

SITE-SPECIFIC REGULATION OF CORTICOSTEROID AND SEROTONIN RECEPTOR SUBTYPE GENE EXPRESSION IN THE RAT HIPPOCAMPUS FOLLOWING 3,4-METHYLENEDIOXYMETHAMPHETAMINE: ROLE OF CORTICOSTERONE AND SEROTONIN

J. L. W. YAU,* J. NOBLE and J. R. SECKL

University of Edinburgh, Department of Medicine, Molecular Medicine Centre,
Western General Hospital, Edinburgh EH4 2XU, U.K.

Abstract—Abnormal interactions between serotonin (5-hydroxytryptamine) and glucocorticoids, notably in the hippocampus, may underpin neuroendocrine, affective and cognitive dysfunction in depression and ageing. Glucocorticoids act via intracellular glucocorticoid and mineralocorticoid receptors, whereas 5-hydroxytryptamine binds to a family of transmembrane sites; both cross- and auto-regulation have been proposed. To determine the roles of 5-hydroxytryptamine and corticosterone in the short-term control of hippocampal receptor gene expression, we used 3,4-methylenedioxymethamphetamine (20 mg/kg), which causes acute release of both 5-hydroxytryptamine and corticosterone. 3,4-methylenedioxymethamphetamine increased mineralocorticoid receptor messenger RNA expression throughout the hippocampus after 16 h. In rats with fixed glucocorticoid levels (adrenalectomy plus corticosterone pellets) this effect was lost in CA1–4, suggesting corticosterone-mediation, but maintained in the dentate gyrus, indicating 5-hydroxytryptamine involvement. In contrast, 3,4-methylenedioxymethamphetamine decreased glucocorticoid receptor messenger RNA expression in the dentate gyrus and CA1 within 4 h, but only in adrenal-intact rats, suggesting corticosterone control. 5-Hydroxytryptamine_{1A} receptor messenger RNA expression was decreased in CA1 in both groups of rats, but increased in the dentate gyrus only in corticosterone-fixed rats, suggesting 5-hydroxytryptamine differentially regulates expression of this gene within hippocampal subfields. 5-hydroxytryptamine_{2C} receptor messenger RNA was decreased in ventral CA1 only in adrenal-intact rats, suggesting a corticosterone effect, and decreased in the subiculum in both groups, indicating 5-hydroxytryptamine mediation.

These results show the complexity and intricate subregional-specificity of 5-hydroxytryptamine and corticosterone interactions upon hippocampal corticosteroid and 5-hydroxytryptamine receptor gene expression. 3,4-Methylenedioxymethamphetamine-induced alterations in hippocampal receptor gene expression may play a role in the mood and behavioural changes associated with this drug of abuse. © 1997 IBRO. Published by Elsevier Science Ltd.

Key words: mineralocorticoid receptor, glucocorticoid receptor, 5-HT receptor, MDMA, hippocampus, gene expression.

Both disordered serotonergic (5-hydroxytryptamine; 5-HT) neurotransmission³⁹ and glucocorticoid excess¹⁵ have been separately implicated in the pathogenesis of depressive illnesses and, to a lesser extent, a decline in cognitive function, particularly with age.⁵⁵ Thus, central interactions between glucocorticoids and 5-HT systems are of considerable interest.³² The hippocampus, which highly expresses the two types of intracellular receptors for corticosterone, glucocorticoid receptors (GR) and mineralocorticoid receptors (MR),¹³ as well as several subtypes of transmembrane 5-HT binding site,² is a key locus for these interactions.

Neurophysiological studies have demonstrated that hippocampal 5-HT inputs from the midbrain raphe nuclei affect corticosterone-concentrating cells.¹ Moreover, corticosterone receptors and 5-HT immunoreactivity are co-localized in the same serotonergic cells within the raphe nuclei.²⁰ These observations provide a key underlying basis for a functional interaction between 5-HT and glucocorticoids. Indeed, accumulating data indicate that the 5-HT innervation is important in the maintenance of hippocampal expression of GR and MR. Chronic lesions of central 5-HT pathways decrease hippocampal corticosterone binding sites⁵⁶ and attenuate expression of MR and GR mRNAs *in vivo*.⁶⁰ Conversely, serotonergic agents, such as antidepressant drugs, increase expression of GR and MR and their encoding mRNAs in the hippocampus *in vivo*,^{50,54} and in primary hippocampal cultures *in vitro*.⁴¹

*To whom correspondence should be addressed.

Abbreviations: GR, glucocorticoid receptor; 5-HT, 5-hydroxytryptamine; MDMA, 3,4-methylenedioxymethamphetamine; MR, mineralocorticoid receptor; SSC, standard saline citrate.

Similarly, glucocorticoids act on the hippocampus to modulate 5-HT turnover¹² and electrophysiological responses to 5-HT.²⁵ The latter may reflect the documented actions of glucocorticoids on the expression of specific 5-HT receptor subtypes.¹⁰

However, investigation of these interactions is complicated. First, control of receptor expression is often specific to particular hippocampal subregions,^{40,61} which have distinct innervations and functions,⁵⁹ making anatomical resolution important. Second, autoregulation of corticosteroid receptors,^{24,49} and 5-HT receptor subtypes,^{4,60} have been reported. Drugs that enhance brain serotonin function increase serum corticosterone concentration in the rat,¹⁷ yet glucocorticoids *per se* may alter 5-HT release.¹² Third, there are substantial diurnal variations in GR, MR and 5-HT_{2C} receptor gene expression.^{22,23} Clearly, determination of receptor regulation *in vivo* must attempt to control for these effects.

