C1

Hippocampal free corticosterone levels show an ultradian rhythm in Wistar rats

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Plasma corticosterone levels in rodents are characterised by a diurnal rhythm with low levels in the morning and markedly higher levels towards the evening, the activity phase of these nocturnal animals. Apart from diurnal changes, plasma corticosterone levels also display an ultradian rhythm [1], possibly resulting from its pulsatile secretion by the adrenal cortex [2]. At present it is unknown whether this ultradian rhythm in glucocorticoid hormone persists across the blood-brain barrier and can be found in the extracellular space of the brain. To clarify this issue, we designed a series of *in vivo* microdialysis studies to measure corticosterone levels in the rat hippocampus, a principal site of glucocorticoid action in the brain. Importantly, because the extracellular fluid is devoid of corticosterone binding proteins, dialysate levels represent the free, i.e. the biologically active fraction, of this glucocorticoid [3].

Male and female Wistar rats were equipped with a guide cannula under isoflurane anaesthesia. Seven days later a microdialysis probe was inserted, under isoflurane anaesthesia, into the hippocampus. After 2 days, collection of dialysate samples was started at 05:00 h and continued for 48 h. Sample intervals were 10 min or 30 min between 08:00-22:00 h and 22:00-08:00 h, respectively. On the second day animals were subjected to novelty (30 min) or swim stress (15 min, 25°C), or left undisturbed in their home cage. A sensitive radioimmunoassay was used to measure dialysate corticosterone. Data were analysed using Pulsar PC and SPSS software.

It was found that hippocampal free corticosterone levels show a clear pulsatile pattern in both male and female rats. Interestingly, in both sexes the pulse amplitude increased towards the evening. A comparison between male (n=15) and female (n=10) rats over a 12 h period (09:00-21:00 h), revealed no significant gender differences ($P > 0.05$, Student's t test) in the total number of pulses (male (m): 14.6±0.5 vs. female (f): 14.5±1.2 pulses) and the pulse amplitude (0.11±0.01 (m) vs. 0.09±0.01 (f) μg/dl). Moreover, the mean corticosterone level was similar in male and female rats (0.14±0.02 (m) vs. 0.11±0.01 (f) μg/dl). While both novelty and swim stress caused a rise in free corticosterone in male and female rats, the ultradian rhythm was rapidly restored after termination of the stressors (maximal effect in male rats: novelty: 0.27±0.08 μg/dl, swim: 0.79±0.34 μg/dl; female rats: novelty 0.27±0.05 μg/dl, swim: 1.27±0.33 μg/dl).

These data demonstrate for the first time the existence of an ultradian rhythm of free corticosterone in the hippocampus of rats. Such an ultradian pattern of hormone levels could be of great significance for glucocorticoid action in the hippocampus during ongoing conditions and for the role of this brain structure in the regulation of the hypothalamic-pituitary-adrenal axis and other glucocorticoid-sensitive systems over the diurnal cycle.

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Sponsored by NCT.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C2

Rapid turnover of nuclear glucocorticoid receptors in the rat hippocampus after pulsatile corticosterone administration

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Glucocorticoids (GCs) act via intracellular receptors which translocate into the nucleus to regulate target genes. There are two types of GC receptors, the high affinity mineralocorticoid receptor (MR) and the low affinity glucocorticoid receptor (GR). The secretion of GCs occurs in distinct pulses depending on numerous physiological factors (Atkinson et al. 2006); however, the functional significance of this pulsatility is unknown. Here, we present two models of corticosterone (cort) presentation in the rat: IP injection to mimic the prolonged cort release of a stress response and IV injection to mimic basal pulsatility. This study aims to determine how different patterns of cort presentation affect GR and MR activity in the hippocampus (HC), an important site for stress-related memory and learning.

Male Sprague-Dawley rats (n=6/group) were anaesthetised with IM injection of Hypnorm (fentanyl citrate 0.252 mg/kg and fluanisone 8 mg/kg) after IP injection of Diazepam (4 mg/kg) then subjected to bilateral adrenalectomy and jugular cannulation (IV). After recovery for 5 days, each was given either 1 bolus IP injection (750 μg cort) or 2 bolus IV injections (100 μg cort) at times 0 and 120 min. Animals were killed at times 0, 10, 15, 30, 60 and 120 min. The HC was rapidly dissected and frozen. Nuclear extracts were prepared and analysed by Western blot with GR or MR antibodies to determine nuclear GR and MR levels.

