PC53

Environmental enrichment decreases the vulnerability to the effects of experimentally induced immune challenge in rats

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Environmental enrichment has been shown to induce positive effects on several physiological functions including modulation of neuroendocrine activity (1). Moreover, environmental enrichment is known to induce neural plasticity. Receptors for glutamate, the main excitatory amino acid in the brain, are thought to play an important role in these processes. Presented studies were aimed at verifying the hypothesis that environmental enrichment reduces vulnerability to adverse events such as immune challenge occurring during the prenatal period or in adulthood. The studies were conducted in male and female Wistar rats and the animals were humanely killed by rapid decapitation at the end of experiments. Immune challenge was performed by subchronic (5 days) administration of lipopolysacharide (LPS) to simulate acute infection. For the enriched environment, 10-12 animals were kept together in huge plexiglas boxes (1 m × 0.5 m × 0.5 m). Variety of items (platforms, wood swings, plastic tubes, beams etc.) were added to the cages and changed three times per week. Statistical evaluation of the data was performed using two-way analysis of variance. Male offspring (n=12 per group) of mothers injected with LPS in daily doses of 20, 20, 40, 40 and 80 μg/kg s.c. on days 15-19 of pregnancy were studied. Maternal exposure to LPS resulted in a reduced body weight gain of the offspring, which persisted until adulthood ($F=4.536$, $p<0.05$). The differences induced by a prenatal immune challenge were normalized by housing rats in an enriched environment for 8 weeks. In a separate study performed in adult males (n=5 per group) without any prenatal interference, repeated administration of LPS in increasing doses (10, 10, 20, 20, 40 μg/kg i.p.) was performed. LPS treatment induced a rise in corticosterone levels in the adrenals and plasma (1.98 ± 0.8 vs. 11.7 ± 1.6 μg/100 ml; $p<0.01$) as well as a transient decrease in body weight in controls housed under standard conditions, but not in rats kept in an enriched environment for 5 weeks (corticosterone: 1.0 ± 0.7 vs. 3.1 ± 2.1 μg/100 ml; N.S.). Enriched housing resulted in an increase in adrenal weights and enhanced gene expression of a glutamate receptor subunit in the hippocampus. Thus, vulnerability to some negative effects of repeated immune challenge may be modified by environmental conditions associated with changes in brain plasticity. The finding that differences in housing conditions change responses to external stressors has to be considered in animal physiology.

Moncek F, Duncko R, Johansson BB & Jezova D (2004). J Neuroendocr 16, 423-431.

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

PC55

Visualisation of hindbrain target neurones for membrane actions of corticosterone

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A specific, high-affinity neuronal membrane receptor for corticosterone (CORT), with binding properties distinct from intracellular glucocorticoid receptors, mediates rapid suppressive effects of stress and CORT secretion on courtship clasping behaviour in the roughskin newt (Taricha granulosa). Our previous studies showed that CORT acts through this membrane receptor to rapidly disrupt clasping by depressing medullary reticular neurone responsiveness to clasp-triggering sensory stimuli (e.g. pressure on the cloaca) and by interfering with the functioning of reticulospinal neurones. While medullary reticular neurones are affected by CORT action, the identity of specific neurones possessing functional CORT membrane receptors is unknown. To address this question, we prepared a conjugate of CORT and the fluorescent dye, Cascade Blue (CB). This conjugate was injected systemically in unanaesthetized newts or applied directly to the medullary surface of newts anaesthetized by immersion in 0.1% MS-222. Reticulospinal neurones were retrogradely labelled with tetramethylrhodamine dextranamine administered to a hemisected spinal cord at the first cervical vertebral level under MS-222 anaesthesia. Epifluorescent microscopic examination of medullary brain sections revealed CORT-CB internalisation as granular fluorescence in the soma and proximal dendrites of a large proportion of neurones. These neurones were widely distributed in the medulla and constituted a diversity of neuronal phenotypes. CORT-CB internalisation was most prominent 30 min after application, and was blocked by pretreatment with unlabelled CORT as well as the kappa-opiate receptor agonist, U-50488, but not by dexamethasone (DEX), results that match the known binding pattern of the CORT membrane receptor. Systemic administration or medullary CORT-CB application rapidly altered neuronal activity and sensory responsiveness similar to effects of unconjugated CORT. Conjugate administration also produced non-granular, nuclear labelling in some neurones, apparently due to intracellular glucocorticoid receptor binding because it was specifically blocked by pretreatment with DEX but not U-50488. This new approach for functional and structural identification of the neurones with CORT membrane receptors should greatly facilitate our understanding of the neural basis for the behavioural effects of the hormone.

