The effects of analgesia on the behaviour of stags at 0, 7 and 24 hours following velvet antler removal

J.C. Pollard, R.P. Littlejohn, K.A. Waldrup, C.G. Mackintosh, J.M. Suttie, A.J.T. Pearse

Abstract

Five groups of 8, two-year-old stags were used to determine whether administration of analgesic (acetyl salicylate, A) reduced behavioural effects of velvet antler removal Treatment at 0 hrs was carried out in a deer crush and comprised ring block application of local anaesthetic then a velveting treatment, either V: application of a tourniquet plus removal of antlers 4 minutes later, or NV: release from the crush. Analgesic treatments were also given via intravenous injection at 0 hrs and at 7 and 24 hrs, and comprised A: 26 mg/kg acetyl salicylate, or S: 15 ml physiological saline. Treatments were allocated within groups so that 4 stags received V and 4 received NV, then within each subgroup of 4, A was given at 0, 7 or 24 hrs or not at all. One week later velveting treatments were crossed over and the trial was repeated. Videotapes were used to provide measurements of activities of each individual for one hour following treatment at 0, 7 and 24 hrs.

At 0 hrs, greater frequencies of ear-flicking, head shaking, nosing the ground, grooming, head scratching and tongue-flicking were seen in V stags compared with NV stags ($P<0.05$), and head shaking and ear-flicking still differed at 7 hrs ($P<0.05$). Various activities (head shaking, drinking, stepping, vertical head movements, licking and being licked) varied ($P<0.05$) with analgesic treatment but no consistent patterns were observed. At 0 hrs, velveted stags given saline scratched their heads on average 2.83 times/hr compared with 1.10 (SED 0 86) times/hr for velveted stags given A, while levels for NV stags were much lower (0.46 for S stags and 0.65 for A stags). Significant interactions between velveting and analgesic treatments were also seen in the way two activities, drinking and being licked on the head by other deer, changed over time at 0 and 24 hrs respectively. However these few interactive effects provided little evidence that acetyl salicylate reduced any discomfort associated with V. This result contrasted with a previous study in which several significant interactions were seen.

Introduction

In December 1991, an investigation was carried out to determine whether providing analgesia following antler removal was beneficial to stags (Pollard *et al.*, 1992). Stags were given an intravenous injection of saline or analgesic (acetyl salicylate) immediately following antler removal, which was carried out in a mechanical deer crush and using local anaesthesia applied in a ring block Intravenous analgesic reduced many of the behavioural changes otherwise seen following velveting. This effect was seen over the full observation period, from 0-4 hours following antler removal, and there was no indication of a change over time which might have been related to effects of the local anaesthetic subsiding. The analgesic had little effect on the behaviour of stags which were not velveted It was concluded that the velveted stags experienced post-operative sensations, which were reduced by acetyl salicylate. These sensations were likely to be unpleasant as salicylate analgesics act through a blocking effect on inflammatory mediators on pain endings in the peripheral nervous system, and also through anti-inflammatory and anti-pyretic actions (Booth, 1982)

Research continued in 1992 with a study of the duration of the effects of antler removal. The behaviour of 16 stags was recorded for one hour, starting at 0, 1, 2, 13, 24 and 48 hours following antler removal (under local anaesthesia but without additional analgesia) from 8 of the stags. Effects of velveting were largely confined to the 0-2 hour period (Pollard *et al.*, 1993).

In the present experiment, acetyl salicylate was provided to stags starting at 0, 7 and 24 hours following antler removal to determine, firstly, whether the same beneficial effects of additional analgesic were seen as in Pollard *et al.* (1992) and secondly, whether any such effects were present outside the four-hour period identified in that study.

