efflux was measured in NAc, serotonin (5-HT) in DRN and noradrenaline (NA) in LC. Monoamine efflux and uptake t½ values were expressed as a percentage of the pre-drug period. Statistical comparisons were made by t-test. The results after 30 min are shown in Table 1.

In control slices monoamine efflux and uptake t½ were constant over the course of the experiments. Dizocilpine (10 µM) significantly increased NA efflux in the LC and 5-HT efflux in the DRN. Monoamine uptake t½ was also constant in control slices. Dizocilpine significantly increased NA uptake t½ in LC and 5-HT uptake t½ in DRN. The effect of dizocilpine on NA uptake was significantly greater than on efflux ($P < 0.05$). Dizocilpine had no effect on DA efflux or uptake t½ in NAc.

	Efflux (%)	Uptake t½ (%)
NA in locus coeruleus		
Control	97 ± 2	94 ± 7
Dizocilpine (10 µM)	249 ± 16***	786 ± 140***
DA in nucleus accumbens		
Control	109 ± 4	103 ± 8
Dizocilpine (10 µM)	103 ± 5	108 ± 4
5-HT in dorsal raphe nucleus		
Control	110 ± 5	106 ± 11
Dizocilpine (10 µM)	158 ± 17*	266 ± 30**

*Means ± s.e.m. (n=4-6). *P<0.05, **P<0.01, ***P < 0.001 vs control (t-test).*

Table 1: Effect of dizocilpine on monoamine efflux and uptake.

These results support previous reports that dizocilpine blocks NA uptake (Rogers & Lemaire, 1991) and further show that 5-HT uptake is also blocked. The lack of effect on DA uptake suggests that the effects are not a neurotoxic action. Although this concentration of dizocilpine exceeds its EC_{50} at NMDA receptors, and that attainable *in vivo* (Gill *et al.*, 1991), it should be noted that such levels are often used *in vitro*. Actions on monoamines are thus likely and should be considered in the interpretation of data.

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163P A SUPERFUSION STUDY ON CARRIER-MEDIATED RELEASE IN CELLS STABLY EXPRESSING THE HUMAN CLONED 5-HT TRANSPORTER

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The aim of the present study was to investigate the mechanism of action of drugs targeting the human plasmalemmal serotonin transporter (hSERT). Human embryonic kidney 293 cells stably transfected with the hSERT were used. For determination of drug effects on uptake, cells were incubated in 24-well plates at RT for 2.5 min with 0.1 µM [^{3}H]-serotonin ([^{3}H]-5-HT) ($5x10^4$ cells/well) and different concentrations of drugs of interest. For superfusion, cells were grown on glass cover slips ($2x10^4$ cells/coverslip), incubated with [^{3}H]-5-HT (37°C, 20 min, 5 µM) and transferred to superfusion chambers (25°C, 0.7 ml min^{-1}). Superfusate was collected in 4 min fractions. Drugs were added from min 12 onwards. Basal efflux during min 4-12 was expressed as percentage of radioactivity present in the cells at min 4 of superfusion. Drug-induced release was calculated from total efflux during min 12 – 20 minus estimated baseline efflux and expressed as percentage of radioactivity present in the cells at the beginning of drug addition.

The maximal specific uptake initial rate was 323 ± 32 pmol min^{-1} 10^{-6} cells (K_M=51 ± 0.02 nM; n=6). The 5-HT releasing drugs p-chloroamphetamine (PCA) and d-fenfluramine as well as the 5-HT uptake inhibitors imipramine, fluoxetine and paroxetine led to a concentration-dependent inhibition of [^{3}H]-5-HT uptake (IC_{50}, nM: PCA, 2466 ± 326; d-fenfluramine, 2779 ± 444; imipramine, 67 ± 11; fluoxetine, 64 ± 19; paroxetine, 9.5 ± 0.3; n=3). When studied in superfusion experiments, all drugs led to a concentration-dependent increase in the efflux of [^{3}H]-5-HT (basal efflux 2.3 ± 0.1% corresponding to 433 ± 98 dpm, n=12) with maximal effects of uptake inhibitors being about a third of those induced by releasers: the E_{max} values for PCA, d-fenfluramine, imipramine and paroxetine were 16 ± 4.5%, 13 ± 1.0%, 3.9 ± 0.2% and 4.3 ± 0.2%, respectively (n=3). EC_{50} values for inducing [^{3}H]-5-HT release (nM; PCA, 1.29 ± 0.16; d-fenfluramine, 13.2 ± 1.00; imipramine, 3.90 ± 0.18; fluoxetine, 4.57 ± 0.23; paroxetine, 4.33 ± 0.21) significantly correlated with IC_{50} values for inhibiting [^{3}H]-5-HT uptake (r^2=0.92). When PCA (10 µM) was tested in the presence of the Na^+/K^+ ATPase inhibitor ouabain (100 µM) an about fourfold enhancement of its effect on release was observed (n=3). By contrast, [^{3}H]-5-HT efflux induced by imipramine was not changed in the presence of ouabain (n=3). Ouabain alone (100 µM) caused a distinct and time-dependent increase in the release of [^{3}H]-5-HT (136 ± 4% of the baseline efflux after 56 min, n=11).

The results suggest that the releasing effect of PCA is due to carrier-mediated outward transport of [^{3}H]-5-HT whereas the observed imipramine-induced increase in [^{3}H]-5-HT efflux is due to interrupted high-affinity re-uptake which is ongoing even under superfusion conditions. (Supported by the Austrian Science Foundation, project P13183).

164P STUDY OF THE ORL_1 RECEPTOR MODULATING K^+-EVOKED [^{3}H]5-HT RELEASE FROM RAT CORTICAL SLICES

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The endogenous agonist for the ORL_1 receptor nociceptin (or orphanin FQ) has a putative inhibitory role in controlling neurotransmission in the brain. Inhibitions by nociceptin of the evoked release of glutamate (Nicol *et al.*, 1996) or noradrenaline (Schlicker *et al.*, 1998) have been reported, and a preliminary study has described a similar effect for 5-HT (Siniscalchi *et al.*, 1999). Here we report on a more extensive investigation on K^+-evoked [^{3}H]-5-HT release from rat cortex slices using the the ORL_1-receptor agonists nociceptin and nociceptin(1-13)NH_2, the potent partial agonist at the ORL_1 receptor in frontal cortex Ac-RYYRWK-NH_2 (Mason & McKnight, 1998), and the purported antagonist [$Phe^1\psi(CH_2\text{-}NH)Gly^2$]nociceptin(1-13)$NH_2$ (Guerini *et al.*, 1998).

1.8mm diameter punches (one per side) were cut from 400µm slices of the frontal cortex (between 2.2 and 2.7mm anterior to bregma) of the brain of male Hooded Lister rats (200-250g). After incubation for 30 minutes at room temperature in aerated artificial cerebro-spinal fluid (aCSF) supplemented with the peptidase inhibitors captopril, bestatin and phosphoramidon (10µM), and containing 0.5 µM [^{3}H]-5-HT (17.5 Ci/mmol), slices were transferred into individual chambers of a Brandel SF-20 superfusion system. Aliquots were collected every 5 minutes (flow rate: 0.55ml/min) after a 40-minute wash period, for the duration of the experiment (60 minutes). A 5-minute pulse of aCSF containing (50mM KCl) was applied to evoke the release of [^{3}H]-5-HT, with drugs or vehicle (containing 0.045% BSA) added 5 minutes before and during the high potassium pulse. Results were expressed as fractional rate of release. The amplitude of the peak of 5-HT release in the presence of the drug was expressed as a percentage of that in the presence of the vehicle. Each observation for concentration of test ligand was the mean from 3-4 separate experiments (4 replicates/experiment).

Exposure to 50mM K^+ increased the fractional release of [^{3}H]-5-HT to 2.49±0.17, against a basal rate of 1.13±0.04. In the presence of nociceptin the evoked release of 5-HT was reduced to a maximum of 47.33±7.79% at 10µM. The inhibitory effect of nociceptin was concentration dependent (Fitted IC_{50} 57.7nM, 95% CI 83nM to 399nM) and with nociceptin(1-13)-NH_2 the effect was the same (IC_{50}=59.27nM, 95% CI 26nM to 134 nM; maximum inhibition 54.23±8.36%). However we failed to see any inhibition of K^+-evoked [^{3}H]-5-HT release with Ac-RYYRWK-NH_2 (100nM-10µM), although otherwise this peptide has acted as a potent partial agonist with high efficacy in our hands. Ac-RYYRWK-NH_2 acted rather as an antagonist, producing an inhibition of the response to 300nM nociceptin (IC_{50}=493nM, 95% CI 0.11µM to 2.2µM) when the two were co-incubated. Similarly, [$Phe^1\psi(CH_2\text{-}NH)Gly^2$]nociceptin(1-13)$NH_2$ was without effect on its own against K^+-evoked [^{3}H]-5-HT release (30nM-3µM), but also produced a concentration-dependent block of the effect of nociceptin (IC_{50}=771nM, 95% CI 16nM to 10µM).

These results suggest a modulatory role for the ORL_1 receptor on serotoninergic transmission in the rat cortex. The agonist action of the natural peptide nociceptin was shared by the N-terminal tridecapeptide nociceptin(1-13)-NH_2, but not by the close analogue [$Phe^1\psi(CH_2\text{-}NH)Gly^2$]nociceptin(1-13)$NH_2$, which was an antagonist. The partial agonist Ac-RYYRWK-NH_2 had affinity, but no efficacy, suggesting a low receptor reserve.

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165P EFFECT OF A SELECTIVE SEROTONIN REUPTAKE INHIBITOR (SSRI) GIVEN IN COMBINATION WITH A 5-HT_{1A} ANTAGONIST ON FOREBRAIN EXPRESSION OF THE IMMEDIATE EARLY GENE *ARC*

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Recent strategies aimed at accelerating the antidepressant response to SSRI administration have focused on the co-administration of 5-HT_{1A} antagonists with SSRIs (Artigas et al., 1994; Gartside et al., 1995). Although SSRI/5-HT_{1A} combinations are known to increase 5-HT release in the forebrain (Gartside et al., 1995), it is not yet clear whether this results in an increase in postsynaptic 5-HT function. *Arc* (activity-regulated cytoskeletal-associated protein) is an immediate early gene, the expression of which is linked to neuronal activity. Recent studies demonstrate that *arc* expression in the forebrain increases in response to increased function of postsynaptic 5-HT receptors of the 5-HT_2 subtype (Pei et al., 1998). Here we have evaluated the effect of an SSRI (paroxetine) given in combination with a 5-HT_{1A} antagonist (WAY-100635) on *arc* mRNA expression in rat forebrain.

Male Sprague-Dawley rats (250-270 g) were injected with WAY-100635 (0.3 mg/kg s.c.) or saline followed 30 min later by paroxetine (5 mg/kg s.c.) or saline. In a separate experiment the 5-HT_2 antagonist ketanserin (2 mg/kg, i.p.) or saline was followed 30 min later by WAY-100635 (0.3 mg/kg s.c.) or saline, and then 30 min later by paroxetine (5 mg/kg s.c.) or saline. In both experiments rats were sacrificed 2 h after the final injection and *in situ* hybridization was carried out as previously reported (Pei et al., 1997). The relative abundance of *arc* mRNA was determined by densitometric quantification of autoradiograms, and expressed as a % of control.

Neither paroxetine nor WAY-100635, when given alone, had a significant effect on the abundance of *arc* mRNA in any region examined (Table 1). However, the combination of WAY-100635 plus paroxetine significantly enhanced *arc* mRNA abundance in the cingulate, frontal and parietal (layers IV and VI) cortices when compared with both saline- and paroxetine-treated animals. In contrast, WAY-100635 plus paroxetine had no significant effect in either hippocampus (CA1) or striatum (Table 1).

Table 1. Effect of SSRI/5-HT_{1A} antagonist combination on abundance of *arc* mRNA.

	Saline + Saline	Saline + Paroxetine	WAY-100635 + Saline	WAY-100635 + Paroxetine
Cingulate Cortex	100.0 ± 14.9	108.9 ± 11.1	105.7 ± 8.8	166.1 ± 11.7[a,b]
Frontal Cortex	100.2 ± 8.5	95.2 ± 6.0	103.0 ± 8.5	132.1 ± 9.4[a,b]
Parietal Cortex				
Layer IV	99.9 ± 5.4	110.2 ± 8.5	131.6± 11.1	177.0 ± 9.7[a,b]
Layer VI	100.0 ± 8.5	113.6 ± 7.7	119.4 ± 10.1	179.2 ± 15.3[a,b]
Striatum	100.0 ± 12.7	117.4 ± 17.0	106.8 ± 11.9	126.6 ± 18.2
CA1	99.9 ± 6.9	89.9 ± 5.8	105.8 ± 5.8	98.3 ± 8 .7

Data are mean ± s.e.mean values (n=5) expressed as a % of control. [a]p<0.01 *vs* saline/saline; [b]p<0.01 *vs* saline/paroxetine (one-way ANOVA followed by Dunnett' s *t*-test).

Ketanserin attenuated the increase in *arc* mRNA induced by WAY-100635 plus paroxetine in cingulate cortex (-63.8%, p<0.01), with a similar trend in frontal cortex, but not in parietal cortex. Ketanserin alone did not change *arc* mRNA expression. In summary, the present data show that an SSRI/5-HT_{1A} antagonist combination induces *arc* mRNA expression in anterior cortical areas. Our experiments with ketanserin indicate that this effect (in specific areas) is mediated through activation of postsynaptic 5-HT_2 receptors.

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166P BELAPERIDONE: ANTIDOPAMINERGIC AND ANTISEROTONERGIC EFFECTS *IN VIVO*

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Belaperidone (BEL) is in phase II of clinical development as an antipsychotic. It has a similar affinity for dopamine D_4 and 5-hydroxytryptamine $5HT_{2A}$ receptors (Ki 3 nM, for both). Compared with clozapine, it has a similar affinity for 5-HT_{2A} and D_2 receptors, but has a 34-fold selectivity for D_4/D_2 receptors (Unger, Bialojan, Gross et al., *this meeting*). In the present study, BEL was compared with clozapine (CLO) and haloperidol (HAL), in classical in vivo models of anti-dopaminergic and anti-serotonergic properties.

Mice (NMRI, 21-26g; female) in groups of 8, received drug (BEL 4.64-46.4 mg/kg; HAL 0.01- 0.464 mg/kg; CLO 1.0 -21.5 mg/kg) or vehicle, orally, 1 h (2 h for HAL) prior to 1.2 mg/kg apomorphine sc.and climbing behaviour was quantified by scoring every 2 min for 33 min, thereafter. Similar groups received drug (BEL 1.0-21.5 mg/kg; HAL 0.021-0.46 mg/kg; CLO 0.64-10 mg/kg) or vehicle 1 h (2 h for HAL) prior to the administration of methamphetamine (MET), 1 mg/kg po. Locomotor activity was recorded in cages equipped with light detector beams (2 mice/cage, 4 cages/dose) for 1 h starting 30 min after MET. Mice (NMRI, male, 22-27 g; n=6/dose) received mescaline, 60 mg/kg po, 1 h after drug (BEL 2.15-21.5 mg/kg; CLO 2.1-21 mg/kg) or vehicle, orally, and scratching attacks of the hind limbs were counted for 10 min, commencing 20 min after mescaline administration. Rats (Sprague-Dawley, female, 150-180g; n=6/dose) received L-5-hydroxytryptophan (5-HTP), 316 mg/kg ip, 1 h after drug (BEL 0.46-4.64 mg/kg; CLO 0.1- 1.0 mg/kg) or vehicle, orally, and forepaw treading and tremor induced were assessed by a score every 10 min, from 10 through 60 min after 5-HTP administration. In all behavioural assays dose responses of 4-6 doses were analysed by linear regression of log dose vs effect to yield ED values with 95% confidence limits. Plasma prolactin was determined by radioimmunoassay (Amersham) in rats (CD, male, 170-250g; n=6/dose) killed 1 h after BEL 3.8-76 mg/kg or vehicle, orally.

BEL showed dose-dependent antidopaminergic as well as anti-serotonergic effects (ED values; table 1). The high anti-serotonergic potency of BEL after oral administration suggests good oral availability and corresponds well to the high affinity for 5-HT_{2A} receptors in vitro. Consistent with BEL's high D_4/5-HT_{2A} affinity, plasma prolactin levels were not significantly increased up to 76 mg/kg po, showed only low potency in the anti-dopaminergic tests in vivo, and failed to induce any catalepsy up to the highest dose tested (215 mg/kg po; Wicke, Bailey, Freeman et al. *this meeting*). In contrast, haloperidol, which has affinity for the D_2, D_3 and D_4 receptors in the same nanomolar range as BEL's affinity for the D_4 receptor is potently anti-dopaminergic (table 1). It can be concluded that classical anti-dopaminergic tests are not suitable to show D_4 antagonism and the observed anti-dopaminergic effects of BEL may be, at least partially, mediated by D_2 receptors.

Table 1: Effect of belaperidone, clozapine, and haloperidol in vivo; ED-values (95% confidence limits) [mg/kg, po]

Compound	ED50 Apomorphine climbing	ED75 MET antagonism	ED50 Mescaline antagonism	ED50 5-HTP antagonism
Belaperidone	18.1 (12.9/27.4)	4.1 (1.98/7.86)	1.0 (0.52/1.56)	6.1 (3.94/8.91)
Clozapine	5.2 (3.85/7.19)	2.45 (1.2/5.7)	0.32 (0.2/0.5)	6.01 (4.98/7.19)
Haloperidol	0.05 (0.03/0.09)	0.07 (0.06/0.09)		

167P BELAPERIDONE: COMPARISON OF ITS RECEPTOR PROFILE WITH CLOZAPINE

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Both the therapeutic efficacy of typical antipsychotics and their propensity to induce extrapyramidal side effects and tardive dyskinesia are mainly attributed to antagonist properties at dopamine D_2 receptors. Clozapine (CLO), the prototype of atypical antipsychotics, has a relatively low affinity for dopamine (DA) D_2 receptors and typically occupies only about 40% of these receptors at therapeutic doses. Its unique properties have been ascribed to its affinity for DA D_4 receptors or, alternatively, to the specific mixture of its affinities for a variety of monoamine receptors. In the present study we have compared the receptor binding profile of belaperidone (BEL), a novel antipsychotic which is in phase II clinical development, with that of CLO.

