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Behavioural and hypothalamic molecular effects of the anti-cancer agent cisplatin in the rat: A model of chemotherapy-related malaise?

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Abstract

Many cancer patients receiving chemotherapy experience fatigue, disturbed circadian rhythms, anorexia and a variety of dyspeptic symptoms including nausea. There is no animal model for this 'chemotherapy-related malaise' so we investigated the behavioural and molecular effects of a potent chemotherapeutic agent, cisplatin (CP, 6 mg/kg, i.p.) in rats. Dark-phase horizontal locomotor activity declined post-CP reaching a nadir on day 3 ($P<0.001$), before recovering after 7 days. CP's effect was most marked in the late part (05.00–07.00) of the dark-phase. Food intake reached a nadir ($P>0.001$) at 2 days, coincident with an increase in gastric contents (cisplatin 9.04±0.8 vs. saline 2.32±0.3 g; $P<0.001$). No changes occurred in hypothalamic mRNA expression for AGRP, NPY, HCRT, CRH, IL-1, IL-6, TNFα, ABCG1, SLC6A4, PPIA and HPRT mRNA but tryptophan hydroxylase (TPH) mRNA was decreased (47%, $P<0.05$) at day 21 post-CP. This shows that despite marked behavioural effects of cisplatin, only a discrete change (TPH) was found in hypothalamic mRNA expression and that occurred when the animals' behaviour had recovered. Findings are discussed in relation to the neuropharmacology of chemotherapy-induced malaise.

Keywords: Illness behaviour; Malaise; Cisplatin; CRF; Agouti-related peptide; Neuropeptide Y; Orexin; Tumour necrosis factor-α; IL-1; IL-6; Tryptophan hydroxylase; Serotonin transporter; Tryptophan transporter; Emesis; Nausea; Food intake; Gastric stasis

1. Introduction

Anti-cancer chemotherapy in either curative or palliative settings is associated with a number of undesirable side-effects which are associated with increased morbidity and reduced quality of life (Tamburini et al., 2000). These effects can include acute and delayed nausea and vomiting (Rudd and Andrews, 2005; Veyrat-Follet et al., 1997; Hesketh et al., 2003), reduced food intake (Hainsworth and Hesketh, 1992), decreased body weight, disrupted gastrointestinal function including dyspepsia and diarrhoea (Kris et al., 1988; Nelson et al., 2002), and fatigue (Stone et al., 1998; Ahlberg et al., 2003).

The fatigue associated with cancer and its treatment has both subjective (self-reported) and objective (reduced physical activity or capacity to undertake physical or mental tasks) dimensions and differs critically from fatigue associated with physical exercise or sleep-deprivation in that the symptoms are not alleviated by rest (Stone et al., 1998). Studies in patients undergoing chemotherapy using Actigraphy to measure physical activity have revealed that not only is physical activity reduced but also that the circadian rhythm is disrupted (Berger and Farr, 1999) and this is related to subjective measures of fatigue, depression and mood (Roscoe et al., 2002). Fatigue is now ranked alongside or above nausea and vomiting as a concern of cancer patients but currently there are no pharmacological therapies targeted at the fatigue as opposed to co-morbidities such as anaemia and depression (Stone et al., 2000; Ahlberg et al., 2003). A recent clinical trial showed that paroxetine treated the depression in cancer patients undergoing chemotherapy for the first time but was without effect on their fatigue (Morrow et al., 2003).

It has been hypothesised that many features of the body's adaptive response to cytotoxic anti-cancer drugs (see above) mimic the responses to orally ingested toxins ("food poisoning") and infection (Andrews and Morrow, 2002). Thus, the sequelae to cytotoxic drug administration share a number of

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features in common with the well characterized "illness behaviour" (including malaise, lassitude, fatigue, reduced appetite, nausea and vomiting) following infection (Konsman et al., 2002). However data are lacking on the changes in locomotor activity following cytotoxic drug administration and hence the validity of the comparison with "illness behaviour" is difficult to assess.

Studies of the effects of cytotoxic anti-cancer agents such as cisplatin usually only investigate a single parameter in isolation (e.g. gastric contents) and have not examined the inter-relationships between a number of parameters in an attempt to provide a more integrated model of the impact of a chemotherapeutic agent upon the body. As part of continuing investigations into the mechanisms underlying the emetic and gastrointestinal effects of anti-cancer chemotherapy we investigated the relationships between spontaneous activity, food and water intake and gastric contents following the administration of a single dose of cisplatin. In addition, the possible molecular correlates of changes in the above parameters were investigated by using real-time RT-PCR measurement of mRNA expression in the hypothalamus at several time points (2, 7 and 21 days) after cisplatin administration. We chose to focus on the hypothalamus because it has been implicated in the genesis of illness behaviour (Konsman et al., 2002), fatigue (see Andrews et al., 2004), gastrointestinal function (Lissander, 1975), nausea (see Rudd and Andrews, 2005 for references) and vomiting (Beleslin et al., 1987).This was investigated by measuring changes in the expression of a number of substances implicated in the regulation of food intake: HCRT (orexin, Sakurai et al., 1998), agouti-related peptide (Wirth and Giraudo, 2000), neuropeptide Y (Billington and Levine, 1992) and illness behaviour: corticotrophin releasing hormone (Swain and Maric, 1995; Burak et al., 2002), IL-1, IL-6 and tumour necrosis factor-α (Konsman et al., 2002). Changes in systems which modulate the turnover of 5-hydroxytryptamine (tryptophan hydroxylase, serotonin and tryptophan transporters) were also measured given the proposed involvement of brain 5-hydroxytryptamine levels in a range of behaviours including food intake (Meguid et al., 2000), circadian rhythm (Sun et al., 2002), fatigue (Blomstrand et al., 1989; Swain and Maric, 1997; Sharpe et al., 1997; Andrews et al., 2004) as well as nausea and vomiting (Minami et al., 2003).

2. Methods

2.1. Animals

Male Wistar rats (330–350 g) were obtained from A. Tuck and Son Ltd (Essex, UK) and housed in a temperature and humidity controlled room with 12 h light:12 h dark cycle (lights on at 07.00 am). The rats were caged individually in clear plastic cages with food (Bantin and Kingman, Hull, UK) and water provided ab libitum. Body weight, food and water were monitored daily and the overall health of the animals assessed twice daily for the entire experimental period. All experiments were performed under the UK Animals (Scientific Procedures) Act 1986.

2.2. Drug administration

Following a 3 day habituation period in the cages, the rats were given an intraperitoneal injection (i.p.) of either saline (dose volume: 0.4 ml/100 g) or cisplatin (6 mg/kg). Previous studies have used higher doses of cisplatin (e.g. 9 mg/kg, Wang and Aggarwal, 1997) but have only studied the animals for shorter times. In addition, higher doses are not acceptable as in pilot studies we found that the effects reached the pre-set humane end points for termination of an experiment. The dose of cisplatin used in the current experiments is comparable to that in the recent study by Rudd et al. (2002). The cisplatin injection was prepared at a concentration of 1.5 mg/ml saline by dissolving 7.5 mg cis-platinum (II) diammine dichloride (Sigma, Poole, UK) in 5 ml sterile saline with 90 s of sonication.

