# UNIVERSIDAD COMPLUTENSE DE MADRID

**FACULTAD DE PSICOLOGÍA**
**Departamento de Psicobiología**

# ESTUDIO CONDUCTUAL Y FISIOLÓGICO DE LA CONDUCTA AGONÍSTICA TRAS EL TRASLADO DE SEIS GRUPOS DE MONOS RHESUS

**MEMORIA PARA OPTAR AL GRADO DE DOCTOR**

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TESIS DOCTORAL

CAROLINA MARÍN MARTÍN

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Dr. Paul E. Honess

2005

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# ESTUDIO CONDUCTUAL Y FISIOLÓGICO DE LA CONDUCTA AGONÍSTICA TRAS EL TRASLADO DE SEIS GRUPOS DE MONOS RHESUS.

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# Resumen

El atractivo turístico de muchas especies, así como la protección de especies amenazadas o los protocolos experimentales, son algunas de las razones que han llevado al aumento del traslado de animales en las últimas décadas. Esta tendencia ha provocado la preocupación entre muchos sectores profesionales sobre cómo llevar a cabo una práctica correcta que procure la protección tanto física como psicológica del animal, evitándose consecuencias negativas futuras.

La ocasión de nuestra presente investigación fue el traslado de una colonia de crianza de macacos rhesus (*Macaca mulatta)* desde *Harlan UK* en Hillcrest, al *Center for Macaques* en Porton Down, con el fin principal de mejorar las condiciones del hábitat. Nuestro objetivo fue medir el impacto que el traslado causó a diferentes niveles; hormonal, inmunológico y conductual en una muestra compuesta de seis grupos (N=34), pertenecientes a esta colonia de crianza.

Para ello se recogieron muestras de sangre y se observó la conducta de los animales, antes del traslado, inmediatamente después y tras siete meses del mismo.

Los principales resultados encontrados fueron: inmediatamente después del traslado se observaron unos niveles significativamente más altos de cortisol, niveles significativamente más bajos de testosterona y una respuesta inmune menor medida a través de la capacidad de los leucocitos de producir radicales libres de oxigeno en respuesta la estimulación *en vitro* por el PMA (Phorbol 12 - Myristate 13 - Acetate).

Con respecto a la conducta, se observaron aumentos significativos en la frecuencia de aquellas conductas relacionadas con auto-cuidado y exploración del medio. Siete meses después del traslado se registró una recuperación de casi todos los indicadores fisiológicos alcanzándose de nuevo, valores basales, mientras que el patrón de conducta observado tras siete meses, cambió registrándose frecuencias más altas de conductas de afiliación relacionadas con cohesión del grupo posiblemente debido a las condiciones del nuevo hábitat, el cual podría ser definido como enriquecido con respecto al anterior.

Estos resultados nos sugieren que, al menos a corto plazo, el traslado provocó en los animales un cierto grado de inestabilidad, reflejado tanto a nivel fisiológico como conductual. Si bien estos resultados podrían ser considerados como mera respuesta inmediata a una situación de inestabilidad, ya que todos los niveles fisiológicos volvieron a los valores basales siete meses después, ofreciéndonos la alentadora información de que, en principio, no aparecerán consecuencias negativas para la salud de los animales.

# Abstract

The translocation of animals has increased during the last two dacades, due to experimental protocols or protection of threatened species. This fact has provoked the concern among different sectors about how to undertake a correct translocation protocol in order to protect the psychological and physical animal wellbeing.

A breeding colony of rhesus macaques (*Macaca mulatta*) was moved from Harlan UK at Hillcrest to CFM *Center for Macaques* at Porton Down to improve habitat conditions.
Our main aim was to assess the impact of translocation on three different levels: hormonal, immunological and behavioural. Blood sample and behavioural data were collected in three different phases; before translocation of the colony, immediately after translocation and relocation in the new facilities at Porton Down, and seven months after translocation and relocation.

Immediately after colony move, It was observed a significant increase in cortisol levels, a significant decrease in testosterone levels and a lower Leukocyte capacity (*LCC)* to produce oxygen free radicals in response to a *in vitro* PMA (Phorbol 12 - Myristate 13 - Acetate) challenge. In relation to the behaviour, it was noted a significant increase in those behaviours connected with self-manteinance and exploration.
However, seven months after translocation all the physiological stress indicators observed in Phase 1 (immediately after translocation) showed a recovery reaching the baseline levels, and it was also noted a significant increase on those behaviours related to group cohesion. The changes obtained seven months after translocation might be explained due to the end of the instable condition provoked by the translocation and the new habitat characteristics which might be defined as enriched in relation to the old one in Hillcrest.

This results suggest that, the translocation caused a certain degree of stress which showed in significant changes in hormonal levels, immunological response and changes in the repertoire of behaviour. However these changes might be considered as a mere short-term response to an unestable situation which might have been interpreted as threatened by the individuals, although due to all the physiological stress indicators showed a recovery to beaseline levles seven moths after translocation we might conclude that the translocation won´t have a long term negative outcomes.

# BLOQUE I: INTRODUCCIÓN/ INTRODUCTION

- INTRODUCCIÓN GENERAL
- GENERAL INTRODUCTION

- OBJETIVOS GENERALES
- GENERAL GOALS

- HIPÓTESIS DE TRABAJO
- HYPOTHESIS

# INTRODUCCIÓN GENERAL

La protección de especies amenazadas, la exhibición de animales salvajes para el atractivo turístico y la mejora en las condiciones del hábitat, son algunas de las razones, por las que la frecuencia en el traslado de algunas especies de animales salvajes ha aumentado en las últimas décadas. Ya sean unas u otras las razones que mueven a los investigadores y/o cuidadores a llevar a cabo el traslado de ciertas especies, las condiciones de dicho traslado deberían ser estudiadas y controladas con el fin de minimizar los posibles efectos físicos y psicológicos que dicho proceso podría causar en los individuos.

Este aumento en la frecuencia de los traslados ha provocado la preocupación entre muchos profesionales dedicados al bienestar de los animales sobre cómo se está llevando a cabo dicha práctica con el fin de controlar las posibles repercusiones negativas, tanto a nivel psíquico (estrés) como físico (alopecia), que podrían ser consecuencia de un mal protocolo de traslado. Estas consecuencias negativas que ciertas situaciones de inestabilidad, ya sea ambiental o social, podrían causar en los animales, tanto a nivel fisiológico como conductual han sido ampliamente estudiadas, tal como se muestra en una amplia revisión sobre el tema recientemente hecha por nosotros (Honess & Marín 2005, Anexo 5). La respuesta de un sujeto ante una situación inestable y experimentada por dicho sujeto como amenazante (estrés) podría expresarse, al menos, a través de uno de los siguientes canales: conductual, autonómico, neuroendocrino e inmunológico (Moberg, 2000). Cada individuo poseería un órgano diana (más débil) a través del cual manifestaría su estado y el cual podría verse afectado si la situación y/o estímulo se repiten con frecuencia o de una forma constante.

Sin embargo, este mecanismo no es tan sencillo y evidente como podría pensarse en un primer momento. Así se ha observado que dos sujetos ante la misma situación podrían tener respuestas de afrontamiento diferentes, reacciones somáticas diferentes o incluso uno de ellos podría considerar la situación como amenazante mientras el otro sujeto no. Por tanto, no podemos olvidar que existen diferencias inter-individuales. También se ha observado que la percepción de una situación como amenazante podría depender del sexo del animal (Coe y cols., 1978) de la edad (Ha y cols., 2000), del estatus (Bercovich and Clarke, 1975; Coe y cols., 1979) para más información ver Honess & Marín, 2005, Anexo 6. Así mismo, podrían influir en la respuesta del individuo el aprendizaje, la habituación y/o sensibilización a un estímulo o situación anteriormente definido como amenazante, encontrándonos por tanto, ante diferencias intra-individuales. Estas diferencias inter e intra-individuales nos alejan mucho de poder dar una definición precisa del concepto de estrés y de estreses: no parece oportuno pues el uso del concepto *a priori*, ya que son muchas las variables que influyen en la respuesta y es ésta en la que tendríamos que centrarnos antes de definir un estímulo como tal.

Desde la primera definición que se conoció de estrés y con la cual hoy en día se sigue trabajando (Selye´s 1946), mucho se ha ampliado este concepto, llegando incluso a hacerse un mal uso del mismo, presentándose en muchas ocasiones como un cajón desastre en donde cualquier cambio fisiológico y/o conductual es etiquetado como estrés. La nuevas “amenazas” en la sociedad actual, hacen al mismo tiempo que el concepto sea, si cabe, más vago, confuso y menos preciso, llegándonos en numerosas ocasiones a preguntarnos qué aparece primero si la etiqueta o los síntomas.

Como se indicó anteriormente, las diferencias inter e intra-individuales de la respuesta a los posibles estímulos licitadores de estrés, ha contribuido a que dicha confusión persista, no pudiendo definir con exactitud cuáles son las respuestas fisiológicas, conductuales y autonómicas necesarias para poder etiquetar a un estímulo o una situación como estresante o meramente adaptativas.

Lo que parece más claro es que aquellos cambios fisiológicos y/o conductuales que persisten con el tiempo dando lugar a enfermedades somáticas son los que hoy en día se llevan la etiqueta clara de estrés, sin embargo, ¿necesitamos esperar que el daño este hecho para identificar un estimulo o situación como estresante? ¿cuáles son las reacciones normales y adaptativas ante una situación inestable estrés? ¿podría ser el tiempo el único factor que indicara el estado como tal? La única manera de abordar el tema del estrés es desde su multidimensionalidad y su variabilidad, aunque esto nos impida el acercarnos a una definición precisa y universal.

Más aún complejo es el tema cuando se estudia en animales. En primer lugar porque el concepto de estrés surgió en primates humanos, en los cuales se puede abordar “más claramente” el concepto de amenaza. Utilizar dicho concepto en primates no humanos nos plantea un principal problema que será tratado con más detenimiento en capítulos posteriores y en la revisión antes mencionada (Anexo 5), a saber: la extrapolación del concepto de amenaza a los animales. En humanos es “fácil” medir dicho concepto a partir de información verbal aportada por el individuo, pero en animales esto no ocurre, quedándonos como único recurso la medición de variables objetivas, como son las variables fisiológicas y conductuales, que nos permitan inferir cómo el sujeto está viviendo la situación. Es por ello que la obtención de información de al menos dos parámetros objetivos serían de suma importancia para valorar la situación como amenazante o no.

Con respecto al tema que aquí tratamos, el traslado de animales, podrían ser citadas muchas lagunas o sesgos interpretativos. La mayoría de los trabajos se centran única y exclusivamente en los aspectos conductuales o en los fisiológicos no pudiendo por tanto tomar ambos indicadores como un conjunto; pocos de ellos llevan a cabo un estudio post-traslado que permita hacer un seguimiento de los indicadores analizando el impacto a largo plazo con el fin de prever consecuencias futuras;  en numerosas ocasiones una vez "medido" el estrés se olvida la situación y/o estímulo causante de dicho estado; y por último, en numerosas ocasiones se crean situaciones experimentales que provoquen estrés con el fin de medir el estrés y de esta forma definirlo;  es decir, se etiqueta como estrés algo que todavía no se ha identificado y se asume que todos aquellos cambios que aparezcan tras esta situación artificial definida con anterioridad como "estresante" será estrés.

Aquí solo se han señalado algunos de los muchos sesgos y errores que suelen cometerse a la hora de definir, medir y abordar el "estrés". Para no caer en los mismos errores anteriormente citados, a lo largo del trabajo se hablará de cambios fisiológicos y de cambios conductuales para definir aquellos cambios aparecidos tras el traslado de los grupos, y que no pueden ser definidos como estrés por si solos, mientras que se hablará de inestabilidad ambiental a la situación referente al traslado y a los procesos que en este se abarcan. Se hablará de estrés única y exclusivamente cuando existan indicadores fisiológicos y conductuales que lo indiquen pudiéndonos de esta forma referir al traslado como un proceso estresante.

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El trabajo que aquí se presenta es parte de un estudio más amplio realizado por el Departamento de Veterinaria de la Universidad de Oxford, en el cual, aprovechando el obligado traslado de una colonia de crianza de rhesus macacos (*Macaca mulatta*) desde "*Harlan*" UK en Hillcrest al *Center for Macaques "CFM"* en Porton Down, con el fin de mejorar el hábitat de la colonia, se llevó a cabo un estudio completo midiéndose variables fisiológicas (hormonales e inmunológicas), conductuales, índices de nacimientos y muertes, condiciones del cuerpo e índices de alopecia, con el objetivo de estudiar el impacto que tuvo el traslado de la colonia en la salud de los animales. Aquí se presentan sólo aquellos datos relativos a las variables fisiológicas y conductuales en las tres fases que componen este proyecto (**FASE** 1: antes del traslado (línea basal), **FASE 2:** inmediatamente después del traslado y a la llegada al nuevo hábitat, y **FASE 3**: siete meses después del traslado).

Nuestro objetivo principal fue estudiar cómo influía en los animales la situación de inestabilidad ambiental, aportando datos conductuales y fisiológicos, tanto a corto como a largo plazo (siete meses), con el fin de:
a) definir dicha situación como estresante o no, b) rellenar algunas lagunas de los estudios hasta la fecha publicados, y c) aportar información para la definición de un protocolo de traslado que ayude al bienestar de los animales. Es importante destacar que la condición que aquí se presenta carece de artificialidad: se trata de una situación oportunista en la que se aprovechó el obligado traslado de esta colonia para estudiar cómo éste afectó en los animales.

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Tras esta introducción general sobre el tema que aquí se plantea, expondremos los objetivos generales y las hipótesis del trabajo. Una vez llegado al trabajo empírico, se mostrarán los resultados de las respuestas fisiológicas (hormonales e inmunológicas) y conductuales, precedidos por una introducción breve sobre el tema concreto abordado en cada capítulo, los objetivos y las hipótesis de trabajo específicas para cada capítulo. En el **Anexo 5** puede verse una amplia revisión sobre el tema del estrés y la agresión tanto en primates humanos como en no humanos. En esta revisión se abordan muchas de las publicaciones sobre el estrés en primates en el último medio siglo, con especial atención a aquellas publicaciones referidas a cambios ambientales y/o sociales, resaltando las variables que más influyen en dicho estado y cómo los diferentes autores abordan el concepto del estrés.

También se añade como **Anexo 6** la introducción a los capítulos 4, 5 y 6. En este Anexo puede verse una publicación referida al “enriquecimiento ambiental y la agresión” donde se aborda cómo los diferentes hábitat afectan al estrés y en concreto a la agresión, como respuesta a una situación estresante. Recordemos que el realojamiento de los monos en el *CFM* en Porton Down, se llevó a cabo con el fin de mejorar sus condiciones de habitabilidad y bienestar. Este nuevo espacio cuadruplicaba en espacio al anterior en *Harlan UK*, y estaba provisto de más juguetes y estructuras colgantes para facilitar a los animales el despliegue de conductas típicas de la especie, de suma importancia para el bienestar de éstos (Honess & Marín, 2005, Anexo 6).

NOTA: Tal como se exige en la normativa reguladora de los estudios universitarios oficiales de Postgrado de la U.C.M. (Real Decreto 56/2005), parte de la presente tesis doctoral se redacta en inglés, una de las lenguas oficiales de la Unión Europea distintas al castellano.

# OBJETIVOS

El objetivo general del trabajo que aquí se presenta consiste en el estudio de las consecuencias, a corto y largo plazo, del traslado de los animales, a nivel fisiológico (medido mediante la respuesta hormonal e inmune) y conductual (medido mediante la observación y el registro de un total de 22 conductas), con el fin de identificar aquellas variables que pudieran ser perjudiciales para el bienestar físico y psicológico de los animales. Así mismo con este estudio se pretende rellenar alguna de las lagunas aún existentes tras los estudios hasta ahora publicados sobre traslado de animales, a saber: la falta de medidas fisiológicas y conductuales en conjunto y el estudio a largo plazo de estas variables.

Para ello se han medido variables fisiológicas (niveles de cortisol y de testosterona) y variables conductuales antes, inmediatamente después y siete meses después del traslado de los animales.

Se pueden destacar los siguientes objetivos del trabajo:

1. Medir el impacto del traslado de los grupos a nivel hormonal, analizando niveles de cortisol y testosterona en sangre, con el fin de conocer cual es la respuesta hormonal a corto y largo plazo.

2. Medir el impacto del traslado de los grupos a nivel inmunológico, estudiando la capacidad de respuesta de los leucocitos en sangre de producir radicales libres de oxígeno ante la estimulación *in vitro* del PMA (Phorbol 12 - Myristate 13 - Acetate), para poder de esta forma comprobar si el traslado de los grupos afecta a la respuesta inmune a corto y largo plazo.

3. Medir el impacto del traslado de los grupos a nivel conductual, con especial atención a aquellas conductas relacionadas con la cohesión del grupo y con la competición, para identificar si el traslado causa cambios en la frecuencia de las conductas.

4. Estudiar si el rango que el animal ocupa en el grupo y el sexo afecta a la hora de interpretar la situación de inestabilidad como amenazante, y si estas variables influyen en el tipo de respuesta y en su intensidad.

# MAIN AIMS

The general aim of this work was to study the short and long term impact that the translocation caused on *physiology* (assessing cortisol and testosterona levels, and the leukocyte capacity to produce oxigen free radical in response to a *in vitro* stimulation by PMA) and on *behaviour* (observing and analysing 22 different behaviours). Physiological and behavioural data were collected in three phases (Phase 1 (Baseline) before translocation, Phase 2: inmediately after translocation and relocation of the animals in the new facilities at Porton Down, and finally Phase 3: seven months after translocation and relocation in the new facilities) in order to identify the variables related to the move that might have caused more stress to the individuals and to fill some gaps found in translcation papers such as lack of a full physiologycal and behavioural data collection and a post-move study monitoring.

In order to carry out this aims, physiological and behabioural parameters were measured pointing out the followed aims:

1. Assess the impact of the translocation on physiological levels, analysing cortisol and testosterone levels in order to study the physiological response to the translocation immediately after and seven months after the animals´ move.

2. Measure the impact of the translocation on immune response, assessing the leukocyte capacity to produce oxigen free radicals in response to an *in vitro* stimulation by PMA (Phorbol 12 - Myristate 13 - Acetate) immediately after and seven months after the animals´ move.

3. Monitor the impact of the translocation on behaviour, specially focusing on those behaviours related to group´s cohesion and competition, in order to identify if the translocation of the animals provoked some changes on the behavioural pettern.

4. Study if there were rank and sex-related differences on the quality and intensity response immediately after and seven months after translocation.

# HIPÓTESIS

## HIPÓTESIS 1: NIVELES DE CORTISOL, Y DE TESTOSTERONA INMEDIATAMENTE DESPUÉS DEL TRASLADO DE LOS ANIMALES

### HIPÓTESIS 1.1.

Los niveles de cortisol aumentarán inmediatamente después del traslado de los animales, como una respuesta del eje hipotálamo-hipofisario-adrenal ante la situación de inestabilidad ambiental provocada por el movimiento de los animales.

### HIPÓTESIS 1.2.

Los niveles de testosterona descenderán inmediatamente después del traslado de los animales, como consecuencia del aumento en los niveles de cortisol y de algunos cambios en patrones en la conducta social, tales como un descenso en la actividad sexual.

## HIPÓTESIS 2: RESPUESTA INMUNE INMEDIATAMENTE DESPUÉS DEL TRASLADO DE LOS ANIMALES.

### HIPÓTESIS 2.1.

La capacidad de los leucocitos de producir radicales libre de oxígeno como respuesta a la estimulación *in vitro* del PMA, conocida como *LCC* (*Leukocyte Coping Capacity*), disminuirá inmediatamente después del traslado de los animales, sugiriendo un aumento del número de leucocitos activos como respuesta a una situación amenazante.

## HIPÓTESIS 3: ESTUDIO DE LA CONDUCTA SOCIAL INMEDIATAMENTE DESPUÉS DEL TRASLADO DE LOS ANIMALES.

### HIPÓTESIS 3.1.

Las conductas relacionadas con cohesión del grupo, tales como acicalar, ser acicalado, juego o conducta sexual descenderán inmediatamente después del traslado, como consecuencia del aumento en las conductas de competición.

### HIPÓTESIS 3.2.

Las conductas relacionadas con competición y agresión aumentarán inmediatamente después del traslado, como consecuencia de la situación de inestabilidad provocada por éste.

## HIPÓTESIS 4: NIVELES DE CORTISOL, TESTOSTERONA SIETE MESES DESPUÉS DEL TRASLADO DE LOS ANIMALES.

### HIPÓTESIS 4.1.

Siete meses después del traslado de los animales (Fase 3), los niveles de cortisol en sangre serán inferiores a los existentes inmediatamente después del traslado (Fase 2), recuperando los niveles obtenidos antes del movimiento de los animales (Fase 1). Este descenso en los niveles de cortisol se produciría como consecuencia del cese de la situación potencialmente amenazante y la adaptación de los animales al nuevo hábitat.

### HIPÓTESIS 4.2.

Siete meses después del traslado de los animales (Fase 3), los niveles de testosterona serán superiores a los existentes inmediatamente después del traslado (Fase 2), como consecuencia del asentamiento y la adaptación de los animales al nuevo hábitat y la recuperación de conductas sociales, tales como actividad sexual y juego.

## HIPÓTESIS 5: RESPUESTA INMUNE SIETE MESES DESPUÉS DEL TRASLADO DE LOS ANIMALES.

### HIPÓTESIS 5.1.

Siete meses después del traslado de los animales (Fase 3), la capacidad de los leucocitos de responder a la estimulación *in vitro* del PMA (*LCC*), será mayor con respecto a los valores obtenidos en la Fase 2, reflejando la existencia de una mayor cantidad de leucocitos activos libres para responder a la estimulación *in vitro* por el PMA.

## HIPÓTESIS 6: ESTUDIO DE LA CONDUCTA SOCIAL SIETE MESES DESPUÉS DEL TRASLADO DE LOS ANIMALES.

### HIPÓTESIS 6.1.

Siete meses después del traslado (Fase 3), la frecuencia de las conductas relacionadas con cohesión del grupo, tales como acicalar, ser acicalado, juego o conducta sexual, aumentará respecto a los resultados obtenidos en la Fase 2, equiparándose a aquellas frecuencias exhibidas antes del traslado (Fase 1). La recuperación de estas conductas reflejaría la adaptación al nuevo hábitat.

### HIPÓTESIS 6.2.

Siete meses después del traslado (Fase 3), la frecuencia de las conductas relacionadas con competición y agresión, descenderán con respecto a la Fase 2 como consecuencia del cese de la situación inestable, potencialmente amenazante, y la adaptación al nuevo hábitat.

# HYPOTHESIS

## HYPOTHESIS 1: CORTISOL AND TESTOSTERONE LEVELS IMMEDIATELY AFTER ANIMALS´ TRANSLOCATION.

### HYPOTHESIS 1.1

Cortisol levels will increase inmediately after animals´ translocation as a hipotalamus-hypofisiario-adrenal axis response to the unstable condition provoked by the animals´move.

### HYPOTHESIS 1.2.

Testosterone levels will decrease inmediately after animals´ translocation, as consequence of the increase in cortisol levels and of some changes in social behaviour such as decreases in sexual behaviour and reproduction.

## HYPOTHESIS 2: IMMUNE RESPONSE INMEDIATELY AFTER ANIMALS´TRANSLOCATION.

### HYPOTHESIS 2.1.

The “*Leukocyte coping capacity*” *LCC* will be lower inmediately after translocation, in relation to baseline levels, due to the higher number of active leukocytes responding to the stress provoked by the animals´ move.

## HYPOTHESIS 3: STUDY OF SOCIAL BEHAVIOUR IMMEDIATELY AFTER ANIMALS´ TRANSLOCATION.

### HYPOTHESIS 3.1

There will be a decrease inmediately after translocation in relation to phase 1, of those behaviours labelled as “affiliative” and related to group cohesion due to the unstable condition provoked by the move and to the increase in agressive behaviors.

### HYPOTHESIS 3. 2

There will be an increase inmediately after translocation in relation to phase 1, of those behaviours related to group competition due to the unstable situation caused by the move.

## HYPOTHESIS 4: CORTISOL AND TESTOSTERONE LEVELS SEVEN MONTHS AFTER ANIMALS´ TRANSLOCATION AND REALOCATION.

### HYPOTHESIS 4.1

Cortisol levels will decrease seven months after translocation, in relation to the Phase 2, reaching the levels obtained in Phase 1 (baseline). This decrease might be produced by the final of the unstable situation and the complete adaptation to the new and enriched hábitat.

### HYPOTHESIS 4.2

Testosterone levels will decrease seven months after translocation, in relation to the Phase 2, due to the total adaptation to the new and enriched hábitat and the recovery of some behaviours frequencies such as sexual behaviour, reproduction and play.

## HYPOTHESIS 5: IMMUNE RESPONSE SEVEN MONTHS AFTER ANIMALS´ TRANSLOCATION AND REALOCATION.

### HYPOTHESIS 5.1

There will be an increase in *LCC,* seven months after translocation, in relation to the Phase 2, reaching the baseline levels, showing a higher leukocyte capacity to produce oxigen free radicals as a response to stimulation *in vitro* by PMA.

## HYPOTHESIS 6: STUDY OF SOCIAL BEHAVIOUR SEVEN MONTHS AFTER ANIMALS´ TRANSLOCATION AND REALOCATION.

### HYPOTHESIS 6.1

There will be an increase seven months after translocation, in relation to the Phase 2, of those behaviours named as “affiliative behaviours” and are related to group cohesion. This increase might be due to the positive adaptation to the new and enriched habitat.

### HYPOTHESIS 6.2

There will be a decrease seven months after translocation, in relation to the Phase 2, of those behaviours related to aggression and competition. This decrase might be explained due to the end of the unestable situation provoked by the move and the positive adapation to the new facilities.

# BLOQUE II: METODO Y PROCEDIMIENTO

- MUESTRA DEL ESTUDIO
- MÉTODOS
- PROCEDIMIENTO

## MUESTRA

La muestra del estudio se compuso de 35 monos rhesus (*Macaca mulatta*) pertenecientes a una colonia de crianza de un total de 200 monos divididos en grupos. Para el estudio de eligieron seis de esos grupos cada uno de ellos compuesto por un macho y cinco hembras, excepto un grupo que constaba de un macho y cuatro hembras. Aunque en algunos grupos había crías, éstas no se tuvieron en cuenta a la hora de la muestra. Todos los individuos comprendidos en el estudio eran adultos, con una media de edad de 14 años.

A lo largo del estudio murieron tres monos y uno de ellos fue cambiado de grupo, por lo que sus datos fueron eliminados de nuestro análisis, quedando como consecuencia una muestra final de 31 individuos en la tercera fase.

Los grupos fueron formados al menos seis meses antes del comienzo del estudio, y alguno de los animales incluso nacieron y se criaron en el grupo donde fueron observados.

En el **Anexo 1** pueden verse todos los detalles de los diferentes individuos que componen la muestra, así como los diferentes rangos y el tipo de captura utilizada para la recogida de la muestra y su traslado.

# MÉTODOS

Se analizó la respuesta hormonal (niveles de cortisol y testosterona) y el registró de la conducta de los animales en tres momentos:

**FASE 1** → antes del traslado de los animales (en “*Harlan*” UK, en Hillcrest)
**FASE 2** → inmediatamente después del traslado de los animales, a la llegada al nuevo hábitat en *CFM* en Porton Down.
**FASE 3** → siete meses después del traslado de los animales y de su llegada al *CFM*.

Las fechas exactas de la recogida de los datos serán indicadas más adelante cuanto sea descrito el procedimiento utilizado.

## a) ANALISIS HORMONAL

Los niveles de cortisol y testosterona en sangre se midieron con la técnica de radioimmunoensayo (RIA) utilizando un Kit exportado de los EEUU (*ICN Pharmaceuticals, Inc. Diagnostics Division, Costa Mesa, California*).
Para llevar a cabo el análisis de las hormonas se necesitaron 0,5 µl de sangre.

## b) ANÁLISIS INMUNOLÓGICO

El método usado para llevar a cabo la respuesta inmune está basado en el estudio de Mian y cols., (2003) y de McLaren y cols., (2003), quienes tras un estrés, midieron la capacidad de los leucocitos de producir radicales libres de oxígeno *in vitro* en respuesta de PMA (phorbol 12-Myriate 13-Acetate; Sigma P8139) (10-3 mol 1-1 y 10-5 mol 1-1). Observaron un aumento en el número de los leucocitos en sangre ante una situación de estrés. Como respuesta a este estrés, los leucocitos producían radicales libres de oxígeno que podían medirse a través de luminosidad (Unidades de luz relativa: RLUs) en un luminómetro portátil (Junior LB 9509 EGy G Berthold, Alemania). A esta respuesta inmunológica al estrés la llamaron "*Leucocyte coping Capacity*" (LCC).

De esta forma, un valor elevado en el luminómetro indicaría un mayor porcentaje de leucocitos activos en sangre, produciendo radicales libre de oxígeno como una respuesta a la estimulación del PMA, mientras que, por el contrario, un valor bajo en el luminómetro indicaba la existencia de un número elevado de leucocitos respondiendo y defendiendo al organismo ante la situación de estrés, y, por tanto, un número menor de leucocitos respondiendo a la estimulación del PMA. Esta respuesta se midió durante 30 segundos, cada 5 minutos, por un periodo total de 45 minutos.

Esta técnica fue anteriormente utilizada por nuestro equipo para medir las diferencias en los niveles de estrés según el tipo de hábitat (jaulas o habitaciones abiertas), ver Anexo 7.

### c) MÉTODO DE OBSERVACIÓN DE LA CONDUCTA

Para el registro de las conductas se utilizó un programa llamado *JWatcher*, el cual asocia una letra o signo de puntuación a una conducta determinada; presionando la letra o signo de puntuación correspondiente es registrada la conducta asociada. La conducta registrada terminaba cuando se presionaba otra letra que significaba el comienzo de la siguiente. Algunas de las conductas tenían asociado un modificador; éste era el caso, por ejemplo de, "evitar agresión" ("*avoid aggression*"), que iba acompañada de un número (asociado a una animal que era el que agredía) y un signo de puntuación (asociado a un tipo específico de agresión: "morder" ("*bites*" (;)) o perseguir ("*chases*" ([). De esta forma, registrábamos todas las conductas realizadas por el animal que estaba siendo observado en ese momento, así como las conductas que recibía de otro miembro del grupo (por ejemplo, acicalado (g) o agresión (f)) y el animal de quien las recibía.

Se utilizó observación focal con registro continuo de la conducta de cada individuo (Altman, 1962).

Se observaron un total de 22 conductas (ver **Anexo 3)** que posteriormente se dividieron en cinco categorías diferentes**.**

d) MEDIDA DEL RANGO

Para obtener información sobre los rangos que cada animal ocupaba en el grupo, se realizó un análisis matricial a partir de la información obtenida por los observadores de los signos de dominancia mostrados por los distintos individuos mientras realizaban sus observaciones diarias, durante la hora de la comida de los animales (p.ej. los cuidadores les daban de comer a los animales y éstos se acercaban a la comida de acuerdo a su rango jerárquico) así como, a través de una media hora extra de observación durante la cual los observadores daban dulces a los monos registrando el orden en que iban acercándose los animales con el fin de coger los dulces.

Esta tarea se llevó a cabo en todas las fases del estudio con el fin de comprobar si habían existido cambios en la jerarquía de cada grupo.

# PROCEDIMIENTO

Como se indicó en la introducción general, este trabajo es parte de un amplio estudio llevado a cabo por el Departamento de Veterinaria de la Universidad de Oxford, cuyo principal objetivo era estudiar el impacto del traslado y la reubicación de seis grupos de macacos rhesus (*Macaca mulatta*) pertenecientes a una gran colonia de crianza. Se trata por tanto de un proyecto oportunista, en el que fueron observadas y medidas diversas respuestas fisiológicas, e inmunológicas, así como patrones conductuales, índices de nacimientos y mortalidad, alopecia y peso corporal, en tres etapas diferentes: antes del traslado, inmediatamente después y a los seis meses del mismo. Dichos datos se midieron tanto en machos como en hembras. También se tuvo en cuenta otras variables que pudieran afectar a los animales, como son las condiciones del transporte o el método de captura, aunque, como se ha indicado anteriormente, no pudieron controlarse absolutamente todas las variables, debido a que se trataba de un proyecto oportunista.

Las tomas de datos fisiológicos fueron realizadas aprovechando el “*B Virus screening”* al que son sometidos todos los animales cada 3-6 meses.

Las fechas en las que se llevaron a cabo la recogida de datos se detallan a continuación:

## FASE 1

**1ª recogida de datos fisiológicos →** en “Harlan UK” **:** 4, 5, 6 de Marzo del 2003

**1er registro de datos conductuales →** Del 7 de Julio al 11 de Julio del 2002 (primer grupo de cada observador) y del 14 de Julio al 18 de Julio del 2002 (segundo grupo de cada observador)

## FASE 2

**2ª recogida de datos fisiológicos:** → a la llegada a “CFM”: 16, 18, 20, 23, 25 y 27 de Junio del 2003

**2º registro de datos conductuales** → Del 16 de Febrero al 20 de Julio del 2003 (primer grupo de cada observador) y del 24 de Febrero al 28 de Febrero del 2003 (segundo grupo de cada observador)

## FASE 3

**3ª recogida de datos fisiológicos** →

**3er registro de datos conductuales** →

Los animales fueron capturados por los cuidadores pertenecientes al centro y ayudados por los veterinarios. Como puede verse en el **Anexo 1**, el método de captura usado difirió según los animales, dependiendo, entre otras razones, de la peligrosidad del animal. Los individuos fueron capturados y sedados usando hydroclorido de ketamina, obtenida en “Fort Dodge Animal Health”, a una concentración de 100 mg/ml, administrando una dosis de 10mg/kg del peso corporal.

Tras la extracción de sangre, todos los animales fueron medidos, pesados, y examinados exhaustivamente por veterinarios. Los índices de alopecia también fueron registrados.

Por lo que respecta al traslado, se anestesió a algunos animales que no fueron trasladados en sus jaulas; todos los demás fueron capturados y trasladados en sus jaulas, siendo sedados únicamente al llegar a CFM, para poder realizar la extracción de sangre.

Aunque el traslado de los grupos fue realizado en diferentes días, todos ellos fueron trasladados en camión a una temperatura constante que oscilaba entre los 17,7 ºC y los 18,1 ºC según los grupos. La duración del viaje osciló entre 3:20 horas y 3:50 horas.

Con respecto a la Fase 3, se siguió el mismo protocolo que para la Fase 1, el método de captura utilizado, puede verse en el **Anexo 1.**

La recogida de sangre en las tres fases fue llevada a cabo por veterinarios pertenecientes al Departamento de Veterinaria de la Universidad de Oxford, entre las 9:00 y la 13:00, tanto antes del traslado como después del mismo. Del total de la sangre extraída de cada animal, 30µl de sangre se utilizó en el mismo momento para medir la respuesta inmune cuyo procedimiento será expuesto en breve, mientras que 0,5µl fueron guardados y congelados a la temperatura de -20ºC y enviados al Laboratorio de Neuroinmunomodulación, Departamento de Bioquímica y Biología Molecular III, de la Facultad de Medicina (UCM), donde se hicieron los análisis pertinentes.

Con respecto a la respuesta inmune se utilizaron 30µl para la medida de la actividad de los leucocitos preparándose tres tubos compuestos por diferentes concentraciones:

- Un tubo control, compuesto de: 10µl de agua destilada + 90µl de luminol + 10µl de sangre.
- Un tubo PMA $10^{-3}$compuesto de**:** 90µl de luminol + 10µl de PMA $10^{-3}$ + 10µl de sangre.
- Un tubo PMA $10^{-5}$compuesto de: 90µl de luminol + 10µl de PMA $10^{-5}$ + 10µl de sangre.

Una vez que los tubos estaban preparados se agitaban bien para mezclar las diferentes concentraciones, y uno por uno se iban introduciendo en el luminómetro durante 30 segundos. Los restantes tubos pertenecientes a ese sujeto (PMA $10^{-3}$ y PMA $10^{-5}$), eran guardados y mantenidos en una incubadora a una temperatura constante de 37ºC, a la espera de ser analizados. El valor representado por el luminómetro tras los 30 segundos se anotaba en una hoja de datos (ver anexo 3), en la que posteriormente se irían recogiendo todas las mediciones de las tres concentraciones durante 45 minutos. Mientras que el tubo control nos ofrecía la medida la actividad basal de los leucocitos, los tubos PMA $10^{-3}$ y PMA $10^{-5}$ medirían la respuesta de los leucocitos a la estimulación *in vitro* del PMA.

Con respecto a la conducta, en la observación y registro de éstas participaron tres observadores. Unas semanas antes de empezar la recogida de datos, se entrenó a todos los observadores a identificar las conductas objeto de estudio con el fin de unificar su criterio de observación. Para ello, se trasladaron durante algunas horas al Departamento de Psicología Experimental de la Universidad de Oxford, donde se encontraba un grupo de macacos rhesus en cautividad. Tras este entrenamiento, se procedió a otro con el fin de tomar contacto directo con los grupos asignados y reconocer a cada uno de los animales del grupo. Ésto se realizó en el mismo lugar donde se llevó a cabo la investigación y con los grupos asignados a cada observador (aunque cada animal llevaba tatuado su nombre o número en el pecho, muchas veces la conducta realizada impedía ver el tatuaje y por tanto reconocer al animal). Los observadores pasaron un día entero reconociendo los animales y las distintas conductas.

Como parte de protocolo de la investigación, se les pedía a todos los observadores que llegaran al lugar de estudio (*Harlan UK* en la primera fase y *CFM,* en la segunda fase) media hora antes de comenzar la recogida de datos con el fin de preparar el material que sería utilizado y tomar un primer contacto con el grupo que iban a observar. Todos los observadores comenzaban el registro de datos a la misma hora, a las 9:00 de la mañana, terminando a las 18:00 de la tarde. Se observaba a cada animal del grupo durante media hora por la mañana y media hora por la tarde es decir un total de cinco horas a la semana. En el **Anexo 4** puede verse el esquema de trabajo que se siguió para la recogida de datos de dos grupos (Blues y Thor).

Con la información obtenida en estudios anteriores sobre la conducta de la colonia y después de una amplia revisión bibliográfica, se consideró oportuno dividir las conductas en cinco categorías:

## 1. CONDUCTA SOCIAL ***(CS)***

### → AGRESION ***(F)***

- Auto agresión
- Evitar agresión
- Agresión

### → CONDUCTA AFILIATIVA ***(A)***

- Intercambiar acicalado
- Acicalar
- Pelea-juego
- Conducta sexual
- Ser acicalado
- Abrazar
- Juego

## 2. CONDUCTA EXPLORATORIA ***(M)***

- Manipular cage
- Observar
- Jugar con objetos

## 3. CONDUCTAS DE AUTO-CUIDADO ***(E)***

- Beber
- Comer o buscar comida
- Auto-acicalado
- Descansar/inactividad

## 4. OTRAS ***(O)***

- Movimiento
- Cuidar de la cría
- Movimientos estereotipados
- Rapid energy expenditure

# BLOQUE III: TRABAJO EMPÍRICO

## CAPÍTULO 1: MEDIDA DE LOS NIVELES HORMONALES INMEDIATAMENTE DESPUÉS DEL TRASLADO DE LOS ANIMALES.

1.1. Introducción
  a) Motivo y objetivos de la medición de los niveles hormonales
  b) Hipótesis

1.2. Resultados
  a) cortisol
  b) testosterona

1.3. Discusión
1.4. Conclusiones

## CAPÍTULO 2: ESTUDIO DE LA RESPUESTA INMUNE INMEDIATAMENTE DESPUÉS DEL TRASLADO DE LOS ANIMALES.

2.1. Introducción
  a) Motivo y objetivos de la medición de la respuesta inmune
  b) Hipótesis

2.2. Resultados
2.3. Discusión
2.4. Conclusiones

CAPÍTULO 3: ESTUDIO DE LA CONDUCTA SOCIAL INMEDIATMENTE DEPUÉS DEL TRASLADO DE LOS ANIMALES

3.1. Introducción
    a) Motivo y objetivos de la medición de la respuesta inmune
    b) Hipótesis

3.2. Resultados
3.3. Discusión
3.4. Conclusiones

CAPÍTULO 4: MEDIDA DE LOS NIVELES HORMONALES SIETE MESES DESPUÉS DEL TRASLADO DE LOS ANIMALES.

4.1. Introducción
    a) Motivo y objetivos de la medición de los niveles hormonales siete meses después del traslado.
    b) Hipótesis

4.2. Resultados
4.3. Discusión
4.4. Conclusiones

CAPÍTULO 5: ESTUDIO DE LA RESPUESTA INMUNE SIETE MESES DESPUÉS DEL TRASLADO DE LOS ANIMALES.

5.1. Introducción
    a) Motivo y objetivos de la medición de los niveles hormonales siete meses después del traslado.
    b) Hipótesis

5.2. Resultados

# *Capítulo 1:*

# *MEDIDA DE LOS NIVELES HORMONALES TRAS EL TRASLADO DE LOS ANIMALES*

1.1. Introducción

- a) Motivo y objetivos de la medición de los niveles hormonales
- b) Hipótesis

1.2. Resultados

- a) cortisol
- b) testosterona

1.3. Discusión
1.4. Conclusiones

## 1.1. INTRODUCCIÓN

Son muchas las razones que explican el aumento en al número de traslados de algunas especies animales en las dos últimas décadas. Entre otras, podrían destacarse la protección de especies amenazadas, el atractivo turístico de animales salvajes, las exhibiciones y los protocolos experimentales. Este aumento ha provocado la preocupación de biólogos, primatólogos, veterinarios y expertos en el bienestar de los animales sobre cómo está siendo utilizada dicha técnica y cuál debería ser el protocolo más adecuado para su transporte y reubicación, con el fin de maximizar las ventajas del traslado y minimizar el posible coste psicológico y físico causado en los animales. Algunos de los aspectos que más preocupan a los estudiosos del tema son aquellos relacionados con el posible estrés sufrido por las colonias o los grupos de animales objeto del traslado y aquellos otros que se refieren a la propagación de enfermedades infecciosas**.**

Aunque existen muchas definiciones sobre el fenómeno del traslado de animales, solo una minoría explicita el objetivo y el protocolo que debería seguir dicho proceso. Por ejemplo: "*deliberate and mediated movement of wild individuals or populations from one part of their rang to another*" (IUCN/SSC, 1995 citado en Ostro y cols., 1999)**;** "*transport and release of wild animals from one location to another with emphasis on a nuisance and damage context*" (Craven y cols., 1998); **"***deliberate movement of wild primates from one natural habitat to another for the purpose of conservation or management*" (Baker, 2002).

En el traslado y reubicación de animales pueden ser identificados varios subprocesos y diversas variables que deberían ser estudiados cuidadosamente con el objetivo de procurar el máximo éxito final. Entre los subprocesos pueden destacarse: el método de captura y el tipo de anestesia , el transporte del grupo o colonia, y la reubicación en el nuevo hábitat.

En cuanto al método de captura, el entrenar al animal para que coopere, por ejemplo, en la extracción de sangre, sería la técnica más costosa, aunque, a cambio, podría aminorar el estrés producido por la captura (Vertein y Reinhardt, 1989; Reinhardt y cols., 1990; Reinhardt y cols., 1991c; Reinhardt y cols., 1995; Reinhardt y Cowley, 1992; Reinhardt, 1991c, 1996). También podrían aconsejarse otras técnicas de “atrape”, tales como el uso de redes, por ser consideradas rápidas para capturar al animal y por lo tanto poco elicitadoras de estrés (Moinde y cols., 2004).

Por lo que a la anestesia se refiere, la *Ketamina* es la más aconsejada para primates no humanos (Crockett y cols., 1993; Clarke y cols., 1996) a una concentración máxima de 100 mg/ml, administrando una dosis de 10mg/kg de peso corporal (Honess y cols., 2004). No obstante, se ha observado que puede provocar cambios en los marcadores fisiológicos relativos al estrés, como es el incremento en los niveles de glucocorticoides.

Respecto al transporte, se ha observado que tanto el transporte por aire (Honess y cols., 2004) como por tierra (Ha y cols., 2000) provoca un cierto grado de estrés en los animales, aunque un exhaustivo control de las condiciones del mismo (temperatura, humedad, duración…) podría aminorar dicho estrés.

Por último, el hábitat al que serán enviados los animales debería ser “conscientemente” elegido con el fin de garantizar una mejora en sus condiciones de habitabilidad, siempre que las condiciones experimentales lo permitieran, deberían estudiarse el tipo (en libertad/semi-libertad, cautividad o jaulas) y las condiciones del nuevo espacio (disponibilidad de comida, tipo de cuidadores, enriquecimiento del medio etc…) para, de alguna forma, anticipar eventuales consecuencias positivas y negativas y así poder potenciar las primeras y buscar posibles soluciones a las segundas.

En muchas ocasiones los investigadores y/o los cuidadores aprovechan el cambio de entorno para realizar cambios sociales en el grupo. Estos cambios sociales podrían llegar a ser la mayor y más significativa fuente de estrés.