3,4-Methylenedioxymethamphetamine (MDMA), a potent psychostimulant and widely used recreational drug of abuse, is a selective 5-HT neurotoxin in animals¹⁹ and possibly man.⁵¹ Repeated administration of MDMA to rats markedly, but selectively, depletes central 5-HT producing degeneration of 5-HT terminals.⁴⁵ Chronic MDMA treatment potentially decrease hippocampal MR and GR, but increase 5-HT_{2C} receptor mRNA expression.⁶⁰ In contrast, a single administration of MDMA selectively increases synaptic 5-HT by inhibiting re-uptake and monoamine oxidase-A.²⁸ These initial "acute" effects last less than 24 h and are followed by longer-term 5-HT depletion.³⁶ MDMA also increases basal glucocorticoid levels, both acutely and chronically.^{43,60} Here we exploit the selective acute action of MDMA to differentiate the short term actions of endogenous 5-HT and glucocorticoids *in vivo* upon hippocampal corticosteroid and 5-HT receptor mRNA expression.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague-Dawley rats (200–250 g) from Harlan, UK, were maintained under controlled lighting (lights on 07.00–19.00) and temperature (22°C) and had access to food and water *ad libitum*. All procedures were carried out in accordance with the U. K. Animals (Scientific Procedures) Act, 1986 and associated guidelines. Rats were given a single injection of MDMA (20 mg/kg, s.c., Sigma, Poole, UK), a dose previously shown to potentially release 5-HT and corticosterone,^{3,43} and were killed by decapitation 4, 9, 16 and 48 h later ($n=6$ /time-point). The time of MDMA administration was adjusted, such that all rats were killed in the morning (08.30–10.00) to avoid any effect of the documented circadian changes in circulating glucocorticoids or receptor gene expression.^{22,23} Control animals ($n=6$) were injected with saline (taken as time-point zero).

To determine whether changes in plasma corticosterone might be responsible for any effects of MDMA in adrenalectomized rats, a group of animals ($n=5$ /time-point) were bilaterally adrenalectomized, via a dorsal approach, under

halothane anaesthesia and implanted with continuous-release corticosterone pellets (50 mg, 21 day pellets, Innovative Research of America). This paradigm produces fixed plasma corticosterone at near basal (morning) levels. MDMA (20 mg/kg, s.c.) was administered six days after surgery, controls were given saline. The injection schedule was the same as adrenal-intact rats, but excluding the 48 h time-point (corticosterone levels have returned to control basal levels at this time-point in adrenal-intact rats). After decapitation, the brains were rapidly removed and frozen. Coronal sections (10 μ m) were cut at the level of the anterior dorsal and posterior hippocampus, thaw-mounted onto gelatin-subbed, poly-L-lysine-coated slides and stored at -85°C . Trunk blood was taken for corticosterone assay.

In situ hybridization

In situ hybridization was carried out as described previously.⁶⁰ Slides were postfixed in 4% paraformaldehyde, washed twice in $2\times$ standard saline citrate (SSC) and hybridized overnight at 50°C using [^{35}S]UTP-labelled cRNA antisense probes transcribed *in vitro* from plasmid vectors containing the appropriate cDNA insert. MR cRNA was transcribed from a 513 base pair EcoRI fragment of rat MR cDNA; GR cRNA from a 674 base pair PstI-EcoRI fragment of rat GR cDNA; 5-HT_{1A} receptor cRNA from a 910 base pair Ball-PvuII fragment of the rat 5-HT_{1A} receptor cDNA; 5-HT_{2A} receptor cRNA from a 1.1 kb HpaI-EcoRI fragment of the rat 5-HT_{2A} receptor cDNA; and 5-HT_{2C} receptor cRNA from a 401 base pair EcoRI-SstI fragment of the rat 5-HT_{2C} receptor cDNA. Probes were denatured and added at a final concentration of 10×10^6 c.p.m./ml (MR, GR and 5-HT_{2A}) and 20×10^6 c.p.m./ml (5-HT_{1A} and 5-HT_{2C}) to hybridization buffer. For the 5-HT receptor detection, a prehybridization step involving addition of 200 μ l of prehybridization buffer (hybridization buffer without dextran sulphate), incubated at 50°C for 3 h, was included. Following hybridization, sections were treated with RNase A (30 μ g/ml, 45 min at 37°C) and washed to a final stringency of $0.1\times$ SSC at 60°C . Slides were dehydrated, dipped in photographic emulsion (NTB-2, Kodak Ltd, UK) and exposed at 4°C for three to five weeks before being developed and counterstained with 1% Pyronin.

Corticosterone assay

Plasma corticosterone was measured using a previously described radioimmunoassay with a highly specific anti-serum (Dr C. Kenyon) and [^3H]corticosterone (88 Ci/mmol, Amersham Int., UK).⁶⁰ Assay sensitivity was 4 pg/ml and intra-assay CV was 5.3%.

Data analysis

Hybridization signal within hippocampal subregions was assessed by computer-assisted grain counting using an image analysis system (Seescan plc, Cambridge, UK). Silver grains were counted over individual neurons within each hippocampal subfield, except the dentate gyrus where it was difficult to define cell boundaries (here a fixed area field covered one cell and a fraction of neighbouring cells). For each animal, the mean grain counts were calculated over 12–18 cells/subregion, after subtraction of background (counted over areas of white matter). Data were assessed by one-way ANOVA followed by the Scheffé *post hoc* test. Significance was set at $P<0.05$. Values are means $\pm$ S.E.M.