Methods

Animals

Three days before the experiment (carried out in December 1993 and January 1994), 40 two-year-old red deer stags with growing velvet antlers were weighed (mean weight was 140 kg, SD 11.1 kg), plastic collars were fitted, and the stags were subsequently confined in five separate groups of eight, with the groups comprising animals with similar antler casting dates (velvet was removed during the experiment at a mean of 56 (SD 5.3) days after casting). The stags were confined at pasture except during observation periods, when they were housed in an indoor pen containing a water trough and 1/5 bale lucerne hay. Once the first group had been treated, pairs of individuals from this group were confined with subsequent groups at pasture. These "spare" individuals were used to accompany the deer during the experiment so that individuals were not isolated.

Treatments

The treatment regime for each animal consisted of a velveting treatment and an analgesic treatment. Animals were treated in groups at 0, 7 and 24 hrs, then one week later velveting treatments were crossed over between individuals and the experiment was repeated. Treatment at 0 hrs was carried out under mechanical restraint in a deer crush and included, for all animals, injection of approximately 20-25 ml local anaesthetic (Lopaine 2%, Troy Laboratories, Auckland) in a ring block around the base of the antler pedicles, using a 22-gauge needle. All stags were also marked with spray raddle at 0 hrs. Subsequent treatments at 7 and 24 hrs were carried out under manual restraint (except for some individuals which were difficult to handle, these were restrained in the crush). Treatments for each group over each 24-hour period were administered by one of two veterinarians.

Each of the eight stags in each group received a different treatment defined by the interaction of velveting and analgesic treatments Velveting treatments were carried out at 0 hrs and consisted of antler removal (V) or no antler removal (NV). Restraint for V lasted approximately 8 (SE 0.2) minutes, and 3 (SE 0.3) minutes for NV For V, a tourniquet was applied to the antler pedicles, then the antlers were removed using a surgical saw, four minutes after completion of administration of local anaesthetic.

The analgesic treatments consisted of intravenous (jugular) injections, using an 18-gauge needle, at 0, 7 and 24 hrs. The injected substance comprised either A: 26 mg/kg acetyl

salicylate (Vetalgine 5.5g; Sanofi Animal Health, France; 14 ml sterile water was added to each vial containing 5.5 g to make a total of 21 ml solution, which was then administered in proportion to the weight of each stag) or S. 15 ml physiological saline. A was administered once, to one each of the V and NV stags, at 0, 7, or 24 hrs, and otherwise S was administered. This treatment regime is referred to hereafter as ASS, SAS and SSA where A was given at 0, 7, and 24 hours respectively, and as SSS when S was administered at each of these times. (For data sampled at 0 hours, the "effective" analgesic treatments are referred to as A or S, and for data sampled at 7 hrs, the effective treatments are referred to as AS, SA, and SS.)

At each of hrs 0, 7 and 24, the group of 8 stags was confined in an indoor observation pen for one hour starting from the time the last individual was treated. Tourniquets were removed from V stags during confinement, approximately 22 (SE 0.7) minutes following V. The deer were released back to pasture following each observation period.

Measurements

Behaviour during the periods of confinement in the observation pen was recorded using a video camera mounted above the pen. The videotapes were used to measure all activities of each individual (Appendix 1) over successive 10-minute periods during the hour following treatment. To expedite this process, five different observers were used, each assigned to record data for one of the five groups of deer

Statistical analysis

Mean and linear contrasts for each behaviour (except tongue-flicking) for each sample period were analysed by analysis of variance, with day within tag within group as the block structure, and day plus velveting treatment, effective analgesic treatment, and their interaction as the treatment structure (It had been established that no crossover effect was in operation.) Data for pacing, ear-flicking, head shaking, vertical head movements and head scratching were log transformed (geometric means are presented).

The percentage of 10-minute samples in which tongue-flicking was observed for each hour of observation was analysed as a binomial generalised linear model, fitting terms for group, day plus velveting treatment, effective analgesic treatment, and their interaction

Results

(a) Means of hourly samples

Velveted stags had significantly greater frequencies of ear-flicking, head shaking, nosing the ground, grooming, scratching the head and tongue-flicking than NV stags in the observation period at 0 hrs ($P<0.05$; Table 1a). In the observation period at 7 hrs, greater frequencies of ear-flicking and head shaking were again found among the V stags ($P<0.05$; Table 1b) No significant differences between means for V and NV were observed at 24 hrs (Table 1c).