2.3. Measurement of activity

A photobeam activity system (AM1053 Amlogger; Linton Instruments, Norfolk) was used to monitor activity for up to 21 days after injection of cisplatin or saline but most studies were terminated at 15 days as it became apparent that by this time-point all parameters in the cisplatin group were not significantly different from the saline group (see Results). In the Amlogger system the rats are placed in clear plastic cages ($50 \times 27 \times 20$ cm) surrounded by an infrared array. This array consists of two levels of infrared beams (3.0 and 11.0 cm from the bottom of the cage) with 24 on each level arranged in an 8×16, 25.4 mm pitched grid. The lower grid measures normal X and Y movement and the upper grid measures rearing movement. Categories of activity measured were horizontal movement: number of seconds where at least one beam was broken on the lower level; rearing: number of seconds where at least one beam was broken on the upper level. Indices of locomotion were measured by mobility: time spent moving at least 50 mm across the lower level and also FR count: the number of times the rat moved from the front to the back of the cage or vice-versa. Activity was measured for 23 h and body weight, food and water intake were measured daily between 10.00 and 11.00. Routine husbandry was undertaken at this time and cages replaced on days 4, 11 and 18 where applicable after cisplatin administration. All times refer to the 24 h clock. Data was collected every hour and processed as 11 h totals for light-phase and dark-phase activities. For total dark-phase activity analysis, data for the last hour of darkness (06.00–07.00) was excluded in order to obtain the same number of hours as day activity for comparison and to enable a day:night activity ratio to be calculated. Preliminary analysis showed that removal of this 1 h of data had neither an effect on the percentage changes in the activities measured nor on the statistical significance of the changes.

Results from the activity study are reported in three ways to illustrate different effects: 1) The averaged light and dark-phase activity for each parameter was quantified over sequential 5 day periods to enhance the detection of any small but persistent

effects which would not necessarily be apparent from the daily values; 2) Dark-phase activity for each of the 15 days following cisplatin or saline was analyzed in detail as this was the time when animals were most active and hence the phase most likely to reveal any effects of treatment; 3) Initial studies identified two surges of nocturnal activity that were analyzed further. These were the 2 h immediately after the lights were turned off (19.00–21.00) and the 2 h prior to the lights being turned on (05.00–07.00).

2.4. Measurement of gastric contents

Gastric contents were removed post mortem and weighed from a group of animals 2 days after cisplatin or saline administration, as this time had been identified as the point at which food intake was at a nadir. These values were used to investigate the relationship between gastric contents, food intake and activity.

2.5. Measurement of expression levels of mRNA

Animals were killed by a rising concentration of CO_2 and cervical dislocation (Schedule 1 method) and placed on a bed of dry ice for tissue removal. Hypothalami were taken from cisplatin-treated rats and saline-treated controls at 2, 7 and 21 days post-injection and snap frozen in liquid nitrogen prior to storage at −70 °C. Total RNA was isolated from the hypothalamus samples using the RNeasy® Mini kit (Qiagen, Crawley, UK) according to the manufacturers instructions. RNAs were quantitated using a SPECTRAmax® Plus spectrophotometer (Molecular Devices Corp., Sunnyvale, CA, USA) and quality confirmed by electrophoresis on a 1% *w*/*v* agarose gel containing ethidium bromide. Relative quantitations of mRNA transcripts were performed using the RT-PCR-based 5′ nuclease assay utilizing gene-specific fluorogenic TaqMan® probes (TaqMan analysis) (reviewed in Bustin, 2000). 0.21 μg of each deoxyribonuclease-treated RNA was reverse transcribed once along with "NoRT" and "NoRNA" controls as described previously (Moore et al., 2001) except that the deoxyribonuclease reaction was performed in 24 μl at 30 °C for 20 min. All completed 20 μl reverse transcription and control reactions were diluted 5.25-fold and stored at −30 °C. TaqMan assay oligonucleotide primers (Proligo France SAS, Paris) and probes (Applied Biosystems, Warrington, UK) were designed (see Table 1 for sequences; those for hypocretin (orexin) were published in Irving et al., 2002) and TaqMan analysis performed as previously described (Moore et al., 2001). For the study samples, equal amounts of cDNA were added to each reaction (equivalent to 10 ng total RNA). Reaction conditions were as follows: 50 °C for 2 min, 95 °C for 10 min then 45–50 cycles of 95 °C for 15 s and 60 °C for 1 min. A 3-fold serial dilution of rat hypothalamus, pituitary, or white adipose tissue total cDNA (equivalent to 150 to 0.0025 ng total RNA) or rat genomic DNA (Clontech at BD Biosciences, Palo Alto, UK; 100 ng to 0.0017, the choice of which was dependent on the mRNA being studied) was assayed for each measurement and used to construct a standard curve.

2.6. Statistical analysis

Results of the behavioural studies are presented as means ± SEM Statistical comparisons of the different treatment groups in the behavioural studies were made by one-way and two-way ANOVA with Bonferroni post-tests using Graphpad Prism software (version 3.02). Results of the mRNA studies are presented as the ratio to the geometric mean of the saline-treated controls at each time point with 95% confidence intervals for the ratio. The transcript data were analyzed using one-way analysis of variance after a $\log_{10}$-transformation followed by pair-wise comparisons with the unrestricted Least Significant Difference (LSD) test (performed in Microsoft® Excel 97; Microsoft Corporation, Redmond, WA, USA) with an add-in toolkit for the analysis of real-time PCR data (PRISM Training and Consultancy Ltd., Cambridge, UK). Differences were considered significant when $P<0.05$.

3. Results

3.1. Effect of cisplatin on activity

3.1.1. The pattern of activity in the first 48 h following cisplatin

Fig. 1 shows the expected circadian pattern of activity in animals treated with saline characterized by an elevated level of horizontal movement in the dark-phase. This pattern is typical of all activity parameters and shows that in saline-treated animals there was a surge in activity at the onset of the dark-phase at 19.00. This was followed by a stable period of activity lasting about 6 h and then a small decline for 4 h, followed by a surge 2 h before the end of the dark-phase. In animals treated with cisplatin at 11.00, horizontal movement also increased at the onset of the dark-phase, but then declined significantly relative to the saline-treated animals between 8 and 10 h into the dark-phase, i.e. between 16 and 18 h after cisplatin administration. Examination of the activity pattern for the next 24 h revealed that whilst daytime activity was not different between the two treatments, nocturnal activity was again reduced in the cisplatin-treated group and of particular note was the reduction in the magnitude of the increased activity seen prior to the end of the dark-phase. This effect is described in detail in Section 3.1.4 below.

3.1.2. Overall effect of cisplatin on activity over 15 days

The saline-treated controls showed a stable circadian pattern for all activity parameters with the greatest levels of activity occurring during darkness (Fig. 2). For horizontal movement the dark/light-phase ratio in the saline group over 15 days was 2.8 ± 0.1 ($N=8$). In the cisplatin-treated group all activity parameters were significantly reduced compared to controls during dark-phase hours for days 1–5 post-injection. Dark-phase horizontal movement, mobility and FR count tended to be reduced for 6–10 days post-cisplatin compared to controls (Fig. 2) although only FR count reached statistical significance ($P<0.05$). During light-phase hours, locomotion

Table 1
Oligonucleotide sequences of gene-specific TaqMan assay primers and probes

