Title:
Decrease of food intake by a melanocortin-4 receptor agonist (MT$_{1\beta}$) in Siberian hamsters in long and short photoperiods

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Running head:
MT$_{1\beta}$ decreases food intake in Siberian hamsters

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Abstract:

We investigated the role of the hypothalamic melanocortin system in the regulation of food intake in the Siberian hamster which shows a profound seasonal decrease in food intake and body weight in short photoperiod (SP). In male hamsters maintained in long photoperiod (LP), icv injection of MT\(_{II}\) just before lights off significantly decreased food intake relative to vehicle treatment over the 6 h observation period. Similar effects were observed in age-matched hamsters after exposure to a short daylength for nine weeks, when body weight had significantly decreased. There was no clear difference in either the magnitude of response or the dose required for half-maximal inhibition of food intake in hamsters in SP compared to those in LP. MT\(_{II}\) significantly increased grooming in both LP and SP. Our results indicate that the melanocortin system is a potent short-term regulator of food intake. However, the lack of differential response or sensitivity to MT\(_{II}\) treatment in the obese (LP) vs lean (SP) states do not support the hypothesis that changes in this melanocortin pathway underlie the long-term decrease in food intake which occurs in this seasonal model.
Introduction

The hypothalamus plays a key role in the regulation of energy homeostasis via its neural and endocrine outputs. While both anabolic and catabolic components of this system have been characterised, most studies have focused upon their involvement in the defence against acute energy deficits (for review see 30). Studies on a number of rodent species, including seasonal animals, provide compelling evidence that body weight is defended, at least in some species, within certain limits. Weight loss during dieting may engage these powerful compensatory mechanisms that increase hunger and reduce metabolic rate, so most dieters return to their original weight in the long term (20). Maintenance of weight loss in the long term may only be possible if therapeutic strategies are developed to counteract these compensatory mechanisms.

Studies from several animal models provide evidence that the level at which compensatory mechanisms operate can be altered. This plasticity is particularly clear in mammals that have evolved under seasonal conditions and consequently display profound annual cycles of weight gain and loss (25). For example, under increasing or long photoperiods the Siberian hamster (*Phodopus sungorus*) defends its maximum body weight, whereas under decreasing or short photoperiods it enters a catabolic state and gradually loses body weight (10, 22, 31), mainly through a reduction in intraperitoneal fat stores (5). The reduction in body weight takes approximately 12 to 15 weeks to reach the maximum weight loss of up to 40% of initial weight (24, 29, 31).

The central mechanisms underlying this transition to a catabolic state are not understood. The aim of the current study was to investigate the role of the melanocortin system in this transition, since this has been shown to be an important component of the appetite regulating system in experimental rodents and man. Neurons expressing pro-opiomelanocortin (POMC) are localised in the ventrolateral region of the arcuate nucleus, and a major cleavage product of POMC,
α-melanocyte stimulating hormone (α-MSH) has been shown to inhibit feeding via an action on the melanocortin-4 receptor (MC4-R). MC4-R deficient mice are hyperphagic and obese (18) and mutations in MC4-R or its associated signalling pathways have been observed in association with extreme obesity in man (33). Intracerebroventricular (icv) injections of α-MSH and Melanotan-II (MTII) a specific synthetic MC3/4-R agonist, potently inhibit food intake in mice and rats (8, 11, 17, 19, 32). MC4-R mRNA is detected in hypothalamic sites which play important roles in the control of feeding behavior, including the ventromedial, lateral, dorsomedial and paraventricular nuclei (26).

We adopted a functional approach to investigating whether changes in melanocortin mechanisms might contribute to the weight loss and reduced food intake which occurs in hamsters in SP. Previous studies in Siberian hamsters have shown that the effectiveness of some putative satiety peptides (bombesin and cholecystokinin) is dependent upon a change in the photoperiod (4), whereas sensitivity to certain orexigenic peptides (NPY) does not change (7). We tested whether icv administration of the MC3/4-R agonist MTII could influence food intake, grooming, resting and other activities in food-deprived and ad libitum fed hamsters. We then tested whether the suppression of food intake by MTII differed between obese (LP) and lean (SP) hamsters, predicting that if increased activity of the endogenous melanocortin system contributes to the generation and maintenance of this catabolic state in SP, then we would observe a diminished response to MTII in SP.
Materials and methods