Several activities differed between analgesic treatments, but there was little evidence of a consistent pattern over all three sampling periods. At 0 hrs (Appendix 2a) head shaking was more frequent among S stags than A stags, and the time spent drinking was greater for A

stags (P<0.05). No significant differences between analgesic treatments were found at 7 hrs (Appendix 2b), but various activities (stepping, vertical head movements, licking and being licked) differed at 24 hrs (P<0.05; Appendix 2c). Of these, being licked by other deer on the body (which was greatest for all stags which had received A) was the only activity to show a similar (non-significant) trend during observations at 0 and 7 hrs.

The only significant interaction between velveting and analgesic treatments at any time was found for scratching the head (with a foot) at 0 hrs (P<0 05). Velveted stags given saline scratched their heads on average 2.83 times/hr compared with 1.10 (SED 0.86) times/hr for velveted stags given A, while levels for NV stags were much lower (0.46 for S stags and 0.65 (SED 0.46) for A stags).

(b) Linear changes within hourly samples

Many activities showed significant changes (P<0 05) in frequency during one or more of the 60 minute observation periods (Table 2) Declines were seen in the frequency of eating, shaking the head and body, nosing the ground, and licking the heads of other deer, and increases occurred in grooming and aggression (including threats) Being licked on the head or body declined during the observation period at 0 hrs only, while nosing the wall and vertical head movements at the wall or door increased during the observation period at 24 hrs only. Stepping and pacing were unusual in that they declined during observation at 0 hrs but increased in the two subsequent observation periods at 7 and 24 hrs (Table 2)

Some differences between treatments in the way frequencies of activities changed over time were seen. The only activity for which slope varied significantly with velveting treatment was grooming at 0 hrs, which increased over the hour at a mean rate of 1.6 for velveted stags compared with 0 2 (SED 0.53) for NV stags. For analgesic treatments, nosing the ground at 0 hrs declined at a mean rate of 0.34 for A stags compared with a decline of 0 09 for S stags (SED 0.117). At 7 hrs pacing and threats received increased over the hour for SA stags but remained fairly stable for other stags (P<0.05), while being licked by others and scratching the head decreased for AS stags but increased for SA stags (P<0.05). There were no significant differences in slope with analgesic treatment at 24 hrs.

Significant interactions between velveting and analgesic treatments in the way frequencies of activities changed over time were seen. At 0 hrs, the time spent drinking increased among NV stags given A, but decreased among V stags given A, while values remained relatively constant for stags given S (P<0.05). At 24 hrs, being licked on the head by other deer increased among velveted stags for SAS and SSA stags but decreased for ASS stags (P<0.05), while SSS and non-velveted stags showed little change in this behaviour.

Discussion

In the present study several significant main effects of the separate velveting and analgesic treatments were found, but few interactions were apparent. Overall these results contrasted with the previous study on analgesia in velveted stags (Pollard *et al.*, 1992), where few main effects of velveting (specifically, head-shaking and changes over time in head-shaking and ear-flicking) and no main effects of analgesic treatment were seen, but many interactions between velveting and analgesic treatments were significant (including nosing the ground, ear-flicking,

attempting to groom, eating, aggression, and position of the head) suggesting that the analgesic treatment might reduce the behavioural effects of velveting

Velveted stags initially shook their heads, flicked their ears, groomed, scratched their heads and flicked their tongues more than intact stags, with only head-shaking and ear-flicking still differing between the two treatments at 7 hrs. Previous comparisons of velveted and non-velveted stags have also found higher frequencies of head-shaking, ear-flicking and grooming, as well as effects on other activities not seen to differ in the present study (resting and eating (Pollard *et al*, 1991; 1993), stepping, vertical head movements, aggression, licking, being licked, and shaking the body (Pollard *et al.*, 1993)) Scratching the head did not differ in the previous study where it was measured (Pollard *et al*, 1993). Thus the specific activities associated with the velveting treatment have been quite variable between studies. The duration of the behavioural differences between V and NV stags seen in the present study were consistent with previous observations; Pollard *et al.* (1991) observed most of the differences in an initial 3-hour observation period, with only eating differing at 9 hours, while Pollard *et al.* (1993) reported differences only at 0-2 hrs, and not at subsequent observations at 13, 24 and 48 hours post-treatment

