Novel restraint system for neuroendocrine studies of socially living common marmoset monkeys

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Summary

We describe a novel soft jacket and sling-harness restraint that permits species-typical postures for small-bodied primates, such as the common marmoset (*Callithrix jacchus*), during long-term (>6 h), continuous restraint. The restraint system is straightforward to use and manipulate, it is easily repaired, and the materials used are readily available. The soft jacket allows for increased versatility and longevity, and the sling-harness provides for greater movement and much longer duration of continuous restraint (up to 3 days) compared to a previously described, more conventional chair restraint for small-bodied primates. The new restraint system prevents the normal diurnal decrease in plasma cortisol levels across the daylight hours; however, it does not disrupt ovulatory cycles. Unlike the previously available techniques, therefore, this new restraint system is applicable to many neurobiological and neuroendocrine studies involving small-bodied, non-human primates and is especially suited to investigations requiring the maintenance of relationships within social groups.

Keywords Marmoset; restraint; long-term sampling; reproductive neuroendocrinology; behavioural adaptation; methods; cortisol

Effective and well-tolerated drug delivery and restraint systems are essential for studies of the neural mechanisms regulating physiology and behaviour. While such systems are available for large primates, such as macaques (Friday & Lipkin 1990) and baboons (Coelho & Carey 1990), suitable delivery and restraint systems for small primates are not as well developed (Blum *et al.* 1983, O'Byrne 1988, O'Byrne & Morris 1988).

Common marmosets, *Callithrix jacchus*, are anthropoid, New World primates and are increasingly being used in biomedical research (Scott 1994, Owen *et al.* 1997, Abbott *et al.* 2003, Mansfield 2003). The marmoset's small size (350–400 g), tractability, high fecundity potential (typically with twin births every 5–6 months) and rapid maturation (adult by 18 months of age) provide clear advantages over the larger, less fecund and slower-maturing macaques

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conventionally used in biomedical research (Abbott 1992). Marmosets make excellent subjects for studies in neurobiology, neuroendocrinology and behaviour because they adapt easily to a variety of experimental procedures, including venepuncture (Hearn 1977, 1983), venous cannulation (O'Byrne 1988), hypothalamic push–pull perfusion (Abbott *et al.* 1998), operant testing (Pryce *et al.* 1993), learning paradigms (Collins *et al.* 1998), functional magnetic resonance imaging (Ferris *et al.* 2001) and social testing procedures (Saltzman *et al.* 1996, Baker *et al.* 1999). These monkeys also appear to be comfortable in small, enclosed spaces (Ferris *et al.* 2001).

Common marmosets share numerous biochemical, physiological and behavioural similarities with humans. For example, marmosets exhibit a 28-day ovarian cycle, establish complex, long-lasting social relationships, and live in small, family-based social units (Abbott 1992). Offspring remain within their natal group into adulthood, and all group members contribute to infant care, including extensive carrying and post-weaning provisioning of infants (French 1997, Tardif 1997). In contrast to humans, however, the behaviourally dominant female in marmoset social groups (the mother in family groups) is usually the only female to conceive and give birth (Abbott 1984, 1987, 1993). At the same time, the ovulatory cycles of subordinate females in social groups are suppressed in response to a combination of behavioural, visual and olfactory cues from the dominant female, so that subordinate females are usually anovulatory and hypo-oestrogenemic (Abbott *et al.* 1988, 1993, 1997, Barrett *et al.* 1990, 1993). The common marmoset therefore provides a specialized primate model for determining mechanisms mediating the social regulation of reproduction.

To investigate the neuroendocrine mechanisms underlying the social control of ovulation in marmosets, we needed to significantly refine previously described restraint techniques to permit long-term (up to 3 days) assessment and manipulation of hypothalamic and pituitary function. An improved methodology would also provide new opportunities for neurobiological investigation in a wide range of studies utilizing common marmosets and other small-bodied primates. Our immediate goal was to develop a well-tolerated restraint system that would permit a moderate degree of movement while enabling repeated intravenous and hypothalamic sampling of the marmoset in close proximity to its social group members. A backpack previously described for use with marmosets did not have the versatility necessary for our proposed studies (Ruiz de Elvira & Abbott 1986) as it was designed to accommodate only osmotic mini-pumps housed in small, fluid-filled (5 ml) chambers. Furthermore, a previously described restraint system for marmosets was limited to a maximum of 6 h of continuous use (O'Byrne & Morris 1988). Since the previous restraint system did not allow for species-typical resting postures, it could not accommodate the minimum of 3 days of continual restraint required for hypothalamic perfusion studies (Gearing & Terasawa 1988).

To permit such long-term restraint, we developed a soft jacket that serves as both (1) a pocket for an indwelling, intravenous cannula and (2) an integral part of a novel, sling-harness restraint. This system permitted such complex techniques as hypothalamic push–pull perfusion with simultaneous sampling from an intravenous cannula. Our findings demonstrated that marmosets quickly adapted to 3 days of continuous restraint in this novel system, and validated the system's usefulness in neurobiological investigations using the marmoset.