RESULTS

Plasma corticosterone

In adrenalectomized rats, injection of MDMA markedly increased basal (morning) plasma corticosterone

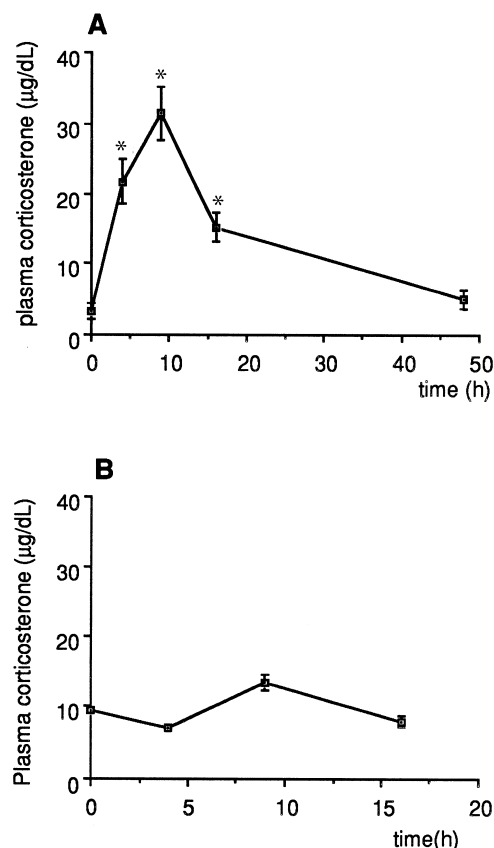


Fig. 1. Plasma corticosterone levels at different time points following a single s.c. injection of MDMA (20 mg/kg) in adrenal-intact (A) and corticosterone-fixed (B) rats. Control rats (time-point zero) were injected with saline (1 ml/kg). * $P < 0.001$ compared to saline control. $n = 6$ /time-point.

levels ($P < 0.001$, compared to saline-injected controls; Fig. 1A). Corticosterone levels peaked 9 h after MDMA at 31 ± 4 µg/dl, levels associated with maximal responses to stress in this strain, and were still significantly elevated 16 h after the single dose of MDMA. Corticosterone pellets in adrenalectomized rats produced slightly higher basal corticosterone levels than control adrenal-intact rats ($P < 0.01$; Fig. 1B). These corticosterone levels were maintained following MDMA although slightly elevated at 9 h ($P < 0.001$, compared to saline-injected controls; Fig. 1B), likely an action of MDMA on remaining accessory adrenal tissue.

Mineralocorticoid and glucocorticoid receptor messenger RNA expression

Both MR and GR mRNA were expressed in all hippocampal subfields with distinctive distributions; MR mRNA was highly and relatively evenly expressed, with highest levels in CA2; GR mRNA was very highly expressed in the dentate gyrus, CA1 and CA2, with lower levels in CA3 and CA4, as previously described.⁶⁰ In adrenalectomized rats, MDMA produced a significant, but delayed (16 h) increase in

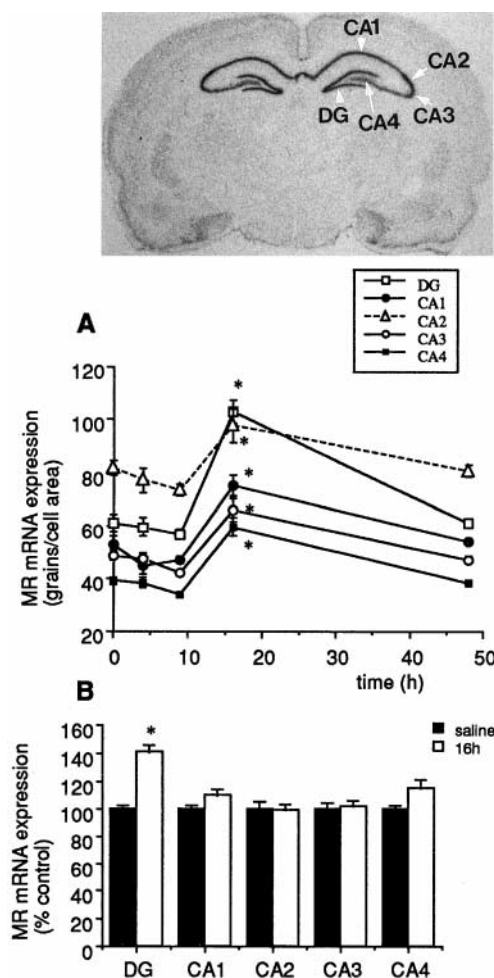


Fig. 2. MR mRNA expression in hippocampal subregions at different time points following a single MDMA injection. Autoradiograph shows MR mRNA distribution in the hippocampus of control animals. $n = 6$ /time-point. * $P < 0.001$ compared to saline control (time-point zero). (A) In adrenal-intact rats, MR mRNA expression is increased in all hippocampal subregions at 16 h; greatest increase 69% in dentate gyrus. (B) Histogram shows MR mRNA expression as % control of saline in all hippocampal subregions at 16 h time-point in corticosterone-fixed rats. No significant effect of MDMA found in any subregion at the other time-points examined (4 h and 9 h). $n = 5$ /time-point. MR mRNA expression is increased in dentate gyrus at 16 h. DG, dentate gyrus.

MR gene expression in all hippocampal subfields (Fig. 2A). The increase, greatest in the dentate gyrus (69% rise; $P < 0.001$, see Fig. 3A, B), had returned to baseline by 48 h.

Since MDMA produced prolonged glucocorticoid hypersecretion, a second group of rats was adrenalectomized and replaced with corticosterone at a fixed level, such that effects due to MDMA-induced secretion of corticosterone could be excluded. Such "corticosterone-fixed" rats were only examined 4, 9 and 16 h after MDMA; times at which plasma corticosterone levels were elevated in adrenalectomized rats (see Fig. 1A). In corticosterone-fixed rats, the