Competition binding experiments used membrane fragments from animal tissues or receptor clones, as follows: D_1 (bovine caudate) labelled with 1 nM [^{3}H]-SCH 23390, defined by 10 µM butaclamol; D_{2low} (human clone) labelled with 0.1 nM [^{125}I]-iodospiperone, defined by 1 µM haloperidol; D_3 (human clone) labelled with 0.1 nM [^{125}I]-iodosulpride, defined by 1 µM spiperone; D_4 (human clone) labelled by 0.05 nM [^{125}I]-iodospiperone, defined by 1 µM haloperidol; 5-HT_{1A} (rat hippocampus) labelled with 0.8 nM [^{3}H]-8-OH-DPAT, defined by 10 µM 5-HT; 5-HT_{2A} (rat cortex) labelled with 1 nM [^{3}H]-ketanserin, defined by 1 µM cyproheptadine; 5-HT_{2C} (pig choroid plexus) labelled by 1 nM [^{3}H]-mesulergine, defined by 1 µM 5-HT; histamine H_1 (guinea pig cerebellum) labelled with 2 nM [^{3}H]- pyrilamine, defined by 1 µM pyrilamine; α_1 adrenoceptor (rat brain) labelled with 0.25 nM [^{3}H]-prazosin and defined by 0.5 µM prazosin and muscarinic receptors (rat cortex), labelled with 0.05 nM [^{3}H]-QNB and defined by 1 µM atropine. Functional interaction of BEL with D_4 receptors was studied in HEK 293 cells stably expressing $D_{4.2}$ receptors with a luciferase gene as a reporter, driven by a cAMP-dependent promoter. Forskolin-induced luminescence is decreased by agonists, and the effect of agonists is inhibited by antagonists; pA_2 values of BEL and CLO were calculated from quinpirole concentration-response curves at different agonist concentrations.

The results of binding are summarised in Table 1. BEL alone did not induce a change in cAMP concentration in HEK 293 cells expressing $D_{4.2}$ receptors, but antagonised the quinpirole effect with a pA_2 value of 7.5 as compared to 7.4 for CLO. BEL is also an antagonist of 5-HT_{2A} receptors (Teschendorf, Needham and Gross, *this meeting*).

The results show that, whilst both drugs have similarly high affinities for 5-HT_{2A} and moderate affinities for D_2 receptors, among the dopamine receptor subtypes, BEL has a higher selectivity for the D_4 receptor than CLO. The more pronounced 5-HT_{2A}/5-HT_{2C} selectivity of BEL may indicate a reduced propensity to cause weight gain and its lower affinity for histamine H_1 and α_1 adrenergic receptors indicates less propensity to induce sedation and orthostatic hypotension, respectively. The lack of affinity for muscarinic receptors may represent a further advantage. These predicted properties have to be verified by the currently ongoing clinical studies.

Table 1. Receptor Binding - Ki (nM) values determined by simultaneous fitting of 2-4 individual binding curves

	D_1	D_{2low}	D_3	$D_{4.2}$	5-HT_{1A}	5-HT_{2A}	5-HT_{2C}	H_1	α_1	Musc
BEL	580	105	2234	3.1	1030	3.3	51	17	19	>1000
CLO	172	139	305	22	81	2.5	13	0.6	3.9	7

168P CHANGE IN 5-HIAA/5-HT RATIO IN THE HIPPOCAMPUS FOLLOWING INTRAVENOUS BUT NOT INTRACEREBROVENTRICULAR INJECTION OF DHEAS

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Neurosteroids have a number of well documented biological effects including sedative, hypnotic (Melchior and Ritzmann, 1992) and anaesthetic properties (Majewska, 1992). These have led to the suggestion that specific neurosteroids could modulate a number of psychopharmacological disorders and behaviours, including, anxiety, stress, depression, memory, sleep and seizures. A number of 3β-hydroxy-Δ5-steroids including Dehydroepiandrosterone (DHEA) and its sulphate, DHEAS, have been found in the brain of several mammalian species (Azuma *et al*, 1993). In the present study we have investigated the effect of intravenous (i.v.) and intracerebroventricular (i.c.v.) injection of DHEAS on amino acid levels and an index of amine turnover (expressed as a ratio of the metabolite over the neurotransmitter) in distinct rat brain regions.

Male Lister hooded rats (300-350g) were housed under a 12 hour light/dark cycle under standard conditions with food and water ad libitum. Rats were randomised and split into two groups. Group 1 (n=30) were administered i.v. DHEAS (0.1, 1, 10mg/kg, body weight) or vehicle (10% β-cyclodextrin). Group 2 (n=27) were anaesthetised (Dormitor, 0.4mls/100g i.m. Sublimaze, 0.9mls/100g i.p.) implanted with i.c.v. cannulae (A/P –0.8mm, L +/- 1.5mm, V –4.1mm) and allowed to recover for 7 days following surgery. Correct implantation of cannula was verified with Angiotensin II drinking test. Animals were then administered i.c.v. DHEAS (0.15, 1.5 and 15µg in 5µl, estimated equivalent doses to those given i.v. assuming an average brain weight of 1.5g) or vehicle. A pre-treatment time of 1 hour was allowed, before animals were killed and brain regions dissected on ice. Brain samples were homogenised for analysis of amino acid content using HPLC-FD. Amines (5-HT and DA) were measured using HPLC-ECD (Routledge *et al*, 1995).

DHEAS (i.v. and i.c.v.) had no significant effect on amino acid levels or dopamine and metabolite levels in the hippocampus. However, i.v. DHEAS significantly ($p<0.05$, one way ANOVA followed by least significant difference test) decreased hippocampal 5-HT turnover (5-HIAA/5-HT) at 10mg/kg (0.69 ±0.06, n=6) as compared to vehicle (0.88 ±0.06, n=7).

The data presented above suggests that DHEAS modulates neuronal 5-HT. The mechanism by which this occurs is unclear. However, the lack of effect of DHEAS on 5-HT turnover following i.c.v. suggests an involvement of peripheral organs e.g. the adrenals in the mechanism of action of DHEAS.

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169P ATYPICAL ANTIPSYCHOTIC PROFILE OF BTS 79 018 MAY BE MEDIATED BY CENTRAL 5-HT$_{1A}$ AGONIST ACTIVITY

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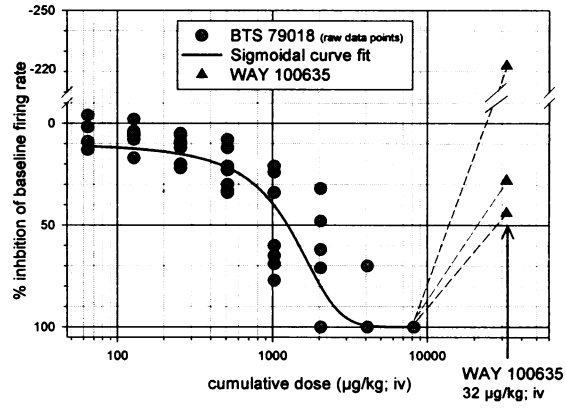
BTS 79 018 ((S)-*N*-(7-Chloro-2,3-dihydro-1,4-benzodioxin-2-ylmethyl)-1-[1-(2-methoxyphenyl)piperid-4-yl]methylamine) potently inhibits dopamine-mediated behaviours, predictive of antipsychotic efficacy, but has no potential to induce extrapyramidal movement disorder side-effects, as judged by its inability to induce catalepsy (Needham et al, 1998). This atypical profile may be mediated in part, by an action at 5HT$_{1A}$ receptors, for which BTS 79 018 has a high affinity, since 5HT$_{1A}$ agonism attenuates dopamine antagonist-induced catalepsy (Needham et al, 1994). In order to characterise the 5HT$_{1A}$ affinity of BTS 79 018, in vivo, its effects on the spontaneous discharge of 5HT neurones in the dorsal raphe was compared with those of the 5HT$_{1A}$ agonists, (±)8-hydroxy-2-(dipropylamino) tetralin (8-OH-DPAT) and buspirone.

Male SD rats (260-320 g; Janvier; n = 6-7) were anaesthetised with choral hydrate (400 mg/kg ip) and dorsal raphe cell firing (DRCF) was measured using standard extracellular recording methods (Aghajanian et al, 1970). One neuron per animal was recorded. The baseline firing rate of each cell was recorded for 5 min. Thereafter, drugs (BTS 79 018, 64-8192 µg/kg; (±)8-OH-DPAT, 0.25-16 µg/kg; buspirone, 1-64 µg/kg) were injected iv in cumulative 2-fold dose increments, until complete inhibition of firing was achieved. In 3 experiments the 5HT$_{1A}$ antagonist, N-{2-[4-(2-methoxyphenyl) piperazin-1-yl]ethyl}-N-(2-pyridyl)cyclohexanecarboxamide (WAY 100635; 32 µg/kg), was administered 1 - 2 min after complete inhibition of firing by BTS 79 018. In all experiments the time interval between the application of 2 consecutive doses was 60-90 s.

BTS 79 018 induced a dose-dependent inhibition of DRCF (Fig 1). Like the high-efficacy partial agonist (±)8-OH-DPAT and buspirone, BTS 79 018 completely inhibited DRCF at high doses, but was much less potent than either (ED_{50}s 0.97 ± 0.07 µg/kg, 4.65 ± 0.38 µg/kg and 1.25 ± 0.10 mg/kg, iv, respectively). The inhibitory effects of BTS 79 018 on DRCF were abolished following the administration of the 5HT$_{1A}$ antagonist WAY 100635 confirming these effects are mediated by the activation of somatodendritic 5HT$_{1A}$ autoreceptors.

Fig. 1. DRCF inhibited by BTS 79 018 and reversed by WAY100635



In conclusion, these data confirm 5HT$_{1A}$ agonist action of BTS 79 018 in vivo, a feature which may contribute to its atypical antipsychotic profile.

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170P INVOLVEMENT OF 5-HT$_{1A}$ RECEPTORS IN OPIOID-INDUCED DESCENDING INHIBITION OF SPINAL REFLEX IN THE DECEREBRATED RABBIT

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Fentanyl given into the fourth ventricle of decerebrated rabbits inhibits reflexes in medial gastrocnemius (MG) motoneurones evoked by electrical stimulation of the sural nerve. This effect is abolished by spinalization but not by the α_2-adrenoceptor antagonist RX 821002 (Clarke *et al*, 1998). Previous work has suggested that descending inhibition activated by opioids is mediated by 5-HT (see Wang & Wessendorf, 1999). In this study we have investigated the possibility that 5-HT$_{1A}$ receptors are involved in the bulbospinal inhibition activated by fentanyl.

Sixteen Dutch rabbits of either sex (1.7 - 2.7 kg) were decerebrated and spinalized at the thoracolumbar junction under halothane (2- 3.5%)/N_2O anaesthesia. Reflexes were evoked by electrical stimulation of the exposed sural nerve at a strength sufficient to excite all myelinated afferent fibres (mean, 23 x threshold), and recorded from the ipsilateral MG nerve. Reflex responses, averaged from 8 stimuli at 1 Hz, were recorded every 2 min. Fentanyl was given by the intraventricular (i.c.v.) route to 8 rabbits in doses of 3, 7 and 20 µg kg^{-1} (all injection volumes <10 µl, 0.19 - 1.9 x 10^{-5} M), doses being separated by intervals of 8 min. After a 1 h recovery period, the selective 5-HT$_{1A}$ receptor antagonist WAY-100635 was given intrathecally (i.th.) at 100 µg (50 µl, 3.7 x 10^{-6} M). After a further 30 min, fentanyl was administered as before. A second group of 8 animals received i.v. fentanyl in doses of 0.3, 0.7, 2, 7 and 20 µg kg^{-1} (all injection volumes <1 ml, 1.9 x 10^{-9} to 1.9 x 10^{-7} M), again dosing at 8 min intervals. As before, this procedure was repeated after WAY-100635, 100 µg i.th.. Drugs were dissolved in Ringer's solution.

I.c.v. fentanyl significantly (Friedman's ANOVA, $p<0.01$) decreased the sural-MG reflex in a dose-related fashion to a median of 34% (inter-quartile range, IQR, 24-51%) of pre-fentanyl values after the highest dose. After WAY-100635 reflexes increased to a median of 137% (IQR 114-171%). In the presence of this drug, i.c.v. fentanyl no longer significantly decreased reflexes (Friedman's ANOVA, $p=0.2$). After the highest dose responses were 83% (IQR 54-102%) of immediate pre-fentanyl levels. I.v. fentanyl also caused a dose-related depression of reflexes, to a median of 17% (IQR 5-27%) of pre-fentanyl levels (Friedman's ANOVA, $p<0.01$). In these animals WAY-100635 increased reflexes to 145% (IQR 127-167%) of pre-drug values. In the presence of the 5-HT$_{1A}$ receptor antagonist, i.v. fentanyl generated significant inhibition of the sural-MG reflex, to a median of 14% (IQR 5-39%) of pre-fentanyl levels, not significantly different from the effect before WAY-100635 (Wilcoxon test, $p=0.4$).

Thus, inhibition of spinal events following intraventricular fentanyl is mediated through activation of 5-HT$_{1A}$ receptors. As WAY-100635 did not reduce the effects of i.v. fentanyl, it appears that the spinal cord is the primary site of action after systemic administration of the opioid (Clarke *et al.* 1998).

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171P OREXIN A AND B INDUCE DIFFERENT EFFECTS ON DOPAMINE AND 5-HT TURNOVER IN VARIOUS RAT BRAIN AREAS

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Orexin A and B are neuropeptides which have been implicated in feeding behaviour and are known to interact with two receptors, termed orexin-1 (OX_1) and orexin-2 (OX_2). Orexin-A and orexin-B have similar affinities for OX_2 receptors, but orexin-A has greater affinity and potency for OX_1 than orexin-B (Sakurai *et al*, 1998). In this study, we have investigated the effects of orexin A and B on dopamine (DA), 5-HT and their metabolites in various rat brain regions in order to further characterise the functions of these neuropeptides.

Male Sprague Dawley rats (350-450g) previously implanted with i.c.v. cannulae were used. At least one week later, correct cannula placement was confirmed by an intense drinking response to angiotensin II (100ng/rat, icv). A minimum of one week was allowed before further use. Rats were pre-treated with either orexin A, B or saline vehicle and 40 min later were decapitated and six brain regions dissected (medial pre-frontal cortex, hypothalamus, nucleus accumbens, striatum, hippocampus and either the occipital cortex or cerebellum) and frozen on dry ice for storage at -70°C. Samples were weighed, homogenised in buffer (0.4M perchloric acid containing 0.1% sodium metabisulphite, 0.01% EDTA and 0.1% cysteine), centrifuged at 10,000 rpm for 10 mins and the supernatant analysed for DA, 5-HT and metabolites by HPLC with electrochemical detection (Routledge *et al.*, 1995). DA and 5-HT turnover are defined as a ratio of DA and 5-HT to their respective metabolites and are shown as mean ± SEM:

DA turnover = ([DOPAC]+[HVA])/[DA];

5-HT turnover = [5-HIAA]/[5-HT].

Data were analysed using analysis of variance with least significant difference test where appropriate.

Orexin A (10 and 30μg/rat, icv) dose-dependently increased DA turnover (vehicle = 0.97 ± 0.06; orexin A (30μg) = 1.63 ± 0.3, n=6 to 8) in the medial pre-frontal cortex. The effect was significant at 30μg. Orexin A had no effect on DA or 5-HT turnover or metabolites in any other brain area. In contrast, orexin B (3, 10 and 30μg/rat, icv) had no effect on DA turnover in any brain region whereas it induced a significant increase in 5-HT turnover in the hypothalamus (vehicle = 0.63 ± 0.4; orexin B (30μg) = 0.75 ± 0.02, n=4 to 6) and striatum (vehicle = 0.90 ± 0.08; orexin B (30μg) = 1.13 ± 0.08, n=4 to 6) having no significant effect in the other four brain areas. These results, together with those of the neuroendocrine study (Ashmeade *et al.*, 1999, this meeting), suggest that orexin A and B induce different effects in the brain that may reflect different physiological roles.

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172P EFFECT OF THE SSRI SERTRALINE ON EXTRACELLULAR DOPAMINE AND DOPAMINE RECEPTOR EXPRESSION IN THE NUCLEUS ACCUMBENS OF THE RAT

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Important differences in the pharmacology of the selective serotonin reuptake inhibitors (SSRIs) are emerging. For example, sertraline (SERT), when compared to other SSRIs, is reported to have a relatively high affinity for the dopamine (DA) transporter. This effect may contribute to the antidepressant efficacy of SERT, especially in cases with psychomotor dysfunction where DA may be of importance (Lane, 1998). Recently, we reported that repeated administration of the SSRI fluoxetine enhances DA D_2 receptor expression in the nucleus accumbens (NAc) (an area linked to psychomotor function), without altering extracellular DA in this region (Ainsworth *et al.*, 1998). Here we report the effect of SERT on extracellular DA and DA receptor expression in the NAc.

All experiments utilised male Sprague-Dawley rats (260-280g). SERT (10 mg/kg i.p.) was administered either as a single dose or twice daily for 14 days. Control animals received 2 % Tween-80 vehicle as appropriate. Microdialysis was used to measure extracellular DA in the NAc of awake rats as described previously (Ainsworth et al., 1998). In brief, probes were surgically implanted under halothane anaesthesia. Following overnight recovery probes were perfused with artificial CSF (2.5 μl/min). Dialysates were collected every 20 min and analysed for DA by HPLC. The abundance of mRNA encoding D_2 and D_1 receptors was determined by *in situ* hybridization histochemistry, and D2-like binding site density by receptor autoradiography using [^{3}H]YM-09151-2 (Ainsworth et al., 1998). Image analysis of autoradiograms was performed on four distinct brain regions; the NAc shell (Shell), NAc core (Core), ventromedial striatum (VMS) and dorsolateral striatum (DLS).