Aunque el estudio sobre el traslado de animales es relativamente reciente, la documentación sobre el tema es amplia (Southwick y cols., 1984; Strum y Southwick, 1986; Garcia-Orduna y cols., 1987; De Vries, 1991; Ha y cols., 2000; Thoisy y cols., 2001; Berman y Li  2002; Strum, 2004), dado el interés de llevar a cabo una práctica correcta, despertado por diferentes especialistas en distintas áreas de estudio.

Para valorar el efecto causado por el movimiento de los animales, numerosos autores han destacado la necesidad de recoger datos conductuales, fisiológicos, sociales y demográficos como los índices de nacimientos y supervivencia durante al menos un año, (Moinde y cols., 2004; Strum, 2004). Sin embargo no faltan lagunas en los estudios sobre el traslado. Por ejemplo, la mayor parte de los trabajos se han centrado en el impacto del traslado a nivel conductual (Ostro y cols., 1999**;** Berman y Li, 2002), sin prestar suficiente atención a otras variables de tipo fisiológico o inmunológico; y sólo una minoría de los estudios publicados han llevado a cabo un estudio post-traslado observando las consecuencias a largo plazo del impacto del traslado **(**Southwick y cols., 1998; Berman y Li, 2002**;** Strum, 2004). Esta importancia del estudio a largo plazo ha sido destacada en los últimos años por muchos autores; por ejemplo, Moinde y cols., (2004) sugiren que la completa adaptación al nuevo hábitat debería ser considerada sólo cuando las relaciones entre los miembros del grupo hayan sido consolidadas y conocidas; sólo de esta forma podría darse por concluido el estudio.

Dos de los <u>cambios conductuales</u> más observados tras el traslado de animales han sido el aumento de encuentros agresivos (Berman y Li, 2002) y la reducción de las conductas de cohesión en el grupo (Johnson y Southwick, 1984**;** Yeager y Silver, 1999). Y aunque no existen muchos datos sobre la respuesta fisiológica de los animales después del traslado, podrían predecirse ciertos patrones fisiológicos relativos al estrés, tales como el incremento en los niveles de glucocorticoides y el descenso en la respuesta inmune.

Respecto a los factores demográficos, se ha observado cómo el traslado podría incrementar los índices de mortalidad, tanto infantil (Berman y Li, 2002) como de adultos (Ha y cols. 2002), y disminuir los índices de nacimientos (Johnson y Southwick, 1984; Berman y Li, 2002)**.** Sin embargo, en otros trabajos no se observaron consecuencias conductuales tras el traslado de una colonia de macacos rhesus (Strum y Southwick, 1986) ni cambios en las tasas de supervivencia o reproducción (Ha y cols., 2000).

Los distintos autores suelen estar de acuerdo en afirmar que los criterios más válidos para evaluar el impacto del traslado en los animales podrían ser la cohesión en el grupo, las respuestas fisiológica e inmunológica, el porcentaje de nacimientos y muertes así como las condiciones del cuerpo (peso, talla, grado de alopecia, etc…) (Strum y Southwick 1986; Strum, 2004).

Una de las lagunas en los estudios sobre el traslado de colonias hasta ahora publicados, es precisamente la medición de las respuestas fisiológicas tras el traslado. La gran cantidad de variables que deben controlarse a la hora de interpretar y considerar los resultados hormonales ha llevado a los investigadores a ignorar los factores fisiológicos, centrándose en aquellos índices más estables y objetivos, como pueden ser la conducta, los índices de supervivencia, y de nacimientos etc… Sin embargo, la amplia bibliografía existente sobre el estrés en primates humanos y no humanos, nos ofrece una gran variedad de respuestas fisiológicas, consideradas en las últimas décadas como indicadores válidos del estrés. Entre éstas cabe destacar: los niveles de cortisol, de la hormona del crecimiento (GH), de testosterona y de prolactina (Browns y cols., 1971; Crockett y cols., 1994**;** Smith y French, 1997)**.**

Aunque existe acuerdo en considerar que dichos marcadores podrían reflejar distintos estados o grados de estrés, las interpretaciones deberían hacerse con mucho cuidado ya que, como se ha apuntado anteriormente, en la respuesta del estrés podrían estar influyendo un gran número de variables entre las que se pueden destacar: variaciones circadianas **(**Rose y cols, 1971**;** Sousa y Ziegler, 1998**;** Mendoza y cols., 2000**;** Theorell, 2003)**,** diferencias inter-individuales (Rose y cols., 1978; Crockett y cols., 1993; Capitanio y cols., 2004**;**), diferencias entre especies de primates (p.ej**.** se ha observado que los monos ardilla presentan valores basales de esteroides superiores a otras especies y que muestran unas considerables diferencias inter/intra individuales en la respuesta hormonal tras el estrés (Coe y cols., 1979**;** Anzenber y cols., 1986**;** Clarke y cols., 1988), edad (Rose y cols., 1978), apoyo social (Maestripieri, 1993**),** estabilidad del grupo (Gordon y cols., 1979), rango (Rose y cols., 1975; Coe y cols., 1979; Bercotvich y Clarke, 1995; Shively, 1998), sexo (Aidara y cols., 1981**;** Crockett y cols., 1993) tipo de estrés (Brown y cols., 1971) y condiciones del hábitat (p.ej. los animales que habitan en libertad y semi-libertad tienen niveles basales de cortisol más bajos que aquellos que viven en cautividad (Suleman y cols., 2002).

Tras cambios sociales (Soltis y cols., 2003) y/o cambios ambientales (Shively, 1998) también se ha observado un incremento en los niveles de hormonas adrenocorticales en muy diversas especies de primates y de otros mamíferos, tales como: monos ardilla (Brown y col., 1971**;** Soltis y cols., 2003)**,** monos talapoin (Eberhart y cols., 1983)**,** monos *Rhesus* (Gordon y Gust 1993)**,** roedores (Jahn y Deis 1986; Almeida y cols., 2000) y humanos (Chatterton y cols., 1997). En algunas ocasiones, sin embargo, los incrementos en los niveles de cortisol son malinterpretados, pues no siempre van asociados con situaciones de estrés. Así, por ejemplo, el ejercicio físico y la práctica deportiva, sin ser necesariamente a nivel competitivo, pueden alterar los niveles hormonales, aumentando los valores de cortisol en sangre (Filaire y cols., 1996; Kane, 2004; Honess y Marín, 2005; )**,** mientras que, por otra parte, los niveles bajos de cortisol se asocian con un bajo grado o ausencia de estrés, si bien podrían también estar reflejando un estrés crónico o postraumático.

La testosterona ha sido ampliamente estudiada en relación con la agresión (Rose y cols., 1971**;** Bernstein y cols., 1974**;** Dixon y Herbert, 1977**;** Gordon y cols., 1979; Ramírez, 2003; Archer, 2005), la reproducción (Gordon y cols., 1976**;** Carlstead y Sheperdson, 1994; Ha y cols., 2000**;** Lynch y cols., 2002) las emociones (Rernhardt, 1997; Mazur y Booth, 1998**)** y las variables cognitivas y la personalidad (Olweus y cols., 1980).

Sin embargo la relación directa entre la testosterona y el estrés parece ser un área de estudio relativamente nueva.

La discrepancia entre los resultados arrojados por los diferentes estudios ha provocado un desacuerdo en lo que se refiere al papel de la testosterona en el estrés. Aunque algunos estudios en el área del estrés indican que los niveles de testosterona podrían disminuir debido a un estrés físico o psicológico en primates humanos (Francis, 1981**;** Spivak y cols., 2003) y no humanos **(**Sapolsky y Donnelly, 1985**;** Dinzburg y cols., 1992)**,** también se han observado aumentos en los niveles de testosterona tras la exposición a estímulos estresantes (Coe y cols., 1979).

Otra de las hormonas utilizadas como indicador de estrés ha sido la prolactina (Suay y cols., 1999). Los niveles de prolactina parecen incrementar tras una situación de estrés agudo (Aidara y cols., 1981; Jahn y Deis, 1986; Almeida y cols., 2000) y crónico (Almeida y cols., 2000) aunque también se ha observado descenso en sus niveles tras situaciones estresantes (Eberhart y cols., 1983). Aunque el papel de la prolactina como indicador del estrés no está muy claro, existe un consenso respecto a que la respuesta de la prolactina podría ser menor y más rápida que la dada por otras hormonas tales como el cortisol, la hormona GH o la testosterona, y que, al igual que las hormonas anteriormente señaladas, dicha respuesta podría depender del sexo del animal (Shively, 1998) y de las condiciones del hábitat (Suleman y cols., 2002).

Una de las variables que podrían afectar a las respuestas hormonales después del estrés sería el rango que el animal ocupa en el grupo. En muchas especies [monos ardilla (Coe y Mendoza, 1979); macacos rhesus (Hayasi y Moberg, 1987); babuinos (Sapolsky, 1993)), se han observado niveles hormonales basales diferentes dependiendo del rango. Estos niveles basales podrían predisponer al animal a ser y/o a actuar de forma diferente al resto del grupo alcanzando de esta forma un rango u otro, por el contrario, las causas de dichas diferencias hormonales basales podrían ser los rasgos conductuales exhibidos por los individuos con rangos diferentes. Estos interrogantes permanecen aún sin resolver y será difícil encontrar una respuesta que satisfaga a todas las especies en todos los momentos. Sin embargo, parece evidente que, si realmente existen diferencias basales, éstas "predispondrían" al animal a responder de una u otra forma ante situaciones de estrés (Coe y cols., 1979; Sapolsky, 1993).

La relación entre niveles de glucocorticoides y dominancia ha sido la más estudiada en diferentes especies; lemures (*Lemur catta*), (Cavigelli, 1999)**,** perros salvajes africanos (Creel y cols., 1996), papiones enanos (Monfort, 1997), babuinos (Sapolsky, 1994), macacos rhesus (Bercotvich y Clarke, 1995). Se pensaba que los subordinados mostrarían niveles más altos de glucocorticoides (Sassenrath, 1970**;** Manogue y cols. 1975**;** Keverne y cols., 1978**;** Keverne y cols., 1982; Sapolsky, 1993) e incluso se sugirió que estas características neurofisiológicas de los subordinados podían predisponer a la depresión (Shively, 1998**).** Sin embargo, algunos trabajos resaltaron el hecho de que en algunas especies los individuos dominantes presentaban niveles más altos de glucocorticoides que sus subordinados, sugiriendo que el beneficio de la dominancia podría tener un hándicap a largo plazo para los "poderosos" (Coe y cols., 1979; Cavigelli, 1999**;** Barret y cols., 2002)

Las diferencias en los niveles de testosterona según el rango se han estudiado del mismo modo (Bercovitch y Clarke, 1995**;** Lynch y cols., 2002**)**, observandose niveles más altos de testosterona en los animales dominantes que en los subordinados. Esto se explica dada la relación entre agresión y testosterona [la mayor parte de los encuentros agresivos en el grupo se dan entre individuos dominantes (Bernstein y cols., 1974; Gordon y Rose, 1976; Dixson, 1977), y entre reproducción y testosterona [los individuos dominantes tienen más acceso a las hembras (Gordon y Rose, 1976**;** Bercovith y Clarke, 1995**;** Lynch, 2002). Sin embargo se ha observado la existencia de otras variables, tales como apoyo social (Eaton y Resko, 1974; Rose y cols., 1978) y la estabilidad del grupo (Sapolsky, 1983), que podrían ser más importantes que la dominancia y la subordinación.

La relación entre el rango jerárquico del animal y los niveles hormonales permanece confusa, si bien los trabajos recientes sugieren que serían los rasgos conductuales propios de cada especie, más que el rango del individuo, los que podrían estar relacionados con los niveles hormonales. De esta forma, aquellas especies en las que el animal dominante y subordinado presente rasgos típicos de conductas específicas, mostrarán una mayor posibilidad de diferencias en los niveles hormonales que aquellas otras especies o grupos cuyos individuos dominantes y subordinados presenten rasgos de conductas similares (Sapolsky, 1993).

Entre las deficiencias más llamativas en el estudio sobre el impacto del traslado de animales, señaladas con anterioridad a lo largo de esta introducción, cabe destacar la falta de medidas fisiológicas y de un estudio detallado de las variables que intervienen previamente al transporte y que podrían estar causando un efecto desencadenante de estrés (como el método de captura y la anestesia). Aunque existe una amplia referencia bibliográfica sobre los indicadores fisiológicos del estrés, tanto en primates humanos como en no humanos, también nos encontramos una gran cantidad de lagunas por lo que se refiere a estos últimos. La mayor parte de los trabajos se han realizado en machos (Bernstein y cols., 1974**;** Rose y cols., 1978; Coe y cols., 1979; Gordon y Gust, 1993; Lynch y cols., 2002), analizándose respuestas conductuales pero ignorando respuestas fisiológicas, en la presunción gratuita de que una conducta observada es consecuencia de un estado emocional, sin presentar ningún indicador fisiológico que nos permita comprobar si realmente existe dicho estado como tal (Das y cols., 1998)**.** Por otra parte, cuando se miden las respuestas fisiológicas, tampoco suele llevarse a cabo un estudio exhaustivo sobre aquellas variables que pudieran estar influyendo en los marcadores fisiológicos. En general, existe una falta de documentación en cuanto al análisis de valores hormonales y conductuales como un todo, tanto en machos como en hembras.

## a) MOTIVO Y OBJETIVOS DE LA MEDIDA DE LOS NIVELES HORMONALES.

Aprovechando el obligado traslado de una colonia de crianza de macacos rhesus (*Macaca mulatta*) desde las instalaciones de Harlan UK, en Hillcrest al *Centre for Macaques (CFM)”*en Porton Down, hemos realizado un estudio bastante completo a nivel conductual, fisiológico e inmunológico, así como de algunas de sus condiciones demográficas, como los índices de nacimiento, de mortalidad y supervivencia, y biológicas, como las condiciones del cuerpo, peso, grado de alopecia, etc … en varios grupos de sujetos.

El trabajo que aquí se presenta mostrará exclusivamente aquellos datos relacionados con las respuestas hormonales niveles de COR, y de testosterona y se expondrán datos de variables relativas a las primeras fases del traslado, como son la captura de los animales y su anestesia, y el tipo y las condiciones del transporte. En apartados posteriores serán presentados los datos inmunológicos y conductuales pertenecientes a las dos primeras fases del estudio y los correspondientes a la última fase.

El objetivo principal del trabajo consiste en aportar una serie de datos que permitan rellenar algunas de las lagunas observadas en los diferentes estudios sobre el tema hasta ahora publicados. En concreto quisiéramos estudiar el impacto a nivel fisiológico del traslado en diversos grupos de macacos rhesus, de ambos sexos, con la esperanza de que ayude a esclarecer, de algún modo, la eventual respuesta hormonal de los individuos tras el traslado, sin asumir estados emocionales, y comprobar si existen diferencias en los niveles hormonales basales según el rango, así como en la respuesta fisiológica tras el transporte en un grupo estable con jerarquía sólida.

## b) HIPÓTESIS

Las hipótesis con las que trabajamos fueron las siguientes:

1. Los niveles de cortisol aumentarán inmediatamente después del traslado de los animales, como una respuesta del eje hipotálamo-hipofisiario-adrenal a la situación de inestabilidad ambiental provocada por el movimiento de los animales.

2. Los niveles de testosterona descenderán inmediatamente después del traslado de los animales, como consecuencia del aumento en los niveles de cortisol y a cambios en patrones en la conducta social, tales como descensos de la conducta sexual.

## 1.2. RESULTADOS

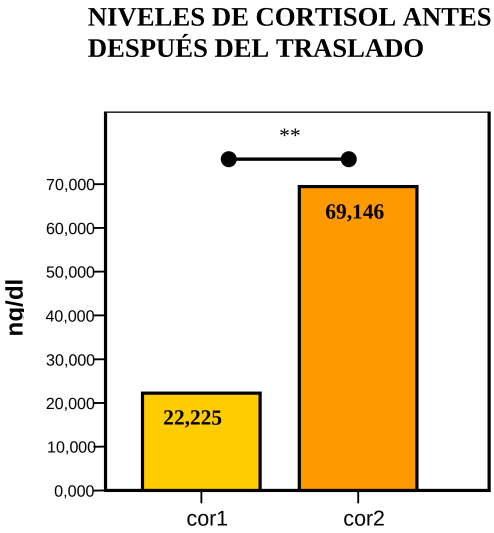
Los niveles de cortisol, testosterona en sangre se midieron antes e inmediatamente después del traslado de la colonia con el fin de observar el posible impacto de dicho proceso a nivel fisiológico. También fueron estudiados los efectos del rango y del sexo en los niveles hormonales basales y en la respuesta hormonal tras el transporte.

### ANÁLISIS ESTADÍSTICO

Para llevar a cabo el analisis estadístico, se utilizo el programa estadistico SPSS 12.0 para Windows. Una vez comprobado el supuesto de normalidad para cada variable a través de la prueba de *Shapiro Wilk*, se utilizaron pruebas paramétricas, cuando las variables cumplían dicho supuesto de normalidad, en concreto se utilizó *Anova de medidas repetidas*, para estudiar las posibles diferencias en los niveles hormonales antes y despues del trasporte y *ANOVA de un factor*, para examinar diferencias relacionadas con el genero y el rango, y no paramétricas, cuando las variables no cumplian dicho supuesto, concretamente *Wilcoxon* para estudiar las posibles diferencias en los niveles hormonales antes y después del transporte, la prueba *U de Mann-Whitney* para examinar las posibles diferencias relativas al sexo y el test *H de Krutal-Wallis* para examinar las posibles diferencias en los valores hormonales basales en relación al rango.

## a) CORTISOL

**GRÁFICO 1**



La prueba de *ANOVA de medidas repetidas* mostró que existían **diferencias significativas** entre los niveles de cortisol antes y después del traslado ***(F=303.081; n=31; p= 0,000)***

**Xcor1** = 22,225; **N** = 31; **DS** = 12,556; **StdE**=2.2552

**Xcor2** = 69,146; **N** = 31; **DS** = 13,375 **StdE**=2.2938

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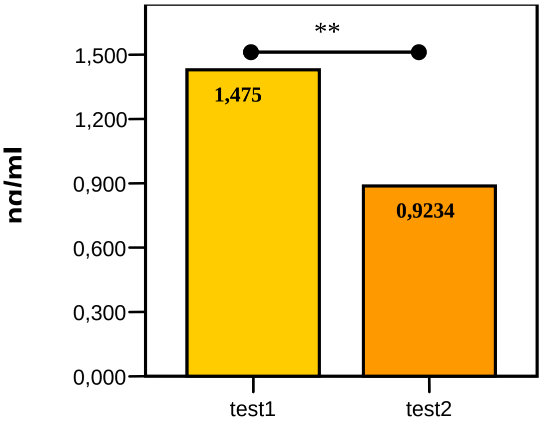
Tras realizar la prueba de *ANOVA de un factor* para estudiar las diferencias en los niveles del cortisol antes y después del traslado en relación con el sexo, no se encontraron diferencias significativas en los valores basales ***(F= 0.214; n = 31; p = 0.647***), ni tras el traslado ***(F=1.445; n = 33; p = 0.238)***. Tampoco se encontraron diferencias significativas en los niveles de cortisol con respecto al rango en la primera fase (***F = 0.396; n = 31; p = 0.847)*** ni en la segunda ***(F = 0.863; n = 34; p = 0.518).***

## b) TESTOSTERONA

**GRÁFICO 2**

**NIVELES DE TESTOSTERONA ANTES Y DESPUÉS DEL TRASLADO**



El *test de Wicoxon* fue utilizado para estudiar diferencias en los niveles de testosterone antes y despues del traslado, encontrándose **diferencias significativas** en los niveles de testosterona antes del transporte y después del mismo. **(*Z =-2,505; n = 28; p = 0,012*)**

**Xtest1** = 1,475; **N** = 28; **SD** = 1,1752 ; **StdE**=0.21456

**Xtest2** = 0,9234; **N** = 28; **SD** = 0,7210 ; **StdE**=0.12746

No se encontraron diferencias en los niveles basales de testosterona dependiendo del sexo **(U = 61; n = 30; p = 0,568)** ni en los niveles de testosterona tras el transporte **(U = 70; n = 32; p = 0,724)**, ni tampoco se encontraron diferencias significativas en ninguna de las dos variables en relación con el rango: ***(H =5,55; n = 30; p = 0,353)***, ***(H = 8,68; n = 32; p = 0,122).***

En la fase del traslado, cuatro individuos tuvieron que ser trasladados en jaulas diferentes y separados del grupo y para ello fueron sedados antes del transporte. Para analizar eventuales diferencias entre estos animales y los que sólo fueron sedados a la llegada a CFM, a través de *la prueba U de Mann-Whitney se* estudiaron las posibles diferencias entre estas dos condiciones experimentales. No se encontraron diferencias significativas ni en los niveles de cortisol ***(U = 47; n = 32 ; p = 0,608)***, ni en los niveles de testosterona ***(U = 43; n =30; p = 0,583)*** ni en los de prolactina ***(U = 37; n = 31; p = 0,316).***

## 1.3. DISCUSIÓN

Como fue apuntado en la introducción, no existen muchos trabajos que examinen el impacto del traslado a nivel fisiológico; la mayoría de estos estudios se han centrado en el impacto a nivel conductual, y/o en el porcentaje de nacimientos, índices de mortalidad y pérdida de peso corporal. Dichos parámetros indican que tras el traslado de la colonia hay un cierto grado de estrés que dependería, entre otras cosas, de las condiciones de captura, traslado y reubicación de los animales.

Aunque existen múltiples lagunas en los estudios sobre el traslado de animales por tratarse de un área de estudio relativamente nueva, mucha es la bibliografía que mide el impacto a nivel fisiológico causado por cambios ambientales y sociales, tales como: la formación de un nuevo grupo (Anzenberg y cols., 1986**),** o la exposición a estímulos nuevos (Coe y cols., 1985; Boccia y cols., 1989). Nuestros resultados, por tanto, serán contrastados con los mostrados en estos trabajos, que nos servirán de marco de referencia.

Se encontraron diferencias significativas en los niveles de cortisol antes y después del traslado de la colonia. El que los niveles de cortisol pueden aumentar tras un estrés físico o psicológico ha sido ampliamente documentado en primates humanos (Chatterton y cols., 1997) y no humanos (Brown y cols., 1971; Gordon y Gust, 1993). Este aumento de los niveles del cortisol tendría como principal efecto una hiperglucemia, transformando las proteínas y grasas del hígado en glucosa y procurando al organismo la energía necesaria para actuar ante una situación amenazante o de competición (Sapolsky, 1993**;** Salvador y cols., 2003; Queyros y Carosi, 2004). Por el contrario, otros estudios destacan la estabilidad en los niveles de cortisol tras la exposición a estresores intensos**.** Muchas son las variables que podrían estar influyendo en los niveles hormonales y que, consecuentemente, podrían explicar la incongruencia arrojada por los diferentes estudios. Por ejemplo, es ampliamente conocido que ritmos circadianos a los que se ven sometidos las hormonas obligan al investigador a ceñirse a los mismos horarios de recogida de la muestra para poder comparar resultados, o bien a tomar varias muestras a lo largo del día y sacar un promedio de dichos valores. Así los niveles de cortisol presentan un pico máximo en las primeras horas de la mañana, alcanzando los valores mínimos al anochecer y estabilizándose a medianoche.

De la misma forma, nos encontramos con resultados contradictorios en cuanto a los niveles basales de cortisol en las diferentes especies. Algunas especies son más sensibles que otras a los cambios hormonales (Coe y cols., 1979; Aidara y cols., 1981; Anzenberg y cols., 1986; Clarke y cols., 1988**;** Lynch, 2002) debido a esas  mencionadas diferencias basales.

Nuestros resultados son congruentes con los arrojados por la mayoría de los estudios sobre estrés en primates no humanos: los niveles de cortisol en sangre aparecen incrementados tras un cambio ambiental **(**Bercovitch y Clarke, 1995; Clarke y cols., 1996; Lynch y cols., 2002)**.** En el caso que aquí se presenta, los animales podrían haber estado expuestos a más de un agente elicitador de estrés. Aunque todas las condiciones hayan sido cuidadosamente diseñadas para minimizar el coste físico y psicológico de los animales, los procesos a los que los sujetos se ven sometidos (captura, anestesia, transporte) provocarían un cierto grado de estrés, que se reflejaría en los niveles de glucocorticoides.

El hecho de que no se encontraran diferencias en los niveles basales en cuanto al rango de los animales, sin embargo, podría ser explicado por la estabilidad jerárquica de los grupos que fueron analizados. Como se apuntó anteriormente, todos los grupos fueron formados al menos seis meses antes de comienzo de la recogida de datos, e incluso algunos de los animales nacieron en el grupo donde fueron observados. La diferencia en los niveles hormonales marcaría las diferencias en los rasgos conductuales que cada rango tiene, más que el rango en sí (Sapolsky, 1993 y Abbott y cols., 2003). Sería necesario mirar los datos conductuales y comprobar si esta falta de diferencia en los niveles hormonales basales se corresponde con una falta de diferencia en los patrones conductuales de los diferentes rangos. Por tanto se podría decir que no existe una predisposición a padecer más o menos estrés fisiológico según el rango. Nuestros resultados son congruentes con los encontrados por otros autores en diferentes especies (Bercovitch y Clarke, 1995 en macacos rhesus; Lynch y cols., 2002, en monos capuchinos) observando que, en grupos estables, no existían diferencias en los niveles hormonales basales entre individuos subordinados y dominantes.

El que tampoco se hayan encontrado diferencias respecto al rango en los niveles de cortisol después del traslado, indicaría que el estrés causado por el traslado del grupo es vivenciado de igual manera por cada sujeto, independientemente de que sea un animal dominante o un subordinado. No existe, por tanto, una predisposición a padecer más o menos estrés según el rango o jerarquía dentro del grupo.

También se ha observado que, durante la inestabilidad ambiental y/o social, animales pertenecientes a grupos con jerarquías poco estables aprovechan dichos periodos de inestabilidad para ascender en la escala jerárquica. Esta lucha por niveles jerárquicos superiores provocaría un aumento de encuentros agresivos, y la ansiedad que acompaña a dichos encuentros podría verse reflejada en los niveles de cortisol. Al poseer los grupos aquí estudiados una jerarquía lineal relativamente sólida formada al menos seis meses antes de comenzar el estudio (como se detalla en el **Anexo 1**), el traslado no sería vivenciado por los individuos como una oportunidad de ascender jerárquicamente. Esta afirmación, sin embargo, deberá ser contrastada con datos conductuales y de dominancia de la segunda etapa, tal como haremos en ulteriores trabajos.

Los cambios en los niveles de testosterona han sido ampliamente estudiados en relación con la agresión (Bernstein y cols., 1974; Rose y cols., 1975; Dixon y Herbert, 1977) en la reproductividad (Gordon y cols., 1976; Barret y cols., 2002), y sólo recientemente también con el estrés (Khaksari y cols., 2005; Ardí y cols., 2005; Kunstmann y Christansen 2004).

El descenso en los niveles de testosterona, observado después del traslado de los animales, reflejaría lo que otros muchos autores han descrito en distintas especies de primates no humanos expuestos a situaciones de estrés físico o psicológico [macacos rhesus**;** Rose y cols., 1971; Hayashi y Moberg, 1987**;** en babuinos: Sapolsky, 1985**;** Dainzburg y cols., 1992; en macacos japoneses (*Macaca fuscata*): Barret y cols., 2002; Chatterton y cols., 1997**,** Schurmeyer y cols., 1984**,** Francis, 1981) tanto en hábitat naturales (Sapolsky, 1985), como en cautividad (Dainzburg y cols., 1992). Tras una situación de estrés aumentan los niveles de cortisol, apareciendo un descenso en el nivel de las hormonas sexuales. La supresión de los niveles de testosterona no tiene que ir siempre correlacionada negativamente con los niveles de COR. También se han observado correlaciones positivas entre ambas hormonas tras estrés (Higley y cols, 1992)**,** e incluso una falta de relación entre ambas hormonas (Ghanadian y cols., 1981**;** Chatterton y cols., 1997**;** Schurmeyer y cols., 1984**;** Bercovitch y Clarke, 1995)**.** Como vimos anteriormente, en la respuesta hormonal y la relación entre hormonas podrían estar influyendo un gran número de variables. Se han observado efectos dependientes del rango (una relación negativa entre niveles de cortisol y testosterona solo en animales dominantes (Barret y cols., 2002)), y del sexo (así Saltzman y cols., 1998 observaron que los niveles de testosterona en hembras después de un estrés no se relacionaban con los de COR).

El descenso de los niveles de testosterona observado en nuestro estudio, puede explicarse por el aumento en los niveles de cortisol; podría ser otra respuesta endocrina debido a la situación estresante. Se ha documentado en primates humanos y no humanos que tras un estrés físico o psicológico se suprime la función reproductora. Éste descenso en la función reproductora se reflejaría a nivel fisiológico en un descenso en los niveles de testosterona. También se ha observado que las hormonas gonadales del sistema reproductivo endocrino podrían ser más susceptibles a los efectos del estrés a largo plazo que las hormonas adrenales (Schurmeyer y cols., 1984). Las hormonas sexuales podrían ser mejores indicadores del estrés que las hormonas adrenales, dado que éstas parecen tener un efecto más sensible y rápido.

Aunque otros autores han encontrado diferencias según el rango en los niveles hormonales (testosterona) después de un suceso estresante (Sapolsky, 1982, 1983**;** Bercovitch y Clarke, 1995), nuestros resultados son congruentes con la mayoría de los trabajos, donde esta diferencia no fue observada (Eaton y Resco, 1974; Dixon y Herbert, 1977). El hecho de que no se encontraran diferencias significativas en los niveles de testosterona según el rango podría sugerir que no existe posibilidad entre los miembros del grupo de ascender en la jerarquía social, ni competitividad entre los miembros del grupo, ya que como se dijo en la introducción, se trata de grupos estables con una jerarquía muy sólida.

El hecho de que tampoco se encontraran diferencias significativas entre aquellos sujetos que no fueron anestesiados para el transporte y los que si lo fueron, podría sugerir, que la alteración de la estabilidad de la colonia estaría causada por el proceso del traslado más que el transporte en sí.

## 1.4. CONCLUSIONES

1. El traslado en camión de una colonia de crianza de macacos rhesus *(Macaca mulatta*), desde *Harlan UK* en Hillcrest al *Centro para Macacos* en Porton Down, produjo una *respuesta fisiológica* en los individuos*,* tal como se refleja en el aumento significativo de los niveles del cortisol y en el descenso significativo de los niveles de testosterona en sangre.

2. La no aparición de diferencias en los niveles hormonales basales de animales de distinto rango sugiere la falta de una predisposición de los individuos dominantes y subordinados a actuar de una forma diferente, y a presentar respuestas fisiológicas distintas tras una situación inestable como la provocada por el traslado.

3. El hecho de que no existan diferencias significativas en las respuestas hormonales en relación con el rango tras el traslado, podría explicar la falta de iniciativa o de predisposición de los individuos a actuar de forma distinta y a convertir la situación de inestabilidad en una situación de competición para ascender en la escala jerárquica.

4. Aunque las variables necesarias para minimizar el posible estrés en los animales sean controladas y estudiadas *a priori*, el traslado de animales supone al menos a corto plazo un gasto físico, reflejado en las respuestas hormonales.

5. Lo que los animales podrían vivenciar como amenazante, no sería tanto la experiencia del transporte en sí, sino el traslado y el abandono de un espacio “familiar” y la consiguiente ruptura de su estabilidad.

# *Capítulo 2:*

# *RESPUESTA INMUNE TRAS EL TRASLADO DE LOS ANIMALES*

2.1. Introducción

a) Motivo y objetivos de la medición de la respuesta inmune
b) Hipótesis

2.2. Resultados
2.3. Discusión
2.4. Conclusiones

## 2.1. INTRODUCCIÓN

Debido a la asociación entre estrés y enfermedad física, la búsqueda de métodos de cuantificación del estrés es considerada una de las tareas principales entre psicólogos, psiquiatras, psicobiólogos y en general, entre los profesionales preocupados por la salud física y mental (Moberg, 1985; Glaser y cols., 1985, 1986; Cohen, 1988; Forsen, 1991).

La psiconeuroinmunología (PNI), ha centrado su interés en el estudio de la interacción entre sistema nervioso central (SNC), sistema endocrino (SE) e inmunológico (SI), y la repercusión que dicha interacción puede tener en la salud. Uno de los objetivos prioritarios de esta nueva disciplina, en las últimas décadas, es la búsqueda de estos métodos de cuantificación relativos al estrés, a través de los cuales trabajar y para prevenir consecuencias negativas. Sin embargo, la gran cantidad de variables (por ejemplo, sexo, edad o apoyo social) que pueden influir en la respuesta al estrés, hacen que este concepto permanezca vago y confuso, dificultando, de esta forma, el alcanzar un consenso sobre los marcadores fisiológicos y conductuales más idóneos que nos permitan una medición precisa de este estado.

De la misma forma, las nuevas demandas que la sociedad actual nos impone y los distintos retos sociales a los que estamos obligados, han hecho que tanto las definiciones, como las clasificaciones del estrés, hayan cambiado con el objetivo de amoldarse a la realidad que nos rodea, resultando más difícil aún, si cabe, el llegar a un acuerdo entre los diferentes sectores. Así, la definición de estrés que Seley aportó en 1946, y su descripción del Síndrome General de Adaptación, dista mucho de lo que hoy en día entendemos por estrés. A pesar de esto, parece existir un acuerdo en afirmar que el estrés conlleva una respuesta conductual, autonómica, endocrina e inmunológica a través de las cuales podría ser valorado.

Muchos autores (Bishop, 1999; Zhukov y Vinogradova, 2002) han apuntado que la respuesta al estrés dependería de la interpretación que el sujeto hace de la situación y por tanto de lo amenazante que la encuentre. Sin embargo, si esto es cierto, ¿podemos hablar de estrés en los animales? ¿cuál sería la mejor medida de las variables cognitivas en los animales? Esta es una de las razones por las que se hace necesario encontrar parámetros conductuales y fisiológicos que nos ofrezcan medidas fehacientes del estado del animal. Llegados a este punto, nos encontraríamos con otros interrogantes: ¿podemos hablar de estrés cuando sólo se ha observado o medido uno de estos parámetros?, ¿es necesario que todos los cambios existan para poder hablar de estrés o serían suficiente meros cambios en uno de estos marcadores para etiquetar este estado como tal?, ¿un cambio fisiológico tiene que verse traducido necesariamente en un cambio conductual, o viceversa? ¿son suficientes los cambios en uno sólo de los parámetros para que exista una repercusión a nivel inmunológico y consecuencias futuras negativas para la salud física?

Como se citó en el primer capítulo, los marcadores fisiológicos más utilizados por muchos autores para medir la respuesta del estrés tanto en primates humanos (Chatterton y cols., 1997) como no humanos (Browns y cols., 1971; Crockett y cols., 1994) han sido los niveles de cortisol, testosterona o prolactina**.** Sin embargo, parece evidente que los cambios en los niveles hormonales representan única y exclusivamente un eslabón de la cadena entre estrés y enfermedad física. La relación entre hormonas y sistema inmune ha sido ampliamente documentada (Casto y cols., 2001; Collazos y cols., 2003). El mantenimiento de ciertos niveles hormonales relativos al estrés (por ejemplo, niveles altos de cortisol) podrían provocar en el sistema inmune serias consecuencias a largo plazo, exponiendo al organismo a enfermedades físicas tales como cáncer de pecho (Forsen, 1991; Mizzen, 1998), psoriasis y dermatitis atípica (Arnetz y cols., 1991) o enfermedades mentales como depresión (Kalin y cols., 1986). Así, se ha observado que la respuesta hormonal e inmunológica tras un estrés agudo se presenta como una respuesta positiva de afrontamiento, preparando al organismo para actuar y defenderse ante la situación amenazante; y, por el contrario, el mantenimiento a largo plazo de estos niveles hormonales, representaría una amenaza para la salud (Padgett y Glaser, 2003). Se ha observado que un estrés agudo a corto plazo incrementaría el número de linfocitos, presentándose como una respuesta de defensa del organismo, mientras que un estrés crónico los reduciría, pudiendo dejar al organismo expuesto a enfermedades. También se ha comprobado que un estrés agudo podría convertirse en crónico, manteniendo, por tanto, los niveles hormonales e inmunológicos alterados de forma constante y exponiendo al organismo a amenazas externas (Gust y cols., 1991; Rinner y cols., 1992).

Aunque la respuesta inmune tras situaciones estresantes ha sido medida en muchas ocasiones a través de del número de células T (Ashwell, 1992), y asesinas (NK) (Clarke y cols., 1996) o de la medida de la inmunoglobulina A (IgA) (Kane, 2004), pocos trabajos han llevado a cabo un estudio longitudinal de esta respuesta. Parece evidente que lo realmente importante para la salud no es la respuesta inmediata de los marcadores fisiológicos, sino la capacidad que dichos marcadores tienen de recuperarse. Esta capacidad de recuperación nos ofrecerá la posibilidad de anticipar consecuencias negativas futuras para la salud.

Sin embargo, debido a la dificultad que en muchas ocasiones supone la medición de marcadores fisiológicos, algunos autores se decantan por la sola observación de cambios conductuales, sin prestar atención a que los cambios de conducta exhibidos en los individuos podrían estar condicionados y determinados por cambios en el entorno físico, así como por cambios en conductas ajenas, sin tener que estar estos cambios de conducta ligados necesariamente a un estado interno del individuo. De igual forma, la observación exclusiva de cambios a nivel conductual podría dar un valor aproximativo del estado interno sin llegar a ofrecernos una valoración de las posibles consecuencias futuras del estrés, ya que no contaríamos con información suficiente para confirmar si dichos cambios conductuales están teniendo repercusiones a nivel inmunológico.

Como se señaló en el punto primero, el interés de diversos sectores, tales como primatólogos y veterinarios, por llevar a cabo el mejor protocolo de traslado de animales que les permita minimizar el posible estrés causado por este proceso, ha hecho que el número de investigaciones sobre el traslado de colonias o grupos de animales haya aumentado en las últimas décadas. Sin embargo, y como también ha sido apuntado con anterioridad, se echan en falta variables fisiológicas que apoyen los resultados conductuales y estudios a largo plazo que nos permitan llegar a conclusiones determinantes sobre las consecuencias del traslado y reubicación de dichos grupos.

En general, existe un consenso entre los expertos sobre la necesidad de utilizar marcadores conductuales y fisiológicos, tanto a corto como a largo plazo, para valorar las consecuencias que el traslado de animales tiene en su salud física y mental.

### a) MOTIVO Y OBJETIVOS DE LA MEDICIÓN DE LA RESPUESTA INMUNE:

Aprovechando el obligado traslado de una colonia de macacos rhesus (*Macaca mulatta)* desde *Harlan UK* en Hillcrest al “*Centro para Macacos*” (*CFM)* en Porton Down, se realizó un estudio en el que se analizó el impacto a nivel inmunológico que dicho traslado tuvo en los animales.

El trabajo que aquí se presenta tiene como objetivo principal aportar al estudio del traslado de animales una medida fisiológica, en concreto, la medición de la respuesta inmune a través del análisis de la actividad de los leucocitos. De esta forma, intentamos rellenar una de las lagunas encontradas en los trabajos hasta ahora publicados sobre este proceso. Otro de nuestros objetivos consiste en validar, y extrapolar a primates no humanos, la fiabilidad de esta técnica de medida de la respuesta inmune, apoyándonos en datos hormonales y conductuales expuestos en otros puntos. El análisis de datos fisiológicos y conductuales, nos ofrece una prueba más fehaciente de que los parámetros que estamos utilizando miden un estado de estrés.

Como objetivos secundarios podrían destacarse el interés por saber si el rango que el animal ocupa dentro del grupo determina o influye en la respuesta fisiológica, así como si el sexo del animal es una variable determinante en dicha respuesta.

### b) HIPÓTESIS

La actividad de los leucocitos de responder a la estimulación *in vitro* del PMA conocida como *LCC* “*Leukocyte Coping Capacity*”, será menor inmediatamente después del traslado de los animales, sugiriendo que existen un número mayor de leucocitos activos respondiendo a una situación amenazante.

## 2.2. RESULTADOS

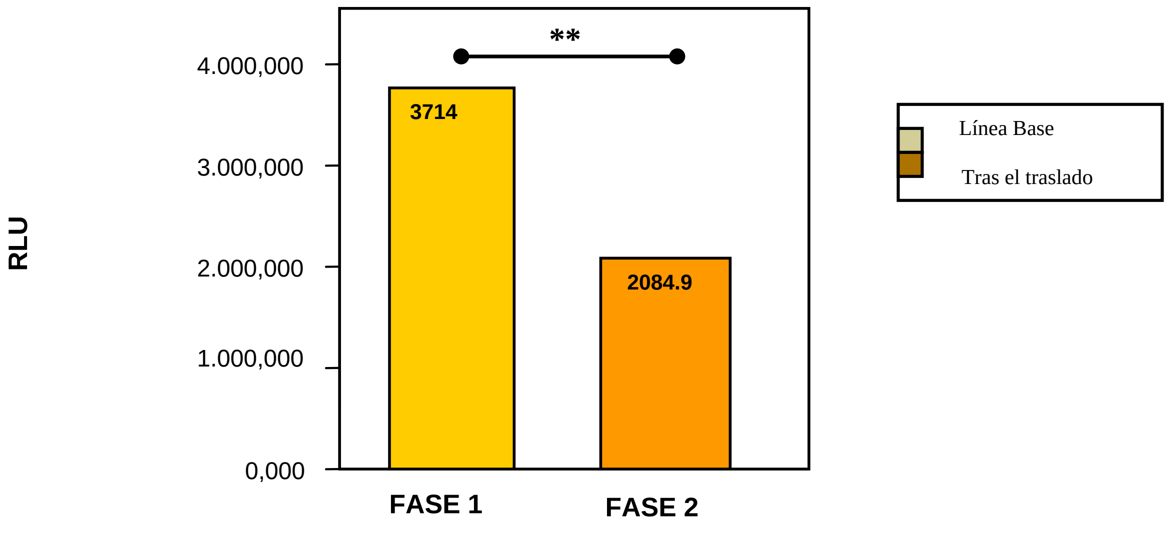
La respuesta inmune se midió a través de la actividad de los leucocitos en sangre, antes del traslado e inmediatamente después del mismo con el fin de observar el posible impacto inmunológico de dicho proceso.

Para el análisis estadístico se utilizaron pruebas no paramétricas. En concreto se utilizó, *la prueba de Wilcoxon* de dos muestras relacionadas, para estudiar posibles diferencias en la respuesta inmune antes del traslado e inmediatamente después del mismo. Así mismo, *la prueba U de Mann-Whitney* fue utilizada para estudiar posibles diferencias entre la respuesta inmune en relación con el sexo, y el *test de Kruskal-Wallis* para analizar diferencias entre la respuesta inmune en relación con el rango.

Se encontraron **diferencias significativas** en la respuesta inmune antes e inmediatamente después del traslado (***Z = -2,043; n = 31; p = 0,041***) ver Gráfico 5

**GRÁFICO 3**

***RESPUESTA INMUNE ANTES DEL TRASLADO E INMEDIATAMENTE DESPUÉS DEL MISMO***



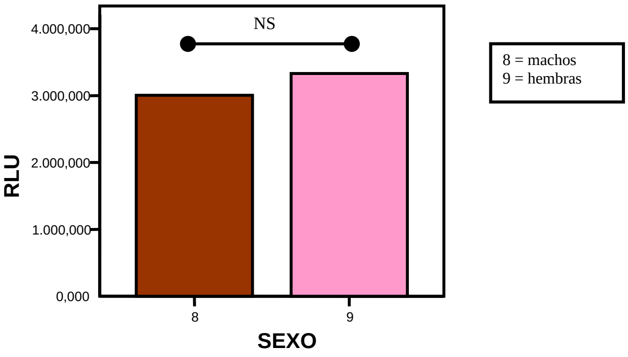
**Xinm1** = 3714; **N** = 35; **DS** = 3662.906; **StdE**=2.2552

**Xinm2** = 2084.919; **N** = 34; **DS** =; 315.053**; StdE**= 1837.063

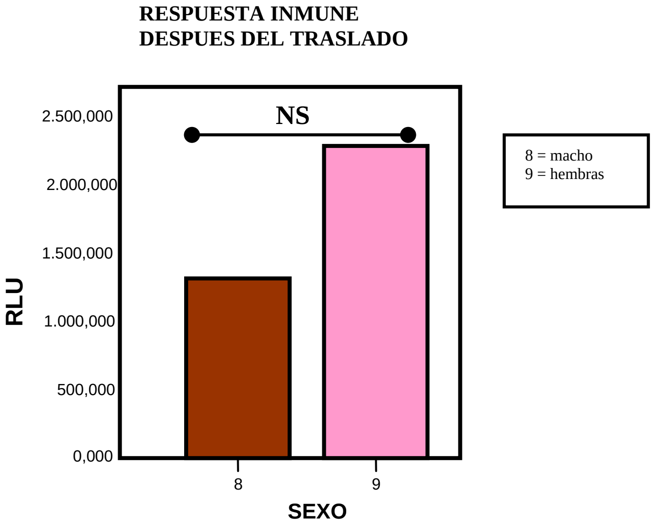
A continuación se presentan los gráficos correspondientes a la medida de la respuesta inmunológica en relación con el sexo del animal.