IP injection caused prolonged high plasma cort levels (466±164 ng/ml at 30 min, 206±31 ng/ml at 60 min) decreasing by 120 min (38±13 ng/ml). Nuclear translocation of both GR and MR in the HC was prolonged (21±4-fold GR, 23±5-fold MR at 30 min, and 14±4-fold GR, 31±5-fold MR at 60 min).

IV injection caused a rapid pulse of GC (883±157 ng/ml) at 1 min. This was rapidly cleared as the half life of cort in blood is 10 min. Nuclear translocation of both GR and MR was observed in the HC at 10 min (19±6-fold GR, 14±3-fold MR) reaching a maximum at 15 min (22±9-fold GR, 19±4-fold MR). The retention time of GR in the nucleus was much shorter in duration than that observed after the IP injection. The depletion of nuclear GR after each pulse was evident by 30 min (9±2-fold) returning to near basal levels by 60 min. MR displayed a much slower nuclear depletion with high nuclear levels of MR throughout the time course decreasing only at 120 min after each pulse.

Cort IP injection provides a model of a stress-like prolonged presence of GR and MR in HC nuclei as described by Kitchener et al. (2004). IV injection provides a temporal model of basal pulsatility with rapid turnover of GR but not MR in HC nuclei. Our findings have significant implications for GR as a sensitive mediator of adrenal GC pulses transmitted to the HC. The highly fluctuating nature of nuclear GR levels (but not MR levels) may be functionally important in allowing dynamic interactions with physiological responses e.g. stressors.

Atkinson HC et al. (2006). J Neuroendocrinol 18, 526-533.

Kitchener P et al. (2004). Eur J Neuroscir 19, 1837-1846.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C3

The role of the vasopressin V1b receptor in the HPA axis response to acute stress: molecular and pharmacological studies

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Vasopressin (VP) is the hormonal regulator of water homeostasis and has major effects on behaviour and vascular tone. In addition to this it is also a key regulator of the hypothalamic-pituitary-adrenal (HPA) axis, an action mediated through the G protein-coupled V1b receptor (R) (or V3R) predominantly found on the corticotrophs of the anterior pituitary. Although corticotrophin releasing hormone (CRH) seems to be the dominant adrenocorticotrophin (ACTH) secretagogue in rodents in response to acute stress (e.g. restraint), VP synergizes with CRH in activating the release of ACTH and may be preferentially released in response to some acute stressors (e.g. insulin-induced hypoglycaemia (1,2)). To further investigate the role of the VP V1bR in the HPA axis response to stress, we compared the effects of a recently described V1bR antagonist (Org 52186 (3)) on the levels of plasma ACTH and corticosterone (CORT) in V1bR knockout mice subjected to acute restraint stress. Org 52186 is a potent antagonist at the human V1bR and exhibits >1000-fold selectivity over the closely related V1a, V2 or oxytocin receptors. Adult male and female V1bR KO mice (and wild-type littermates) were administered s.c. with vehicle or Org 52186 (10 or 30mg/kg) 2h prior to acute restraint stress (30min in a 50ml 'Falcon' tube). Both doses of the V1bR antagonist significantly reduced stress-induced plasma ACTH and CORT levels in wild-type mice (ACTH: male wild-type restraint 129±16pg/ml, n=4, vs male wild-type restraint + Org52186 (30mg/kg) 45±7.87pg/ml, n=5; $p<0.05$). The HPA axis response to restraint was decreased in the V1bR KO (ACTH: male wild-type restraint 129±16pg/ml, n=4, vs male knockout + restraint 63±14pg/ml, n=5; $p<0.001$ - pretreatment with Org 52186 did not significantly effect the stress-induced hormone levels in these mice. Similar results were obtained in the female knockout and wild-type mice (e.g. vehicle + restraint 73±8pg/ml, n=7, vs Org 52186 (30mg/kg) + restraint 21±5.6pg/ml, n=6; $p<0.001$). In contrast to some previous studies on the VP-deficient Brattleboro rat (4), our studies clearly demonstrate that the V1bR is important for a normal acute restraint stress-induced ACTH and CORT response, and suggest that it is unlikely that possible compensatory/developmental changes resulting from the ablation of the V1bR gene play an important role in the HPA axis response to acute restraint in the V1bR KO.