Animals and housing

A colony of Siberian hamsters (*Phodopus sungorus*) was maintained in a temperature controlled room (21 ± 1°C) on a 16 h light / 8 h dark cycle (LP; lights on from 20.00 h to 12.00 h) with red light (~10 lux) during the dark period. At a minimum age of 8 weeks, gonadally-intact male Siberian hamsters weighing 35-45 g were separated into single transparent cages and kept individually during all experimental treatments. Two groups of animals used in Experiments 1 and 2 stayed in LP. A third group of hamsters used in Experiment 3 was transferred to a short photoperiodic room on a 8 h light / 16 h dark cycle (SP; lights on from 04.00 h to 12.00 h) with red light (~10 lux) during the dark period. Lab chow and water were allowed *ad libitum* until the start of each experiment. For Experiment 1, food was withheld for 24 h before icv vehicle or MTII injection. All animal procedures were carried out in accordance with the UK Home Office Animals Scientific Procedures Act 1986 (Project Licence no. PPL 80/1463) and are consistent with the guiding principles for research of the American Physiology Society (3).

Intracerebroventricular (icv) cannulation

After 7 weeks in LP or SP, hamsters were anesthetized with a mixture of ketamine (0.4 mg/kg) and xylamine (2 mg/kg, i.p.) and placed in a stereotaxic frame with height of the incisor bar set at the intra-aural level. A hole was drilled in the skull on midline at the point of Bregma and a 22 gauge stainless steel cannula guide measuring 10 mm lowered to 6.5 mm below the surface of the dura, after deflection of the superior mid-sagittal sinus. This guide cannula was cemented to stainless steel screws attached to the skull.
An obturator (29 gauge stylet) was inserted into the guide cannula to maintain patency. After the completion of each experiment, icv cannula placement was verified by histological examination. Only the animals that showed appropriate placement of the guide cannula into the third ventricle were included in the analysis.

**Acclimation**

Before any experimental trials were conducted, hamsters were allowed to recover from surgery for 2 weeks, during which time they were handled daily. Three days before the first icv vehicle or MT$_{II}$ injection, all hamsters were acclimated to the icv injection procedure and to the food test regimen. Just before the beginning of the dark phase, the stylet was removed to check cannula patency and to simulate the injection procedure in order to reduce stress on the experimental days. At the beginning of the dark phase, a known weight of food pellets were given to the hamsters and the amount of food removed was measured 6 h later (see measurement of food intake).

**Peptide, peptide administration and doses injected**

Ac-Nle-Asp-His-D-Phe-Arg-D-Trp-Lys-NH$_2$ (Melanotan-II; MT$_{II}$) was purchased from Bachem (UK). MT$_{II}$ was dissolved in PBS (0.01M phosphate-buffered 0.9% saline, pH = 7.4).

Intracerebroventricular infusions were given to hamsters in their home room during the 45 min prior to the beginning of the dark phase (11.15-12.00 h). Infusions were carried out over 1 min in conscious unrestrained hamsters, via a 29 gauge injection cannula inserted in the guide cannula, connected to a 10 µl Hamilton syringe with fine polyethylene tubing. A Harvard infusion pump delivered the vehicle or test substance at a rate of 1 µl per minute. The injection cannula was left
in place for 3 min to allow diffusion of the injected solution. Animals were injected with 1 µl of vehicle (PBS) or MT\textsubscript{II}. The cannula stylet was replaced immediately after withdrawal of the infusion cannula. Each hamster received all the treatments in random order; those which did not receive all the treatments were excluded from the study. Each hamster did not receive more than one injection per week.

In Experiment 1 (n = 12), hamsters maintained in LP were food-deprived for a 24 h period and infused with 1 µl of vehicle (PBS) or MT\textsubscript{II} (5 µg/µl). In Experiment 2 (n = 10), \textit{ad libitum} fed Siberian hamsters in LP were injected with 1 µl of vehicle or MT\textsubscript{II} (0.3, 0.6, 1.25, 2.5 or 5 µg/µl). In Experiment 3 (n = 12), \textit{ad libitum} fed Siberian hamsters in SP were injected with 1 µl of vehicle or MT\textsubscript{II} (0.3, 1.25 or 5 µg/µl) and all injections were carried out between week 9 and week 13 in SP.