Rat mRNA (RGD gene symbol)	Accession number (amplicon position in parentheses)	Primer or probe	Primer or probe sequence
Abcg1	AJ303374	Forward (sense)	5′-ACACACTGGCCCTGGATGA-3′
	(2392–2512)	Reverse (antisense)	5′-TGAGAGGAACTTTAGAAGGCTGAGA-3′
		Fluorogenic probe	FAM-5′-ACAGTCGTCAGGCGGAGTCCCTAAGAC-3′-TAMRA
Agrp	AF206017	Forward (sense)	5′-GTTCTCCGCGTCGCTGTG-3′
	(124–216)	Reverse (antisense)	5′-AGAAGCGGCAGTAGCACGTG-3′
		Fluorogenic probe	FAM-5′-TCGCAGCAAGGTACCTGTTGTCCCAA-3′-TAMRA
Crh	X03036	Forward (sense)	5′-GCAGATGGAAGTCACCCAGTTG-3′
	(1045–1136)	Reverse (antisense)	5′-AGAGCTTACACATTTTGTCCTAGCCA-3′
		Fluorogenic probe	FAM-5′-CCCTCAAGAATGAATTCTCTTACACTGTGTCTCCA-3′-TAMRA
Hprt	M63983	Forward (sense)	5′-GGTGAAAAGGACCTCTCGAAGTG-3′
	(590–683)	Reverse (antisense)	5′-ATAGTCAAGGGCATATCCAACAACA-3′
		Fluorogenic probe	FAM-5′-CCAGACTTTGTTGGATTTGAAATTCCAGACAA-3′-TAMRA
Il6	M26744	Forward (sense)	5′-CCCAACTTCCAATGCTCTCC-3′
	(565–692)	Reverse (antisense)	5′-GCCGAGTAGACCTCATAGTGACC-3′
		Fluorogenic probe	FAM-5′-TCTTGGTCCTTAGCCACTCCTTCTGTGACT-3′-TAMRA
Npy	M20373	Forward (sense)	5′-GTCTGCCTGTCCCACCAATG-3′
	(416–486)	Reverse (antisense)	5′-ATACAACGACAACAAGGGAAATGG-3′
		Fluorogenic probe	FAM-5′-CCACCACCAGGCTGGATTCCGA-3′-TAMRA
Ppia	M19533	Forward (sense)	5′-ATGAGAACTTCATCCTGAAGCATACA-3′
	(296–401)	Reverse (antisense)	5′-TCAGTCTTGGCAGTGCAGATAAA-3′
		Fluorogenic probe	FAM-5′-CCTGGCATCTTGTCCATGGCAAATG-3′-TAMRA
Slc6a4	M79450	Forward (sense)	5′-CCGTGGACTCTCAGACATGCT-3′
	(2256–2357)	Reverse (antisense)	5′-TGACTGGGTGGCGTTCATC-3′
		Fluorogenic probe	FAM-5′-TCCTTCCACTCCCTGACGTGTCCAAG-3′-TAMRA
Tnf	X66539	Forward (sense)	5′-CCCAGACCCTCACACTCAGATC-3′
	(224–332)	Reverse (antisense)	5′-GCCACTCCAGCTGCTCCTC-3′
		Fluorogenic probe	FAM-5′-TAGCCCACGTCGTAGCAAACCACCAAG-3′-TAMRA
Tph	X53501	Forward (sense)	5′-GCTCACAACCATCCGTCACTC-3′
	(2722–2812)	Reverse (antisense)	5′-TGTATTTCTATCTCTGCACCACTTGC-3′
		Fluorogenic probe	FAM-5′-TCCAATGTTTTCTGGCCTCCACAGATAGC-3′-TAMRA

FAM: fluorogenic probe reporter dye 6-carboxyfluorescein; TAMRA: fluorogenic probe quencher dye 6-carboxytetramethylrhodamine; RGD: rat genome database (http://rgd.mcw.edu/); Abcg1: ATP-binding cassette transporter G1 (homologue of *Drosophila* white gene encoding tryptophan transporter); Agrp: agouti-related protein; Crh: corticotropin releasing hormone (CRF); Hprt: hypoxanthine guanine phosphoribosyl transferase; Il6: interleukin 6; Npy: neuropeptide Y; Ppia: peptidylprolyl isomerase A (cyclophilin); Slc6a4: solute carrier family 6, member 4 (serotonin transporter, SERT); Tnf: tumor necrosis factor (TNFα); Tph: tryptophan hydroxylase. Primer and probe sequences for Il1b (interleukin 1β) from Li and Wang, 2000.

during 1–5 days post-cisplatin was significantly reduced (mobility: $P<0.05$ and FR count: $P<0.001$). A small number of animals were studied for an additional week (i.e. to 21 days) but their activity was not statistically different from the animals at 15 days, irrespective of the parameter, and hence longer time periods were not studied further (data not shown).

The ratios of dark-phase to light-phase activity (dark/light ratios) were calculated for each activity type over the entire 15 day observation period. The ratios were significantly different between cisplatin and saline for horizontal movement (2.2 ± 0.1 vs. 2.8 ± 0.1; $P<0.001$), mobility (3.5 ± 0.1 vs. 4.2 ± 0.2; $P<0.01$), and rearing (4.1 ± 0.2 vs. 5.3 ± 0.3; $P<0.01$) but not FR count. When analyzing the dark/light ratios over 5 day periods, horizontal movement and mobility were significantly different between cisplatin and saline on day: 1–5 ($P<0.01$) and 6–10 ($P<0.01$) but not 11–15; rearing was significantly different between cisplatin and saline on days 6–10 ($P<0.01$) and 11–25 ($P<0.05$). In contrast, dark/light ratios for FR count were not significantly different between cisplatin and saline.

3.1.3. Dark -phase activity following cisplatin

In saline-treated animals all dark-phase activity parameters remained stable over the entire 15 day study period (Fig. 3) In cisplatin-treated animals all dark-phase activity parameters, except rearing, were significantly reduced in the first 24 h after cisplatin administration and reached a nadir in 3 days. The following rank-order of changes was apparent when expressing the activity value differences at 3 days in the cisplatin group as a percentage of the saline-treated group: horizontal movement (−52%); rearing (−55%); mobility (−64%); FR count (−84%). Following this nadir, all parameters gradually recovered and whilst statistically significant differences from the saline-treated animals were lost between 6 (rearing) and 8 days (FR count) depending upon the parameter, the graphs in Fig. 3 show that full convergence with the saline-treated groups occurs later.

3.1.4. Effect of cisplatin on early (19.00–21.00) and late (05.00–07.00) dark-phase activity

Detailed analysis of circadian activity patterns (Fig. 1) revealed that although cisplatin reduced total nocturnal activity,