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

PC56

Genomic and proteomic analysis of the plasticity within the rat hypothalamo-neurohypophyseal system

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The neuropeptide hormone arginine vasopressin (VP) is produced in the magnocellular neurons of the hypothalamic supraoptic (SON) and paraventricular (PVN) nuclei and stored in the posterior pituitary (PP). Dehydration evokes an up-regulation of the expression of the VP gene in magnocellular neurons and a massive release of the peptide from the PP in the circulation to promote water conservation at the level of the kidney. In parallel, a functional remodelling of the hypothalamo-neurohypophyseal system (HNS) is observed but not totally understood. To investigate this activity dependent plasticity of the HNS in terms of coordinated action of cellular and gene networks, we have used transcriptomic (microarray) and proteomic (2D fluorescence difference gel electrophoresis combined with MALDI mass spectrometry) approaches to identify mRNAs and proteins that change in abundance in the SON and the PP from rat (humanely killed at the end of the experiments) as a consequence of 3 days of dehydration. Real-time quantitative PCR and semi-quantitative Western-blotting were used to confirm the data. Using these approaches, we have identified the isoform ζ of the ubiquitous adapter protein family 14-3-3, known to modulate interactions/functions of components involved in cell signalling and cell cycle control, as being up-regulated in the SON by 1.88 (± 0.27, n=4) -fold at the mRNA level and by 3.38 (± 0.93, n=3) -fold at the protein level. At the same time, levels of RNAs encoding members of the NR4A family of orphan nuclear receptor transcription factors, known as products of immediate early genes induced through multiple signal transduction pathways, appear to be coordinately reduced in PP by 4.16-fold for NR4A1 and 3.76-fold for NR4A2.

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

PC57

Effect of acute stress in the day of proestrus on sexual behaviour and ovulation in female rats: participation of angiotensinergic system

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Physical or emotional stress can affect reproductive function. Angiotensin II (AII) is a hormone that participates in the stress response and also in the control of reproductive hormones. The study aimed to evaluate the effects of acute stress in the morning and afternoon of proestrus on the sexual behaviour and ovulation in female rats and also the participation of AII on the stress-induced effects.

Adult female Wistar rats (n=193) had the oestrous cycle monitored daily. Rats with three regular cycles were used. We tested different paradigms of stress in the morning (10h) and in the afternoon (16h) of proestrus: control (no intervention) (n=17/n=17); restraint stress 10min (n=13/n=17); restraint stress 1h (n=15/n=15) and ether stress (n=14/n=13), respectively. We analysed sexual behaviour and ovulation. We also tested the participation of AII on the effects of stress (restraint 1h) in the morning of proestrus by injecting AII receptor antagonists: peripheral (i.p.) injection (control, n=10; saline + stress, n=8; losartan + stress, n=7; PD + stress, n=7; losartan/PD + stress, n=8) and central (i.c.v.) injection (control, n=8; stress, n=8; saline + stress, n=8; losartan + stress, n=8). We analysed ovulation. For central injection an i.c.v. guide cannulae was stereotactically implanted under ketamine/xylasine anaesthesia (100/50 mg/kg, i.m.). Peripheral (saline, 0.9%, losartan (AT1 antagonist), 10mg/kg and PD123319 (AT2 antagonist), 3mg/kg) and central (saline, 0.9% and losartan, 5μg/2μl) injections were performed 15min before stress. Number of lordosis/mounts (lordosis quotient) was recorded for 15min. The number of oocytes was counted in the morning of oestrous. All animals were humanely killed at the end of the experiments. All data (mean±S.E.M.) were compared by one-way ANOVA followed by Newman-Keuls test.