**GRÁFICO 4**

**RESPUESTA INMUNE ANTES DEL TRASLDO SEGÚN EL SEXO**



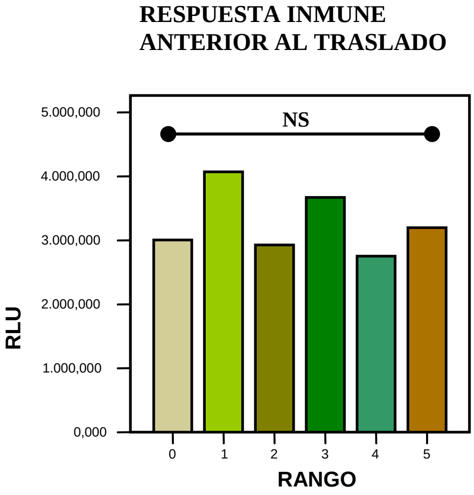
Tras realizar el análisis estadístico utilizando el test no paramétrico *Mann-Whitney,* no se encontraron diferencias significativas, en lo que respecta a la primera fase (linea base) en la respuesta inmune dependiendo del sexo (***U = 62; Z = -0,994; n = 34; p =0,341***).



## GRÁFICO 5

Tampoco se encontraron diferencias significativas en la fase 2 (inmediatamente después del traslado), (***U = 51; Z = 0,341; n = 33; p = 0,173***)

A continuación se presentan los gráficos correspondientes a la respuesta inmune estudiando las posibles diferencias dependiendo del rango que el animal ocupa dentro del grupo.

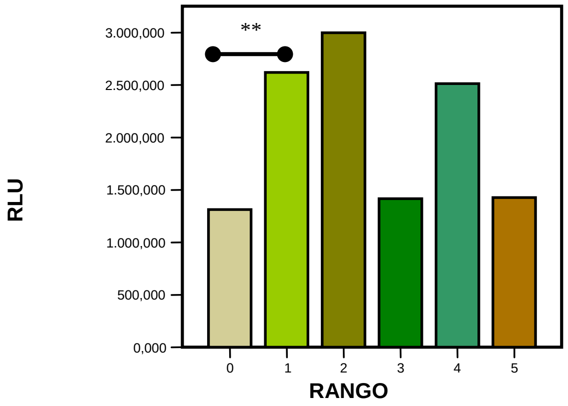


## GRÁFICO 6

Tras realizar el test no paramétrico de *Kruskal Wallis,* no se observaron diferencias significativas en la respuesta inmune dependiendo del rango ocupado por el animal dentro del grupo, en la primera fase ***(H = 6,41; n = 12; p = 0,426).***

**RESPUESTA INMUNE**
**TRAS EL TRASLADO**

**GRÁFICO 7**



**Sin embargo si se encontraron diferencias significativas** entre la respuesta inmune de los machos y la de las hembras dominantes inmediatamente después del traslado (***H = 5,769; n = 12; p = 0,016***).

La prueba *U de Mann-Whitney*, permitió analizar si existían o no diferencias entre aquellos animales que fueron sedados durante el transporte y los que no lo fueron. No encontrándose diferencias significativas entre ambos grupos ***(U = 30; n = 31; p = 0,173).***

## 2.3. DISCUSIÓN

Existen muchas investigaciones que demuestran que el estrés puede causar una gran cantidad de cambios que afectarían funciones biológicas, incluyendo la respuesta inmune (Moberg, 1985; Maule y Vanderkooi, 1999**;** Raberg y cols., 1998). Que el estrés agudo puede aumentar el número de leucocitos en sangre, su activación, así como su composición se ha documentado tanto en primates humanos (Goebel y Mills, 2000; Ellard y cols., 2001; Mian y cols., 2002, 2003) como no humanos (Honess y cols., 2005) así como en otras especies, por ejemplo en tejones (Montes y cols., 2004).

Que el estrés provoca cambios hormonales e imunológicos ha sido sobradamente documentado, aunque la relación entre ambas variables permanece todavía confusa. Aquí nos hemos planteado el reto de estudiar los cambios inmunológicos tras el transporte de una colonia de crianza de rhesus macacos, para comprobar si dicho proceso tiene un impacto a nivel inmunológico en los animales y compararlo con los datos hormonales y conductuales. Los resultados encontrados en nuestro estudio demuestran la existencia de diferencias significativas entre la respuesta inmune antes del traslado y después del mismo. Parece que el traslado, o un subproceso del mismo, tiene un impacto significativo en la respuesta inmune, como también ha sido documentado en otras especies. En efecto, se ha observado una mayor activación de los leucocitos en los tejones que fueron transportados en relación con aquellos otros que no lo fueron o aquellos que, tras ser transportados, pudieron descansar (McLaren y cols., 2003 y Montes y cols., 2004).

Otros estudios que han medido otro tipo de estreses también confirman nuestros resultados. Por ejemplo, Gordon y Gust (1993)**,** observaron que, tras la separación de sus crías, las madres presentaban niveles de cortisol más elevados acompañados de un descenso en el número de células T, presentando los niveles más altos de cortisol y los más bajos de células T aquellos sujetos que recibieron más agresión y que por tanto estuvieron sometidos a una situación de estrés más aguda. Los presentes datos también confirman previos resultados de nuestro propio equipo (Honess y cols., 2005): utilizando idéntico procedimiento observamos niveles más altos de estrés en aquellos en macacos rhesus (*Macaca mulatta*), que vivían en jaulas comparados con los grupos de animales que vivían en habitaciones abiertas.

El que se observaran diferencias significativas entre los machos y las hembras dominantes en la respuesta inmune tras el traslado indicaría que los machos estarían viviendo la situación del traslado como más amenazante que las hembras dominantes, aunque para afirmar esto con seguridad, deberíamos encontrar datos hormonales y/o conductuales que apoyaran éstos resultados inmunológicos. En algunas especies se ha observado, que el macho dominante en el grupo podría ser considerado el animal más activo en situaciones de amenaza externa, mientras que por el contrario, la hembra dominante asumiría este papel en situaciones “amenazantes” o “inestables” surgidas dentro del grupo.

El que no se hayan encontrado diferencias significativas de la respuesta inmune dependiendo del rango, indica que al menos este tipo de estrés es vivenciado por igual por todos los sujetos, independientemente del lugar jerárquico que éstos ocupen en el grupo. Por tanto, no existiría una amenaza añadida a la situación del traslado en sí. Sin embargo, otros autores (Rose y cols., 1974; Gust y cols., 1991) si encontraron diferencias en la respuesta inmune de los animales dependiendo del rango: los animales de bajo rango presentaban repuestas fisiológicas mayores, incrementando los niveles hormonales y descensos en la respuesta inmune.

## 2.4. CONCLUSIONES

1- El traslado de la colonia tuvo un impacto a nivel inmunológico, reflejado a través de una menor capacidad de los leucocitos en sangre de producir radicales libres de oxigeno en respuesta a la estimulación *in vitro* de PMA.

2- No se encontraron diferencias significativas en relación con el sexo del animal ni con el rango.

3- Los machos, tras el traslado, presentan un mayor grado de respuesta fisiológica relativa a la actividad inmune que las hembras dominantes, reflejado en una respuesta menor de los leucocitos a la estimulación del PMA.

# Capítulo 3:

# ESTUDIO DE LA CONDUCTA SOCIAL ANTES Y DESPUÉS DEL TRASLADO DE LOS ANIMALES.

3.1. Introducción

  a) Motivo y objetivos del estudio de la conducta social tras el traslado
  b) Hipótesis

3.2. Resultados
3.3. Discusión
3.4. Conclusiones

## 3.1. INTRODUCCIÓN

El gran reto de la psicología es el estudio de la conducta humana. Sin embargo, la dificultad de controlar la gran cantidad de variables que se quedan fuera del alcance del investigador ha obligado a los expertos al uso de modelos animales, los cuales podrían ofrecernos muchas ventajas (Shively, 1998; Ramírez, 2000). El estudio del comportamiento en animales permite observar la conducta *in situ*, ofreciéndonos, de esta forma, la oportunidad de obtener información de las conductas que la preceden y que siguen.

En primates humanos, la mayoría de las veces resulta difícil observar una conducta que está siendo desplegada de forma espontánea (p.ej. agresión). Por este motivo, los investigadores suelen crear una situación artificial en la que observar el comportamiento que les interesa, o se decantan por estudiar la personalidad o las consecuencias de dicho comportamiento en vez de la propia conducta. Así por ejemplo, si queremos estudiar la conducta agonística, como en la mayoría de las ocasiones el investigador no está presente cuando ésta ocurre, debe trabajar con la persona que realiza la acción, más que con la acción en sí.

Otra ventaja del estudio en animales, consiste en que  nos permite controlar, o al menos tener información relativamente completa, sobre la historia personal de los animales que componen la muestra. Esto nos ofrece la posibilidad de obtener información sobre los procesos de aprendizaje. Así, por ejemplo, las colonias de crianza, donde los animales nacen y se crían dentro del grupo, ofrecen la oportunidad de estudiar patrones conductuales y poder comparar animales con historias personales semejantes pudiendo, de esta forma, generalizar resultados.

El estudio con primates no humanos nos ofrece información fehaciente a partir del estudio de conductas y marcadores fisiológicos, pudiéndonos dar pistas sobre dónde seguir profundizando en la investigación con primates humanos (Ramírez, 2000), dada su cercanía específica, su capacidad de vivir en grupos sociales, la presentando un repertorio de conductas sociales muy extensas, la presencia de procesos cognitivos muy complejos y lo parecido del funcionamiento del SNC.

Sin embargo, como se dijo anteriormente, la recogida única y exclusivamente de datos conductuales, ya sea en primates humanos o no humanos, no nos asegura la posibilidad de inferir un estado interno, aunque la mayoría de los autores se han inclinado por la observación de los cambios en los patrones conductuales tras situaciones de inestabilidad, debido a las dificultades que conlleva, en muchas ocasiones, la medición de marcadores fisiológicos (Ostro y cols., 1999; Berman y Li, 2002; Ha y cols., 2002). Si bien es verdad que la conducta refleja la relación que el animal tiene con el entorno y a partir de este reflejo se podrían inferir *ciertos* estados, no se debería correr el riesgo de extrapolar dicha inferencia, asumiendo que los cambios conductuales siempre son causas o consecuencias de los estados internos. En muchas ocasiones los cambios conductuales pueden no estar asociados, al menos explícitamente, a cambios a nivel fisiológico.

La conducta observada, además de una reacción espontánea a “amenazas” del medio, podría ser interpretada como una consecuencia de sus demandas y posibilidades, sin que tenga que estar necesariamente, siempre, relacionada con un cambio interno. De esta forma, los patrones conductuales son vulnerables a las posibilidades del medio sin que esto signifique un estado emocional. Así mismo, las conductas de un individuo pueden ser la respuesta o la reacción a una conducta ajena, sin tener que relacionarse tampoco con un cambio a nivel interno.

A pesar de todos estos sesgos interpretativos, la dificultad de controlar todas las variables que pueden influir a la hora de utilizar marcadores fisiológicos, (p.ej. los ritmos circadianos de las hormonas, las diferencias entre especies), ha llevado a muchos autores a estudiar la relación entre conductas y estados emocionales, con el fin de encontrar marcadores conductuales que nos informen sobre el estado del animal**.**

Sin embargo, a la hora de interpretar estados internos a partir de conductas observadas, deberíamos resaltar la elección y la definición de las conductas a observar. En los modelos animales, a la hora de definir y clasificar una conducta, es importante recordar que cada especie se caracteriza por una forma específica de relacionarse y enfrentarse al medio en el que habita (Clarke y Mason 1988; Anzenber y cols., 1986) y que, por lo tanto, conductas "anormales" en unas especies, podrían considerarse "normales" o adaptativas en otras. Por ejemplo, se ha comprobado que los macacos rhesus son una de las especies que muestran más agresión en grupo, tanto en situaciones de estabilidad o de inestabilidad (Southwick, 1967). De la misma forma, las condiciones del hábitat también deberían ser analizadas a la hora de definir conductas. Así, se ha observado que los animales en cautividad muestran un repertorio de conductas muy diferente a aquellos que viven en libertad (Southwick, 1967), como evidencia de la sugerencia de Sackett, (1968) varias décadas atrás de que las condiciones de crianza del animal (p.ej. en grupo, aislado, por cuidadores etc…) también podrían influir en las conductas que se desarrollarían más tarde.

A la hora de elegir qué conductas conviene observar en animales que viven en grupos sociales, como por ejemplo los primates no humanos, es necesario contar con un conocimiento extenso sobre el entorno social del grupo, llegando a un acuerdo sobre qué llamamos conductas socialmente aceptadas *por el grupo* y qué se llama conductas socialmente no aceptadas. Muchas veces, se comete el error de observar conductas en los animales e interpretarlas desde el punto de vista antropomórfico. Así por ejemplo, unos autores asocian la agresión a situaciones de inestabilidad tanto social como ambiental, interpretando dicha conducta como una reacción de estrés del animal ante la situación inestable**,** mientras que otros autores la interpretan como una manera de comunicación entre los miembros del grupo.

Tras lo apuntado con anterioridad, parece evidente pensar que, más que conductas específicas que se relacionan siempre con los mismos estados internos emocionales, debemos trabajar sobre las posibilidades sociales y ambientales que se le ofrecen al grupo y/o colonia que estudiamos, a través de las cuáles se podrán definir y clasificar aquellas conductas como adaptativas, “normales” o “anormales”, no siendo posible la elección de dichas conductas *a priori* a partir de los resultados observados por otras especies y en otras situaciones. De la misma forma, es importante señalar la necesidad de estudios de la conducta a largo plazo, ya que conductas que en principio parecen “anormales” o “no adaptativas**”** al medio, con el tiempo pueden convertirse en normales.

La recogida de información basal sobre el repertorio conductual de los animales que componen la muestra nos permitirá controlar todas las variables anteriormente mostradas. También nos ofrecerá información del patrón conductual basal más que de una conducta en sí. En muchas ocasiones, tras una situación de inestabilidad social o ambiental, lo que cambia es el patrón conductual más que una conducta concreta; pueden desaparecer conductas anteriormente expresadas y ser sustituidas por otras. La observación y recogida de información de más de una conducta facilitará llegar a conclusiones sobre los cambios en los patrones conductuales más allá de los cambios en conductas concretas.

En primates no humanos la conducta social comprende más del 70% de las conductas expresadas a lo largo del día. La relación entre los miembros del grupo, así como la relación del grupo con el medio, dará información para valorar ciertos estados. Los macacos rhesus son una de las especies más estudiadas debido a su capacidad de adaptación, a su riqueza en el repertorio conductual, su capacidad de vivir en grupos sociales y a su cercanía filogenética con los humanos, a pesar de ser considerada por algunos autores como una de las especies más agresivas, tanto en cautividad como en libertad.

Parece existir un consenso general en afirmar que la conducta social se ve afectada tras una situación de inestabilidad, apareciendo aumentos de los encuentros agresivos entre los miembros del grupo (Berman y Li, 2002), en muchas ocasiones debido a la competición entre los individuos por llegar a ser el animal dominante o, simplemente, por ascender en la jerarquía, así como descensos de actividades relacionadas con cohesión del grupo, como acicalado, juego o conducta sexual.

## a) MOTIVO Y OBJETIVOS DEL ESTUDIO DE LA CONDUCTA SOCIAL TRAS EL TRASLADO.

Con motivo del traslado de la colonia de macacos rhesus desde *Harlan UK* (Hillcrest) a *CFM* en Porton Down, con el fin de estudiar el impacto que el traslado y reubicación en el nuevo entorno, tuvo en la conducta social del grupo se observó la conducta de los animales antes del traslado e inmediatamente después del mismo.

Otro de los objetivos del estudio consistió en comprobar si los cambios a nivel conductual se mostraban en la misma dirección que los observados desde el punto de vista hormonal e inmunológico.

## b) HIPÓTESIS

1. Las conductas relacionadas con cohesión del grupo tales como acicalar, ser acicalado, juego o conducta sexual descenderán inmediatamente después del traslado, como consecuencia del aumento en las conductas de competición.

2. Las conductas relacionadas con competición y agresión, aumentarán inmediatamente después del traslado como consecuencia de la situación de inestabilidad provocada por éste.

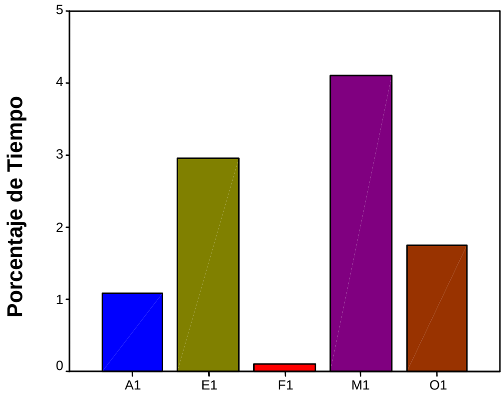
## 3.2. RESULTADOS

Para estudiar el impacto que el traslado de la colonia tuvo en la conducta, se utilizaron medidas parametricas y no paramétricas dependiendo si las variables cumplian o no el supuesto de normalidad. En concreto se utilizó *ANOVA de medidas repetidas* y la *prueba de Wilcoxon* para ver si existían diferencias significativas entre cada una de las categorías de conducta antes y después del traslado, *ANOVA de un factor* y la *U de Mann-Whitney* para estudiar si existían diferencias significativas entre los distintos patrones de conducta dependiendo del sexo, y la *prueba H de Kruskal Wallis* para analizar las posibles diferencias en las conductas en relación con el rango que el animal ocupaba en el grupo.

Se estudiaron los patrones de conducta exhibidos por los animales antes y después del traslado de la colonia.

**PATRON DE CONDUCTA ANTES DEL TRASLADO**

**GRÁFICO 8**

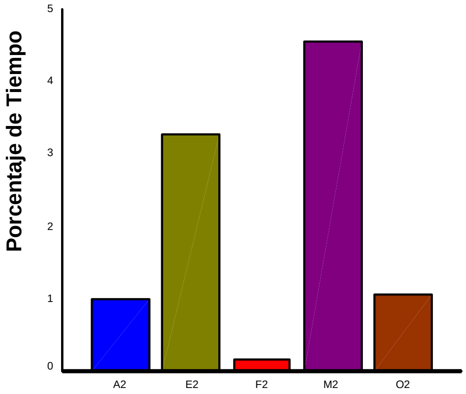


**A**→ Conducta afiliativa
**E**→ Conductas de auto-mantenimiento
**F** → Conducta agresiva
**M**→ Conducta de exploración
**O** → Otras

Como puede verse en el gráfico 10, antes del traslado los animales pasan la mayor parte del tiempo mostrando conductas de exploración (M) y de auto-mantenimiento (E).

**GRAFICO 9**

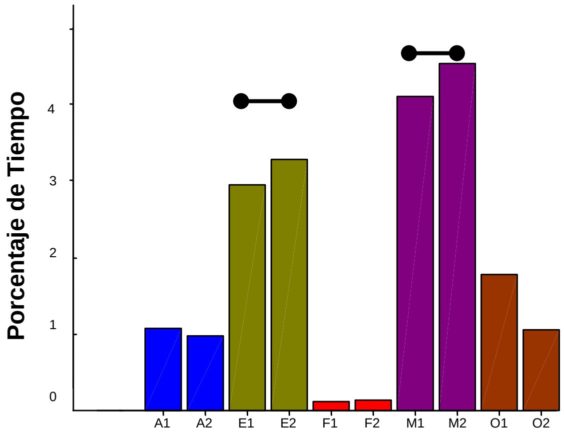
**PATRON DE CONDUCTA**
**DESPUES DEL TRANSLADO**



**A**→ Conducta Afiliativa
**E**→ Conductas de Auto mantenimiento
**F** → Conducta agresiva
**M**→ Conducta explorative
**O** → Otras

**GRAFICO 10**

**PATRON DE CONDUCTA ANTES Y TRAS**
**EL TRASLADO**



No se encontraron diferencias significativas entre A1*A2 *(F=-0.742; n=33; p=0.458)*, tampoco se encontraron diferencias en relación con el genero A1 *(U=-1.310;n=33;p=0.190)* A2 *(U=-0.700; n=33; p=0.430)* ni con el rango A1 *(H=4.373; n=34; p=0.497)* A2 (*H=4.025; n=33; p=0.546).*

Sin embargo se **encontraron diferencias** significativas entre las conductas de auto-mantenimiento (E) antes y después del traslado.

**E1*E2** ***(F=4.003; n=33; p=0.054)***. Podemos apreciar un incremento después del traslado de aquellas conductas relacionadas con el auto-mantenimiento. Sin embargo, no se encontraron diferencias significativas en relación con el genero E1 *(F=0.430; n=33; p=0.517)* E2 *(F=0.308; n=33;*

*p=0.583)* ni con el rango E1 *(F=0.178; n=33; p=0.968),* E2 *(F=0.802; n=33; p=0.588).*

No se encontraron diferencias significativas en la conducta agonística F1*F2 *(F=-1.385; n=33; p=0.166)* y tampoco se encontraron diferencias significativas en relación con el genero F1 *(U= -1.175; n=33; p=0.240)* F2 *(U=-0.700; n=33; p=0.484)* ni con el rango F1 *(U=3.577;n=33;p=0.612),* F2 *(U=5.463; n=33; p=0.362).*

Se encontraron **diferencias significativas** en la conducta de exploracion (M) entre antes y después del traslado **M1*M2** ***(F=7.530; n=33; p=0.010)***. Sin embargo no se encontró efectos del genero M1(F=-1.646;n=33; p=0.209), M2 (F=-0.544; n=33; p=0.467) ni del rango M1 (F=0.486; n=33; p=0.784) M2 (F=0.356; n=33; p=0.874).

También se encontraron diferencias significativas en la categoría de otras conductas donde estaban incluidas, cuidado de la cría o estereotipias O1*O2 (F= -4.011; n=33; p= 0.000), apareciendo un descenso de estas conductas tras el transporte **(Ver tabla 1)**

## TABLA 1

**Descriptive Statistics**

| | N | Minimum | Maximum | Mean | | Std. |
|---|---|---|---|---|---|---|
| | Statistic | Statistic | Statistic | Statistic | Std. Error | Statistic |
| A1 | 34 | .0431 | 3.1206 | 1.082344 | .105380 | .6144661 |
| A2 | 33 | .0098 | 3.0859 | .982091 | .112959 | .6489013 |
| E1 | 34 | 1.2314 | 5.0925 | 2.955035 | .167436 | .9763100 |
| E2 | 33 | 1.4994 | 5.1493 | 3.274955 | .179444 | 1.0308257 |
| F1 | 34 | .0125 | .3301 | .105868 | .013061 | .0761584 |
| F2 | 33 | .0123 | .5348 | .146036 | .019019 | .1092534 |
| M1 | 34 | 2.5879 | 5.6107 | 4.101612 | .143398 | .8361461 |
| M2 | 33 | 2.7847 | 6.8315 | 4.535742 | .169399 | .9731247 |
| O1 | 34 | .5447 | 3.2080 | 1.753759 | .123131 | .7179733 |
| O2 | 33 | .3537 | 3.6831 | 1.061156 | .122914 | .7060855 |
| Valid N (listwise) | 33 | | | | | |

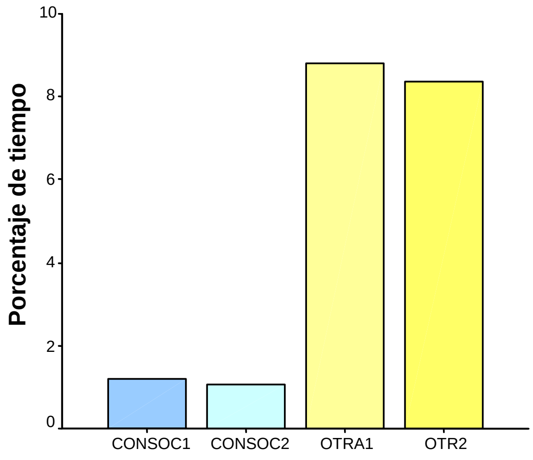
También se analizaron las posibles diferencias en los patrones conductuales entre aquellos sujetos que habían sido sedados durante el transporte y los que no. Para ello se utilizó el *test de Mann-Whitney*.

Tampoco se encontraron diferencias significativas en ninguna de las categorías ni antes ni después del traslado.

Fueron analizadas las posibles diferencias en la conducta social en general (Afiliativa*Agresion) entre antes y después del traslado.

**CONDUCTA SOCIAL ANTES Y DESPUES DEL TRASLADO DE LOS GRUPOS**

**GRÁFICO 11**



No se encontraron diferencias significativas en la conducta social antes y después del traslado (*Z=-0.950; n=33; p=0.342)*

## Tabla 2

**Descriptive Statistics**

| | N | Minimum | Maximum | Mean | | Std. |
|---|---|---|---|---|---|---|
| | Statistic | Statistic | Statistic | Statistic | Std. Error | Statistic |
| CONSOC1 | 35 | .0766 | 3.1486 | 1.172654 | .106134 | .6279000 |
| CONSOC2 | 35 | .0000 | 3.1349 | 1.063663 | .111701 | .6608314 |
| Valid N (listwise) | 35 | | | | | |

## 3.3. DISCUSIÓN

En numerosas ocasiones y en diferentes especies se ha documentado que, tras situaciones de inestabilidad social o ambiental, podrían aparecer aumentos en la frecuencia de las conductas de competición (Southwick 1967;

Bernstein y cols., 1979)**,** así como descensos en la de las conductas de cohesión del grupo, tales como acicalado, juego o conductas sexuales.

Jonson y Southwick, (1984) y Yeager y Silver (1999) observaron una falta de cohesión en el grupo, bajos índices de nacimientos y tasas altas de mortalidad infantil tras el traslado de una colonia. Sin embargo, estos últimos autores sugirieron que la causante de dichos cambios, sería el cambio en las condiciones del nuevo hábitat más que el traslado de la colonia. En concreto, éste aumento en la frecuencia de encuentros agresivos en el grupo, sobre todo dirigido a las crías, parecía provocado por la dificultad de accesibilidad a la comida.

Nuestros resultados no apoyan los encontrados por estos autores. Una semana después del traslado de los grupos, no se encontraron diferencias significativas ni en la conducta afiliativa (A) ni en la conducta agonistica (F). El traslado de los animales no afectó a la relacion entre los miembros del grupo: después del traslado los animales, siguieron exhibiendo un alto porcentaje de conductas afiliativas aunque ligeramente en menor frecuencia y un bajo porcentaje de conductas clasificadas como agonísticas aunque ligeramente mayor que antes. Como se apuntó anteriormente, son muchos los estudios que observaron aumentos de la conducta agresiva tras una situacion de inestabilidad. Este incremento de la agresion podría haber sido una consecuencia del estrés o el reflejo de una situación de competición propiciada por la situación de inestabilidad. El que no se encontraran aumentos en la frecuencia de las conductas agresivas podría explicarse debido a la estabilidad y la solidez de los grupos estudiados, como lo muestra el hecho de que el traslado de los animales y el realojamiento de los grupos en las nuevas habitaciones no fuera aprovechado por los individuos para ascender en la jerarquía. De esta forma se aminoraría el estrés provocado por esta situación de competitividad.

Otra de las razones por la que no se encontraron diferencias en la conducta agonística podría haber sido debido a las características del nuevo espacio: el *CFM* de Porton Down, además de presentar mejores condiciones de habitabilidad (más juegos, estructuras colgantes para el despliegue de conductas típicas de la especie), cuadruplica en extensión al hábitat anterior en *Harlan UK*. En numerosas ocasiones se ha observado la relación entre “espacio” y agresión o “densidad” y agresión (Judge, P. et al.,; 1993 Judge, P.G., et al., 1997; de Waal et al., 2000). Muchos autores han documentado que los espacios pequeños aumentan la probabilidad de que aparezcan encuentros agresivos entre los miembros(Faucheaux et al., 1978; Howell et al., 1993; Kitchen and Martin, 1996), aunque también se ha observado que esta relación es muy dependiente de las características de la especie; mientras que las especies agresivas tenderían a aumentar sus encuentros agresivos bajo situaciones de hacinamiento, las especies que se caracterizan por mostrar un amplio espectro de conductas de afiliación, aumentarían dichas conductas afiliativas (Alexander & Roth, 1971). En nuestro estudio, aunque los encuentros agresivos pudieran haber aumentado, el mayor espacio existente en el nuevo hábitat podría haber frenado el encuentro agresivo entre los miembros del grupo, ofreciendo más posibilidades de escape y evitando conductas agresivas. Esto podría también explicar el aumento en la frecuencia de conductas de exploración y manipulación del medio exhibido por los animales en la segunda fase. Debido a las condiciones del nuevo hábitat su realojamiento propició el incremento en la frecuencia de las conductas de exploración evitando, de alguna forma, la aparición de nuevos encuentros agresivos consecuencia del estrés. Incrementos en la frecuencia de conductas de exploración y en general de la actividad, ha sido también observado por otros autores (Kerl and Rothe, 1996; Kitchen and Martin, 1996)

Así mismo, otros autores han comprobado que los aumentos en la conducta agresiva o competitiva, más que provocados por los cambios ambientales, son causados por cambios sociales (Southwick, 1967), los cuales no afectarían a todos los animales por igual: aquellos miembros pertenecientes a rangos dominantes se verán más amenazados que los individuos subordinados. Sin embargo, los cambios ambientales suponen la misma amenaza para todos, pudiendo ser ésta aprovechada o no por los animales subordinados.

También se encontraron diferencias significativas en las conductas de auto-cuidado antes y después del traslado; por ejemplo en auto-acicalado. Aunque futuros análisis estudiarán esta conducta de forma aislada debido a su relevancia y su “contradictorio” significado según distintos autores, aunque parece existir un acuerdo en que el auto-acicalado esta relacionado con el estrés; los animales exhibirían auto-acicalado como forma de aminorar tensiones, las conductas que claramente denotarían este estado serían las auto-lesivas, las estereotipias y el auto-acicalado compulsivo definido como “self-plucking” (Honess &Marín, 2005, Anexo 7)

El que no se hayan encontrado diferencias significativas dependiendo del rango, parece sugerir que, al menos en los grupos estudiados, no existe un patrón característico de conducta determinado por el rango y, por tanto, no existe una predisposición por parte de los animales de actuar de una u otra forma.

## 3.4. CONCLUSIONES

1. Tras el traslado de la colonia no se observaron cambios en la conducta social exhibida por el grupo. El traslado de la colonia y el reciente realojamiento no afectó la relación entre los miembros del grupo.

2. Tras el traslado de la colonia no se observaron cambios en conductas relacionadas con competición y/o agresión entre los miembros del grupo.

3. Tras el traslado de la colonia se observaron diferencias significativas en la conducta de exploración, apareciendo un incremento en todas estas conductas relacionadas con la manipulación del medio.

4. Tras el traslado de la colonia se observaron aumentos en aquellas conductas relacionadas con auto mantenimiento.

5. En general el trasporte de los individuos no causó efecto en la dinámica del grupo, aunque si aparecieron incrementos en aquellas conductas relacionadas con exploración y en las relacionadas con el auto-cuidado.

# Capítulo 4:

# MEDIDA DE LOS NIVELES HORMONALES SIETE MESES DESPUÉS DEL TRASLADO DE LOS ANIMALES

4.1. Introducción
  a) Motivo del estudio a largo plazo de los niveles hormonal
  b) Hipótesis

4.2. Resultados
  a) Cortisol
  b) Testosterona

4.3. Discusión
4.4. Conclusiones

## 4.1. INTRODUCCIÓN

En este capítulo se presentarán los resultados de los niveles hormonales (cortisol y testosterona) obtenidos siete meses después del traslado de los animales y la reubicación al nuevo hábitat. Como se comentó en la introducción general y en los capítulos anteriores, el seguimiento y la medición de los niveles hormonales a largo plazo tras una situación de inestabilidad potencialmente amenazante para el individuo, sería básica para la determinación del verdadero impacto que ésta tiene sobre la salud y el bienestar del individuo.

Muchas son las enfermedades físicas y los trastornos psicológicos que podrían apuntar al estrés como la causa predominante, pudiendo destacar la alopecia (Honess y cols. 2005), la dermatitis y la soriasis (Arnetz y cols., 1991), la depresión (Kalin y cols., 1986; Sapolsky, 1996, 2003) e incluso algún tipo de cáncer (Sapolsky and Donnelly, 1985; Forsen, 1991; Mizzen, 1998; Shively y cols., 2004), en la mayoría de los casos, esta causa es conocida hipotéticamente y muy a posteriori, cuando el daño es irreversible. Esto podría ocurrir por que se subestima el verdadero y dañino efecto del estrés debido al mal uso de dicho concepto, tan cotidiano en la vida moderna. El seguimiento de ciertos parámetros nos ayudará a frenar, o al menos a prever las posibles consecuencias negativas para abordarlo con más rapidez.

Como se dijo anteriormente, existen pocos trabajos sobre el traslado de animales que estudien a largo plazo las consecuencias de dicho proceso, A consecuencia de esta falta de datos, no resulta fácil valorar las verdaderas consecuencias que el movimiento de animales pudo causar en la salud y el bienestar de los individuos. En nuestro caso concreto, debido a los resultados obtenidos en la segunda fase, los cuales apuntaban hacia un cierto grado de amenaza y posible estrés, reflejado tanto en los indicadores fisiológicos como conductuales, nos decidimos hacer un seguimiento a largo plazo, con el fin de asegurar el bienestar de los animales.

## a) ESTUDIO A LARGO PLAZO DE LOS NIVELES HORMONALES

Nuestro principal objetivo fue llevar a cabo un estudio post-traslado tanto a nivel conductual como fisiológico, con el fin de añadir información a los estudios sobre el traslado publicados hasta el momento y medir el verdadero impacto a corto y largo plazo del transporte y sus posibles efectos sobre la salud de los animales.

En este capítulo examinaremos el impacto a largo plazo (siete meses después) que el traslado y el realojamiento en el nuevo espacio tuvo sobre los niveles hormonales de los animales, estudiando si existen diferencias entre machos y hembras y si el rango es una variable influyente para la posible recuperación de los niveles hormonales tras el impacto y para el mejor asentamiento en el nuevo hábitat.

## b) HIPÓTESIS

Trabajaremos con las siguientes hipótesis:

1. Siete meses después del traslado de los animales, los niveles de cortisol en sangre serán menores que los encontrados en la Fase 2, recuperando los niveles obtenidos antes del traslado. Este descenso en los niveles de cortisol se produciría como consecuencia del cese de la situación potencialmente amenazante y la adaptación de los animales al nuevo hábitat.

2. Siete meses después del traslado de los animales, los niveles de testosterona serán mayores que los obtenidos en la Fase 2, como consecuencia del asentamiento y la adaptación de los animales al nuevo hábitat y la recuperación de conductas socales tales como actividad sexual y juego.

## 4.2. RESULTADOS

Se analizaron las diferencias en los niveles hormonales siete meses después del traslado y a la largo de las tres fases del estudio. Se utilizaron medidas paramétricas, *ANOVA de una entrada y ANOVA de medidas repetidas*, cuando las variables cumplían el supuesto de normalidad, y medidas no paramétricas: *el test de Wicoxon*, *U de Mann-Whitney* y *Kruskal Wallis*, cuando las variables no presentaban una distribución normal.

a) CORTISOL

A continuación se presentan los niveles de cortisol obtenidos en la FASE 2 y en la FASE 3, para mostrar los niveles de cortisol de las tres fases y su comparación en el gráfico 14.

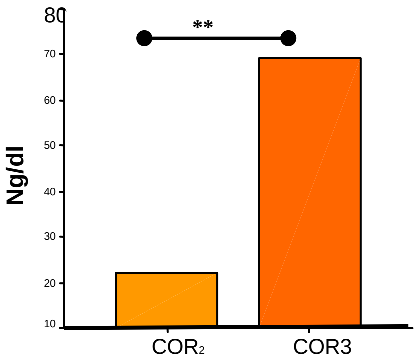
**Descriptive Statistics**

| | N | Minimum | Maximum | Mean | | Std. |
|---|---|---|---|---|---|---|
| | Statistic | Statistic | Statistic | Statistic | Std. Error | Statistic |
| COR1 | 31 | .625 | 51.945 | 22.22595 | 2.25521 | 12.556497 |
| COR2 | 34 | 47.200 | 102.440 | 69.14626 | 2.29380 | 13.375055 |
| COR3 | 31 | 3.945 | 45.723 | 23.43580 | 2.04708 | 11.397675 |
| Valid N (listwise) | 28 | | | | | |

No se encontraron diferencias significativas en los niveles de COR3 ni en relación con el sexo (*F=0.876; n=30; p=0.357*), ni en relación con el rango (*F=1.351; n=33; p=0.276).*

## GRÁFICO 12

**NIVELES DE CORTISOL INMEDIATAMENTE DESPUÉS DEL TRANSPORTE Y SIETE MESES DESPUÉS DEL TRANSPORTE**



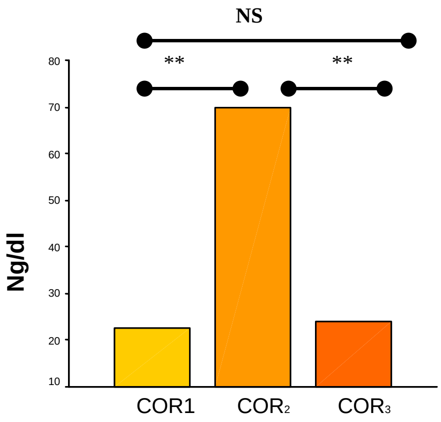
| | Mean | Std. Deviation | N |
|---|---|---|---|
| COR2 | 69.50171 | 13.383346 | 31 |
| COR3 | 23.43580 | 11.397675 | 31 |

Se encontraron **diferencias significativas** entre los niveles de cortisol inmediatamente después del traslado y siete meses después

***(F=303.081; n=33; p=0.000)***.

## GRÁFICO 13

**CAMBIOS EN LOS NIVELES DE CORTISOL DURANTE LAS TRES FASES**



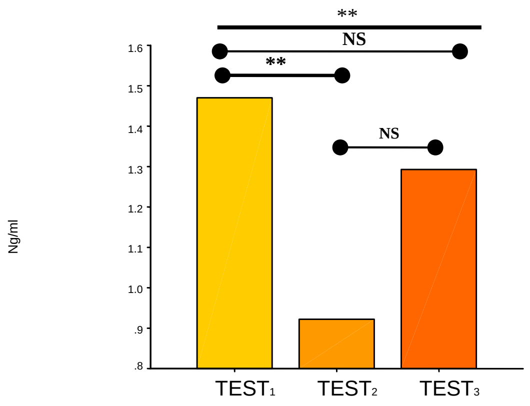
No se encontraron diferencias significativas entre COR1*COR3 *(F=0.143; n=28: p=0.708)*, sin embargo **si se encontraron diferencias a través del tiempo** COR1*COR2*COR3 ***(F=155.384; n=28; p=0.000)***

## b) TESTOSTERONA

No se encontraron diferencias significativas en los niveles de testosterona TES2*TES3 *(Z=-0.113; n=33; p=0.910)*, ni en los niveles de testosterona TES1*TES3 *(Z=-1.548; n=33; p=0.122),* pero, sin embargo, sí se encontraron ***diferencias significativas*** en los niveles de testosterona a través del tiempo ***(CH= 5.846; n=26; p=0.054)*** gráfico 14.

**NIVELES DE TESTOSTERONA**
**A LO LARGO DE LAS TRES FASES**

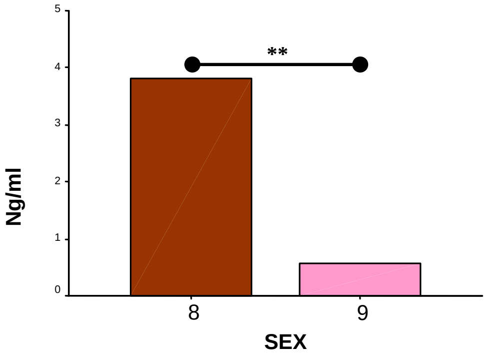
**GRÁFICO 14**



Se encontraron **diferencias significativas** en los valores de testosterona dependiendo del género ***(Z=-3.766; n=29; p=0.000)*** y del rango ***(H=18.879; n=29; p=0.002)***

### NIVELES DE TESTOSTERONA DEPENDIENDO DEL EL SEXO. FASE 3.

### GRÁFICO 15



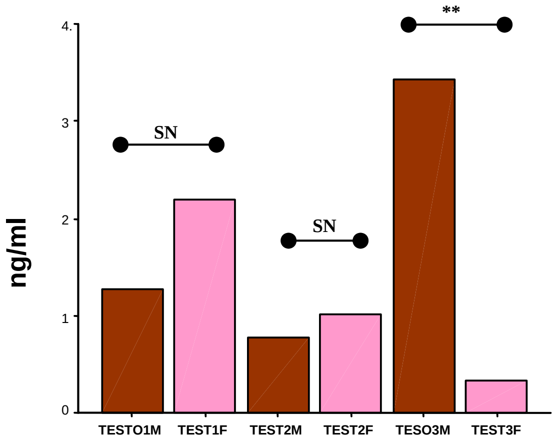
**8** → male
**9** → female

Siete meses después del traslado de los animales, encontramos diferencias significativas en los niveles de testosterona de los machos y las hembras.

Los machos presentan niveles más altos que las hembras, aunque en las primeras etapas no se observó esta diferencia.

### DIFERENCIAS EN LOS NIVELES DE TESTOSTERONA EN LAS TRES FASES

### GRÁFICO 16



**8** → male
**9** → female

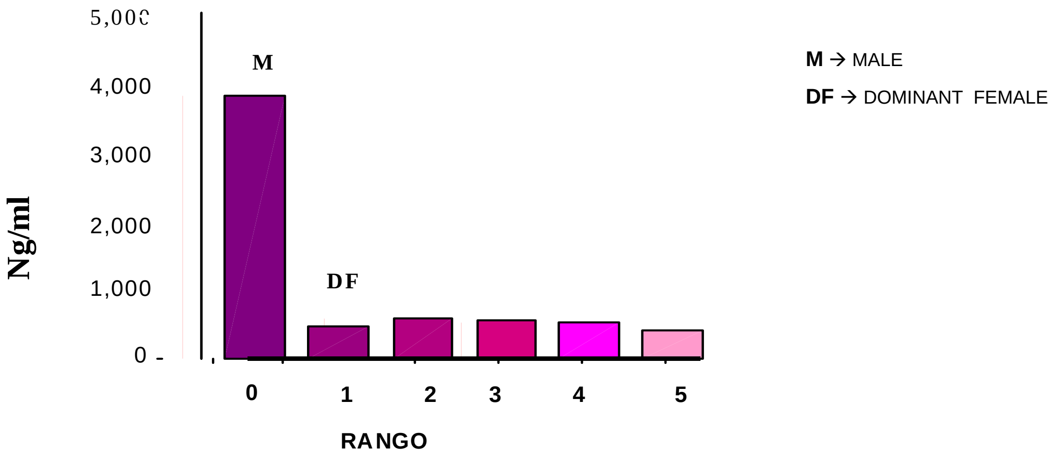
Como puede verse en el grafico, no se encontraron diferencias significativas entre los machos y las hembras en las dos primeras fases, sin embargo, se observó un aumento en lo niveles de testosterona en los machos, siete meses después del transporte y realojamiento en el nuevo hábitat

**Descriptive Statistics**

| | N | Minimum | Maximum | Mean | | Std. |
|---|---|---|---|---|---|---|
| | Statistic | Statistic | Statistic | Statistic | Std. Error | Statistic |
| TESTO1M | 6 | .3540 | 2.9920 | 1.277833 | .411325 | 1.0075373 |
| TEST2M | 6 | .4250 | 1.2280 | .777333 | .129150 | .3163528 |
| TESO3M | 6 | .2990 | 6.2860 | 3.435333 | .874027 | 2.1409213 |
| TEST1F | 24 | .3540 | 6.2360 | 1.516208 | .252183 | 1.2354391 |
| TEST2F | 26 | .1860 | 3.1630 | .943346 | .154639 | .7885090 |
| TEST3F | 26 | .2000 | 1.1880 | .558962 | .052910 | .2697881 |
| Valid N (listwise) | 5 | | | | | |

## NIVELES DE TESTOSTERONA EN RELACIÓN CON EL RANGO

**GRÁFICO 17**



Como puede verse en el gráfico 19, la diferencia en los niveles de testosterona encontrada en relación con el rango, puede explicarse debido a la gran diferencia encontrada entre los machos y las hembras, no existiendo tal diferencia cuando se analizan los niveles de esta hormona solamente en las hembras (*CH= 1,086; n=28; p=0,897*).

## 4.3. DISCUSIÓN

Parece lógico pensar que todos y cada uno de los procesos a los que se ven sometidos los animales que son trasladados podrían conllevar por sí mismos una carga importante de amenaza para los individuos y por tanto potencialmente estresante. Véase por ejemplo, la captura, la sedación, el transporte, y en general el abandono de un espacio familiar y la adaptación a nuevos entornos. Que el estrés, o mejor dicho, todos los tipos de estrés son malos, ha sido discutido por numerosos autores durante muchas décadas, hoy en día puede decirse que ciertos indicadores de lo que llamamos estrés, podrían ser absolutamente necesarios para el enfrentamiento a la situación amenazante, y lo que llamamos "diestrés" sería aquel que manteniéndose los niveles fisiológicos y/o autonómicos anormales (ya sean más altos o más bajos de lo considerado normal) durante mucho tiempo, podrían causar serios daños para la salud tanto física como psíquica del individuo.