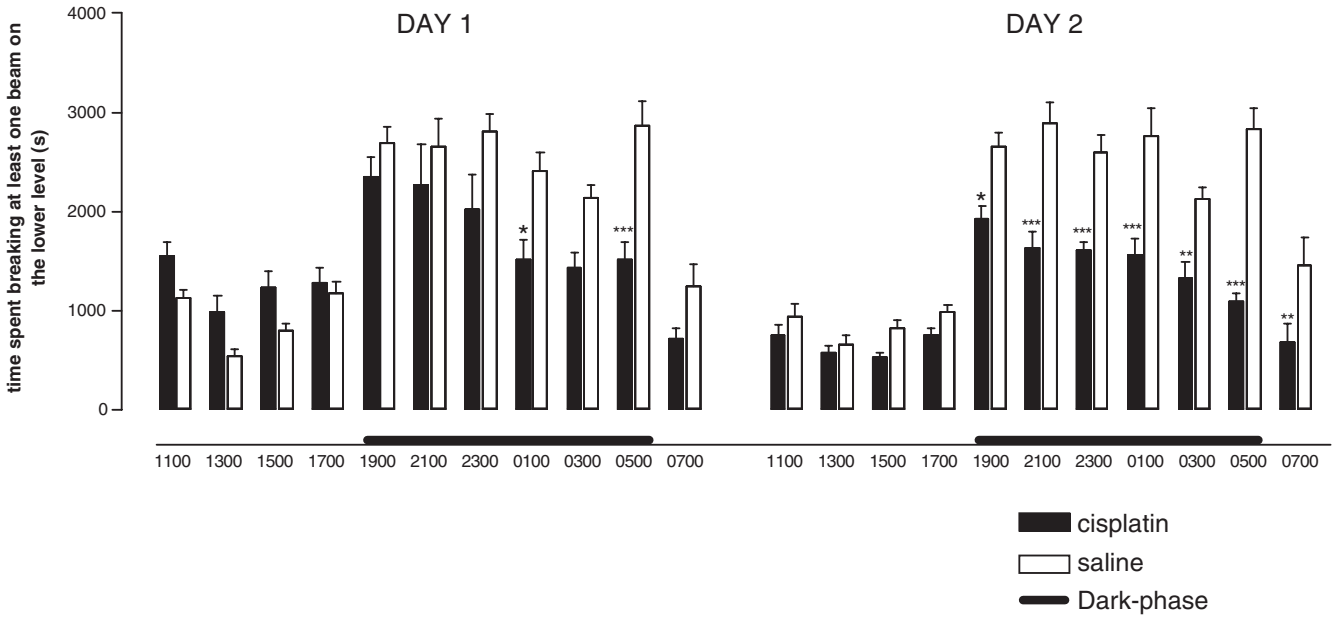

Fig. 1. The effect of cisplatin (6 mg/kg i.p.) on horizontal movement for 48 h post-injection. Cisplatin (6 mg/kg; i.p.) or saline (0.9% *w*/*v*; i.p.) was given at 11.00 on day 1. Bars represent the sum of 2 h bins of horizontal movement expressed as mean ± SEM (s). Horizontal movements of cisplatin-treated rats significantly different from that of saline-treated rats are indicated as $*P<0.05$, $**P<0.01$, $***P<0.001$; $N=8-12$ (Two-way ANOVA with Bonferroni post-tests). At the bottom of the figure, horizontal dark bars represent the dark-phase.

the effect appeared most marked at the onset of the dark-phase (19.00–21.00) and prior to the end of the dark-phase (05.00–07.00). These periods of early (Fig. 4a) and late (Fig. 4b) dark-phase activity were plotted over 15 days post-injection (horizontal movement, mobility and rearing shown only; Fig. 4). In cisplatin-treated animals the early dark-phase surges in horizontal movement, mobility, FR count and rearing were unaffected on the first night after cisplatin treatment but declined on the second day and was absent on days 3 and 4 post-injection (Fig. 4). There were no significant reductions beyond night 4, with nocturnal activity gradually returning to normal levels over the subsequent days. In contrast, the late dark-phase surges (Fig. 4b) in horizontal movement, mobility, FR count and rearing were markedly reduced on the first night following cisplatin and remained significantly below the saline-treated group for between 4 days (rearing) and 9 days post-cisplatin (horizontal movement, mobility and FR count; Fig. 4).

3.2. Effect of cisplatin on food and water intake and gastric contents

Fig. 5a shows that administration of 6 mg/kg i.p. cisplatin significantly reduced food intake on the first day following cisplatin, reaching a nadir at 2 days and progressively recovering thereafter. Water intake was significantly reduced by day 3 post-cisplatin ($P<0.05$) and then showed a progressive increase, being significantly different from saline-treated animals 6 days after cisplatin treatment, after which it remained elevated for the rest of the study (Fig. 5b). In saline-treated rats, food and water intake were tightly coupled with a ratio of close to one, but in cisplatin-treated rats this ratio was significantly reduced compared to controls for almost the entire study period ($P<0.001$; Fig. 5c). Body weight was reduced (Fig. 5d) reaching a nadir at 6 days and then gradually began to increase with the rate of weight gain appearing to be the same as the saline-treated animals 9 days after cisplatin. By 15 days after cisplatin administration body weight was still significantly different but the sub-group group studied for 21 days the significant difference in body weight between saline and cisplatin-treated groups was lost at day 19 although the mean weight for the cisplatin group was still below that for the saline treated group (data not shown).

In rats killed at day 2 post-cisplatin, the stomach content weights were significantly higher than saline-treated rats (9.04 ± 0.8 vs. 2.32 ± 0.3 g; $P<0.001$).

3.3. Correlation of activity parameters with indices of feeding

In the cisplatin-treated animals, the relationship between the daily measures of food and water intake and the various activity parameters were investigated. All activity parameters (global activity, mobility, FR count and rearing) were highly significantly correlated with food intake ($P<0.001$) with *r*-values between 0.94 (FR count) and 0.98 (mobility). Water intake was also significantly correlated ($P<0.01-P<0.001$) with *r*-values between 0.65 (global activity) and 0.81 (FR count). The stability of all parameters in the saline-treated group means that it is not possible to undertake a meaningful correlation analysis. However, plotting the food intake and activity values for the saline-treated animals on the same graph as the cisplatin values, revealed that they are all located along the same line and with the saline values clustering at the higher end of food intake (Fig. 6a). In contrast water intake in the saline-treated animals does not form part of the same population as the cisplatin-treated animals (Fig. 6b). None of the activity parameters or food intake measured over the first