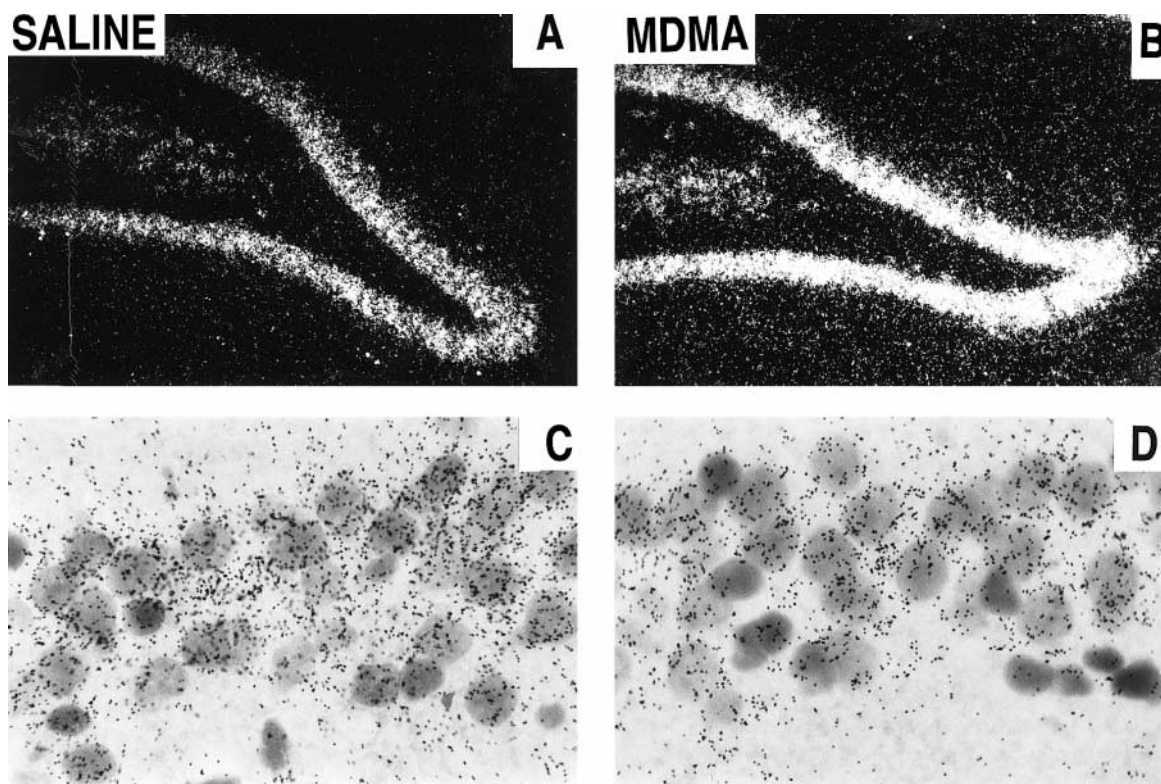


Fig. 3. Dark-field ($\times 43$) and bright-field ($\times 428$) photomicrographs of *in situ* hybridization showing MR mRNA expression in the dentate gyrus (A, B) and GR mRNA expression in the CA1 pyramidal cells (C, D) from saline- and MDMA-treated rats. Note the increased MR mRNA expression 16 h (autoradiographic silver grains appear white under dark-field) and decreased GR mRNA expression 4 h (autoradiographic silver grains appear black under bright-field) following MDMA.

MDMA-related induction of MR gene expression at 16 h was abolished in all hippocampal subregions except the dentate gyrus. Here a 41% rise persisted, similar to that observed in adrenalectomized rats (Fig. 2B). No changes in MR mRNA expression were found in any hippocampal subregion for the other time-points (data not shown).

In contrast to the delayed increase in MR mRNA expression, MDMA produced a more rapid (by 4 h) decrease in GR gene expression in adrenalectomized rats, selectively in the dentate gyrus (33% fall) and CA1 (42% fall) subfields (see Figs 3C, D, 4A). This fall persisted for 16 h in both subregions. No effects were found in CA3 or CA4. In corticosterone-fixed rats, the fall in GR mRNA expression in the dentate gyrus and CA1 were not observed at any time (Fig. 4B).

5-Hydroxytryptamine_{1A} receptor messenger RNA expression

5-HT_{1A} receptor mRNA was highly expressed throughout the hippocampus, with highest levels in dentate gyrus granule cells and lowest expression in CA3, in agreement with previous reports.²⁴ In adrenalectomized rats, MDMA decreased 5-HT_{1A} receptor mRNA expression selectively in CA1 at 4 h

(36% fall, $P < 0.001$, e.g., see Fig. 6A, B) and 9 h (20% fall, $P < 0.001$), returning to control levels by 16 h (Fig. 5A). No change was seen in other subfields. The fall in 5-HT_{1A} receptor mRNA expression in CA1 following MDMA was also observed in corticosterone-fixed rats (Fig. 5B). In addition, the corticosterone-fixed animals showed a small rise in 5-HT_{1A} receptor gene expression in the dentate gyrus 9 and 16 h after MDMA (22% rise, $P < 0.05$, e.g., see Fig. 6C, D), an effect not seen in adrenalectomized rats.

5-Hydroxytryptamine_{2C} receptor messenger RNA expression

5-HT_{2C} receptor mRNA was measured in the posterior hippocampus where this transcript is considerably more widespread than in dorsal hippocampus (where expression is restricted to scattered cells in the stratum oriens and stratum radiatum of CA3). High expression of 5-HT_{2C} receptor mRNA was found in ventral CA1 and the subiculum, with much lower expression in posterior CA1, as previously described.²⁴ In adrenalectomized rats, MDMA acutely decreased 5-HT_{2C} receptor gene expression in ventral CA1 and subiculum (see Figs 7A, 8), but not posterior CA1, an effect maximal at 16 h (38% fall,