Materials and methods

Animals

Subjects were 17 captive-born adult (2–8 years of age) female common marmosets (*Callithrix jacchus*) housed at the National Primate Research Center, University of Wisconsin (NPRC), as previously described (Saltzman *et al.* 1998). NPRC is fully accredited by AAALAC as part of the University of Wisconsin–Madison Graduate School. Animal protocols and experiments were approved by the Graduate School Animal

Care and Use Committee of the University of Wisconsin, Madison. The animals were maintained according to recommendations of the Guide for the Care and Use of Laboratory Animals (US National Research Council) and the United States Animal Welfare Act with its subsequent amendments.

The 11 ovary-intact and six bilaterally ovariectomized female subjects were either pair-housed with males or housed in social groups (Abbott 1984, Saltzman *et al.* 1994). Ovary-intact females exhibited regular ovulatory cycles. Bilateral ovariectomy was performed using a ventral midline approach 129 ± 43 days (mean ± SEM) prior to first restraint. We formed social groups by introducing four unrelated adults of each sex into a large observation room (363 × 212 × 218 cm) containing nest boxes and tree branches. Individuals were removed in cases of behavioural incompatibility (usually within 3–14 days), and the resulting stable group comprised one dominant female and 1–3 subordinate females along with 1–3 males, as previously described (Saltzman *et al.* 1998). Social groups remained together, with a stable hierarchy, for 2 months to over 3 years, as described in an earlier study (Abbott & George 1991).

Sling-harness restraint

Jacket for sling-harness restraint

The jacket was substantially improved from an initial backpack system designed for marmosets by Ruiz de Elvira and Abbott (1986). The outer layer of heavy sailcloth (Gallagher Tent and Awning, Madison, WI, USA) was lined with brushed flannel, and a 5 cm wide Velcro strap fastened the back flap. The new materials now provided the pliability, versatility and longevity absent from previous designs.

This new version of the jacket incorporated the addition of a sailcloth sleeve onto the back flap of the jacket (Fig 1) containing

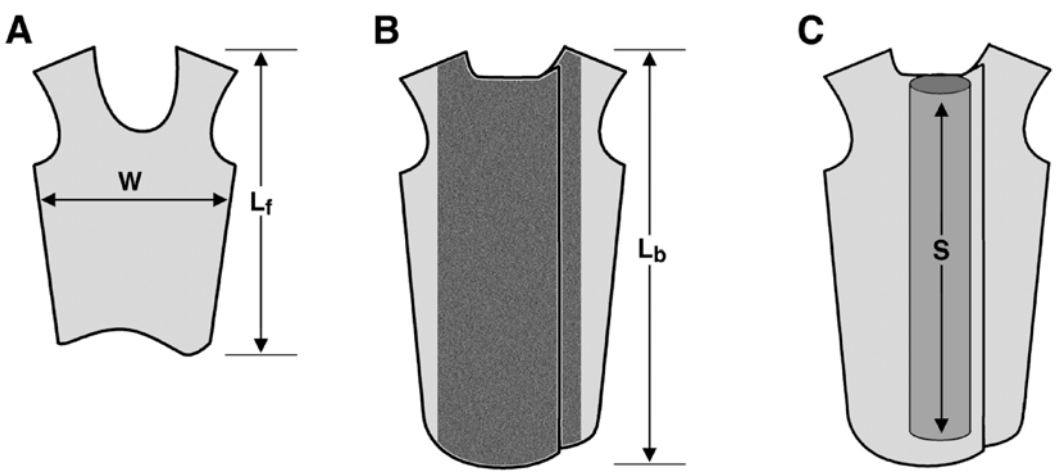

Fig 1 Design of the jacket. (A) Front view, including the standard frontal length (L_f = 7 cm) width (W = 8 cm) measured at a point about 5 mm below the bottom of the spaces for the arms, with a slightly tapered lower portion to accommodate the width of the monkey's abdomen. (B) Back view illustrating the jacket configuration that houses an intravenous/atrial cannula without the cylindrical sleeve for attachment to the restraint apparatus. The dotted area represents the Velcro attachment on the underside of the outer flap affixed to Velcro sewn on to the back of the jacket, thus permitting an adjustable width across the back. The standard length (L_b) of each back section is 12 cm. The part of the jacket underneath the Velcro fastening (dotted area) also includes the pouch (approximately 2.5 cm × 4.0 cm) for housing an intravenous/atrial cannula. (C) Back view illustrating the jacket configuration with the additional cylindrical sleeve for attachment to the restraint apparatus. The design is identical to that housing the intravenous/atrial cannula, except that an additional, cylindrical sleeve (S = length, 12.5 cm; diameter: 1.5 cm) was sewn onto the outer flap of the back of the jacket to accommodate attachment to the angled support arm on the sling-harness restraint (Fig 2)

a thin aluminium bar (11.5 × 1.8 × 0.2 cm). The bar provided increased stability and rigidity to the sleeve when it was fitted on to the angled part of the support bar of the sling-harness restraint (Fig 2). The new jacket still contained a pocket on the back for housing the exteriorized portion of an intravenous cannula, similar to that described by Ruiz de Elvira and Abbott (1986). To fit the jacket to an adult marmoset, one person manually restrained the marmoset using leather gloves while another slipped the open jacket up the animal's forelimbs and over the shoulders, fastening the Velcro attachment around the animal's back (Fig 1).