Como ha sido mostrado en este capítulo, se observó una recuperación de los niveles hormonales siete meses después del traslado de los animales, tanto en los niveles de cortisol como en los de testosterona. Esta recuperación nos estaría indicando que, aunque el traslado hubiera supuesto una situación de inestabilidad y probablemente de amenaza para los sujetos, debido al abandono del espacio familiar, los indicadores fisiológicos respondieron ante dicha amenaza como forma de afrontamiento, recuperándose los valores normales una vez que cesó la situación inestable. Esta recuperación de los niveles de cortisol podría haber estado propiciada por las condiciones del nuevo hábitat, como puede verse en la revisión sobre enriquecimiento ambiental (Anexo 6): existe evidencia empírica que indica que un ambiente enriquecido favorecería el bienestar de los animales, propiciando conductas típicas de la especie, así como en general un bienestar físico y psicológico que podría verse traducido a nivel fisiológico (Honess y Marín, 2005, Anexo 6).

El aumento en los niveles de cortisol, tras una situación inestable, vivida como una amenaza y por tanto potencialmente estresante, ha sido estudiado ampliamente tanto en primates humanos (Chatterton y cols., 1997) como no humanos (Brown y cols., 1971, Gordon y Gust 1993), como fue expuesto en el capítulo 1, aunque también se ha observado que en condiciones normales dichos niveles vuelven a recuperarse acercándose o alcanzándose los niveles basales

Como puede leerse en nuestra revisión sobre el tema (Anexo 5), en el comúnmente llamado estrés crónico, dichos niveles se mantendrían durante largos periodos de tiempo, pudiendo de esta forma tener una repercusión en la actividad inmune y finalmente en la salud.

La falta de diferencias significativas en relación con el sexo y el rango en los niveles de cortisol de la tercera fase, podrían preverse, ya que tampoco fueron encontradas dichas diferencias en las dos primeras fases.

En cuanto a la testosterona, el aumento en los niveles de testosterona siete meses después del traslado podría estar indicando la recuperación en la frecuencia de ciertas conductas como son conducta sexual, tema que trataremos en las conclusiones finales. De la misma forma podría estar indicando una frecuencia más alta de conductas de competición y agresivas, una vez que el individuo se ha asentado en el nuevo hábitat, este podría estar exhibiendo un mayor porcentaje de conductas de competición.

Se encontraron diferencias significativas con respecto al sexo en los niveles de testosterona encontrados siete meses después del traslado de los animales. Los machos alcanzaron niveles de testosterona significativamente más altos que las hembras siete meses después del movimiento. Según los datos obtenidos y analizados hasta el momento, parece probable que la testosterona responde ante situaciones de amenaza como un indicador fisiológico de ésta, al igual que ha sido observado con el cortisol.

Sin embargo, del mismo modo ha sido ampliamente estudiado que la testosterona está ligada a otras variables tales como conducta reproductora y agresión. Tras una situación inestable y potencialmente estresante, la testosterona podría estar más íntimamente ligada al estrés que a las conductas anteriormente citadas. De ahí que no existieran diferencias significativas en las dos primeras fases, apareciendo estas diferencias una vez que los animales se habían asentado en el nuevo espacio y comenzaran a exhibir sus conductas típicas.

Como puede verse en el gráfico 18, los niveles de testosterona en las hembras van en grado decreciente según avanzan las fases, mientras que en los machos van en orden creciente, notándose un bajón significativo en la segunda fase.

## 4.4. CONCLUSIONES

1. Siete meses después del traslado los niveles de cortisol eran inferiores a los niveles encontrados en la Fase 2, recuperando los valores obtenidos antes del movimiento de los animales.

2. No se encontraron diferencias significativas con respecto al sexo ni al rango en los niveles de cortisol, siete meses después del traslado.

3. Siete meses después del traslado los niveles de testosterona aumentaron con respecto a los valores obtenidos en la Fase 2, aunque no llegaron a alcanzar los niveles obtenidos e la linea base (Fase 1).

4. Siete meses después del traslado se observaron diferencias significativas con respecto al sexo en los niveles de testosterona. Los machos presentaron niveles significativamente más altos que las hembras, hecho que no se observó en ninguna de las fases anteriores.

# *Capítulo 5:*

# *RESPUESTA INMUNE SIETE MESES DESPUÉS DEL TRASLADO DE LOS ANIMALES*

## 5.1. INTRODUCCIÓN

Se han llevado a cabo muy pocos estudios con seguimiento a largo plazo, en primates humanos y no humanos, sobre la respuesta inmune tras una situación amenazante. En especial, se echan en falta estudios que den información conjunta de la respuesta fisiológica y la inmunológica tanto a corto-medio como largo plazo.

Los resultados que aquí se exponen se obtuvieron tras medir la actividad inmune siete meses después del traslado de los animales y su reubicación en el nuevo hábitat. La respuesta inmune a largo plazo nos permitirá obtener información relevante sobre el impacto del movimiento de los animales sobre el sistema inmune.

### a) MOTIVO DE LA MEDICIÓN DE LA RESPUESTA IMMUNE SIETE MESES DESOUÉS DEL TRASLADO DE LA COLONIA.

El objetivo principal fue determinar si la actividad inmune encontrada en la segunda etapa seguía apareciendo siete meses después del traslado, o si por el contrario, se observaba una recuperación de dicha actividad, debido a la situación ofrecida en el nuevo hábitat.

### b) HIPÓTESIS

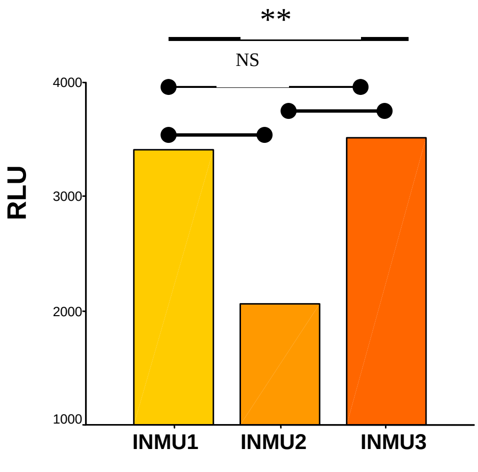
La capacidad de los leucocitos de producir radicales libres de oxígeno en respuesta a la estimulación *in vitro* del PMA (*LCC*), será mayor siete meses después del traslado de los animales, con respecto a los valores obtenidos en la Fase 2, reflejando una mayor cantidad de leucocitos activos libres para responder a la estimulación *in vitro* por el PMA.

## 5.2. RESULTADOS

Tras utilizar la prueba de Shapiro-Wilk para estudiar si las variables cumplían el supuesto de normalidad y observar que no lo cumplían, se analizó la actividad de los leucocitos en las diferentes fases utilizando pruebas no paramétricas. Concretamente, a través del *test de Wilcoxon* estudiamos las diferencias entre las fases 2-3, fases 3-1 y fases 1-2-3; con el *test de Mann-Whitney* las diferencias según el sexo; y con la *prueba de Kruskal-Wallis* si existían diferencias en la actividad de los leucocitos dependiendo del rango que el animal ocupaba en la jerarquía.

**ACTIVIDAD DE LOS**
**LEUCOCITOS EN LAS**
**DIFERENTES FASES**

**GRÁFICO 18**



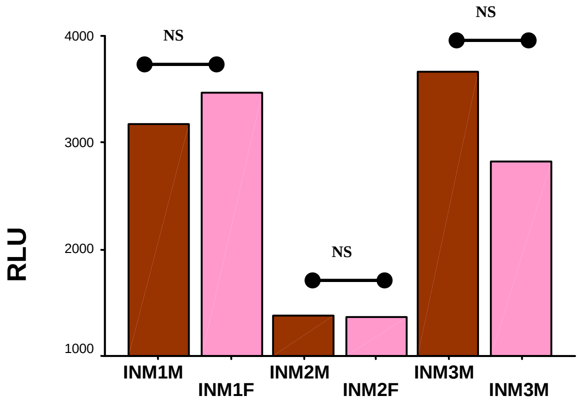
FASE 1
FASE 2
FASE 3

Se encontraron **diferencias significativas en INM2*INM3** ***(Z=-3.057; n=31; p=0.002)***, no se encontraron diferencias significativas en INM1*INM3 *(Z=-0.524; n=32; p=0.601)*, pero si en la actividad de los leucocitos a través del tiempo INM1*INM2*INM3 ***(Ch=12.194; n=31; p=0.002)***

También se estudió la respuesta inmune en relación con el sexo y con el rango, no encontrándose diferencias significativas en ninguna de las variables *(U=75.000; n=32; p=0.885), (H=2.535; n=32; p=0.771)*, aunque, como puede verse en el grafico 20, la respuesta inmune de los machos y las hembras fue diferente a través del tiempo. Las hembras presentaron unos valores basales superiores a los machos, siendo más pronunciado el descenso en la segunda etapa.

**DIFERENCIAS ENTRE MACHOS Y HEMBRAS**
**EN LA RESPUESTA INMUNE**

**GRÁFICO 19**



**INMU1M → respuesta inmune de los machos en la fase 1**
**INMU1F → respuesta inmune hembras en la fase 1**
**INMU2M → respuesta inmune de los machos fase 2**
**IMNU2F → respuesta inmune hembras fase 2**
**INMU3M → respuesta inmune de los machos en la fase 3**
**INMU3F → respuesta inmune hembras en la fase 3**

**Descriptive Statistics**

| | N | Minimum | Maximum | Mean | | Std. |
|---|---|---|---|---|---|---|
| | Statistic | Statistic | Statistic | Statistic | Std. Error | Statistic |
| IMUN51M | 6 | 1751.71 | 5062.64 | 3003.8837 | 485.7306 | 1189.792 |
| IMUN52M | 6 | 961.72 | 1752.69 | 1312.6293 | 138.2989 | 338.76184 |
| IMUN53M | 6 | 1977.55 | 6701.18 | 3415.1858 | 755.5373 | 1850.681 |
| IMUN51F | 29 | 704.72 | 10968.35 | 3392.0829 | 522.1841 | 2812.047 |
| IMUN52F | 28 | 741.09 | 8582.15 | 2236.3955 | 376.6386 | 1992.984 |
| IMUN53F | 27 | 1012.20 | 14346.59 | 3527.1025 | 505.9187 | 2628.831 |
| Valid N (listwise) | 5 | | | | | |

## 5.3. DISCUSIÓN

Siete meses después del traslado, y tras el uso de la técnica expuesta en el apartado de Métodos y procedimiento, encontramos que la capacidad de los leucocitos de producir radicales libre de oxígeno tras la estimulación *in vitro* por el PMA (*LCC*), fue significativamente más alta que la registrada en la Fase 2 (inmediatamente después del traslado de la colonia). Como fue explicado en el mencionado apartado, esta técnica mide el número de leucocitos activos que responden a la estimulación del PMA *in vitro* a través de luminiscencia, produciendo radicales libres de oxígeno. Ante una situación de estrés, el número de leucocitos que responden a esta estimulación sería menor puesto que la mayoría de estos leucocitos estaría respondiendo a la situación de estrés como defensa del organismo. Como puede verse en los gráficos y tras el análisis estadístico realizado, podemos afirmar que inmediatamente después del traslado los animales presentaban una respuesta inmune mayor (representada con luminiscencia como un valor menor) a lo que ahora podríamos llamar estrés, no siendo así en la primera y la última fase, donde la LCC sería mayor, representando un menor estrés.

Esta técnica ha sido llevada a cabo en otros estudios en primates no humanos (Honess y cols., 2005, Anexo 7) y en tejones (McLaren y cols., 2003; Montes y cols., 2004). Recientemente también ha sido utilizada en humanos para medir la reacción de los estudiantes ante una situación potencialmente estresante como es un examen (Ellard y cols., 2001; Mian y cols., 2003)

Aunque no se encontraron diferencias significativas con respecto al sexo, según puede verse en la figura 20, los niveles basales de los machos y de las hembras fueron diferentes, igualándose estos valores inmediatamente después del traslado y volviendo a ser diferentes en la Fase 3. Podría deberse a la amenaza percibida por las hembras. Recordemos que aunque se trata de una jerarquía estable, las hembras ante una situación de inestabilidad aumentarían el temor a ser derrocadas y perder su rango. Esto podría explicar esta diferencia de sexos, aunque como ha sido indicado con anterioridad dichas diferencias no fueron significativas.

## 5.4.CONCLUSIONES

1. Siete meses después del traslado de los animales y su reubicación al nuevo espacio, la *LCC* “*Leukocyte Coping Capacity*” aumentó significativamente con respecto a la respuesta registrada inmediatamente después del traslado de los animales. Sería debido al cese de la amenaza por parte de los animales y a su adaptación al nuevo hábitat.

2. No se observaron diferencias significativas en la respuesta inmune entre los machos y las hembras, aunque los machos tuvieron una *LCC* superior a las hembras. Esto podría interpretarse como más estrés.

3. No se observaron diferencias significativas con respecto al rango en la *LCC* en la tercera fase.

# Capítulo 6:

# CAMBIOS CONDUCTUALES SIETE MESES DESPUÉS DEL TRASLADO DE LOS ANIMALES

## 6.1. INTRODUCCIÓN

Los cambios en los patrones de conducta tras una situación y/o estímulo amenazante, es uno de los indicadores más utilizado por muchos autores para medir el grado de estrés. No obstante, como fue apuntado en la introducción del capítulo 3 y se verá a lo largo de este capítulo, las conductas están íntimamente ligadas a las condiciones del entorno, dificultando de esta forma la interpretación de dichos cambios como meros correlatos fisiológicos cuando, como es el caso que nos ocupa, el espacio es una variable variante.

Como se señaló en la introducción general, el espacio en el CFM de Porton Down, a donde fue traslada la colonia de macacos *rhesus*, poseía un gran número de novedades, tales como su mayor espacio, cuadruplicando al anterior en Harlan UK, en Hillcrest, juguetes como pelotas o gomas elásticas, y estructuras colgantes para facilitar la aparición de conductas típicas de la especie, de suma importancia para el bienestar de ésta.

En este capítulo se expondrán y analizarán aquellos cambios conductuales observados en la fase 3, en relación con las fases precedentes, con el fin de determinar si el traslado de los animales afectó, y cómo lo hizo, a los nuevos patrones de conducta una vez establecidos en el nuevo hábitat. Se hará una especial referencia, como ya se hizo en la fase 2, a la conducta social.

## a) MOTIVO DEL ESTUDIO DE LA CONDUCTA SOCIAL SIETE MESES DESPUES DEL TRASLADO Y UBICACIÓN EN EL NUEVO ESPACIO.

Se realizó un estudio de la conducta social siete meses después del traslado de los animales y a la ubicación al nuevo hábitat con el fin de explorar dos condiciones. La primera, cómo las condiciones del nuevo espacio afectaron al patrón de conducta en general y a la conducta social en particular; y la segunda, observar si los cambios ocurridos en la fase 2 se habían prolongado en el tiempo, estableciéndose como un patrón estable, o, por el contrario, si esos cambios habían supuesto una mera reacción adaptativa tras la situación de inestabilidad.

## b) HIPÓTESIS

1. Siete meses después del traslado de los animales aumentará la frecuencia de las conductas relacionadas con cohesión del grupo -tales como acicalar, ser acicalado, juego o conducta sexual-, con respecto a los resultados obtenidos en la Fase 2, equiparándose a aquellas frecuencias exhibidas antes del traslado. La recuperación de estas conductas reflejaría la adaptación al nuevo hábitat.

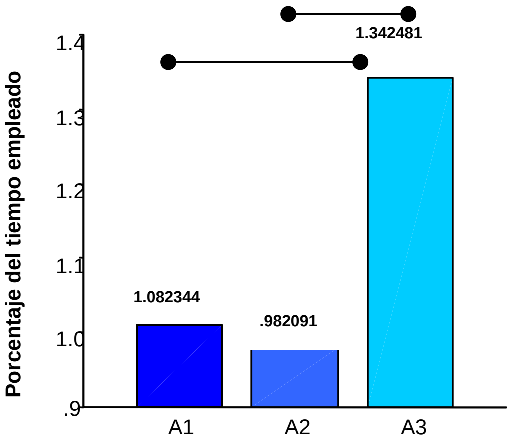
2. Siete meses después del traslado de los animales disminuirá la frecuencia de las conductas relacionadas con competición y agresión, con respecto a la Fase 2, como consecuencia del cese de la situación inestable, potencialmente amenazante, y de la adaptación al nuevo hábitat.

## 6.2. RESULTADOS

Se estudiaron los cambios conductuales siete meses después del traslado de la colonia, con especial atención a la conducta social. Para el análisis estadístico de las distintas categorías conductuales se utilizaron pruebas paramétricas y no paramétricas. Así mismo se estudiaron las posibles diferencias según el sexo del animal y según el rango que ocupaba dentro de la jerarquía.

**CONDUCTA AFILIATIVA**

**GRÁFICO 20**



Se observó un aumento significativo de las conductas afiliativas siete meses después del traslado de la colonia A2*A3 ***(Z=-2.293; n=31; p=0.022)*** A1*A3 ***(Z=-2.293; n=31; p=0.022)***.

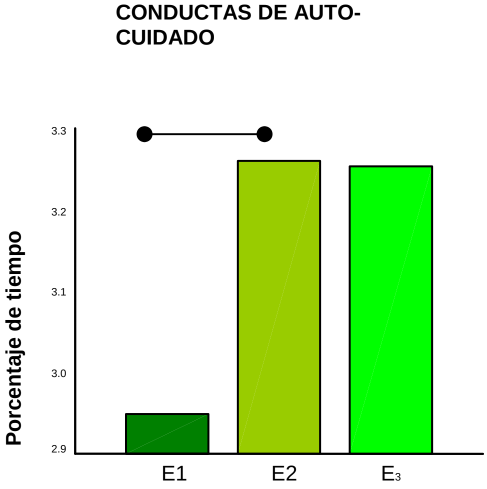
Sin embargo no se encontraron diferencias significativas ni en relación con el sexo *(U=-0.700; n=31; p=0.484)*, ni con el rango *(H=4.373;*

| | N | Mean | Std. Deviation | Minimum | Maximum |
|---|---|---|---|---|---|
| A2 | 33 | .982091 | .6489013 | .0098 | 3.0859 |
| A3 | 31 | 1.342481 | .7790803 | .0591 | 3.5015 |

| | N | Mean | Std. Deviation | Minimum | Maximum |
|---|---|---|---|---|---|
| A1 | 34 | 1.082344 | .6144661 | .0431 | 3.1206 |
| A3 | 31 | 1.342481 | .7790803 | .0591 | 3.5015 |

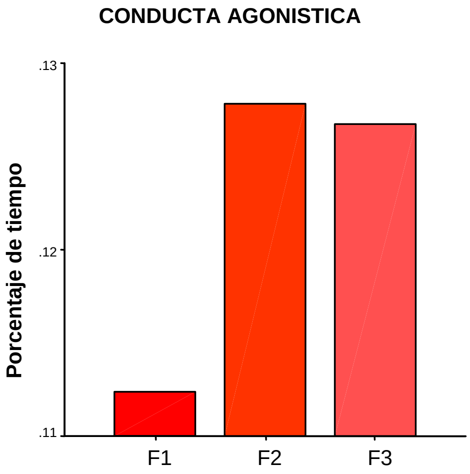
Con respecto a la categoría de conductas de auto-cuidado (E), no se observaron diferencias significativas entre la frecuencias exhibidas en la segunda etapa y en la tercera E2*E3 *(F=2.251; n=30; p=0.144),* ni en las exhibidas en la primera etapa y la tercera E1*E3 *(F=0.001; n= 30; p= 0.973)*. (Grafico 22). Tampoco se observaron diferencias significativas en la tercera etapa en relación con el sexo *(F=1.056; n=30; p=0.313*), ni con el rango *(F=0.537; n=30; p=0.747).*

**GRÁFICO 21**



Como puede apreciarse en el grafico, el aumento más significativo de las conductas de auto-cuidado se observó tras la llegada de la colonia al nuevo hábitat, sin embargo estas conductas se mantuvieron siete meses después.

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| E1 | 34 | 1.2314 | 5.0925 | 2.955035 | .9763100 |
| E2 | 33 | 1.4994 | 5.1493 | 3.274955 | 1.0308257 |
| E3 | 31 | 1.5963 | 5.2531 | 3.253329 | .9023638 |
| Valid N (listwise) | 31 | | | | |



## GRÁFICO 22

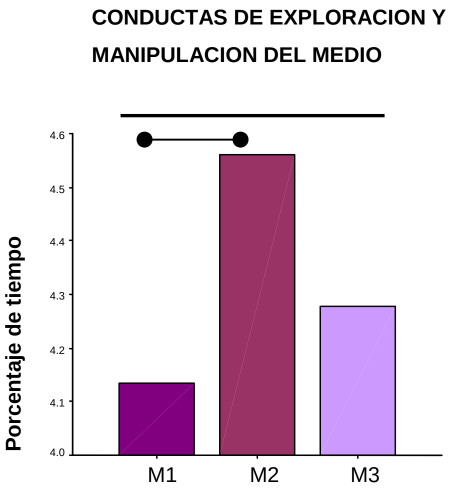
No se encontraron diferencias significativas en ninguna de las fases F2*F3 *(Z= -0.372; n=31; p=0.710)* F1*F3 *(Z=-0.154; n=31; p=0.877)* F1*F2*F3 *(Ch=0.97; n=31; p=0.629)*. Tampoco se notaron diferencias significativas en relación con el género *(U=-0.500; n=30; p=0.617)* ni con el rango del animal *(H=3.030; n=30; p=0.695).*

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| F1 | 34 | .0125 | .3301 | .105868 | .0761584 |
| F2 | 33 | .0123 | .5348 | .146036 | .1092534 |
| F3 | 31 | .0158 | .4367 | .126703 | .1076349 |
| Valid N (listwise) | 31 | | | | |

Con respecto a las conductas de exploración y manipulación del medio (M), no se observaron diferencias significativas entre las etapas M2*M3 *(F=2.394; n=30; p=0.132),* ni entre las etapas M1*M3 *(F=0.392; n=31; p=0.536)*, sin embargo si se observaron diferencias a través de las tres etapas ***M1*M2*M3 ( F=7.530; n=31; p=0.010)***.

No se observaron diferencias significativas en la categoría M3 con respecto al sexo *(F=0.270; n=31; p=0.607),* ni el rango *(F=0.440; n=31; p=0.816)*

**GRÁFICO 23**

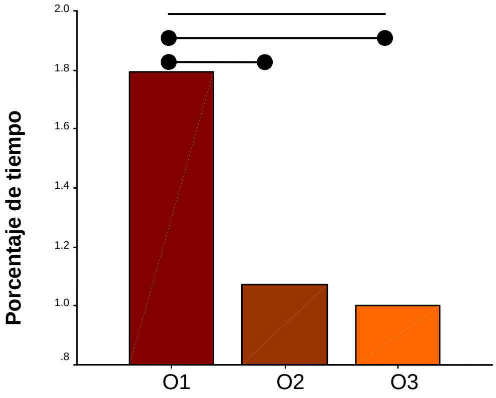


**Descriptive Statistics**

| | Mean | Std. Deviation | N |
|---|---|---|---|
| M1 | 4.134965 | .8372024 | 31 |
| M3 | 4.277048 | 1.1125014 | 31 |

Por ultimo, con respecto al resto de conductas analizadas, no se observaron diferencias significativas en O2*O3 *(Z=-0.039; n=31; p=0.969),* aunque si se observaron diferencias significativas en ***O1*O3 (Z= -3.919; n=31; p=0.000)*** y a través del tiempo **O1*O2*O3** ***(Ch=21.353; n=31; p=0.000)***

**GRÁFICO 24**



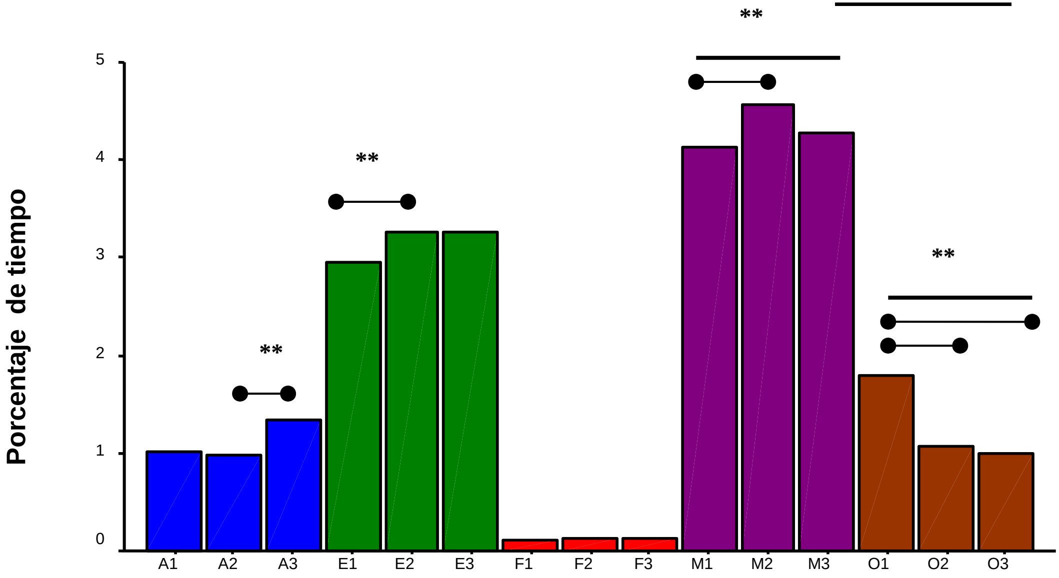
No se observaron diferencias significativas ni en relación con el sexo *(U=-0.400; n=31; p=0.689),* ni con el rango *(H=4.994; n=31; p=0.417).*

En el grafico 26 pueden verse el patrón de conducta en las diferentes fases.

|  | N | Mean | Std. Deviation | Minimum | Maximum |
|---|---|---|---|---|---|
| O1 | 34 | 1.753759 | .7179733 | .5447 | 3.2080 |
| O3 | 31 | 1.000400 | .5093692 | .3313 | 2.9610 |

## FRECUENCIA EN LOS PATRONES DE CONDUCTA EXHIBIDOS DURANTE LAS TRES FASES

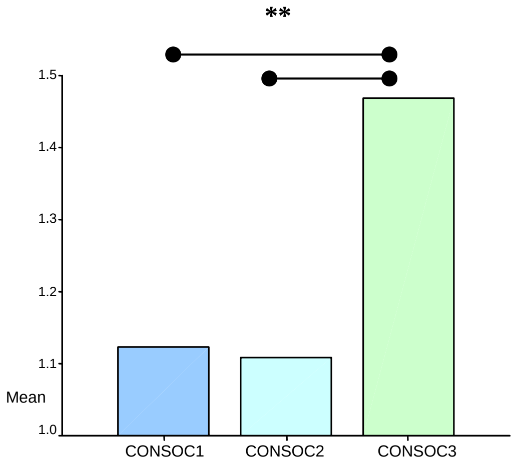
**GRAFICO 25**



Como puede apreciarse en el gráfico, la única categoría que no sufrió ninguna variación a lo largo de las fases fue la conducta agonística.

Como se hizo en las dos primeras etapas, también se estudió la conducta social en general (afiliativa y agonística) en relación con las otras conductas.

## CONDUCTA SOCIAL (AFILIATIVA Y AGONÍSTICA) A LO LARGO DE LAS TRES FASES



### GRÁFICO 26

Se encontraron diferencias significativas en la conducta social ***CS2*CS3 (Z=-2.195; n=31; p= 0.028)*** y entre ***CS1*CS3 (Z=-2.317; n= 31; p=0.018)***.

## 6.3. DISCUSIÓN

Enriquecimiento ambiental y bienestar físico y psicológico está siendo debate entre profesionales desde hace algunas décadas. El hacinamiento de animales salvajes en jaulas está provocando serias disputas entre los expertos preocupados por la calidad de vida de aquellos animales destinados a fines experimentales y/o turísticos, pues lo limitado del espacio hace que el número de conductas desplegadas sea menor de lo normal. En una previa publicación nuestra, presentada en este trabajo como Anexo 6, puede verse una revisión amplia sobre el tema que aquí nos ocupa.

Los resultados obtenidos en esta tercera fase pueden deberse principalmente a dos variables altamente determinantes: una de ellas sería el cese de aquellos cambios provocados por la situación de inestabilidad del traslado, y otra sería el gran cambio en cuanto a enriquecimiento del medio aportado por las nuevas facilidades en el CFM en Porton Down. Sobre este tema volveremos en las conclusiones generales.

Los resultados más significativos encontrados en esta fase estuvieron relacionados con aquellas conductas comprendidas en la categoría de afiliación, íntimamente relacionadas con la cohesión del grupo. Se observaron aumentos significativos en la frecuencia de estas conductas con respecto a las otras fases. Se ha observado que la conducta afiliativa podría ser un buen indicador del estrés (Honess y cols., 2004), pareciendo estar ambas variables inversamente relacionadas. Este hecho podría haber sido favorecido por las condiciones favorables del nuevo hábitat pudiendo afirmar que las características del nuevo espacio podría haber mejorado significativamente esta dinámica grupal.

Con respecto a la frecuencia de las conductas relacionadas con auto-cuidado, parece que se mantuvieron con el tiempo, estableciéndose definitivamente siete meses después del traslado. Recordemos que estas conductas aumentaron significativamente en la fase 2 (inmediatamente después del traslado y realojamiento) en relación con la fase 1 (línea base).
Una de las hipótesis relaciona las conductas de auto-cuidado con el estrés: las conductas de auto-acicalado estarían relacionadas con situaciones de ansiedad, en las que el animal despliega con alta frecuencia este tipo de conductas con el fin de relajarse, aunque más recientemente se ha observado que serían las conductas auto-lesivas y las estereotipadas las que estarían más claramente relacionadas con el estrés (Honess & Marín, 2005, Anexo 6). En nuestro caso y a la vista de los otros resultados, parece que las conductas de auto-cuidado y más concretamente las de auto-acicalado y aunque sufrieron un aumento significativo inmediatamente después del traslado, parece que, al menos a largo plazo, esta más relacionadas con bienestar que con estrés.

No se encontraron diferencias significativas en la frecuencia de las conductas relacionadas con competición y agresión, y tampoco se encontraron diferencias significativas entre las fases 2 (inmediatamente después del traslado) y la fase 3 (siete meses después del traslado) en las frecuencias de aquellas conductas relacionadas con exploración y manipulación del medio. El primer aspecto podría explicarse debido a la baja frecuencia y el bajo porcentaje, en relación con las otras categorías, de las conductas agresivas registradas en la fase 1 (línea base), posiblemente por lo estable de la jerarquía y la solidez de los grupos.

Con respecto a las conductas relacionadas con exploración y manipulación del medio, el hecho de que, tras siete meses desde la llegada al nuevo espacio, haya descendido la frecuencia con que se expresan estas conductas, sugeriría la adaptación de los animales al nuevo hábitat, el cual empezaría a ser visto como familiar.

Con respecto a la conducta social, su frecuencia aumentó de modo significativo siete meses después del traslado de los animales. Este aumento muestra una exhibición más frecuente de aquellas conductas relacionadas con la cohesión del grupo en el nuevo hábitat que antes o inmediatamente después del traslado, sugiriendo que el nuevo espacio afectó positivamente a la conducta socia y, en general, a la dinámica de grupo.

## 6.4. CONCLUSIONES

Siete meses después del traslado y de la ubicación de los animales en el nuevo espacio:

1. Se observaron aumentos significativos con respecto a la fase 1(línea base) y la fase 2 (inmediatamente después del traslado), en la frecuencia de aquellas conductas relacionadas con afiliación y que están estrechamente relacionadas con la cohesión del grupo.

2. No se observaron cambios ni con respecto a la fase 1, ni a la fase 2, en la frecuencia de aquellas conductas relacionadas con competición y agresión.

3. No se observaron cambios en las conductas relacionadas con auto-cuidado, aunque la frecuencia de estas conductas se mantuvo con respecto a la Fase 2, mostrando una diferencia significativa con respecto a la Fase1.

4. Se observó un descenso en la frecuencia de aquellas conductas relacionadas con exploración y manipulación del medio, con respecto a la Fase 2, si bien este descenso no fue significativo.

5. Por último, se observó un aumento en la frecuencia de aquellas conductas etiquetadas como sociales (afiliativa + competitiva/agresión) con respecto a las Fases 1 y 2, relacionado posiblemente, con las conductas afiliativas, ya que las conductas competitivas y agonísticas no sufrieron variación alguna a través del tiempo.

# CONCLUSIONES GENERALES

Inmediatamente después del traslado de los animales se observó:

1. Un aumento significativo de los niveles de cortisol en sangre, con respecto a la Fase 1 (línea base).

2. Un descenso significativo de los niveles de testosterona en sangre, con respecto a la Fase 1 (línea base).

3. Un descenso significativo en la capacidad de los leucocitos de producir radicales libres de oxígeno en respuesta al PMA *in vitro* con respecto a la Fase 1 (línea base).

4. Un aumento significativo en la frecuencia de las conductas de auto-cuidado como son: beber, comer o buscar comida, auto-acicalado y descansar, con respecto a la Fase 1 (línea base).

5. Un aumento significativo en la frecuencia de las conductas relacionadas con exploración y manipulación del medio: manipulación del medio, observar y juego con objetos, con respecto a la Fase 1 (línea base).

Tanto las variables fisiológicas como las conductuales (aunque no se corroboró ninguna de las hipótesis de trabajo correspondientes a la conducta) sugirieron que el traslado de la colonia fue vivenciado por los animales como un suceso amenazante, pudiendo ser etiquetada dicha situación como estresante.

Siete meses después del traslado de los animales se observó:

1. Un descenso significativo de los niveles de cortisol en sangre, con respecto a la Fase 2 (inmediatamente después del traslado), recuperándose los valores encontrados en la Fase 1 (línea base).

2. Un aumento, aunque no significativo, de los niveles de testosterona, con respecto a la Fase 2 (inmediatamente después del traslado), si bien no llegaron a recuperarse los valores encontrados en la Fase 1 (línea base).

3. Un aumento significativo de la *LCC ”Leukocyte Coping* Capacity*”,* con respecto a la Fase 2 (inmediatamente después del traslado), recuperándose los valores registrados en la Fase 1 (línea base).

4. Un aumento significativo de las conductas de afiliación; relacionadas con la cohesión del grupo (auto-acicalado, ser acicalado, intercambio de acicalado, pelea-juego, conducta sexual, abrazos, juego, con respecto a las Fases 1 (línea base) y 2 (inmediatamente después del traslado),.

5. Un aumento significativo de las conductas de auto-cuidado: bebida, comida o su búsqueda, auto-acicalado y descanso, con respecto a la Fase 1 (línea base).

6. Un descenso, aunque no significativo, de las conductas de exploración y manipulación del medio con respecto a la Fase 2 (inmediatamente después del traslado), y un aumento, igualmente no significativo, con respecto a la Fase 1 (línea base).

Todos aquellos indicadores fisiológicos alterados inmediatamente después del traslado de los animales, recuperaron sus niveles basales, o al menos mostraron una tendencia hacia dichos niveles, como fue el caso de la testosterona, sugiriendo que el traslado no resultó estresante a largo plazo.

A la vista de los resultados anteriormente citados podríamos concluir diciendo que:

1. El traslado de los animales supuso un estrés a corto plazo para los individuos, tal como se reflejó tanto a nivel fisiológico como conductual. Sin embargo no se observaron consecuencias negativas a largo plazo en ninguno de los niveles anteriormente citados.

2. Ni el transporte de los animales ni los distintos métodos de captura utilizados fueron los procesos más estresantes para los individuos, Como sugerencia, el estrés encontrado inmediatamente después del traslado parece haberse debido a un efecto sumatorio de todos los procesos comprendidos en el traslado y del posible abandono del espacio “familiar”.

3. El porcentaje de conductas agonísticas observadas en los distintos grupos fue mínimo en relación con las otras conductas exhibidas, e incluso en relación con las conductas de afiliación. Esto podría explicarse dada la estabilidad de la jerarquía y a la solidez de los grupos observados.

4. Ni el rango ni el sexo de los animales fueron variables influyentes que afectaran ni al tipo ni a la intensidad de las respuestas registradas en ninguna de las tres fases.

5. Las características del nuevo hábitat (enriquecido) podrían haber favorecido la recuperación de los niveles fisiológicos y el despliegue de un mayor número de conductas de afiliación y de auto-cuidado.

En general, las observaciones realizadas siete meses después del traslado de la colonia -un aumento de la frecuencia de conductas sociales y del auto-cuidado- nos permiten concluir afirmando que a largo plazo el traslado a un hábitat mejor ha sido un proceso positivo para los macacos, pues se han visto beneficiados por un aumento en la frecuencia de aquellas conductas relacionadas con la cohesión del grupo.

Por tanto, podríamos concluir sugiriendo que el traslado de animales no significa un peligro para la salud física y psíquica de los individuos a largo plazo, siempre y cuando las condiciones del traslado sean cuidadosamente elegidas, se realice un seguimiento de aquellas variables alteradas inmediatamente después del traslado, y se les reubique en espacios enriquecidos, tanto cuantitativamente como cualitativamente.

# GENERAL CONCLUSIONS

Immediately after animals´ translocation was noted:

1. A significant increase in cortisol levels in relation to Phase 1 (Baseline).
2. A significant decrease in testosterone levels in relation to Phase 1 (Baseline)
3. A significant decrease in relation to Phase 1 (Baseline), in the Leukocyte capacity to produce oxygen free radicals "*LCC*" in response to a stimulation *in vitro* by PMA .
4. A significant increase in relation to Phase 1 (Baseline), in the frequencies of those behaviours related to self-maintenance such as drink, eat, forage or self-grooming.
5. A significant increase in relation to Phase 1 (Baseline), in the frequencies of those behaviours related to exploration such as environmental manipulation, observation or play with objects.

Physiological and behaviour indicators would suggest that the translocation provoked an unstable situation causing a certain grade of threatened and so could be labelled as stressful condition.

Seven months after translocation and relocation in the new facilities was noted:

1. A significant decrease in cortisol levels, in relation to Phase 2 (immediately after translocation and relocation in the new facilities), recovering the cortisol levels and reaching the values registered in the Phase 1 (Baseline)

2. An increase but not statistically significant, in testosterone levels in relation to Phase 2 (immediately after translocation and relocation in the new facilities), although this values did not reach the baseline levels.

3. A significant increase in the "*LCC" Leukocyte Coping* Capacity*,* in relation to Phase 2 (immediately after translocation and relocation in the new facilities) recovering the values obtained in Phase 1 (Baseline).

4. A significant increase in relation to Phase 1 (Baseline) and Phase 2 (immediately after translocation and relocation in the new facilities) in the "affiliative behaviour" category; related to group´s cohesion (being groomed, exchange grooming, jostle (play fighting), sexual behaviour and play).

5. A significant increase in relation to Phase 1 (Baseline), of those behaviours related to self-maintenance such as drink, eat, forage, self-grooming or resting.

6. A decrease, but not statistically significant, in relation to phase 2 (immediately after translocation and relocation in the new facilities) of explorative behaviours and an increase, neither significant, in relation to Phase 1 (Baseline).

As it can be noted from the results above indicated, all the physiological indicators which were altered immediately after translocation (Phase 2), seven months after the move and relocation in the new facilities, recovered the baseline levels or at least showed a prone to do it, suggesting that the translocation was not a long term stressful event, or at least, it did not caused long-term negative outcomes.

We can conclude pointing out:

The translocation of the colony caused a short-term stress response reflected on physiological and behavioural parameters. However it was not noted long-term (seven months after translocation) negative outcomes in none of the indicators above indicated.

1. Neither the transport nor the different methods of capture used were the main cause of the stress observed immediately after translocation, we might suggest that the stress noted in Phase 2 may be due to a the added effect of the different stressors during the translocation and more concretely may be due to the living of “familiar” habitat.

2. The average of agonistic behaviours observed during the three phases was very low in relation to the other behaviours and even in relation to the behaviours labelled as “affiliative”. This might be explained due to the group and hierarchy stability.

3. It was not noted neither rank nor sex-related differences. Neither the type of response nor the intensity depended on those variables.

4. The new habitat characteristics might have been the cause of the quick recovery in physiological indicators and the appearance of the increase in behaviours related to group’s cohesion.

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# General References

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- ***ANEXO 2: Hoja de recogida de datos inmunológicos***
- ***ANEXO 3: Etograma***
- ***ANEXO 4: Esquema de trabajo para la recogida de datos conductuales.***
- ***ANEXO 5:*** Behavioural and physiological aspects of stress and aggressión in nonhuman primates”. ***Neuroscience and Biobehavioural Reviews xx*** (2005) 1-23
- ***ANEXO 6-*** “Enrichment and aggression in primates: A review”. ***Neuroscience and Biobehavioural Reviews xx*** (2005): 1-24
- ***ANEXO 7-*** Assessment of stress in non-human primates: the application of a novel technique”. ***Animal Welfare 14 xxx-xxx (2005)***

# ANEXO 1:

## COMPOSICIÓN DE LA MUESTRA

## STUDY ANIMALS

## 1.-GRUPO DE BLUES

| NOMBRE | Año de nacimiento | RANk | METODO DE CAPTURA | METODO DE CAPTURE 2 | METODO DE CAPTURA 3 |
|---|---|---|---|---|---|
| **Blues** | **01.01.89** | **0** | entrenado | entrenam+sedado+entrenado | entrenado |
| 139 | 05.05.89 | 1 | red | Red + entrenado | red |
| 152 | 15.11.89 | 2 | red | Red + entrenado | red |
| Beth | 12.03.90 | 3 | entrenado | Red + entrenado | entrenado |
| 138 | 01.05.89 | 4 | red | Red + entrenado | red |
| Josie |  | 5 | entrenado | Red + entrenado | entrenado |

## 2.-GRUPO DE BAZIL

| NOMBRE | Año de nacimiento | RANK | METODO DE CAPTURA 1 | METODO DE CAPTURA 2 | METODO DE CAPTURA 3 |
|---|---|---|---|---|---|
| **Bazil** | **11.09.93** | **0** | entrenado | Aprision+sedado + aprisionado | entrenado |
| Hannah2 | 05.05.90 | 1 | entrenado | red + aprisionado | entrenado |
| Olivia | 29.05.91 | 2 | red | red + aprisionado | red |
| Fleur | 02.06.90 | 3 | red | red + aprisionado | red |
| Crystal | 03.06.91 | 4 | red | red + aprisionado | red |
| Balbina | 21.05.86 | 5 | red | red + aprisionado | red |

## 3.-GRUPO DE DOYLE

| NOMBRE | Año de nacimiento | RANK | METODO DE CAPTURA 1 | METODO DE CAPTURA 2 | METODO DE CAPTURA 3 |
|---|---|---|---|---|---|
| **Doyle** | **01.01.89** | **0** | red | Aprisi+Sedado+aprisionado | red |
| Charlotte | 20.01.92 | 1 | red | Entrena+Sedado +entrenado | red |
| Maggie | 14.01.92 | 2 | red | Red+entrenado | red |
| Nova | 17.04.92 | 3 | red | Red+entrenado | red |
| 192 | 20.06.91 | 4 | entrenado | Red+entrenado | entrenado |
| 156 | 21.03.90 | 5 | red | Red+entrenado | red |

## 4.-GRUPO DE THOR

| NOMBRE | Año de nacimiento | RANK | METODO DE CAPTURA 1 | METODO DE CAPTURA 2 | METODO DE CAPTURA 3 |
|---|---|---|---|---|---|
| **Thor** | **08.08.88** | **0** | aprisionado | Red + aprisionado | red |
| 123 | 21.06.88 | 1 | aprisionado | Red + entranado | red |
| 117 | 23.12.87 | 2 | aprisionado | Red + entranado | red |
| Eileen | 02.06.83 | 3 | aprisionado | Red + entranado | red |
| Wendy | N/A | 4 | aprisionado | Red + entranado | red |
| 149 | N/A | 5 | aprisionado | Red + entranado | red |

## 5.-GRUPO DE BARNEY

| NOMBRE | Año de nacimiento | RANK | METODO DE CAPTURA 1 | METODO DE CAPTURA 2 | METODO DE CAPTURA 3 |
|---|---|---|---|---|---|
| **Barney** | | **0** | Entrenado | Red+aprisionado | entrenado |
| Tess | 31.05.93 | 1 | red | Red+red | red |
| Sheba | 27.05.93 | 2 | entrenado | Red+entrenado | entrenado |
| Frieda | 30.07.85 | 3 | entrenado | Red+red | entrenado |
| Beryl | 22.06.85 | 4 | red | Red+entrenado | red |
| Robin | 28.07.83 | 5 | red | Red | red |

## 6.-GRUPO DE JIM

| NOMBRE | Año de nacimiento | RANK | METODO DE CAPTURA 1 | METODO DE CAPTURA 2 | METODO DE CAPTURA 3 |
|---|---|---|---|---|---|
| **Jim** | **20.05.92** | **0** | entrenado | Red+entrenado | entrenado |
| Imogen | 01.06.93 | 1 | entrenado | Red+entrenado | entrenado |
| Zoe | 20.06.83 | 2 | entrenado | Red+entrenado | entrenado |
| Dora | 03.05.905 | 3 | entrenado | Red+entrenado | entrenado |
| Ella | 25.05.90 | 4 | entrenado | Red+entrenado | entrenado |

**1 Linea Base (antes del traslado de la colonia)**
**2 Llegada a Porton Down (Inmediatamente después del traslado de la colonia)**

- “entrenado” → el animal era entrenado para presentar su brazo a miembros del equipo y de esta forma era sedado.
- “red” → el animal era capturado con una red contra su voluntad y era sedado por los veterinarios.
- “aprisionado” → el animal era aprisionado por los miembros del equipo y sedado.