Several activities were found to differ between analgesic treatments, namely head shaking, drinking, stepping, vertical head movements, licking and being licked by other deer There was little consistency in these effects over the three sample periods so it is difficult to speculate why the differences occurred. In the previous study where acetyl salicylate was administered (Pollard *et al*, 1992) no behavioural effects were seen. However observations in that study were restricted to 0-4 hours post-treatment whereas many of the differences between S and A treatments seen in the present study (aside from head-shaking and drinking) were found 24 hours after the first analgesic treatment was given.

A significant interaction between the velveting and analgesic treatments was seen at 0 hrs, when the analgesic appeared to reduce head-scratching in the velveted stags back towards the lower frequency seen in the non-velveted stags. Thus acetyl salicylate may have reduced some irritation resulting from velveting The only other interactions found were in the way two activities changed over time, namely drinking at 0 hrs and being licked on the head by other deer at 24 hrs. These and the main effects of the analgesic treatment indicate some effect of acetyl salicylate administration on fluid balance, and ingestion and availability of substances (possibly including blood) from other deer.

In summary, there was little evidence that acetyl salicylate reduced behavioural effects of velveting at any time following treatment, therefore there was little indication of a need to provide additional analgesia to velveted stags. This discrepancy between the present study and the findings of Pollard *et al* (1992), where the use of additional analgesia was implicated (for stags of the same age and stage of antler development), deserves close scrutiny. One difference between the two trials was the concentration of acetyl salicylate in the intravenous injection; in the present study 14 ml was added to vials containing 5.5 g acetyl salicylate, whereas in the previous study 40 ml was added to the vials. (The smaller amount was used to facilitate administration of the drug.) However in both cases the salicylate had dissolved prior to injection, therefore it is unlikely that it was ineffective in the present trial

A more convincing reason for the discrepancy between the results presented here and those

of Pollard *et al.* (1992) is evident in the data from that study. Effects of the additional analgesic were apparent immediately, when the local anaesthetic would have been expected to be fully effective. Further, there was no evidence of changes over time consistent with an increasing need for analgesia as effects of the local anaesthetic subsided (Pollard *et al* , 1992) This suggests that the local anaesthetic had not provided full analgesia prior to antler removal, either because the substance itself, or the method of application, was ineffective In turn, this raises the possibility that there is variability between operators in the degree of analgesia achieved through ring block application of local anaesthetic.

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Table 1a Mean frequencies of activities following V and NV, and SEDs, at 0 hrs following velveting treatments Significance of differences between V and NV is indicated (ns not significant, * P<0 05, ** P<0 01, *** P<0 001)

(a) 0 Hrs	Treatment			
Activity (frequency/hr)	V	NV	SED	
Steps	661	661	31 3	ns
Paces	43 7	46 5	9 44	ns
Ear-flicks	61 2	21 6	10 8	***
Headshakes	12 2	3 6	1 20	***
Bodyshakes	1 32	1 08	0 232	ns
Nosing the ground	25 5	30 6	2 32	*
Nosing the wall	37 7	40 2	3 36	ns
Vertical head movements	8 2	10 5	3 04	ns
Grooming	7 3	3 5	1 37	**
Att groom	1 01	0 99	0 347	ns
Licking-instigator-head	12 2	12 6	2 24	ns
Licking-instigator-body	23 6	27 3	4 05	ns
Licking-recipient-head	13 1	11 0	1 81	ns
Licking-recipient-body	16 1	17 9	3 57	ns
Scratching head	2 30	0 51	0 31	ns
Eating	11 1	9 7	3 07	ns
Drinking	0 47	0 41	0 185	ns
Aggression-instigator	1 4	2 6	2 13	ns
Aggression-recipient	3 1	1 8	1 56	ns
Threats-instigator	3 6	2 7	1 14	ns
Threats-recipient	3 5	3 4	1 10	ns
Mounting-instigator	0 0	0 0	0 0	ns
Mounting-recipient	0 0	0 0	0 0	ns
Head held at shoulder (s)	4 7	6 3	3 65	ns
Eating (s)	5 2	4 2	1 04	ns
Drinking (s)	2 8	2 4	1 22	ns
Tongue-flicking (%)	74 6	55 0	5 97	**