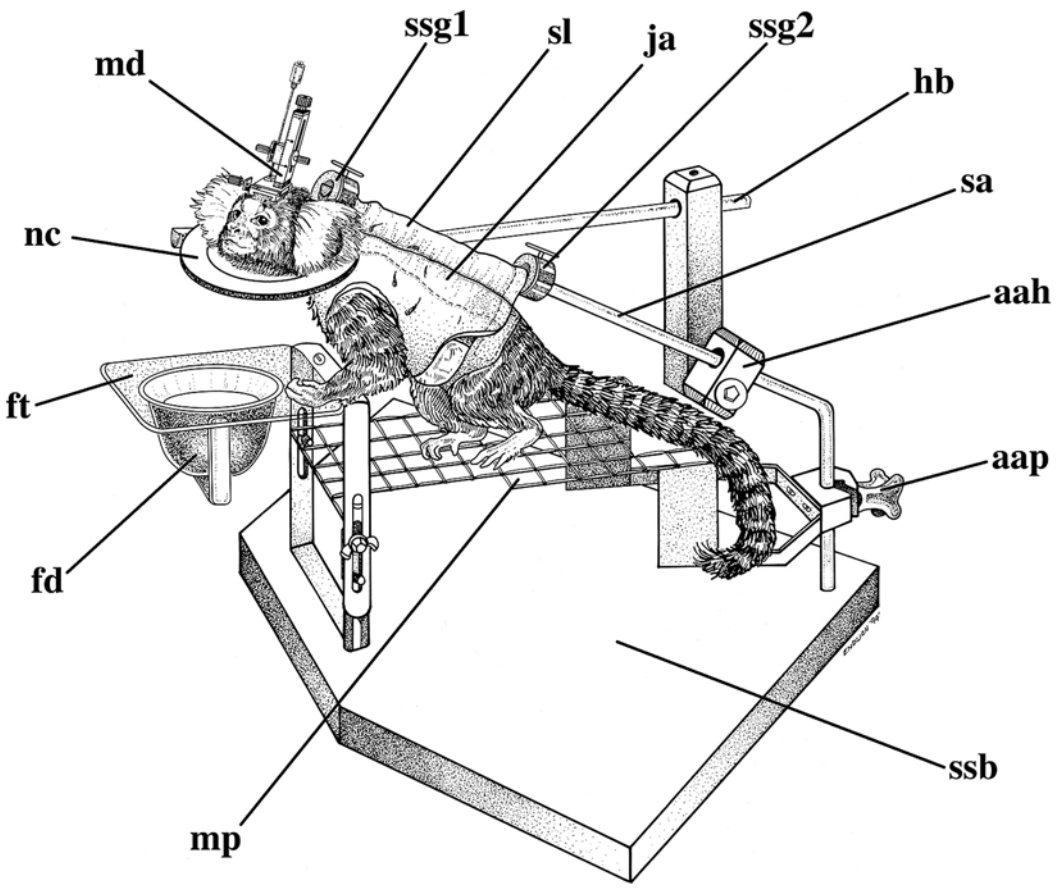

Fig 2 Side view of a marmoset in the sling-harness restraint. The entire restraint is supported by a stainless steel base (ssb; length of the right side of the base: 35 cm). The neck collar (nc) is attached for head restraint during hypothalamic push–pull perfusion, and the monkey has an intracranial cannula maintained in precision placement by a small microdrive unit (md; Narishige Scientific Instrument Laboratory, Tokyo, Japan). The jacket (ja) shown is the configuration for sling-harness restraint (Fig 1C). The sleeve (sl) on the back of the jacket has been slipped down the angled part of the support arm (sa), and stainless steel grips have been fastened above (ssg1) and below (ssg2) the sleeve to hold it in position. Immediately prior to hypothalamic push–pull perfusion, the neck collar is attached to a stainless steel holding bar (hb) by two nuts and bolts placed through pre-drilled 0.5 cm holes (not shown) in the side of the neck collar. The holding bar has an adjustable attachment (aah) on the angled part of the support arm below the jacket attachment. The monkey's feet are supported by a stainless steel, mesh platform (mp) that has an adjustable attachment (aap) near the base of the vertical part of the support arm. The monkey's left hand is resting on the food tray (ft) that holds a food dish (fd; 5 cm in diameter and 6 cm deep)