# ANEXO 2:

# HOJA DE RECOGIDA DE DATOS INMUNOLÓGICOS

**Name**: ________________ **Sex**: M / F **Housing**: Caged / Open **Time**: __ ______

| **Tube #** | **1** | | **2** | | **3** | |
|---|---|---|---|---|---|---|
| **Reading** | **Control** | **Luminom reading** | **$10^{-3}$M** | **Luminom reading** | **$10^{-5}$M** | **Luminom reading** |
| 1 | T0$^{C}$ | | T0$^{3}$ | | T0$^{5}$ | |
| 2 | T0$^{C}$+5 mins | | T0$^{3}$+5 mins | | T0$^{5}$+5 mins | |
| 3 | T0$^{C}$+10 | | T0$^{3}$+10 | | T0$^{5}$+10 | |
| 4 | T0$^{C}$+15 | | T0$^{3}$+15 | | T0$^{5}$+15 | |
| 5 | T0$^{C}$+20 | | T0$^{3}$+20 | | T0$^{5}$+20 | |
| 6 | T0$^{C}$+25 | | T0$^{3}$+25 | | T0$^{5}$+25 | |
| 7 | T0$^{C}$+30 | | T0$^{3}$+30 | | T0$^{5}$+30 | |
| 8 | T0$^{C}$+35 | | T0$^{3}$+35 | | T0$^{5}$+35 | |
| 9 | T0$^{C}$+40 | | T0$^{3}$+40 | | T0$^{5}$+40 | |
| 10 | T0$^{C}$+45 | | T0$^{3}$+45 | | T0$^{5}$+45 | |

**Name**: ________________ **Sex**: M / F **Housing**: Caged / Open **Time**: __ ______

| **Tube #** | **4** | | **5** | | **6** | |
|---|---|---|---|---|---|---|
| **Reading** | **Control** | **Luminom reading** | **$10^{-3}$M** | **Luminom reading** | **$10^{-5}$M** | **Luminom reading** |
| 1 | T0$^{C}$ | | T0$^{3}$ | | T0$^{5}$ | |
| 2 | T0$^{C}$+5 mins | | T0$^{3}$+5 mins | | T0$^{5}$+5 mins | |
| 3 | T0$^{C}$+10 | | T0$^{3}$+10 | | T0$^{5}$+10 | |
| 4 | T0$^{C}$+15 | | T0$^{3}$+15 | | T0$^{5}$+15 | |
| 5 | T0$^{C}$+20 | | T0$^{3}$+20 | | T0$^{5}$+20 | |
| 6 | T0$^{C}$+25 | | T0$^{3}$+25 | | T0$^{5}$+25 | |
| 7 | T0$^{C}$+30 | | T0$^{3}$+30 | | T0$^{5}$+30 | |
| 8 | T0$^{C}$+35 | | T0$^{3}$+35 | | T0$^{5}$+35 | |
| 9 | T0$^{C}$+40 | | T0$^{3}$+40 | | T0$^{5}$+40 | |
| 10 | T0$^{C}$+45 | | T0$^{3}$+45 | | T0$^{5}$+45 | |

# ANEXO 3

## ETOGRAMA

## ETHOGRAMME

**AVOID AGGRESSION (A)**: Evasive action taken to avoid another that is showing aggression (including latent). Aggressive behaviour is described with a modifier.

**BEING GROOMED (B)**: Receiving grooming from one or more individuals.

**DRINK (D)**: Drinking water from the ad lib water spouts.

**EAT / FORAGE (E)**: All food related behaviour: Looking for / gathering / extracting / processing food and / or chewing / consuming / ingesting food.

**AGGRESSION (F):** Display of aggression described with a range of modifiers of the behaviour and the object of the aggression.

**GROOMING (allo) (G)**: Giving grooming to another. Add ‘Hair pulling’ for plucking.

**HUG (H):** Ventral-ventral hug / clasp may be accompanied by lip-smacking or fear grimacing.

**INACTIVE/ RESTING (I)**: sitting / lying / asleep / resting without active behaviour (e.g. glancing around, active watching etc)

**PLAY FIGHT / JOSTLE (J)**: More energetic version of play, which may be accompanied with play-biting and jostling and open mouth display. Often ends in tears.

**SELF HARM (K):** Chewing digits etc and self hair pulling.

**LOCOMOTING (L):** General locomotion activity around the cage (walking, climbing, short jumps etc.).

**MANIPULATE CAGE (M):** Fiddling with / chewing etc. any part of the fixed surroundings / environment.

**OBSERVATION (O)**: Watching / actively following with eyes.

**PLAY (P)**: Energetic social interaction, often with open mouth display, between individuals not related to maintenance, reproduction or aggression.

**STEROTYPIC MOVEMENT (Q):** Inappropriate, afunctional behaviour e.g. circling, head flicking etc

**RAPID ENERGY EXPENDITURE (R):** Running, leaping around the cage.

**SELF GROOM (S): S**cratching, picking (e.g. feet), biting nails, rubbing eyes, hands etc, self-suckling.

**PLAY WITH OBJECT (T):** Playing with loose objects (non-food) e.g. toys.

**EXCHANGING GROOMING (V):** Trade grooming with at least one other individual or in the middle of a triad of groomers (receiving and giving grooming with different animals).

**INFANT CARE (W):** Includes behaviours such as gathering up the infant etc. Suckling is assumed with younger infants when mother is inactive.

**SEXUAL BEHAVIOUR (X):** Mating, same sex mounting and masturbation.

**NOT SEEN (Z):** Animal is out of view.

**Modifiers**

Error (0)
Animal identity (1-6)
Infant (any) (7)

Staff/observer(9)

**Aggression modifiers**

Bark (,)
Jaw dropped (.)
Canines exposed (/)

Bites (;)
Chases ([)
Threatens (])
Grabbing (-)
Pushing (=)
Hair pulling (`)

# ANEXO 4

## ESQUEMA DE TRABAJO PARA LA RECOGIDA DE DATOS CONDUCTUALES

## BEHAVIORAL DATA COLLECTION TIMETIBLE

## Blues group

| Time | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|---|---|---|---|---|---|
| **09:00-09:30** | 152 | 139 | 138 | Josie | Blues |
| **09:40-10:10** | 139 | Blues | Josie | Beth | 138 |
| **10:20-10:50** | Blues | 138 | Beth | 152 | Josie |
| | | | | | |
| **11:10-11:40** | 138 | Josie | 152 | 139 | Beth |
| **11:50-12:20** | Josie | Beth | 139 | Blues | 152 |
| **12:30-13:00** | Beth | 152 | Blues | 138 | 139 |
| | | | | | |
| **14:00-14:30** | 152 | 139 | 138 | Josie | Blues |
| **14:40-15:10** | 139 | Blues | Josie | Beth | 138 |
| **15:20-15:50** | Blues | 138 | Beth | 152 | Josie |
| | | | | | |
| **16:10-16:40** | 138 | Josie | 152 | 139 | Beth |
| **16:50-17:20** | Josie | Beth | 139 | Blues | 152 |
| **17:30-18:00** | Beth | 152 | Blues | 138 | 139 |

## Thor's group

| Time | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|---|---|---|---|---|---|
| **09:00-09:30** | 117 | 149 | Thor | Wendy | 123 |
| **09:40-10:10** | 149 | 123 | Wendy | Eileen | Thor |
| **10:20-10:50** | 123 | Thor | Eileen | 117 | Wendy |
| | | | | | |
| **11:10-11:40** | Thor | Wendy | 117 | 149 | Eileen |
| **11:50-12:20** | Wendy | Eileen | 149 | 123 | 117 |
| **12:30-13:00** | Eileen | 117 | 123 | Thor | 149 |
| | | | | | |
| **14:00-14:30** | 117 | 149 | Thor | Wendy | 123 |
| **14:40-15:10** | 149 | 123 | Wendy | Eileen | Thor |
| **15:20-15:50** | 123 | Thor | Eileen | 117 | Wendy |
| | | | | | |
| **16:10-16:40** | Thor | Wendy | 117 | 149 | Eileen |
| **16:50-17:20** | Wendy | Eileen | 149 | 123 | 117 |
| **17:30-18:00** | Eileen | 117 | 123 | Thor | 149 |

# *ANEXO 5:*

## Behavioural and physiological aspects of stress and aggressión in nonhuman primates”.

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Review

# Behavioural and physiological aspects of stress and aggression in nonhuman primates

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## Abstract

There is considerable interest in the study of stress and aggression in primates as a model for their interpretation in humans. Despite methodological and interpretational problems associated with behavioural and physiological measurement and definition, a considerable body of literature exists on these phenomena in primates. In the course of reviewing this literature we examine examples of many of the sources of variation in stress and aggression, including species identity, sex, age, breeding and social status, individual temperament, background, learning and resource distribution. This is followed by an examination of the interaction between stress and aggression before reviewing the most important areas in which changes in both stress and aggression are measured. In particular we examine those studies covering social aspects of an animal's life, specifically relating to social isolation, crowding as well as group formation, composition and instability. This review reveals the complex and often contradictory nature of relationships, not just between an animal's physiology and its behaviour, but between its stress status and display or receipt of aggression.

*Keywords:* Stress; Aggression; Primates; Behaviour

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## Contents

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## 1. Introduction

Since the earliest beginnings of man he has been fascinated with aggression as exhibited both by his own species and other animals and many of his efforts to distinguish himself from other animals centre on attempts to demonstrate that violence and aggression in man is, unlike in non-human animals, discriminate, measured and reasoned (Durbin and Bowlby, 1939). Towards the mid 1900s our closest relatives; the nonhuman primates, were frequently viewed as living in peaceful, egalitarian societies (de Waal et al., 2000), a perspective only countered by more recent, long-term studies (e.g. Goodall, 1986). Increasingly large numbers of primates have been maintained in captivity, initially for exhibition, amusement and curiosity and more recently primarily for biomedical research but also education and conservation. Together with long-term field studies captivity has enabled detailed observations such as those of warfare, cannibalism and infanticide in chimpanzees, *Pan troglodytes* (Goodall, 1977; Goodall et al., 1979), which have dispelled many of the myths of peaceful primate societies. Furthermore it is now clear that due to the high potential cost of physically aggressive behaviour, most animal aggression occurs in often highly ritualised contexts designed for maximum effect and minimum risk (Bernstein and Gordon, 1974; de Waal, 1989; Tinbergen, 1968, 1969).

While we now have a better understanding of the often sophisticated and complex constraints on aggressive behaviour in primates we can also trace a primitive root in apparently discriminate and reasoned human aggression. Lorenz (1966) in his classic text *On Aggression* identifies what he calls ‘militant enthusiasm’ that can be harnessed in the aggressive defence of high ideals or cultural values but which nevertheless has its origins in the communal defence response of our prehistoric ancestors. Tinbergen (1968) sees the origin of warring aggressive behaviour in man as deriving from group territorial defence and points to within group aggression, such as exists within a nation and is mediated by the rule of law, as being homologous with that which exists within animal groups and is associated with determining a ‘peck order’. Tinbergen was however at the time incorrect in ascribing warring behaviour and mass ‘murder’ uniquely to man; we now know of the existence of very similar behaviour in at least one animal species: chimpanzees where, in the wild, entire communities have been eradicated by their neighbours (Goodall et al., 1979; Wilson and Wrangham, 2003).

Primates are intensely social animals and most species form groups. Group-living with a number of conspecifics whose ecological and reproductive needs match your own has clear competitive consequences, but there are also significant advantages to forming such groups and these cost and benefits of group living are reviewed elsewhere (e.g. Krebs and Davies, 1993; Slater, 1999). However conflict is almost inevitable under these conditions and a considerable body of research has been generated examining what factors (environmental and social) regulate this conflict and its resolution that acts to repair relationships and maintains cooperative, cohesive group life (for extensive review see e.g. Aureli and de Waal, 2000; Aureli et al., 2002; de Waal, 1989).

In contrast to studies of aggression and its resolution, stress in primates is a relatively new area of research. In man stress is perceived as a condition particularly associated with the modern way of life. The demonstration of tangible health consequences for stress sufferers e.g. heart conditions (Hamm et al., 1983; Kaplan et al., 1982; Kaplan et al., 1983), carcinogenesis (Forsen, 1991; Sapolsky and Donnelly, 1985; Shively et al., 2004), long-term mental illness (Sapolsky, 1996; Sapolsky, 2003) has increased research into primate models of stress examining causal factors as well as physiological and behavioural symptoms and consequences.

This article will review literature available on the relationship between stress and aggression in primates examining environmental, social and other influences on the interaction of the two phenomena. We begin this examination of stress and aggression firstly with an overview of each, before detailing key sources of their variation, important aspects of their interaction, and finally some of the most important investigations of primate responses to changes in the physical or social environment that may be expected to cause or alleviate stress or aggression.

The greater body of published work involves the study captive primates, primarily macaques (genus: *Macaca*), particularly the rhesus (*M. mulatta*), long-tailed (*M. fascicularis*) and pig-tailed (*M. nemestrina*) species, as large numbers of these are held in biomedical research facilities.

## 2. Stress

The earliest investigations of stress in animals concentrated on the examination of the response of laboratory animals to either novel or aversive stimuli, but in the 1960s it expanded to include studies of the impact of modern intensive farming techniques on livestock species (Perry, 1975). More recently stress research has become an important part of the assessment of the impact of husbandry practices, experimental protocols and environmental conditions on animals maintained in captivity for agricultural production, research, education, entertainment or companionship (Broom, 2001; Broom and Kirden, 2004; Moberg, 1985; Wolfensohn and Honess, 2005; Wolfensohn and Lloyd, 2003). Techniques validated for use in captivity are now increasingly being used to assess the effect of social, environmental and management variables on wild or free-ranging animals (e.g. Bonacic and Macdonald, 2003; Cavigelli, 1999; Creel et al., 1996; Goymann et al., 2001; Muller and Wrangham, 2004).

Much of stress research is founded on Selye, 1946 description of the General Adaptation Syndrome which identifies the three components of the stress response as being the alarm reaction, followed by the phases of resistance and exhaustion. The latter two stages (the acute stress response) form the focus of most experimental studies with the first component, the alarm reaction, being dominated by the HPA (hypothalamic pituitary adrenal) system and its influence on the diversion of resources away from non-critical functions, such as reproduction, to those enabling a return to homeostasis for the animal and in extreme situations enable its survival (Mendoza et al., 2000; Moberg, 1985; Moberg, 2000). It is beyond the scope of this review to detail the neuroendocrine pathways associated with the stress response and these may be found reviewed elsewhere (e.g. Dixson, 1998; Sapolsky, 1987; Warburton, 1990; and papers in Moberg and Mench, 2000).

### 2.1. Physiological and behavioural assessment of stress

An animal's biological responses to stressors may be directed through one or more of four channels: behavioural, autonomic, neuroendocrine and immunological (Moberg, 2000). Not all individuals respond in the same way (or through the same channel) to the same stimulus and different stimuli may provoke different responses by the same individual. It is therefore important when examining stress in animals or changes in stress levels due to modification of their environment, husbandry regime or research protocol that a combination of objective, quantifiable measures are used, including both behavioural and physiological (Crockett et al., 2000; Moberg, 2000; Rushen, 2000).

Significant problems surround stress research not least the search for a satisfactory definition of stress encompassing physiological, psychological and behavioural symptoms and consequences, accompanied by methodological problems associated with its detection and measurement. Moberg (1985) suggests that many of these problems stem from a tendency to use the word ‘stress’ to describe a wide range of states from environmental discomfort (e.g. high temperatures) to a range of psychological states from basic dissatisfaction with life through to emotional conflict. It is also clear that the condition of stress can manifest itself through a range of symptoms from the physiological (e.g. elevated heart rate, blood pressure and/or adrenal corticosteroids) to the behavioural (e.g. hair-pulling, self-harming, stereotypic pacing).

Although in humans questionnaires and interviews may be used to detect and investigate stress, traditionally, in animals, stress has been quantified via physiological parameters such as the measurement of adrenocortical response through the detection of cortisol levels either in the plasma, faeces, urine or saliva (Mendoza et al., 2000; Theorell, 2003) or monitoring autonomic responses such as changes in heart rate or blood pressure (Clarke et al., 1994; Line et al., 1989a, Line et al., 1989; Porges, 1985). Both routes of investigation have problems but benefit over questionnaires and interviews, not possible in animals anyway, by not being prone to exaggeration or denial (Theorell, 2003) although they still present their own interpretational problems.

Changes in heart rate and blood pressure can be measured using harness-mounted (e.g. Anzenberger et al., 1986) or implantable (e.g. Aureli et al., 1999; Boccia et al., 1995; Clarke et al., 1994; Line et al., 1989 a, b) telemetry devices. However, the implantation of these could compound and confound stress measures, as well as any scientific procedure which an animal may be undergoing. Major constraints exist on experimental design when using cortisol measures since faecal or urinary cortisol requires the accurate identification of the individual that has produced a given sample and cortisol levels are subject to natural circadian variation (Mendoza et al., 2000; Rose et al., 1971, 1978b; Sousa and Ziegler, 1998; Theorell, 2003) and considerable inter individual variation (Rose et al., 1978b). Cortisol is not alone; testosterone, serum levels of which, may also be measured in studies of reproduction or aggression is also subject to circadian cyclicity (coinciding with light changes) and individual variation such that the recommended way to determine a treatment effect on levels of these hormones is to use animals as their own control with repeated measures being compared against a baseline (Bernstein et al., 1974, Rose et al., 1978b).

A number of biological factors can modify cortisol levels including differences in species identity, population, genetics, individual temperament, social status, reproductive condition, developmental history, age, physical health, social environment and support, season and climate. Further problems exist in the interpretation of cortisol results beyond individual differences in response, with some non-stress stimuli (e.g. exercise) producing elevated levels and on the other hand some stress responses not resulting in cortisol increases (Moberg, 2000). The former is the result of cortisol's well-defined role in the production of energy in the liver from glycerol through gluconeogenesis and in muscle from glycerol derived from fatty acids released by lipolysis of adipose tissue (Devlin, 1992). This forms an important part of the stress response as energy release is central to a stress-induced 'fight or flight' response. There are a number of cases in the literature where this metabolic role of cortisol may be seen, or interpreted to confound raised cortisol levels as a direct response to primarily psychological stress. In wild populations negative correlations have been found between cortisol level and food availability or feeding effort (Cavigelli, 1999; Muller and Wrangham, 2004) and this relationship is associated with seasonally decreased food availability, the end of gestation and increased predation risk (Cavigelli, 1999). Therefore in these instances heightened cortisol may be more reflective of metabolic rather than psychological stress. Furthermore, the higher cortisol level in dominant rather than subordinate squirrel monkeys may reflect the energy cost of maintaining dominance status (Leshner and Candland, 1972). While high cortisol levels therefore may not indicate psychological stress equally low cortisol levels, typically associated with low stress, may be misleading; as chronically, rather than acutely, stressed primates are known to exhibit hypocorticolism (Mendoza et al., 2000).

Interpretation of cortisol measures may be further complicated by the subjects' response to stressful aspects of the sampling method (Boccia et al., 1995). Animals that are experienced and conditioned to capture and venipuncture may show insignificant cortisol increase in response to sampling (Rose et al., 1978b). However, it has been demonstrated across a range of primate species that techniques for immobilisation, restraint and sampling, that includes separation from social partners, can result in heightened levels of glucocorticoids that may mask the effect of any stressor under investigation. These techniques include darting (e.g. Sapolsky, 1982, 1986, 1987), confinement to transport cages (e.g. Line et al., 1987), handling or physical restraint (e.g. Brown et al., 1970; Crockett et al., 1993; Hayashi and Moberg, 1987; Manogue et al., 1975), chemical restraint (e.g. Crockett et al., 1993, 2000; Manogue et al., 1975) or combinations of restraint techniques (e.g. Coe et al., 1978, 1979; Steklis et al., 1986). Confounding effects may even be seen in animals trained to present a limb for sampling (Herndon et al., 1984). Therefore where levels of other hormones are the subject of investigation care should be taken to control for the fact that the elevation of stress, including cortisol increase, is implicated in effecting levels of other hormones (e.g. testosterone: Hayashi and Moberg, 1987; Sapolsky, 1982, 1983, 1986; luteinizing hormone: Hayashi and Moberg, 1987; Sapolsky, 1987).

In addition to the established effect of some sedatives, e.g. ketamine, in elevating cortisol levels (Gosselin et al., 1983; Puri et al., 1981), the duration of a stressful sedation and sampling procedure, e.g. for blood, may produce a cortisol response that varies between individuals, which, in male squirrel monkeys (*Saimiri sciureus*), was not found to be related social rank (Steklis et al., 1986). This is not however surprising, as the disorienting effect of the sedative/anaesthetic is the likely major stressor in the capture-sedation-sampling process and this would be a constant irrespective of the duration of the sampling and in addition any rank-related effect on cortisol and testosterone response may only be detected within the first 30 min following a stressor (Sapolsky, 1982, 1986).

The problems outlined above have contributed to an increasing dissatisfaction with the use of cortisol measures to assess stress, particularly where it is the sole measure employed. This combined with the continued search for a single, unequivocal and universal measure of stress has resulted in a plethora of biological measures available to the modern stress researcher including other hormonal measures (e.g. growth hormone: Brown et al., 1971; luteinizing hormone: Hayashi and Moberg, 1987; prolactin: Keverne et al., 1982; serotonin: Raleigh et al., 1984), neutrophil activity (Ellard et al., 2001), body weight (McLaren et al., 2004) and lymphocyte (e.g. Alberts et al., 1992) and differential cell counts (e.g. Dhabhar, 1998; Dhabhar et al., 1995).

Behavioural measures of stress are also not without interpretational difficulties, although there are a number of very distinctive, though in normal instances rare, behaviours associated with extreme levels of stress in primates (e.g. pacing stereotypies, rocking and self-injurious behaviours such as self-mutilation and hair-plucking). Many studies of stress and aggression have focused on specific abnormal behaviours that are unequivocally indicative of high levels of stress. However changes (proportional or qualitative) in the animal's overall behavioural repertoire, particularly in levels of aggressive and affiliative behaviours, may provide a valuable non-invasive indicator of stress (Honess et al., 2004). Disruption of the normal circadian pattern of activity and rest, revealing how well individuals are adapted to their environment (Rushen, 2000), may also indicate stress, for example raised heart rate is associated with disturbed sleep (Boccia et al., 1995).

Many factors in captivity are known to produce a measurable behavioural or physiological stress response in primates and some relating to restraint have been mentioned above. Among the others are: routine husbandry events (e.g. Brown et al., 1970; Clarke et al., 1994; Line et al., 1989a), the presence of care staff and anticipation of feeding (Line et al., 1989b; Waitt and Buchanan-Smith, 2001), intense sound and learning during aversive conditioning (Brown et al., 1971), predation threat (e.g. Cavigelli, 1999; Manogue et al., 1975; van Schaik and Mitrasetia, 1990; Vogt et al., 1981), resource scarcity (e.g. Cavigelli, 1999; Muller and Wrangham, 2004; Southwick, 1967), an unstimulating environment (e.g. Clarke and Mayeaux, 1992), solitary housing (e.g. Boccia et al., 1995; Crockett et al., 1994), cage (Anzenberger et al., 1986) and group composition changes (e.g. Alberts et al., 1992), and social dominance rank (e.g. Coe et al., 1979; Keverne et al., 1983, Sapolsky, 1982, 1987).

### 2.2. Consequences of stress

Research demonstrates that stress levels may cause changes in animals that affect a number of aspects of their biology (see contributions in Balm, 1999) but specifically including their immune competence (e.g. Braude et al., 1999; Coe et al., 2002; Maule and VanderKooi, 1999; Moberg, 1985; Raberg et al., 1998), coronary health (Hamm et al., 1983; Kaplan et al., 1982, 1983) and brain structure and function (Sapolsky, 1996, 2003; Uno et al., 1989).

Even brief subjection to a relatively mild stressor, such as a routine husbandry procedure, can cause either a disruption of critical biological events or a reallocation of an animal's resources away from other biological functions to coping with the resulting stress (Moberg, 2000; Rowell and Hinde, 1963). Stress may interfere with the hormonal pathways controlling the timing of maturation and ovulation and, particularly in seasonal breeders, may result in a female missing a reproductive opportunity (Ha et al., 2000; Moberg, 2000). More chronic levels of stress may cause such a high level of redistribution of resources that biological functions such as reproduction and growth are more permanently affected (e.g. Ha et al., 2000; Moberg, 2000). For example the advantage of dominance status in allowing preferential access to resources may result in more rapid return to reproductive condition, reduced infant mortality and earlier onset of breeding in offspring, but has stress-associated costs in gaining and maintaining this status such as an increased risk of miscarriage and, in some instances, reduced fertility (Paker et al., 1995).

Primates are particularly susceptible to psychological stress; being known to possess a cognitive sophistication second only to that of humans. They are particular prone to suffering stress and anxiety as not only are they affected by real stressors, but their stress response can also be initiated simply in anticipation of a stressful event that may, or may not, subsequently take place (Sapolsky, 1991, 2003) for example: during aversive conditioning (Brown et al., 1971), and in anticipation of intragroup aggression (Aureli et al., 1999; Baker and Aureli, 1997; Maestripieri, 1993).

## 3. Aggression

Since the earliest systematic studies of primate social behaviour researchers have been aware of the importance of aggression as a key element of primate social behaviour (Bernstein and Gordon, 1974; Deag, 1977; Hall, 1964). In a mid 20th Century world that had just suffered two world wars and was then in the Cold War under the threat of imminent nuclear destruction there was an intensified interest in efforts to explain and defuse man's aggressive behaviour and one route was to use ethological approaches to explore aggression in man as well as to seek evolutionary/adaptive insights through the study of this behaviour in animals particularly primates (Tinbergen, 1968). The validity of using an ethological approach and animal models for the study of human aggression is now well established (Kalin, 1999; Lorenz, 1966; Ramirez, 2000; Symons, 1978; Tinbergen, 1968; Wilson and Wrangham, 2003).

Unlike stress research, which has almost exclusively been conducted in captive primates due to the requirement for measurement of physiological parameters, aggression research has been conducted in a variety of settings from wild/free-ranging through to laboratories. However studies of wild primates that measure physiological parameters as well as the more usual behavioural ones remain rare (e.g. Barret et al., 2002; Cavigelli, 1999; Muller and Wrangham, 2003, 2004; Sapolsky, 1982, 1983, 1986, 1987, 1991). A focus of laboratory-based studies has been the examination of changes in the incidence and pattern of aggressive behaviours resulting from the manipulation of the physical, social or hormonal environment whereas field studies have concentrated on the influence of seasonal changes, resource distribution, age/sex parameters and social status on aggression.

Aggressive behaviour occurs in a number of contexts in primates: Intergroup resource defence, antipredator behaviour, predation, and intragroup social contexts such as dominance contests (for food, mates, status, etc.) and reproduction as well as pathological self-directed aggressive behaviour such as self-injurous behaviours (SIBs). For the purposes of this article we will concentrate on intragroup displays of aggression and the literature that examines the relationship between these and stress.

A number of important reviews have been written on the function of aggression in primate societies (e.g. Bernstein, 1976; Bernstein and Gordon, 1974; Deag, 1977; de Waal, 1989; Hall, 1964). They examine the role of aggression in the protection of the group and its resources from both predators and competing groups of conspecifics and in maintaining social order. Some (e.g. Bercovitch et al., 1987; Bernstein and Gordon, 1974; de Waal, 1989) examine the apparent contrary functions of aggression in producing cohesive and repulsive forces within a social group. However, Bernstein and Gordon (1974) hint at a group-selection approach which appears to struggle with the existence of potentially damaging levels of intragroup aggression that may threaten the survival of the group. More recent work (e.g. Carr and Macdonald, 1986; Lee, 1994; Wrangham, 1980) would point to the pattern of intra- and intergroup aggression as being a sum of selfish resource acquisition and defence overlaid with patterns of kin selection as well as individual and species-specific reproductive strategies. It is now widely accepted that intragroup aggression is moderated by taking place in the context of an hierarchical dominance structure of differential status and moderated by social rules and furthermore that these hierarchies are widespread in primate societies (Deag, 1977) and not simply an artefact of stress characterising captive primates (e.g. Rowell, 1967).

Whilst intrasexual aggression, particularly associated with reproductive competition, as highlighted above is reasonably well understood, the existence of intersexual aggression, particularly associated with reproduction, seems especially perplexing as it might be thought that the requirement for physical proximity would select against repulsive behaviour. However, Eaton et al. (1981) suggest that male on female aggression, particularly during the mating season, may be part of a male reproductive strategy related to intersexual selection, 'through intensifying the social bonds necessary to achieve reproductive success' (pg. 61). As a precedent that aggression can intensify bonds they cite Sackett et al.'s (1967) findings' that rhesus infants raised by abusive mothers showed stronger attachment to their mothers than those raised by normal mothers. Barret et al., (2002) also suggest that female directed contact and non-contact aggression by male Japanese macaques during the mating season form part of the male reproductive strategy and represent sexual coercion and solicitation respectively. However, Bercovitch et al. (1987) dismiss the role of intersexual aggression as a male reproductive strategy noting that in captive rhesus macaques it is rarely followed by copulation, instead they conclude it represents an individual spacing and dominance assertion mechanism. However the existence of intersexual aggression may contribute to patterns of stress and aggression that vary with breeding season and between mixed and single-sexed groups discussed later.

### 3.1. Aggression and dominance behaviour

A frequent problem encountered when trying to compare studies of primate aggression is the oversight of many authors in not providing a clear definition of the term 'aggression'. Whilst some restrict its use to describe physical attack, others include aspects of more mild threatening behaviour which is a more common form of the communication and maintenance of dominance status (e.g. Deag, 1977; Southwick, 1967; Stavisky et al., 2001). It is, in fact, clear that there are different types of aggression characterised by differing contexts as well as different neural and hormonal activity. Hence it can be valuable to broadly divide aggressive states into those that are defensive (e.g. responsive, threatening gestures such as the lower jaw drop of rhesus macaques) and those that are offensive (e.g. spontaneous attack of one adult male by another). The former being characterised by increased activity in the right frontal area of the brain and high plasma cortisol levels (Kalin, 1999), and the latter by elevated plasma testosterone (Kalin, 1999; Rose et al., 1974; Sapolsky, 1983; Steklis et al., 1986), low serotonin levels in cerebrospinal fluid and high plasma cortisol levels (Kalin, 1999; Steklis et al., 1986).

Dixson (1980) points out there is also little consistency in the way that 'dominance' is defined or measured, although a considerable body of literature exist that clarifies the concept and presents methods for its assessment (e.g. Boyd and Silk, 1983; Martin and Bateson, 1993; Richards, 1974). Mazur (1976) in distinguishing between aggressive and dominance behaviour is clear that dominance behaviour may be, but is not necessarily, aggressive and equally aggressive behaviour need not be related to dominance. Nevertheless in some instances dominance status may be determined by analysing the frequency and direction of either subtle behaviours such as simple threats, avoidance and displacement or using less subtle behaviours such as contact aggression, or a combination of the two. However, dominance hierarchies constructed using different intensities of aggressive behaviours in the same animals (e.g. the use of presenting displays versus feeding priority: Green et al., 1972) may not be the same and there is therefore a danger in using relative dominance status as an index of aggression (Dixson, 1980). Furthermore Bernstein et al. (1983) point out that the concept of a social rank number may be simply be a human construct having no meaning to the animals; an 'alpha' male simply submits to no one in the group. Equally, the lowest-ranked animal may, whilst obeying social rules, very rarely be attacked by more dominant group-mates and may be well tolerated by the most dominant as it (similar to infants and juveniles) is not perceived as a threat.

### 3.2. Aggression and testosterone

The role of hormones in causing or facilitating aggressive behaviour has been studied across a range of animal species (Leshner, 1975). Of great importance in the study of primate aggression has been the realisation of the importance of reproductive hormones. Several excellent reviews exist of the role of androgens in primate aggression and sexual behaviour, most notably Bercovitch and Zeigler (1990, 2002), Dixson (1980, 1998), Kling (1975), Michael and Zumpe (1993) and Rose et al. (1974), and therefore we shall only present an overview of this area as background to investigation of the mechanisms and expression primate aggressive behaviour.

It is difficult to separate the impact of testosterone on sexual and aggressive behaviour as both are closely linked to brain systems androgens are known to effect (Kling, 1975).

It has become increasingly clear that the relationship of testosterone to these aspects of an animal's life is extremely complex and it can prove almost impossible to discern a generalised pattern across primate species (Bercovitch and Zeigler, 2002; Dixson, 1980, 1998). There appears to be a phylogenetic pattern in how tightly all aspects of aggressive and reproductive behaviour are controlled by androgens, with a clear-cut effect in 'lower' animal orders becoming less simple in primates, where complex behavioural repertoires, behavioural plasticity, and a high dependence on learned behaviour exist and the emotional response to hormones is variable (Dixson, 1980; Klopper, 1964).

Mazur (1976) proposes a seemingly contradictory model for the complex interaction of testosterone, dominance and aggression in males. In this model testosterone levels are related to activities concerned with achieving or maintaining status though not directly related to status or aggressiveness with dominance activity being facilitated by an increase, and inhibited by a decrease, in testosterone. However, he further proposes that an increase in dominance activity will trigger an increase in testosterone thereby facilitating yet further dominance behaviour and, conversely, a decrease in dominance activity will reduce testosterone levels, thereby inhibiting further dominance behaviour.

Despite the absence of evidence for any clear causal relationship between testosterone and aggressive behaviour in primates, nevertheless many studies have examined the relationship between the two in a range of developmental and social contexts and are able to demonstrate the importance of testosterone in priming this behaviour. Some of the key findings relating to the role of androgens in primate aggression and sexual behaviour help to elucidate the important points in an animal's life at which changes in testosterone levels, naturally or experimentally produce physiological, anatomical or behaviour changes that differentiate the sexes or influence rank status.

Sexual dimorphism in the development of behaviour not only informs us about the ontology of aggressive behaviour and its link with sexual variation in hormone levels but also about general intersexual differences in the intensity and nature of aggressive behaviour. Dimorphism in the timing of the appearance of aggression has been demonstrated in experiments that controlled for learnt behaviour by removal from adult group members. Under these conditions more sexual, threat and play-fighting behaviour (e.g. Goy and Phoenix, 1972; Harlow, 1965) and independence from and intolerance of mothers (Jensen et al., 1966) is developed sooner in male infants than females. As Dixson (1980) and Rose et al. (1978) point out this is not necessarily indicative of what may happen under more naturalistic conditions, where more sexual, threat and play behaviour has been noted in male Japanese macaque (*Macaca fuscata*) infants and more grooming in females (a difference that later disappeared) (Eaton et al., 1990), but no significant differences in the onset or frequency of play behaviour in male and female infant rhesus (Hinde and Spencer-Booth, 1967).

It has proved possible to influence these patterns of development by making changes in the developing animal's hormonal environment. Artificially boosting testosterone levels in females pregnant with genetically female foetuses results in the production of pseudohermaphrodite daughters which exhibit threat and play behaviour levels intermediate between males and females and sexual behaviour more similar to that of males as well as masculinized genitalia (Young et al., 1964) and both of the latter may persist into adulthood (Eaton et al., 1973).

Injecting testosterone proprionate into intact female rhesus infants produced long-lasting increases in aggressive and dominance behaviour causing reversal of dominance over males, although play and sexual behaviour were not masculinized (Joslyn, 1973). These females doubled the weight gain of males and control females and were, at the end of the hormone treatment, becoming on average 28% heavier than the males, an increase, which in the absence of adults to enforce inherited rank (Hinde, 1983) may have been a confounding factor in assessing the relationship between testosterone, aggressive behaviour and dominance. A similar elevation in dominance behaviour towards males can be seen in ovariectomised adult females treated with testosterone (Trimble and Herbert, 1968).

Altering testosterone levels in males has also been shown to influence behaviour. Castrated common marmosets (*Callithrix jacchus*) display aggression towards females but not to intact males, but when testosterone levels were raised to those of intact males they are more aggressive towards males than females (Dixson, 1993). The same procedure in male talapoin monkeys resulted in more aggression towards subordinate males but not towards more dominant males or females (Dixson and Herbert, 1977). Green et al. (1972) found that in similarly treated squirrel monkeys' behaviours changed but rank did not and that changes in the order of feeding were simply a result of hormone-dependent changes in appetite.

Wilson and Vessey (1968) observed that eight of ten castrated rhesus macaques reintroduced to their natal groups associated preferentially with other castrates and none became high ranking. Although one continued to copulate for seven years after castration, the subjects displayed less sexual behaviour than intact males.

## 4. Sources of variation in stress and aggression

From the literature it is clear that patterns of stress (response and baseline levels) as well as aggression vary with a number of aspects including taxonomic identity, individual status and characteristics, life history stage, and environment. Examples of many of these sources of variation are examined below as their investigation is important for the accurate interpretation of data in studies of both stress and aggression.

### 4.1. Species

Whilst it is unsurprising that very different species may exhibit markedly different stress responses, for example the different responses of titi (*Callicebus moloch*) and squirrel monkeys to chronic social stress (Mendoza et al., 2000), and the acute stress of stranger confrontation (Anzenberger et al., 1986), even closely related species and subspecies may exhibit different responses. Differences in stress-related heart rate changes in response to both cage change and restraint have been found in three macaque species; in order of decreasing response from long-tailed macaques to bonnet macaques (*M. radiata*) and the least response in rhesus macaques (Clarke et al., 1994). Long-tailed macaques also show a greater stress response than pig-tailed macaques (husbandry practices: Crockett et al., 2000), which in turn show a greater response than bonnet macaques (maternal separation and water test: Boccia et al., 1995).

It should be noted that basal levels of physiological stress indicators may vary considerably between species. A number of species characteristics which affect an animal’s energy budget and opportunity for social stress (e.g. such as diet, foraging style, home range size, social proximity and life history parameters) have been shown to influence HPA activity and behaviour and therefore to account for genus and species level differences in baseline stress physiology (Smith and McCallister, 2005). In additional to relative differences between species some, such as squirrel monkeys, naturally have unusual high basal plasma cortisol (Anzenberger et al., 1986; Brown et al., 1970; Coe et al., 1978; Coe et al., 1979) and blood serotonin (Steklis et al., 1986) levels.

While there can be clear differences in the way that different species respond to stressors, indicating differences in temperament, these differences extends to displays of aggression, with different species demonstrating differing levels of overt aggression in intragroup contexts. For example de Waal and Johanowicz (1993) note a difference between rhesus and stump-tailed (*M. arctoides*) macaques in the aggressiveness with which conflicts are resolved and demonstrate that, when they are co-housed, it is possible for the more aggressive and less reconciliatory rhesus to learn less aggressive resolution techniques from the stump-tailed macaques.

Changes in hormone levels may have different consequences for different species due to their variation in sensitivity to changes; therefore a greater magnitude of change or higher threshold level may be required to produce a behavioural or physiological change in different species. For example the threshold level of exogenous testosterone needed to increase ejaculatory activity is eight times higher in long-tailed than rhesus macaques (Zumpe and Michael, 1985). Nevertheless there were no significant effects of these very high testosterone levels on agonistic or other social behaviour apart from grooming which increased in females and decreased in males. This higher sensitivity to testosterone relates to the rhesus macaque being a seasonal breeder, unlike the long-tailed macaque, and experiencing testicular regression during the non-breeding season (Herndon et al., 1996).

### 4.2. Sex

There is only limited evidence of a sex-dependent stress response in primates, however an examination of the stress response of squirrel monkeys to capture and ether anaesthesia found a lower cortisol (as well as testosterone and ACTH) response in females than males (Coe et al., 1978) and further that high levels of cortisol were not accompanied by raised ACTH levels, not supporting the hypothesis of Brown et al. (1971) that high cortisol levels are due to raised ACTH.

As Reinhardt (1987) points out it is commonly held that male primates are more aggressive than females. Whilst this appears true for some macaque species, such as the Japanese macaque (Alexander and Roth, 1971; Eaton et al., 1981), this observation is not supported by work on captive rhesus macaques in which aggressiveness is considered more of an individual trait than a sex- or rank- dependent one (Reinhardt, 1987) or where males actually show a greater tendency towards restorative behaviour following aggressive food competition events than females (de Waal, 1984). It has however been found that male rhesus are more likely to exhibit self-directed aggressive behaviour in the form of SIBs (Lutz et al., 2003b). Michael (1998) found that female pair-housed common marmosets were consistently more aggressive than their male mates in food acquisition trials.

### 4.3. Age

Evidence of age-dependent stress responses in primates is limited although a higher mortality among older animals following the translocation of a breeding colony (Ha et al., 2000) and reports of typically high basal cortisol levels and slower poststress recovery in neonatal common marmosets (Pryce et al., 2002a). However, there is more evidence of age-related effect on patterns relating to reproductive maturation and age-related dominance. In gibbons (*Hylobates spp*.) maturing males suffer an increase in the level of aggression from their fathers due to their increase in testosterone levels, which may function to encourage them to leave the family group (Carpenter, 1940). The movement of males between breeding groups in other primates is also known to predominantly take place during times of heightened aggression such as the breeding season (Altmann, 1962; Wilson and Boelkins, 1970). In Japanese macaques male rank, and therefore the initiation or receipt of aggression, appears to a large extent to be a product of the animal’s age (Johnson et al., 1982). Age-related secondary sexual characteristics are associated with higher testosterone levels in dominant males across a range of species (e.g. mandrill: Dixson, 1998; orang utan: Kingsley, 1982) and the development of these may or may not be correlated with increases in dominance behaviour towards subordinates.

### 4.4. Reproductive status

It has long been established that a wide range of primate species are seasonal breeders (Dixson, 1998). This is particularly true among those whose wild habitat lacks a circum annual distribution of sufficient resources necessary for breeding, particularly the lactational transfer of energy from mother to infant. Reproductive status may therefore change on a seasonal or year round basis and may have a substantial effect on social and physiological aspects of an animal's life. It may also influence basal cortisol measures and there is a well established and predictable association between female reproductive status and cortisol in many primates with levels peaking during the peri-ovulatory phase and being at their lowest during mid-luteal phases (e.g. Saltzman et al., 1998; Ziegler, 1995). Furthermore, heightened levels have been reported in pregnant females in squirrel monkeys (Vogt et al., 1981), cotton-top tamarins (*Saguinus oedipus*: Ziegler et al., 1995) and ringtailed lemurs (*Lemur catta*: Cavigelli, 1999), though not in rhesus macaques (Challis et al., 1975). However heightened cortisol at a time of increased metabolic demand might not be surprising considering its metabolic role. While stress has been implicated in the delay of maturation in female rhesus macaques, the possibility that raised cortisol levels might do the same in males through suppression of reproductive hormones such as luteinizing hormone and thereby testosterone has been discounted (Bercovitch and Clarke, 1995).

A number of changes appear to take place in the sexually mature male in the breeding season and these changes may be accompanied by a significant increase in aggression. An increase in aggressive behaviour during the mating season has been reported across a range of species in free-ranging or semi free-ranging populations (rhesus macaques: Wilson and Boelkins, 1970; Japanese macaques: Eaton et al., 1981; Enemoto, 1981; Johnson et al., 1982; Rostal et al., 1986; squirrel monkeys: Baldwin, 1968; ringtailed lemurs: Cavigelli and Pereira, 2000) although this change was not always found by those studying captive animals (e.g. Mendoza et al., 1978) and the converse is reported for the brown lemur (*Eulemur fulvus*), found to be more aggressive during the breeding season in semi-free-ranging conditions than in the wild (Kaufmann, 1996). These heightened seasonal levels of aggression may also be associated with an increase in testosterone levels in the breeding season (rhesus macaques: Gordon et al., 1976; Herndon et al., 1996; Japanese macaques: Rostal et al., 1986) and have been demonstrated to exist even under controlled environmental conditions (Michael and Zumpe, 1978). Some species, however, show no seasonal fluctuation in aggressive behaviour or testosterone (e.g. stump-tailed macaques: Nieuwenhuijsen et al., 1987).

This pattern of seasonal fluctuation of testosterone is established in male rhesus macaques by four years of age old (although timing may be influenced by the number of adult males present) but, at this stage, is not accompanied by any increase in aggressive behaviour (Rose et al., 1978a). While some (Gordon et al., 1976; Keverne et al., 1978) make a case for these seasonal increases resulting from the presence of receptive females others present contrary evidence (Herndon et al., 1996). Further evidence suggests that this seasonal pattern may actually result from increased intrasexual competition for mates (Cavigelli and Pereira, 2000; Michael and Zumpe, 1978; Muller and Wrangham, 2003; Rose et al., 1978b). This evidence would tend to support the challenge hypothesis (Wingfield et al., 1990) which suggests that testosterone levels rise as a result of increases in competitive interactions, particularly in unstable social contexts, associated with reproduction rather than changes in reproductive physiology.