Table 1b Mean frequencies of activities following V and NV and SEDs, at 7 hrs following velveting treatments Significance of differences between V and NV is indicated (ns not significant * P<0 05, ** P<0 01 *** P<0 001)

(b) 7 Hrs	Treatment			
Activity (frequency/hr)	V	NV	SED	
Steps	465	480	31 4	ns
Paces	21 6	18 9	6 07	ns
Ear-flicks	24 7	16 0	3 72	*
Headshakes	3 5	2 0	3 51	**
Bodyshakes	0 63	0 62	0 170	ns
Nosing the ground	20 9	20 9	2 69	ns
Nosing the wall	29 8	31 4	2 54	ns
Vertical head movements	7 6	6 7	1 77	ns
Grooming	5 0	5 4	1 13	ns
Att groom	1 23	1 13	0 292	ns
Licking-instigator-head	8 4	8 6	1 16	ns
Licking-instigator-body	22 0	22 4	2 73	ns
Licking-recipient-head	7 3	7 6	0 94	ns
Licking-recipient-body	14 1	16 1	2 08	ns
Scratching head	1 92	1 91	0 038	ns
Eating	9 3	9 1	2 14	ns
Drinking	0 28	0 25	0 118	ns
Aggression-instigator	6 7	6 0	1 82	ns
Aggression-recipient	4 2	7 5	2 54	ns
Threats-instigator	7 5	7 6	0 94	ns
Threats-recipient	7 1	8 1	1 37	ns
Mounting-instigator	0 12	0 18	0 100	ns
Mounting-recipient	0 05	0 08	0 067	ns
Head held at shoulder (s)	16 2	10 4	6 75	ns
Eating (s)	5 0	4 8	0 98	ns
Drinking (s)	1 30	1 38	0 678	ns
Tongue-flicking (%)	55 0	51 7	6 06	ns

Table 1c Mean frequencies of activities following V and NV, and SEDs, at 24 hrs following velveting treatments Significance of differences between V and NV is indicated (ns not significant, * P<0 05, ** P<0 01, *** P<0 001)

(c) 24 Hrs	Treatment			
Activity (frequency/hr)	V	NV	SED	
Steps	497	499	27 9	ns
Paces	16 5	16 5	4 64	ns
Ear-flicks	15 6	11 4	2 19	ns
Headshakes	2 0	1 8	0 41	ns
Bodyshakes	0 33	0 33	0 094	ns
Nosing the ground	16 8	17 6	2 83	ns
Nosing the wall	28 7	28 8	2 96	ns
Vertical head movements	5 4	5 8	1 40	ns
Grooming	4 3	3 5	0 80	ns
Att. groom	0 73	0 80	0 228	ns
Licking-instigator-head	8 6	8 5	1 07	ns
Licking-instigator-body	21 0	22 1	2 64	ns
Licking-recipient-head	8 4	7 8	0 90	ns
Licking-recipient-body	14 9	15 4	1 84	ns
Scratching head	1 88	1 83	0 035	ns
Eating	7 5	6 0	1 77	ns
Drinking	0 15	0 08	0 080	ns
Aggression-instigator	7 3	8 4	2 98	ns
Aggression-recipient	7 8	6 2	1 55	ns
Threats-instigator	8 1	7 5	1 23	ns
Threats-recipient	7 6	6 7	0 70	ns
Mounting-instigator	0 18	0 20	0 113	ns
Mounting-recipient	0 13	0 18	0 127	ns
Head held at shoulder (s)	10 9	8 1	3 67	ns
Eating (s)	4 3	3 3	0 94	ns
Drinking (s)	0 55	0 60	0 382	ns
Tongue-flicking (%)	55 4	48 8	6 49	ns