Sling-harness restraint apparatus

The sling-harness restraint consisted of a stainless steel base (approximately 35 × 35 cm) upon which an angled support bar (1.2 cm diameter, stainless steel, flattened on one side) was mounted (Fig 2). A marmoset wearing the jacket was fitted to the restraint by slipping the sleeve of the jacket over the angled part of the support bar until the top of the support bar protruded approximately one centimetre above the top of the sleeve (Figs 2 and 1C). An adjustable stainless steel grip (which had been slipped over the support bar prior to attachment of the sling-harness jacket) was then moved up the support bar and tightened into position at the base of the jacket's sleeve to prevent the jacket sliding farther down the support bar (Fig 2). An additional stainless steel grip was then placed over the top one centimetre of the support bar and tightened into position to prevent the jacket sliding off the top of the support bar. The marmoset was then free to move into a variety of postures, including standing, squatting or huddling, as its feet were supported by an adjustable stainless steel mesh platform (25.5 × 16.0 cm) attached to the lower (vertical) part of the support bar.

A food tray, adjustable for both height and distance from the restrained animal, was affixed to the front of this platform. The food tray and platform were moved as necessary to accommodate the many positions assumed by marmosets, including those during sleep. Soft towels were positioned on the platform to facilitate a huddling posture, and, during periods of dim lighting when the main room lights were off (18:00–06:00 h), a soft, folded towel was positioned on top of the cleaned food tray to provide ventral support to the head and neck. A huddled body posture is one of many that are typical and well-described for common marmosets (Stevenson & Rylands 1988).

To minimize the marmoset's ability to manipulate an acutely implanted hypothalamic cannula, a neck collar was fitted to the marmoset when the animal was first placed in the sling-harness restraint. The two halves of the circular plastic neck collar (outer diameter: 10 cm; inner diameter: 3.5 or 3.1 cm, depending on the neck size of the marmoset; depth: 0.5 cm) were held together by an 'O-ring'. A tongue-in-groove arrangement enabled the two halves of the collar to be slotted together. To minimize head movement during hypothalamic push–pull perfusion, the neck collar was attached to a stainless steel holding bar (the position shown in Fig 2) by placement of two nuts and bolts through pre-drilled 0.5 cm holes in one side of the neck collar. The holding bar had an adjustable attachment to the angled support bar at a point below the marmoset's jacket and above the platform's attachment to the base of the restraint. An adjustable hinge on the attachment of the holding bar to the support arm provided the needed flexibility for setting a locked position of the neck collar that was well-tolerated by the marmoset.

Training of animals in the sling-harness restraint

Monkeys commenced their adaptation to the sling-harness restraint well before (220 ± 69 days) they were first used in a hypothalamic push–pull perfusion experiment. Training comprised a series of trials of increasing duration and was performed in a procedure room separate from the animals' regular housing rooms. During these trials, restrained animals remained in full view of their cage mates, which had been placed inside a small cage (61 cm × 46 cm × 61 cm; equipped with perches, sleeping box and bedding) positioned to within 0.3 m of the front of the sling-harness restraint. Training began with one hour of restraint on day 1, 4 h on day 2, 8 h on day 3 or 4, and overnight restraint (24 h) starting on day 7 or 8. This schedule facilitated optimal adaptation to the restraint. During training, marmosets learned to feed themselves and to drink fluids offered manually from a syringe. Once adapted to the restraint (see Results), each marmoset underwent an additional 8 h restraint session once every 6 months to maintain adaptation. The head was not restrained during training.

Maintenance of animals in the sling-harness restraint

Food and fluids were offered hourly to restrained animals during lights-on (06:00–18:00 h). Food items included a specialized marmoset diet (ZuPreem, Shawnee Mission, KS, USA), marmoset jelly (Mazuri, Purina Mills International (PMI), St Louis, MO, USA), a variety of fruit (apples, bananas or grapes), miniature marshmallows, and liquid Ensure (a human dietary supplement rich in calories, vitamins and minerals; Abbott Laboratories, Columbus OH, USA). We assessed the fluid intake supplied by the fluids or fruits offered (as derived from Bogert, Briggs and Calloway, 1973): saline, Plasmalyte, 10% dextrose solution (all from Baxter Health Care Corp., Deerfield, IL, USA), tap water, and Ensure were recorded as specific millilitres of fluid; one cm^3 piece of apple or one grape was equated to 5 ml of fluid; and one cm^3 piece of banana or one piece of marmoset jelly was equated to 2 ml of fluid.

Monitoring of animals in the sling-harness restraint

Observations were recorded at least hourly during lights-on (06:00–18:00 h) and once (at 22:00 h) during dim lighting, when the main room lights were off. At each timed observation we recorded (1) the amount of food and fluid intake, (2) behaviour and posture, (3) deposition of urine and faeces, and (4) ambient temperature. Comments on the animal's posture, occurrence of struggling, and vocalizations were also recorded on a behavioural assessment sheet and were later ranked on a scale of 1 through 8 (with 1 rated the least and 8 rated the most agitated; see Table 1). These behavioural assessments were used to gauge the adaptation of the marmosets to the restraint.