Seasonal changes in aggression are not limited to males. Higher levels of female aggression are reported during the birth season in both Japanese (Eaton et al., 1981) and rhesus (Wilson and Boelkins, 1970) macaques, though in the latter the increase was not statistically significant. Male directed aggression may be higher than expected out of the breeding season when females are unreceptive, and is associated with the rejection of male mounting attempts (Michael and Zumpe, 1970). Males are less aggressive and more tolerant of increased female aggression during pregnancy and of female presence during consortship.

Heightened sociosexual behaviour around mating time has caused some species to develop strategies to enable the reduction of other parts of their behavioural repertoire to accommodate this. In two notable species high-ranking males accumulate fat deposits prior to the mating season; as a result they can reduce feeding time during the mating season, drawing resources from their fat deposits, and focus on reproductive behaviour (rhesus macaques: Bercovitch, 1992, 1997; squirrel monkeys: Coe et al., 1985). This increase in reproductive behaviour at the expense of feeding time is also seen in males of other species (e.g. Japanese macaques: Barret et al., 2002). While, during the mating season, these males have elevated levels of cortisol, testosterone and oestrogen, care should be taken in interpreting these heightened cortisol levels solely as an indication of reproductive stress when it may in fact reflect the role of cortisol in energy mobilisation as described earlier in this article. This strategy may still carry consequences in terms of immunosuppression that may shorten lifespan in the same way as if these cortisol levels resulted from psychosocial stress (Bercovitch and Ziegler, 2002). This evidence also appears to counter that of various authors who note an inverse relationship between testosterone and cortisol levels (see below); implying that stress may have a suppressive effect on reproduction (e.g. O'Byrne et al, 1988; Paker et al., 1995).

### *4.5. Social status and individual temperament*

A number of authors have demonstrated that personality features including aggressive behaviour are individual characteristics (e.g. Capitanio, 1999; Clarke and Boinski, 1995; Maestripieri, 2000; Reinhardt, 1987) and that these may vary with age, sex, social status and social group composition and stability (McGuire et al., 1994). Baseline stress levels and the magnitude of the stress response can also vary with individual characteristics such as temperament (e.g. Clarke and Boinski, 1995; Maestripieri, 2000) and social status (Bercovitch and Clarke, 1995; Clarke and Boinski, 1995; Coe et al., 1979; Abbott et al., 2003; Creel et al., 1996; Sapolsky, 1982, 1983, 1986, 1987, 1993). Clarke and Boinski (1995) review the factors that may influence an animal's temperament, for example in its reactivity, whether it is confident/fearful, active/slow or social/solitary, including species identity, population of origin, rearing history, housing condition. The influence of these and other factors produces an almost infinite variation among individuals the manifestation of which in terms of stress and aggression are beyond the scope of this article to cover. However, Clarke and Boinski (1995) do importantly indicate trends at different levels from the species down to the individual and they point to the role of temperament in predisposing an animal to higher or lower dominance status and the way in which, for example those exhibiting a lower-key behavioural response to stressors seem better able to distinguish between levels of stressors and maintain a lower baseline cortisol level.

Goymann and Wingfield (2004) propose that allostatic load predicts relative glucocorticoid levels in dominant and subordinate animals; a higher allostatic load in dominant animals will correspond to higher glucocorticoid levels and vice versa, and that this is influenced by a number of factors including reproductive and foraging strategies and the social and ecological environment. Abbott et al. (2003) have identified precise situations that predict when subordinate primates will exhibit higher basal cortisol levels than dominant animals, i.e. with higher rates of stressors and decreased social support. Indeed many studies indicate that dominance is generally associated with less variable (Bercovitch and Clarke, 1995) or lower (e.g. baboons: Sapolsky, 1982, 1983, 1987; squirrel monkeys: Coe et al., 1979, 1983; Mendoza et al., 1979; Steklis et al., 1986; talapoins: Keverne et al., 1982) stress hormone levels, although for some species the converse, or no relationship at all, has been shown (e.g. long-tailed macaques: Stavisky et al., 2001; ringtailed lemur: Cavigelli, 1999; Japanese macaques: Barret et al., 2002; male rhesus macaques: Bercovitch and Clarke, 1995; chimpanzees: Muller and Wrangham, 2004) and for some species there is contradictory evidence. In squirrel monkeys, Manogue et al. (1975) found low cortisol was related with dominance, contrary to the evidence of other investigators (Coe et al., 1979, 1983; Mendoza et al., 1979; Steklis et al., 1986), but this may be explained by differences in housing and the presence of other stressors, such as crowding or living in large groups (Coe et al., 1983). Similarly, the finding of a positive correlation between cortisol and dominance rank during the mating season in wild male Japanese macaques (Barret et al., 2002) is contrary to the findings for a number of species in captivity. This is attributed to an elevation of stress in captive subordinates that cannot avoid the stressful, aggressive behaviour of more dominant animals when, in the wild, they tend to occupy a position peripheral to the group. There may also be an effect related to rank-related mating strategies: the higher stress of dominant's consort versus a subordinate's 'sneaking' mating (Virgin and Sapolsky, 1997).

Dominant males have also been shown to exhibit a smaller stress response than subordinates in some instances (e.g. capture or restraint: Coe et al., 1979) and a higher stress response in other (e.g. during group formation: Mendoza et al., 1979; predator threat, anaesthesia and restraint: Manogue et al., 1975; darting: Sapolsky, 1982; Sapolsky, 1983). Under some conditions, particularly social instability, any relationship between status and stress may be lost or reversed (e.g. squirrel monkeys: Mendoza et al., 1979; long-tailed macaques: Kaplan et al., 1982; Sapolsky, 1983; Talapoins: Keverne et al., 1982; rhesus macaques: Gust et al., 1993).

A major factor contributing to levels of aggression exhibited in a social group is the stability of its dominance hierarchy (Altmann, 1962). Instability itself may be the result of a number of events including environmental disruption, maturation, immigration or emigration of individuals, and increased competition for scarce resources. An animal's success in aggressive encounters has measurable outcomes seen in patterns of aggressive behaviour as well as hormonal profile. Failure in conflict leads to a drop in testosterone (e.g. rhesus macaques: Bernstein et al., 1983; Rose et al., 1972, 1975; pig-tailed macaques: Bernstein et al., 1979) which may be adaptive; reducing the likelihood of further defensive aggression that could result in a more injurious attack (Mazur, 1976; Rose et al., 1972). This is confirmed by the others whose work indicates an inverse relationship between testosterone and cortisol (e.g. Barret et al., 2002; Hayashi and Moberg, 1987; Keverne et al., 1983, Sapolsky, 1983), particularly with cortisol increases in response to a stressor (e.g. Keverne et al., 1983; Sapolsky, 1983, 1987). However Bercovitch and Clarke (1995) found no negative correlation between these hormones in rhesus macaques and Sapolsky, 1986, 1987) points out that dominant male baboons react hormonally under stress quite differently to subordinates. Following darting, sedation and a resulting cortisol elevation, he found that dominant males produced an initial surge in testosterone during the first hour compared to a fall in subordinate males.

This post-stressor testosterone boost in dominant males is possible despite the typically inverse relationship between testosterone and cortisol as their testes are less sensitive to the suppressive effect of glucocorticoids and produce this brief increase via the sympathetic nervous system (Sapolsky, 1987). Simultaneously high testosterone and cortisol levels also occur in dominant male squirrel monkeys (Coe et al., 1983).

Other physiological parameters also vary with dominance: In male vervet monkeys (*Cercopithecus aethiops*) there is a positive correlation between dominance and blood serotonin and isolation of dominant animals reduces serotonin levels to those of subordinates, indicating that high levels are maintained through active occupation of most dominant rank (Raleigh et al., 1984). It is suggested that serotonin may provide a better indicator of social tension than either cortisol or testosterone as it is not subject to circadian or annual cycles. Indeed, Steklis et al. (1985) found no correlation between rank and testosterone in the same species, although higher testosterone levels in dominant males did correlate with aggression, indicating again the dissociation between some measures of dominance and aggression discussed earlier.

Many of the complexities in associating social status, testosterone and aggression have come to light during efforts to determine the effect of social and environmental variables on testosterone levels, particularly in males and this is typified by the important series of experiments of Bernstein, Gordon, Rose and colleagues (Bernstein et al., 1974, 1983; Gordon et al., 1976; Rose et al., 1971, 1972, 1975, 1978, 1978b) summarised by Bernstein et al. (1983). By manipulating the social, physical and hormonal environment, primarily of rhesus macaques, and monitoring behavioural and hormonal responses they examined a range of influences on testosterone levels and determine relationships between rank and testosterone (Rose et al., 1971), between victory and briefly raised testosterone (Bernstein et al., 1974, 1983; Rose et al., 1971), defeat/submission and extended, lowered testosterone and sexual dominance or mating behaviour (Bernstein and Gordon, 1974; Rose et al., 1972). The authors themselves admit that the apparent relationship between rank and testosterone in males found by Rose et al. (1971) may simply have reflected the distribution of success and failure in dominance contests during the group's establishment as this relationship was not found in the same animals six months later (Bernstein et al., 1983).

Further investigation showed that changes in an animal's rank without aggressive interaction, caused by the removal and later reintroduction of higher ranking males, produced the anticipated changes in testosterone level indicative of a positive relationship between testosterone and rank (Bernstein et al., 1974). However in rhesus males surges in testosterone brought about either through natural changes at around 3 years of age (Rose et al., 1978a) or artificially using human chorionic gonadotropin (Bernstein et al., 1974; Gordon et al., 1979) produced no observable increase in aggressive behaviour but the subjects in the latter case were found to attract more aggression from other males which perhaps suggests a more discrete response than was being measured; possibly even an olfactory one. Gordon et al. (1979) later noted that artificially boosted testosterone seemed to have an effect on a range of social behaviours similar to that seen during breeding season changes amounting to a general intensification of existing social relationships. The only animal that showed a post-treatment increase in aggressive behaviour was one with a pre-existing high level of aggression. In extending their studies to other species these researchers were suspicious that low testosterone levels recorded for some (e.g. mandrill, *Mandrillus sphinx*, and Celebes macaques, *M. nigra*) were an artefact of the use of chemical restraint not employed in their previous studies of rhesus macaques (Bernstein et al., 1974).

Cross-species patterns of the relationship between testosterone levels and aggression remain unclear. So while Sapolsky, 1982, 1987) found that testosterone levels correlated with aggression in male olive baboons, no correlation was found with aggression or dominance in bonobos (*Pan paniscus*: Sannen et al., 2004), rhesus (Bercovitch, 1993; Gordon et al., 1976; Turner et al., 1989), stump-tailed (Nieuwenhuijsen et al., 1987) or Japanese macaques (Eaton and Resko, 1974).

In the same species, under different conditions, results may appear contradictory and a relationship between testosterone and aggression, under certain conditions, may only be found if different aspects of aggressive behaviour are examined. For example in wild male Japanese macaques during the mating season, where neither aggressive nor copulatory behaviour correlated with dominance, non-contact aggression towards females was found to correlate with testosterone levels (Barret et al., 2002). In the same species under both laboratory and semi-free ranging conditions, where testosterone did not, aggression did correlate positively with dominance (Eaton and Resko, 1974). Although in the latter case sampling-related stress is claimed not to confound testosterone measures in either housing condition as no sampling-related drop in testosterone was evident in the laboratory, this overlooks that the response of the laboratory animals, that may be more habituated to people and possibly to handling but potentially more chronically stressed by single housing, is likely to be quite different from group-living, corral-housed animals.

## 4.6. Background

Specific aspects of an individual's background are known to influence its stress response, including its source (wild-caught versus captive-bred) (Crockett et al., 2000; Ha et al., 2000; Paulk et al., 1977) and its rearing conditions (Boccia et al., 1995; Dettling et al., 2002; Moberg, 1985; Pryce et al., 2002b; Sackett et al., 1973; Shannon et al., 1998), whether suffering social separation (e.g. McKinney, 1974; Mendoza et al., 2000) and whether housed socially or not. The company of appropriate conspecifics has been shown to have a buffering effect on the cortisol response of squirrel monkeys presented with a snake (Vogt et al., 1981) and on the behavioural response of rhesus macaques (Rowell and Hinde, 1963) and cortisol response of female black tufted-ear marmosets (*Callithrix kuhli*) (Smith and French, 1997) to social stressors. Stressful maternal separation experiments may lead to psychological and behavioural problems that persist into adulthood, where effected animals may play less and have fewer, less complex relationships (Boccia et al., 1995). When presented with a novel stimulus these individuals exhibited the same cortisol levels as but more self-directed and stereotypic behaviour than normally reared individuals.

Apart from the consequences relating to stress and the development of inadequate and pathological behaviours, isolation rearing can affect hormonal responses to social stimulation. For example, male rhesus macaques raised in isolation do not experience the same testosterone increase as socially raised males when introduced to females (Rose et al., 1972).

### *4.7. Learning/habituation*

Learning through habituation can change the response to a stressful stimulus and may result in a drop in physiological indicators of stress, as seen in squirrel monkeys that learnt to bar-press to avoid a shock (Brown et al., 1971). Also habituation to restraint and blood sampling over a 40 day period is indicated in previously naïve Japanese macaques by a negative correlation between time and faecal cortisol levels (Barret et al., 2002). Further evidence of physiological habituation to stress can be seen in rhesus macaques, experienced with capture and blood sampling, which experience a stress response, reflected in cortisol levels, insufficient to cause a significant reduction in testosterone levels (Rose et al., 1978).

Experience with stressful procedures may lead to animals adopting strategies to reduce their exposure to stress. There is evidence that among animals exposed to a number of procedures over time the order in which they self-select when being removed for testing reflects their age rather than dominance (Boccia et al., 1995). The older ones had more experience of experimental protocols were their more rapid self-selection is reinforced by the understanding that this results in being returned to their home cage and cage-mates sooner and that lengthening the process causes them more stress, illustrated by a positive relationship between cortisol and the time taken to get the blood sample. It has also been demonstrated that using positive reinforcement training techniques to encourage primates to cooperate with research and husbandry procedures can reduce their physiological stress response to these activities (e.g. Bassett et al., 2003; Reinhardt, 2003).

### *4.8. Resource distribution*

The distribution of food in provisioned groups of primates can influence levels of behaviour with dispersed food resulting in less aggressive behaviour than that provided in clumps (Boccia et al., 1995; de Waal, 1984; Gore, 1993; Southwick et al., 1976). While creating a 25% food surplus in a captive group of rhesus macaques produced a non significant reduction in aggressive interactions; a five-day 50% food shortage did produce a significant decrease in aggression (Southwick, 1967). This decrease in response to shortage was associated with a reduction in other behaviours but an increase in investigative behaviour. Animals of low social status, and therefore access to resources, suffered most with lethargy similar to that seen in starving humans.

Enrichment, be it social, behavioural or environmental can have an effect on aggression levels (for a review see Honess and Marin, 2005). An example is that the provision of cover can allow animals to remove themselves from socially tense situations and to hide from a pursuing aggressor; however the effective of this provision in diffusing aggression may vary between stable and unstable social groups (e.g. Erwin et al., 1976).

## **5. Interaction of stress and aggression**

Any direct relationship between stress, or its indicators, and aggression remains as clouded as that between androgens and aggression or dominance. A number of the sources of variation described above and variation in the classification of aggressive behaviours frequently confound efforts to elucidate clear patterns of cause and effect. We will highlight some of the results, where both stress and aggression are assessed, that are behind our current understanding of their interaction. We will conclude by reviewing a range of studies that examine social and environmental influences on stress and aggression.

One association in which there is some clarity is that between stress hormone levels (particularly glucocorticoids) and either levels of, or the receptiveness to, androgens (Barret et al., 2002; Dixson, 1998; Keverne et al., 1978; Sapolsky, 1987) and as such it has become more common for studies of either stress or aggression to measure levels of both androgens and stress hormones (e.g. Barret et al., 2002; Coe et al., 1983; Kalin, 1999; Keverne et al., 1978; Mendoza et al., 1979; Rose et al., 1978b; Sapolsky, 1982). In the context of this now established relationship it is also common to measure just behaviour, for example using levels of aggression as an index of stress. This is often the case in studies attempting to treat behavioural pathologies (inappropriate levels or direction of aggressive behaviour may be classed as such) in captivity or to adjust behavioural repertoires to more closely resemble those of their wild conspecifics (Honess and Marin, 2005).

Certain self-directed behaviours such as autogrooming, scratching, body-shaking and yawning have often been interpreted as displacement behaviours indicating an animal's frustration and even state of stress arising from aspects of social tension (e.g. Aureli and van Schaik, 1991; Baker and Aureli, 1997; Das et al., 1998; Kutsukake, 2003; Kutsukake and Castle, 2001; Maestripieri, 2000; Maestripieri et al., 1992; Manson and Perry, 2000; Rowell and Hinde, 1963), whereas behaviour such as allogrooming, that may be part of reconciliation strategies, may act to reduce stress (e.g. Aureli et al., 1999; Boccia et al., 1995; Kutsukake and Castle, 2001). A number of studies of anxiety and mild self-directed behaviour in primates have used these behaviours as an index of anxiety and stress. However, Maestripieri (2000) cautions against the over-interpretation of such behaviour as, having other functions, they are not exclusive exhibited in anxiety/stress contexts.

It has been shown that increased stress does not necessarily affect different types of aggression in the same way. Lutz et al., 2003a found that male rhesus macaques placed in stressful conditions, such as the presence of a stranger, showed increases in cortisol and aggression levels but there was no significant relationship with self-directed aggressive behaviour, in the form of SIBs, which is often used as an index of stress. However, reducing the anxiety levels using anxiolytics (e.g. tryptophan) in rhesus macaques exhibiting self-biting has demonstrated a link between cerebrospinal serotonin levels and SIB (Weld et al., 1998). This treatment decreased SIBs and increased serotonin, an effect on behaviour and serotonin that was not found in subjects without a history of SIBs. Tryptophan also decreases aggressiveness during feeding competition in vervet monkeys (Chamberlain et al., 1987). Increases in mild forms of self-directed behaviour (e.g. scratching and autogrooming) also occur in incidences that indicate increased anxiety, particularly associated with social tension such as increased proximity of conspecifics in rhesus macaques and capuchins (Maestripieri, 2000; Manson and Perry, 2000), or following vocal displays between group-housed chimpanzees as an anticipation of an intragroup aggressive response (Baker and Aureli, 1997) and in long-tailed macaques following receipt of aggressive behaviour (Aureli and van Schaik, 1991).

As with the relationships between hormones and behaviour, species identity and social status are important variables in the relationship between stress and aggression. Steklis et al. (1986) found no relationship between rank and testosterone or cortisol levels in male squirrel monkeys, but did find that whole blood serotonin was positively related to rank when they were subjected to capture stress. High serotonin was correlated with aggressiveness in dominant animals, and in subordinates increases in cortisol correlated with aggression received. Subordinate male talapoin monkeys produced a higher cortisol response to increased aggression associated with group-housing with other males and ovariectomised females (Eberhart et al., 1983). When some females were made attractive with oestradiol, changes resulted in levels of some aggressive behaviour and all males showed increases in cortisol. Sassenrath (1970) also found a heightened stress response in subordinate rhesus macaques. Conversely, however, faecal cortisol levels correlate with dominance in wild Japanese macaques (Barret et al., 2002) and in female ring-tailed lemurs dominance and the initiation of aggression are positively correlated with cortisol levels (Cavigelli, 1999). In the latter case interpretation is complicated by ecological and reproductive stressors that are also shown to correlate with cortisol. There may be an additional reaction between the nature of the cortisol response and dominance status depending on the nature of the stressor e.g. snake presentation versus chemical versus physical restraint (Manogue et al., 1975).

Animals may adopt behavioural strategies to reduce the potentially harmful effects of highly stressful situations as is illustrated by long-tailed macaques in stressfully unstable groups, where a relationship, uninfluenced by rank, was found between enhanced immune competence and the displaying more affiliative than aggressive behaviour (Kaplan et al., 1991).

## 6. Investigations of influences on stress and aggression

As described above there is a considerable body of literature that describes either stress, aggression or the interaction of stress and aggression in primates. In this section we will examine the literature that measures stress and aggression responses to changes made, or observed in their environmental or social conditions. Key aspects of these relate to the availability and distribution of resources such as food and space, as well as social housing, the extent of crowding and instability resulting from the fission and fusion of groups.

### *6.1. Resource availability*

A common strategy when attempting to determine the dominance status of animals in a group is to produce a degree of instability in which animals challenge or displace each other for access to a presented, favoured food (e.g. Honess et al., 2004) or, following a period of deprivation, to water or fluid (e.g. Boccia et al., 1995; Cords and Aureli, 1993; de Waal and Johanowicz, 1993; Fornasieri et al., 1990). The pattern of aggression in this competitive context reveals relative dominance status.

Natural or experimental variation in the availability or distribution of food can affect stress and aggression responses. A decrease in food availability and increased predation risk for female ringtailed lemurs at the end of the Malagasy dry season results in increased feeding effort (Cavigelli, 1999). This, which is associated with increased cortisol levels, in turn correlated with dominance and the initiation of aggressive behaviour. In an experiment, presenting food in clumps to captive bonnet macaques also increased aggression and immunological parameters indicated stress (Boccia et al., 1995).

Cage size might intuitively be expected to affect stress and aggression levels, but results can be complicated by sources of variation such as an animal's history. For example when singly-housed, wild born and raised adult rhesus macaques exhibit less self-directed aggression and stereotypies than identically housed, laboratory raised adults and increasing their cage size from 0.288 $m^3$ to 6.144 $m^3$ had no effect on non-locomotory abnormal behaviour but did produce a reduction in stereotypic movement (Paulk et al., 1977). For singly-housed females of the same species research shows that increasing cage size by as little as 40% produced no significant change in rates of aggressive or submissive behaviour or in the stress measure of heart rate (Line et al., 1989b), supporting other findings that changes in size alone of what still remain small cages, does not affect cortisol levels (Crockett et al., 1993, 2000). While cage size on its own is important the way that the space is enriched (perches, toys etc.) is of particularly importance and more likely to affect psychological well-being and it is also important that species- and age-specific requirements are met (Honess and Marin, 2005; Wolfensohn and Honess, 2005).

### 6.2. Social density

#### 6.2.1. Isolation

In captivity primates can suffer isolation in a number of ways: It may be a brief event associated with husbandry or veterinary practicalities or more long-term being singly-housed, with or without sensory contact with conspecifics, as a result of either experimental constraints or insufficient social provision. It is the more prolonged isolation to which we refer here.

The literature on the behavioural effects of social isolation in primates is extensive (for a brief review see McKinney, 1974). Sackett et al. (1973) have demonstrated that peer-raised rhesus macaques had lower basal cortisol levels than those raised in isolation. They showed marked differences when exposed to a complex, novel stressor (a playroom); isolation-raised animals showing fear-disturbance behaviour and peer-raised ones showing more social and physical exploration behaviour. However, both showed a similarly low level of aggression-threat behaviour and cortisol levels that, while raised, were not significantly different to basal levels, though measures did reach the maximum significance possible for a sample size of four per treatment. The interpretation of these results is complex as a greater cortisol response would be expected in the isolates. It is possible that their already high basal levels may constrain the magnitude of a further response, limiting damagingly high concentrations of cortisol, or the response in the more physically active, peer-reared animals may reflect the metabolic role of cortisol and that in the less active isolates, a truer stress response.

A comparison of between long-term group- and singly-housed Peruvian squirrel monkeys found that cortisol levels differed between the housing conditions, being higher in the group-housed animals and among these there were relationships between dominance and both cortisol (positive) and catecholamine (negative) levels (Leshner and Candland, 1972). High catecholamine levels in subordinates are interpreted as reflecting the stress of the receipt of aggressive dominance behaviour, and raised cortisol in dominants as being less related to stress than to the energetic requirement for the aggressive maintenance of their dominant status.

It has also been found that body weight and reactivity to a novel environment, rather than cortisol response and testosterone level, proved good indicators of eventual dominance in male long-tailed macaques moving from single- to group-housing (Morgan et al., 2000). This was despite testosterone correlating with body weight and suggests a lack of causal relationship between testosterone and dominance. Cortisol but not testosterone did increase under group-housing but neither correlated with aggressive behaviour.

Whilst there are many negative consequences of social isolation in primates of all ages (McKinney, 1974) it should not be forgotten that there are also negative consequences for animals associated with social housing, particularly for those not at the top of the dominance hierarchy (see above). In addition, Baker and Aureli (1997) found that single-housed chimpanzees showed less stress-related displacement behaviour than those that were group-housed when exposed to noisy displays of conspecifics, indicating an anxious anticipation of intra- rather than inter-group aggression.

#### 6.2.2. Crowding

There has been considerable interest in the impact on, and response of primates to, social crowding with a view to addressing questions about the potential consequences of high densities in human populations. de Waal et al. (2000) report the ambiguity of evidence in primates for increasing aggression under increasing density. They report, among rhesus macaques, increased affiliative and submissive/appeasement behaviour in male-male and male–female interactions under increased crowding while there was an increase in aggressive female–female interactions. Affiliative and appeasement behaviour remained constant between females of the same matrilines but increased between females from different matrilines. These changes in behaviour to crowding stress indicate a strategy for coping with crowding and reducing potentially injurious disputes.

Evidence of a coping strategy for stress is also found in captive chimpanzees (de Waal et al., 2000). Without sensory contact with noisy or displaying neighbouring conspecifics, chimpanzees housed in small or large spaces have the same stress levels (self-scratching and faecal cortisol). However, when perceiving provocative displays by neighbours those in small spaces exhibit more stress, but no increase in aggression, and were three times less likely to respond than those in larger spaces. It is the anticipation and anxiety of subsequent intragroup aggression that is likely to govern the suppression of any response (Baker and Aureli, 1997).

Increasing the density of socially housed primates is clearly different from simply increasing cage size, particularly for singly-housed animals, most importantly it increases social tension and stresses by reducing inter-individual spacing. It is possible to change crowding in group-housed animals by either changing their space provision, or maintaining the space provision and changing the number of animals: Nagel and Kummer's (1974) 'spatial density' versus 'social density' experiments. Many of the general principles and much of the literature on crowding in primates is covered elsewhere (e.g. de Waal et al., 2000; Eaton et al., 1981; Judge and de Waal, 1997). However, a number of studies give an insight into the stress and aggression responses of primates to crowding.

Among the earliest of spatial density experiments was that of Southwick (1967) who found that halving space provision for a group of rhesus macaques produced a marked increase in aggressive (mild more than severe) and submissive behaviour. Alexander and Roth (1971) found a similar response in a group of 84 Japanese macaques when briefly moved to smaller housing, increasing density from 0.01 to 0.45 animals/m$^2$; both mild and severe aggressive behaviour increased mostly among males. On their return to their previous, larger housing severe aggression disappeared and mild aggression returned to baseline levels and therefore severe aggression is interpreted as an artefact of unfamiliar housing and mild aggression as a direct response to crowding. During short-term increased crowding of 61 rhesus macaques, in their home accommodation, from 0.76 to 2.54 animals/m$^2$ there were increases in mild aggression, submissive behaviour and rank-related pacing indicative of anxiety or stress as well as the only two observations of SIB during the study (Judge and de Waal, 1993). The absence of an increase in severe aggression supports claims that this occurs as a response to unfamiliar housing. Grooming, however, decreased under crowding in a pattern of changes in behaviour that the authors term a 'conflict avoidance strategy'.

Judge and de Waal (1997) also examined the behaviour of rhesus macaques under a range of long-term crowding densities ranging from 0.002 animals/m$^2$ (island free-ranging) through 0.046–0.057 animals/m$^2$ (corral) to 0.842–0.824 animals/m$^2$ (zoo enclosure). With increased crowding; grooming and huddling, but not aggression increased in males, and aggression and affiliative behaviour increased between females. Increases in the grooming, huddling and appeasement behaviour of females towards males became indicative of a coping strategy for aggression reduction. In other chronically crowded rhesus macaques (0.23 animals/m$^2$) stress levels (corticosteroid response to ACTH injection) were found to be inversely related to dominance (Sassenrath, 1970). By manipulating group structure by firstly isolating individuals, secondly removing the dominant animal and then removing the most subordinate it was determined that the greatest contribution to the higher stress response of subordinates was from fear- or anxiety-evoking aspects of social interactions.

Investigation of the effects of increasing social density by adding animals to a group would be confounded by the aggressive responses primates typically exhibit to conspecific strangers (see below). Therefore Eaton et al. (1981) monitored the responses of a Japanese macaque group in a 0.81 ha. corral that recruited new members though natural reproductive events, expanding from 107 to 192 animals over five years (0.013–0.024 animals/m$^2$). Over this period of increasing density only the adult males increased aggressive behaviour while this decreased in adult females.

### 6.3. Creating and changing social groups

Group formation describes composing a new group from a number of previously singly- or pair-housed animals. Whereas group enlargement involves adding one or more unfamiliar animals to an established group either sequentially or simultaneously, and group merging, happens when two, or more, established groups are brought together. Pair formation does not constitute a group in most primate species (Wolfensohn and Honess, 2005) and is therefore dealt with separately.

#### 6.3.1. Group formation

During the first hour after introduction of 11 previously singly-housed, mixed sex and age rhesus macaques levels of both aggressive and sexual behaviours were substantially higher than after the group settled (20 and 15 times higher respectively), reflecting arousal, or stress of the novel situation (Bernstein and Mason, 1963). Threats and attacks during group formation were not indiscriminate, being directed at similar-sized and smaller individuals, reinforcing dominance status, which seemed to be determined visually, based on physical attributes, posture and the deference of other animals. Gust et al. (1996), in a new mixed sex group of pig-tailed macaques formed from singly-housed animals, there was no wounding or serious aggression, but significant stress was indicated by raised cortisol levels and decrease in T cell counts. Further impact of social instability stress on the immune system is illustrated by Capitanio et al. (1998) where rhesus macaques in frequently recomposed groups of two to four members had higher levels of aggression, lower basal cortisol levels and subsequent shorter survival following inoculation with SIV than similar sized stable groups.

Although squirrel monkeys given sensory but not physical contact with stranger conspecifics showed increased male–male aggression but no notable increase in heart rate (Anzenberger et al., 1986) stress was indicated by cortisol increases in singly-housed males of this species during group formation followed by the introduction of females (Mendoza et al., 1979).

### 6.3.2. Group enlargement

Bernstein (1964) showed how disruptive introducing stranger rhesus macaques into an established captive group can be: Enlarging a group of rhesus macaques by sequentially introducing nine new animals to an established group (of one male and three females) resulted in elevated levels of aggression. This aggression primarily involved the females and was pronounced in three of the introductions. Aggression increasing rapidly during the first 20 min and normal activity patterns resumed only after the second day, although affiliative behaviour took longer to recover. Southwick (1967) described additions to a group of rhesus macaques of firstly two juveniles, then two adult females and finally two adult males (all strangers) which all caused significant increases in aggression (fourfold, tenfold and sixfold respectively). All additions except the juveniles were removed after two days due to injuries suffered in attacks lead by their equivalents in the resident group.

Schaffner and French (1997) found that the response to introduced strangers of female but not male black tufted-ear marmosets varied with group size; those in larger groups being more aggressive. The immigration of an aggressive male into a wild group of yellow baboons (*Papio cynocephalus*) was associated with increase cortisol levels in the residents, especially the females and lower lymphocyte levels in those that were the particular attention of his aggression (Alberts et al., 1992). The new male also had high cortisol and low lymphocyte levels together with testosterone levels three times that of average males.

### 6.3.3. Group merging

Southwick (1967) found that in merging 10 juveniles and seven adults it took four weeks for agonistic behaviour to level reach a baseline. During the introductions some severe aggression occurred, one adult male died as a result but, in contrast to the sequential introductions (see above), all other introduced animals were assimilated into the group, indicating that simultaneous introductions to stable groups are less disruptive than those of individuals conducted sequentially.

Merging two mixed sex groups of either rhesus or pig-tailed macaques results in agonistic defeat of the smaller group with defeated males showing a drop in testosterone levels and victorious ones, an increase (Bernstein et al., 1974, 1979; Rose et al., 1972). The same research demonstrated that if males were introduced into a group containing males of which they had prior experience, they were integrated with very low aggression (and no significant changes in testosterone levels) and assumed subordinate positions, irrespective of their previous rank relative to the resident males.

Clarke et al. (1996) examined baseline and post-introduction levels of cortisol, reproductive hormones, immune function and behavioural patterns in nine small groups of rhesus macaques being merged into one large group of 49 and compared these measures to a stable, similar sized control group. In the merged group there was more noncontact aggression, less affiliative behaviour, lower male testosterone, lower cortisol (but only in the best integrated females) and higher immune function, which became lower than the control group's after a period following the merger.

Westergaard et al. (1999), in an effort to reduce wounding, examined both the timing (rapid versus staged) of introductions and the structure of the housing (divided versus undivided) in which introductions of 190 rhesus macaques (23 males and 167 females) were made. The animals, originally from free-ranging groups, were singly- or group-housed (1 male: 8 females) for one year during health screening. Four breeding groups (two per treatment) were then formed of 16–31 animals either by all animals being introduced at one time (rapid) or by first introducing the males, allowing them to establish a dominance hierarchy and then phasing the introduction of each male's group of females in descending male dominance order (staged). Introductions were in either undivided corrals or those divided midway by a wall with doors. Wounding and reproduction rates were measured over the following two years revealing that staged introductions into divided corrals had the lowest level of wounding, which was particularly high in males in undivided corrals (no hiding places), and also in the first year of the rapid introductions. No differences reproduction rates were found.

### 6.3.4. Pair formation

When forming same sex pairs in long-tailed macaques it has been found that females showed more affiliative behaviour and less contact aggression than males (Crockett et al., 1994). When their cortisol levels were compared before and after pairing males had higher levels when paired and females had higher levels when housed alone.

## 6.4. Social instability

Social instability has well known effects on hormonal levels (Sapolsky, 1991). Under instability, dominant males have higher basal testosterone and aggression levels (Cavigelli and Pereira, 2000; Coe et al., 1979; Keverne et al., 1978; Mendoza et al., 1979; Rose et al., 1975; Sapolsky, 1983), higher basal cortisol levels and a lower stress response (Sapolsky, 1983).

As discussed earlier there are many factors at play that act to hold a social group together despite the competitive/selfish forces acting to drive individuals apart. As such complex and species variable systems have evolved to enable group members to patch up relationships following aggressive interactions that take place in the context of the group's dominance structure in what can be described as a particular dominance style (de Waal, 1989). Reconciliation also plays an important role in the reduction of post-conflict stress, for example allogrooming reduces heart rate (Aureli et al., 1999).

Splitting and then reforming groups through the removal and reintroduction of individuals enables the assessment of the effects of social disruption. Vessey (1971) found that removing animals, even the dominant male, from their group had no effect on group home range size or intergroup dominance in free-ranging rhesus macaques. However, although only one of nine separated females failed to reassimilate, almost 50% of reintroduced males failed to rejoin their original group becoming subordinate in another group or solitary. Furthermore while lower levels of aggression were found upon reintroducing adult male pig-tailed macaques to their group than was exhibited at original group formation (Bernstein et al., 1979), juvenile rhesus macaques, peer-housed during 18 weeks of separation, showed an increase in cortisol and decrease in immune function on reintroduction to their natal group that correlated with the amount of aggression they received (Gordon and Gust, 1993). The mothers of the separated juveniles produced a higher cortisol response to the reintroduction than other mothers in the group.

Stavisky et al. (2001) found that cortisol levels in female long-tailed macaques were lower eleven months following group formation than at 4.5 and 7.5 months, illustrating an attenuation of stress response as social stability increases. Although no relationship was found between cortisol and rank in these small groups the pattern of aggressive and submissive behaviour, up to 13 months following group formation, conformed to the expected with dominant animals displaying more aggressive and less submissive behaviour than subordinates.

## 7. Conclusion

The numerous sources of variation associated with the stress and aggression, together with inconsistencies in the definitions used by authors point towards the importance of controlling for these in future studies as it is clear that variation in these between studies has frequently confounded results and led to contradictory findings. Further work is needed to examine the influence of such factors as age and sex on the stress response and to elucidate any causal relationship behind the exhibition of aggressive behaviour as a response to stress.

It is clear that the relationships between stress and aggression and in particular the hormonal changes that are associated with them are highly complex in primates. Their reliance on learnt behaviour and the importance of appropriate social interactions in the context of complex social relationships plays a key role in this complexity. As Bercovitch and Ziegler (2002) remark primates have evolved 'a flexible physiological system responsive to the social environment. Hormones and behaviour are inextricably intertwined in a feedback relationship in which each regulates the other.'

However, a number of relationships are already very clear, not least the consequences for animals of elevated stress levels in terms of their health, development and reproductive success and the need to manage this stress is important in determining social and reproductive strategies. It is also important to consider that some of the important consequences of stress discussed earlier, particularly those deriving from conditions and practices associated with captivity, have clear implications for immunological and neuroscience research that assumes the primate model being used is as natural, and therefore, as valid as possible.

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- ***<u>ANEXO 6</u>***

“Enrichment and aggression in primates: A review”.

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Review

# Enrichment and aggression in primates

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## Abstract

There is considerable evidence that primates housed under impoverished conditions develop behavioural abnormalities, including, in the most extreme example, self-harming behaviour. This has implications for all contexts in which primates are maintained in captivity from laboratories to zoos since by compromising the animals' psychological well-being and allowing them to develop behavioural abnormalities their value as appropriate educational and research models is diminished. This review examines the extensive body of literature documenting attempts to improve living conditions with a view to correcting behavioural abnormalities and housing primates in such a way that they are encouraged to exhibit a more natural range and proportion of behaviours, including less self-directed and social aggression. The results of housing, feeding, physical, sensory and social enrichment efforts are examined with specific focus on their effect on aggressive behaviour and variation in their use and efficacy. It is concluded that while inappropriate or poorly distributed enrichment may encourage aggressive competition, enrichment that is species, sex, age and background appropriate can dramatically reduce aggression, can eliminate abnormal behaviour and substantially improve the welfare of primates maintained in captivity.

*Keywords:* Environmental enrichment; Aggression; Abnormal behaviour; Primates; Welfare

## Contents

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## 1. Introduction

The field of environmental enrichment is still a relatively young one and the scientific study of its effects and efficacy in improving the psychological well-being of captive animals is younger still. Environmental enrichment forms just one part of any coherent strategy that cares for the lives of animals held in captivity either to meet the interests of man or, increasingly, as part of ex situ conservation efforts. Caring for an animal’s non-psychological health is of equal importance and there are times when efforts to optimise both physical and psychological well-being can conflict, for example outdoor enclosures may have some clear behavioural benefits (see below) but also hold added disease and climatic exposure risks for the animals (Wolfensohn and Honess, 2005).

Whilst the general principles of environmental enrichment apply to all contexts in which animals are kept in captivity, we are specifically concerned here with nonhuman primates (hereafter referred to as simply primates). Whilst zoos have long been aware of the paying public’s concern over the welfare of the animals they exhibit (Young, 2003) the drive to carry out environmental enrichment in research facilities (experimental and breeding) is more recent and to an extent is driven either by the concern of animal care staff working in them or by force of legislation (Wolfensohn and Honess, 2005), this being despite the consequences for research models of animals that are stressed and in poor psychological health (see Honess and Marin, 2005). The greater body of literature we examine derives from laboratory housing contexts but it is important to point out that the findings are relevant for all captive primate contexts. It is clear that zoo housing may provide conditions that allow animals to exhibit behavioural profiles and time budgets that may be very similar to those of wild conspecifics (Hosey, 2005), nevertheless they do form part of a continuum of conditions in which primates live that spans barren, solitary caging through to undisturbed natural habitat. Hosey (2005) in his excellent review of the effects of zoo environments on primate behaviour identifies three dimensions on which to distinguish zoo from other captive environments: The chronic presence of human visitors; restricted space; and being managed. Although quantities and qualities of these dimensions may differ between primate housing contexts all have a great or lesser impact on primates, and their welfare, in all captive contexts and therefore, his approach is also extremely valuable and valid for non-zoo environments. It is also worth pointing out that while there may be a general perception that zoos provide better conditions for their primates than laboratories or primate centres, it is of course true that not all zoos are better than all labs and we consider the following review relevant to all housing contexts and not solely the laboratory one in which most of the research was conducted.

A number of definitions exist for the term *environmental enrichment* but few are very concise (for examples see Young, 2003). A practical definition that covers all major aspects of environmental enrichment and relates it to behaviour in the wild is that of Carlstead and Sherpherdson (1994) who define it as ‘A practice aiming to provide environments of greater physical, temporal and social complexity that affords animals more of the behavioural opportunities found in the wild’. Aggression as considered here includes that which is more intense such as contact aggression between individuals, more mild such as non-contact aggression (such as threat displays) and varying degrees of intensity of self-directed aggression as is often exhibited as part of a suite of behavioural pathologies by animals suffering significant stress or psychological problems. The major concepts surrounding aggression in primates are covered elsewhere (e.g. see Honess and Marin, 2005).

Enriching the environment of captive primates reduces the amount of time in which animals are inactive. Stimulating them and occupying their minds reduces boredom and helps to prevent the development of abnormal behaviour, which includes stereotypic behaviour, self-injurious behaviour and even natural behaviour that occurs at an unnatural frequency, intensity or duration, such as grooming and displays of aggression. Producing an environment that encourages animals to indulge in an appropriately balanced repertoire of natural behaviours that resembles as close as possible that of wild conspecifics will result animals that are significantly more psychologically healthy than those with restricted, disproportionate repertoires (Mason, 1991). This will in turn result in healthier animals through a reduction of injury associated with excessive aggression and self-injurious behaviours, lower stress levels and associated vulnerability to opportunistic infection and neurological damage (see Honess and Marin, 2005), a more accurate model for research, a more natural, species-typical exhibit for zoos with better reproduction for breeding programmes and increased likelihood of success for conservation or reintroduction projects.

Young (2003) provides an extremely thorough review of the history of environmental enrichment and the philosophical foundations underpinning it. He also describes and reviews the success of a wide range of environmental enrichment across a range of species in different captive situations. Here, we will concentrate on examining methods used to enrich the lives of captive primates with special reference to its effects on levels of aggression, but it is important to note that the order in which different types of environmental enrichment are discussed does not reflect the authors' view of their relative importance to the animals. Where a mixture of types of enrichment are used and it is not always possible to separate their contribution to any success in enrichment these are dealt with separately at the end.

## 2. Housing enrichment

Although many states have regulated minimum standards for housing there is little consistency in cage size, temperature and other housing conditions (Poole, 1995; Reinhart et al., 1996). Many researchers have stressed the importance of space provision (quantity and quality) as well as appropriate temperature, humidity and light conditions to promote species-typical behaviour and thereby improve the psychological well-being of captive primates. Space provision should be adequate to allow the display of a wide range of species-typical behaviour exhibited by wild conspecifics and increasing space for group-housed animals will assist subordinate individuals in avoiding aggression displayed by more dominant animals (e.g. Reinhardt, 1992b).

### *2.1. Cage size*

Zoos generally provide for space requirements better than laboratories. Many guidelines which specify dimensions for housing laboratory primates use body weight as the main criterion, assuming that lighter animals have a lower space requirement, and making little allowance for body size (linear measurements), species, sex, age and individual differences in energy budget, activity patterns, and space use (Buchanan-Smith et al., 2004; O'Neill et al., 1991; Wolfensohn and Honess, 2005). The regulations stipulating *minimum* cage size are inconsistent: USA regulations recommend $0.28\ \mathrm{m}^2 \times 0.76\ \mathrm{m}$ high for an animal of approximately 2.5 kg., UK*:* $0.60\ \mathrm{m}^2 \times 1.00\ \mathrm{m}$ high and *EU:* $0.35\ \mathrm{m}^2 \times 0.75\ \mathrm{m}$ (Poole, 1995). However even the largest of these (UK legislation) limits the ease and comfort with which an animal can move. This inconsistency may reflect a lack of understanding of the needs of captive primates or of enrichment programme goals. Significant space is required if animals are to display natural behaviour including general and more rapid locomotion such as chasing, an important component of normal aggressive or play behaviour. Where insufficient space is provided, in addition to hampering the expression of natural behaviour, stereotypic and other abnormal behaviour may develop and be exhibited in stressful situations (Chamove, 1989a,b; Draper and Bernstein, 1963; Paulk et al., 1977). Research into, and the promotion of, larger cage sizes has been limited both due to contradictory findings and the substantial resource (financial cost) implications (Wilson, 1982; Woolverton et al., 1989). Here, we outline some of this contradictory evidence with specific reference to the effect of cage size on aggressive behaviour.

Larger housing not only improves reproduction (Boot et al., 1985) it also avoids the problems of reduced growth and, over the long term, muscle atrophy associated with small caging (Faucheux et al., 1978). Neither stress (Crockett et al., 1993a,b; Crockett et al., 2000; Reinhardt, 1991; Schapiro et al., 1993a) nor autonomic response (Line et al., 1989a; 1990a) are heightened in animals in small cages suggesting that size is not a special source of stress, but there are indications of behavioural benefits through the promotion of natural behaviour as a measurable indicator of psychological well-being. Care needs to be taken in interpreting the results of some studies (e.g. Paulk et al., 1977) as isolated animals may have been used and the apparent deterioration in behaviour found in restricted space may be confounded by social and developmental factors (Ruppenthal and Sackett, 1979).