A single injection of SERT caused an increase in dialysate DA levels when compared to the effect of vehicle control (two-way ANOVA, $P<0.001$, N=5/group). This effect was maximal 60 min post-injection (+ 57 % above pre-drug levels). The day after the last of the twice daily injections, the mean basal level of DA in SERT-treated rats was not different from that of vehicle-treated controls (0.06 ± 0.01 versus 0.07 ± 0.007 pmol DA / 50μl sample, N=8/group). However, in both groups, DA increased in response to a challenge injection of SERT (+53 % and +60 % above pre-drug levels, respectively; one-way ANOVA, $P<0.035$ and $P<0.009$, respectively). Twenty four h after repeated SERT, increased abundance of D_2 mRNA was found in the NAc (shell + 53 %, core + 58 %) but not VMS or DLS (Table 1). Repeated SERT also increased D2-like binding site density in the NAc (shell only; + 28 %). The abundance of D_1 mRNA was not altered by SERT in any region examined.

	Shell	Core	VMS	DLS
D2 mRNA				
Vehicle	42.2±5.0	34.3±3.7	40.7±6.1	49.6±6.9
SERT	64.7±9.1*	62.8±7.0*	61.0±7.5	73.2±9.6
D2 binding				
Vehicle	115.8±6.3	94.8±4.3	139.4±10.0	186.4±13.8
SERT	148.4±12.2*	107.6±9.1	163.8±11.7	191.8±6.5
D1 mRNA				
Vehicle	32.2±2.5	22.8±2.3	27.6±2.1	24.8±2.8
SERT	38.0±3.3	23.8±2.2	31.5±3.9	24.8±2.5

Table 1. Effect of repeated SERT on abundance of D_2 and D_1 mRNA (μCi/g tissue) and D2-like binding site density (fmol/mg tissue). Data are expressed as mean ± s.e.mean values (n=6) and were analysed for significance using Student's unpaired *t*-test. * $P<0.05$ *vs.* vehicle control.

In summary, the present results show that repeated administration of SERT increases both extracellular DA and D_2 receptor expression in the NAc of the rat. In similar experiments, fluoxetine increased D_2 receptor expression but not extracellular DA (Ainsworth *et al., 1998* and unpublished data). The effect of other SSRIs on NAc DA are, as yet, unknown. An increase in DA transmission through a combined action at both pre- and postsynaptic sites may be relevant to the therapeutic effects of SERT in major depression.

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173P STUDIES ON THE RELATIONSHIPS BETWEEN EXTRACELLULAR DOPAMINE IN THE RAT NUCLEUS ACCUMBENS AND QUANTITATIVE EEG

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The mesolimbic-mesocortical dopaminergic system plays an important role in the mediation of locomotor activity as well as affective and rewarding functions and is involved in the pathophysiology of schizophrenia and addiction.
In order to study regulatory mechanisms in the nucleus accumbens (NAc) dopamine receptor agonistic and glutamate receptor antagonistic substances were applied by microdialysis to freely moving rats with simultaneous recording of the extracellular dopamine (DA) level and the EEG as functional output. The microdialysis method is described by Krügel et al. (1999). For telemetric EEG recording (Kittner et al., 1999) an electrode was implanted together with the microdialysis guide cannula into the NAc of male Wistar rats (280-320g). The electrode was connected with a transmitter that allowed a parallel monitoring of the EEG and the extracellular dopamine level. Each substance was applied to 5 animals. The most prominent changes of the EEG were found in direct recordings from the NAc. All results presented are statistically significant at $P<0.05$ (ANOVA for repeated measures). Compounds with DA releasing properties such as amphetamine (1 mM) and 2-methylthio ATP (1mM for 20min each) elevated the extracellular level of DA up to 230±35% and 215±22% of its original value, respectively. This was accompanied by changes of the EEG. The EEG showed the classical signs of desynchronization with a selective elevation of the α-band power of the EEG spectrum up to 249±63% compared to the pretreatment period. With respect to EEG studies in humans (Machleidt et al. 1994) this may suggest an enhancement of intentional and hedonistic behaviour. Moreover, the D2-receptor agonist quinpirole (100μM for 60min) also increased the α-band power to 231±53% but decreased the DA level to 56±4% of the respective control values. The P2-purinoceptor antagonist pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS; 30μM for 120min) decreased the extracellular level of DA to 32±4% whereas the combination of the ionotrophic glutamate antagonists 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP; 100 μM) and 6-cyano-7-nitroquinoxaline-2,3 dione (CNQX; 300 μM for 60min each) caused only a weak elevation of DA up to 123±10%. However, these substances changed the EEG-spectra in a similar manner as compounds which act at the dopaminergic system with an enhancement of the α-band power to 243±60% and 404±109%, respectively.
Taken together, opposing changes of the DA level can be accompanied by an elevation of the α-band power in the EEG which seems to be correlated with the activity of NAc GABAergic neurons projecting to various brain regions involved in the generation of locomotor function. The activity of these neurons is mainly regulated by interactions of dopaminergic and glutamatergic inputs. The combined approach of microdialysis and EEG gives the possibility to investigate the correlation between transmitter concentration and functional outcome.

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174P *IN VITRO* EFFECT OF CYTOKINES ON NEUROPEPTIDE Y RELEASE FROM HYPOTHALAMIC SLICES

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Hypothalamic neuropeptide Y (NPY) is involved in the regulation of energy balance through its orexigenic activity and the ability to inhibit brown adipose tissue (BAT) thermogenesis. Hypothalamic NPY activity is strongly activated in states of negative balance, such as periods of dietary restriction or starvation (Kalra *et al*, 1991). In animal models of cancer cachexia, when there is a significant reduction in body weight as a result of reduced appetite, there is no significant augmentation in the activity of the NPY system (Chance *et al*, 1994; Bing *et al*, 1999). We have therefore examined whether cytokines, interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumour-necrosis factor-α (TNF-α; cachectin) which were found to be elevated in cancer patients (Noguchi *et al*, 1996), can attenuate NPY release from hypothalamic slices *in vitro*. Adult male Wistar rats (250-300g) were killed by CO_2 inhalation and their brains quickly removed. Hypothalamic blocks were dissected free and placed in previously gassed (95% O_2/5% CO_2) cold Kreb's bicarbonate buffer (KRB)(pH 7.4, 4°C). Slices (0.1 mm^3) were prepared by cross-chopping the hypothalami using a razor blade and preincubating them in warm, gassed KRB (0.4 ml; 37°C) for 1 h with four changes of fresh buffer. Slices were then incubated for four periods of 15 min each in fresh KRB (0.4ml) slices and then depolarised for 15 min with KRB containing 60 mM high potassium concentration followed by three recovery periods. Cytokines, TNF-α (50 nM), IL-1β (40 nM) and IL-6 (35 nM) or a non-selective calcium channel antagonist NiCl (30 μM), were added to period 3 and were present throughout the remaining incubation periods. NPY concentrations in the samples were measured by radioimmunoassay, using a high affinity polyclonal antibody. NPY concentrations in the slices were also measured by tissue sonication at the end of the experiment. Potassium dependent release was approximately 2 times basal release. Potassium-stimulated NPY release was significantly reduced by the 30 μM NiCl (-62%) without any effect on basal release (basal release (pg/15 min); control = 67 ± 5; plus 30 μM NiCl = 83 ± 4: n = 4: stimulated release; control = 82 ± 12; plus 30 μM NiCl = 31 ± 9* $P<0.05$; Student t-test, mean ± S.E.M). None of the cytokines altered either the basal or stimulated NPY release from the hypothalamic slices (basal release; control = 76 ± 3; plus 50 nM TNF-α = 72 ± 5; n = 4: stimulated release; control = 83 ± 23; plus 50 nM TNF-α = 129 ± 10). We have demonstrated that NPY release from hypothalamic slices is calcium-dependent, but is not affected by the presence of cytokines. Therefore, the reduction of appetite in states of cancer cachexia may not be attributed to a cytokine-induced reduction in neurotransmitter release.

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175P AN INHIBITORY ACTION OF NEUROPEPTIDE Y (NPY) ON NEURONES IN THE RAT SPINAL CORD SUPERFICIAL DORSAL HORN (LAMINAE I-IV) *IN VITRO*

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NPY is colocalised in a subpopulation of GABA containing neurones in the dorsal horn which form both presynaptic axo-axonic and axo-dendritic synapses (Doyle and Maxwell, 1993). Some of these terminals are preferentially targeted on neurones expressing neurokinin 1 receptors (Polgar et al 1999) and intrathecal application of NPY produces behavioural analgesia in the rat (Hua et al., 1991). Surprisingly, the actions of this peptide on dorsal horn neurones do not appear to have been investigated and hence, the present study was carried out.

Rats (12-18 days old) were anaesthetised with ether, decapitated, and their lumbar spinal cords dissected. A sagittal slice of the L3-L5 segments of the lumbar spinal cord was cut maintaining the sciatic and sural nerves in functional continuity. The slice was perfused with a Kreb's solution, gassed with 95% O_2 -5% CO_2 at room temperature. Sharp electrode, intracellular recordings were made from neurones in LI-IV and their membrane properties and responses to peripheral nerve stimulation (50 mV, 0.5 ms, recruiting both A- and C- fibres), were measured. The effect of bath application of NPY on these parameters was examined.

Application of NPY (0.5–1.0μM) induced a hyperpolarisation, which averaged 5.9 ± 1.2 mV (n=11), in peak amplitude whilst in 6 neurones it had no effect. None of the cells tested (n=17) at this dose range of NPY were depolarised. NPY also decreased neuronal excitability as revealed by (a) a significantly reduction in the input resistance (17.6 ± 3.0%. P< 0.05; n=8) (b) an increase in the threshold current (depolarising step) (0.26 ± 0.03nA to 0.35 ± 0.35 nA, mean ± s.e.m., a 36% increase) required for cell firing (c) a decrease in the number of spikes elicited by a depolarising step from a fixed membrane potential. Application of NPY (0.5-1.0 μM) attenuated the mean amplitude of the evoked EPSPs by 21.7 ± 2.3 % (P < 0.01) in 7 out of 9 neurones responding to peripheral nerve stimulation.

In the spinal cord superficial dorsal horn (laminae I-IV), NPY reduced the intrinsic excitability of many neurones and reduced their synaptic responses to primary afferent activation. These actions are compatible with the known anatomy of NPY synapses in this region which suggest that both pre-synaptic and post-synaptic effects would be observed. They also suggest that the co-release of GABA and NPY in the dorsal horn would have similar and possibly synergistic actions. These inhibitory actions of NPY could explain its anti-nociceptive action and indicate that NPY may have analgesic action. Further study is required to identify the specific NPY receptor type(s) involved.

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176P STUDIES ON THE DESENSITISATION OF HUMAN RECOMBINANT NOCICEPTIN RECEPTORS EXPRESSED IN CHINESE HAMSTER OVARY CELLS

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Nociceptin (NC) is the endogenous ligand for the opioid receptor like-1 receptor (NCR). NCR displays some structural similarity to opioid receptors and is negatively coupled to adenylyl cyclase via a pertussis toxin sensitive G-protein (Meunier, 1997). It has been reported that chronic exposure of opioids leads to desensitization resulting in a diminished ability to inhibit cAMP accumulation (Yabaluri, et al., 1997). In the present study we examine the effects of NC pretreatment on NC induced inhibition of cAMP formation in Chinese hamster ovary cells (CHO) expressing approximately 1.7 pmol/mg protein (Okawa, et al., 1999) of the human NC-receptor.

CHO cells were maintained in DMEM/F12 (50/50) supplemented with foetal calf serum (5%), hygromycin B (200μg ml^{-1}) and G418 (200μg ml^{-1}) as described previously (Okawa, et al., 1999). Monolayers were pretreated with 1nM NC in the presence of the peptidase inhibitors; amastatin, bestain, captopril and phosphoramidon (30μM each) for 2 to 48 hours. For the longer incubations culture medium was changed every 24 hours. Following pretreatment cells were rapidly harvested with EDTA-containing HEPES buffer and washed three times with ice-cold Krebs/HEPES buffer. cAMP formation was then measured in 0.3 ml volumes of whole cell suspensions in Krebs/HEPES buffer with BSA (0.5%), IBMX (1mM), forskolin (FSK, 1μM) and NC (10^{-6} -10^{-12}M), pH7.4. After 15 min of incubation at 37°C, reactions were terminated and cAMP measured as described (Okawa, et al., 1999). All data are expressed mean±SEM (n=4-6) and were analyzed by ANOVA with post hoc Bonfferoni's test.

NC produced a concentration dependent inhibition of forskolin stimulated cAMP formation in control and pretreated cells (Figure1). Following ≥24h pretreatment the concentration response curves were shifted to the right. pIC_{50} values were 9.40±0.07 (control), 9.54±0.17 (2h), 9.25±0.09 (6h), 8.98±0.06 (24h: p=0.0008 vs control) and 8.73±0.04 (48h: p<0.0001 vs control, p=0.025 vs 24h) (Fig. 1). In addition, NC-pretreatment increased both basal and FSK stimulated cAMP values to more than double the control (untreated) levels (Table 1).

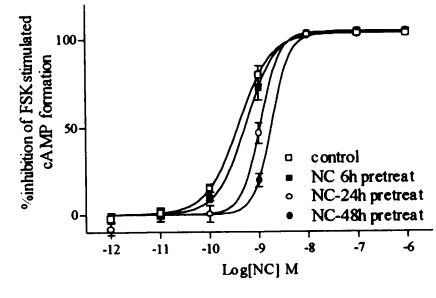
The magnitude of desensitization was dependent on the duration of pretreatment requiring in excess of 6 hours. The observation that absolute cAMP levels increased following 24 and 48 hours of pretreatment indicates that at 1.7pmol/mg protein the receptor may be constitutively active. These data are similar to those reported for opioid receptors (Pak, et al., 1996; Yabaluri, et al., 1997). Further studies are required to determine why desensitization takes so long to develop although this may be related to high levels of expression.

Table 1. Basal and FSK-stimulated cAMP mass (pmol/mg protein)

Pretreat (h)	0	24	48
Basal	8.9±0.6	23.7±2.1*	24.9±0.4*
FSK	227±12	506±34*	537±21*

*p<0.0001 compared with 0h

Fig. 1 Effects of NC pretreatment on FSK stimulated cAMP formation



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177P THE EFFECT OF *RHAYZA STRICTA* STRONGLY BASIC ALKALOIDAL FRACTION ON RAT BRAIN CATECHOLAMINES

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Rhazya stricta (RS) is a widely used medicinal plant in the Arabian Peninsula. Previously, its lyophilised crude leaf extract has been shown to posses a sedative effect (Ali *et al*, 1995), an antidepressant-like activity in the forced swimming test (Ali *et al*, 1998), a hypotensive action and other effects (Tanira *et al*, 1996). The activity-guided phytochemical analysis indicated that an alkaloidal fraction (AF) is the most active fraction. Since the antidepressant activity can be mediated by changes in brain catecholamines levels, we studied the effect of RS-AF on the brain content of these neurotransmitters.

Male Wistar rats weighing 230-250 g were used. RS strongly basic AF was given orally (200 mg kg^{-1}). After 1h animals were decapitated and their brains were collected. In one experiment, catecholamine (CA) contents were measured in the whole brain tissue, whereas in another, CAs were separately measured in the cerebrum, cerebellum, pons and medulla and the remaining part of the brain. The brain tissue was weighed, homogenised in 0.1 M of perchloric acid 10% w/v at 2-4°C then, centrifuged (7000 rpm; 5400 G) at 2 °C for 20 mins. The supernatant liquid was aspirated for HPLC analysis using electrochemical detector. The system was calibrated using standard CAs [noradrenaline (NA), adrenaline (Ad), dopamine (DA) and 5-hydroxytryptamine (5-HT)]. The obtained values were expressed as ng mg^{-1} tissue weight. Differences between group means (n=6 for each group) were calculated by unpaired Students t test, $p < 0.05$ was considered significant.

The strongly basic RS-AF caused a reduction in all measured CAs concentrations. Adrenaline concetration was the most affected (reduced to less than 2% of untreated animals). The whole brain tissue showed statistically significant reductions in all CAs (Ad -99%, NA -80%, DA -90% and 5-HT -87%). The pons and medulla were, generally, least affected. The cerebrum and the remainder part of the brain tissue values showed a variable response but significant reductions were observed with NA in the cerebellum (-72%) and DA in the cerebrum (-69%).

It is concluded that at least part of the pharmacological activity of the strongly basic RS-AF in the rat may be a result of a central action mediated by changes in CA brain concentrations. Other fractions or other doses of the same fraction may act by other mechanism(s).

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178P EFFECTS OF LOCAL ANAESTHETIC AGENTS ON [^{3}H]NISOXETINE BINDING TO RECOMBINANT HUMAN NOREPINEPHRINE TRANSPORTERS

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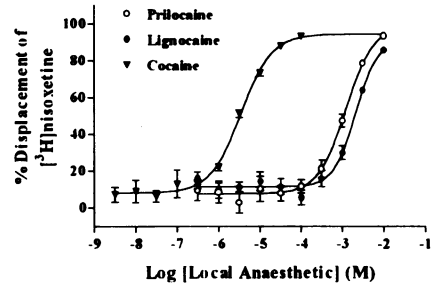
Intravenous regional guanethidine is often used to treat reflex sympathetic dystrophy (Hannington-Kiff. 1977). Guanethidine is taken up via the neuronal norepinephrine transporter (NET) and displaces norepinephrine from release vesicles (Maxwell. 1982). Clinically a local anaesthetic (LAs) is co-administered to reduce the discomfort of administration. As we have previously demonstrated that LA's inhibit norepinephrine uptake. (Joyce *et al.*, 1998a) the validity of this coadministration is debatable. LAs may inhibit norepinephrine uptake by competing with the substrate or interacting at the uptake recognition site. In this study using recombinant human NET (hNET) expressed in human embryonic kidney cells (293-hNET) we have examined the effects of cocaine. lignocaine and prilocaine on the binding of [^{3}H]nisoxetine to the uptake recognition site.

293-hNET cells were maintained as described previously (Joyce *et al.*, 1998b). Cell suspensions were homogenised in buffer of the following composition: Tris (50mM). NaCl (120mM). pH7.4. The homogenate was then centrifuged at 16,500g for 10 mins and washed with ice cold homogenisation buffer. This procedure was repeated twice more with the final pellet resuspended in incubation buffer of the following composition Tris (50mM). NaCl (300mM). pH7.4. The K_d and B_{max} of [^{3}H]nisoxetine binding was determined by isotope dilution of ~0.5nM [^{3}H]nisoxetine with 0.2-25nM unlabelled nisoxetine. Incubations were performed in 0.5ml volumes for three hours at 0°C in the dark. Non-specific binding was defined in the presence of 10μM imipramine. In displacement studies ~0.5nM [^{3}H]nisoxetine was incubated in the presence of cocaine ($3x10^{-9}$-10^{-2}M), prilocaine and lignocaine ($3x10^{-8}$-10^{-4}M). Bound and free radioactivity were separated by rapid vacuum filtration using a Brandell harvester. The pK_d and B_{max} of nisoxetine binding and the concentration of local anaesthetic producing 50% inhibition of uptake (pIC_{50}) were estimated by non-linear regression. pIC_{50} values were corrected for the competing mass of [^{3}H]nisoxetine to yield pK_i. All data are mean±s.e.mean (n≥4).