Blood sampling

Cortisol levels have served as an index of generalized stress in marmosets (Smith & French 1997). In order to evaluate stress in our animals during long-term restraint for hypothalamic push–pull perfusion, four subjects (three ovary-intact females in the mid-follicular phase of the ovarian cycle and one ovariectomized female) underwent catheterization of the right internal jugular vein (modified from O'Byrne (1988); polyethylene PE10 tubing; Becton Dickinson, Sparks, MD, USA) under Saffan anaesthesia (20 mg/kg, i.m.; alphaxolone:alphadolone 15 mg:5 mg; Veterinary Drug Co., Dunnington, York, UK) at least one week prior to hypothalamic push–pull perfusion. After catheterization, the subjects returned to their home cages wearing a jacket specifically designed to hold a dorsally exteriorized, intravenous cannula tubing inside a Velcro-sealed pocket. To maintain cannula patency, the implanted polyethylene tubing was completely filled with heparinized saline (30 U/ml) and flushed daily. Blood samples (0.1 ml) were collected from each of the four marmosets at approximately hourly intervals (between 12:00 h and

Table 1 Assessment scale for behavioural responses of marmosets restrained in the sling-harness assembly

Score	Behavioural category
1	Quiet: marmoset was calm and moved body in a relaxed manner
2	Mostly quiet, agitated only initially involving mild struggling
3	Mostly quiet, with brief, intermittent mild agitation
4	Became quiet after initial struggle, then became increasingly agitated over time
5	Mild agitation for about half of the restraint period
6	Moderate agitation during half of the restraint period
7	Restless and agitated during most of the restraint period
8	Extremely agitated during most of the restraint period

22:00 h) during hypothalamic push–pull perfusion on the third consecutive day in the sling-harness restraint and were assayed for cortisol. All blood samples were immediately placed on ice and centrifuged at ~2000 rpm for 10 min, and the plasma fraction was removed and stored at −20°C until assayed.

To monitor ovarian cycles, blood samples (0.1–0.3 ml) were collected twice weekly from the femoral vein of three ovary-intact females during brief (~2–5 min) restraint without anaesthesia (Hearn 1977, Saltzman *et al.* 1994) for analysis of plasma progesterone concentrations. All blood samples were immediately placed on ice and centrifuged at ~2000 rpm for 10 min, and the plasma fraction was removed and stored at −20°C until assayed.

To control the onset of ovarian cycles in intact females, cloprostenol sodium (a prostaglandin F2-alpha analogue, 1.0 μg, IM, Estrumate, Shering-Plough Corp., Kenilworth, NJ, USA) was injected for 1–3 consecutive days during the luteal phase (14–39 days following ovulation) in order to induce luteolysis and terminate the luteal phase or early pregnancy (Summers *et al.* 1985). In our marmoset colony, ovulation usually occurs 8–12 days after cloprostenol treatment (Saltzman *et al.* 1998). In the present study, subjects received cloprostenol 4–5 days prior to a push–pull perfusion session. Plasma progesterone determinations from the twice-weekly blood samples taken after cloprostenol injection and before push–pull perfusion confirmed that each ovary-intact subject responded to cloprostenol and was in the follicular phase of the ovarian cycle (plasma progesterone <30 nmol/l; Harlow *et al.* 1983). Ovulation occurs on the day before a sustained rise of plasma progesterone above 30 nmol/l (Harlow *et al.* 1983). Follicular phase duration was determined as the time from the day plasma progesterone levels fell below 30 nmol/l until the day of ovulation.

Hormone assays

Plasma cortisol levels were measured in duplicate aliquots using a GammaCoat radioimmunosssay kit (DiaSorin Inc., Stillwater, MN), as described previously (Saltzman *et al.* 1998). Assay sensitivity, and inter- and intra-assay coefficients of variation (CVs) were 0.041 nmol/tube, 7.5% and 3.3%, respectively. Plasma from twice-weekly blood samples was assayed in duplicate for progesterone using an enzyme immunoassay (Saltzman *et al.* 1994). Assay sensitivity, and inter- and intra-assay CVs were 7.95 pmol/well, 12.9% and 4.6%, respectively.

Statistical analysis

Data are expressed as mean ± SEM unless otherwise noted. The behavioural scores of restrained marmosets, volume of fluid intake, and rates of urine and faecal excretion were analysed utilizing the Friedman non-parametric ANOVA, followed by the Wilcoxon signed rank test for pairwise comparisons. One-way ANOVAs with repeated-measures design (Sokal & Rohlf 1995) were used to analyse (1) the log-transformed plasma cortisol values and (2) the differing durations between cloprostenol injection and estimated day of ovulation before, during and after hypothalamic push–pull perfusion. A probability value of 0.05 (two-tailed) was used to determine significance.