Stress in the morning of proestrus induced a reduction in the number of oocytes in all stress groups when compared with control group: control (11.7±0.6), 10min restraint (7.5±0.7), 1h restraint (7.7±0.5) and ether (8.7±0.8) ($p<0.05$). No difference was found in the lordosis quotient. Stress in the afternoon of proestrus induced a reduction in the lordosis quotient when 1h restraint (0.81±0.05) was compared with control (0.96±0.01) ($p<0.05$). We found no difference in the number of oocytes in this experiment. Peripheral losartan (10.5±0.7) and losartan + PD (11±0.3) partially reverted the stress-induced effects on ovulation when compared with control (12.6±0.5) and saline (6.4±0.6) ($p<0.05$). PD (6.6±0.5) injected alone had no effect. Central losartan (8.5±0.8) also partially reverted the stress-induced effects on ovulation when compared with control (11.6±0.5) and saline (6.0±0.8) ($p<0.05$). The results indicate that acute stress in the morning of proestrus alters mechanisms that control ovulation, as shown by the reduction in the number of oocytes, while stress in the afternoon alters mechanisms that control sexual behaviour. The stress-induced reduction in the ovulation is mediated, at least in part, by the angiotensinergic system through AT1 receptors.

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PC58

5-HT modulates mouse hypothalamic arcuate RipCre neuronal excitability in a 5-HT2 receptor-independent manner

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Hypothalamic arcuate neurones that express the gene for pro-opiomelanocortin (POMC) form a distinct neuronal popula-

tion that controls energy homeostasis. Many neuromodulators affect the excitability of these neurones and specifically, serotonin (5-HT) may modulate these neurones via activation of 5-HT_{2C} receptors (Heisler et al. 2002). Recently we have identified a novel arcuate neuronal population in mice, which regulate energy homeostasis, by the expression of green fluorescent protein (GFP) induced by the rat insulin 2 promoter (RipCre) transgene (Choudhury et al. 2005). To determine if 5-HT could modulate these neurones, whole cell current-clamp recordings were made from hypothalamic arcuate neurones (GFP positive) identified by epifluorescence and differential interference contrast microscopy, as previously described (Choudhury et al. 2005). Mice (8-16 weeks) were humanely killed. All data are expressed as means ± S.E.M.

RipCre neurons had a mean resting potential of -50 ± 1 mV (n = 32) and spontaneously fired sodium-mediated action potentials. Application of 5-HT to the perfusion solution (1 μM) or ejection (2 μM) directly above the recording neurone had a mixed affect on neuronal excitability. In the majority of recordings (44 %) 5-HT hyperpolarized RipCre neurones from -52 ± 2 mV to -69 ± 2 mV (n = 14/32). Alternatively 5-HT either failed to induce a response (34 %), or depolarized neurones from -48 ± 2 mV to -43 ± 2 mV (n = 7/32). It has been suggested that 5-HT_{2C} receptors play an important role in hypothalamic control of energy homeostasis. However, the non-specific 5-HT_2 receptor agonist, α-methyl-5-HT (α -me-5-HT; 1 μM) failed to induce any changes in neuronal excitability (n = 5). At higher concentrations (5 - 10 μM), α-Me-5-HT mimicked the effects of 5-HT (n = 9); yet these, and 5-HT, responses could not be inhibited by the 5-HT_2 antagonist ketanserin (10 - 100 nM; n = 4), or by selective 5-HT_{2B} (SB204741, 10 nM; n = 2) or 5-HT_{2C} (SB242084, 100nM; n = 2) receptor antagonists. These data strongly suggest that the effects of high concentrations of α-me-5-HT are probably non-specific. In contrast, 5-carboxamidotryptamine (5-CT, 1 μM) hyperpolarized RipCre neurones from -49 ± 1 to -68 ± 8 mV (n = 3/4). These data suggest that 5-HT 1, 5, 6 or 7 receptors may modulate the 5-HT response in RipCre neurons. However, the 5-HT_{1A} receptor agonist, 8-hydroxy-di-n-propylamino tetralin (8-OH DPAT, 1 μM), failed to alter excitability (n = 4).