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Org52186 Patent no. WO/2006/095014; 2-(4-0X0-4H-QUINAZ0LIN-3-YL) Acetamides and their use as Vasopressin V3 antagonists.

Zelena D *et al.* (2004). *Brain Research Bulletin* **63**, 521-530.

This work is supported by grants from the Wellcome Trust, UK.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C4

Central vasopressin mechanisms contribute to the mediation of the cardiovascular response to stress

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Emotional stress may contribute to the development of hypertension. However, the central mechanisms involved in the mediation of the cardiovascular response to emotional stress have not been fully investigated [1]. With the latest discovery of a new vasopressin V1b antagonist with potent anxiolytic activity [2], we were prompted to investigate the contribution of central vasopressin mechanisms in the cardiovascular response to emotional stress. Experiments were performed in conscious rats equipped with left lateral intracerebroventricular (icv) cannula (under 0.4 ml, 10% ketamine plus 0.1 ml, 2% xylazine, i.p. anesthesia) for drug injection and left femoral arterial catheter (under 4% concentration percentage for induction and 2% for maintenance of halothane anesthesia) for arterial pressure recording. Equidistant sampling allowed direct spectral analysis (fast Fourier transform) of SBP and HR in very low (VLF: 0-0.2Hz), low (LF: 0.2-0.8Hz) and high frequency (HF: 0.8-3Hz) domains. Rats were submitted to two models of stress: air-jet (by blowing air into the nose of a rat for 2 min, n=6) and immobilization (by covering rat with Plexiglas restrainer for 15 min, n=6). Air-jet induced a sharp rise in SBP (168±3mmHg, $p<0.01$) and HR (525±10bpm, $p<0.01$) and gradual recovery with appearance of sympathetically mediated LF BP variability. V1a (SR49059, 100ng, 500ng, icv) and V1b (SSR149415, 100ng, 500ng, icv) antagonists (n=6/each group) did not modify basal values but did reduce the increase of the area under the SBP curve (sum of SBP values, $3.6x10^5$±0.1mmHg, $p<0.05$ in nontreated rats,

$3.3x10^5$±0.2mmHg, p>0.05 in 100ng V1a PT rats, $3.4x10^5$±0.2mmHg, p>0.05 in 500ng V1a PT rats, $3.2x10^5$±0.2mmHg, p>0.05 in 100ng V1b PT rats and $3.3x10^5$±0.1mmHg, p>0.05 in 500ng V1b PT rats), the increase of HRmax (488±10bpm, p<0.05 and 469±14bpm, p<0.05, respectively) during exposure to air-jet and shortened the recovery period of SBP (361±22s in nontreated rats, 206±46s, p<0.001 in 100ng V1a PT rats, 113±15s, p<0.001 in 500ng V1a PT rats, 138±42s, p<0.001 in 100ng V1b PT rats, and 60±8s, p<0.001 in 500ng V1b PT rats) and HR (378±18s in nontreated rats, 216±43s, p<0.05 in 100ng V1a PT rats, 138±21s, p<0.001 in 500ng V1a PT rats, 175±57s, p<0.001 in 100ng V1b PT rats and 140±26s, p<0.001 in 500ng V1b PT rats) and prevented the appearance of LF-SBP. The V1b also reduced the SBPmax increase during exposure to stress (145±9mmHg, p<0.05 in 100ng V1b PT rats and 151±5mmHg, p<0.05 in 500ng V1b PT rats). Immobilization induced a rise of SBP (153±4mmHg, p<0.01), LF-SBP and respiratory-related HF-SBP variability; it did not affect HR but did enhance the vagally mediated HF-HR variability. Both V1a and V1b (n=6/each group) reduced the evoked increases in SBP and SBP variability during immobilization. The V1a PT rats submitted to immobilization now exhibited significant tachycardia (495±65bpm, p<0.05) and failed to increase HF-HR variability. The results suggest that both vasopressin V1a and V1b receptors are involved in the central mediation of the cardiovascular response of rats exposed to emotional stress.

McDougall SJ et al.(2005). Auton Neurosci 123, 1-11.

Serradeil-Le Gal C et al.(2005). CNS Drug Reviews 11, 53-68.