Results

Responses of marmosets to sling-harness restraint

All 17 female marmosets adapted well to the sling-harness restraint. The most reactive behavioural response score of '8' (Table 1) was achieved during the first hour of training. This score was attained by only one female and was well above the mean score of 4.1 (±0.6) for this session. The behavioural response scores in this first training session exceeded ($P < 0.015$) those in all subsequent restraint periods (Fig 3). The highest scoring female in the first training session also achieved the highest scores in all succeeding sessions: '6' during the 4 h and 8 h training sessions (mean values of 2.4 (±0.4) and 3.2 (±0.3), respectively) and '5'

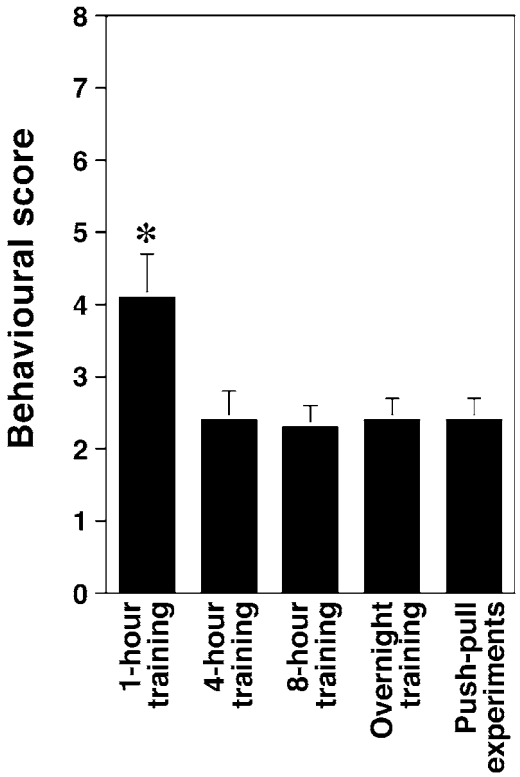

Fig 3 Behavioural responses (mean ± SEM) of 17 female marmosets undergoing sling-harness restraint training or hypothalamic push–pull perfusion. Behavioural definitions are given in Table 1. *$P < 0.015$ versus all other training sessions and push–pull perfusion (Friedman one-way ANOVA; *post-hoc* Wilcoxon paired *t*-test)

during overnight training and push–pull experiments (mean values of 2.4 (±0.3) and 2.0 (±0.3), respectively; Fig 3). As indicated by the mean scores, the majority of animals adapted more easily to the restraint system than the continually highest scoring female. All 17 animals, however, did successfully complete the training schedule and were used subsequently in hypothalamic push–pull perfusion sessions (2.2 ± 0.3 sessions per animal; range: 1–4 sessions per animal). During the actual push–pull perfusion procedure, subjects appeared to be mostly calm and alert. The subjects visually and vocally interacted with their group mates, ate, napped, and slept during the night.

Fluid consumption during restraint training and long-term restraint stabilized at approximately 4–5 ml/h. These values were within the daily fluid maintenance requirements for captive adult marmosets (1 ml per kcal of dietary energy intake: Kerr 1972, Panel on Nonhuman Primate Nutrition 1978). During the initial one hour of training, however, restrained marmosets consumed a larger volume of fluid (9.6 ± 0.6 ml) because of frequent (every 5–10 min) offerings of Ensure and fruit employed as positive reinforcement. Urine was voided approximately every 1–2 h, and faeces were deposited every 3–10 h, throughout all restraint periods, reflecting a relatively stable excretion and defaecation rate.

Plasma cortisol levels during restraint for hypothalamic push–pull perfusion

As an assessment of stress in female marmosets undergoing continuous restraint, hypothalamic push–pull perfusion and repeated blood sampling from an intravenous cannula, we determined plasma cortisol levels on the third day of restraint during push–pull perfusion. Data from ovary-intact females in the early follicular phase and the single ovariectomized female are shown separately (Fig 4), as circulating cortisol levels in ovariectomized females can be lower than those in ovary-intact females (Saltzman *et al.* 1998). Plasma cortisol levels in ovary-intact females (5.14 [5.05–5.23] μmol/l; back-transformed mean of all time points combined [95% CI]) did not change significantly ($F[7,14] = 0.88$, NS) during the hours sampled (13:00–22:00 h) and approximated those found during morning (08:45–09:15 h and ~11:45 h) sampling of ovary-intact females under baseline conditions (Saltzman *et al.* 1994; Fig 4). Nevertheless, plasma cortisol levels in restrained females did not show the expected decrease in values across the day (Fig 4; Smith & French, unpublished data). Cortisol levels during restraint and push–pull perfusion, however, failed to reach the high values exhibited by females wounded during the establishment of the social dominance hierarchy in the 2 days following group formation (Fig 4).