Choudhury et al. (2005). J Clin Invest 115 (in press)

Heisler et al. (2002). Science 297, 609-611.

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

that express pro-opiomelanocortin (POMC). α-MSH binds to melanocortin (MC) 3 and 4 receptors and reduces body weight and food intake. Conversely, a different arcuate neuronal population make and release agouti-related peptide (AGRP), an inverse agonist to MC 3/4 receptors. Recently we identified a novel arcuate neuronal population that regulates energy homeostasis, by the expression of green fluorescent protein (GFP) induced by the rat insulin 2 promoter (RipCre) transgene (Choudhury et al. 2005). Here we demonstrate that RipCre neurones lie within the hypothalamic melanocortin neuronal network.

Hypothalamic arcuate neurones were identified in acute brain slices by epiflorescence and differential interference contrast microscopy. Mice (8-16 weeks) were humanely killed. Whole cell current-clamp recordings were made from GFP positive neurones driven by POMC or RipCre promoters, as previously described (Choudhury et al., 2005). All data are expressed as means ± S.E.M and statistical significance determined by paired t-test.. RipCre and POMC neurones spontaneously fired sodium-mediated action potentials at resting membrane potentials (-50 ± 1 mV, n = 62 & -50 ± 1 mV, n = 50 respectively). A mixed MC3/4 receptor agonist (MTII, 100 nM) depolarized RipCre (-53 ± 3 to -48 ± 2 mV, $P < 0.01$; n = 9) and POMC (-53 ± 3 to 49 ± 3 mV, $P < 0.01$; n = 6) neurones and this was associated with an increase in action potential firing. In contrast, AGRP (10 nM) reduced spike firing frequency in RipCre and POMC neurones by cellular hyperpolarization (RipCre: -47 ± 4 to -63 ± 5 mV, $P < 0.04$, n = 4; POMC: -46 ± 2 to -48 ± 1 mV, $P < 0.01$, n = 7). The selective MC3 (γ-Trp-MSH, 10 nM) and MC4 (Cyclo (β-Ala-His-D-Phe-Arg-Trp-Glu)-NH2; 10 nM) receptor agonists depolarized POMC (MC3: -44 ± 2 to -40 ± 3 mV, $P < 0.03$, n = 4; MC4: -49 ± 1 to -47 ± 1 mV, $P < 0.04$, n = 4) and RipCre (MC3: -54 ± 5 to -45 ± 5 mV, $P < 0.04$, n = 5; MC4: -54 ± 3 to -51 ± 3 mV, $P < 0.07$, n = 6) neurones. Note that RipCre neuron depolarization by the MC4 receptor agonist was not statistically significant at the 95% level, although firing frequency increased from 3.2 ± 0.7 to 4.0 ± 0.5 Hz ($P < 0.04$). These data suggest that although expression of MC4 receptors is low in the arcuate nucleus (Liu et al., 2003), both MC 3 and 4 receptor subtypes are functional in these neurones.

Choudhury et al. (2005) J. Clin. Invest.115 (in press)

Liu et al. (2003) J. Neurosci. 23:7143-7154.