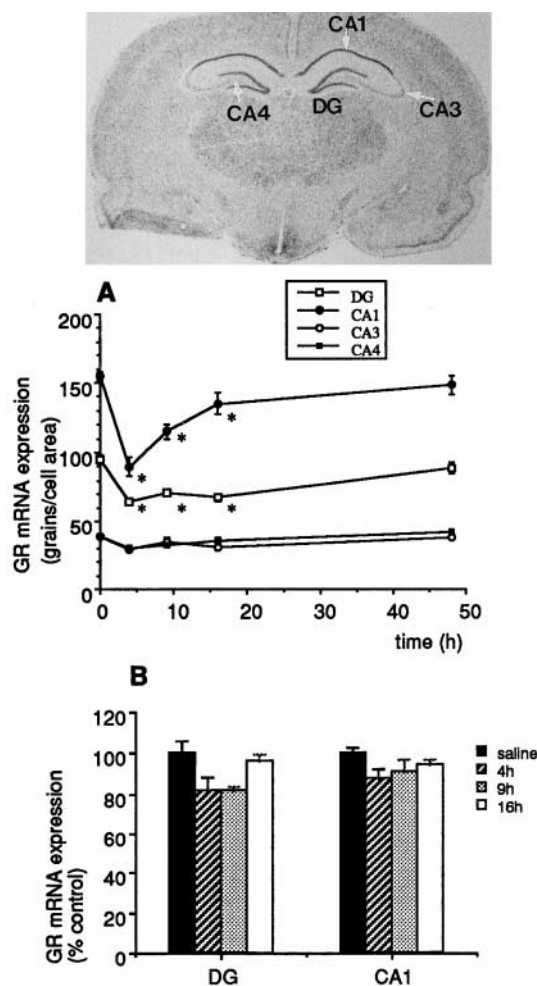


Fig. 4. GR mRNA expression in hippocampal subregions at different time-points following a single MDMA injection. Autoradiograph shows GR mRNA distribution in the hippocampus of control rats. $n=6$ /time-point. $*P<0.001$ compared to saline control. (A) In adrenal-intact rats, GR mRNA expression is decreased in dentate gyrus (33% fall) and CA1 (42% fall) at 4 h, remaining decreased up to 16 h. (B) Histogram shows GR mRNA expression as % control of saline in the dentate gyrus and CA1 from corticosterone-fixed rats. No significant effect of MDMA found in dentate gyrus and CA1 or in the other subregions at any time-point examined. $n=5$ /time-point. DG, dentate gyrus.

$P<0.001$). In the subiculum, 5-HT_{2C} receptor gene expression became elevated 48 h after MDMA (38% rise, $P<0.001$), whilst in ventral CA1 expression returned to control levels (Fig. 7A). The acute decrease in 5-HT_{2C} receptor mRNA expression in the subiculum following MDMA was maintained in corticosterone-fixed rats (Fig. 7B). In contrast, the effects of MDMA on 5-HT_{2C} receptor gene expression in ventral CA1 was abolished in corticosterone-fixed rats.

DISCUSSION

A single 20 mg/kg injection of MDMA increased MR mRNA expression in all hippocampal subfields with a 16 h delay, decreased GR and 5-HT_{1A}

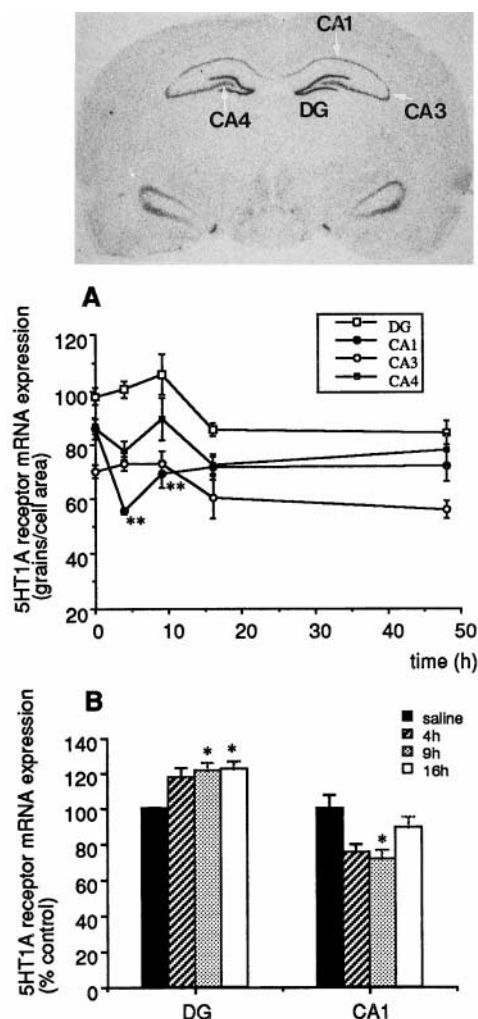


Fig. 5. 5-HT_{1A} receptor mRNA expression in hippocampal subregions at different time-points following a single MDMA injection. Autoradiograph shows 5-HT_{1A} receptor mRNA distribution in the hippocampus of control rats. $n=6$ /time-point. $*P<0.05$, $**P<0.001$ compared to saline control (time-point zero). (A) In adrenal-intact rats, 5-HT_{1A} receptor mRNA expression is decreased in CA1. (B) Histogram shows 5-HT_{1A} receptor mRNA expression as % control of saline in the dentate gyrus and CA1 from corticosterone-fixed rats. 5-HT_{1A} receptor mRNA expression is increased in the dentate gyrus (22% rise; $P<0.05$) and decreased in CA1 (29% fall; $P<0.05$). No significant effect of MDMA found in the other subregions at any time-point examined. $n=5$ /time-point. DG, dentate gyrus.

receptor mRNA expression within 4 h and 5-HT_{2C} receptor mRNA by 16 h, although only in specific hippocampal subfields. This contrasts with the chronic MDMA injection schedule, which resulted in decreased hippocampal MR and GR, but increased 5-HT_{2C} receptor mRNA expression,⁶⁰ a likely consequence of the neurotoxic effects of MDMA on 5-HT nerve terminals. The acute effects of MDMA in the present study may result from direct interactions with postsynaptic 5-HT receptors or indirectly from drug-induced presynaptic release of 5-HT from serotonergic terminals.⁵⁸ The poor affinity of