\textit{Measurement of food intake}

Immediately before drug infusion, the used sawdust was replaced in each cage in order to eliminate any food hoards. To reduce novelty induced-stress, we kept the used paper bedding in each cage. Food was removed from the hopper, and dry food was soaked in tap water for 30 min. Wet food has the advantage of higher palatability than dry pellets, while its consistency reduces spillage and prevents hamsters from storing food in their cheek pouches (Schuhler and Ebling, unpublished observations). In order to estimate the reduction in weight of the test meal through water evaporation, pre-weighed dishes containing wet pellets were also placed in control cages alongside the experimental hamsters. All studies were carried out at the beginning of the dark phase (12.00 h). Six hours later (at 18.00 h), all dishes containing the food were collected and weighed. The calculation of the amount of food taken by each hamster during 6 h included the deduction of the mean evaporation of water of 4 controls adjacent to the experimental
In Experiments 2 and 3, we used this protocol on the injection day (day 1; D1), and on the two following days (day 2; D2 and day 3; D3).

**Behavioral scores**

On the injection day, under red light of approximately 10 lux at cage level, each hamster was observed in its home cage for 5 seconds in every minute for periods of 30 minutes, as indicated below. Each behavior was scored as being present or absent in the 5 second period and recorded in a table. Six hamsters were observed in Experiment 2 (*ad libitum* fed hamsters in LP) and 8 in Experiment 3 (*ad libitum* fed hamsters in SP). Five behavioral categories (whose definitions are based on reference 15) were used in this study: 1) Feeding (biting, gnawing or swallowing food from wet mash dish), 2) drinking (licking the water bottle spout), 3) in activity (locomotion, sniffing and rearing), 4) grooming (scratching, licking or biting of the fur, whiskers, feet or genitals) and 5) resting (sitting or lying in a relaxed position). Three periods of 30 min behavioral observation were studied: 1) First period: from 12.00 h to 12.30 h (immediately after lights off); 2) Second period: from 14.45 h to 15.15 h (in between the first and the third period); 3) Third period: from 17.30 h to 18.00 h (prior to the weigh of the food).

**Statistical analysis**

Statistical analyses of food intake measures were carried out using Prism 2.01 (GraphPad Software, San Diego, CA) statistical software. Data are presented as group mean values ± S.E.M. A Student’s *t*-test was used for two group comparisons (Experiment 1). For experiments with greater than two study groups (Experiments 2 and 3), a two-factor ANOVA with repeated measures was used initially to determine the effects of photoperiod and treatment, and the interaction between photoperiod and doses.
of MT$_{II}$ on food intake and behavioral scores (Statview, Calabas, USA). Subsequently, within each photoperiod, MT$_{II}$-treated groups were compared with the vehicle group using a Dunnett’s procedure for post-hoc comparisons. A P value < 0.05 between group mean values was considered statistically significant.
Results

Experiment 1: **Effect on food intake of icv injection of MT\textsubscript{II} in 24h-food deprived Siberian hamsters in LP**

Food intake in the 6 hour post infusion period was significantly reduced by 55 % after icv injection of 5 µg/µl of MT\textsubscript{II} compared to vehicle injection (Fig. 1; t-test: $P < 0.0001$).

- Fig. 1-

Experiment 2: **Effect on food intake of icv injection of MT\textsubscript{II} in ad libitum fed Siberian hamsters in LP.**

On the day of injection (D1), MT\textsubscript{II} at the dose of 5 µg/µl significantly reduced food intake by 83% relative to the vehicle control treatment (Fig. 2; $P < 0.01$). Mean food intake was 18 % less than the vehicle control treatment on the second day after the injection (D2), and was 24 % higher than the control day on the third day after the injection (D3, Fig. 2). MT\textsubscript{II} inhibited food intake in a dose-dependent manner (Fig. 3). Four doses of MT\textsubscript{II} (0.6, 1.25, 2.5 and 5 µg/µl) led to a significant reduction in food intake (49 %, 68 %, 79 % and 83 % respectively; $P < 0.01$). The lowest dose (0.3 µg/µl) did not significantly reduce food intake (31 % less than vehicle treatment).

- Figs. 2 and 3-
Experiment 3: Effect on food intake of icv injection of MT\textsubscript{II} in ad libitum fed Siberian hamsters in SP.