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

PC59

Electrophysiological evidence for functional melanocortin 3 and 4 receptors in mouse hypothalamic arcuate neurones

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Alpha-melanocyte stimulating hormone (α-MSH) is made and released from a population of hypothalamic arcuate neurones

PC60

Urocortin 2 increases c-Fos expression in serotonergic neurons in the dorsal raphe nucleus

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Corticotropin-releasing factor (CRF) is a 41-amino acid neuropeptide that is known to play a critical role in the regulation of stress-related behaviour and the hypothalamic-pituitary-adrenal (HPA) axis. The CRF family of neuropeptides also includes urocortin 1 (Ucn 1), urocortin 2 (Ucn 2), and urocortin 3 (Ucn 3).

The role of these other CRF-related neuropeptides in the regulation of stress-related behaviour and the HPA axis activity is unclear. Ucn 2, acting on CRF2 receptors, may influence anxiety states through effects on brainstem neuromodulatory systems such as the serotonergic system. CRF2 receptor mRNA expression is abundant in the dorsal raphe nucleus (DR) suggesting that this may be an important site of action for Ucn 2 or other CRF2 receptor ligands (Day et al. 2004).

To investigate the effects of CRF2 receptor activation on topographically organized subpopulations of serotonergic neurons in the DR and associated behavioural responses adult male Wistar rats were anaesthetized with 0.15 ml Hypnorm, (0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone; i.m.) and 0.15 ml diazepam (10 mg/2 ml i.p.) and fitted with a single I.C.V. guide cannula. One week following surgery rats received I.C.V. injections of vehicle, 0.01, 0.1, or 1.0 ug mouse Ucn 2 (mUcn 2). Home cage behaviours were recorded for 30 min prior to and 2 hours following drug treatment. Two hours following drug treatment, rats were anaesthetized with 0.5 ml Euthatal (200 mg/ml sodium pentobarbital), transcardially perfused with fixative, and brain tissues were processed for immunohistochemistry.

mUcn 2 had no effect on most behavioural endpoints studied, however, mUcn 2 consistently increased c-Fos expression in subpopulations of serotonergic neurons identified by either tryptophan hydroxylase or serotonin immunostaining within topographically organized subdivisions of the DR, articularly the dorsal region of the middle and caudal DR (−7.64, −8.18, −8.54 and −9.16 mm bregma), regions that provide the majority of serotonergic innervation of stress-related or anxiety-related brain regions including the central nucleus of the amygdala (Abrams et al. 2004). With the exception of actions on drinking-related behaviour, the effects of mUcn 2 on c-Fos expression in serotonergic neurons within the DR were not associated with acute behavioural responses. mUcn 2 actions on serotonergic systems described here may contribute to delayed behavioural effects of Ucn 2 described previously, including orexigenic, locomotor, and anxiety-related effects in a variety of behavioural tests as well as potentiation of conditioned fear and induction of escape deficits in a model of learned helplessness.

Abrams JK, Johnson PL, Hollis JH & Lowry CA (2004). Ann NY Acad Sci 1018, 46-57.

Day HE, Greenwood BN, Hammack SE, Watkins LR, Fleshner M, Maier SF & Campeau S (2004). J Comp Neurol 474, 364-378.

Hammack SE, Schmid MJ, LoPresti ML, Der-Avakian A, Pellymounter MA, Foster AC, Watkins LR & Maier SF (2003). J Neurosci 23, 1019-1025.

Jacobs BL & Azmitia EC (1992). Physiol Rev 72, 165-229.

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

PC61

The transcription factor JunD is up-regulated in the endocrine hypothalamus at both the mRNA and protein level in water-deprived rats

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In an attempt to understand the molecular basis of hypothalamic regulation of blood volume homeostasis we are interested in the regulation of transcription factors. JunD is a member of the family of activator protein-1 (AP-1) transcription factors. While past studies have demonstrated a change in the expression of transcription factors such as c-Fos in the paraventricular (PVN) and supraoptic nucleus (SON) (Morien *et al.* 1999), no study to date has examine changes in the expression of JunD in response to water deprivation, despite its reported presence within the rat hypothalamus (Herdegen *et al.* 1995).