Table 2a Mean slope for linear changes within hourly samples for each activity at 0 hrs following velveting treatments Levels of significance are indicated (ns not significant * P<0 05, ** P<0 01, *** P<0 001)

(a) 0 Hrs	Treatment		
Activity (frequency/hr)	Slope	SE	
Steps	-22 6	5 590	***
Paces	-0 18	0 158	ns
Ear-flicks	0 01	0 105	ns
Headshakes	-0 40	0 077	***
Bodyshakes	-0 20	0 045	***
Nosing the ground	-1 49	0 474	**
Nosing the wall	-0 40	0 661	ns
Vertical head movements	0 11	0 108	ns
Grooming	0 91	0 265	***
Att. groom	0 09	0 063	ns
Licking-instigator-head	-0 73	0 309	*
Licking-instigator-body	-0 30	0 538	ns
Licking-recipient-head	-0 81	0 281	**
Licking-recipient-body	-1 32	0 386	***
Scratching head	0 10	0 073	ns
Eating	-1 14	0 298	***
Drinking	-0 04	0 040	ns
Aggression-instigator	0 44	0 241	ns
Aggression-recipient	-0 39	0 224	ns
Threats-instigator	0 54	0 226	*
Threats-recipient	0 14	0 169	ns
Mounting-instigator	0 00	0 000	-
Mounting-recipient	0 00	0 000	-
Head held at shoulder (s)	0 31	0 377	ns
Eating (s)	-0 45	0 152	**
Drinking (s)	-0 25	0 223	ns

Table 2b Mean slope for linear changes within hourly samples for each activity at 7 hrs following velveting treatments Levels of significance are indicated (ns not significant, * P<0 05, ** P<0 01, *** P<0 001)

(b) 7 Hrs	Treatment		
Activity (frequency/hr)	Slope	SE	
Steps	15 5	4 89	**
Paces	0 12	0 134	ns
Ear-flicks	-0 08	0 121	ns
Headshakes	-0 15	0 048	**
Bodyshakes	-0 20	0 046	***
Nosing the ground	-0 82	0 328	*
Nosing the wall	-0 02	0 476	ns
Vertical head movements	0 05	0 095	ns
Grooming	0 33	0 150	*
Att groom	0 08	0 078	ns
Licking-instigator-head	-0 19	0 252	ns
Licking-instigator-body	0 43	0 591	ns
Licking-recipient-head	-0 25	0 221	ns
Licking-recipient-body	0 42	0 475	ns
Scratching head	-0 002	0 005	ns
Eating	-0 31	0 438	ns
Drinking	0 05	0 016	**
Aggression-instigator	2 36	0 520	***
Aggression-recipient	2 17	0 415	***
Threats-instigator	2 02	0 231	***
Threats-recipient	1 75	0 360	***
Mounting-instigator	0 07	0 022	**
Mounting-recipient	0 02	0 022	ns
Head held at shoulder (s)	0 80	0 738	ns
Eating (s)	-0 30	0 196	ns
Drinking (s)	0 21	0 125	ns

Table 2c Mean slope for linear changes within hourly samples for each activity at 24 hrs following velveting treatments Levels of significance are indicated (ns not significant, * P<0 05, ** P<0 01, *** P<0 001)