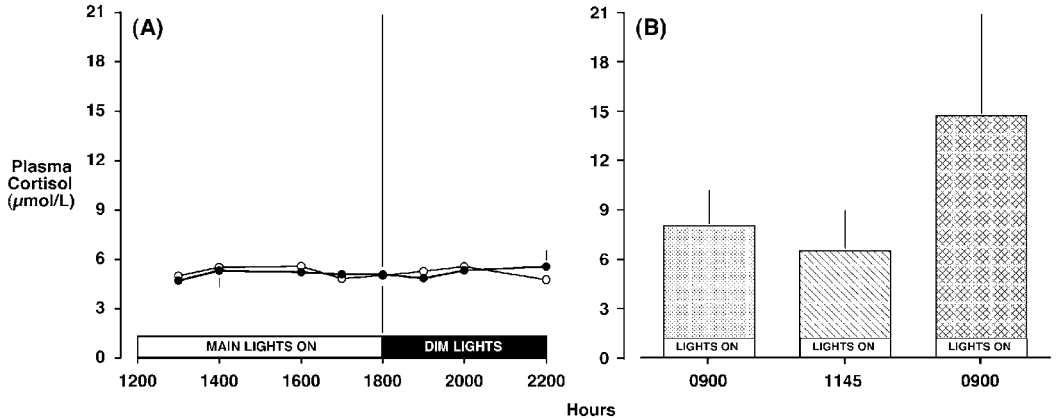

Fig 4 Plasma cortisol levels (A) on the third day of continuous restraint during hypothalamic push–pull perfusion (three ovary-intact females [solid circles; back-transformed means (95% confidence interval)] and one ovariectomized female [open circles; individual values] from 13:00–22:00 h, and (B) in morning (~09:00 h [stippled bar; mean (95% confidence interval)] and ~11:45 h [diagonal-lined bar]) baseline samples during the early follicular phase in ovary-intact female marmosets, and following aggression and wounding during the morning of the second day following social group formation (~09:00 h; hatched bar) in ovary-intact females (data for (B) are modified from Saltzman *et al.* 1994). Note that marmoset plasma cortisol values are typically much higher than those found in Old World primates (Coe *et al.* 1992)

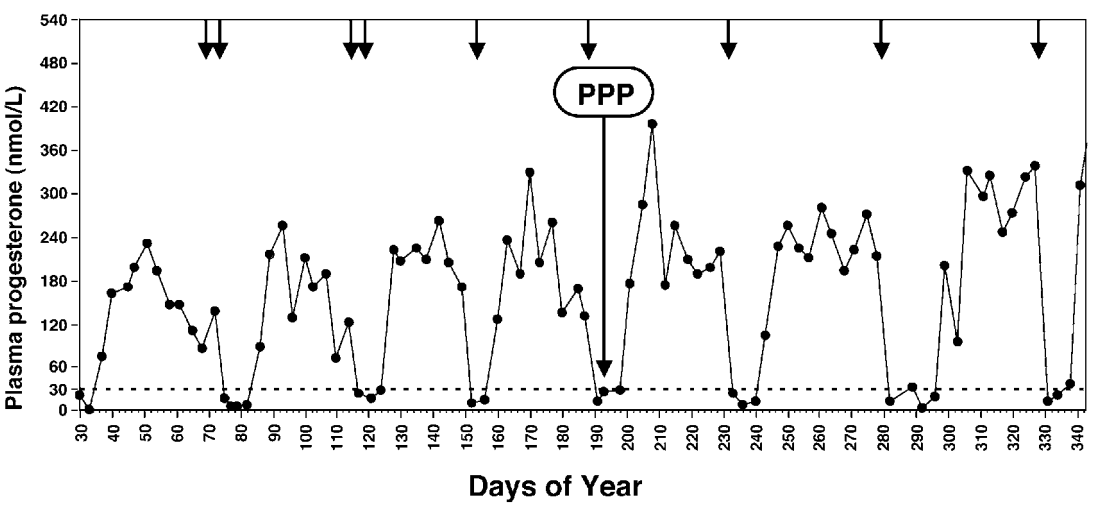

Fig 5 Plasma progesterone concentrations in twice-weekly blood samples from a representative ovary-intact female before, during and after restraint for hypothalamic push–pull perfusion (PPP). Note the maintenance of regular ovulatory cycles from 4 months before through 2 months after restraint and PPP. Female marmosets in our colony usually ovulate within 8–12 days of a prostaglandin analogue (cloprostenol) injection (Saltzman *et al.* 1998). We considered animals to have ovulated on the day immediately preceding a sustained elevation of plasma progesterone above 30 nmol/l (dotted horizontal line). The arrows indicate the days on which cloprostenol was given to terminate the luteal phase or early pregnancy and initiate a new follicular phase

Table 2 Duration of follicular phase before, during and after restraint for hypothalamic push–pull perfusion (PPP) in three female marmosets

Timing of ovulatory cycle	Follicular phase duration (days*)
Immediately before PPP	8.3 ± 0.7
During PPP	9.3 ± 1.8
Immediately after PPP	10.7 ± 1.7

*Mean ± SEM

Ovarian function during restraint for hypothalamic push–pull perfusion

Restraint and push–pull perfusion did not result in any obvious disruption of ovulatory cycles in the three ovary-intact females that were monitored during their ovulatory cycles immediately before, during and immediately after restraint and push–pull perfusion. Figure 5 illustrates a representative example of the reliable and regular ovulatory cycles and plasma progesterone levels shown by one of these three females. Over all the cycles monitored, follicular phase duration ranged from 7 to 14 days, fairly typical for marmosets in this colony (Saltzman *et al.* 1998). There was also no significant ($F[2,4] = 0.54$, NS) delay in the time taken to ovulate during the cycle involving restraint for hypothalamic push–pull perfusion (Table 2).