All icv injections were carried out between week 9 and week 13 in SP, at a time when the hamsters’ body weight had decreased. A two factor ANOVA revealed an overall significant effect of the MT\textsubscript{II} dose (F=41.2, $P < 0.001$), but no significant effect of the photoperiod nor any interaction between dose and photoperiod was observed. Post-hoc tests revealed that MT\textsubscript{II} at the dose of 5 $\mu$g/\mu l significantly decreased food intake by 70 % relative to the vehicle treatment (Fig. 4; $P < 0.01$). Compared to vehicle, the mean food intake was 19 % lower on D2 and 8 % higher on D3, but these values were not significantly different from the control period. MT\textsubscript{II} inhibited food intake in a dose-dependent manner (Fig. 5). MT\textsubscript{II} at doses of 5 and 1.25 $\mu$g/\mu l led to significant reductions in food intake (70 % and 61 % respectively; $P < 0.01$). At a dose of 0.3 $\mu$g/\mu l, MT\textsubscript{II} was also able to decrease food intake significantly (32 %; $P < 0.05$).

Behavioral scores

In the first period of behavioral observation shortly after treatment (Fig. 6A), two factor ANOVA revealed an overall significant effect of the MT\textsubscript{II} dose (F=6.19, $P < 0.001$) and of the photoperiod (F=5.92, $P < 0.05$) on the proportion of time spent feeding (Fig. 6A). However, there was no interaction of the two main effects, thus the dose–dependent suppression of time spent engaged in feeding behavior did not differ between long and short days. No drinking behavior was observed when hamsters were provided with a test meal, i.e. hamsters did not approach their water bottles. MT\textsubscript{II} dose-dependently increased the time spent grooming (F=7.82, $P < 0.001$), but no significant effect of the
photoperiod nor any interaction between dose and photoperiod was observed on grooming (Fig. 6A). MT$_{II}$ dose-dependently reduced the proportion of time spent resting ($F=10.8$, $P<0.0001$), but no significant effect of the photoperiod was observed. MT$_{II}$ did not significantly affect the proportion of time that the hamsters were engaged in other types of activity (Fig. 6A).

In the second period of behavioral observation, approximately three hours after treatment (Fig. 6B), two factor ANOVA revealed that MT$_{II}$ dose-dependently decreased the proportion of time spent feeding ($F=3.28$, $P<0.05$). No significant effect of the photoperiod or any interaction between dose and photoperiod was observed on the time spent feeding. MT$_{II}$ dose-dependently increased the time spent grooming ($P<0.05$), but there was no significant effect of the MT$_{II}$ treatment on the proportion of time spent engaged in other activity or resting, during this observation period (Fig. 6B).

In the third period of behavioral observation, approximately six hours after treatment (Fig. 6C), MT$_{II}$ had no significant effect on the proportion of time spend feeding or grooming, and there were no significant effects of photoperiod on these two parameters, nor were there significant interactions between MT$_{II}$ treatment and photoperiod. However, there was a significant effect of MT$_{II}$ on the proportion of time spent engaged in other activity ($F=5.43$, $P<0.005$) and conversely on the proportion of time spent resting ($F=3.75$, $P<0.05$). There was also a significant overall effect of photoperiod on these two parameters, but no significant interaction between dose and photoperiod, indicating that the effect of the MT$_{II}$ treatment on general activity and rest did not differ between the photoperiodic conditions.