The binding of [^{3}H]nisoxetine to 293-hNET cell membranes was concentration dependent and saturable with pK_d, slope and B_{max} values of 8.35±0.05, 1.33±0.11 and 6.48±0.40pmol mg protein^{-1} respectively. In addition, cocaine, prilocaine and lignocaine inhibited the binding of [^{3}H]nisoxetine with pK_i values of 5.49±0.01, 2.92±0.03, 2.68±0.04 respectively, Figure 1.

Figure 1. Effect of cocaine, prilocaine and lignocaine on [^{3}H]nisoxetine binding in 293-hNET membranes.



In this study, we have demonstrated that cocaine. prilocaine and lignocaine inhibit binding of [^{3}H]nisoxetine to hNET. These values are similar to those reported for the inhibition of norepinepherine uptake (pIC_{50}:cocaine, 6.44±0.09. prilocaine 3.19±0.05 and lignocaine 2.89±0.07, Joyce *et al.*, 1998b and unpublished) indicating that uptake inhibition may be mediated, in part, via an interaction with the recognition site.

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179P THE ROLE OF INTERCELLULAR GAP-JUNCTION COMMUNICATION ON BICUCULLINE-INDUCED EPILEPTIFORM EXCITABILITY IN DISSOCIATED RAT HIPPOCAMPAL NEURONES *IN VITRO*: EFFECTS OF CARBENOXOLONE

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The $GABA_A$ receptor antagonist bicuculline (BIC) has been shown to induce synchronised bursting activity in monolayer hippocampal neuronal networks grown on multiple microelectrode arrays (MMEAs) *in vitro* (Sokal *et al.*, 1998). The role of intercellular gap-junctional communication in synchronised bursting in this system was investigated using the glycyrrhetinic acid derivative, carbenoxolone (CBX). CBX has been shown to block intercellular junctional communication (Davidson *et al.*, 1986). A 64-channel MMEA was utilised to examine the effects of changing the recording medium from Neurobasal media with B27 serum-free supplement to artificial cerebrospinal fluid (ACSF) and of CBX on BIC-synchronised epileptiform bursting within hippocampal neuronal networks.

Primary dissociated Wistar rat hippocampal neurones from E18 fetuses were cultured directly onto MMEAs for 21 days in Neurobasal medium with B27 serum-free supplement. Multiple single-unit discharge activity was captured using a multi-channel neuronal acquisition processor (Plexon Inc, Texas, USA). Data was analysed off-line using *SpikeWorks* (Plexon Inc), then further processed using multiple spike train analysis software (*NeuroEXplorer*, NEX Technologies, USA). Synchronisation between cells within the network was assessed using cross-correlation analysis between a reference cell and the others recorded on the array. Data are expressed as mean±s.e.mean. Statistical differences between groups were measured using a Student's paired t-test.

Between 12-16 cells were recorded simultaneously per MMEA. All cells recorded exhibited spontaneous extracellular discharge activity at 37°C in Neurobasal medium, expressing firing rates of 1.1-37.4Hz (10.4±1.3Hz). Changing the recording medium to ACSF decreased firing in all cells recorded (3.5±0.5Hz; $p<0.001$). BIC (10μM) increased firing rate (0.6±0.1Hz to 1.5±0.2Hz; $p<0.001$) and burst rate (3.7±0.5 to 9.1±0.9 bursts min^{-1}; $p<0.001$) in all cells recorded (n=43 cells/3 MMEAs); a burst was defined as a minimum of three spikes with interspike intervals <0.01s. Bursting in the presence of BIC was highly synchronised between all cells recorded. Subsequent addition of CBX (100μM) significantly decreased the firing rate to basal levels (1.5±0.2Hz to 0.7±0.1Hz; $p<0.001$). Burst rate was also significantly reduced with CBX (9.1±0.9Hz to 2.8±0.4 bursts min^{-1}; $p<0.001$) however the level was significantly higher ($p<0.01$) than basal values. CBX had no effect on the synchronisation of firing between cells

Extracellular firing rates in ACSF were significantly lower than those seen in Neurobasal culture medium. This could, in part, be a reflection of the elevated potassium concentration in Neurobasal medium (5.4mM) compared to ACSF (4.5mM). In these monolayer hippocampal networks CBX reversed bicuculline-evoked epileptiform activity, decreasing both burst and firing rates, but had little effect on the synchronisation of bursting between cells in the network.

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180P PARADOXICAL EFFECTS OF THE NON-OPIOID PEPTIDE NOCICEPTIN (ORPHANIN FQ) ON NOCICEPTION IN RATS

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Nociceptin (orphanin FQ) is an endogenous ligand for the orphan opioid-like receptor (Reinscheid et al, 1995; Meunier et al, 1995). When given i.c.v. this peptide apparently produces hyperalgesia. At the spinal level it has been reported either to produce analgesia or have no effect. Nociceptin has also been claimed either to reverse or potentiate morphine analgesia. We studied the spinal effects of nociceptin in the rat during restraint stress-modulated nociception, when both analgesia and hyperalgesia are produced in the same individual, depending on the noxious stimulus (Pilcher & Browne, 1983).

Male Sprague-Dawley rats (300-350g) were spinally catheterised under pentobarbitone anaesthesia according to the procedure of Yaksh & Rudy (1976). Two days were allowed for recovery. Peptides were administered in a volume of 10μl (i.t.). Other drugs were given by s.c. injection. Control animals received vehicle only. Stress was induced by confining rats in restraint cages for 15 min. Nociceptive responses to heat and pressure were measured using tail-immersion (52°C) and paw-pressure tests, respectively. In all tests there were at least 7 rats per group. Statistical analysis was by one-way ANOVA and two-tailed t-tests.

With noxious heat, nociceptin (5-40μg) dose-dependently elevated response thresholds. This effect was maximal at 20μg (30%; $p< 0.01$) and not significantly antagonised by naloxone at 1mg kg^{-1}, the dose used throughout. Restraint alone produced naloxone-reversible increases in latency (23%; $p<0.01$). Nociceptin given prior to restraint had an additive effect, raising thresholds by 44% ($p<0.01$). However, it antagonised the analgesic effects of morphine at 5 and 10 mg kg^{-1} by 91% and 44% respectively ($p<0.01$).

With noxious pressure, nociceptin at lower doses (1-10μg) produced a weak but significant reduction in response thresholds (-12%; $p<0.05$), which was reversed by naloxone. Restraint alone also slightly lowered thresholds (-14%; $p<0.05$), in a naloxone-reversible manner. In combination with restraint nociceptin did not lower thresholds below those of restraint alone. Morphine at 5 and 10 mg kg^{-1} raised thresholds by 191% and 281% respectively ($p<0.001$). This analgesia was not affected by the peptide. Both the analgesic and hyperalgesic actions of nociceptin (10μg) were fully reversed by the nociceptin-receptor antagonist [Phe^1-Ψ(CH_2-NH)-Gly^2]nociceptin (1-13)-NH_2 (1 μg given 10 min prior to the agonist).

This study confirms that nociceptin has analgesic activity at the spinal level against noxious heat. In contrast, it produces mild hyperalgesia with pressure. Thus, the spinal action of nociceptin in nociception depends on the nature of the noxious stimulus. Paradoxically, nociceptin antagonised morphine-induced analgesia but enhanced that produced by stress.

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181P GALANIN FACILITATES THE RESPONSES OF DORSAL HORN WIDE-DYNAMIC NEURONES IN THE ANAESTHETIZED RAT

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The aim of this study was to observe the contribution of the peptide, galanin on the processing of somatosensory inputs at the spinal cord level.

Galanin (porcine) was evaluated on the electrically evoked responses of dorsal horn wide-dynamic range (WDR) neurones in the anaesthetized rat. Male Sprague Dawley rats (200-220g) were anaesthetized with enflurane in a mixture of O_2/N_2O (33/66% respectively) and held in a stereotaxic frame. The lumbar segments (L_1-L_3) were removed via a laminectomy to expose the spinal cord. The cord was then clamped rostral and caudal to the laminectomy for stability. The peripheral receptive field was stimulated via transcutaneous electrical stimulation to evoke Aβ-, Aδ, C-fibre, post-discharge and wind-up responses of the WDR-neurones. Galanin (0.15-15nmol/50µl, n=9, obtained from Bachem) was applied intrathecally onto the exposed spinal cord, in a cumulative manner, followed by the non-selective galanin antagonist, (D-Thr^6, D-Trp^{8,9}, 15-ol)-galanin (1-15) (30nmol, obtained from Sigma). Each dose was followed for one hour. Galanin and (D-Thr^6, D-Trp^{8,9}, 15-ol)-galanin (1-15) were made up in saline and frozen until required. Results are expressed as percentage of control ± 1 s.e. mean. Statistical analysis was carried out using a Student's t-test, paired, 2 tailed, and significance set at $P<0.05$.

Galanin had no effect on the Aβ-fibre evoked responses compared with controls, see Table 1.

In contrast the Aδ- and C-fibre evoked responses tended to be enhanced by 1.5 and 15nmol galanin. However, only the C-fibre evoked responses were significantly facilitated with 15nmol galanin ($P<0.05$). The enhanced neuronal responses of post-discharge and wind-up were facilitated significantly with both 1.5 and 15nmol galanin ($P<0.05$) as were the acute C-fibre evoked responses, see Table 1. The antagonist (D-Thr^6, D-Trp^{8,9}, 15-ol)-galanin (1-15) (30nmol/50ul) did not attenuate the facilitations produced by galanin.

	0.15	1.5	15nmol Galanin
Aβ	89±12	90 ±12	93±13
Aδ	76±12	117±9	132±11
C	105±11	121±12	126±9
Acute C-fibre	116±13	143±7	151±18
PD	176±34	207±25	222±32
Wind-up	128±23	171±22	192±21

Table 1: Effects of 0.15, 1.5 and 15nmol galanin on the Aβ-, Aδ, C-fibre, acute C-fibre, post-discharge (PD) and wind-up. Results are expressed as % of control ±s.e.m.

This study demonstrates an involvement of the peptide galanin in the processing of noxious inputs in the dorsal horn of the spinal cord. The resultant facilitation of dorsal horn wide-dynamic range neurones may result in hyperalgesia in an awake animal.

182P CHARACTERISATION OF THE BINDING OF THE ANTAGONIST RADIOLIGAND [^{3}H]-ZM241385 TO A_{2A} ADENOSINE RECEPTORS IN PARTICULATE AND SLICE PREPARATIONS FROM PORCINE STRIATUM

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The antagonist ZM241385 has proved useful as a means of identifying A_{2A} adenosine receptor-mediated responses both *in vivo* and *in vitro* (Ralevic & Burnstock, 1998). Binding of a tritiated version of this ligand has recently been examined in rat brain homogenates (Alexander, 1998). Here we report binding of [^{3}H]-ZM241385 to particulate and slice preparations from porcine striatum.

Particulate preparations from porcine striatum (dissected from whole brain rapidly transported in ice from the abattoir) were obtained and used in binding assays, as previously described (Alexander *et al.* 1998). Saturation analysis was conducted over the nominal radioligand concentration range of 0.19 - 16 nM; competition curves (antagonist range 10^{-12} – 10^{-5} M; agonist range 10^{-8} – $10^{-3.5}$ M) at 0.6 nM. Binding of [^{3}H]-ZM241385 to intact slices (350 x 350 µm) was conducted in quadruplicate over 30 min at 37°C, in Krebs-Henseleit buffer (pH 7.4) containing 1 U.mL^{-1} adenosine deaminase (Alexander *et al.*, 1994). Saturation analysis was conducted over the nominal radioligand concentration range of 0.5 - 22 nM; competition curves at 2.5 nM. In both instances, non-specific binding was defined by 5 mM theophylline. Data reported are means ± SEM of 3-6 separate experiments.

Analysis of [^{3}H]-ZM241385 saturation isotherms in particulate preparations showed the radioligand to have a K_d of 0.26 ± 0.03 nM, with a B_{max} of 941 ± 13 fmol.mg protein^{-1}. At 0.4 nM radioligand, non-specific binding was 11 ± 2 % total binding. Analysis of saturation data generated using striatal slices showed that [^{3}H]-ZM241385 bound specifically, with a calculated K_d value of 7.0 ± 2.3 nM. A B_{max} value of 742 ± 137 fmol.mg protein^{-1} could be calculated, on the assumption of 1 mg protein per 25 µL of packed brain slices (Alexander *et al.*, 1994). At a concentration of 5 nM radioligand, non-specific binding was 48 ± 5 % total binding.

Analysis of ligand competition for [^{3}H]-ZM241385 binding to striatal membranes showed that curves fitted to two sites failed to fit better than those to a single site (F-test, GraphPad Prism). The rank order of adenosine receptor antagonist affinities was (pK_i values, Hill slopes): SCH58261 (8.39 ± 0.01, -1.13 ± 0.17) > XAC (7.50 ± 0.08, -1.22 ± 0.12) > DPCPX (7.16 ± 0.06, -1.45 ± 0.20). Agonist competition curves showed the rank order (pK_i values, Hill slopes): NECA (7.20 ± 0.05, -1.08 ± 0.05) > CGS21680 (7.05 ± 0.10, -0.86 ± 0.08) > IB-MECA (5.83 ± 0.03, -1.14 ± 0.07) > CCPA (5.33 ± 0.18, -0.99 ± 0.11). In striatal slices, NECA (over the range $10^{-7.5}$ – 10^{-3} M) competed for [^{3}H]-ZM241385 binding with a calculated pK_i value of 5.23 ± 0.11 (Hill slope -0.61 ± 0.10).

These data indicate that the novel antagonist radioligand [^{3}H]-ZM241385 binds with high affinity and low non-specific binding to A_{2A} adenosine receptors in the porcine striatum. The reduced affinity of [^{3}H]-ZM2141385 and NECA in the intact slice preparation is distinct to the maintained affinities of agonists and antagonists at A_1 receptors in guinea-pig cerebral cortex slice and particulate preparations (Alexander *et al.*, 1994). Whether factors such as the distinct intracellular and extracellular components or temperature lead to these differences awaits further investigation.

We thank Tocris-Cookson for provision of the radioligand.

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183P GLUTAMATE RELEASE FROM RAT BRAIN SLICES IN RESPONSE TO HYPOXIA/AGLYCAEMIA: INHIBITION BY CLOMETHIAZOLE

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There is substantial evidence that clomethiazole (CMZ) is neuroprotective in animal models of acute ischaemic stroke (Green, 1998) and CMZ improved functional outcome in patients whose clinical description suggested a large stroke (Wahlgren *et al.*, 1999). Baldwin *et al.* (1994) found no effect of a high concentration of CMZ (1 mM) on glutamate release from cortical slices *in vitro* over a 12 min hypoxic period. Here we used a hypoxic/aglycaemic (H/A) challenge and a longer incubation period.

Equivalent aliquots of cerebro-cortical slices (350 μm^2) from adult female Wistar rats (200-250 g) were incubated at 37°C for 30-45 min in mesh baskets immersed in Hepes-buffered saline (HBS; 2 ml). Different aliquots were incubated in either control HBS (10 mM glucose, normoxic), H/A HBS (no added glucose, bubbled with N_2 for >2 h), or H/A-HBS containing varying concentrations of GABA or CMZ. In some experiments, aliquots were transferred to fresh HBS at 5 min intervals to determine a time course for glutamate release. Released glutamate was measured fluorimetrically using the reduction of $NADP^+$ by glutamate dehydrogenase (Nicol *et al.*, 1996). Significance was assessed by paired Student's t-tests ($p<0.05$).

Glutamate release increased modestly over time in control HBS (1.18 fold after 30 min, n=8, p=0.09) and release increased significantly in H/A conditions (2.4 fold after 30 min, n=8, p=0.003). In a second series of experiments, uninterrupted incubation in H/A-HBS gave a similar increase in release (Figure 1) which was depressed by either CMZ (10-300 μM; Fig 1) or GABA (10-300 μM; data not shown). Thus, a substantial fraction of H/A-induced glutamate release in this system was inhibited by either CMZ or GABA. This may be relevant to the neuroprotective effects of CMZ *in vivo*.

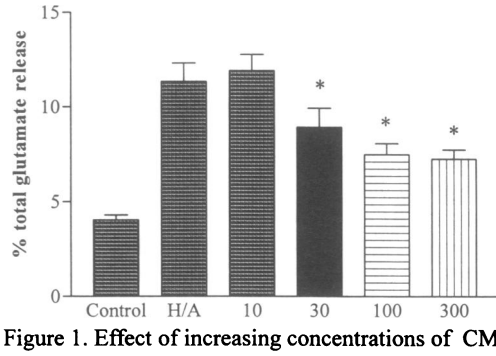

Figure 1. Effect of increasing concentrations of CMZ (μM) on the H/A-induced release of glutamate from cortical tissue slices after 30 min incubation (mean + s.e.mean, n=3; *Different from H/A, $p \leq 0.05$).

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184P INHIBITION OF THE BRADYKININ-INDUCED SENSITIZATION OF HEAT ACTIVATED CURRENTS IN DORSAL ROOT GANGLION CELLS BY THE ANTI-HYPERALGESIC COMPOUND GABAPENTIN

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Applications of noxious heat are transduced into inward currents in nociceptive nerve terminals. These responses often show sensitization following repeated applications of a painful stimulus. Although the exact mechanisms of transduction and sensitization in response to noxious heat are not fully understood, it has been suggested that activation of PKC and subsequent phosphorylation of the channel or an intracellular mediator cause the sensitization of the heat response following bradykinin application (Cesare & McNaughton. 1996). In the present study the whole cell patch clamp technique was used to examine the effects of the anti-hyperalgesic drug Gabapentin (Neurontin) on bradykinin sensitized heat currents.