The effects on aggression caused by crowding either through reducing space or increasing numbers are well known and reviewed briefly in Honess and Marin (2005). It is frequently the case that crowding results in an increase in low intensity aggression. Under conditions of restricted space higher levels of aggression and stereotypic behaviour have been reported compared with larger caging, zoo or free-ranging conditions (Faucheux et al., 1978; Howell et al., 1993; Kitchen and Martin, 1996). Restricted space has also been demonstrated to result in a significant decrease in locomotion (including stereotypic) levels in long-tailed (*Macaca fascicularis*) and pig-tailed macaques (*Macaca nemestrina*) (Crockett et al., 1995, 2000), but it should be noted that the smallest ($0.08\ \text{m}^2 \times 0.43$ m) cages used in these studies, at just 20% of the USDA regulation floor area, were too small to allow any natural locomotion. When cage size and complexity are increased, general levels of activity also increase (Kerl and Rothe, 1996; Kitchen and Martin, 1996) and levels of maternal aggressive punishment of infants are lower but more successful in redirecting the infant's behaviour (Ruppenthal and Sackett, 1979).

Evidence which does not support the use of expanded space alone to improve psychological well-being in primates tends to highlight the importance of environmental complexity and social factors, as well as pointing to threshold levels of change in space provision below which the are no discernable benefits. This was certainly the case in studies where rhesus macaques (*Macaca mulatta*) placed in different sized cages (smallest (m): $0.61 \times 0.66 \times 0.81$, largest (m): $0.86 \times 0.66 \times 0.81$) showed no changes in aggressive behaviour (Line et al., 1989a) or heart rate (Line et al., 1990a). It was concluded that modest size increases will not measurably improve well-being and that social factors, such as the presence of cage-mates, were more important in decreasing stereotypic locomotion.

Comparing different environmental enrichment for gorillas (*Gorilla gorilla*) and orang-utans (*Pongo pygmaeus*), Wilson (1982) found that cage size did not affect activity levels and that environmental complexity provided better enrichment than changes in feeding frequency, the total size or usable surface area of enclosures. Adding extra space in the form of environmentally complex playgrounds has been shown to have positive effects in promoting species-normal behaviour and increasing activity. Qualitative and quantitative reductions were reported in the aggressive and abnormal behaviour of gorillas and orang-utans moved from barren to enriched, naturalistic accommodation (Maple and Finlay, 1987). When chimpanzees (*Pan troglodytes*) are allowed to use playground enclosures, increases in activity, environmental manipulation and species-typical behaviours (e.g. foraging, nest building, locomotion and object use) are observed and this may be coupled with a decrease in abnormal and self-directed behaviour (Brent et al., 1991; Warniment and Brent, 1997). While in some instances playground use is expected to be associated with a decrease in aggression (Eichberg et al., 1991) in others there is evidence that levels of social aggression as well as affiliative and sexual behaviour remain unaffected (Brent et al., 1991).

A simple increase in cage size can be associated with a decrease in stereotypic and other abnormal behaviour and an increase in normal locomotion indicates that stereotypic behaviour may be a reaction to spatial restriction (Draper and Bernstein, 1963). However, simply increasing cage size has been shown to be an unsuccessful therapy in attempts to decrease stereotypic or abnormal behaviour already present in an animal's behavioural repertoire, particularly where these have developed as a result of poor social or environmental conditions (Crockett and Bowden, 1994; Line et al., 1989a, 1990a, 1991a). Although when enlargement is combined with complexity, stereotypic behaviour and abnormal behaviour have decreased (Leu et al., 1993).

In summary, most researchers that have examined the effect of cage size increases have concluded that the extension of space itself is insufficient to increase species-typical normal behaviour and decrease abnormal levels of some behaviour (e.g. grooming and aggression). They point to the need to improve the quality, or increase the complexity, of that space to make it useable and useful (e.g. Brent et al., 1991; Kaplan and Lobao, 1991; Kerl and Rothe, 1996; Line et al., 1989a, 1990a; Röder and Timmermans, 2002; Wilson, 1982). Reinhardt et al. (1996) note that adequate size cage should be determined by behavioural criteria and that, particularly for primates, an arboreal dimension is a necessary requirement. The nature of any expanded space is important too, particularly the extent to which it creates vertical space that can be enriched with furniture for climbing as most primate species are primarily arboreal and will preferentially use elevated rather than floor space (e.g. Buchanan-Smith, 1991; Buchanan-Smith et al., 2002; Buchanan-Smith et al., 2004; Goff et al., 1994; O'Neill-Wagner, 1994; Reinhardt, 1992b; Taylor and Owens, 2004). This is especially important for those species that are naturally more arboreal (for example marmosets compared to rhesus macaques) (Wolfensohn and Honess, 2005). Increased availability of vertical space can also reduce stereotypies as in alarming situations (including potential conflict with cage-mates) animals can retreat up and away to a safer location, displaying escape behaviour and thereby reducing stress levels (Draper and Bernstein, 1963; Reinhardt, 1992b; Röder and Timmermans, 2002). Dividing existing space with visual barriers may assist in reducing aggressive encounters facilitating avoidance of aggressive individuals (e.g. Chamove et al., 1984). A strong case can be made that significant housing size increases above the minimum required by regulation should not be looked on as enrichment as these increases are essential for psychological well-being covering the most basic movement and activity needs of the animal (Reinhardt and Reinhardt, 2001).

### 2.2. Light, temperature and humidity

Variation in light, temperature and humidity as a means of enrichment has received little consideration in the literature. Minimum provision is regulated for research animals kept indoors under controlled conditions designed to assist in the maintenance of physically healthy animals (e.g. sufficient lighting for inspection of sick animals and a source of vitamin $D_3$) (Poole, 1995; Wolfensohn and Honess, 2005). Light can affect physiology, morphology and behaviour, and its intensity, duration of exposure, and the pigmentation of the animals are important when planning an enrichment programme. Photoperiod is a critical regulator of reproductive behaviour in many species (Brainard et al., 1986; Cherry, 1987) and can also alter body-weight gain and food intake (Tucker et al., 1984). Poor lighting can result in impaired fertility in female primates but this can be returned to normal with improved lighting; more than doubling breeding rates (Heger et al., 1986).

Light is generally provided on a 12h on, 12 h off cycle, even where there is access to ambient light, as at higher latitudes day length varies more than in the tropical regions from where most primates originate. Banks of lighting may be phased to roughly mimic dawn and dusk. On the same basis adequate temperature fluctuation on a 24-h cycle may help encourage natural behaviour such as taking a siesta during the hot part of the day and huddling at nights when it is cooler (Honess et al., 2004).

Research on the effect of different coloured light on human moods indicates warm colours such red or yellow increase excitement and cool colours, particularly green, reduce anxiety (Jacobs and Suess, 1975; O'Connell et al., 1985). Fritz et al. (1997) found that different coloured lights had different effects on chimpanzee behaviour, in particular red intensified aggression and blue and green reduced anxiety-related pacing. They concluded that coloured lights might be a useful environmental enrichment tool to alleviate anxiety-related behaviours.

### 2.3. Indoor/outdoor facilities

Outdoor environments provide primates with more sensory enrichment opportunities than indoor ones, with visual, olfactory and auditory stimulation and greater opportunities for exploration and manipulation (O'Neill et al., 1991). There should, however, be no underlying assumption that the provision of outside areas is automatically an improvement. Bearing in mind that primates are often kept outside their normal geographical range important factors that may militate against outdoor provision are ambient temperatures, precipitation and wind levels, insufficient shelter and the opportunity for infection from vermin and wildlife. The latter is of key importance to biomedical facilities aiming to maintain SPF (Specific Pathogen Free) animals (Wolfensohn and Honess, 2005).

Studies of the effect of outdoor facilities generally support their use as enrichment for psychological well-being. Lowered cortisol in common marmosets (*Callithrix jacchus*) given access to an outdoor enclosure indicates a decrease in stress (M. Pines, in litt., 4th April 2005) and even singly-caged rhesus macaques housed indoors showed higher activation of the hypothalamic-pituitary-adrenal (HPA) system than those undercover but outdoors (Schapiro et al., 1993a). Behavioural effects can also be marked: sifakas (*Propithecus sp.*) showed improved levels of reproduction and activity with access to a small outdoor enclosure used preferentially (90% of the time) to a larger indoor room (Pereira, 1991). Similarly rhesus macaques moved to outdoor areas showed an increase in activity, and exploration and less self-oral (O'Neill and Price, 1991; O'Neill et al., 1991) and self-aggressive behaviour (Schapiro et al., 1995a). These improvements disappeared when animals were returned indoors with decreased activity and increased resting time (O'Neill and Price, 1991; O'Neill et al., 1991). Levels of aggression have been shown to increase among animals moved from outdoor to indoor accommodation for the winter (Nieuwenhuijsen and de Waal, 1982).

A group of previously singly-housed chimpanzees briefly placed in a naturalistic environment reacted with a decrease in stereotypic and self-directed behaviour and an increase in manipulative activities (Clarke et al., 1982). The absence of any change in social behaviour suggests that the decrease in abnormal behaviour may be the first step towards improving social behaviour in previously isolated animals.

### 2.4. Conclusion: Efficacy of housing enrichment

The majority of this section has dealt with the effect of increasing space availability as reported results of the effect of other aspects of housing enrichment are limited or absent. Although the negative effects of small cage sizes have been known for some time (e.g. Draper and Bernstein, 1963; Faucheux et al., 1978) efforts to improve the psychological well-being of primates with small increments to caging that is already too small to adequately meet their behavioural needs have largely been unsuccessful (e.g. Crockett et al., 2000; Line et al., 1989a; Paulk et al., 1977). Whilst it is not always possible to provide the degree of naturalism in captive environments advocated by some authors (e.g. Clarke et al., 1982) it is nevertheless possible, as shown above, to reduce the incidence of abnormal behaviour, including aggression, and promote more species-typical behaviour by increasing available space provided that space is suitably structured to meet the varying requirements of animals of different species, sex, age and personality (Buchanan-Smith et al., 2004; Reinhardt and Reinhardt, 2001).

## 3. Feeding enrichment

In the wild primates spend the majority of their day in feeding activities, including searching for food as well as food selection, processing and ingestion behaviour (Oates, 1987). In captivity the animals' time and energy allocation to feeding can be very low as food may be presented in a processed form (e.g. pellets) and in a temporally and spatially clumped manner, removing the need for animals to search for their food or to spend significant time processing it. An effective strategy for occupying otherwise inactive captive primates is to promote foraging and feeding behaviour and here we examine the results of some of the many studies using a variety of feeding enrichment techniques to promote foraging behaviour and increase the time spent in food acquisition. As Young (2003) notes it is important to be aware that increasing foraging time represents only part of the natural feeding behaviour of primates and species-specific food acquiring behaviour must also be encouraged (e.g. fruit peeling, dehusking, extractive foraging etc.), particularly if animals are to be reintroduced to the wild or to maximise their educational value in zoos.

There is a wide range of feeding enrichment techniques that promote species-typical behaviour, including variation in food presentation, diet composition, treats and hidden food. Foraging programmes encourage the animals to ‘work’ for food, increase processing time and novelty of food (Champoux et al., 1993; Laule and Desmond, 1998; Reinhardt, 1993a,b; 1994a; Schapiro et al., 1991; Schub and Eisenstein, 2003; Wolfensohn and Honess, 2005). They should be designed carefully to avoid creating an unbalanced diet through the introduction of excess calories or deficiencies in vital nutrients due to the over-consumption of supplements and treats (Bloomsmith et al., 1988; Wolfensohn and Honess, 2005).

The simplest ways to enrich the feeding of animals that requires no extra equipment is to vary their feeding routine, specifically the type of food and its temporal and spatial distribution. The introduction of fresh fruit or green forage (as appropriate for a species’ diet) not only adds variety in taste, texture and processing, but can contribute valuable trace elements and vitamins to the diet (Wolfensohn and Honess, 2005).

### *3.1. Variation in normal diet and treat provision*

Novel foods and varied feeding routines can be valuable enrichment tools but treats may have nutritional implications, and potentially increase stress and intragroup aggressive competition (Wolfensohn and Honess, 2005; NRC, 1996).

Foraging time and natural foraging behaviour can be increased by simply presenting food or edible items that require more extensive processing than standard primate food such as monkey chow. Beirise and Reinhardt (1992) reported an increase in species-typical behaviour, foraging and food processing time when group-housed rhesus macaques were given unhusked corn, peanuts in the shell and cardboard boxes (torn up and chewed). Frozen fruit can also increase eating time (by about six times) and reduce aggression by a factor of four (Chamove et al., 1982).

The presentation of novel foods (peanut butter, mealworms and grubs) in novel ways has been shown to increase foraging time, exhibit use and locomotion and also to reduce the amount of threatening behaviour towards visitors in zoo-housed cotton-top tamarins (*Saguinus oedipus*) despite the fact that the novel foods were not in all cases eaten (Glick-Bauer, 1997). In laboratories, singly-housed rhesus macaques given treats (Purina Monkey Yums) by technicians that also mimicked macaque submissive behaviour, showed a significant decrease in stereotypic and other abnormal behaviour, including a small though not significant decrease in self-directed behaviour (including hair plucking and self-biting), compared to baseline measures (Bayne et al., 1993a). Varying the time at which animals are fed may also have some benefits, however, delays and departures from a regular feeding regime can increase self-directed and abnormal behaviour associated with heightened anticipation stress (Waitt and Buchanan-Smith, 2001).

### *3.2. Simple foraging tasks*

Foraging tasks can even be created under conditions where animals are held on mesh floors (e.g. for experimental reasons) by using a container such as a rubber feeding bowl to present a forage mix. This simple measure was shown to decrease aggressive behaviour and prevent boredom in a breeding colony of rhesus macaques (Davys, 1995). Replacing the mesh floor with a metal tray also allows forage to be offered to animals in a substrate (Harris, 1988; Spector et al., 1994). In circumstances where animals are housed on solid floors it is possible to provide them with a simple foraging task by mixing particulate food (e.g. mixed grain) into a forage substrate (e.g. woodchips) and spreading it on the cage or enclosure floor (Chamove et al., 1982; Wolfensohn and Honess, 2005). This was shown to reduce the frequency of social aggression in group-housed stump-tailed macaques (*Macaca arctoides*) by almost 50% in adults and 90% in juveniles compared to when forage was offered without substrate. It also reduced self-directed aggression and the monopolisation of food by dominant animals (Anderson and Chamove, 1984; Chamove and Anderson, 1979; Chamove et al., 1982, 1984). Similar reductions in aggressive behaviour associated with a woodchip forage substrate containing grain or mealworms were also demonstrated for red-bellied tamarins (*Saguinus labiatus*), as well as stump-tailed macaques and five other primate species in a zoo: moustached guenons (*Cercopithecus cephus*), vervet monkeys (*Cercopithecus aethiops*), squirrel monkeys (*Saimiri sciureus*), common marmosets and ring-tailed lemurs (*Lemur catta*). However capuchins (*Cebus apella*), did not show a decrease in aggression but were nevertheless less inactive and more playful (Chamove et al., 1982). Forage substrate also increased foraging time and decreased abnormal (including self-aggressive) behaviour in long-tailed macaques (Bryant et al., 1988) and chimpanzees where it also decreased aggressive displaying and increased play and locomotion (Baker, 1997).

Similar results were found with a new foraging task (sunflower seeds spread through woodchip forage substrate) given to a group of pig-tail macaques; producing a decrease in aggressive and stereotypic behaviour (e.g. hair-pulling) and an increase in social and exploratory behaviour (Boccia, 1989a, 1989b; Boccia et al., 1995). These results were compared to the impact of the same foraging task on another group of the same species which had a different behaviour pattern including a lower baseline level of aggression (Boccia and Hijazi, 1998). The second group also showed the same changes in abnormal and exploratory behaviours but there was no drop in an already low level of aggressive behaviour. It was concluded that the effect of the foraging task may be related to the original characteristics of each group and that differences in some behavioural responses appeared to be age-related (Boccia and Hijazi, 1998; Boccia et al., 1995).

An equally simple method of presenting food that extends foraging time is for it to be scattered on the mesh top of the cage encouraging the animals to manipulate the food through the mesh in order to eat it (Reinhardt and Garza-Schmidt, 2000; Reinhardt, 1992a, 1993c). Reinhardt (1993c) found that in grouped-housed male rhesus macaques this technique resulted in them spending more time working for food in this way than for that given by the caretaker.

The use of woodchip litter, despite contamination with faeces, urine and overlooked or discarded food, has been shown to both reduce unpleasant smells (Chamove and Anderson, 1979) as well as, after an initial increase, to inhibit bacteria in the forage substrate and reduce the risk of infection (Chamove et al., 1982). However, there is evidence that where pine wood shavings are used for an extended period there may be an increase in aggression and stereotypic behaviour (Novak et al., 1995) and the long-term health effects clearly need further investigation.

### 3.3. Feeding enrichment devices

Various devices exist that have been specifically designed to encourage animals to spend longer foraging and display more natural species-specific foraging behaviour. These include a number of ingenious approaches that for example promote behaviour mimicking termite fishing for chimpanzees (Maki et al., 1989) and gnawing wood for gum in marmosets using a gum-feeder (McGrew et al., 1986) the latter being shown to be successful in reducing stereotypic behaviour and inactivity (Roberts et al., 1999). An extensive range of feeding enrichment devices are described and reviewed elsewhere (e.g. Reinhardt, 1993b; Röder and Timmermans, 2002). Here, we will concentrate on two of the most commonly-used categories of device: Foraging/grooming boards and puzzle feeders.

#### 3.3.1. Foraging/grooming boards

These devices, attached to the side of a cage, consist of a tray or board on which a substrate (e.g. artificial fleece or artificial turf) is fixed and through which the animal must search to find concealed, small, desirable food items (e.g. seeds). Their use has been shown to significantly reduce abnormal behaviour and promote natural behaviour in singly-housed baboons, *Papio spp*. (Pyle et al., 1996) and similarly housed rhesus macaques (Bayne et al., 1991a, 1992a) with animals directing as much as 52% of their time to the foraging task. Different substrates can produce different effects: a fleece foraging/grooming board produced a decrease in abnormal behaviour including self-directed, stereotypic and cage directed behaviour (Bayne et al., 1991a) and an artificial turf board reduced stereotypic but not self- (including self-directed aggression) or cage-directed behaviours (Bayne et al., 1992a). These changes were not eroded over time indicating they were not simply a response to novelty, however abnormal behaviour did return to baseline levels when the foraging boards were removed. Several advantages of the turf over the fleece were apparent in these studies including ease of cleaning and re-filling as well as safety of operation (Bayne et al., 1992a). Singly-housed long-tailed macaques responded even more dramatically to a fleece pad with greater reductions in abnormal behaviour with stereotypic behaviour decreasing by 73%, being replaced by pad grooming (Lam et al., 1991). A much-reduced effect was found when forage was added to the fleece and the authors recommend the provision of separate foraging and grooming tasks. Whilst this difference may reflect a transformation of the pad from a grooming to a foraging task, no distinction is made in this study between ‘foraging’ and ‘grooming’ behaviour, and so the difference in duration of pad-directed behaviour may simply reflect extended searching time (without forage).

Foraging boards are not always totally successful in reducing abnormal behaviour. An artificial turf foraging board did not reduce abnormal (including self-aggressive) behaviour in singly-housed year-old rhesus macaques (Schapiro et al., 1996a) nor aggressive or stereotypic behaviour in pair-housed squirrel monkeys (Fekete et al., 2000). However, in the latter case it did result in more natural behaviours and postures seen during use of the board.

#### 3.3.2. Puzzle feeders

Puzzle feeders present a cognitive and manipulative challenge to animals generally involving the manipulation of food items through a series of small holes to a position where they can be removed and eaten. They vary considerably in complexity and design from simple clear plastic sheets, placed on a cage, with a number of different-sized holes through which food must be manipulated from below, to complex flexible plastic mazes around which food items must be negotiated in order to release them.

The use of puzzle feeders by a range of primate species in captivity produces an increase in foraging time (e.g. chimpanzees: Bloomsmith et al., 1988; Bloomstrand et al., 1986; Brent and Eichberg, 1991; long-tailed macaques: Holmes et al., 1995; Murchison, 1991; Watson, 1992; rhesus macaques: Bloom and Cook, 1989; Novak et al., 1998; Meunier et al., 1989; Reinhardt, 1994a; stump-tailed macaques: Reinhardt, 1993a; pig-tailed macaques: Murchison, 1994; Murchison and Nolte, 1992; baboons: Pyle et al., 1996; and common marmosets: De Rosa et al., 2003; Roberts et al., 1999). While results across studies are not entirely consistent, nevertheless it is clear that the general increase in foraging time can be associated with a reduction in aggressive (Bloomsmith et al., 1988; Brent and Eichberg, 1991; Watson, 1992), abnormal (Bloomsmith et al., 1988) and stereotypic behaviour (Meunier et al., 1989; Novak et al., 1998; Pyle et al., 1996; Roberts et al., 1999). Other benefits include a reduction in inactivity (Murchison, 1994; Roberts et al., 1999), the provision of cognitive stimulation (Murchison, 1991; Murchison and Nolte, 1992) and the promotion of species-specific foraging behaviour (e.g. Maki et al., 1989; Novak et al., 1998; Reinhardt, 1993a). Puzzle feeders can, on the other hand, increase levels of aggression, resulting from increased competition, but this can be reduced where several devices are available or more than one animal can use the device at a time and where it is elevated and animals below can collect dropped or discarded food (Bloomstrand et al., 1986; Maki et al., 1989).

Animals can be encouraged to use more of their cage space by changing the location of foraging tasks but this is not always successful in reducing aggression. For example Lutz and Novak (1995) found a slight, but statistically not significant increase in aggression when rhesus macaques were presented with varied amounts of food in plastic cups mounted in racks that could be placed at different heights on the cage, introducing unpredictability. However, there were more positive behaviours, locomotory activity and a change in feeding patterns when foraging racks were available. The use of three different devices also produced no significant decrease in aggression between paired female common marmosets irrespective of whether they were presented as foraging tasks or, without food, simply as novel objects (Majolo et al. 2003a), although they were quicker to examine and spent longer at the foraging tasks. An absence of effect on self-directed behaviour was also noted by Gullekson et al. (1991) in singly-housed long-tailed macaques using a seed feeder to obtain sunflower seeds, nevertheless foraging time was increased.

### 3.4. Training to reduce aggressive encounters during feeding time

Some of the highest level of aggression in groups of captive primates can be observed at feeding times and the periods immediately preceding them. Much of this is a result of dominant individuals' efforts to monopolise, or have priority of access to, resources. Although Howell et al. (1993) found no relationship between novelty food (varieties of fruit) and pre-feeding aggression in groups of chimpanzees. Where aggression does occur in this context it is possible to dramatically reduce it using positive reinforcement training (PRT) (Bloomsmith et al., 1994). 'Cooperative feeding' (Laule and Desmond, 1998), in which dominant animals are positively reinforced for permitting subordinates to feed and the latter rewarded for bravery in feeding in the presence of the dominant individual, is valuable for mitigating dominance-related problems and reducing aggression during feeding.

### 3.5. Conclusion: Efficacy and variation in use of feeding enrichment

It is important to note that there are a number of sources of variation in the use by animals of feeding enrichment that can affect the distribution of behavioural changes and the efficacy of feeding enrichment programmes. These include social housing conditions (De Rosa et al., 2003), species identity (Boccia et al., 1995; Chamove et al., 1982; Gilloux et al., 1992; Gore, 1993), sex (Baker, 1997; Brent and Eichberg, 1991; Michels, 1998; Murchison, 1994; Murchison and Nolte, 1992), age (Boccia and Hijazi, 1998; Majolo et al., 2003a; Murchison, 1994; Murchison and Nolte, 1992; Plesker et al., 2001), experience (Bloomstrand et al., 1986; Lloyd et al., 2005), motivation (hunger) (De Rosa et al., 2003; Reinhardt, 1994a), type of behavioural disorder (Pyle et al., 1996), and dominance status (Bloomstrand et al., 1986; Gilloux et al., 1992; Lutz and Novak, 1995). Where there is an apparent absence of any effect on the overall rate of intragroup aggression this may conceal significant increases or decreases in this behaviour on an individual level (Bloomstrand et al., 1986) or a pre-existing low level of aggression (Boccia and Hijazi, 1998; Boccia et al., 1995).

Despite variation in its use feeding enrichment is clearly valuable for adjusting behavioural patterns and frequently for reducing aggressive and abnormal behaviour in captive primates. There is evidence from a number of studies that these effects can be long-lasting with feeding enrichment (particularly forage substrate and puzzle feeders) remaining useful in encouraging time-consuming feeding behaviour beyond its initial novelty value (e.g. Boccia, 1989b; Boccia and Hijazi, 1998; Brent and Eichberg, 1991; Bloomsmith et al., 1988, 1986; Maki et al., 1989) although decay of interest has also been noted (Holmes et al., 1995). Optimal effects may be achieved by increasing the variety of feeding enrichment devices either in a rotational programme (Holmes et al., 1995), or when combined (e.g. food requiring time-consuming processing, standard ad lib food, a widely dispersed, particulate foraging task and food puzzles: Bloomsmith et al., 1988). This both avoids decay of interest and stimulates different behaviours or the same behaviours in different ways.

## 4. Physical enrichment: Furniture, toys and other objects

Young (2003; p.143) provides a list of desirable behaviours and associated items of furniture, toys and other objects that facilitate their expression in captive animals. The importance of the approach he advocates is that enrichment with such devices ‘should be both species-specific and goal-specific’. Different items encourage different behaviours in the same species and the same item may encourage different behaviour in different species. A range of physical enrichment devices is reviewed by Schapiro et al. (1991). This section examines a number of studies that demonstrate the success or otherwise of enrichment of the physical environment in reducing aggressive behaviour.

### *4.1. Structural enrichment*

### *4.1.1. Perches and other devices to promote arboreal behaviour*

As pointed out above, the vertical component of space and its accessibility not just through climbing apparatus but through the location of other devices is of great importance to primates (e.g. nest boxes for callithricids: Hosey et al., 1999). Young (2003) devotes a considerable portion of his chapter on ‘Furniture, toys and other objects’ to the design, for captive arboreal primates, of furniture that replicates some of the key functional features of a forest habitat, including structural complexity and how this encourages different locomotor, perceptual and cognitive behaviour. He further points out that artificial alternatives are no substitute for the complexity of a natural tree, and materials used to construct alternatives need serious consideration (e.g. with respect to toxicity, hygiene, strength etc.). Nevertheless a flexible arrangement of climbing furniture that can be rearranged encourages spatial learning and the development of spatial memory (Young, 2003) and reduces boredom and habituation (Wolfensohn and Honess, 2005).

Providing structures that enable the full use the vertical dimension of housing is one of the basic goals of introducing climbable furniture that may be fixed or moveable, rigid or flexible. The space created may be used in different ways depending on species identity (e.g. Chamove and Goldsborough, 2004; Smith et al., 2003) and social status as dominant individuals tend to occupy higher vantage points (giving a good view of the group) and more subordinate animals, lower ones (Wolfensohn and Honess, 2005; Reinhardt, 1992b). The availability of a variety of levels is important in assisting in the avoidance of possible aggression from dominant animals and promoting different behaviours which may be exhibited at different heights (O’Neill-Wagner, 1994; Williams et al., 1988).

Appropriate climbable furniture includes perches, other static structures and swings. Perches have even been designed that fit in laboratory cages and allow the continued operation of a squeeze-back mechanism (Watson, 1991). Several studies have shown that where both perches and swings are available a clear preference may be shown for static over dynamic furniture, perhaps as the latter afford a more relaxed posture (e.g. Kopecky and Reinhardt, 1991; Schaefer and Nash, 2004; Taylor and Owens, 2004; Williams et al., 1988). Installing perches can reduce aggression in group-housed primates (e.g. Japanese macaques, *Macaca fuscata*: Nakamichi and Asanuma, 1998; grey-cheeked mangabeys, *Lophocebus albigena*: Neveu and Deputte, 1999). Swings, on the other hand, produced no decrease in abnormal behaviour, including stereotypies and self-directed behaviour, among singly-housed rhesus macaques (Dexter and Bayne, 1994).

### *4.1.2. Visual barriers*

Visual barriers help reduce contact agonistic behaviour by providing an opportunity for a threatened animal to hide from its aggressor and may improve relationship quality when animals can choose to be alone (Reinhardt and Reinhardt, 1991). A barrier may be a simple privacy panel inserted into laboratory caging (e.g. Reinhardt and Reinhardt, 1991), a concrete cylinder (e.g. Erwin et al., 1976), hanging sections of PVC pipe (Ricker et al., 1995), or a dividing wall with pop-holes or doors (e.g. Westergaard et al., 1999).

Reinhardt and Reinhardt (1991) noted a decrease in aggressive interactions, from 2.2 to 0.3/h, and an over 10% increase in affiliation time when visual barriers were provided for established pairs of female rhesus macaques. Where primates are kept in groups several authors also report a decrease in aggression associated with visual barriers. Although only non-contact aggression decreased in rhesus macaques provided with barriers (McCormack and Megna, 2001), all types of aggression decreased in pig-tailed macaques (Erwin, 1977; Erwin et al., 1976; Maninger et al., 1998), and wounding from fights dropped 60% in squirrel monkeys (Ricker et al., 1995). Erwin et al. (1976) however, found that barriers failed to prevent increased aggression when new individuals were introduced to already unstable pig-tailed macaque groups. Nevertheless aggression during group formation may still be lower with, rather than without, barriers as illustrated by Westergaard et al. (1999) who found, when forming new groups of rhesus macaques, that levels of traumatic wounding, primarily among males, were significantly lower with, rather than without, a visual barrier. As a result of the demonstrated success of visual barriers several other researchers also recommend their use to enable animals to conceal themselves and reduce the risk of attack (e.g. Miller-Schroeder and Paterson, 1989; Reinhardt, 1992b; Stoinski et al., 2004).

#### 4.1.3. Water baths

Shallow water troughs are known to encourage tool use (e.g. cup-like containers) and food processing (e.g. soaking) in captive primates while not being associated with any increase in aggression (e.g. Parks and Novak, 1993). Water baths, tanks or pools provide an additional structural element to primate housing and have proved valuable in encouraging species-specific play behaviour particularly among species (e.g. macaques) that appear to enjoy swimming (Anderson et al., 1992, 1994; Gilbert and Wrenshall, 1989), but are not of interest to others, such as marmosets (Hazlewood, 2001). Additionally, incidents of aggression directed from dominant animals around the edge to swimming subordinates can increase in association with water baths (Anderson et al., 1992).

### 4.2. Manipulable objects

Manipulable objects may function to reduce abnormal behaviour and decrease inactivity by replacing it with other species-typical behaviours such as play, exploration or manipulation. They may even be used as tools, although this may be positive such as the use of plastic containers by capuchins to collect water or carry objects (Westergaard and Fragaszy, 1985) or negative such as their incorporation into routines of self-harm and as targets of threat and aggression (Anderson and Stoppa, 1991; Bayne, 1989).

A wide variety of manipulable objects have been tested as enrichment devices including wooden sticks (e.g. Anderson and Stoppa, 1991; Champoux et al., 1987; Reinhardt et al., 1987b), those commonly used for other species e.g. Kong toys (Crockett et al., 1989; Line et al., 1991b), nylon balls (Brent and Stone, 1996; Renquist and Judge, 1985) and children's toys (Cardinal and Kent, 1998; Paquette and Prescott, 1988), as well as miscellaneous objects (Brinkman, 1996).

#### 4.2.1. Trees, logs, branches and sticks

Where space permits uprooted trees have proved valuable in eliciting species-typical behaviour from chimpanzees with little habituation (Maki and Bloomsmith, 1989). Branches or small logs are readily used by a number of species including baboons (Hienz et al., 1998) where they increase activity in briefly socially deprived animals but not those in sensory contact (Hienz et al., 2000, 2002), and rhesus macaques where use seems irrespective of age or sex and with little sign of habituation (Reinhardt et al., 1987b). The use of branches or sticks has been shown to reduce self-directed behaviour (including self-aggression) in singly-housed primates either when used alone (e.g. Champoux et al., 1987) or in combination with other objects (Bryant et al., 1988; Anderson and Stoppa, 1991).

Further study of the effect of sticks on behaviour in rhesus macaques also report widespread use, limited habituation and a decrease in self-directed behaviour but no change in observer-directed aggression (Champoux et al., 1987). Unfortunately no detail is presented of which self-directed behaviours (e.g. self-aggression) were alleviated and others (e.g. Line et al., 1991b) are critical of the determination of stick use in this instance, i.e. via a reduction (>2%) in stick volume (derived from linear measurements) or stick-rolling observed at least twice a fortnight. This is indeed a generous definition as determining stick volume from linear measurement is likely to be wildly inaccurate due to consistent wear patterns (see Line and Morgan, 1991) and also such limited rolling may simply reflect the stick being moved when obstructing another activity.

#### 4.2.2. Toys

Line et al. (1991b) found that neither sticks, Kong toys (for description see Crockett et al., 1989) nor hard nylon balls had any effect on levels of self-aggression (including self-biting and fur plucking) or stereotypic behaviour in singly-housed, old (over 20 year) but healthy rhesus macaques. It is suggested that low use and rapid habituation to these objects was related to the animals' old age and the authors also report greater object use by captive-as opposed to wild-born subjects but no difference in use of those items that were natural (stick) or artificial (Kong toy, nylon ball) (Line et al., 1991b). However, more use of sticks than nylon balls was found in similarly-housed younger adults without, again, any decrease in abnormal or self-aggressive behaviour (Line and Morgan, 1991) and pair-housed stump-tailed macaques did show a preference for a branch over a PVC pipe perch (Reinhardt, 1990a).

Studies of nylon ball use in chimpanzees report differing results with it either not being related to age, sex or housing (Brent and Stone, 1996) or being greater in younger animals and those in traditional rooms (Bloomsmith et al., 1990a) as well as increasing aggressive competition for the ball. Individual variation in ball use has also been found both within and between macaque species (long-tailed, rhesus and stump-tailed) and although reductions in abnormal behaviour were found some animals also incorporated the ball into stereotypic or self-aggressive displays (Bayne, 1989).

There are mixed results with Kong toys too with them reducing pathological behaviour (including self-aggression) in singly-housed chimpanzees (Brent et al., 1989a), and having some positive effects on non-aggressive behaviour, either when presented alone (Bayne et al., 1993b) or with other objects to singly-housed rhesus macaques (Bayne et al., 1992b). Other evidence indicates Kong toys had no effect on abnormal or aggressive behaviour in macaques (long-tailed, pig-tailed and rhesus macaques: Crockett et al., 1989). Additionally it was found that the animals rapidly habituated to Kong toys, although filling them with frozen juice temporarily revived interest (Crockett et al., 1989). There also appeared to be greater toy use by pig-tailed compared to long-tailed macaques, however this may be affected by more of the former being captive-born and therefore toy-experienced. No other differences were found relating to sex or background (wild- or captive-born). Furthermore, Kong toy had no effect on levels of abnormal or aggressive behaviour in group-housed chimpanzees and were less attractive and subject to more rapid and long-term habituation than wrapping paper, which could be torn-up and chewed (Pruetz and Bloomsmith, 1992). The paper resulted in less grooming behaviour than the Kong, an effect which could help reduce over-grooming (although no data are presented to allow assessment of the benefit to the animals over baseline levels), but agonistic, abnormal, affiliative, inactive and sexual behaviour remained unchanged.

More complex toys (a plastic teething ring, rubber ‘tuffy’, Teddy bear) have been found to increase play time and dominance interactions, while decreasing foraging and self-grooming in paired juvenile pig-tailed macaques (Cardinal and Kent, 1998). A similar effect was found when a more substantial array of durable children’s toys was presented to group-housed chimpanzees: Aggressive behaviour was unaffected despite dominant animals tending to monopolise toys when few were available, but within the group there was an increase in manipulation, decrease in inactivity and self-grooming and the almost total elimination of abnormal behaviour in one individual (Paquette and Prescott, 1988). Competition for toys may be counteracted where there is an excess as shown by Brent and Belik (1997) presenting 21 items (7 nylon bones, 7 Kong toys and 7 Plaque Attackers) to 14 enclosure-housed olive baboons (*Papio anubis*) resulting in decreased abnormal, cage-directed and inactive behaviour.

### *4.3. Conclusion: Efficacy and variation in use of physical enrichment*

It therefore appears that enrichment using perches and visual barriers clearly decreases aggression and/or abnormal behaviour, but that some toys, particularly in limited numbers, may even increase these behaviours, and the evidence for the success of sticks, Kong toys and nylon balls in affecting these behaviours remains ambiguous. However, while it is clear that some manipulable enrichment objects are successful in reducing abnormal and aggressive behaviour in some species, most studies qualify this in one way or another on the basis of practical and safety issues, habituation and transient effects, or variation in object use.

In some instances whole or parts of enrichment objects may block and damage drainage systems (wood splinters: Champoux et al., 1987; nylon balls: Bayne, 1989; Renquist and Judge, 1985) or may be used to create unpleasant levels of noise (Bayne, 1989; Renquist and Judge, 1985). There are also concerns about the effect on the animals’ health of ingesting wood or rope used to enhance the structural environment (Eckert, 1999; Eckert et al., 2000) and of transmission of infection persisting on toys (Bayne et al., 1993b), although there may also be dental benefits of gnawing on wood (Reinhardt, 1990a).

Other ways in which the efficacy of manipulable devices may be limited include the high levels of habituation noted in many studies (e.g. Bloomsmith et al., 1990a; Cardinal and Kent, 1998; Crockett et al., 1989; Line et al., 1991b) and preferences and variation in their use. Toys are not suitable for all species and those with reduced manipulatory skills may show a preference for structural enrichment (e.g. Garnett’s bushbaby, *Otolemur garnettii*: Watson et al., 2002), but even among those that use manipulable objects there can be differences in their use relating to species (e.g. Bayne, 1989; Crockett et al., 1989) and individual identity (e.g. Cardinal and Kent, 1998; Line and Morgan, 1991), dominance rank (e.g. Paquette and Prescott, 1988), experience (e.g. Crockett et al., 1989), upbringing (e.g. captive or wild-bred: Line et al., 1991b; isolation or surrogate reared: Sackett et al., 1973), age (e.g. Bloomsmith et al., 1990a; Brent et al., 1989a; Line et al., 1991b; Maki and Bloomsmith, 1989; Plesker et al., 2001; Taylor and Owens, 2004), sex (e.g. Williams et al., 1988) and toy type (e.g. Brent and Stone, 1996). Little or no change in abnormal behaviour has been reported by some investigators with some toys e.g. Prima Hedrons (e.g. Plesker et al., 2001), nylon balls (e.g. Bayne, 1989; Line and Morgan, 1991; Line et al., 1991b), and Kong toys (e.g. Crockett et al., 1989; Pruetz and Bloomsmith, 1992), yet these continue to be amongst the most common enrichment objects found in the cages of laboratory primates.

Key features which appear to contribute to the success of this type of enrichment include the variety and control afforded by objects (e.g. being manipulable and portable: Bayne, 1989; Westergaard and Fragaszy, 1985), that they be provided in sufficient number to exceed the number of animals and thereby not encourage aggressive competition (e.g. Bloomsmith et al., 1990a, Paquette and Prescott, 1988), and that to reduce habituation and maximise results their exposure should be intermittent (Crockett et al., 1989; Hienz et al., 2000; Paquette and Prescott, 1988), and on rotation (Cardinal and Kent, 1998; Paquette and Prescott, 1988). To balance this it should be noted that animals may become possessive and protective towards their enrichment objects and therefore stressed at their removal (Bayne, 1989). Furthermore the removal of successful enrichment items may cause abnormal or aggressive behaviour to reappear (Weld and Erwin, 1990) and therefore, it needs careful consideration where rotation or rationing of devices is part of an enrichment strategy (Bayne et al., 1992b).

## 5. Sensory enrichment

Sensory enrichment focuses on stimulating animals' senses (Harris et al., 1999). Perhaps most important to most primate species are their senses of sight and hearing and as such we shall concentrate on examining efforts to enrich captive primates through audio and visual media. Ideal captive environments should incorporate complexity to allow animals some control in obtaining rewards for appropriated actions (Washburn et al., 1991) and include some unpredictability (Poole, 1991) and appropriate sensory stimulation aims to add just such a level of complexity.

### *5.1. Visual enrichment*

Primates in general, and Old World monkeys in particular, rely heavily on their visual senses as illustrated by their brain structure and visual communication systems (Platt and Novak, 1997). Technological advances have allowed the use of increasingly sophisticated videostimulation to examine aspects of their cognitive abilities and preferences (e.g. Deaner et al., 2005; Waitt et al., 2003; Washburn et al., 1991) and enrich their captive lives (Platt and Novak, 1997). In a number of instances videostimulation has successfully been used in a programme of mixed enrichment (see later section) (e.g. video or television, additional space, manipulable toys, foraging: Schapiro and Bloomsmith, 1995; Schapiro and Bushong, 1994; Schapiro et al., 1996a; Turner and Grantham, 2002).

#### *5.1.1. Computer 'games'*

Elder and Menzel (2001) noted that a joystick-operated computer task presented to an orang-utan increased some frustration-related behaviours but reduced stress (salivary cortisol) levels when the animal had the chance to performance the task. However, this may simply illustrate raised stress when the animal is unable to use the device. The same device used by mixed-sex orang-utan pairs resulted in increased contact and non-contact aggression through monopolisation of the device by one of the pair, frequently the female (Tarou et al., 2004). Species differences in competitive behaviour in this context may exist as the same task produced no increase in aggressive behaviour in socially-housed chimpanzees or rhesus macaques (Bloomsmith et al., 2000; Platt and Novak, 1997).

The use of computer task has been associated with reductions in abnormal (stereotypic and self-directed) behaviours in rhesus macaques (Washburn and Rumbaugh, 1992), but apes show increases in anxiety-related self-directed behaviour, such as self-scratching (Elder and Menzel, 2001; Tarou et al., 2004), though this is likely to be related to the increasing difficulty of the task (Leavens et al., 2001). Washburn et al. (1991) noted that performance on computer game-like, food-rewarded tasks was improved when rhesus macaques could select from a range of tasks of which they had previous experience. While they preferred the easiest task they did occasionally choose those that were more difficult and the animals' perception of choice was anecdotally suggested to have improved their welfare. Computer enrichment may prove valuable as part of a long-term strategy, although in view of the increased aggression and anxiety that it may create in some instances precautions need to be taken to ensure either sufficient of these or other devices are available to help reduce tension.

#### *5.1.2. Video and television*

Primates can be attentive to television or videotapes, for example nature films or film of primates behaving normally, (e.g. Bloomsmith and Lambeth, 2000; Harris et al., 1999; Mahoney, 1992; Schapiro and Bloomsmith, 1995), but several researchers indicate that while they may affect other behaviours (e.g. observational behaviour and some stereotypies), on their own they have little or no effect on social- or self-aggression (Brent et al., 1989a; Plesker et al., 2001). The value of such visual stimulation to the animal remains questionable as those given the opportunity to bar-press to turn on videoclips or to advance still images do not appear to readily do so (Harris et al., 1999) unless they have been trained perhaps for a visual discrimination research project (Kiyama et al., 2003). However, in those that do learn this there is evidence to suggest that they find it enriching (e.g. Harris et al., 1999; Kiyama et al., 2003). Unfortunately, Harris et al. (1999) excluded four out of eight subjects from their study of singly-housed rhesus macaques for exhibiting 'unabated repetitive behavior', which included saluting and rocking so it is unknown what any later learning of videoclip activation would have had on these abnormal behaviours.

Primates also respond well to being able to see what is going on outside their room: singly-housed rhesus macaques in cages next to windows exhibit less abnormal behaviour (O'Neill, 1989) and chimpanzees' fear response to the sound of chainsaws is alleviated by being shown closed-circuit television images of this work going on outside their accommodation (Rumbaugh et al., 1989).

### *5.2. Music*

Evidence exists that loud noise is stressful to primates (e.g. Peterson, 1980; Pines et al., 2004) and that white noise may aid concentration for cognitive tasks (Carlson et al., 1997) or have a calming influence during blood sampling (Kawakami et al., 2002), yet there has been only limited investigation of the positive affects that music may have on captive primates. This remains true despite it being common for music or radio to be played by animal care staff in facilities, particularly laboratories (Reinhardt, 2004).

There is mixed evidence as to the extent to which playing music or other sounds to primates is a good source of sensory enrichment (Newberry, 1995) and different music may have different effects on activity levels in primates (e.g. Harvey et al., 2000). Brent and Weaver (1996) were able to demonstrate that a radio playing 'oldies' tunes reduced heart rate, but had no effect on behaviour, in singly-housed baboons, though it was felt that the absence of behavioural effects may be a product of non-social housing. Behavioural changes have, however, been observed in rhesus macaques with light jazz increasing affiliative behaviour (Novak and Drewsen, 1989) and classical music decreasing stereotypic and self-mouthing behaviour (O'Neill, 1989), and reducing concentration in baboons during cognitive tests (Carlson et al., 1997). Importantly, Howell et al. (2003) found that music reduced agitation and aggression in socially-housed chimpanzees, particularly in all-male groups and in the morning when disturbance and activity in the colony was at its highest. Social behaviour, primarily play, increased as did active exploration, particularly by females. In some studies additional control has been given to the animals by enabling them to turn the music or radio on or off, under such conditions they may play music for about 50% of the time (Markowitz and Line, 1989) and have reduced levels of stress and abnormal behaviour (Line et al., 1991a), and increased affiliative behaviour (Novak and Drewsen, 1989).