- Fig. 6-
Discussion

Our results show that intracerebroventricular administration of MT$_{II}$ (a MC3/4-R agonist) was able to exert a potent anorexigenic effect in Siberian hamsters in the three conditions tested. When the animals were in a hungry state after being deprived of food for 24 h, MT$_{II}$ at the dose of 5 $\mu$g/$\mu$l significantly decreased food intake by 55 %. Although it is common practise to test the anorectic potential of compounds in food-restricted rodents, hypothalamic gene expression is modified by short-term food restriction in Siberian hamsters as in other species (1, 23, 24, 28, 29), so in subsequent experiments we chose to investigate the melanocortin system in animals fed ad libitum. Hamsters were studied at the start of the dark phase when their circadian system would dictate a hungry state - a more physiological condition of hunger in which short-term compensatory responses to complete dietary restriction would not be able to influence the response to a melanocortin agonist. In such ad libitum fed hamsters maintained in LP, food intake after vehicle injection was lower than in the food-restricted group, and the significant decrease of food intake induced by 5 $\mu$g/$\mu$l of MT$_{II}$ was greater than in the first experiment (83 %). In ad libitum fed hamsters maintained in SP, food intake after vehicle injection was slightly higher over the first 6 hours of the dark phase than in ad libitum fed hamsters maintained in LP, and was similar to the food intake in the restricted group in LP (Experiment 1). It is our previous experience that food intake over 24 hours is reduced by approximately 20% in hamsters in SP, either when measured chronically (Ebling et al., 1998), or in acute food intake trials (Reddy et al., 1999). Nevertheless, MT$_{II}$ at the dose of 5 $\mu$g/$\mu$l was able to decrease significantly food intake by 70 %. In both ad libitum fed groups (LP and SP), the food intake on the second day after the injection (D2) tended to be lower than the control period, which
may indicate that MT$_{II}$ has a prolonged duration of action and is consistent with previous studies in rats (11, 32).

In *ad libitum* fed hamsters in both LP and SP, MT$_{II}$ led to a dose-dependent reduction in food intake with doses as low as 0.3 µg/µl in SP and 0.6 µg/µl in LP. ANOVA revealed no significant interaction between dose and photoperiod. We therefore infer that there is no differential sensitivity between hamsters in LP and SP. Although the post-hoc tests indicate that the effect of 0.3 µg/µl treatment was not significant in LP, the magnitude of the effects of MT$_{II}$ is broadly similar in LP and SP (reduction of 31 % and 32 % respectively) as it is the case for 1.25 µg/µl MT$_{II}$ (reduction of 68 % in LP and 61 % in SP).

Suppression of food intake can occur for many reasons, including nausea, malaise, sedation, or induction of other behaviors which conflict with ingestion. In rats, administration of MT$_{II}$ into the third ventricle was found to induce conditioned taste aversion (32). This effect is believed to be due to the action of MT$_{II}$ on MC3-R rather than on MC4-R (6). In hamsters, we can not exclude that the reduction of food intake induced by MT$_{II}$ may be a consequence of a taste aversion. In rats, MT$_{II}$ significantly reduced water intake (27). In our study, we never observed any drinking behavior in vehicle or MT$_{II}$-treated hamsters. Hamsters are omnivores that have evolved under arid conditions, and are rarely observed drinking from water bottles, particularly when their food supply has a significant water content. The moisture content of the test meal may have masked any effect of MT$_{II}$ on drinking per se, but represents a biologically relevant test. The reduction of food intake by MT$_{II}$ may well relate to the induction of grooming by this agonist, because in the first and the second periods of behavioral observation, MT$_{II}$ significantly increased the proportion of time spent grooming. As a probable consequence of this increase of grooming, the time spend resting was
decreased significantly. The induction of grooming behavior by melanocortin receptor agonists was also reported in rats (2). Indeed excessive grooming can be induced by icv injections of α-MSH in the rat, and this effect can be blocked by MC4-R antagonists, suggesting that melanocortin-induced grooming is mediated by the MC4-R (2). Intravenous injections of MTII were also able to induce grooming in the rat and this effect was blocked by SHU9119, a MC3/4-R antagonist (2). The significance of MC4-R activation for physiologically evoked grooming is still unclear.

The potency of administration of MTII into the third ventricle of hamsters is consistent with an intrahypothalamic site of action of MC3/4-R agonists. In mice and rats, MTII or α-MSH causes significant and long lasting reductions in food intake when injected in the third ventricle (11, 32) or into the paraventricular nucleus of the hypothalamus (9, 12, 21, 34). Moreover, in the rat, icv injections of MTII induced c-Fos expression in the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala (32) and MC4-R mRNA was detected in the ventromedial, lateral, dorsomedial and paraventricular nuclei (26). Fibers containing POMC are also found in these nuclei (16).

In Siberian hamsters, Mercer et al. (24) detected MC3-R mRNA in the arcuate nucleus and in the ventromedial nucleus, and MC4-R gene expression was found in the PVN.

In addition to actions within the hypothalamus, our results do not rule out the possibility that MTII may also act in lower brainstem sites, as compounds delivered into the third ventricle will clearly reach regions adjacent to the fourth ventricle. Indeed, MC4 receptors are widespread in the caudal brainstem (26) and melanocortin receptor ligands delivered to the caudal brainstem have clear effects on food intake in rats (13, 14).