This work was supported by Wellcome Trust.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C5

Hippocampal neurogenesis in the prenatal stress rat is enhanced by agomelatine treatment. Functional implications for anxiety behaviour

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Prenatal stress (PS) in the rat is a well documented model of early stress that has high face and predictive validity as animal model of depression (Maccari et al. 2003; Morley-Fletcher et al. 2004). Indeed, PS rats present a life span reduction of hippocampal neurogenesis (Lemaire et al. 2000), increased anxiety and impairment of the feedback inhibition of the hypothalamus-pituitary adrenal axis. We here evaluated the effect of a chronic treatment (6 weeks, 40 mg/kg i.p. daily) with the new antidepressant agomelatine, a melatonin agonist with 5-HT2C antagonist properties, on hippocampal neurogenesis in PS male adult rats and, on PSA-NCAM expression, a marker of neuroplasticity. To investigate also the functional, behavioural impact of neurogenesis, we tested animals in the elevated-plus maze test to assess their anxiety-like response. To evidence neurogenesis and cell survival, the thymidine-analogue bromodeoxyuridine (BrdU, 75 mg/kg i.p. twice daily for 4 days) was injected after 3 weeks of the agomelatine treatment which was then continued for an additional 3 weeks. The results indicate a markedly reduced neurogenesis in the dentate gyrus of PS rats and an enhanced PSA-NCAM expression (ANOVA, group by treatment interaction, $F(1,21) = 10.53$, $P < 0.01$). The effects of PS were reversed by the chronic agomelatine treatment Agomelatine's effect on survival was selectively observed in the ventral part of the dentate gyrus (ANOVA region by group by treatment interaction, $F(1,26) = 4.73$, $P < 0.05$), a brain region specifically involved in anxiety (Kjesltrup et al. 2002). Moreover in PS animals agomelatine did not modify the ratio between neurons and glial cells assessed by NeuN and GFAP labelling. Behaviourally, PS rats treated with agomelatine spent more time on the open arms of the elevated plus maze, (ANOVA, group by treatment interaction, $F(1,27) = 7.06$, $P < 0.05$) suggesting a possible causal link between increased hippocampal neurogenesis and attenuated anxiety-like behaviour in a validated model of depression. The results obtained with agomelatine provide further evidence of neuroplasticity as one of the targets of antidepressants and further reinforce the high predictive validity of the PS rat as animal model of depression.

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Morley-Fletcher S, Darnaudery M, Mocaer E, Froger N, Lanfumey L, Laviola G et al. (2004). Neuropharmacology 47, 841-847.

This study was supported by the University of Lille I and IRIS.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C6

Differences in subregion-specific translocation patterns of mineralocorticoid and glucocorticoid receptors in rat hippocampus revealed by immunohistochemistry

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Activation of glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) by corticosteroids results in nuclear translocation of the receptor-ligand complex. There, the receptors can bind to DNA for transcriptional regulation. Though this cellular mechanism is well established in cell lines, very little is known about the subcellar behaviour of the receptors in the brain and the associated consequences for DNA binding and gene expression. The aim of this study was to examine the translocation patterns of MR and GR in the different subfields of the rat hippocampus in detail by using immunohistochemistry and confocal imaging. Based on studies that have described differences in receptor expression pattern in the hippocampus and affinities for corticosterone, we hypothesize (1) differences in translocation patterns for the different hippocampal subregions and (2) different translocation speed for MR and GR within one area.

Here, we present data on the translocation of MR and GR in hippocampal subregions CA1, CA2, CA3 and dentate gyrus after a single high corticosterone pulse. In order to prevent translocation induced by endogenously synthesized corticosterone, adrenalectomised male Sprague-Dawley rats (n=3) were terminally anaesthetised at 0, 30, 60, or 120 min after i.p. 3 mg/kg corticosterone complexed to HBC to increase solubility. Animals were anesthetized using 0.4 l/min isoflurane intra-nasally. Primary antibodies for MR (MR 1D5 1-18, Gomez-Sanchez et al. 2005) and GR (H300, Santa Cruz Biotechnology) were used for immunofluorescence and confocal microscopy to visualize and quantify the subcellular distribution of the receptors.
Results indicate (1) large subregion-specific differences in translocation patterns and (2) different behaviours for MR and GR within areas. Indeed, a strong increase in nuclear immunoreactivity for both GR and MR was observed in CA1 ($p<0.001$ vs. $p<0.01$) and CA2 ($p<0.01$ vs NS) after corticosterone administration. Interestingly, in the DG, a markedly increase was only observed for nuclear GR ($p<0.05$) while nuclear MR did not change after steroid treatment. The results of this study suggest a revision of the view that MR and GR uniformly translocate to the nucleus upon corticosterone administration. Even though the consequences of this differential translocation in the hippocampus on gene expression and function have to be investigated in more detail, the data suggest that for MR mediated effects ligand availability is more of a limiting factor in the CA1 area, while in other hippocampal subfields the MR signal may be modulated primarily by receptor number or posttranslational modifications. The next step is to study these region-specific translocation patterns of MR and GR in a relevant physiological context, like the ultradian release of corticosterone.
Gomez-Sanchez CE et al. (2006). Endocrinology 147, 1343-1348.