Whatever value music may have as sensory enrichment for primates it may not be appropriate for all captive contexts, for example in zoos. However, as an alternative habitat sounds or the vocalisations of conspecifics may be played to captive primates and it has been demonstrated that this may have enrichment value. For example recordings of tropical forest sounds played to gorillas were found to increase activity in adults and to decrease clinging behaviour in infants, while on the other hand an increase in stress-related behaviours was associated with sounds of staff and bonobo (*Pan paniscus*) vocalisations (Ogden et al., 1994). The vocalisations of conspecifics may have a more positive effect and gibbon duets calls played to Lar gibbons (*Hylobates lar*) increased both activity and vocalisation, which is important in forming and maintaining pair-bond and therefore may encourage more affiliative behaviour (Shepherdson et al., 1989).

Novak and Drewsen (1989) express some caution on the value of music (or other sound) for enrichment noting it should be compared with other stimulation that can be turned on and off. Reported benefits may derive as much from control aspects as from those relating to the masking of environmental noise reported by some authors (e.g. Carlson et al., 1997; Howell et al., 2003; Kawakami et al., 2002; Ogden et al., 1994).

### *5.3. Conclusion: Efficacy and variation in use of sensory enrichment*

The value of sensory stimuli for enrichment, particularly for reducing aggressive behaviours remains unclear. Indeed some efforts in this area may actually increase aggression and measures need to be taken to avoid this if other benefits are to be gained. There are also issues relating to patterns of use and effect that relate to sex (Howell et al., 2003; Platt and Novak, 1997; Plesker et al., 2001; Tarou et al., 2004), and age (Platt and Novak, 1997; Plesker et al., 2001) that may indicate this enrichment is of different value and efficacy to different individuals. Contradictory evidence also exists on whether housing conditions affect patterns of television use (Bloomsmith and Lambeth, 2000) or not (Brent and Stone, 1996) and while there is some evidence of habituation to video stimulation (Bloomsmith et al., 1990b; Bloomsmith and Lambeth, 2000; Brent et al., 1989a; Harris et al., 1999; Platt and Novak, 1997) this is much less the case with computer-assisted tasks due to changeable levels of difficulty (Platt and Novak, 1997; Tarou et al., 2004).

## 6. Social enrichment

It is well-established that most primates, particularly monkeys and apes are intensely social animals that in the wild live in family or extended social groups. They have evolved to cope with the tensions inherent in living with conspecifics, but also benefit from the social support, learning opportunities and added vigilance that are among the many benefits of group-living (e.g. see contributions in Smuts et al., 1987). In addition to learning how to manage social relationships with conspecifics it is also important that captive primates have positive relationships with the humans around them as this will reduce tension related to husbandry and management tasks (Reinhardt, 1997; Waitt et al., 2002). It is known that the presence of people particularly in large numbers at zoos (Chamove et al., 1988; Hosey, 2005; Skyner et al., 2004) or related to negative stimuli (e.g. Pond and Rush, 1983) can have a negative effect on primates. The possibility and evidence that positive interactions and training in particular can have an enriching effect on primates will not be discussed here. However it is important to note that primates can readily be trained to cooperate with a range of husbandry, veterinary and research procedures (e.g. Bassett et al., 2003; Rasmussen, 1991; Reinhardt, 1997; 2003, 2004; Scott et al., 2003) which can reduce the expression of aggression towards staff (e.g. Heath, 1989; Knowles et al., 1995; Savastano et al., 2003), reduce the use of sedatives and anaesthesia (e.g. Colahan and Breder, 2003; Laule et al., 2003; Wolfensohn and Honess, 2005), reduce stress for the animal (e.g. Bassett et al., 2003; Reinhardt, 2003; Savastano et al., 2003) and is relatively cheap (e.g. Luttrell et al., 1994; McKinley et al., 2003).

### *6.1. Aggression and social/solitary housing conditions*

Reinhardt (2004) points out that where primates are kept in cages, it practically impossible to provide a 'species-adequate living environment' and it seems likely that truly replicating a species' natural environment in captivity with all the opportunities and dangers it presents will never be possible or even desirable. Even in zoos, enclosures that are more natural to the visitor's eye may not elicit behavioural repertoires and patterns that are identical to those of wild conspecifics (Young, 2003). It is also true that the most basic, simple way of improving a primate's psychological well-being is to provide it with a partner or place it in an appropriate social group, preferably conspecific, enabling it to express at least some natural social behaviour (Bernstein, 1991; Novak and Drewsen, 1989; Novak and Suomi, 1988; Novak and Suomi, 1991; Reinhardt, 2004; Wolfensohn and Honess, 2005). Indeed unless a special case can be made (e.g. in laboratory primates certain protocols may alter the behaviour of the animal so making it socially incompatible: Bayne, 1991) the default position should be that all primates should be housed in pairs as a minimum and in appropriate groups where possible (Mason, 1991; Novak and Suomi, 1991; Reinhardt, 2004; Wolfensohn and Honess, 2005). It should be noted that while mixed species groupings occur in the wild (e.g. among some callithrichids and some guenons) and can be successful and even beneficial in captivity (e.g. for educative purposes in zoo), evidence suggests that forming mixed species groups can be problematic and lead to substantial problems (Bernstein, 1991).

The consequences of single-housing and other forms of social deprivation/separation for primates, particularly in a research setting, are discussed extensively elsewhere (e.g. Baker, 1996; Bicknese et al., 1989; Boccia et al., 1995; Gwinn, 1996; Mason, 1991; Novak and Suomi, 1991; Reinhardt, 2004; Ruppenthal and Sackett, 1979; Schapiro and Bushong, 1994; Woolverton et al., 1989; Young, 2003). In this section we confine ourselves to examining efforts to address levels of self-aggressive behaviour seen in singly-housed primates through social enrichment highlighting changes in the intensity and direction of aggression.

It may be expected that moving an animal from solitary to pair or other social housing may increase at least one aspect of aggression-that which is directed at another individual. While there may have been an opportunity to express this behaviour between animals housed in sensory contact (through aggressive gestures or vocalisations) social housing presents an opportunity for contact aggression. Anderson and Chamove (1980) maintain that, in a research setting, 'self-aggression might be considered preferable to social aggression in some instances', however, even though some individuals even in the wild may occasionally exhibit unusually high levels of social aggression a certain amount of aggression remains a natural part of the social life of primates (e.g. Bernstein, 1991; 2003; Honess and Marin, 2005; Majolo et al., 2003b; Novak and Suomi, 1991). Anderson and Chamove's point does not hold if, on the other hand, self-aggression reflects an animal that is a psychologically, neurologically and physiologically compromised or flawed scientific model and therefore the whole reason for maintaining it under the very conditions that cause this behavioural pathology is brought into question.

Anderson and Chamove (1980) describe self-aggression as behaviour that is 'abnormally frequent and/or intense threats or physical attacks directed towards one's own body which may or may not result in tissue damage' and while the *causes* of this behaviour remain unclear (see e.g. Reinhardt and Rossell, 2001) it nevertheless appears to be more common in singly-housed individuals and those that experienced socially restricted development (Anderson and Chamove, 1980, 1985; Brent et al., 1989b; Chamove et al., 1984; Gluck and Sackett, 1974; Pond and Rush, 1983; Reinhardt and Rossell, 2001; Woolverton et al., 1989). Furthermore this behaviour can be accentuated by a number of conditions including frustration (Gluck and Sackett, 1974), self-reward (Anderson and Chamove, 1980), excessive management disturbance (Jorgensen et al., 1998), withdrawal of enrichment (Bryant et al., 1988; Chamove et al., 1984), tension or anxiety related to human presence (Pond and Rush, 1983; Skyner et al., 2004) and social contexts (Anderson and Chamove, 1980; Erwin et al., 1973). It has been suggested that it arises as the result of the absence of 'appropriate social aggression targets' during development when young animals would otherwise be involved in play-fighting (Anderson and Chamove, 1980), of self-stimulation when in an otherwise unstimulating environment (Chamove et al., 1984), or of the development of a coping strategy to reduce arousal in distressing situations (Novak, 2003). The most extreme form of self-aggression is self-injurious behaviour, resulting in damage to the part of the body at which the behaviour is directed. It is relatively common among laboratory housed primates (e.g. Lutz et al., 2003a; Reinhardt, 2004; Reinhardt and Rossell, 2001) but is rare in zoo-housed species (e.g. Hosey, 2005; Hosey and Skyner, in press; Skyner et al., 2004) and in the wild (e.g. Grewal, 1981). It is uncorrelated (Lutz et al, 2003b) or even negatively correlated (Chamove et al., 1984) with other aggressive behaviour (social or externally directed).

Initial approaches to pairing or grouping animals were rather ad hoc, random and were frequently associated with unacceptable levels of incompatibility, serious injury and even death the response to which was a widespread trend towards single-housing of primates in laboratories and breeding centres (Bayne, 1991). In addition to management convenience this conservative approach has been supported by concerns about possible adverse effects of social housing including excessive aggression (wounding and death), social consequences for subordinate animals, disease and partner boredom (Reinhardt, 1990b; Reinhardt et al., 1995). However, basic research and systematic approaches towards determining compatibility before introductions have helped reduce risks associated with the process of social enrichment. Simple non-contact familiarisation between potential pair-mates may not be successful in avoiding aggression upon introduction and may, in some instances, actually encourage it (Bernstein, 1991). More complex approaches that examine general levels of aggression (Line et al., 1990b), partner preference (Crockett et al., 1994) or unilateral demonstrations of dominance before full introduction (Reinhardt, 1988; 1989, 1992c, 1994b; Reinhardt et al., 1995), may be needed to improve and better predict compatibility of partners. Reinhardt (1992c) points out that relationships may change and as a result initially successful pairings may still require separation at a later stage.

There is a considerable body of evidence of successful programmes for a range of species of removing animals from solitary to social housing, in pairs or into groups, with little or no serious aggression (e.g. Bayne et al., 1991b; Bernstein, 1991; Eaton et al., 1991; Gust et al., 1996; Kessel and Brent, 2001; Line et al., 1990b; Majolo et al., 2003b; Reinhardt, 1989, 1990b, 1992c, 1994b, 1994c; Reinhardt et al., 1988, 1989; Weed et al., 2003). However, not all introductions are so aggression-free as demonstrated by Line et al. (1990c) who, when pairing rhesus macaques that had been singly-housed for at least ten years, found that subsequent to social pairing and then grouping, the majority (10 of 13) sustained injury and one animal died. This was despite examining compatibility through unidirectional demonstrations of dominance beforehand. Nevertheless, with aggression dropping once the group was split in two, this social enrichment was still considered a success.

Abnormal behaviour, particularly self-aggression, can become deeply engrained in an animal's behavioural repertoire but can be reduced following social enrichment although it may prove difficult (Chamove et al., 1984; Kessel and Brent, 2001; Reinhardt, 2004; Warniment and Brent, 1997) although not impossible, to eliminate (Line et al., 1990b; Reinhardt, 1999, 2002; Weed et al., 2003).

### 6.1.1. Pair formation for enrichment

Most studies of social enrichment examine the affect of placing animals in isosexual pairs primarily to avoid unwanted breeding (Weed et al., 2003). Where breeding is not a problem adult males and females can be paired without severe social aggression and even a reduction of previous exhibited abnormal behaviour (Kessel and Brent, 2001). Where it is not desirable vasectomised males can successfully be paired with adult females and this has proved useful for successfully treating self-injurious behaviour in male rhesus macaques (Weed et al., 2003). However, this does involve anaesthesia and an invasive procedure, which carries a degree of risk for the animal and it may therefore not be desirable where other socialisation options exist. While pregnancy may be prevented male mating attempts during the non-breeding season may not and this is known to be related to increased male-directed aggression (Michael and Zumpe, 1970).

A considerable body of literature exists on successful pairing for social enrichment, particularly on laboratory housed macaque species. Some mixed results are reported with long-tailed macaques for example Line et al. (1990b) had over 83% (of 6 pairs) success, accompanied by a disappearance in self-aggressive behaviour, in creating all female macaque pairs, whereas Crockett et al. (1994) found compatibility between all 15 pairs of females but only in just over half of the same number of males, those that were not compatible were separated due to high levels of aggression including injury. Pre-introduction preference tests were no more able to predict compatible pairings than random selection (Crockett et al., 1994). Most of the studies of isosexual pairing of rhesus macaques for social enrichment have been conducted by Reinhardt and colleagues. Reinhardt (1992c) using the unidirectional dominance system to predict compatibility found high success (85% of 68 pairs) with isosexual pairing of previously singly-housed adult rhesus macaques for up to five years following pairing. In only one instance did incompatibility result from aggressive behaviour; in the other nine incompatible pairs this was due to inadequate food sharing. In another study (Reinhardt, 2002) a compatibility rate of 92% was achieved across a range of age and sex pairings (excluding adult male/adult female) the lowest success being in adult male pairs (87%) and highest in juvenile pairs (100%). Eaton et al. (1994) also found very infrequent aggressive behaviour in paired female rhesus macaques, which spent less time in abnormal behaviour than singly-housed. Isosexual pairing of stump-tailed macaques has proved to be equally successful and aggression-free at 88% (of 17 pairs: Reinhardt, 1990a) and 100% (of 8 pairs: Reinhardt, 1994c) and only very limited aggression in one of the total of 25 pairs.

Some studies have used a combination of adult isosexual pairing and adult-infant pairing reporting equally successful results with low levels of aggression. Reinhardt et al. (1987a) had a 90% success rate, lasting at least 11 months, with 29 mixed-and same-sex pairings of adult with infant (12–18 months) rhesus macaques, where the infants appeared to inhibit adult aggression and compatibility was not dependent on the adult's or the infant's origin, age or sex (Reinhardt et al., 1988). Further work by Reinhardt and colleagues (Reinhardt et al., 1989) in socialising unrelated rhesus macaques found low aggression (2% of all 176 animals were wounded) and high, long-lasting compatibility (ranging from 80–87%) in adult female-infant, adult male-infant, adult female and adult male pairs. Based on the success of these studies a substantial social enrichment (pairing) programme was implemented for a large colony of rhesus macaques at a major US primate research institution (Reinhardt, 1994b). This study reported similar results to others with high, long-lasting compatibility (from 80–93.8%) in adult isosexual and adult-infant pairs. Only eight out of 175 pairs formed from previously singly-housed animals were deemed incompatible due to high levels of aggression and this only resulted in serious wounding to three individuals (1 male and two females). Majolo et al. (2003b) even without pre-introduction familiarisation and predicting compatibility purely on pairing older with younger animals, report a success rate of almost 80% (of 28 pairs) in female common marmosets. Two pairs were incompatible due to wounding and a further seven were separated due to high levels of aggression.

### 6.1.2. Group formation for enrichment

Aggression associated with group formation is covered more thoroughly in Honess and Marin (2005), but a few brief examples will be covered here of studies where groups have been formed from previously singly-housed individuals. Just as with pair formation there are a number of examples where group formation is achieved with relatively low levels of social aggression. An absence of aggression-related problems has been reported in association with mixed sex group formation in both juvenile (Schapiro et al., 1994) and sub-adult rhesus macaques (Wolff and Ruppert, 1991). Indeed the addition of two adult male rhesus macaques to a group was not only free of serious aggression but these males also exhibited reduced levels of stereotypic and self-aggressive behaviour (Missakian, 1972). Gust et al. (1996) reported that eight previously singly-housed adult female pig-tailed macaques were successfully formed into a group with a male without an increase in aggression and no wounding despite increased stress compared to singly-housed controls. Only low levels of aggression (2 woundings) were associated with the formation of three groups of three baboons, both instances in the only all male group (Kessel and Brent, 2001). Bayne et al. (1991b) though finding no overall significant decrease in self-aggressive behaviour in seven (4 adult males, 2 adult females and 1 yearling female) capuchin monkeys (*Cebus apella*) nevertheless report that in only one (the yearling) did self-aggression increase, in one other it remained almost the same, there was a considerable decrease in two and it disappeared altogether in three.

### 6.2. Conclusion: Variation in efficacy of social enrichment

Despite individual variation in response to social enrichment (e.g. Bayne et al., 1991b) possibly relating to age/sexual maturity (Majolo, 2003b), temperament (Boccia et al., 1995), and sex (Crockett et al., 1994), it is nevertheless clear from the large number of successful introductions that social enrichment is both a feasible and practical alternative to single housing primates. Pairing should therefore be viewed as the minimum degree of social housing for primates and where possible this should be extended to group-housing, providing species-specific variation in natural group structure is considered. It is a common theme in the literature that this can be achieved at relatively low cost (e.g. Line et al., 1990b; Reinhardt, 2002) and, provided a systematic approach is taken to determining likely compatibility introductions, it need not be accompanied by undue or wounding aggression and may be prove valuable as behavioural therapy for animals that exhibited self-aggressive behaviour when housed alone.

Species-appropriate social housing has a number of benefits: in zoos it allows animals to be exhibited in a way that enhance their educative value through the display of species-typical natural behaviours and the creation of a more natural social context in which valuable lessons can be learnt that enhance an animal's survival chances should it form part of a reintroduction programme. Where animals are kept for research, social housing does not interfere with research protocols (blood and urine collection, drug administration, tethered blood collection, food intake studies and cranial implants: Reinhardt, 2002) and even produces a better research model due to lower disease (Reinhardt, 1990b; Schapiro and Bushong, 1994) and while some individuals of some species may appear less stressed when housed socially (e.g. Mendoza, 1991; Mendoza et al., 2000) others may suffer increased stress (e.g. Crockett et al., 1994; Gust et al., 1996) which may be ameliorated by social buffering (Boccia et al., 1995; Mendoza, 1991; Vogt et al., 1981) and affiliative behaviour (e.g. Boccia et al., 1989).

Reinhardt et al. (1995) present an ethological approach to pair formation that is designed to significantly increase the chances of success through minimising or even avoiding aggression. The likelihood of successful, compatible social enrichment may also be increased by reducing aggression through grouping or pairing animals of a similar history and avoiding socialising those previously housed in proximity where they were able to display dominance behaviour without consequences as they may be more aggression than with strangers (Bernstein, 1991; Ruppenthal and Sackett, 1979). Success may be further improved by housing same sex pairs away from members of the opposite sex to avoid sexual competition (Majolo et al., 2003b; Reinhardt, 1992c).

## 7. Mixed programmes of enrichment

In a number of instances researchers have examined the impact on the behaviour of captive primates of a mixture of enrichment strategies drawn from some or all of the categories discussed above. Frequently this is simply to investigate the effect of enrichment per se on the animals rather than the value of specific enrichment items or the features of items that make them enriching. A number of examples have already been discussed where the effect of combinations of enrichments have been examined for positive effects on primate behaviour (e.g. Brent and Stone, 1996; Brent et al., 1989a). In this section a number of key studies that test a more complex array of enrichment will briefly be outlined.

As discussed earlier, simply adding space to animal's accommodation is rarely sufficient to encourage them to exhibit a better balanced behavioural repertoire including more species-typical behaviour or to affect a reduction in the levels of abnormal or aggressive behaviour. In adding furniture and or moveable objects to this space an effect may be achieved but the influence of specific items may be difficult to isolate (e.g. Brent et al., 1991; Wilson, 1982). Indeed while it might not always be possible to attribute effects it is still possible to examine preferences for different items under these conditions, for example Reinhardt

(1990a) noted that paired stump-tailed macaques presented with PVC perches and sticks still showed a preference for interacting with their cage mate.

Although in some mixed programmes of enrichment (e.g. Turner and Grantham, 2002) it may not be possible to separate the effect of individual items either from each other or from the effect of their combination, some studies (e.g. Schapiro and Bushong, 1994; Schapiro et al., 1996b) have incorporated experimental design that allows this. Schapiro and Bushong, (1994) found that a range of physical, feeding and sensory enrichment whilst having a positive effect on behaviour made no difference to physical health as measured by the amount of veterinary attention required, however enriched animals took longer to recover once they had developed a problem. The degree of social housing did affect health with more veterinary attention being required by singly- (diarrhoea treatment) and group-housed (trauma) animals than by those that were pair-housed. Using a similar array of enrichment devices Schapiro et al. (1996b) compared the effects of social and inanimate environmental enrichment on year-old rhesus macaques and found that inanimate enrichment had no effect on aggression levels in group-housed subjects compared to unenriched controls. However, there were some positive behavioural changes (increase in play and exploration) in singly-housed subjects related more to feeding and physical devices than with sensory (video) devices, yet the greatest positive effect on behaviour was produced by the social enrichment.

This demonstrates that the first option for enriching the lives of captive primates is social enrichment. However even where that might not be an option, a mixed programme of physical, feeding and sensory enrichment can have a dramatic effect in preventing behavioural pathologies often associated with single-housing. Turner and Grantham (2002) showed with a mixed programme of enrichment for singly-housed long-tailed macaques including toys, novel food and forage, television, and additional space that over just five weeks resulted in an absence of self-inflicted trauma compared to a 25% occurrence in unenriched controls. A mixed enrichment programme also positively affected singly-housed male capuchins (Boinski et al., 1999): foraging boxes and plastic toys presented separately and together were associated with increases in normal and decreases in abnormal behaviour (including aggression towards staff, other animals and self). Importantly the benefits of this enrichment were supported by plasma cortisol measures of stress which decreased as normal behaviour increased in response to changing levels of enrichment.

## 8. General discussion

In examining the literature it becomes clear that living in impoverished or unenriched environments has a number of consequences, including (in the case of young animals) the increased likelihood of becoming subordinate (Ruppenthal and Sackett, 1979) and that these effects are most pronounced where animals are socially restricted. It is not surprising therefore that environmental enrichment can have such dramatically positive effects both on levels of aggression and abnormal behaviour as demonstrated in many of the studies examined above and that the positive effect of increasing enrichment are supported by lowered stress levels (Boinski et al., 1999). Efforts are not always straight forward with evidence of both a wide variation in the uptake and efficacy of specific enrichment (depending on species, sex, age, rearing history and personality) and, where it has been shown to reduce aggression there is also evidence of effect reversal once the enrichment is removed (e.g. Bayne and Dexter, 1992; Bryant et al., 1988; Neveu and Deputte, 1999; Weld and Erwin, 1990).

Given a choice animals may not always show a preference for an enriched area over a barren one, however what interaction they do have with the enriched area may still be successful in reducing abnormal behaviour (Bayne et al., 1992b). Where they are presented with a range of options social enrichment appears to be preferred to, and more effective than, inanimate enrichment (e.g. Reinhardt, 1990a; Schapiro et al., 1995b; 1996b); providing the most appropriate stimulation in what is otherwise a socially unstimulating environment (Reinhardt et al., 1995). The value of social enrichment in reducing and even eliminating self-directed aggression, including extreme self-injurious behaviour, is clearly demonstrated. Provided a systematic approach is taken, such as that of Reinhardt et al. (1995), it is remarkable the extent to which previously singly-housed primates can be paired or grouped with only very low levels of social aggression.

Despite the fact that appropriate environmental enrichment has been demonstrated to be both effective and cheap (e.g. Beirise and Reinhardt, 1992; Dexter and Bayne, 1994; Reinhardt, 1994d; Reinhardt, 2004) there are those who advocate caution or are prescriptive about enrichment based largely on arguments relating to financial cost (e.g. Woolverton et al., 1989) or the safety of animals (Eckert, 1999; Eckert et al., 2000; Schapiro and Bushong, 1994) and staff (e.g. Dexter and Bayne, 1994). These concerns are outweighed by the clear benefits demonstrated in the studies examined in this article, including: improved validity of research models (more reliable, accurate and therefore cost effective science), generally lower veterinary interventions (in enriched group-housed animals with less disease and self-injury), avoidance of possible euthanasia and expensive replacement of research subjects (with accompanying loss of research time and data), and not least the reduction of contingent suffering which makes justifiable research programmes easier for society to support. However, it should not be overlooked that increased environmental enrichment is likely to increase some levels of variability in research subjects and that efforts to control for this may ultimately lead to a more primates being used in research

(Novak and Suomi, 1991). In a review of this nature which necessarily paints a poor, but improvable, picture of the lives of captive primates, it is important to stress that considerable progress has been made in the provision of more welfare friendly environments for captive primates, particularly those housed in laboratories (Box and Scott, 1995). An example of this is that there is now an increasing acceptance that it is even possible to socially-house primates with cranial (Reinhardt, 1991; Reinhardt et al., 1989; Roberts and Platt, 2004; Wolfensohn and Peters, 2005) and telemetric implants (Honess and Macdonald, 2003; Rasmussen, 1991).

It may be unnecessary and to an extent impossible and undesirable to reproduce an environment for captive primates that exactly replicates all aspects encountered by wild conspecifics (e.g. presence of predator and disease pressure), but this is rarely what is being advocated. At the other extreme however, the parameters set down in regulations governing the maintenance of captive animals and environments are all too often seen as goals, rather than the minimum standard which all should be exceeding. The demands of captive contexts may be very different: zoos needing to maintain and exhibit animals in a representative environment that enhances their educational value; and laboratories needing to maintain and use animals that are valid models of their species, and its biological processes, that enhances their scientific value. While these ends are quite different the means are not dissimilar as both require animals that are physically and psychologically healthy, exhibiting behavioural repertoires that are as natural as possible and without behavioural pathologies. Although zoo environments do not necessarily have a negative effect on primate welfare (Hosey, 2005) captivity does present challenges for primates and there is a need for continuing progress in developing programmes of enrichment if boredom and habituation are to be continually overcome and animals remain healthy. Conducting rigorous research in some areas of enrichment (e.g. using an A–B–A design: Young, 2003) may create an ethical dilemma. With strong circumstantial evidence that an enrichment option is beneficial perhaps in reducing aggressive or abnormal behaviour it is not easy to justify its removal to measure a post-exposure effect. However, it is important that enrichment research is systematic, hypothesis and prediction driven and incorporates suitable controls if its value and impact are to be maximised (Newberry, 1995; Young, 2003). It needs to continue to examine and develop different enrichment options that address the specific needs of different taxa, sexes, ages, backgrounds and personalities if its effects are to be maximised (e.g. Box and Scott, 1995; Poole, 1991). For a truly successful programme of enrichment to be designed, tested and implemented it is important that it complements, or at least does not conflict with, research or education protocols (Spinelli and Markowitz, 1985) and that a full range of expertise is brought to bear; a suitable team would include primatologists, veterinarians, animal care staff, engineers, physiologists, facility managers, occupational health and health and safety professionals (Spinelli and Markowitz, 1985; Wolfensohn and Honess, 2005).

Although it has been claimed that ‘there are few if any demonstrable adverse effects of individual housing in adult primates’ (Woolverton et al., 1989) the evidence examined above and by Honess and Marin (2005) clearly indicates otherwise in relation to both stress and aggression, and although there is a measure of contrary or ambiguous evidence that highlights some of the mistakes made in enrichment and cage expansion exercises (see Woolverton et al., 1989) these do not of themselves warrant abandoning the process of refinement of living conditions for captive primates through enrichment.

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## *ANEXO 7*-

# Assessment of stress in non-human primates: the application of a novel technique”.

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# Assessment of stress in non-human primates: application of the neutrophil activation test

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## Abstract

*A technique measuring leukocyte (neutrophil) activity was used to examine differences between stress levels in a breeding colony of rhesus macaques housed in either a traditional caging system or open-rooms. The leukocyte activation test measured the degree to which blood from the two treatment groups could launch a further neutrophil response (superoxide production) to an* in vitro *challenge. Animals housed in a traditional caging system produced a significantly lower leukocyte response than animals housed in open-rooms, indicating that there was a higher level of stress associated with caged housing than open-room housing. This was not influenced by whether animals were physically restrained or trained to stand for a sedating injection. No differences were found between treatment groups in leukocyte numbers or composition. This study validates the use of the leukocyte activation test to assess physiological stress levels in non-human primates and demonstrates the animal welfare benefits of open-room housing over traditional laboratory caging systems.*

**Keywords**: *animal welfare, housing refinement, leukocyte activity,* Macaca mulatta*, rhesus macaques, stress*

## Introduction

### Caging and stress

A considerable body of literature exists on the influence of housing conditions on the physiological measures of primate stress (eg Crockett *et al* 2000; Mendoza *et al* 2000; Honess & Marin 2005). Research demonstrates that stress levels cause changes that affect a number of biological functions, including immune competence (Moberg 1985; Raberg *et al* 1998; Maule & VanderKooi 1999), brain structure and function (Uno *et al* 1989; Sapolsky 1996, 2003), and reproduction (Carlstead & Shepherdson 1994; Pottinger 1999; Ha *et al* 2000).

There is a clear effect of overall cage size (eg Draper & Bernstein 1963; Paulk *et al* 1977), and its structural complexity (eg Reinhardt 1996; Röder & Timmermans 2002; Buchanan-Smith *et al* 2004; Wolfensohn & Honess 2005), on the psychological well-being of primates. An increase in space and its enrichment allows increases in socialisation and creates opportunities for training animals to cooperate with husbandry and veterinary procedures (Wolfensohn 2004). This has clear implications for the maintenance of captive animals for research and for breeding.

### Measurement of stress

Traditional measurements of physiological stress have focused on the detection of cortisol levels in the blood plasma, faeces, urine or saliva (Mendoza *et al* 2000; Theorell 2003), or on monitoring autonomic responses, such as changes in heart rate or blood pressure (Porges 1985; Line *et al* 1989a,b). The implantation of telemetry devices to measure cardiovascular parameters may compound and confound stress measures and less invasive methods of monitoring are ethically more acceptable. However, the collection of faeces, urine or saliva samples for cortisol assay is practically complex, particularly from group-housed animals on a forage substrate. Furthermore, the interpretation of cortisol assay results is complicated by considerable individual variation (Montane *et al* 2002), a natural circadian variation in cortisol levels (Sousa & Ziegler 1998; Mendoza *et al* 2000; Theorell 2003), the fact that a cortisol response is associated with some non-stress stimuli, and that some stress responses may not involve elevated cortisol levels (Moberg 2000). Although low concentrations of cortisol are typically associated with low stress, chronically stressed primates are known to exhibit hypocorticolism (Mendoza *et al* 2000). These problems have contributed to an increasing dissatisfaction with the use of cortisol to measure stress levels (Crockett *et al* 2000; Moberg 2000).

This study monitors the activity of leukocytes, which have been shown to increase oxygen uptake in response to bacterial challenge in order to produce oxygen free radicals that kill bacteria (Hu *et al* 1999; Halliwell & Gutteridge 2000). Psychological stress produces the same response (Ellard *et al* 2001) and can also influence the number and distribution of leucocytes and the expression of their

**Table 1 Analysis of leukocyte activity (relative light units [RLUs]) in rhesus macaques kept in a traditional caging system versus open room housing conditions. Figures in brackets are one standard error, ns = non significant, ** = $P < 0.01$.**

| | Leukocyte activity | | | Leukocyte numbers and composition | | | |
|---|---|---|---|---|---|---|---|
| | PMA $10^{-3}$ mol $l^{-1}$ | PMA $10^{-5}$ mol $l^{-1}$ | Unchallenged | Leukocytes $10^{9}$ $l^{-1}$ | % Neutrophils | % Lymphocytes | Neutrophil-lymphocyte ratio |
| Caged (n = 10) | 7960.68 (1178.46) | 2069.49 (400.33) | 1923.25 (402.11) | 9.76 (1.32) | 58.30 (6.68) | 40.50 (6.51) | 2.48 (0.77) |
| Open room (n = 43) | 16157.11 (2840.28) | 3258.02 (525.09) | 1605.92 (150.86) | 9.38 (0.55) | 62.47 (2.43) | 36.37 (2.40) | 2.28 (0.24) |
| Mann-Whitney *U* | 97.00 | 184.00 | 190.00 | 215.00 | 186.50 | 185.00 | 185.00 |
| P value (2-tailed) | 0.007** | 0.481 ns | 0.570 ns | 1.000 ns | 0.517 ns | 0.495 ns | 0.495 ns |

**Table 2 Analysis of the effect of capture technique (standing for injection versus being netted) on leukocyte activity (RLUs) in rhesus macaques in open rooms. Figures in brackets are one standard error, ns = non significant.**

| | PMA $10^{-3}$ mol $l^{-1}$ | PMA $10^{-5}$ mol $l^{-1}$ | Unchallenged |
|---|---|---|---|
| Standing (n = 13) | 24129.67 (8933.49) | 3473.01 (773.25) | 1759.42 (301.11) |
| Netted (n = 22) | 13766.96 (1219.71) | 3856.59 (884.81) | 1668.72 (222.37) |
| Mann-Whitney *U* | 136.0 | 134.0 | 131 |
| P value (2-tailed) | 0.811 ns | 0.759 ns | 0.682 ns |

adhesion receptors (Dhabhar *et al* 1995; Kang *et al* 1996; Goebel & Mills 2000). While changes in cell numbers and adhesion molecules are not exclusively associated with altered cellular activity and function (Mian & Marshall 1993), an extensive range of physical and psychological stressors do produce a rapid response in leucocytes that can affect the immune system's ability to cope with an ongoing or potential challenge (Dhabhar *et al* 1995).

This technique has already been demonstrated as an appropriate measurement of psychological stress in humans (Ellard *et al* 2001; Mian *et al* 2003) and in wild badgers in the field (McLaren *et al* 2003; Montes *et al* 2004). This study aims to examine its use in non-human primates by investigating the stress levels of rhesus macaques (*Macaca mulatta*) housed in different conditions (a traditional caging system versus open-rooms) and the extent to which any difference in stress levels is affected by capture method (physical restraint versus trained standing for injection).

## Materials and methods

### Study subjects

The animals used in this study were part of the Oxford University rhesus macaque breeding colony housed at Harlan, UK. This specific pathogen free colony consisted of approximately 200 monkeys including 16 single male breeding groups of 4–11 females and their unweaned infants, which were transferred to peer groups at 12–18 months. All macaques were group-housed indoors in either a traditional caging system of three linked modules of typical reinforced stainless steel two-tier laboratory cages (each module: 1.20 × 1.20 × 2.0 m, width × depth × height; total: 2.88 × 3 = 8.64 $m^3$), or in open-rooms (approximate average total of 22.8 $m^3$ per group) containing either these cages with the fronts removed or no caging at all. These different housing conditions resulted from a progressive move toward all open-room housing as part of a programme of refinement of the colony's husbandry and management (Wolfensohn 2004).

All housing conditions had a woodchip forage substrate spread on the floor. Each room contained a flexible arrangement of wooden furniture, wooden shelves and hanging items (eg tyres), had ceramic tiles or forex sheeting (Lonza Ltd, Basle, Switzerland) on the walls, protected light and electrical fittings, and a secondary door. Waste solids were removed every two days and the substrate changed weekly; rooms were thoroughly washed every 10–14 days, and cages were thoroughly washed every 7–10 days. The animals were fed three times per day: in the morning with monkey chow pellets (Harlan Teklad, Madison, Wisconsin, USA); around noon with foraging mixture (as of Davys 1995); and mid-afternoon with whole, fresh fruit. Water was available *ad libitum*. The animals were maintained under a 12:12h light:dark lighting regime at a constant temperature (15–24°C) and humidity (45–65%), in accordance with the UK Home Office Code of Practice for the Housing and Care of Animals in Designated Breeding and Supplying Establishments (Home Office 1995).

### Capture, sedation and blood sampling

This study used 53 adult rhesus macaques aged between 8 and 20 years: 10 from traditional cages (3 males, 7 females) and 43 from open-rooms (6 males, 37 females). These animals had been primarily raised in breeding units, having been weaned at 6–12 months and group-housed for more than 5 years. Those housed in traditional cages were sedated by injection following physical restraint using a squeeze-back mechanism; those in open-rooms were either trained to stand and present their hind-quarters for injection when a technician entered the room (Wolfensohn 2004) or were caught and physically restrained using a net before being injected.

Sedation was via an intramuscular injection of ketamine hydrochloride at a concentration of 100 mg ml$^{-1}$ , and at a dose of 10 mg kg$^{-1}$ (Ketaset, Fort Dodge, USA). Blood samples were obtained during routine health screening carried out under the Veterinary Surgeons Act 1966 (available at http://www.rcvs.org.uk/Templates/Internal.asp?NodeID=89679) by needle venepuncture of the femoral vein into an EDTA Vacutainer (BD Vacutainer Systems, Plymouth, UK). 30 µl of this blood was taken immediately for the leukocyte assay; the remainder was used for measuring haematology parameters (Celltac MEK–5108K: Kohden, Japan) and differential cell counts from alcohol-fixed blood smears using May-Grunwald and Giemsa stains.

### Measurement of leukocyte activity

As described by McLaren *et al* (2003), luminol (5–amino–2, 3–dihydrophthalzine [Sigma A8511]: Sigma-Aldrich Company Ltd, Poole, Dorset, UK) fluoresces in the presence of oxygen free radicals and is therefore used to measure blood chemiluminescence levels. The method used in this study is based on the studies by Mian *et al* (2003) and McLaren *et al* (2003). Appropriate concentrations of luminol ($10^{-4}$ mol l$^{-1}$) and the microbial product PMA (phorbol 12–myristate 13–acetate [$10^{-3}$ mol l$^{-1}$ and $10^{-5}$ mol l$^{-1}$] [Sigma P8139]: Sigma-Aldrich Company Ltd, Poole, Dorset, UK) were used to measure luminescence (relative light units [RLUs]) in a portable chemiluminometer (Junior LB 9509: EG & G Berthold, Germany) for 30 s, every 5 min, over a 45 min period. Three tubes were prepared for each animal's sample: an unchallenged sample (10 µl of blood and 90 µl of luminol), and two with 10 µl of blood, 90 µl of luminol and 10 µl of PMA at each of the two concentrations (high concentration = $10^{-3}$ mol l$^{-1}$ and low concentration = $10^{-5}$ mol l$^{-1}$). Tubes were agitated to mix the contents; when not in the luminometer tubes were maintained at 37°C in a water bath. Blood was challenged with two concentrations of PMA to control for any differential response to a weak or strong concentration. An unchallenged sample allowed assessment of background leukocyte activity.

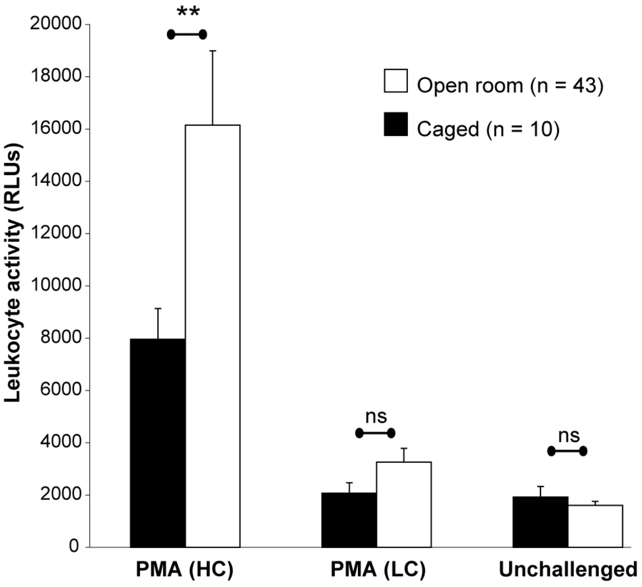
### Statistical analyses

Leukocyte activity data were derived from calculating the area under the response curve and dividing by the number of neutrophils, obtained from the differential cell count, to produce a response value that reflected the total response per neutrophil. Data from the two groups (a traditional caging system versus open-rooms) were compared using the non-parametric Mann-Whitney *U* test, using SPSS 11.5® for Windows®.

## Results

Comparison of the leukocyte activity of differently housed rhesus macaques revealed that macaques housed in a traditional caging system produced a significantly lower response than those housed in open-rooms to challenge with PMA at high concentration (Mann-Whitney $U = 97.0$, $P = 0.007$) (see Figure 1 and Table 1). A lower response was also found for caged monkeys with the low concentration of PMA, but this was not significant ($U = 184.0$, $P = 0.481$). There was no significant difference in background, unchallenged activity ($U = 190.0$, $P = 0.570$) (see Table 1), and no significant differences were found between the two housing types in the number or composition of leukocytes (see Table 1).

**Figure 1**



Leukocyte activity in blood challenged with two different concentrations of PMA (HC: $10^{-3}$ mol l$^{-1}$; LC: $10^{-5}$ mol l$^{-1}$) in rhesus macaques housed in a traditional caging system versus open rooms. Bars represent the mean total leukocyte activity (+ standard error of the mean) over 45 min, divided by the number of neutrophils ($10^9$ l$^{-1}$); ns = non significant, ** = $P < 0.01$.

As this result could be confounded by differential response to capture technique, ie standing for injection versus physical restraint (net or squeeze-back mechanism), the data from open-room-housed animals, where the capture method (netting or standing) was known, were tested. No significant difference was observed in the leukocyte response to challenge with PMA (see Table 2).

## Conclusions

There is considerable evidence to suggest that stress may reduce the effectiveness of the immune system, therefore increasing the risk of infection or disease (Dhabhar *et al* 1995; Kang *et al* 1996; Raberg *et al* 1998). Research suggests that even short-term stress can produce demonstrable and immediate physiological changes in heart rate, blood pressure and the activation of leukocytes (Ellard *et al* 2001, Mian *et al* 2003); these changes depend on the nature and intensity of the stressor (Willard *et al* 1989; McLaren *et al* 2003). In humans, epidemiological studies support the idea that individuals who are more stressed have a suppressed immune system (eg Graham *et al* 1986); stress has also been linked to disease in farm animals (eg Koolhaas *et al* 1999). Therefore, a reduction of immune reactivity as an indicator of stress is not a novel observation (Murata 1989; Haigh *et al* 1997; Maes *et al* 1998). Indeed

McLaren *et al* (2003) recently reported that in the absence of other underlying pathology an alteration the leukocyte activity is a good indicator of stress.

The animals used in this study were of an extremely high health status, being part of a specific pathogen free colony, where they were subjected to regular health screening and continuous surveillance by trained staff. These screenings indicated that there was no difference in the underlying pathology between the treatment groups. All other parameters for the treatment groups were the same as they were housed under regulated conditions and received the same nutrition. This, coupled with the evidence of accommodation size-related stress (see *Caging and stress*), and clear differences in the space provision in this study, strongly supports that a reduction in the leukocyte responsiveness is an indicator of differences in stress levels, as supported by the published literature (eg Willard *et al* 1989; McLaren *et al* 2003; Mian *et al* 2003; Montes *et al* 2004; Mian *et al* 2005).

This study shows that the leukocyte activation test is appropriate for the measurement of stress in non-human primates, specifically in rhesus macaques. Lower leukocyte response to *in vitro* PMA challenge in animals housed in a traditional caging system, compared with open-room housed animals, demonstrates that cage housing is associated with higher levels of physiological stress. Results also show that this effect was not affected by whether individuals were physically restrained or trained to stand for a sedative injection. A significant difference in the response of the treatment groups to only one of the two PMA concentrations suggests that the leukocyte population may contain functionally different types of neutrophils, responding to different levels or nature of challenge. Where those types that respond to less stressful stimuli have already been used, a further mild challenge may not result in a significant response. Eliciting a further response may, as in this case, require a more powerful stimulus, eg a higher concentration of PMA. The composition of the neutrophil population by type may vary following an acute stressor or as a result of living in chronically stressful conditions.

Unlike previous studies using this technique (ie McLaren *et al* 2003; Montes *et al* 2004), differences in leukocyte activity between the treatment groups were not accompanied by differences in leukocyte number or composition. This may be attributable to differing demographics of the leucocyte populations in the two treatment groups. Stressed animals may be recruiting more immature leukocytes (ie band neutrophils) to replace those used in the stress response, which are represented in cell counts but are not sufficiently mature to emit superoxide in response to a challenge. Therefore, more and less stressed animals may have the same leukocyte population sizes, but more stressed animals may have a greater proportion of neutrophils that are too immature to respond to a further challenge; therefore the total neutrophil response would be less.

## Animal welfare implications

This study provides evidence that open-rooms are less stressful than a traditional caging system for groups of rhesus macaques. This leukocyte activation test can be used to indicate the immune competence of animals, highlighting any requirement for additional health monitoring or modification of management timetables, which may include further potentially stressful events (eg change in group composition, weaning of offspring etc). The combination of additional veterinary attention and deferral of further stressful events will optimise the physical and psychological health of an animal, which may otherwise be compromised by compounding an already heightened stress level. This has implications for the animal's reproductive potential as well as its value as an experimental model, as high stress levels are well known to negatively impact on both these factors.

The leukocyte activation test also allows the measurement of physiological stress using much smaller blood samples than for traditional blood cortisol measures (30 μl versus approximately 2 ml). This small amount can be obtained by capillary sampling from a heel prick on trained animals, avoiding the need for sedation and the possible negative consequences (eg haematoma, phlebitis) associated with needle venepuncture sampling. This technique confirms the refinement of housing in the study colony, allows refinement of the management of the animals, and is conducive to the refinement of the sampling technique, with a decrease in blood sample size to approximately 30 μl.

It should, however, be remembered that this technique may not be practical in some situations, for example, in some zoos and sanctuaries where blood sampling may be acceptable for veterinary/diagnostic purposes but not for research purposes where, in the UK, a Home Office licence would be required.

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