In conclusion, the current studies demonstrate that the melanocortin agonist MTII is a potent suppressor of food intake in the Siberian hamster in LP (after 24h food deprivation and ad libitum fed) and in SP (ad libitum fed). Our results do not provide
clear evidence of differential response or sensitivity in the obese (long day) vs lean (short day) states. The behavioral observations show that MT\textsubscript{II} influences processes other than food intake, including grooming, which is consistent with the view that in addition to direct effects on satiety, melanocortins affect multiple systems that ultimately influence energy balance.
Acknowledgements

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Abbreviations

α-MSH: α-melanocyte stimulating hormone
AGRP: agouti-related peptide
Icv: Intracerebroventricular
LP: Long photoperiod
MC3-R: Melanocortin-3 receptor
MC4-R: Melanocortin-4 receptor
MT-II: Melanotan-II
PBS: Phosphate-buffered saline
POMC: Pro-opiomelanocortin
SP: Short photoperiod
PVN: Paraventricular nucleus
References


Figure Legends

Fig. 1: Food intake over 6 hours after icv injection of vehicle or MT$_{II}$ (5 µg/µl) in 24 h food-deprived Siberian hamsters in long photoperiod at the start of the dark phase (n = 12). Values are expressed as mean ± SEM; **$P < 0.0001$ vs vehicle.

Fig. 2: Food intake over 6 hours after icv injection of MT$_{II}$ (5 µg/µl) or vehicle in *ad libitum* fed Siberian hamsters in long photoperiod at the start of the dark phase (n = 10). **$P < 0.01$ vs vehicle. D1: day of injection; D2: second day after the injection; D3: third day after the injection.

Fig. 3: Dose-response relationship between doses of MT$_{II}$ and the amount of food taken on the 6 hour post treatment period in *ad libitum* fed Siberian hamsters in long photoperiod at the start of the dark phase (n = 10). **$P < 0.01$ vs vehicle.

Fig. 4: Food intake over 6 hours after icv injection of MT$_{II}$ (5 µg/µl) or vehicle in *ad libitum* fed Siberian hamsters in short photoperiod at the start of the dark phase (n = 12). **$P < 0.01$ vs vehicle. D1: day of injection; D2: second day after the injection; D3: third day after the injection.

Fig. 5: Dose-response relationship between doses of MT$_{II}$ and the amount of food taken on the 6 hour post treatment period in *ad libitum* fed Siberian hamsters in short photoperiod at the start of the dark phase (n = 12). All icv injections were carried out between week 9 and week 13 in SP. *$P < 0.05$ and **$P < 0.01$ vs control.
Fig. 6: Behavioral effects of MT_{II} (0.3, 1.25 and 5 μg/μl icv) or vehicle in _ad libitum_ fed Siberian hamsters in long photoperiod (n = 6) and short photoperiod (n = 8). *P < 0.05 and **P < 0.01 vs vehicle. Three periods of 30 min behavioral observation were studied: (A) First period: from 12.00 h to 12.30 h (immediately after lights off); (B) Second period: from 14.45 h to 15.15 h (mid-way between the first and the third period); (C) Third period: from 17.30 h to 18.00 h (prior to the measurement of food intake).
Fig. 1

![Graph showing food intake comparison between Vehicle and MT$_{II}$ (5µg/µl).](image)

- Food intake (g)

Vehicle

MT$_{II}$

(5µg/µl)
Fig. 2

Food intake (g)

Vehicle  D1  D2  D3

**

MT (5µg/µl)
Fig. 3

Percentage of food intake (%)

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** Significant difference from Vehicle

MTII (µg/µl)
Fig. 4

Food intake (g)

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Food intake (g)
Fig. 5

Percentage of food intake (%) vs. MT$_{II}$ (µg/µl)
Fig. 6

A  
Period 1

B  
Period 2

C  
Period 3

**Legend**

- Resting
- Grooming
- Active
- Feeding

**Graphs**

- Period 1: Vehicle, MT II (0.3), MT II (1.25), MT II (5)
- Period 2: Vehicle, MT II (0.3), MT II (1.25), MT II (5)
- Period 3: Vehicle, MT II (0.3), MT II (1.25), MT II (5)