Supported by NWO grant 017.002.021.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C7

Opioids and suppressed hypothalamo-pituitary-adrenal (HPA) axis responses in late pregnant rats to central neuropeptides signalling metabolic state

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In pregnancy metabolic actions of stress-stimulated glucocorticoids may compromise energy supply to the fetuses. I.C.V. injection of orexin-A or neuropeptide Y (NPY), signalling energy lack, stimulates the HPA axis in virgin but not late pregnant rats [1,2]. Here we studied responses to I.C.V. ghrelin, which acts like orexin via NPY neurones, and to insulin-induced hypoglycaemia (IIH). We tested roles of endogenous opioid [3] in suppressing responses to NPY and orexin. Rats (n=5-7/group) were implanted (halothane anaesthesia) 5 days before experiment (pregnancy day 21) with a jugular vein cannula, and an I.C.V. cannula if required for 2 µl aCSF ± peptide injections. Rats were housed singly, in standard conditions (lights on 07.00h, off 19.00h), and cannulae connected *ca* 07.30h, 2h before experiment. Blood samples (replaced by 0.9% saline) were taken for ACTH and corticosterone assay pre- and post-treatment; 240 or 90 min later brains were collected after decapitation or formaldehyde fixation-perfusion (pentobarbitone anaesthesia, 42 mg/kg I.P.), for quantitative in situ hybridisation or Fos immunocytochemistry on coronal cryostat or frozen sections, respectively. Data were analysed by ANOVA. In virgins I.C.V. ghrelin (2 nmol) increased plasma ACTH and corticosterone concentrations by 38% and 65%, respectively (at 10-30 min, n=6, $p<0.05$), but had no significant effects in late pregnant rats; similarly, ghrelin increased parvocellular paraventricular nucleus (pPVN) Fos expression (3.9x, $p<0.05$) only in virgins. As expected, NPY (1 nmol I.C.V.) alone only activated the HPA axis in virgins (increased plasma ACTH, pPVN CRH and vasopressin (VP) mRNA [see 2] and Fos (3.5x) expression). Naloxone (5 mg/kg I.V.) had no effect in virgins, but after naloxone in pregnant rats, NPY increased plasma ACTH 2.7x at 15 min, pPVN CRH and VP mRNA expression 1.6x and 15.2x, and Fos counts by 2.3x (all $p<0.05$). I.C.V. orexin-A (1.4 nmol) increased pPVN Fos counts (2.1x) and plasma ACTH (1.9x at 15 min; both $p<0.05$) only in virgins [1]. Naloxone had no effects in virgins, but in pregnant rats it restored orexin responses: increased plasma ACTH (1.6x, $p<0.05$), and increases in CRH and VP mRNA expression in the pPVN (2.2x and 5.5x vs. orexin alone, respectively, $p<0.05$). Thus endogenous opioid suppresses actions of NPY, and hence of orexin, and maybe ghrelin (since it acts via NPY), on the HPA axis in late pregnancy. In contrast, IIH induced by I.V. insulin injection (10 U/kg) in virgin and pregnant rats similarly reduced blood glucose (to ca 2 mmol/l), increased plasma ACTH (by, mean±s.e.m., 46.1±17.8 and 35.6±7.8 pg/ml) and pPVN VP mRNA expression (by 3.3x and 2.3x; all $p<0.05$). Evidently activation of the HPA axis by IIH is intact in late pregnancy, and is does not appear to be mediated by NPY, orexin or ghrelin.
Brunton PJ *et al.* (2003). *J Neuroendocrinol* **15**, 633-637.

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Brunton PJ *et al.* (2005). *J Neurosci* **25**, 5117-5126.

Supported by BBSRC and MRC (J.B.).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C8

Maternal stress alters endocrine function of the feto-placental unit in rats

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Prenatal stress (PS) can cause early and long term developmental effects mainly through altered maternal glucocorticoid status.