For immunohistochemistry, male Sprague-Dawley rats (200-300g, n=12) were randomly assigned to two groups, a control and a 72-hour water-deprived group. Following water deprivation, rats were deeply anaesthetised with pentobarbitone sodium (100mg/kg i.p.), transcardially perfused with phosphate buffered saline and 4% paraformaldehyde and the brains processed for immunohistochemical detection of JunD. For mRNA quantification (n=4), the SON was excised from fresh hypothalamic slices and the total RNA was extracted and quantified using real-time PCR.

Control, euhydrated rats exhibited very little expression of JunD-like immunoreactivity, where only very few cells within the SON (34±11) and PVN (22±7) showed any staining. In contrast, there was a significant (P<0.05, unpaired t test) increase in the number of JunD-like immunoreactive cells (SON, 128±13; PVN, 116±24) in the water-deprived rats. Moreover, quantitative RT-PCR revealed a significant increase (fold change 1.58±0.27) of JunD mRNA expression in the SON after 3 days of dehydration (P<0.05).

This study provides the first demonstration of the up-regulation of JunD mRNA within the SON and changes at the protein level in both the SON and PVN following water deprivation. These results suggest a role for JunD in the regulation of magnocellular neurones for body fluid homeostasis.

Herdegen T *et al.* (1995). *J Comp Neurol* 354, 39-56.

Morien A *et al.* (1999). *Brain Res* 816, 1-7.

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

PC63

Stress responses of the HPA axis: relevance of time of day and basal pulsatility in male rats

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Corticosterone (CORT) secretion in the rat is characterised by discrete pulses that vary in amplitude and frequency to produce a diurnal variation in circulating levels (Seale et al, 2004). Furthermore the phase of a pulse can influence the response of the hypothalamic-pituitary-adrenal (HPA) axis to a mild stress such as noise (Windle et al, 1998). This study examines the CORT response of male rats to noise stress at a time when there are no endogenous CORT pulses (early light phase) and when there are hourly CORT pulses (early dark phase).

Male rats (14L:10D) were anesthetised and the jugular vein cannulated. Four days later cannulae were connected to an automated blood-sampling system (Windle et al, 1998; Seale et al,

2004). Blood samples were collected at 5 or 10 min intervals commencing at lights on or at lights off. Activation of a white noise generator in the room exposed all animals to 100 dB for 10 min. Animals remained in their home cages from the day of surgery and throughout blood-sampling. CORT levels were measured by radioimmunoassay. Animals were humanely killed at the end of the experiment.

During the early light phase (morning), as expected, there was no evidence of endogenous CORT pulsatility. In response to the 10 min noise stress, CORT levels rose rapidly reaching peak values 5-10 min after the noise had ceased, thereafter CORT levels decreased logarithmically at a rate similar to that of the half-life of corticosterone (10 min). Application of a second noise 80 min later, resulted in a similar shape CORT response; however, the total response determined by AUC was significantly lower (82 ± 4%; mean ± sem; n=15; P<0.001; paired t test) than for the first noise exposure.

During the early dark phase (evening), CORT pulses were observed prior to noise exposure. There was a clear response to the stress with CORT levels reaching a peak 5-10 min after the noise had ceased. In the evening, however, the decline in CORT levels only lasted 25-30 minutes before the resumption of pulsatility. Application of second noise, 80 min later, resulted in a similar CORT profile to the first noise exposure. Comparison of the AUCs revealed no evidence of adaptation (paired t test).

Exposure to noise elicits a reproducible corticosterone profile. Repeating the stress results in adaptation in the morning but not in the evening. The brief secretory response to the stressor is followed by a falling phase that is similar to the hormones half-life, indicative of a lack of secretion during this time. In the evening these periods of HPA inactivity only last 25-30 min whereas in the morning the period of HPA inactivity is prolonged.

Seale et al. (2004). J Neuroendocrinol 16, 516.

Windle et al. (1998). Endocrinology 139, 443.