(c) 24 Hrs	Treatment		
Activity (frequency/hr)	Slope	SE	
Steps	10 2	4 511	*
Paces	0 44	0 156	**
Ear-flicks	-0 10	0 074	ns
Headshakes	-0 13	0 054	*
Bodyshakes	-0 09	0 026	***
Nosing the ground	-0 55	0 356	ns
Nosing the wall	1 58	0 414	***
Vertical head movements	0 37	0 098	***
Grooming	0 29	0 123	*
Att groom	0 07	0 057	ns
Licking-instigator-head	-0 37	0 143	*
Licking-instigator-body	-0 39	0 395	ns
Licking-recipient-head	0 02	0 188	ns
Licking-recipient-body	-0 38	0 287	ns
Scratching head	-0 01	0 033	ns
Eating	-1 38	0 266	***
Drinking	0 01	0 008	ns
Aggression-instigator	1 80	0 274	***
Aggression-recipient	1 86	0 259	***
Threats-instigator	2 09	0 256	***
Threats-recipient	1 72	0 186	***
Mounting-instigator	0 04	0 028	ns
Mounting-recipient	0 04	0 030	ns
Head held at shoulder (s)	1 41	0 758	ns
Eating (s)	-0 73	0 137	***
Drinking (s)	0 05	0 050	ns

Appendix 1 Activities measured over six successive 10-minute periods for each stag at 0, 7 and 24 hrs after velveting treatments

Activities measured per 10-minute period

No steps
No paces (steps parallel to, 0 5 m of, a wall or door)
No earflicks
No head shakes
No body shakes
No nose→ground
No times nosed wall/door
No head bobs/head side-side @ wall/door (refered to in text as vertical head movements)
No times groomed self
No times attempted to groom self
No times licked/nosed others head
No times licked/nosed others body
No times licked/nosed by others head
No times licked/nosed by others body
No times scratched head with foot
No times ate (ingested hay)
No times drank
No aggressive interactions instigated
No aggressive interactions received
No threats instigated
No threats received
Whether tongue-flicking seen (Y/N)
No times sat→stood
Time spent sitting (seconds)
Time spent standing idle - head down (s)
Time spent standing idle - head held at shoulder (s)
Time spent eating (s) (ingesting and chewing hay)
Time spent drinking(s)
No times mounted others
No times mounted by others
No seconds visible for
No times removed from pen

Appendix 2a Mean frequencies of activities at 0 hrs, following A and S with SEDs between treatments Significance of differences between treatments is indicated (* P<0 05)

(a) 0 Hrs	Treatment			
Activity (frequency/hr)	A	S	SED	
Steps	625	673	66 1	ns
Paces	53 1	42 4	19 7	ns
Ear-flicks	33 1	37 9	9 80	ns
Headshakes	4 0	8 0	1 69	*
Bodyshakes	0 79	1 33	0 343	ns
Nosing the ground	27 1	28 4	4 25	ns
Nosing the wall	38 1	39 2	7 48	ns
Vertical head movements	6 4	10 5	3 62	ns
Grooming	4 24	5 77	1 626	ns
Att groom	0 98	1 01	0 379	ns
Licking-instigator-head	11 9	12 6	3 11	ns
Licking-instigator-body	19 8	27 3	6 29	ns
Licking-recipient-head	11 4	12 3	1 98	ns
Licking-recipient-body	21 5	15 5	3 59	ns
Scratching head	0 86	1 37	0 53	ns
Eating	8 9	10 9	2 82	ns
Drinking	0 73	0 34	0 232	ns
Aggression-instigator	1 0	2 4	2 33	ns
Aggression-recipient	1 88	2 61	1 582	ns
Threats-instigator	2 49	3 41	1 898	ns
Threats-recipient	2 58	3 73	1 271	ns
Mounting-instigator	0 00	0 00	-	ns
Mounting-recipient	0 00	0 00	-	ns
Head held at shoulder (s)	3 0	6 4	3 34	ns
Eating (s)	4 10	4 94	1 613	ns
Drinking (s)	5 29	1 71	1 494	*
Tongue-flicking (%)	55 8	67 8	7 12	ns

Appendix 2b Mean frequencies of activities at 7 hrs, following AS, SS and SA with SEDs between treatments