PC2

Forced swimming-induced behavioural immobility involves chromatin remodelling in dentate granule neurons via recruitment of the NMDA/MAPK/ERK/MSK pathway

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Behavioural adaptation in response to stressful events may involve gene transcription associated epigenetic mechanisms in the brain. We have previously shown that forced swimming (FS)-induced phosphorylation and phospho-acetylation of histone H3 in adult dentate gyrus (DG) granule neurons is associated with the acquisition of the behavioural immobility response [1]. However, the intracellular molecular mechanisms underlying this learned behavioural response have hardly been investigated. Therefore, we investigated the signalling pathways that mediate the FS-induced increase in histone H3 phospho-acetylation and the acquisition of the immobility response. In particular, we examined the role of the NMDA receptor (NMDAR), the mitogen-activated protein kinase kinase (MEK) and mitogen- and stress-activated protein kinase (MSK).

Male Wistar rats were pre-treated with the NMDAR antagonist MK-801 (100μg/kg i.p.; n=6) or the MEK inhibitor SL327 (50mg/kg i.p.; n=6) before subjecting them to a FS session (15 min in 25°C water). We also subjected wild-type (n=6) and MSK1/2 double knock-out (KO; n=6) mice to FS. Respective control groups were not forced to swim. Animals were then killed after 2 h for immunohistochemical determination of histone H3 phospho-acetylation in dentate neurons (the numbers of positive neurons were counted and expressed as number/DG/10μm section/animal. In other experiments, animals were pre-treated as above, subjected to a FS session (15 min in 25°C water) followed by a second FS session (5 min in 25°C water) 24 h later to examine the behavioural immobility response. The immobility, swimming and struggling behaviour of the animal was scored in the test and the re-test.

Our results show that FS induces significant increases in histone H3 phospho-acetylation in the DG of rats (vehicle/control: 10.5±0.8 (n=6); vehicle/FS 20.5±0.5 (n=6)) and wild-type mice (control: 4.6±0.3 (n=6); FS: 8.7±0.8 (n=6)), $P<0.05$, post-hoc Bonferroni tests. The FS-induced histone H3 modifications could be blocked by pre-treatment with MK-801 (MK-801/control: 8.2±0.7 (n=6) ; MK-801/FS 12.3±1.0 (n=6)), and SL327 (SL327/control: 5.0±0.9 (n=6); SL327/FS: 9.4±0.8 (n=6)), as well as double genetic deletion of MSK1/2 (KO/control: 0.1±0.1 (n=6); KO/FS: 0.2±0.1 (n=6), $P<0.05$). Moreover, blockade of the forced swimming-induced epigenetic response resulted in an impaired acquisition of behavioural immobility as measured in the re-test (mean immobility scores (arbitrary units): control: 19.6±1.0; MK-801: 9.9±1.7; SL327: 15.7±0.6; wild-type: 24.7±1.4; KO: 14.5±1.0; all manipulations: $P<0.05$). Thus, acquisition of behavioural immobility after FS appears to involve phospho-acetylation of histone H3 in DG neurons via activation of the NMDA/MAPK/ERK/MSK pathway.

Bilang-Bleuel A et al. (2005). Eur J Neurosci 22(7), 1691-1700.

Supported by the Medical Research Council, UK.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC3

Mental Instability related to ECG

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TITLE ONLY

PC4

Expression of organic cation transporter 3, a corticosterone-sensitive monoamine transporter, in neural systems regulating the hypothalamo-pituitary-adrenocortical axis

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Organic cation transporters (OCTs) are multispecific, bidirectional, corticosterone-sensitive transporters, primarily studied in the periphery, substrates of which include serotonin (5-HT), noradrenaline (NA), and dopamine (DA). Our previous studies demonstrated OCT3 expression in the rat dorsomedial hypothalamus (Gasser et al. 2006), a region that accumulates 5-HT, NA and DA in response to acute stress or corticosterone administration (Lowry et al. 2001, 2003). These studies also demonstrated bidirectional, corticosterone-sensitive transport of OCT substrates by medial hypothalamic tissue in vitro (Gasser et al. 2006). We have hypothesized that inhibition of OCT-mediated monoamine transport represents a general mechanism by which corticosterone rapidly modulates physiological and behavioural responses. In order to begin to understand the importance of OCT3 in the regulation of monoaminergic neurotransmission in hypothalamic and extrahypothalamic brain regions, we used immunohistochemical methods to characterize the distribution of OCT3-like immunoreactive (OCT3-ir) cells in the rat brain.