Dorsal root ganglion neurones from 2-5 day old Sprague-Dawley rats were enzymatically dissociated and cells maintained in culture for 48-96hr before recordings were performed. Cells were superfused ($0.5ml.min^{-1}$) with a solution containing (in mM): NaCl 130; KCl 4; $CaCl_2$ 5; $MgCl_2$ 1; HEPES 10; D-glucose 4. Whole cell patch clamp recordings were made with electrodes containing (in mM): KCl 135; $CaCl_2$ 0.2; $MgCl_2$ 1.6; EGTA 2; MgATP 2.5; LiGTP 0.2; HEPES 10. All solutions were adjusted to pH 7.3. Small diameter cells (<30μm) were held at –80mV and inward currents were evoked by rapidly applying 1s pulses of bathing solution at 55°C using an automated multipipe solution changer (Biologic RSC-100). Solution temperature was monitored by a thermocouple placed immediately before the solution entered the recording chamber. All data presented are mean±s.e.mean of the number of observations given in parentheses.

Pre-treating the cells with bradykinin before the heat application could sensitize the response to noxious heat. A 4min application of bradykinin (1μM) evoked an inward current in 40% of the cells tested, which declined to zero by the end of the bradykinin application. After 1min of bradykinin application the response to noxious heat had increased by 46±12% (n=20) and sensitization of the heat response was still observed even when the bradykinin current had returned to zero.

Pre-treatment of the cells with Gabapentin, for up to 10min, had no effect on the holding current of the cells or the amplitude of the currents evoked by the application of noxious heat. However, Gabapentin inhibited the bradykinin induced sensitization of the heat current in a concentration-dependent manner (10nM-100μM) with an IC_{50} of 120nM, a Hill slope close to unity and maximum inhibition produced by 100μM. A 5min pre-application of Gabapentin (100μM) significantly reduced the bradykinin sensitization by 79±7% (n=15, $p<0.05$, Student's unpaired t-test).

These results demonstrate that responses to noxious heat can be sensitized by bradykinin application. This sensitization is not simply due to activation of a membrane current by bradykinin as sensitization was still observed after the bradykinin induced current had returned to zero. Gabapentin significantly inhibits the bradykinin-induced sensitization of the heat current, possibly accounting for some of its anti-hyperalgesic effects.

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185P ALTERED THERMOREGULATORY RESPONSE TO HIGH AMBIENT TEMPERATURE (30 °C) IN MDMA-PRETREATED RATS

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Administration of 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy") to rats produces acute hyperthermia and long term neurotoxic loss of 5-hydroxytryptamine (5-HT) in several brain regions (see O'Shea *et al.*, 1998). Recently Dafters & Lynch (1998) using radio-biotelemetry found that MDMA-treated female Wistar rats showed a prolonged hyperthermic response following a thermoregulatory challenge of exposure to high ambient temperature. We have now examined whether Dark Agouti rats also show this altered response to elevated ambient temperature using conventional rectal temperature measurement.

Male adult (200-260g) Dark Agouti rats were used. An established neurotoxic dose (O'Shea *et al.*, 1998) of MDMA (12.5 mg kg^{-1} i.p.) at 10.00h produced a rapid and sustained hyperthermia lasting >3.5 h. 24 h post-injection the temperature of the MDMA-treated group was similar to the saline-injected group and remained so (measured at 10.00 h) over the following 3 weeks.

Four to 6 weeks post treatment both saline- and MDMA- treated groups were exposed to an ambient temperature of 30°C for 1h and then returned to a room at 20°C. During the high temperature exposure the rectal temperature of both groups rose approx. 1°C. When returned to normal ambient temperature conditions, the rectal temperature of the control group returned to normal within 1.5 h while the MDMA-treated group remained elevated above control for >2.5h (Figure), consistent with the findings of Dafters & Lynch (1998).

No difference was found between the 2 groups in the hypothermic response following 8-OH-DPAT (0.15mg kg^{-1}s.c.) [Saline- treated: -2.0 ± 0.1°C; MDMA-treated: -2.1 ± 0.1°C, measured 25 min post 8-OH-DPAT injection; n = 12 each group].

The data suggest that a neurotoxic dose of MDMA impairs the ability of rats to thermoregulate both during and following exposure to a high ambient temperature and this change does not result from a change in the response of the 5-HT_{1A} receptor.

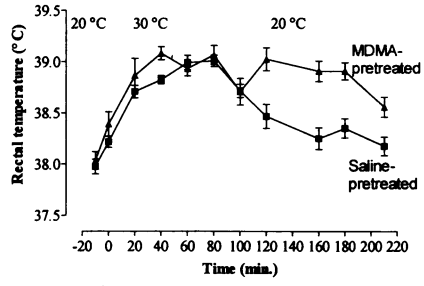

Figure. Effect on rectal temperature of exposure of rats (kept at ambient temperature of 20°C) to an ambient temperature of 30°C for 60 min. MDMA-pretreated group (n=12) diff from control group (n=12) during t_0-t_{40}: $F(1,22)=8.99$, $P<0.05$; t_{60}-t_{210}: $F(1,22)=20.89$, $P<0.001$ (ANOVA with repeated measures, followed by Bonnferoni post-hoc test).

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186P OXIDATIVE STRESS AND LOSS OF [^{3}H]PAROXETINE BINDING IN RAT CORTEX FOLLOWING CHRONIC 3,4-METHYLENEDIOXYMETAMPHETAMINE (MDMA) ADMINISTRATION

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Chronic administration of 3,4-methylenedioxymethamphetamine (MDMA) is widely accepted to cause degeneration of 5-HTergic neurones in rodents (Battaglia *et al.*, 1987). More recently, oxidative stress has been indirectly suggested to play a role in this degeneration, since the damage can be prevented by spin trap agents such as α-phenyl-N-tert-butyl-nitrone (Colado & Green, 1995). The aim of the present study was to examine more directly whether oxidative stress was involved, by investigating whether specific biomarkers of lipid and protein oxidative damage were elevated in the rat brain following chronic MDMA administration.

Male Sprague Dawley rats (280-300g) were treated chronically with MDMA (10 mg / kg i.p; n=5), or vehicle (n=5), at 12 h intervals for a total of 4 injections. Locomotor activity was monitored following each dose to confirm the efficacy of MDMA. Approximately 6 h after the last injection the animals were killed by cervical dislocation, their brains removed and the hemispheres separated. One hemisphere was frozen intact, then cryostat sectioned and used for [^{3}H]paroxetine autoradiography (Meoni *et al.*, 1997) to monitor the level of 5-HT uptake sites. Cortex, brainstem (containing raphe nuclei) and cerebellum (control region) were dissected from the other hemisphere, snap frozen and prepared for biochemical assays of malondialdehyde (MDA), an end product of lipid peroxidation, and protein carbonyls, an index of oxidative damage to proteins. The MDA assay utilised standard detection of thiobarbaturic acid reactive substances (TBARS) with additional HPLC in order to specifically isolate the MDA-TBA complex. Both this and the protein carbonyl assay were performed as previously described (Lyras, *et al.*, 1997). Statistical significance between MDMA and vehicle treated groups was assessed, for all parameters, using a Student's unpaired t-test (significance level $p<0.05$).

MDMA administration produced a significant locomotor increase compared to vehicle, confirming the efficacy of the MDMA dose used. Chronic administration of MDMA significantly reduced specific [^{3}H]paroxetine binding in the hippocampus (51.0 %) and cortex (56.3%) compared to vehicle, but not in the brainstem or cerebellum (control region). The data for lipid peroxidation appear in Table 1. MDA levels were significantly increased in the cortex, but not in the brainstem or cerebellum following chronic MDMA. Protein carbonyls were also elevated by approximately 34 % in the cortex, but this failed to reach significance. No changes in protein carbonyl levels were noted in either the brainstem or cerebellum.

Brain Area	Vehicle (4 x 1ml/kg i.p)	MDMA (4 x 10mg/kg i.p)
Cortex	21.81 ± 1.36	24.10 ± 1.47 *
Cerebellum	19.82 ± 3.45	19.79 ± 3.18
Brainstem	17.16 ± 2.29	20.47 ± 3.91

Table 1: Effect of chronic administration of MDMA or vehicle on levels of MDA (μM/mg tissue). Data are mean ± s.e.m. *Significant difference between MDMA and vehicle ($p<0.05$, unpaired t-test).

In conclusion, these data confirm that chronic administration of MDMA in the rat produces a loss of 5-HT uptake sites in the cortex and hippocampus, but not in brainstem, indicating degeneration of 5-HT neuronal terminals. Although not yet analysed in the hippocampus, the significant increase in the biomarker of lipid peroxidation in the cortex and the trend for increased oxidative protein damage in this region, indicate that oxidative stress may underlie at least some of the neurodegenerative effects of MDMA.

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187P AN *IN VITRO* MODEL OF INFLAMMATORY NEURODEGENERATION AND ITS NEUROPROTECTION BY ANTIOXIDANTS

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In the past few years, inflammation has been been implicated in disorders such as Alzheimer's disease. In this disease the inflammatory processes have been shown not only to result from neurodegenerative effects but also to contribute to these effects (Pasinetti, 1996). To investigate the primary effect of inflammatory processes on neuronal survival a co-culture system of neuronal cells and monocytic cells was set up.

SH-SY5Y human neuroblastoma cells were differentiated for two weeks into postmitotic neuronal cells in the presence of 10μM retinoic acid. These were co-cultured with monocytic cells from the THP-1 cell line in a HEPES-based buffer (Munir et al., 1995). THP-1 cells were activated with a combination of phorbolmyristateacetate (PMA, 10ng/ml) and lipopolysaccharide (LPS, 20μg/ml) present in the buffer (Gatanaga et al., 1991). After 5 days of co-culture, neuronal cell death was determined with the LDH-assay.

Protective effects of the antioxidants ascorbic acid and glutathione were investigated by addition to the co-culture in concentrations ranging from 0-200μM for ascorbic acid and 0-2mM for glutathione. Again cell death was determined after 5 days. Data were analysed for significance using one-way ANOVA.

The co-culture of activated THP-1 resulted in a significant increase of neuronal cell death compared to the co-culture of neuronal cells without activation (Table 1). The co-application of ascorbic acid to the co-culture reduced the cell death due to the activated THP-1 highly significantly. Glutathione also reduced the cell death caused by activated THP-1 but to a lesser extent than ascorbic acid; this also demonstrated a toxic effect in the unactivated co-culture ($p<0.05$). The application of PMA and LPS to neuronal cultures in the absence of THP-1 cells did not result in any increased cell death over the course of 5 days.

Table 1: Neuronal cell death in the presence of THP-1 monocytes

	unactivated	activated
co-culture	25.4±5.6	38.4±5.4*
+ ascorbic acid (10μM)	25.5±2.4	20.6±8.0^{+}
+ glutathione (500μM)	34.4±5.7	31.0±3.0^{+}

Values are percent of maximal cell death expressed as mean±s.d.
* Significant versus unactivated monocytes ($p<0.001$, n=8).
$^{+}$ Significant versus activated monocytes without antioxidants: ascorbic acid ($p<0.001$, n=3) and glutathione ($p<0.05$, n=3).

The co-culture of SH-SY5Y cells and activated THP-1 provides a useful model for the study of the involvement of inflammation in neuronal cell death. As shown here, activated monocytes can increase cell death in neuronal cell culture. Cell death was reduced by the co-application of antioxidants such as ascorbic acid, implicating reactive oxygen species in the inflammatory neurotoxicity seen here. These first results provide the basis for further investigation of the involvement of inflammation in neurodegeneration and its prevention. This model could be useful for the examination of the effects of potential antiinflammatory and neuroprotective drugs.

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Pasinetti, G.M. (1996) *Neurobiol. Aging*, 17, 707-716

188P PROTECTIVE EFFECTS OF CLYCLOOXYGENASE INHIBITION AGAINST MPTP TOXICITY

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Salicylic acid and acetylsalicylic acid showed a pronounced protection against MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induced striatal dopamine depletion in mice [Aubin *et al.*, 1998]. In the present study we investigated the effects of the preferential cyclooxygenase-2 (COX-2) inhibitor meloxicam in comparison with the preferential COX-1 inhibitor acetylsalicylic acid on MPTP-induced dopamine depletion and locomotor activity.

Male C57BL/6 mice (22-25g, n = 82) were injected with a single dose of acetylsalicylic acid (10, 50, 100 mg/kg i.p.) or meloxicam (2, 7.5, 50 mg/kg i.p.) immediately before administration of MPTP (30 mg/kg s.c.) or saline. One day before and one day after MPTP or saline treatment locomotor activity was counted using activity cages with automatically counting photobeam interruptions in a 60 min test session. One week after MPTP treatment mice were sacrificed and striatal dopamine and its metabolites were analysed using HPLC.

In non-MPTP treated animals neither acetylsalicylic acid nor meloxicam altered dopamine, serotonin and metabolite levels. MPTP treatment alone led to a significant depletion of dopamine to 2.2 ± 0.4 ng/mg, compared with 13.9 ± 0.7 ng/mg in saline treated controls. Acetylsalicylic acid and meloxicam significantly attenuated the MPTP-induced dopamine depletion by 20 % (ANOVA, Duncan-test) (Fig. 1). One day after MPTP treatment acetylsalicylic acid and meloxicam significantly attenuated MPTP induced hypomotility (ANOVA, Duncan-test).

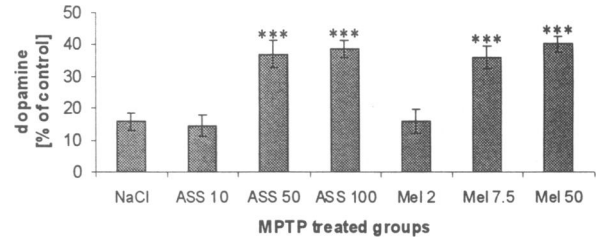

Fig.1: Effects of acetylsalicylic acid (ASS, mg/kg) and meloxicam (Mel, mg/kg) treatment on MPTP-induced depletion of striatal dopamine levels. Data are mean ± S.E.M. of n = 6 – 10 animals. The saline + saline treated group served as control and was set at 100 % (*** $P < 0.001$, ANOVA with subsequent Duncan-test for multiple comparisons).

In conclusion, MPTP-induced dopamine depletion was partly antagonised by acetylsalicylic acid and meloxicam. Furthermore, both drugs enhanced functional recovery indicated by improved locomotor activity. Our data demonstrate that inhibition of either COX-1 or COX-2 leads to a similar degree of attenuation of MPTP-induced neurotoxicity.

This work was supported by grants of the DFG (Fe 465/1-2 and 1-3) and the Hertie-Foundation (Frankfurt a.M.).

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189P PARTIAL AGONIST-INVERSE AGONIST BEHAVIOUR OF 6'-AZIDOHEX-2'-YNE-Δ^8-TETRAHYDROCANNABINOL AT CANNABINOID CB AND CB_2 RECEPTORS

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We have shown previously that 6'-azidohex-2'-yne-Δ^8-tetrahydrocannabinol (O-1184) behaves as a potent and selective cannabinoid receptor antagonist in the myenteric plexus-longitudinal muscle preparation of guinea-pig small intestine and that it readily binds to cannabinoid receptors on membranes obtained from this tissue and on guinea-pig forebrain membranes (Ross *et al.*, 1998). More recently, Griffin *et al.* (1999) concluded from results they obtained in experiments with [^{3}H]CP55940 and [^{35}S]GTPγS using rat cerebellar membranes, that O-1184 is a partial agonist for the cannabinoid CB_1 receptor. This investigation was directed at characterizing O-1184 more fully and at comparing it with CP55940, an established agonist for cannabinoid CB_1 and CB_2 receptors (Ross *et al.*, 1999).

Chinese hamster ovary (CHO) cells expressing human CB_1 or CB_2 receptors were used to perform binding experiments with [^{3}H]CP55940 and cyclic AMP assays as described by Ross *et al.* (1999). Cyclic AMP production was stimulated by 2 μM forskolin and normalized to 100%. Experiments were also performed with vasa deferentia (MVD) from adult MF1 mice (35 to 52 g). Tissues were mounted in 4 ml organ baths at an initial tension of 0.5 g and stimulated supramaximally with 0.5 s trains of 3 pulses (train frequency 0.1 Hz; pulse duration 0.5 ms). Isometric contractions were recorded. The baths contained Mg^{++}-free Krebs solution kept at 37°C and bubbled with 95% O_2 and 5% CO_2 (Griffin *et al.*, 1997). Concentration-response curves were constructed cumulatively using 30-min dose cycles. Drugs were dissolved in ethanol (CHO cells) or mixed with two parts of Tween 80 by weight and dispersed in saline (MVD). pK_i, pEC_{50} and E_{max} values were calculated using the equation for a sigmoid log concentration-response curve (GraphPad Prism; Ross *et al.*, 1999). Values are expressed as means and variability as s.e.mean or 95% confidence limits.

O-1184 readily displaced [^{3}H]CP55940 from specific binding sites on membranes of CB_1 (pK_i = 8.28±0.07; n=5) and CB_2 cells (pK_i = 8.13±0.01; n=3). In CB_1 cells, both CP55940 and O-1184 depressed cyclic AMP production with pEC_{50} values of 9.27±0.17 and 9.51±0.20 respectively (n=6). The maximal effect of CP55940 exceeded that of O-1184, the E_{max} values of these cannabinoids being 98.2% (78.5 to 117.9%) and 64.7% (56.8 to 72.7%) respectively. In CB_2 cells, O-1184 enhanced cyclic AMP production by 1.61±0.10 fold (pEC_{50} = 8.20± 0.38; n=4) and at 100 nM, it decreased the pEC_{50} of CP55940 for inhibition of cyclic AMP production from 8.61±0.16 to 7.42±0.17 ($p<0.01$; unpaired t test; n=3). In MVD, O-1184 inhibited electrically-evoked contractions with pEC_{50} and E_{max} values of 9.08±0.46 (n=20) and 21.1% (14.0 to 28.1%). At 1 nM, which induced 9.1±4.4% inhibition of evoked contractions, O-1184 decreased the pEC_{50} of CP55940 in MVD from 9.98±0.20 to 8.70±0.48 ($p<0.05$; unpaired t test; n=7) without affecting the E_{max} values of CP55940: 95.1% (84.5 to 105.8%) and 96.4% (67.6 to 125.2%) respectively.

In conclusion, O-1184 behaved in MVD and at CB_1 receptors as a partial agonist with less efficacy than CP55940 and mixed agonist-antagonist properties and, at CB_2 receptors, as an inverse agonist.