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

PC64

Hypothalamic-pituitary-adrenocortical axis changes evoked by long-term voluntary exercise in rats

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Studies in rats and mice have shown that voluntary exercise has positive effects on various physiological, metabolic and neurobiological processes. There is evidence that voluntary exercise results in an improved coping with stressful challenges of which the underlying neurobiological mechanisms are, however, still unclear. We studied changes in physical parameters and the hypothalamic-pituitary-adrenocortical (HPA) axis in male Sprague Dawley rats (n=12 per group) after giving them access to a running wheel in their home cage for 4 weeks. The rats used the wheel immediately and ran approximately 6 km per night. All animals were humanely killed at the end of the experiment. In general, the exercising rats showed less weight gain (ANOVA with repeated measures: interaction of time and wheel: $F(1,22)=8.5$, $p<0.05$; overall effect of wheel: $F(1,22)=12.5$, $p<0.05$) and substantially less abdominal fat tissue (2.0 ± 0.1 g) than control rats (2.8 ± 0.1 g; $p<0.05$, Student's t test). The thymus weight was reduced in the exercising rats (351 ± 21 mg vs. 474 ± 20 mg; $p<0.05$, Student's t test) and the adrenal weight was unchanged (50.2 ± 1.4 mg vs. 50.2 ± 1.4 mg; n.s., Student's t test). The early morning baseline and forced swimming-induced levels of plasma corticosterone were increased in the exercising rats as compared to the controls (baseline: 29 ± 6.5 ng/ml vs. 13.63 ± 1.8 ng/ml; $p<0.05$, Student's t test; forced swim: 631.4 ± 89.4 ng/ml vs. 314.3 ± 51.7 ng/ml; $p<0.05$, Student's t test) whereas ACTH levels were similar (baseline: 76.73 ± 6.79 pg/ml vs. 81.8 ± 6.4 pg/ml; n.s., Student's t test; forced swim: 600.4 ± 49.1 pg/ml vs. 577.2 ± 56.1 pg/ml; $p<0.05$, Student's t test) measured by radioimmunoassay. Tyrosine hydroxylase (TH) mRNA in the adrenal medulla was measured as an index for the sympathoadrenomedullary activity in these rats. Exercising rats showed higher TH mRNA levels in the right adrenal gland compared to control rats (7715 ± 701 vs. 5581 ± 551 area (square pixel/1000)); two-way ANOVA followed by post-hoc test with contrasts. Furthermore, corticotropin-releasing hormone and arginine-vasopressin mRNA levels were determined in the paraventricular nucleus of snap-frozen brain tissue sections. So far our results indicate that long-term voluntary exercise evokes distinct changes in HPA axis regulation and body composition in rats.

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

PC65

The effects of stress on extracellular levels of GABA in the hippocampus: an *in vivo* microdialysis study in the rat

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Gamma-aminobutyric acid (GABA) has been implicated in the central mechanisms controlling emotion and in the response to stress. An important brain structure involved in behavioural and neuroendocrine responses to stress is the hippocampus. There is, however, little known about the actual extracellular GABA concentrations in this brain structure under stressful conditions. The aim of the present study is therefore to characterise the effects of different types of stress on GABA levels in the hippocampus of the rat.

In vivo microdialysis was performed to monitor extracellular GABA levels under baseline and stress conditions. Rats were equipped with a guide cannula (surgery under ketamine/xylazine anaesthesia, 100 and 5 mg/kg, respectively, i.p.) and after a 7 day recovery period, a microdialysis probe was inserted through the guide cannula into the hippocampus (under halothane anaesthesia). The microdialysis experiments started on the second day after implantation of the microdialysis probe. During the baseline period, six 10 min samples were collected, after which the animals were exposed to a stressful challenge. At the start of the stress paradigms the sampling time was 5 min for a 30 min period followed by the collection of 10 min samples for another 2.5 h. Rats were

exposed to either novelty or forced swim stress (5-7 animals per group). For the novelty exposure rats were placed in a clean cage (similar to their home cage) for 30 min. For the forced swim stress rats were placed in a large beaker glass containing water at a temperature of 25°C for a period of 15 min. The behaviours displayed by the animals were scored throughout the microdialysis experiments, including the novelty and swim stress period. We have established a sensitive HPLC/electrochemical detection method (adapted from Smith & Sharp, 1994) for the analysis of GABA concentrations in dialysates of short sample duration. All animals were humanely killed at the end of the experiment.