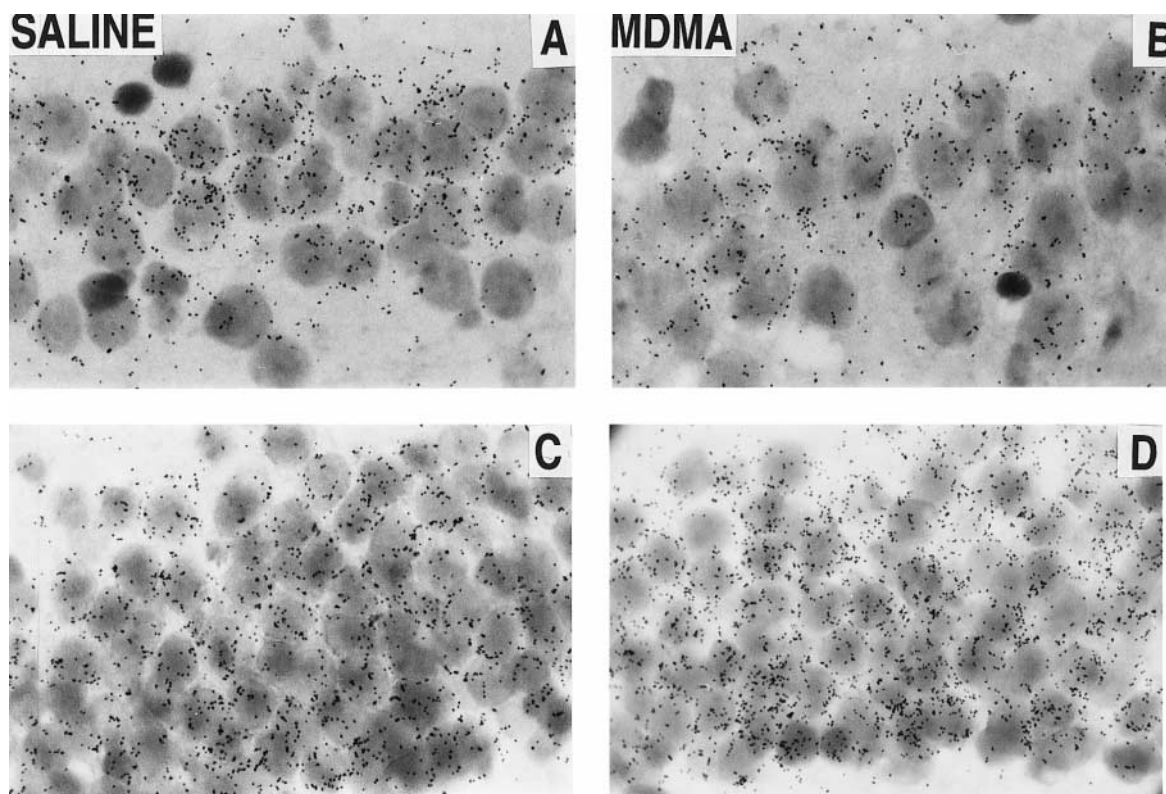


Fig. 6. Bright-field photomicrograph ($\times 428$) of *in situ* hybridization showing 5-HT_{1A} receptor mRNA expression in the pyramidal cells (A, B) and the dentate gyrus granule cells (C, D) from saline and MDMA treated rats. Autoradiographic silver grains appear black. Note the decreased 5-HT_{1A} receptor mRNA expression in CA1 4 h after MDMA from adrenal-intact rats and the increased expression in the dentate gyrus 9 h after MDMA from the "corticosterone-fixed" rats.

MDMA for 5-HT₁ and 5-HT₂ receptor subtypes³¹ supports 5-HT efflux as the most likely mechanism, although other potential MDMA-induced changes to other (as yet unidentified) neuromodulators might also contribute to the altered hippocampal receptor gene expression. Acutely, MDMA also releases corticosterone and, since GR, MR and 5-HT_{2C} receptor mRNA expression show substantial diurnal variation in the hippocampus,^{22,23} several potential mechanisms might be involved. The design of this study excluded diurnal effects, since all rats were killed in the morning (the nadir of motor activity and plasma corticosterone). However, MDMA caused prolonged elevation of basal plasma corticosterone, consistent with previous studies.^{34,43} The mechanism is likely to be mediated via activation of 5-HT_{2A/2C} receptors, as ketanserin antagonises this effect.⁴³ Interestingly, the rise in corticosterone may play a role in the development of chronic MDMA neurotoxicity upon hippocampal 5-HT systems, since adrenalectomy is protective, an effect reversed by concurrent corticosterone administration.²⁶

Hippocampal mineralocorticoid and glucocorticoid receptor messenger RNA expression following acute 3,4-methylenedioxymethamphetamine

The MDMA-induced rise in hippocampal MR mRNA expression was lost in CA1–4 in rats with

fixed corticosterone levels, suggesting that, in these regions, it reflected the elevation of plasma corticosterone *per se*. Consistent with this, corticosterone directly induces transcription from the MR gene promoter in primary hippocampal neurons *in vitro*.⁸ In contrast, in the dentate gyrus, the marked rise in MR mRNA expression persisted in rats unable to exhibit a corticosterone response to MDMA. In these animals, the potential mechanism for MDMA's effects is most likely a consequence of the increased carrier-mediated efflux of 5-HT from presynaptic terminals. This is also thought responsible for the behavioural effects of MDMA, which are maximal 3–6 h after drug administration.⁵³ The delay is consistent with the effects of monoaminergic agents such as antidepressants *in vivo*, which increase MR (and GR) in rat hippocampus over days,^{50,54} and with 5-HT in primary hippocampal cells *in vitro*, which similarly increases GR expression only after several days.⁴¹ Although the mechanisms of delayed and subregionally specific control of MR expression in the hippocampus remain unclear, various transcription factors may require to be expressed before MR (and other) gene activation can occur, and these are likely to differ between subfields.⁴⁷

GR mRNA expression in contrast, was rapidly attenuated by MDMA in the dentate gyrus and CA1. This effect was abolished in corticosterone-fixed rats