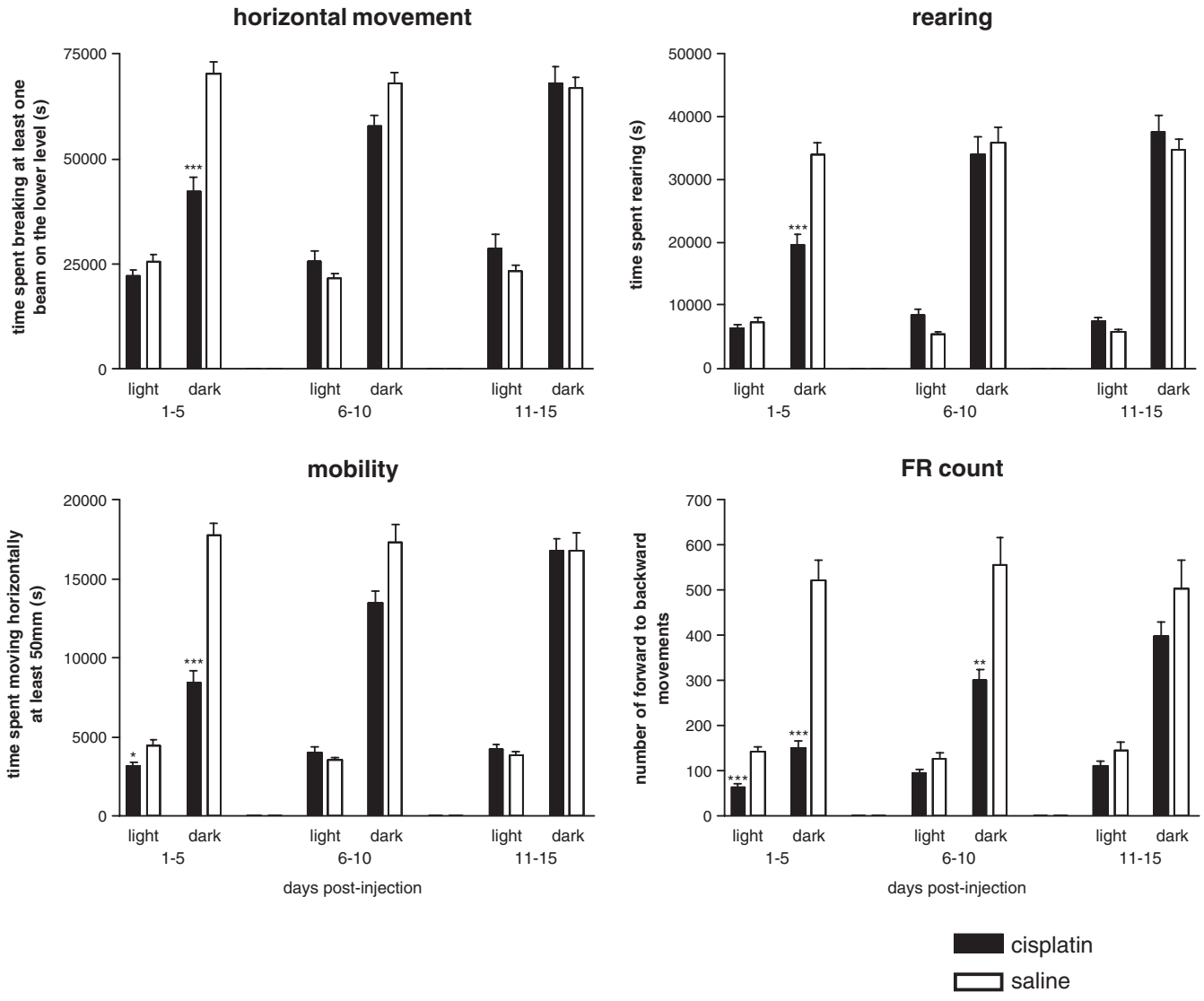

Fig. 2. The effect of cisplatin (6 mg/kg i.p.) on activity over 15 days post-injection. After administration of cisplatin (6 mg/kg; i.p.) or saline (0.9% *w*/*v*; i.p.) activity was monitored continuously for 15 days (23 h from 11.00 to 10.00 each day). Bars represent the sum of 11 h bins of dark-phase or light-phase activity (horizontal movement, mobility, rearing or FR count) totaled over 5 days and expressed as mean ± SEM. Activity parameters for cisplatin-treated rats significantly different from that of saline-treated rats as indicated by $*P<0.05$, $**P<0.01$, $***P<0.001$; $N=8-12$ (Two-way ANOVA with Bonferroni post-tests).

48 h were correlated with the weight of stomach contents measured at 48 h (*r*-values 0.14 to 0.43).

3.4. Effect of cisplatin on expression levels of mRNA

There was no significant difference between hypothalamic mRNA levels of cisplatin- and saline-treated animals for AGRP, NPY, HCRT, CRH, IL-1, IL-6, TNFα, ABCG1, SLC6A4 or the housekeeping genes PPIA and HPRT at 2, 7, or 21 days post-injection (Table 2). There was a significant changes in mRNA expression for TPH at day 21 post-injection (decrease of 47%, $P<0.05$; Table 2).

4. Discussion

In the present study, administration of cisplatin to the rats produced a combination of effects with similarities to those experienced by humans receiving chemotherapy. In particular a reduction of food intake and body weight, disordered gastric motility and a reduction in spontaneous locomotor activity. These behavioural effects will each be discussed separately in relation to the results from the molecular study.

4.1. Effects of cisplatin on ingestion and gastric function

Cisplatin reduced food intake and the stable relationship between food and water intake was altered. Another sign of disordered gastric function was that cisplatin-treated rats culled at 2 days post-injection exhibited delayed gastric emptying as demonstrated by an increased wet weight in stomach content in consistent with previous studies (Wang and Aggarwal, 1997). Reduced food intake or food rejection is associated with nausea (Halford et al., 1998; Pelchat and Rozin, 1982; Richards and

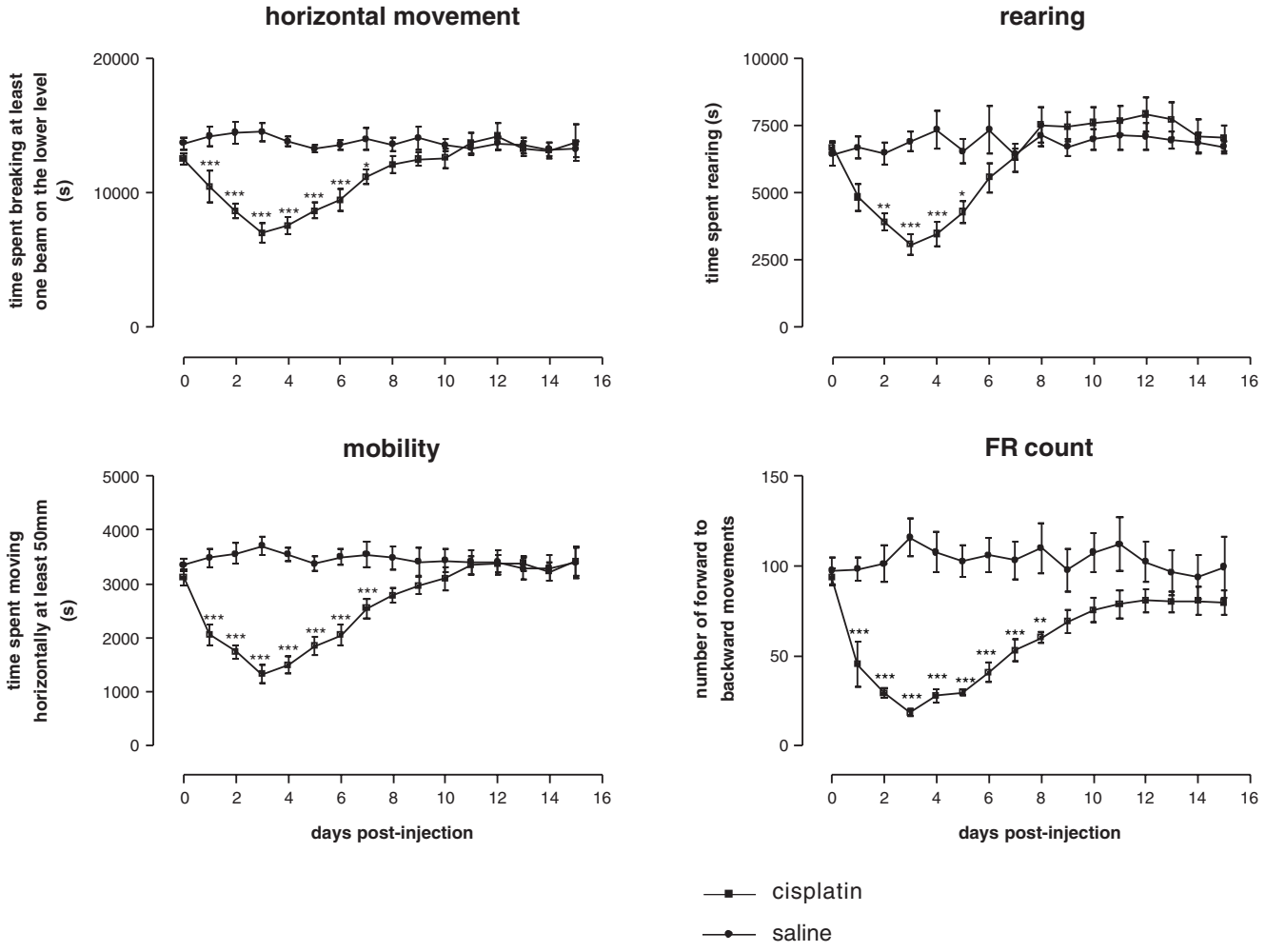

Fig. 3. The effect of cisplatin (6 mg/kg i.p.) on dark-phase activity over 15 days post-injection. After administration of cisplatin (6 mg/kg; i.p.) or saline (0.9% *w*/*v*; i.p.) activity was monitored continuously for 15 days (23 h from 11.00 to 10.00 each day). Data represent the total dark-phase activity parameter (horizontal movement, mobility, rearing or FR count) for each post-injection day expressed as mean ± SEM. Nocturnal activity parameter for cisplatin-treated rats significantly different from that of saline-treated rats as indicated by $*P<0.05$, $**P<0.01$, $***P<0.001$; $N=8-12$ (Two-way ANOVA with Bonferroni post-tests).