pups (lactation day 4-6) were exposed for 30 min to a conspecific female intruder (weight matched; n=8) or left unchallenged (control; n=7; cage disturbance controlled for) and behavioural measurements were recorded. The rats were then left undisturbed for a further 60 min before being deeply anaesthetized (Pentobarbitone, Sagatal, 50mg/kg i.p.) and then transcardially perfused with 4% paraformaldehyde. Brains were removed and coronal, free-floating sections were cut at 52 μm and processed for Fos immunohistochemistry. Intruders elicited aggressive behaviour in all residents. In these aggressive rats, the numbers of Fos-immunoreactive cell nuclei per section (mean±SEM) were significantly higher in the lateral septal nucleus (228.3±10.2 vs. 163.6±19.2), bed nucleus of the stria terminalis (BnST; 70.6±6.5 vs. 40.7±9.8), supraoptic nucleus (36.1±8.9 vs. 9.3±2.2), parvocellular paraventricular nucleus (pPVN; 59.7±10.2 vs. 26.2±4.2) amygdala (medial amygdala, 83.7±10.4 vs. 46.3±9.8; central amygdala, 63.8±11.1 vs. 32.6±7.5; cortical amygdala, 40.9±4.2 vs. 27.7±3.3) and ventromedial hypothalamus (31.2±3.1 vs. 20.3±1.6), as compared to controls (Student's t test, $P<0.05$). Double immunocytochemistry revealed activation of corticotropin-releasing factor (CRF) immunoreactive neurones (double-labelled cells per section; mean±SEM; Student's t test, $P<0.05$) in the central amygdala (8.07±1.49 vs. 2.14±0.46) and BnST (12.86±2.82 vs. 4.64±1.27). There was no significant increase in CRF cell activation within the pPVN (7.61±2.33 vs. 3.11±0.60). In the supraoptic and paraventricular nuclei, both vasopressin and oxytocin neurones were double-immunostained for Fos (Student's t test; $P<0.05$). Thus, we have identified several populations of neurones, including those in the limbic system, which appear to be involved in maternal aggression. Moreover the present results provide evidence for the participation of the central CRF, oxytocin and vasopressin systems in the regulation of maternal aggressive behaviour in the rat.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC16

Hippocampal and hypothalamic nociceptin (NOP) receptor and pre-pro-nociceptin gene expression following acute stress in rats

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Nociceptin and its NOP receptor may play an important role in neuronal systems that process stress-related stimuli. Their importance in the regulation of basal activity of the hypothalamo-pituitary-adrenal (HPA) axis has recently been demonstrated (Leggett et al. 2006,). However the effect of stress on the endogenous nociceptin/NOP system is poorly understood, as is the significance of the NOP receptor in the context of acute stress. We investigated the effects of restraint stress or an inflammatory stimulus (lipopolysaccharide, LPS) on pre-pro-nociceptin (ppNOFQ) and NOP mRNA expression in the rat hypothalamus and hippocampus using RT-PCR. Conscious male Sprague-Dawley rats (200-225 g) were exposed to acute restraint (60 min or homecage control) or an intraperitonneal injection of LPS (250 μg in 0.5 ml/rat or sterile 0.9% saline vehicle). Rats were killed 2 and 4 h following the onset of restraint or 4 h following LPS injection. Total RNA was extracted from hypothalamus and hippocampus using standard protocols. In semi-quantitative RT-PCR assays glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA was an internal control. Data for ppNOFQ and NOP mRNA were normalised by reference to G3PDH mRNA, determined in the same sample (n=6-8 rats/group). A Mann-Whitney U test showed a significant decrease in hippocampal ppNOFQ mRNA in rats killed 2 h after restraint (79 ± 2.4% of homecage control level, $^*P<0.05$). Restraint also caused a significant decrease in expression of hippocampal NOP mRNA 4 h after stress onset (78 ± 5.2% of homecage control level, $^*P<0.05$). In the hypothalamus restraint had little effect on ppNOFQ mRNA level; however, NOP mRNA level was significantly reduced 2 h following stress onset (71 ± 5.3% of homecage control level, $^*P<0.05$). In a separate study, LPS treatment caused a pronounced increase in ppNOFQ mRNA in the hypothalamus 4 h after injection (150 ± 11.1% of saline control level, $^{**}p<0.01$)), but had no effect on hippocampal ppNOFQ mRNA. Unlike following restraint, NOP mRNA level was not significantly changed in either brain region 4 h after LPS injection. These results suggest that changes in ppNOFQ and NOP receptor gene plasticity may reflect adaptive responses to HPA axis activation. Furthermore, the apparent differential effect of psychological or inflammatory stressors warrants further investigation of the interaction between the limbic nociceptin system and the stress response.