(b) 0-7 Hrs	Treatment				
Activity (frequency/hr)	AS	SA	SS	SED	
Steps	398	495	498	67 3	ns
Paces	14 6	36 3	17 7	15 54	ns
Ear-flicks	14 6	19 8	23 2	6 80	ns
Headshakes	2 1	2 3	3 3	0 82	ns
Bodyshakes	0 35	0 72	0 72	0 157	ns
Nosing the ground	19 0	19 4	22 6	4 32	ns
Nosing the wall	26 7	34 2	30 7	6 88	ns
Vertical head movements	4 4	10 4	7 5	3 72	ns
Grooming	4 71	4 52	5 84	1 218	ns
Att groom	0 75	0 75	1 60	0 497	ns
Licking-instigator-head	7 96	7 95	9 00	1 699	ns
Licking-instigator-body	17 7	24 9	23 2	5 59	ns
Licking-recipient-head	7 88	7 44	7 22	1 07	ns
Licking-recipient-body	17 1	16 3	13 6	3 01	ns
Scratching head	0 83	0 56	0 48	0 254	ns
Eating	6 7	7 6	11 2	3 68	ns
Drinking	0 45	0 15	0 23	0 223	ns
Aggression-instigator	3 72	5 59	8 00	5 620	ns
Aggression-recipient	3 4	13 4	3 3	6 52	ns
Threats-instigator	8 31	6 34	7 78	6 585	ns
Threats-recipient	4 34	11 78	7 21	2 978	ns
Mounting-instigator	0 05	0 20	0 18	0 173	ns
Mounting-recipient	0 15	0 10	0 00	0 070	ns
Head held at shoulder (s)	11 1	5 9	18 1	6 49	ns
Eating (s)	3 18	3 12	6 73	2 382	ns
Drinking (s)	2 65	0 45	1 13	1 111	ns
Tongue-flicking (%)	45 8	51 7	57 9	7 48	ns

Appendix 2c Mean frequencies of activities at 24 hrs, following ASS SAS SSA and SSS with SEDs between treatments Significance of differences between treatments is indicated (* P<0 05)

(c) 0-24 Hrs	Treatment					
Activity (frequency/hr)	ASS	SAS	SSA	SSS	SED	
Steps	454	493	418	628	72 4	*
Paces	14 3	39 0	5 5	21 9	12 14	ns
Ear-flicks	9 7	13 9	19 0	12 3	5 92	ns
Headshakes	1 5	1 5	3 2	1 7	0 82	ns
Bodyshakes	0 20	0 36	0 60	0 16	0 196	ns
Nosing the ground	16 6	14 6	16 4	21 1	4 09	ns
Nosing the wall	28 1	29 9	19 4	37 4	7 97	ns
Vertical head movements	2 8	10 0	3 7	8 5	2 73	*
Grooming	2 50	2 90	5 12	4 99	1 533	ns
Att groom	0 30	1 10	0 90	0 75	0 326	ns
Licking-instigator-head	9 11	6 49	12 14	6 51	1 880	*
Licking-instigator-body	21 2	24 2	20 7	20 0	4 84	ns
Licking-recipient-head	9 81	7 59	8 67	6 36	1 443	ns
Licking-recipient-body	17 7	15 1	17 2	10 6	2 42	*
Scratching head	0 19	0 38	0 06	0 46	0 19	ns
Eating	6 85	4 84	4 83	10 45	3 306	ns
Drinking	0 10	0 05	0 00	0 30	0 117	ns
Aggression-instigator	4 0	8 9	1 9	16 6	7 82	ns
Aggression-recipient	5 21	16 45	3 39	3 01	8 660	ns
Threats-instigator	9 72	7 06	8 51	5 90	7 142	ns
Threats-recipient	4 71	9 51	6 25	8 05	2 674	ns
Mounting-instigator	0 00	0 35	0 000	0 40	0 212	ns
Mounting-recipient	0 25	0 15	0 05	0 15	0 176	ns
Head held at shoulder (s)	3 4	12 5	11 3	10 7	4 99	ns
Eating (s)	3 56	2 39	2 22	7 09	2 172	ns
Drinking (s)	0 70	0 15	0 00	1 45	0 848	ns
Tongue-flicking (%)	50 8	44 2	47 5	65 8	9 03	ns