Andrews, 2004) and gastric distension can cause nausea and vomiting (Lang, 1990; Ladabaum et al., 1998). Whilst the gastric stasis could contribute to the reduction in food intake via the activation of abdominal vagal afferents and the release of endogenous satiety hormones (e.g. CCK) the degree of stasis was not correlated with either food intake or activity. However food intake and activity correlate with each other in the cisplatin treated animals. This argues that cisplatin must also be reducing food intake by a mechanism either not activated by gastric distension or activated by a threshold level of distension exceeded in all the animals in the present study. It is worth noting that in the first few days after cisplatin administration, when food intake is reduced and gastric stasis is detected, rats will ingest several grams of kaolin if it is available (Rudd et al., 2002; Liu et al., 2005). This suggests that the reduction in food intake is not solely due to a general reduction in ingestive behaviour secondary to malaise. The hypothalamus plays a major role in regulation of food intake and the pathways are particularly well characterized in rodents (Meguid et al., 2000; Cowley, 2003). Quantitative real-time RT-PCR did not reveal any significant changes in hypothalamic mRNA levels between saline- and cisplatin-treated rats for AGRP, NPY and orexin at any time-point, suggesting that cisplatin was not decreasing feeding through a major effect at the central pathways involving these neuropeptides. These negative findings must be treated with caution as small changes in sub-nuclei could have been obscured by analysis of whole hypothlami.

Although food intake was reduced, water intake was elevated for the majority of the study. This phenomenon has previously been reported and is at least in part secondary to the nephrotoxicity associated with cisplatin (Gordon et al., 1982).

4.2. Effects of cisplatin on activity

Although previous studies in rats have investigated the effects of cisplatin, this is the first to characterise locomotor activity continuously over a period comparable to that of a clinical “cycle” of cisplatin chemotherapy (McKeage et al., 1995). The single dose of cisplatin used produced a suppression of locomotor behaviour, especially nocturnal (although because of the low level of daytime activity reductions in this period may have been harder to detect), up to 8 days with

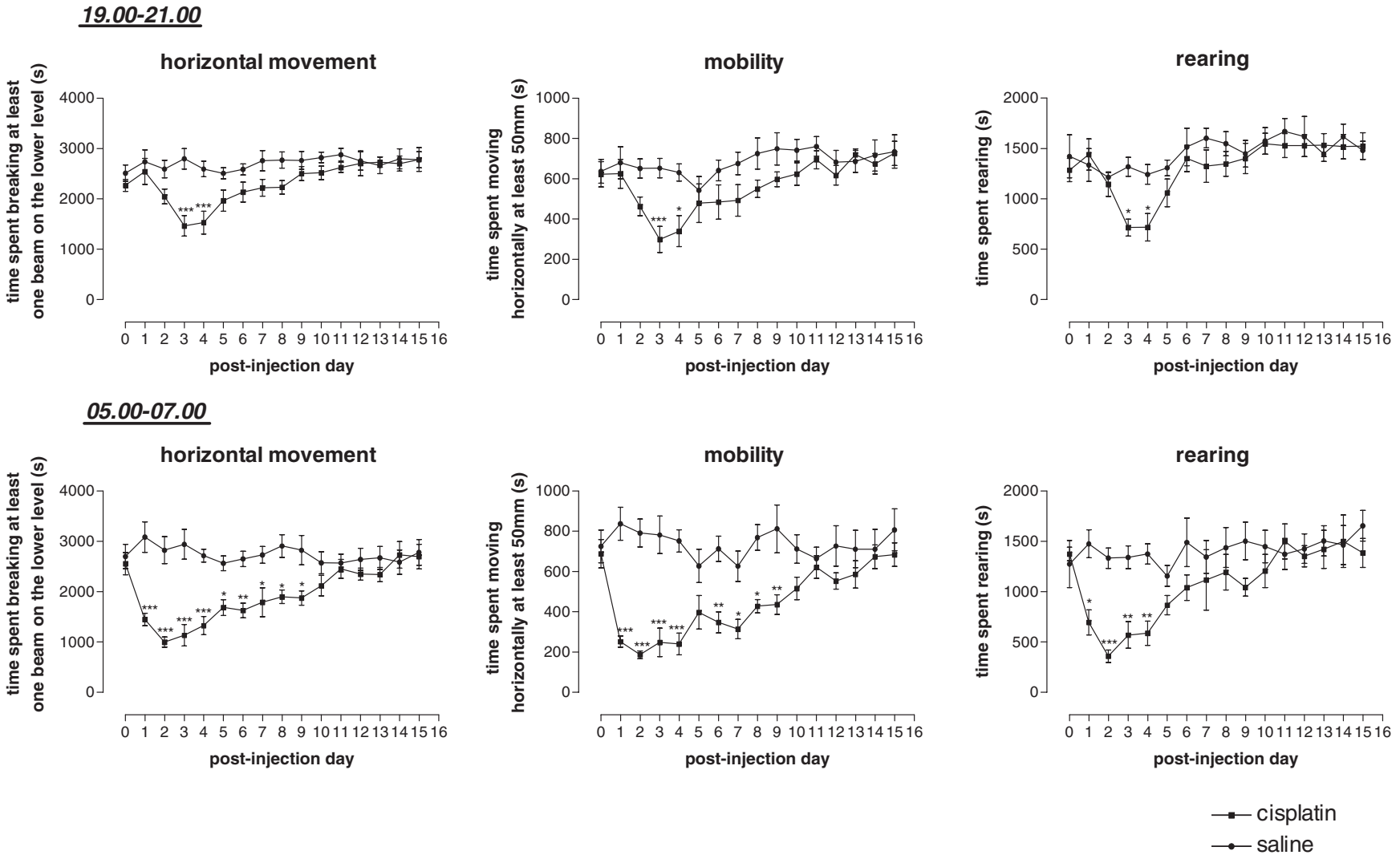

Fig. 4. The effect of cisplatin on the 19.00–21.00 and 05.00–07.00 surges over 15 days. After administration of cisplatin (6 mg/kg; i.p.) or saline (0.9% *w*/*v*; i.p.) activity was monitored continuously for 15 days and nights (23 h from 11.00 to 10.00 each day). Data represent horizontal movement, mobility and rearing summed over 19.00–21.00 and 05.00–07.00 for each day and expressed as mean ± SEM. Activity in these periods on each post-injection day for cisplatin-treated rats significantly different from that of saline-treated rats as indicated by $*P<0.05$, $**P<0.01$, $***P<0.001$; $N=8–12$ (Two-way ANOVA with Bonferroni post-tests).