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190P AGONIST-INDUCED INHIBITION OF CELL SURFACE CANNABINOID RECEPTOR EXPRESSION IN CULTURED HIPPOCAMPAL NEURONES

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Many of the central effects of cannabis and cannabinoid agonists have been shown to be mediated by activation of the CB_1 subtype of cannabinoid receptors. These receptors are expressed mainly on neurones and are at their highest density in the hippocampus, basal ganglia and cerebellum. Using a polyclonal antibody raised against the N-terminus of the rat cloned CB_1 receptor (Tsou *et al.*,1998), we have previously demonstrated that cell surface CB_1 receptors are localised as discrete puncta on fine axonal neurites in living cultured hippocampal neurones (Coutts *et al.*,1998). In the present study, we have investigated the effects of the cannabinoid receptor agonist, (+)-WIN55212, its inactive (–)-enantiomer and the selective CB_1 receptor antagonist, SR141716A (Pertwee, 1997) on the expression of cell surface CB_1 receptors levels on these neurones.

Cultures of rat hippocampal neurones were prepared from 1-2 day old pups as described by Irving *et al.*,(1992). Cells, cultured for 6-8 days, were pretreated overnight at 37°C with (i) (+)-WIN55212, (ii) (–)-WIN55212, (iii) (+)-WIN55212 in the presence of SR141716A or (iv) SR141716A alone. All compounds were applied at a concentration of 1 μM. Hippocampal cultures were incubated for 60 min at room temperature with CB_1 antibody and the pretreatment drug(s) and then fixed for 10 min with 4% paraformaldehyde. The CB_1 receptor immunostaining was then visualised by the addition of Cy^3-conjugated goat anti-rabbit secondary antibody (Jackson ImmunoResearch Labs.). Levels of glutamic acid decarboxylase (GAD) immunoreactivity in these cultures were used as controls. For dual labelling with GAD, cells were permeablised with 0.1% Triton X-100 for 5 min and then exposed to a monoclonal anti-GAD antibody (Boehringer Mannheim Ltd.) followed by an alexa 488-conjugated goat anti-mouse secondary antibody (Molecular Probes, Europe). A laser-scanning confocal imaging system (Bio-Rad MicroRadiance) was used for image acquisition and processing. Images were obtained by Kalman-averaging of 7 individual scans. The intensities of CB_1 receptor and GAD labelling, on at least 9 randomly selected fibres from each of 3 experiments were determined. The labelling intensity after all treatments (i - iv) were compared with each other (ANOVA and Newman-Keul's test); $P<0.05$ was considered significant. Values are expressed as means and variability as s.e.mean.

The intensity of CB_1 receptor labelling of cells in the presence of (+)-WIN55212 was reduced by 84.1±2.2% ($P<0.001$) compared with those pretreated with (–)-WIN55212 whereas the intensity of GAD immunoreactivity was not significantly different ($P> 0.05$). Treatment of cells with SR141716A alone or together with (+)-WIN55212 had no significant effect on CB_1 receptor labelling of cells ($P>0.05$).

In conclusion, sustained pharmacological activation of CB_1 receptors results in a loss in the expression of cell surface CB_1 receptors in hippocampal neurones. Whether this phenomenon is due to internalisation of the receptors or to a reduction in receptor synthesis remains to be elucidated.

We thank the Wellcome Trust (Grants 047368 and 55437) for financial support.

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191P LACK OF RESPONSE SUPPRESSION FOLLOWS REPEATED CANNABINOID APPLICATION IN RAT VENTRAL TEGMENTUM

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There is growing evidence linking the mesolimbic dopamine (DA) system with the behavioural, biochemical and physiological actions of cannabinoid compounds. Cannabinoids have been reported to excite ventral tegmental neurons through activation of CB_1 receptors (French, 1997). Recent biochemical and whole-cell voltage clamp studies carried out on CB_1-transfected AtT-20 cells have shown a rapid desensitisation of these receptors following activation of protein kinase C by 4-α-phorbol (Garcia *et al*, 1998). To investigate the possible physiological correlates of this phenomenon we have studied the effects of repeated cannabinoid treatment on ventral tegmental area (VTA) dopaminergic neuronal firing *in vitro*.

Brain slices containing the VTA from male Wistar or Lister-hooded rats were used for *in vitro* single-unit extracellular recordings. Their responses were characteristic of VTA DA neurons (Koob, 1992) exhibiting long duration (>2 ms) positive-negative action potentials and firing rates<10 spikes/s, in addition to being excited by NMDA (25 μM) and inhibited by DA (30 μM). Neurons with these criteria were allowed to recover until reaching basal firing then exposed to repeated administrations (4 min each, minimum 10 min interval between applications) of the cannabinoid agonist HU210 (5 μM). Data was collected on a PC and plotted online as integrated firing rate histograms. A total of 8 neurons (1 per animal) were assessed.

Two exposures to HU210 produced significant ($p < 0.01$) increases in cell firing rate in the VTA with a maximum 3.5-fold increase over basal (1.66 ± 0.9; Hz ± SEM) for the first administration and a 3-fold increase for the subsequent exposure. Nevertheless, the duration and onset of excitation produced by the cannabinoid differed significantly between the first and second exposures, with the first excitation lasting significantly longer than the second one (481.86 ± 4.09 and 357.28 ± 3.3; s ± SEM respectively: $p < 0.05$) and requiring less time to reach a comparable change in firing rate (55.37 ± 0.63 and 139.07 ± 0.8; s ± SEM respectively: $p < 0.001$). The increases in firing rate (7.19 ± 3.4 and 6.57 ± 4.8; Hz ± SEM) and the time to return to basal firing (129.51 ± 2.1 and 117.06 ± 2.6; s ± SEM) were not significantly different between exposures.

These data fail to support previously reported findings using cell preparations and repeated cannabinoid administration. The results from this preliminary *in vitro* study show that cannabinoids markedly and persistently increase the firing rate of mesolimbic dopamine neurons. Since these dopaminergic circuits are known to play a major role in mediating the reinforcing effects of most drugs of abuse (Koob, 1992), the maintained increase in dopamine drive elicited by the potent cannabinoid agonist HU210 could explain some of the behavioural properties of marijuana.

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Koob, G.F. (1992) *Trends. Pharmacol. Sci.* **13**, 177-184

192P THE EFFECT OF OLEAMIDE ON THE PATTERN OF C-FOS IMMUNOREACTIVITY IN THE RAT

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Ethanolamides of fatty acids bind to the cannabinoid receptors of the central nervous system and can be considered as endogenous ligands of these receptor. Simple amides of fatty acids such as oleamide (cis-9-octadecenamide) are also endogenous bioregulators which can induce sleep (Cravatt et al. 1995) and is itself a sleep-inducing factor

In the present study c-fos-like immunoreactivity in the rat brain was used to determine the pattern of neuronal activation in selected brain regions in response to oleamide administration.

Male Wistar rats (BMSU Nottingham University Medical School) 250-300g were treated with either oleamide (10mg/kg i. p.) or vehicle (Intralipid 1ml/kg i. p.). One hour later animals were deeply anaesthetised (sodium pentobarbitone, 60 mg/kg i.p.) and trans-cardially perfused with saline then 4% (w/v) paraformaldehyde (PFA, pH 7.4, 60 ml each). Brains were removed and fixed for 24 h in PFA at 4 °C. Coronal sections (100μm) were then processed for c-fos-like immunoreactivity (Genosys) and quantified by counting the number of positive cell bodies within brain regions using a 400x400 μm grid (3 per section)

Oleamide treated animals displayed a significantly higher number of immuno-positive cells in several areas compared to vehicle controls (Figure 1). These included the paraventricular nucleus of the hypothalamus and the central, basolateral and lateral subdivisions of the amygdala. Oleamide was without effect in the striatum, hippocampus, globus pallidus and substantia nigra

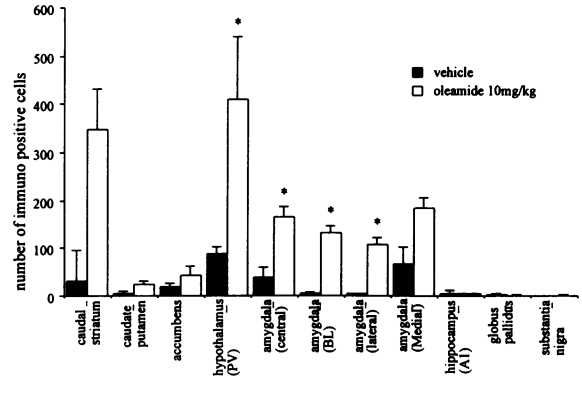

Figure 1 Number of c-fos immunoreactive cell bodies per 400x400 μm grid (mean ±s.e.mean *p<0.05 control vs oleamide. Neuman-Keul's test n=3-4).

These results indicate that oleamide can induce fos-like immunoreactivity in discreet regions of the brain including the hypothalamus and amygdala This pattern of activation is similar to that produced by other cannabinoids but without the characteristic activation of the striatum and nucleus accumbens (McGregor et al. 1998) It remains to be determined whether the responses to oleamide are mediated by cannabinoid receptors.

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193P THE EFFECT OF CANNABINOID LIGANDS ON [^{35}S]GTPγS BINDING IN THE RAT BRAIN

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Central cannabinoid receptors (CB_1) are seven trans-membrane spanning G protein-coupled receptors. They are localised in most brain regions and exhibit a distribution which is consistent with the behavioural effects of cannabinoids including hypolocomotion, hypothermia, antinocioception cognitive impairment and anxiogenesis (Dewey 1986, Deadwyler et al. 1995).

The present study used [^{35}S]GTPγS autoradiography in brain sections to examine receptor-activated G-proteins in response to stimulation with cannabinoid ligands. These include anadamide, HU210, oleamide and the cannabinoid antagonist SR141716A.

Sections were prepared according to the method of Sim et al. (1996). Briefly, male Wistar rats (BMSU Nottingham University Medical School) 250-300g were decapitated and the brain immersed in isopentane. 20μm frozen sections were attached to slides and incubated in GDP buffer containing 0.04Nm [^{35}S]GTPγS with (stimulated) or without (basal) oleamide (10^{-4}M), HU210 (10^{-6}M), anandamide (10^{-5}M) and SR141716A (10^{-6}M). Non-specific binding was determined with GTPγS. 10μm Slides were then exposed to ^{35}SβMax film for 48 hours. The images were digitised on a flat-bed scanner and the optical densities in discreet brain regions (striatum, hippocampus, nucleus accumbens, paraventricular hypothalamus, amygdala and cerebellum) analysed using NIH image.

All the cannabinoids elicited a significant increase in [^{35}S]GTPγS binding in the paraventricular nucleus of the hypothalamus which was reversed by the antagonist SR141716A (figure 1). Of the other regions examined all exhibited increases in response to cannabinoid stimulation which could be reversed with SR141716A except oleamide which failed to produce any change in the striatum, hippocampus or nucleus accumbens.

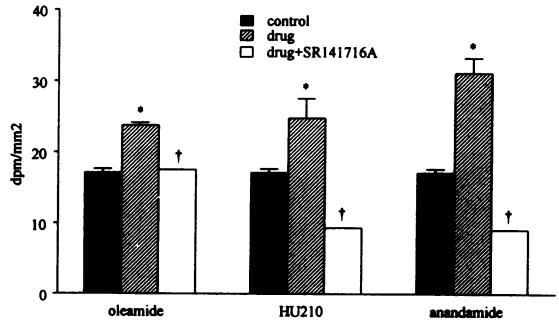

Figure 1 Amount of [^{35}S]GTPγS binding in the paraventricular hypothalamus in response to oleamide, HU210, anandamide and SR141716A. Mean ±sem *,.p<05 vs control, † vs drug (Student's t-test n=4-5)

These results indicate that different cannabinoid ligands display similar regional patterns of receptor-activated G-proteins as revealed by [^{35}S]GTPγS autoradiography. Furthermore these findings agree with studies (Beckett et al. 1999) which show that oleamide appears to be without effect in the nucleus accumbens, striatum or hippocampus, and suggest that the oleamide responses might not be CB_1 receptor-mediated.

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194P REVERSE PHASE-HPLC ANALYSIS OF CLONIDINE-DISPLACING SUBSTANCE PRESENT IN NG108-15 CELLS

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The NG108-15 cells line has been shown to express the proposed endogenous imidazoline (I) ligand, clonidine-displacing substance (CDS; Ernsberger *et al.*, 1989). We have shown CDS to be found in the bovine lung, adrenal gland and brain, by using the technique of reverse phase-HPLC (RP-HPLC; Parker *et al.*, 1999, in press). We now report the existence of a biologically purer form of CDS in NG108-15 cells, by eluting a crude extract through a RP-HPLC column.

NG108-15 cells were cultured according to the methods of Ernsberger and coworkers (1989). Cells were harvested from confluent layers (passages 38-44 for radioligand binding, and passage 16 for RP-HPLC). Crude CDS was extracted from the cells as in Ernsberger *et al* (1989). These extracts were fractionated by C-18 RP-HPLC eluting with a linear gradient of aqueous methanol (5-65%; 1 ml.min^{-1} flow rate) over 50 minutes and collected at 1 minute intervals and subsequently freeze-dried. CDS activity was analysed *via* the technique of radioligand binding, in which the ability of both the crude NG108-15 CDS extract and each individual fraction to displace specific [^{3}H]clonidine (3 nM) bound to α_2-adrenoceptors in rat brain membranes was determined (Hudson *et al.*, 1992). Rauwolscine (10 μM) was used to define non-specific binding. Crude CDS was also analysed for its ability to displace [^{3}H]clonidine (3 nM in the presence of rauwolscine (10 μM)) and [^{3}H]2-BFI (2-(2-benzofuranyl)-2-imidazoline; 1 nM) from binding to I_1-sites and I_2-sites present in rat kidney and brain membranes, respectively (for methods see Hudson *et al.*, 1999).

One unit of crude CDS extracted from the NG108-15 cells ($54x10^6$ cells in 1.6 ml ultrapure water) was defined as the amount required to displace 50% [^{3}H]clonidine bound to α_2-adrenoceptors in rat brain membranes (table 1). However, less than one unit of crude CDS was required to displace 50% of both [^{3}H]2-BFI (I_2-sites) and [^{3}H]clonidine (I_1-sites; see table 1). RP-HPLC of CDS extracted from NG108-15 cells ($500x10^6$ cells in 1.4 ml ultrapure water) exhibited an absorbance peak at 21 minutes, similar to our previous observations (Parker *et al.*, 1999), however, this fraction only displaced a small level of [^{3}H]clonidine bound to α_2-adrenoceptors. Furthermore, a fraction corresponding to a peak eluting at 24 minutes displaced over 45% of bound radioligand ([^{3}H]clonidine).

	α_2-Adrenoceptors	I_1-sites	I_2-sites
NG108-15	1*	0.09 ± 0.02	0.19 ± 0.06

Table 1: Number of units of crude CDS (including s.e.mean) required to displace 50% radioligand bound to α_2-adrenoceptors, I_1- or I_2-sites. Where one unit of CDS (*) is defined as the amount required to displace 50% [^{3}H]clonidine (3 nM) bound to α_2-adrenoceptors in rat brain membranes. Data were obtained from 6-10 separate experiments performed in triplicate.

Collectively, these results confirm the presence of CDS in NG108-15 cells. Furthermore, RP-HPLC analysis suggests the presence of slightly different CDS than that observed previously in our studies, since the biological activity of the NG108-15 CDS eluted with a more hydrophobic gradient than CDS extracted from the bovine lung, brain or adrenal gland (Parker *et al.*, 1999). Therefore, the potent activity contained within the HPLC fraction eluting at 24 minutes requires further investigation.

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Parker, C.A. *et al.* (1999) *Annals N.Y. Acad. Sci.* 881, 92-96.

195P COCAINE-INDUCED HYPOPHAGIA AND LOCOMOTOR HYPERACTIVITY: A COMPARISON OF THE EFFECTS OF DOPAMINE 'D1-LIKE' AND 'D2-LIKE' RECEPTOR SUBTYPE BLOCKADE

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The study was designed to examine the roles of dopamine 'D1-like' and 'D2-like' receptor subtypes in cocaine's effects on food intake and locomotor activity. Cocaine (10 mg/kg, i.p.) was administered to food-deprived rats (thirty-two male Sprague-Dawley; eight per experiment) trained to eat a palatable, sweetened mash. Food intake and locomotor activity were recorded over a 30 min period. The dishes and their contents were weighed at the beginning and end of the test session; locomotor activity was measured using photo-cell beams and then analysed in 5 min intervals using a purpose-written data extraction programme. Cocaine produced two distinct behavioural effects: hypophagia (mean food intake = 10.4 g ± 2.2 g for all groups receiving cocaine vs. mean = 20.5 g ± 1.2 g for all saline-control rats) and locomotor hyperactivity (mean = 106.4 ± 16.0 beam crossings vs. mean = 25.1 ± 5.3 for saline-control rats). The effects of a series of D1-like and D2-like antagonists given as pre-treatments to cocaine were then compared. All were dissolved in saline and administered i.p. 30 min prior to the tests. Data are expressed as Mean ± S.E.M., analysed by One-way and Two-way ANOVA for respective study, with Dunnett's t-test for comparisons. The selective D1-like antagonist SCH 39166 ((-) trans-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-N-methyl -5H-benzo[d]naptho-{2,1-b}azepine) at 0.1 mg/kg reversed cocaine-induced hypophagia (18.7 g ± 2.5 g; $P<0.05$ vs. cocaine alone) and cocaine-induced hyperactivity (49.9 ± 7.2 beam crossings; $P<0.01$ vs. cocaine alone). In contrast, dopamine D2-like receptor antagonists with differing selectivities for D2, D3 and D4 receptors produced different outcomes. The D2/D3 antagonist raclopride (0.1 mg/kg) produced only a marginal attenuation of cocaine's effects on activity (food intake: 9.9 g ± 1.8 g; beam crossings: 92.1 ± 20.8: significant effect in the first 10 min for locomotor activity, $P<0.05$). The D3 antagonist U-99194A (5,6,-Dimethoxy-2-(di-n-propylamino) indan maleate; 3.0 mg/kg) produced negligible effects on cocaine-induced hypophagia (8.9 g ± 1.5 g), but prolonged cocaine's hyperactivity profile over the test session (129.6 ± 2.2 beam crossings; significant at the 4th and 5th time intervals, $P<0.05$). This result implies that indirect stimulation of D3 receptors by cocaine may inhibit activity, and removing this effect by blocking the D3 receptor may prolong cocaine-induced hyperactivity. Finally, the selective D4 antagonist, L-745,870 (3-([4-chlorophenyl-piperazin-1-yl] methyl)-1H-pyrrolo [2,3-b] pyridine; 1.0 mg/kg) failed to alter any of the behavioural effects induced by cocaine (food intake: 11.3 g ± 2.6 g; beam crossings: 108.9 ± 26.2; non-significant compared to cocaine alone). The results suggest that indirect stimulation of receptors of the D1-like subfamily may be more important than stimulation of receptors of the D2-like subfamily for these behavioural effects of cocaine, and support the view that D3 receptors might exert an inhibitory effect on locomotor activity.