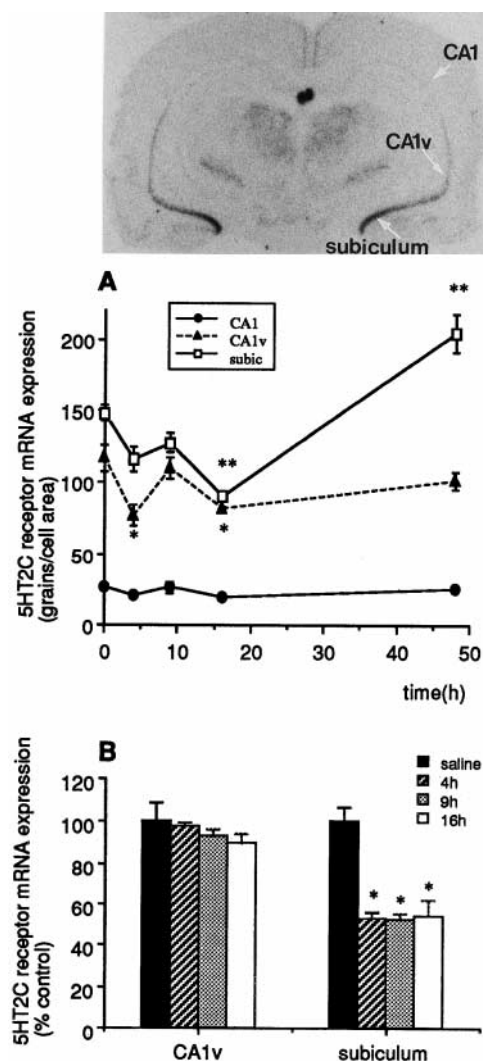


Fig. 7. 5-HT_{2C} receptor mRNA expression in posterior hippocampal subregions at different time-points following a single MDMA injection. Autoradiograph shows 5-HT_{2C} receptor mRNA distribution in the posterior hippocampus of control rats. $n=6$ /time-point. ** $P<0.001$, * $P<0.01$ compared to saline control (time-point zero). (A) In adrenalectomized rats, 5-HT_{2C} receptor mRNA expression is decreased in the subiculum at 16 h and increased by 48 h. Receptor gene expression is also decreased in CA1v. (B) Histogram shows 5-HT_{2C} receptor mRNA expression as % control of saline in the subiculum and CA1v from corticosterone-fixed rats. 5-HT_{2C} receptor mRNA expression is decreased in the subiculum. No significant effect of MDMA found in the other subregions at any time-point examined. $n=5$ /time-point. CA1v, ventral posterior CA1.

suggesting mediation by the MDMA-induced rise in corticosterone and is consistent with autoregulation of the GR gene,⁷ although the precise molecular mechanism(s) remain unclear. The lack of significant changes in the other subregions may be due to the low expression of GR mRNA; interestingly, adrenalectomy-induced increases in GR mRNA expression were also found in the dentate gyrus and CA1.²¹ Endogenous corticosterone therefore acutely

regulates hippocampal MR and GR mRNA expression in opposite directions. Activation of MR and GR in the hippocampus frequently has opposing electrophysiological²⁵ and behavioural effects³³ and disparate control of the receptor genes is thus not surprising. In contrast to our data, a recent study using the 5-HT neurotoxin para-chloroamphetamine reported decreased hippocampal MR and GR binding sites after 3 h.⁴⁴ However, the necessity to adrenalectomize animals for at least 12 h prior to such radioligand binding studies, to remove endogenous steroids, makes the acute regulation of corticosteroid receptors difficult to determine by this approach. It may also be argued that removal of diurnal corticosterone secretion in the corticosterone-fixed rats disrupts the normal rhythmic modulation of gene expression by glucocorticoids, thus producing abnormal patterns of expression. Future studies where rats are killed at different times of the day following MDMA or saline could determine whether regulation of gene expression by MDMA varies with the diurnal rhythm.

Hippocampal 5-hydroxytryptamine_{1A} and 5-hydroxytryptamine_{2A} receptor messenger RNA expression following acute 3,4-methylenedioxymethamphetamine

Hippocampal 5-HT_{1A} receptor mRNA expression was decreased 4 h after drug administration in both the adrenal-intact and corticosterone-fixed rats, selectively in CA1 pyramidal cells. This effect therefore appears to reflect MDMA-induced 5-HT release. Consistent with a 5-HT induced down-regulation, decreased hippocampal 5-HT_{1A} receptor mRNA expression and binding sites was observed following chronic antidepressants,⁶ psychosocial stress in subordinate tree shrews¹⁶ and chronic social stress in colonies of rats;³⁷ all show increased extracellular concentrations of serotonin in the hippocampus. In glucocorticoid-fixed, but not adrenalectomized rats, MDMA also increased 5-HT_{1A} receptor gene expression in the dentate gyrus, an effect therefore relating both to 5-HT release and the glucocorticoid status of the animals. As both dentate and CA1 regions highly express GR and MR, this regionally-distinctive regulation of 5-HT_{1A} receptor mRNA expression could be due to differential control by local hippocampal circuits.⁵⁷ Interestingly, chronic treatment with buspirone, an anxiolytic which is a partial 5-HT_{1A} receptor agonist, also selectively increases 5-HT_{1A} receptor mRNA expression in the dentate gyrus and decreases expression in CA1.¹¹

Previous studies suggest that the markedly increased corticosterone levels following MDMA would tend to decrease 5-HT_{1A} receptor mRNA expression selectively in the dentate gyrus.^{38,40} This is in accordance with a negative transcriptional regulation of hippocampal 5-HT_{1A} receptor mRNA by corticosteroids.⁶² In contrast, 5-HT released by MDMA in adrenalectomized rats increased 5-HT_{1A}