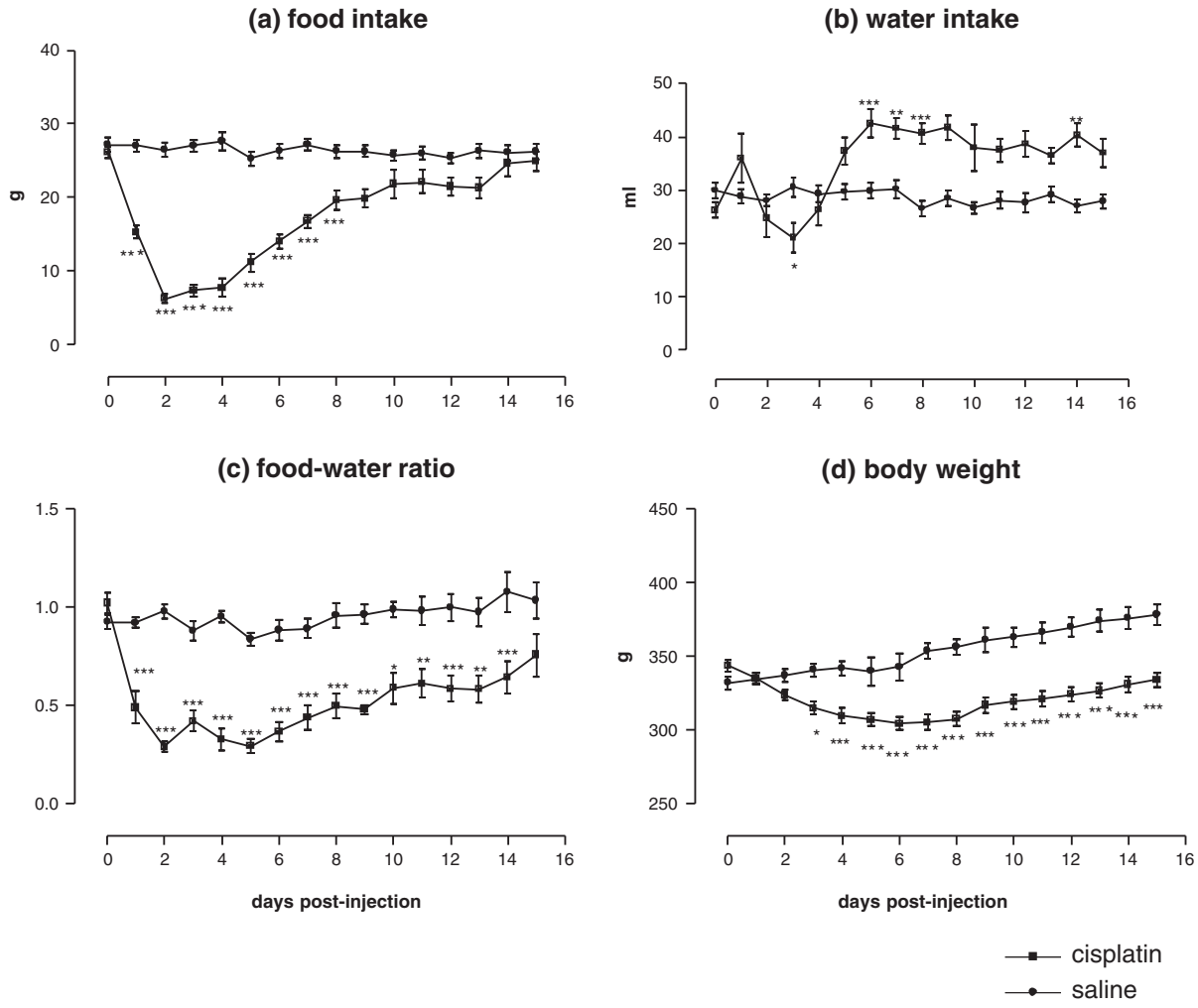

Fig. 5. The effect of cisplatin (6 mg/kg i.p.) on body weight and feeding parameters. After administration of cisplatin (6 mg/kg; i.p.) or saline (0.9% *w*/*v*; i.p.) food intake, water intake and body weight was monitored continuously for 15 days post-injection. Data represent food intake, water intake and body weight on each day post-injection, expressed as mean ± SEM. Food intake, water intake and body weight for cisplatin-treated rats significantly different from that of saline-treated rats as indicated by $*P<0.05$, $**P<0.01$, $***P<0.001$; $N=8-12$ (Two-way ANOVA with Bonferroni post-tests).

individual components being affected to different degrees and having differing rates of recovery. The disruption of the normal circadian rhythms as demonstrated by the changes in dark/light ratios is reminiscent of the changes seen in the circadian rhythm of activity in breast cancer patients following chemotherapy (Roscoe et al., 2002) and which correlates with measures of fatigue and depression. This raises the possibility that the reduction in activity in the rats may provide insights into similar phenomena occurring in cancer patients but clearly further studies need to be undertaken before more meaningful comparisons can be made. Another feature of post-cisplatin activity was that it was not reduced until 8–10 h after the lights were turned off on the day of the injection (i.e.16–18 h after drug injection). This long latency contrasts with an increase in abdominal vagal afferent discharge beginning about 10 min after systemic cisplatin administration in the rat (Hillsley and Grundy, 1999; Horn et al., 2004). Abdominal vagal afferents have been implicated in the genesis of sickness behaviour in the rat (Konsman et al., 2002) but the substantial difference in the latency between the activation of vagal afferents and the expression of a behavioural effect may indicate that either a considerable period of afferent stimulation is required or the abdominal vagal afferents play a minor role.

It could be argued that the effects of cisplatin on food intake is a potential confounding factor in using it to study fatigue-like behaviour. This is suggested by (a) the high degree of correlation between daily measures of food intake and the daily level of activity in cisplatin-treated animals; and (b) the pattern of "early" (19.00–21.00) and "late" (05.00–07.00) dark-phase activity that coincide with the characteristic pattern of nocturnal food intake in rats (Le Magnen and Devos, 1980; Demaria-Pesce and Nicolaidis, 1998). However, the significant increase in water intake 6 days post-cisplatin to some extent, offsets the reduction in activity. Moreover, previous studies in rats have shown that feeding occupies only 5% to 11% of dark-phase time (Meguid et al., 1990; Glendinning and Smith, 1994; Demaria-Pesce and Nicolaidis, 1998). In the present study, the reductions in dark-phase activity from baseline at 3 days post-cisplatin were considerably larger (horizontal movement: −44%; rearing: −54%; mobility −58%; FR count: −81%)