196P FAILURE OF SELECTIVE DOPAMINE$_2$ RECEPTOR ANTAGONISTS TO ATTENUATE 7-OH-DPAT-INDUCED INHIBITION OF PROGRESSIVE RATIO RESPONDING IN THE COMMON MARMOSET *CALLITHRIX JACCHUS*

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The dopamine D_3 receptor agonist 7-OH-DPAT inhibits food-reinforced operant responding in a progressive ratio (PR) paradigm in the rat (Depoortere et al., 1996) and more recently the marmoset (Smith et al., 1999). In order to further examine the role of dopamine D_2 and D_3 receptor subtypes in reinforced behaviour in the marmoset we investigated the effects of the D_2 receptor antagonist haloperidol and D_2/D_3 receptor antagonists (-)sulpiride and raclopride on 7-OH-DPAT-induced reduction in PR responding.

Subjects were five (3 male, 2 female) adult (290-380g) marmosets (*Callithrix jacchus*) trained to lever press for a liquid reward (0.5 ml milk) in daily 30 min sessions on a PR schedule with exponentially increasing response requirements (as previously described Smith et al., 1999). The break point was defined as the ratio value corresponding to the final reinforcer delivery. In preliminary studies (-)sulpiride (5-10 mg/kg), raclopride (6-25 μg/kg), haloperidol (0.01-0.1 mg/kg) or vehicle (0.9% saline) were given by subcutaneous (s.c.) injection (1 ml/kg) 30 min prior to testing. Doses of antagonists which had no significant effect on PR responding alone were given 30 min prior to treatment with 7-OH-DPAT (5 μg/kg)/vehicle, followed 20 min later by testing as above. Data are shown as mean ±s.e.m. break point (BPt.), analysed by one-way ANOVA with *post-hoc* Dunnett's t-test for dose-response data and Fischer's test for antagonist studies.

Administration of (-)Sulpiride (5-10 mg/kg) [$F(2,14)=28.9$, $p<0.001$], raclopride (6-25 μg/kg) [$F(3,19)=6.6$, $p<0.01$] and haloperidol (0.005-0.1 mg/kg) [$F(4,24)=5.5$, $p<0.01$] significantly attenuated lever pressing in a dose-dependent manner. The inhibition of lever pressing induced by 7-OH-DPAT (5 μg/kg) was not antagonised by (-) sulpiride (5 mg/kg), raclopride (6 μg/kg), or haloperidol 0.01 mg/kg (Table 1.).

In contrast with the ability of the dopamine D_3 receptor antagonist U99194 to reverse 7-OH-DPAT-induced inhibition of PR responding in the marmoset (Smith *et al.*, 1999) the failure of D_2/D_3 receptor antagonists at doses with selective affinity for D_2 receptors (Malmberg *et al.*, 1993) to block this response suggests that 7-OH-DPAT-induced inhibition of reinforcement may be mediated by an action at D_3 receptors (Depoortere *et al.*, 1996). In addition, high doses of antagonists reduced PR responding. This may be a non-specific effect due to inhibition of locomotor behaviour in the marmoset, reportedly arising from blockade of postsynaptic D_2 receptors (Löschman *et al.*, 1991).

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Löschman, P-A.,, Smith, L.A., Lange, K.W. *et al.*, (1991) *Psychopharmacol.* 105:303-309.
Malmberg, Å., Jackson, D.M., Eriksson A. *et al.* (1993) *Mol. Pharmacol.* 43: 749-754.
Smith, A.G., Neill, J.C., Costall, B., Shahid, M. (1999) *Br. J. Pharmacol.* 126: 235P.

Table 1. Effect of dopamine receptor antagonists on PR responding and 7-OH-DPAT-induced inhibition of PR responding

pretreatment	vehicle	(-)sulpiride (mg/kg)			raclopride (μg/kg)				haloperidol (mg/kg)			
treatment		0	5	10	0	6	12	25	0	0.01	0.05	0.1
vehicle	61±16	83±15	84±18	36±12†††	79±20	85±27	55±21†	31±8††	83±15	79±19	18±5††	1±0.2†††
7-OH-DPAT 5μg/kg	18±12††	-	50±15*	-	-	53±13*	-	-	-	45±10*	-	-

Data are expressed as mean±sem Bpt. Effect of drug compared to vehicle; significant reduction †-†††, $p<0.05$-0.001 (Dunnett's test). Effect of antagonist/7-OH-DPAT compared to antagonist alone, significant reduction * $p<0.05$ (Fishers test).

197P INESCAPABLE ELECTRIC SHOCK AND CONDITIONED FEAR INFLUENCE THE RELEASE OF AMINO ACIDS IN THE LOCUS COERULEUS

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The locus coeruleus (LC) has been implicated in stress response (Singewald and Philippu, 1998,Singewald *et al.*, 1998). We investigated the effects of chronic inescapable electric shock and conditioned fear on the release of amino acids in the LC. Under anaesthesia (sodium pentobarbital 40 mg/kg and ketamine 50 mg/kg, i.p.) a push-pull cannula was inserted in the LC and an implant in the iliac artery for telemetry recordings of blood pressure (BP), heart rate (HR) and motility (MO). The LC of conscious rats was superfused with artificial cerebrospinal fluid at a rate of 20 µl/min and the superfusate was collected continuously in time periods of 5 min. Amino acids were determined in the superfusate by HPLC. Rats were placed in a chamber provided with a grid floor and exposed to noise (N; 80 dB), light (L; 15 W, intermittently applied for 500 ms/s) and electric shock (S; 0.5 mA, intermittently applied for 1s/2s) for 20s/100s over a conditioning session of 5 min. Three to four N+L+S sessions were performed daily for 4 days. Superfusion of the LC was carried out on naive rats and on conditioned animals on the fifth day before and during 6 alternating exposures to N+L+S or N+L. Data are expressed as mean ± s.e.m. Statistical analysis was carried out by Friedman's analysis of variance followed by Wilcoxon's signed rank test for paired data. For comparison of values between naive rats and conditioned rats, data were analysed by Mann-Whitney test.

The basal release rates of amino acids in the LC were (fmol/min, n=6-13): glutamate (GLU) 2453 ± 564, asparate (ASP) 470 ± 62, taurine (TAU) 1895 ± 591, GABA 312 ± 49, serine (SER) 2174 ± 357, glutamine (GLN) 11973 ± 6701, arginine (ARG) 692 ± 144. Placement of naive or conditioned rats in the grid floor cage elevated the release rate of GLU. In conditioned rats (n=6-14) BP was increased by 10±2 mm Hg ($p<0.05$). In naive (n=6-10) and conditioned rats the MO was increased (4±1 events/5 min, $p<0.05$) in the first 5 min after starting exposure to the new environment. In conditioned rats exposure to N+L+S led to a more than twofold sustained increase in the release rates of all amino acids ($p<0.05$). BP (14±3 mm Hg, $p<0.05$), HR (38±10 bpm, $p<0.05$) and MO (7±1 events/5 min, $p<0.01$) were also elevated. The effects of N+L+S in conditioned animals were more pronounced and/or lasted longer than in naive rats ($p<0.05$).

The conditioned stimuli N+L applied for 5 min enhanced the release of TAU (127±10%, $p<0.05$) ASP (151 ± 19, $p<0.05$) and ARG (137±15%, $p<0.05$) in conditioned rats. The increased release of the inhibitory amino acid TAU lasted at least 30 min. In conditioned rats N+L also increased BP (4±1 mm Hg, $p<0.05$) and HR (17±4 bpm, $p<0.05$) but MO was not changed. In naive rats release of amino acids, HR, BP and MO were not influenced by N+L.

The findings show that novelty does not virtually influence the release rates of the amino acids studied. Electric shock enhances the release of several amino acids in the LC. Conditioned fear elicited by N+L leads to a long-lasting increase in the release of the inhibitory amino acid TAU. Additionally, conditioned fear enhances the release of the excitatory amino acid ASP and of ARG. Hence, excitatory and inhibitory amino acids within the LC seem to be implicated in conditioned fear.

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198P THE EFFECT OF *d*-AMPHETAMINE ON THE BEHAVIOUR OF RATS IN AN OPERANT TIMING SCHEDULE

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d-Amphetamine (*AMPH*) disrupts timing behaviour in rats; it has been proposed that this effect reflects a reduction of the period of a dopaminergically-regulated endogenous 'pacemaker' that is purported to underlie time perception (see Meck, 1996). In a previous communication (Chiang *et al.*, 1999a), we reported that *AMPH* (0.2-0.8 mg kg^{-1}, i.p.) dose-dependently disrupted performance on the interval bisection task, a test of time discrimination. Here, we report the effect of *AMPH* on another timing task, the free-operant psychophysical procedure (Stubbs, 1976).

23 female Wistar rats (250-300 g) received daily 50-min sessions in operant conditioning chambers, in which they pressed levers for a sucrose reinforcer (50 µl, 0.6 M). Daily sessions consisted of fifty 50-s trials in which reinforcers were provided on a variable-interval 30-s schedule; in the first 25 s of each trial reinforcers were only available for responses on lever A, whereas in the last 25 s reinforcers were only available for responses on lever B. Data were collected from 'probe' trials (4 per session) in which no reinforcers were delivered. For 11 rats, the first response on lever B in each trial resulted in removal of lever A from the chamber ('constrained switching'); for 12 rats, no restriction was placed on switching between the two levers ('unconstrained switching'). When steady-state performance had been attained, *AMPH* SO_4 (0.2, 0.4, 0.8 mg kg^{-1}) or 0.9% NaCl vehicle (V) was given i.p. 10 min before the sessions. For each treatment condition, responding on lever B, expressed as a percentage of overall response rate (%B), was plotted against time from the onset of the trial (t); logistic functions ($\%B=100/[1+\{t/T_{50}\}^{\varepsilon}]$) were fitted to the data, and the 'indifference point', T_{50}, and the slope parameter, ε, were estimated (Chiang *et al.*, 1999b).

The values of T_{50} and ε are shown in Table 1. In both versions of the task, *AMPH* reduced the slope of the logistic timing function (i.e. ε became less strongly negative) and reduced the indifference point, T_{50} (ANOVA, with repeated measures: *constrained switching*: ε, $F_{3,30}=10.2$, $p<0.01$; T_{50}, $F_{3,30}=23.6$, $p<0.01$; *unconstrained switching*: ε, $F_{3,33}=25.7$, $p<0.01$; T_{50}, $F_{3,34}=4.2$, $p<0.02$).

Table 1: Effects of *AMPH* SO_4 (dose in mg kg^{-1}) on parameters of logistic timing functions (mean ± s.e.mean)

	Slope, ε				T_{50} (s)			
Timing task	V	0.2	0.4	0.8	V	0.2	0.4	0.8
i. constrained switching	-4.9 ±0.4	-4.4 ±0.6	-2.8* ±0.3	-2.4* ±0.4	16.4 ±1.3	13.9 ±1.2	11.9* ±1.8	8.8* ±1.5
ii. unconstrained switching	-3.0 ±0.3	-2.1* ±0.3	-1.5* ±0.2	-1.4* ±0.1	20.8 ±1.2	17.6 ±1.5	13.9* ±2.4	15.7* ±3.5

Significance of difference from vehicle (V): * $p<0.05$

The effect of *AMPH* on ε confirms the disruptive effects of this drug on timing behaviour (see Meck, 1996), and is consistent with our previous findings with the interval bisection task (Chiang *et al.*, 1999a). However, in the present experiment *AMPH* reduced the value of T_{50}, an effect that was not seen in the case of the interval bisection task (Chiang *et al.*, 1999a). The finding that *AMPH* altered T_{50} in both versions of the free-operant psychophysical task indicates that *AMPH*'s effect on T_{50} was not mediated by a change in switching rate (see Chiang *et al.*, 1999b). The results provide further evidence for the suggestion that different mechanisms may be involved in timing tasks which entail discrimination of the durations of external stimuli (e.g. the interval bisection task), and those which entail temporal regulation of the organism's own behaviour (e.g. the free-operant psychophysical task) (see Chiang *et al.*, 1999b).

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199P EFFECT OF THE DIHYDROPYRIDINE CALCIUM CHANNEL ANTAGONIST, NIMODIPINE, ON ETHANOL CONSUMPTION AFTER LONG TERM ACCESS TO ALCOHOL

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Dihydropyridine calcium channel antagonists reduce alcohol consumption; the dihydropyridine calcium channel blocker, nimodipine decreased the operant self-administration by rats of low concentrations of alcohol (Smith et al., 1997).

The present study followed the paradigm of Wolffgramm and Heyne (1995), in which male Wistar rats were given 63 weeks continuous access to: tap water, 5%, 10%, and 20% alcohol (v/v in tap water), and investigated effects of nimodipine on the ethanol consumption after this 63 week access. Two groups of rats were then treated in the following ways (i) uninterrupted free-access to alcohol and (ii) withdrawal from alcohol for 2 weeks followed by reintroduction to the four drinking fluids. A third group of age-matched control animals were not allowed prior experience with alcohol but were given access to the four fluids for the first time.

During the 63 weeks of either free access to alcohol (2 groups) or no alcohol (control group) animals were caged in groups of four. After this they were all singly housed, with the same four bottle fluid choice. Individual alcohol intake was measured once daily for the 23 days of the experiment. The animals were first left undisturbed for three baseline days (Days 1 to 3). One of the groups receiving alcohol was withdrawn from ethanol for Days 4 to 17, then reintroduced to the original choice of four fluids on Day 18, while the other had continuous access to the fluid choice. The control animals were introduced to the three alcohol concentrations for the first time on Days 18 to 23. From Day 10, all groups received daily intraperitoneal injections of either nimodipine (Nim) 5 mg/kg or 20 mg/kg, or the tween vehicle (Tween 89 0.5% in distilled water). The results were compared pairwise by Student's t-test (n=7 per treatment group).

Alcohol intake of animals after continuous access to alcohol plus 20 mg/kg nimodipine was significantly lower on day 18 than for control animals given 20 mg/kg nimodipine ($P<0.02$) but did not show significance compared with vehicle. There were no other significant differences in ethanol consumption (Table 1), ethanol preference or total fluid intake ($P>0.05$).

Table 1: Ethanol consumption (g/kg/24h, mean ± s.e.m., $^{*}P<0.02$ compared with ethanol naive plus 20 mg/kg nimodipine)

	Ethanol Naïve	Withdrawn	Continuous Access
Tween	4.1 ± 1.0	2.7 ± 0.2	2.1 ± 0.7
Nim 5 mg/kg	4.8 ± 0.6	3.5 ± 0.4	3.0 ± 0.7
Nim 20 mg/kg	3.6 ± 0.5	3.6 ± 0.7	*1.8 ± 0.4

These results are not in agreement with the previously reported effects of dihydropyridines on alcohol intake and preference. However as the rats were 15 months old at the test time, it is possible that age may alter the role of the calcium system in the modulation of alcohol intake.

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200P ANTINOCICEPTIVE EFFECTS OF MORPHINE-6-GLUCURONIDE IN P-GLYCOPROTEIN KNOCKOUT AND WILDTYPE MICE IN THE HOTPLATE TEST

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Morphine-6-glucuronide (M6G) is a major metabolite of morphine with agonist opioid-receptor activity. M6G is a substrate of P-glycoprotein (P-gp) actively pumping M6G that has crossed the blood brain barrier back into the systemic circulation. Inhibition of P-gp was reported to result in higher brain uptake of M6G and may thereby enhance central opioid effects mediated by M6G.

In an observer-blind, placebo controlled study, the antinociceptive effects of M6G were studied in P-gp knockout (mdrla(-/-)) and wildtype mice using the hotplate test. M6G was injected intraperitoneally (i.p.) at doses of 0, 0.5, 1, 2.5, 5, and 10 mg/kg. Eight P-gp knockout and eight wildtype mice were studied per dose.

The hot plate test was performed before and 5, 157 30, 60, 90, 120, and 150 min after administration of M6G. In a second experiment, plasma concentrations of M6G, morphine, and morphine-3-glucuronide (M3G) were measured after i.p. administration of 5 mg/kg M6G in another 14 P-gp knockout and 14 wildtype mice.

Measured antinociceptive effects increased significantly with higher doses of M6G. The same dose of M6G resulted in similar antinociceptive effects in P-gp knockout and wildtype mice. However, the same dose of M6G resulted in a tendency towards higher plasma concentrations in P-gp knockout mice. Absence of P-gp did not enhance antinociceptive effects of M6G in the hotplate test.

201P CHANGES IN PHENCYCLIDINE-INDUCED BEHAVIOUR FOLLOWING ISOLATION REARING IN THE RAT

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The social environment during development can influence brain maturation and the behaviour of the adult animal. Social isolation from weaning in the rat has been reported to cause permanent behavioural changes in the adult comparable to those seen in schizophrenia suggesting the possibility of using isolation rearing as a non pharmacologic model for the disease (Bakshi *et al.*, 1998).

Phencyclidine hydrochloride (PCP), an NMDA antagonist, and amphetamine can induce psychosis in humans resembling schizophrenia. Both drugs reproduce the positive symptoms although only PCP mimics the negative symptoms (Sams-Dodd, 1998). This study investigates the effects of repeated PCP treatment on isolation-reared rats as there is little information on the effects of isolation on the central glutamatergic systems. The effects of PCP on hole poking, locomotor (LMA) and rearing activity were monitored.