Discussion

This novel, long-term restraint system provides a practical solution to the challenges posed by increasingly complex neurobiological and neuroendocrinological research performed on common marmosets. The restraint system is particularly useful for studies in which little disturbance of the social environment can be permitted, including investigation of the neural mechanisms regulating social control of female reproductive neuroendocrinology (Abbott *et al.* 1998) and the neuroendocrine basis of parental behaviour (Pryce 1993).

The previously described backpack for marmosets (Ruiz de Elvira & Abbott 1986) could not be modified to accommodate long-term restraint as it was not sufficiently pliable and produced axillary chafing (D. H. Abbott, unpublished results). In contrast, the jacket described here is highly versatile and pliable, enabling the development of a long-term restraint system for assessment of neuroendocrine mechanisms either directly (e.g. hypothalamic push–pull perfusion; Abbott *et al.* 1998) or indirectly from dynamic changes in circulating hormone concentrations (e.g. plasma luteinizing hormone [LH] levels). The jacket and sling-harness restraint are applicable to other neurobiological procedures, including microdialysis or electrophysiological recording from specific brain regions. The jacket materials (sailcloth, brushed flannel, and Velcro) are easily obtained by most laboratories and, together with the relatively simple jacket design, will enable neurobiological and neuroendocrine studies in marmosets and other small, nonhuman primates in a variety of laboratory settings.

The previously described marmoset restraint system (O'Byrne & Morris 1988) did not readily facilitate neuroendocrine studies of marmosets. It restrained these small, arboreal primates in an upright, sitting posture that is not species-typical for marmosets. The restraint was based on a design for macaques, which are more terrestrial, have ischial callosities, and can readily adopt an upright, sitting posture. The previous restraint also permitted a maximum of only 6 h of continuous restraint (O'Byrne 1988). This time period is far shorter than the length of restraint (>2 days) required for long-term assessment of neuroendocrine function (Gearing & Terasawa 1988). Our new jacket and sling-harness restraint system provides up to 3 days of continuous restraint for assessment of neuropeptide and neurotransmitter release (Abbott *et al.* 1997, 1998).

Restraint and hypothalamic push–pull perfusion did, however, cause some activation of the hypothalamic–pituitary–adrenal (HPA) axis. Although plasma cortisol levels on the third day of continuous restraint for push–pull perfusion and jugular cannulation sampling were similar to those found previously in morning basal samples from female marmosets (Saltzman *et al.* 1994,

Abbott *et al.* 1997, Saltzman *et al.* 1998), they did not exhibit the expected decrease across the daytime hours (Saltzman *et al.* 1994, unpublished data, Smith & French 1997). This absence of a diurnal decrease in circulating cortisol levels suggests at least partial activation of the HPA axis in female marmosets restrained for hypothalamic push–pull perfusion. Adaptation of the monkeys to the restraint (Wade & Ortiz 1997), the amount of movement permitted while in the restraint (Wade & Ortiz 1997), and the close visual proximity of cage mates (Tilbrook *et al.* 1999) may all minimize the elevation of circulating cortisol levels in restrained animals. These restraint-associated elevations in circulating cortisol levels were of a substantially smaller magnitude than those observed in female marmosets following wounding incurred during establishment of intra-sexual social dominance hierarchies over 3 days of new group formation (Saltzman *et al.* 1994). The latter duration was similar to that experienced during restraint for push–pull perfusion. Cortisol elevations during long-term restraint in other laboratory-housed animals are usually not as muted as those demonstrated by female marmosets (e.g. rhesus monkeys: Norman *et al.* 1994; sheep: Tilbrook *et al.* 1999; rats: Tan & Nagata 2002).

Activation of the HPA axis, however, was not associated with any significant inhibition of ovulatory cycles in restrained female marmosets. Regular cycles were maintained in ovary-intact females before, during and after restraint for hypothalamic push–pull perfusion, in contrast to the inhibitory effects of long-term restraint on ovulatory function observed in female rhesus monkeys (Chen *et al.* 1992). Furthermore, we have already demonstrated that our restraint and hypothalamic push–pull perfusion system does not inhibit hypothalamic GnRH release in female marmosets (Abbott *et al.* 1997, 1998), in contrast to the inhibitory contributions made by other restraint systems towards the release of pituitary LH in female marmosets (O'Byrne *et al.* 1989), rhesus monkeys (Norman *et al.* 1994), sheep (Turner *et al.* 2002) and rats (Kam *et al.* 2000). It is still not clear, however, whether activation of the HPA axis by restraint is causally involved in suppression of reproductive function (Norman 1994, Cameron 1997, Tilbrook *et al.* 2002). Thus, our novel restraint system appears to be appropriate for investigating the neuroendocrine regulation of female reproduction, but not adrenal function, in common marmosets.

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