Leggett JD, Harbuz MS, Jessop DS & Fulford AJ (2006). Neuroscience 141, 2051-2057.

We acknowledge the support of the BBSRC.

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PC17

Proteasome-dependent rapid downregulation of activated glucocorticoid receptor in the nucleus of glucocorticoid target cells in the rat hippocampus

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Glucocorticoids (GCs) are known to act via binding to and activating intracellular GC receptors (GR) expressed by target cells. The activated GR then translocates from the cytoplasm into the nucleus to regulate target gene transcription. GCs readily enter the brain and are known to regulate many aspects of neuronal function. Therefore intracellular regulation of GR activity may potentially play an important role in modulating the effects of GCs in this central target tissue. We report that after a single bolus I.V. injection of corticosterone (cort) in the rat, activated GR rapidly translocates from the cytoplasm into the nucleus of cells in the hippocampus (HC) and pre-frontal cortex (PFC). The appearance of GR within the nucleus is rapidly followed by a clearance of GR from the

nucleus. This appears to be a proteasome-dependent machanism because pretreatment with the specific irreversible 26S proteasome inhibitor MG132 abolishes the decrease in nuclear GR levels.

Male Sprague-Dawley rats (n = 3/timepoint/group) were anaesthetised with I.M. injection of Hypnorm (fentanyl citrate 0.252 mg/kg and fluanisone 8 mg/kg) after I.P. injection of Diazepam (4 mg/kg) then subjected to bilateral adrenalectomy, jugular cannulation, and I.C.V. cannulation into the lateral ventricle. After recovery for 5 days, rats were infused via the I.C.V. cannula with either 1 µl of 100 µM MG132 in 1% DMSO/saline or 1% DMSO/saline as vehicle alone, at 5pm. After 16 h, the rats were given a bolus I.V. injection of 100 µg cort. Animals were killed at times 0, 15 and 120 min. The HC was rapidly dissected and frozen. Nuclear extracts were prepared and analysed by Western blot with the GR M20 antibody (Santa Cruz) to determine nuclear GR levels throughout the timecourse in the presence and absence of the inhibitor.

I.V. injection of 100 µg cort resulted in a rapid increase in plasma GC levels at 1 min (883±157 ng/ml), with subsequent rapid decrease consistent with the 10 min half-life of cort in blood. Subsequent nuclear translocation of GR in the HC occurred maximally at 15 min after injection (13.1±3.5-fold). Interestingly, the retention time of GR in the nucleus was short-lived, with levels decreasing at 30 min and reaching near-baseline levels by 60 min. In contrast, in the rats treated with MG132 there was little decrease in levels of nuclear GR in the HC, with levels staying high at 120 min after I.V. bolus injection of cort. Densitometry analysis revealed levels of GR detected in the nucleus to be 9.0 ± 2.6-fold higher than baseline (time 0) in the presence of MG132, compared to 0.9 ± 0.1-fold in the absence of MG132 (significant difference in unpaired t-test, p=0.0199).

Our results suggest that the 26S proteasome is involved in rapidly degrading activated GR after it translocates into the nuclei of GR-expressing cells in the HC. The functional significance of proteasome-dependent rapid turnover of activated GR in the nucleus may be to allow dynamic and continuous responses to the highly fluctuating hormone levels observed in pulsatile GC secretion.

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PC18

Intra-amygdala injection of GABAA agonist, muscimol, reduces tachycardia and modifies cardiac sympatho-vagal balance during restraint stress in rats

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TITLE ONLY

PC19

High post-partum levels of corticosterone given to dams influence postnatal hippocampal cell proliferation and behaviour of offspring: A model of post-partum stress and possible depression

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PC20

The microtubule-associated protein DCL controls retrograde transport of the glucocorticoid receptor in neuronal progenitor cells

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PC21

Temporal dynamics of salivary cortisol

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