Male Lister hooded rats (24) were housed for 8 weeks either singly or in groups (6/cage) after weaning (21 days) and were then injected daily for 3 days with PCP (2mg/kg, s.c.) or 0.9% saline. Rats were placed in the photocell activity cages 30 min before injection and 120 min post injection. Activity was counted as beam breaks per 5 min in the bottom, middle and top fields, which measured hole poking, locomotor, and rearing behaviour, respectively, and data were analysed using ANOVA.

PCP induced hyperactivity in the rat as seen by the significant increase in LMA (Table 1). There was no significant difference between the two rearing groups. PCP also significantly increased the hole poking, an exploratory activity, in isolation but not group-reared rats. There was no significant difference in rearing activity between the groups.

The data support previous studies reporting PCP-induced locomotor hyperactivity. Interestingly, PCP also selectively increased hole poking in isolation-reared rats. This effect was not observed in a previous study looking at the effect of amphetamine (Lapiz *et al.*, 1999). This raises the possibility that this behaviour could be associated with NMDA receptor activity and might indicate isolation-induced changes in the glutamatergic system. Further studies need to be undertaken to identify in details the neurotransmitter basis for this behaviour.

Table 1: Mean (±SEM) photocell beam breaks per 5 min before (pre) and after (post) PCP administration (2mg/kg, s.c.).

	Hole Poking		LMA		Rearing	
	Pre	Post	Pre	Post	Pre	Post
G-S	5 (±1)	2 (± 0)	28 (±6)	10 (±1)	24 (±4)	12 (±2)
G-PCP	5 (±1)	6 (±1)	19 (±3)	40 (±7)***	17 (±3)	14 (±2)
I-S	6 (±1)	2 (±0)	40 (±7)	15 (±2)	37 (±6)	13 (±2)
I-PCP	4 (±1)	20 (±1)***	34 (±7)	44 (±4)***	22 (±6)	11 (±1)

Legends: G=Group-reared; I=Isolation-reared; PCP=Phencyclidine; S=Saline
*** $P<0.001$ ANOVA using Tukey's Multiple Comparison test.

MDSL holds a University of Nottingham and an ORS scholarship.

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202P STIMULATION OF LEPTIN BY 2-DEOXY-D-GLUCOSE INDEPENDENCE FROM ADIPOSITY AND INVOLVEMENT OF FEEDING

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Leptin levels are increased by insulin, corticosteroids and feeding but are inhibited by sympathetic nerve system (SNS) stimulation (Kolaczynski *et al.*, 1997; Muller *et al.*, 1998). To elucidate the role of these influences, we studied the effects on plasma leptin of 2-deoxy-D-glucose (2-DOG), a competitive inhibitor of glucose metabolism, which induces neuroglucopenia and stimulates both feeding and the SNS. Male Wistar rats were given 2-DOG (300 mg/kg, i.p.) either once, or three times over 6 hrs; or once daily (09:00) for 6 days. Plasma analytes were determined using commercially available kits (Cai *et al.*, 1999). A single injection of 2-DOG had no effect on plasma leptin or insulin levels. Daily 2-DOG administration significantly increased leptin levels with no changes in plasma insulin and glucose. Light-phase food intake increased significantly (2-DOG vs control: 4.93 ± 0.16 vs 1.68 ± 0.14 g, $P<0.001$) but there were no changes in 24-hr food intake (28.2 ± 1.58 vs 27.9 ± 1.95 g, $P>0.05$). Plasma corticosterone levels were raised significantly above control. Three injections of 2-DOG over 6 hr increased food intake 3-fold and significantly increased insulin and leptin levels in fed rats but not in fasted rats. Plasma glucose levels were significantly higher in both fed and fasted rats with glucoprivation. 2-DOG administration also increased corticosterone concentrations in both fed and fasted rats with greater levels in the fasted group. In conclusion, we have demonstrated that 2-DOG increases plasma leptin *in vivo*. This effect is independent of adiposity and may be related to hyperphagia perhaps with hyperinsulinemia as a link. Corticosterone has no independent effect on leptin. Therefore SNS activation is over-ridden by effects of feeding.

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Table: Body weight, food intake, plasma glucose and hormones

	Body weight(g)	Food intake (g) Over 6 hrs	Fat mass(g)	Insulin(ng/ml)	Leptin(ng/ml)	Glucose(mM)	Corticosterone (ng/ml)
Single injection							
Control	301 ± 11	1.40 ± 0.31	1.51 ± 0.16	19.7 ± 1.4	4.01 ± 0.57	9.0 ± 0.2	121.2 ± 20.3
2-DOG	301 ± 6	2.12 ± 0.22	1.55 ± 0.12	24.5 ± 4.1	4.72 ± 0.34	10.7 ± 0.2	108.9 ± 20.6
2-DOG + fasted	292 ± 12	0	1.58 ± 0.15	16.3 ± 1.5	3.84 ± 0.43	10.3 ± 0.3	151.4 ± 38.1
Daily injections							
Control	244 ± 8	0.12 ± 0.02	1.95 ± 0.14	17.8 ± 1.2	2.77 ± 0.24	9.3 ± 0.9	59.3 ± 15.9
2-DOG	242 ± 13	2.63 ± 0.26**	1.84 ± 0.16	22.6 ± 2.4	5.09 ± 0.30**	11.2 ± 0.8	199.3 ± 34.3*
Three injections							
Control	240 ± 9	1.25 ± 0.21	1.25 ± 0.11	17.9 ± 0.9	2.19 ± 0.21	10.9 ± 0.5	161.6 ± 37.6
2-DOG	238 ± 11	4.71 ± 0.25**	1.57 ± 0.13	28.5 ± 2.3**	4.14 ± 0.37**	18.9 ± 2.1*	277.7 ± 50.6†
2-DOG + fasted	234 ± 9	0	1.03 ± 0.03	18.3 ± 1.1	2.38 ± 0.19	18.1 ± 1.0*	501.0 ± 32.1**

One-way ANOVA: *$P<0.01$; **$P<0.001$ compared with control; †$P<0.01$ compared with fasted group

203P EFFECT OF SOCIAL ISOLATION ON HIPPOCAMPAL 5-HT $_{1A}$ RECEPTOR ACTIVITY IN THE LISTER HOODED RAT

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It has been demonstrated that isolation-rearing produces behavioural disturbances and causes neurochemical changes in the adult animal which include functional alterations in central dopaminergic and serotonergic systems in rats (Jones *et al.*, 1992). The enhancement of 5-HT (5-hydroxytryptamine) release in the hippocampus by aversive conditions is reduced in isolation reared rats compared to group-reared controls (Bickerdike *et al.*, 1993) indicating pre-synaptic serotonergic function is reduced with a possible concomitant increase in post-synaptic serotonergic receptor function. This study examined the effects of isolation rearing on post-synaptic 5-HT$_{1A}$ receptor sensitivity in the rat hippocampus, *ex vivo*.

Male Lister-hooded rats obtained at weaning (21-25 days postnatal), were housed either singly or in groups of six for eight weeks. At the end of the isolation period, single unit extracellular CA1 hippocampal neuronal activity was recorded using an *in vitro* brain slice preparation. Basal firing rate in hippocampal neurones is low (~1-2 Hz) (Pontzor *et al.*, 1992) so was elevated using carbachol (CCh 1 μM) in order to examine the inhibitory effect of 5-HT and the selective 5-HT$_{1A}$ agonist 8-OH-DPAT (8- hydroxy-2-(di-*n*-propyramino) tetralin).

Application of CCh (1 μM) produced a significant increase ($P < 0.001$) in the basal firing rate of CA1 neurones in both group-reared (n = 57 cells, 11 rats) and isolation-reared rats (n = 53 cells, 11 rats). Both 5-HT and 8-OH-DPAT produced a concentration-dependent (0.1-10 μM) suppression of CCh-evoked firing in group-reared (n = 14 cells, 8 rats; n = 11 cells, 5 rats, respectively) and isolation-reared rats (n = 13 cells, 8 rats; n = 8 cells, 6 rats, respectively); there was no significant difference in either 5-HT or 8-OH-DPAT evoked responses between the two rearing conditions (Student's unpaired *t*-test). The similar concentration-response profiles for both 5-HT and 8-OH-DPAT indicates that the responses of both drugs were mediated by 5-HT$_{1A}$ receptors.

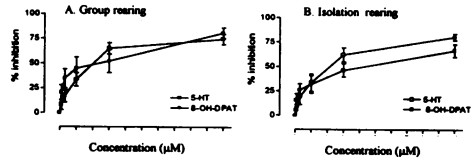

Figure 1 Effect of 5-HT and 8-OH-DPAT on CA1 neurones.

These results indicate that post-synaptic 5-HT$_{1A}$ receptor sensitivity in hippocampus is unaltered by isolation rearing supporting the view that the main effect of isolation rearing on 5-HT neurotransmission in the rat hippocampus may be a decrease in pre-synaptic 5-HT function (Bickerdike *et al.*, 1993).

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204P INVESTIGATION INTO THE CLOZAPINE-INDUCED HYPOTHERMIA IN MICE

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It has been well documented that clozapine induces a dose-dependent hypothermia in mice (Menon *et al.*, 1988; Salmi *et al.*, 1994). However, the mechanism for this hypothermia has not been satisfactorily established. It has been reported that chlorpromazine and haloperidol also induce hypothermia in mice, and that this effect is reversible by the 5-HT$_{2A}$ receptor agonist 1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane(DOI; Yamada *et al.*, 1995). Since these two classic neuroleptics and clozapine have been shown to be 5-HT$_{2A}$ receptor antagonists (Meltzer *et al.*, 1989), it is possible that the clozapine-induced hypothermia is mediated through antagonism of this receptor.

The aim of this experiment was to investigate whether the clozapine-induced and haloperidol-induced hypothermia could be reversed by administering the 5-HT precursor, 5-hydroxy-tryptophan (5-HTP). The effects of the 5-HT$_{1A}$ receptor antagonist N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride (WAY 100635) and the mixed 5-HT$_{1D/2}$ receptor antagonist, metergoline, were also investigated.

Mice weighing 20-30 g (n=5 for all groups) were used and pretreated with WAY 100635 (0.25 mg/kg), metergoline (0.1 mg/kg) and/or carbidopa (25 mg/kg) 30 min before clozapine (1-40 mg/kg), haloperidol (0.2-5 mg/kg) and/or 5-HTP (3-20 mg/kg). All drugs were administered i.p. and injected in a volume of 10 ml/kg. Metergoline (0.1 mg/kg) was dissolved in an acidic solution (pH=5), while all other drugs were dissolved in distilled water. Core temperature was measured by a thermister probe inserted 2 cm into the rectum at various time intervals over a 5 h period, including before and after drug treatment.

Statistical comparison of the change in body temperature, to that of respective control groups, was performed using a One Way Analysis of Variance ($P<0.05$).

Haloperidol (2 mg/kg) induced a hypothermic response (the maximal decrease being 1.8 (0.2(C), which was reversible by administration of 5-HTP (10 mg/kg). Clozapine (3-40 mg/kg) also induced a dose-dependent reduction in body temperature of mice, with maximal decrease following 40 mg/kg clozapine being 8.1 ± 0.1 °C occurring 90 min after administration. The addition of 10 mg/kg 5-HTP, a dose which had no effect on body temperature, potentiated the clozapine (3 mg/kg)-induced hypothermia, with the maximal decrease being increased from 2.8 ± 0.3 °C (clozapine alone) to 5.5 ± 0.3 °C (with 5-HTP). WAY 100635 did not modify the 5-HTP potentiation of the clozapine-induced hypothermia. Metergoline induced a slight non-significant potentiation of the clozapine-induced hypothermia.

These results indicate that, unlike the haloperidol-induced hypothermia, the clozapine-induced hypothermia does not appear to be mediated through antagonism of 5-HT receptors, since this effect was not reversible by 5-HTP: instead it was potentiated. In addition, the failure of WAY 100635 to attenuate the hypothermic effect suggests that presynaptic 5-HT$_{1A}$ receptors are not involved. Further experimentation is required to elucidate the mechanism for the clozapine-induced hypothermia.

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205P THE EFFECT OF Δ^9-TETRAHYDROCANNABINOID AND SR 141716 ON THE APPETITE OF RATS

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Δ9-Tetrahydrocannabinol (Δ9-THC) has been reported to posses inconsistent appetite modulating effects. Studies in humans have reported that cannabis has an enhancing effect on the sensory appeal of food as well as increasing appetite. However in animals there are many reports of a decrease in food intake following Δ9-THC. The increase in appetite in humans has been referred to as the 'munchies', and has been used clinically to stimulate appetite in patients receiving cancer chemotherapy. CB_1 receptors have been reported to be present in several hypothalamic nuclei and are suggested to be involved in appetite modulation. However, some aspects of the Δ9-THC-induced effect on appetite are still not well understood. There have been limited studies to date that specifically have considered the effect of Δ9-THC on appetite and the role of CB1 receptors. In view of this, we investigated the effect of Δ9-THC on consummatory behaviour. The CB_1 antagonist SR141716 (N-piperidine-5-(4-chlorphenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (Compton *et al.*, 1996), was employed to determine the involvement of CB_1 receptors.

Male albino Glaxo-Wistar rats (n=60, 200-350 g) were kept in conditions of constant room temperature (22 °C) and controlled lighting with a 12h light/dark cycle. The animals were adapted to a restricted availability of food paradigm with food being available for 6h (07.00-13.00) commencing 2h after lights on. After eating had stabilised (day 9) animals had a catheter implanted into the external jugular vein (metho-hexitone (18 mg/kg i.p.)/amylobarbitone (30mg/kg i.p.) anaesthesia) in order to allow intravenous (i.v.) drug administration. After two days recovery drug testing occurred. All animals were randomly assigned to receive either vehicle (PVP), 1 ml/kg intraperitonial (i.p.), Δ9-THC (i.v.), 2.5 mg/kg SR 141716 (i.p.) alone or SR 141716 followed 30 min later by Δ9-THC. Comparison of the hourly food intake of groups of drug-treated rats versus their respective control group was performed using a One Way Analysis of Variance and the Student-Newman-Kuels multi-comparison test. Animals treated with Δ9-THC (i.v.) showed a dose dependant inhibition of standard rat food intake, with doses greater than 1 mg/kg Δ9-THC eating significantly less than the control group for the last 5 hours after administration (50% of control intake at 1300h, $p<0.05$, n=5). When administered 30 minutes post access to food, SR 141716 alone significantly decreased food intake after the first hour of feeding (47% of control intake at 1300 h, $p<0.05$, n=5). SR 141716 did not modify the effect of 0.1 and 0.2 mg/kg Δ9-THC except after 4 h of access to food rats pretreated SR†141716 prior to 0.2 mg/kg (9-THC ($p<0.05$, n=5).

The results of the present investigation demonstrate that Δ9-THC produces a dose-dependent inhibition of food intake of partially satiated animals. The inhibition of food intake within the first hour of feeding induced by SR 141716 is noteworthy. Although SR 141716 has previously been described as being devoid of any 'intrinsic' activity, it appears to have activity in our experiments. The time of administration of SR 141716 may be an important factor with respect to its effect on appetite. Alternatively, SR 141716 may block an endogenous cannabinoid system, causing the resultant decrease in food intake. This would assume that an endogenous cannabinoid system stimulates appetite, which is contrary to the effect of the doses of the exogenous cannabinoid used here. In summary, these results suggest that SR 141716 may be acting by blocking an endogenous system (as a CB1 antagonist) as well as antagonising the Δ9-THC-induced effects.

Compton, *et al.*, 1996, J Pharmacol Exp Ther, 277, 586-94.

206P MULTICHANNEL EXTRACELLULAR RECORDINGS FROM A HIPPOCAMPAL NEURONAL NETWORK CULTURE: A DEMONSTRATION

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The use of a Multichannel Acquisition Processor system (MAP, Plexon Inc.) allows large scale real-time neuronal waveform recording and spike sorting from multichannel microelectrode devices. These include microwire bundles, solid-state microprobe arrays or multi-microelectrode culture plates (MMEPs). These technologies provide a powerful tool for studying the physiology and pharmacology of excitable tissues, including neuronal ensembles *in vivo* (Nicolelis *et al.*, 1998), *in vitro* (Gross & Schwalm, 1994) or cardiac myocytes (unpublished data). We will demonstrate its use in recording from hippocampal neuronal networks in culture and its application to studies of epilepsy.

Primary dissociated Wistar rat hippocampal neurones from E18 fetuses were cultured directly onto planar 64-channel MMEPs (Gross & Schwalm, 1994). For recording, the MMEP is connected via two 32-channel amplifiers to signal input boards which provide programmable gain, filtering and analogue-to-digital conversion. A/D conversion is performed by simultaneously sampling 12-bit converters at 40KHz per channel. Signals are then routed to multiple digital signal processor boards (Motorola 56002 DSPs running at 40MHz) for computer-controlled spike waveform capture and sorting. The control software, *RASPUTIN (Real-time Acquisition Systems Programs for Unit Timing in Neuroscience,* Plexon Inc.), for the MAP is implemented in a Server/Client architecture running on a host Pentium PC under Microsoft Windows NT4. During data collection the Server runs a series of on-line clients, with *Sort Client* allowing automatic spike discrimination on all channels with the capability to isolate up to four single-units per microelectrode, principal component analysis of waveforms and recording external events (e.g. drug applications). Additional on-line clients include *NeuroEXplorer* (NEX) and *Graphical Activity Client* which enable on-line data analyses (e.g. integrated firing rate, burst rate, inter-spike interval histograms and cross-correlation analysis) simultaneously with spike capture. On-line data from the Server can also be sent to any other PC either via a local network via *Network Client* and *Data Replicator.* Off-line, data can be manipulated in a number of ways with the spike waveforms re-sorted or the sorted waveforms analysed within NEX, which provides multiple spike-train data analysis package with a very rich set of analysis options and functions. It also provides an open analysis environment to interact with MATLAB, Excel and other mathematical and statistical packages.

In addition to electrophysiological studies of neuronal networks, the system has potential applications in high throughput screens for drug assessment and as a cell-based biosensor (Gross *et al.*, 1997).

Gross GW, Harsch A, Rhoades BK and Gopel W (1997) *Biosensors and Bioelectronics* **12**, 373-393.
Gross GW and Schwalm FU (1994) *J. Neurosci. Methods* **25**, 73-85.
Nicolelis MAL, Stambaugh CR, Brisben A and Laubach M (1998) In *Methods for neural ensemble recordings*, Eds. Nicolelis MAL, CRC Press, p121-156.

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