Exposure to a novel environment caused a moderate increase in hippocampal GABA to 120% basal levels that lasted throughout the exposure period and returned gradually back to baseline after return of the rat to the home cage. Forced swim stress resulted in a profound decrease of GABA levels with a minimum of 70% of baseline reached 15 min after the rats had been taken out of the water. Our preliminary data show that novelty and forced swimming, two different types of stressors that have primarily psychological (emotional) and combined psychological and physiological components, respectively, cause differential changes in hippocampal GABA levels. We conclude that in vivo microdialysis using short sampling times is a powerful method that can contribute to our understanding of the role of GABAergic neurotransmission under stressful challenges.

Smith & Sharp (1994). J Chromatogr B 652, 228-233.

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

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Anxiogenic drug treatment increases serotonin metabolism in specific anxiety-related forebrain circuits

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FG-7142, a benzodiazepine receptor inverse partial agonist, elicits anxiety-like behaviour in rats (Dorow et al. 1983). FG-7142 and other anxiogenic drugs have been shown to induce c-Fos expression in a distributed anxiety-related neural system, including a select subpopulation of midbrain neurons in the dorsal raphe nucleus (Singewald & Sharp, 2000; Singewald et al. 2003). The present study was designed to test the hypothesis that serotonin metabolism is altered in forebrain regions identified as targets of anxiogenic drugs based on these previous studies.

Wistar rats were given one of four treatments (vehicle, 1.675, 3.75, or 7.5 mg/kg FG-7142; intraperitoneal injection, n=5). One hour following treatment, rats were humanely killed and brains were dissected and frozen on dry ice. Brains were sectioned at 300 μm and 16 forebrain regions were microdissected and analysed for tryptophan, serotonin and 5-hydroxy-3-indoleacetic acid (5-HIAA) concentrations using high pressure liquid chromatography with electrochemical detection. Multifactorial repeated measures ANOVA revealed a main effect of dose on tryptophan ($p = 0.009$), serotonin ($p = 0.048$) and 5-HIAA ($p = 0.045$) concentrations and region on tryptophan ($p < 0.001$), serotonin ($p < 0.001$) and 5-HIAA ($p < 0.001$) concentrations. Post-hoc pair-wise comparisons of means revealed the most robust effects in the prelimbic cortex with the highest dose of FG-7142 resulting in elevated 5-HIAA ($p = 0.028$; 122.9% baseline), serotonin ($p = 0.021$; 134.6% baseline), and tryptophan ($p = 0.009$; 127.0% baseline). In addition, FG-7142 (7.5 mg/kg) increased tryptophan concentrations in the primary motor cortex ($p = 0.015$; 138.2%), medial amygdaloid nucleus ($p = 0.016$; 131.3%), and the dorsomedial hypothalamus ($p = 0.039$; 138.4%). An intermediate dose of FG-7142 (3.8 mg/kg) elevated serotonin concentrations in the infralimbic cortex ($p = 0.028$; 140.3%) and dorsomedial hypothalamus ($p = 0.049$; 139.6%). These data demonstrate that FG-7142 alters serotonin metabolism in select forebrain regions previously identified as sites for convergent actions of multiple anxiogenic drugs. In addition, these data highlight the effects of FG-7142 on tissue concentrations of tryptophan and serotonin metabolism in the prelimbic region of the prefrontal cortex. In the context of anxiety, the medial prefrontal cortex has been shown to play a critical role in mediating the differential effects of controllable and uncontrollable stress on stress-related behavior and brainstem serotonergic systems implicated in the potentiation of conditioned fear (Amat et al. 2005).

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