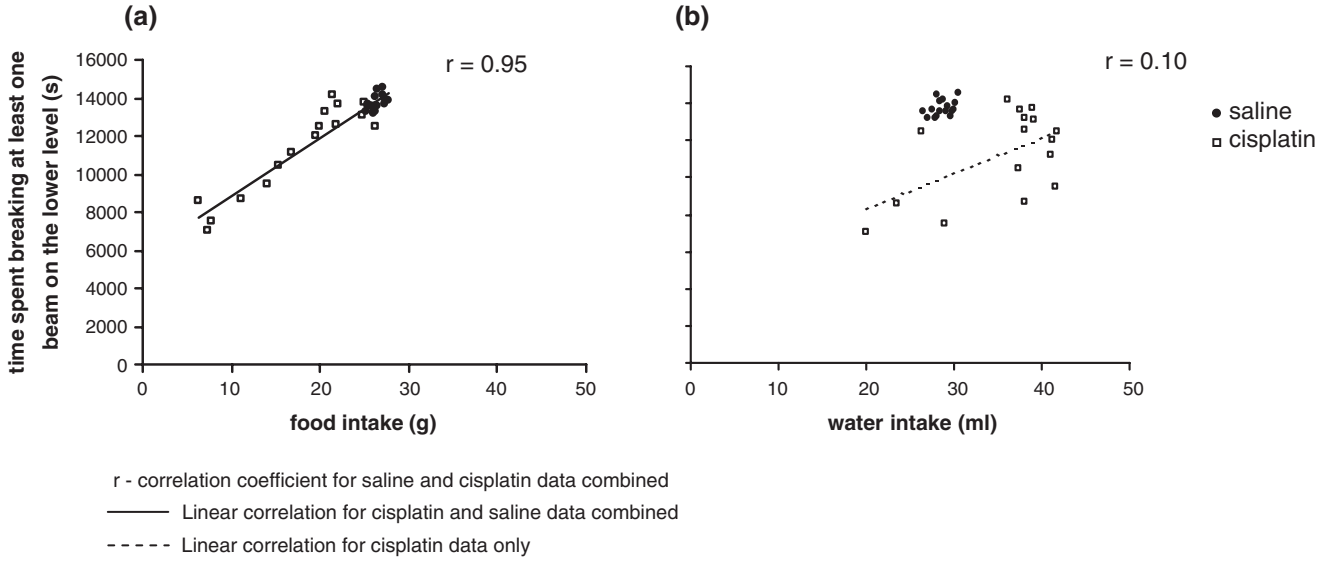

Fig. 6. Horizontal movement over 15 days post-injection versus (a) 15 day food intake and (b) 15 day water intake. Linear correlation was performed for either cisplatin (□) and saline (●) data combined (——) or cisplatin data alone (- - - -). Food/water intake and activity measurements were taken over up to 15 days. In (a) the correlation value r is for cisplatin and saline data combined and in (b) is for cisplatin data only.

making it highly unlikely that the reduction in food intake could account for the majority of activity reduction. It is also noteworthy that whilst there was a transient reduction over two nights of the "early" (19.00–21.00) dark-phase activity the "late" (05.00–07.00) dark-phase activity was reduced for 9 days. Thus cisplatin appears to have a differential effect on "early" and "late" dark-phase activity.

In this study the animals were fed on a pelleted diet which arguably may require more energy expenditure to ingest than a liquid diet and hence could have further added to any reduction in activity due directly to the cisplatin itself. A study using a liquid diet would also be of interest as it is likely that the magnitude of the gastric stasis will also be reduced by use of such a diet.

Table 2
Regulation of hypothalamic mRNA expression following cisplatin treatment of Wistar rats

(a)

Group	Agrp	Npy	Hcrt (orexin)	Crh (CRF)
Saline-treated controls at 2, 7 or 21 days post-treatment	1	1	1	1
Cisplatin-treated animals at:				
2 days post-treatment	1.32 (0.87–1.99)	1.19 (0.90–1.57)	0.99 (0.71–1.39)	0.87 (0.60–1.26)
7 days post-treatment	1.12 (0.75–1.66)	1.21 (0.93–1.58)	0.92 (0.67–1.28)	1.31 (0.92–1.87)
21 days post-treatment	1.08 (0.69–1.70)	0.97 (0.72–1.32)	0.83 (0.58–1.20)	0.83 (0.56–1.24)

(b)

Group	Fold-change in expression level of mRNA (RGD symbol)							
	Il1b	Il6	Tnf (TNFα)	Abcg1	Tph	Slc6a4	Ppia (cyclophilin)	Hprt
Saline-treated controls at 2, 7 or 21 days post-treatment	1	1	1	1	1	1	1	1
Cisplatin-treated animals at:								
2 days post-treatment	0.78 (0.50–1.22)	2.86 (0.74–10.96)	1.05 (0.67–1.65)	1.08 (0.83–1.39)	0.97 (0.59–1.62)	0.51 (0.21–1.26)	1.09 (0.85–1.41)	1.17 (0.91–1.51)
7 days post-treatment	0.83 (0.54–1.28)	1.86 (0.51–6.76)	0.84 (0.55–1.29)	1.13 (0.89–1.45)	0.96 (0.59–1.56)	1.06 (0.45–2.50)	1.07 (0.84–1.37)	1.04 (0.81–1.32)
21 days post-treatment	0.90 (0.55–1.47)	1.51 (0.32–7.18)	0.94 (0.58–1.53)	1.00 (0.76–1.32)	0.53 (0.30–0.92)*	1.01 (0.38–2.67)	1.01 (0.77–1.34)	0.87 (0.66–1.14)

A single dose of either saline or cisplatin (6 mg/kg) was administered to male Wistar rats and hypothalamus mRNA measurements performed in animals 2, 7 and 21 days post-treatment. Data are expressed as the ratio to the geometric mean of the saline-treated controls (95% confidence intervals for the ratio) at each timepoint with $n=4$ to 7. Post-hoc assessment for significant differences was performed using the unprotected least significant difference test, where $*P<0.05$. For each mRNA, the gene symbol as stated in the Rat Genome Database (RGD; http://rgd.mcw.edu/) was used. Hcrt: hypocretin (orexin). See Table 1 legend for other gene names.

Taken together, these observations strongly suggest that the cisplatin-induced reduction in locomotor activity and other behaviours described here has features in common (e.g. reduction in spontaneous locomotor activity, disrupted circadian rhythm) with the fatigue reported in cancer patients undergoing chemotherapy.

In the present study, hypothalamic RNA levels of CRF were not significantly different from those in saline-treated animals. This is perhaps surprising as Burak et al. (2002) using a bile-duct resection (BDR) rat model have proposed that defective corticotrophin-releasing hormone (CRH) release contributes to cholestasis-associated fatigue. Hypothalamic 5-HT is implicated in sleep, food intake, mood and central fatigue (Wilson and Maughan, 1992; Jakeman et al., 1994; Marvin et al., 1997; Bakheit et al., 1992). An interesting finding in the present study was that following cisplatin treatment, tryptophan hydroxylase (the rate limiting enzyme for the synthesis of 5-HT) mRNA expression was decreased by almost 50% compared to controls in the hypothalamus at 21 days but was no different to controls at 2 and 7 days post-cisplatin. Whilst all behavioural parameters had returned to control levels by 21 days after cisplatin, this result is the first time that evidence for a long-term alteration in serotonin metabolism in the hypothalamus has been demonstrated following a single dose of a cytotoxic drug. The functional significance, if any, remains to be elucidated.

A number of the responses to cisplatin (e.g. reduced food intake, reduced spontaneous activity, decreased gastric emptying) are also features of the "sickness-behaviour" which follows an infection. There is a considerable body of evidence implicating pro-inflammatory cytokines such as interleukins and tumour necrosis factor α in the genesis of sickness behaviour (Konsman et al., 2002). In a lipopolysaccharide-induced sickness-behaviour model in the mouse, brain expression of IL-1 was higher than that of TNF-α suggesting only a minor role for TNF-α in sickness-behaviour compared to IL-1 (Bluthe et al., 2000). However, in the present study, RT-PCR did not show a change in either hypothalamic TNF-α, IL-1 or IL-6 mRNA expression. The preliminary molecular studies suggest that whilst there are superficial similarities between the adaptive behaviours to an infection and those to a cytotoxic drug the involvement of hypothalamic cytokines may differ between the two situations. Further studies including the direct measurement of cytokine levels are required.

4.3. Summary

It is not possible to identify whether cisplatin has a primary effect on reducing food intake (e.g. reduced appetite, induction of nausea) which results in a decrease in activity, whether either the cisplatin reduces locomotor activity which results in a decreased food intake or whether cisplatin has independent actions on food intake and activity. Studies in humans of the effect of cytotoxic drugs have not examined the relationships between locomotor activity, food intake, nausea and gastric function so we do not know the impact of one upon the other in the genesis of the pattern of symptoms which follow cytotoxic drug administration. These studies in the rat provide a baseline for investigation of these relationships which may lead to the development of ways of protecting against a spectrum (nausea, disrupted GI function, reduced food intake, fatigue) of adverse effects of cytotoxic anticancer therapy.

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