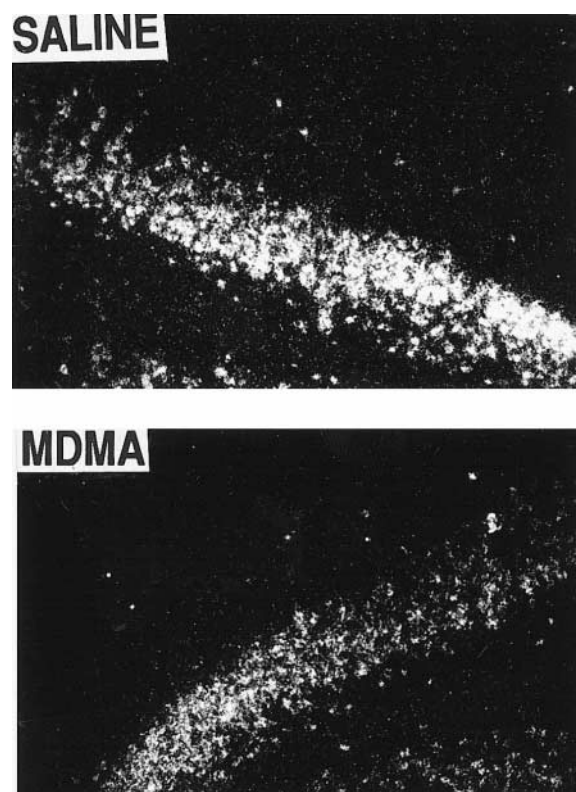


Fig. 8. Dark-field photomicrograph ($\times 85$) of *in situ* hybridization showing 5-HT_{2C} receptor mRNA expression in the subiculum of saline and MDMA treated rats (16 h time-point). Autoradiographic silver grains appear white. Note the decreased expression 16 h after MDMA.

receptor gene expression in the dentate gyrus.³⁸ The lack of overall change in the dentate gyrus in the adrenalectomized rats likely reflects the sum of these divergent effects. In CA1, the 5-HT effect predominates. Not all studies, however, agree with a glucocorticoid regulation of 5-HT_{1A} receptor mRNA expression restricted to the dentate gyrus. Adrenalectomy has also been shown to increase 5-HT_{1A} receptor mRNA expression in all hippocampal subregions,^{9,30} or to have no effect.²⁴ The basis for these discrepancies remains obscure but may, in part, reflect differences in genetic background or environment. Thus the inbred Han Wistar rat showed no glucocorticoid regulation²⁴ while the outbred Harlan Sprague-Dawley rat, as used in the present study, showed adrenalectomy-induced up-regulation of 5-HT_{1A} receptor binding sites and mRNA in the hippocampus.⁵

MDMA acutely decreased 5-HT_{2C} receptor mRNA expression in ventral CA1, an effect abolished in corticosterone-fixed rats suggesting corticosterone mediation and consistent with the documented glucocorticoid control of this gene.²⁴ In contrast in the subiculum, 5-HT_{2C} receptor mRNA expression decreased 16 hours after MDMA, effects maintained in corticosterone-fixed rats suggesting mediation by 5-HT release. The greater decrease in corticosterone-fixed rats compared to intact rats may be due to underlying induction of 5-HT_{2C} receptor

mRNA expression in the subiculum by an adrenal product. The increase in 5-HT_{2C} receptor mRNA expression in the subiculum at 48 h likely represents the long-term phase of MDMA action when 5-HT levels are reduced, and is consistent with previous findings.⁶⁰ No effects at 48 h were seen in ventral CA1, emphasizing the subregional selectivity in 5-HT receptor gene regulation.

The acute MDMA effects appears to be selective for the 5-HT_{1A} and 5-HT_{2C} receptor subtypes, since hippocampal 5-HT_{2A} receptor mRNA expression was unaltered by MDMA at any time-point measured (data not shown); this is consistent with previous reports of a lack of 5-HT or glucocorticoid regulation of this receptor subtype in the hippocampus.^{24,60}

Functional implications of altered hippocampal receptor gene expression

Several studies have reported alterations in hippocampal MR or GR mRNA expression selectively either in the dentate gyrus, CA1 or both,^{21,47,61} consistent with the subregional selectivity of the observed changes in receptor mRNA expression in the present study. The functional significance of this hippocampal subregional specificity is unclear. It is likely that alterations in electrophysiological parameters within these subregions via corticosteroid

or 5-HT receptors, will in turn influence hippocampal function through the hippocampal trisynaptic circuit. Glucocorticoids influence long-term potentiation, an electrophysiological correlate of memory, via MR and GR, in CA1¹⁴ and in the dentate gyrus.⁴⁸ MR mRNA expression in CA1 correlates positively with spatial memory in the Morris watermaze⁶¹ and central administration of MR antagonists impairs spatial memory when given both acutely⁴⁶ and chronically (Yau J. L. W. and Seckl J. R., unpublished observations). 5-HT modulates LTP in the dentate gyrus via the 5-HT_{1A} receptor in an inhibitory manner.⁵² If the mRNA changes are indeed reflected in the protein, then it is possible that altered receptor gene expression in the dentate gyrus and CA1 may affect memory and contribute to the cognitive effects of this drug.²⁹

Both 5-HT_{1A} receptors and 5-HT_{2C} receptors have been implicated in the control of anxiety and mood.^{27,42} Hippocampal MR and GR modulate mood, behaviour, neuroendocrine function and memory.³³ Mood alteration and visual hallucinations are common psychiatric complications of acute MDMA use.³⁵ Marked glucocorticoid excess in sensitive subjects (pre-existing affective disorder) produces potent short-term dysphoric and other ef-

fects on mood.¹⁸ We therefore speculate that acute MDMA-induced glucocorticoid release, coupled with changes in hippocampal corticosteroid and 5-HT receptor gene expression, play an important role in the changes in mood and behaviour associated with this drug of abuse.

CONCLUSION

The data from these studies using the amphetamine drug, MDMA, illustrate the complex and site-specific control of corticosteroid and 5-HT receptor mRNA expression in the hippocampus, with both glucocorticoids and 5-HT exerting potent regulatory effects on their own receptors and heterologous receptors at the level of mRNA expression. In conclusion, there are clearly, intricate interactions between the 5-HT and glucocorticoid systems in the hippocampus; the implications for neuronal function and behaviour merit further detailed investigation.

Acknowledgements—We thank Drs R. Evans, J. Arriza, R. Miesfeld, O. Civelli, D. J. Julius and Professor P. H. Seeburg for kindly supplying the cDNA clones used in this study. This research was supported by a Wellcome Trust Senior Research Fellowship (JRS).

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(Accepted 3 September 1996)