Use of Nonhuman Primates in Research in Sweden: 25 Year Longitudinal Survey

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Summary
All scientific studies published in journals in which research was performed on nonhuman primates in Sweden between 1979 and 2003 (N=1038) were identified and sampled according to species used, area of research, research class and geographic location. Results showed that nonhuman primates were used in various scientific disciplines and that the number of articles published peaked in the early 1990s. Macaca fascicularis and M. mulatta were the most commonly used species. During the sample period projects related to genetics, HIV, and neuroscience increased, whereas projects related to dentistry, ophthalmology and physiology seem to have disappeared.

Zusammenfassung: Einsatz von nicht-menschlichen Primaten in der Forschung in Schweden: eine Übersicht über 25 Jahre
Für diese Übersicht wurden alle Studien über Forschungen mit nicht-menschlichen Primaten ermittelt, die in Schweden zwischen 1979 und 2003 (N=1038) durchgeführt und publiziert wurden. Die Kategorisierung erfolgte nach Arten/Gattungen, Forschungsbereichen, Forschungsklassen und geografischer Lage. Die Ergebnisse zeigten, dass nicht-menschliche Primaten in zahlreichen wissenschaftlichen Bereichen verwendet wurden und dass die Anzahl der Publikationen einen Höhepunkt in den frühen 90er Jahren erreichte. Die in der Forschung am häufigsten verwendeten Arten waren die Macaca fascicularis und M. mulatta. Während der untersuchten Periode nahm die Zahl der Forschungsprojekte in den Bereichen Genetik, HIV und Neurologie zu, während es in der Zahnheilkunde, Ophthalmologie und Physiologie praktisch keine Projekte mehr gibt.

Keywords: nonhuman primates, Sweden, literature survey

1 Introduction

Nonhuman primates (NHPs) have been used as models for humans in many areas of biomedical research for many years. Proponents of the use of NHPs tend to emphasise that because humans share many anatomical, physiological, behavioural and genetic characteristics with other primate species, NHPs will continue to serve a critical role in many biomedical research areas in the years ahead (Kaup and Schwibbe, 2002). Opponents to the use of NHPs for scientific purposes in research tend to stress both moral and scientific concerns (Goodman and Check, 2002).

There is extensive literature on the ethics of using NHPs in research (e.g. Beck, 2001; Boyd Group, 2002). There is however no consensus on this among all stakeholders worldwide. However, many agree that, provided NHPs should be used at all in invasive research, their use should be minimised. This is for example the basis for legislation for the protection of all vertebrate animal species used for scientific purposes in the EU (86/609/EEC, 1986) and in Sweden (Djurskyddslagen, 1988). The present paper will not assess whether it is ethical to use NHPs in research or not. The focus is on the research that was carried out in Sweden during the past 25 years.

Recent use of different species in different areas of research in Sweden has been described in a cross sectional survey (Hagelin, 2004a). Results suggested that Sweden accounted for 3% of articles using live NHPs or some lower level of biological material derived from NHPs in Europe. *Chlorocebus aethiops* (25%) and *Macaca spp.* (54%) were involved in three out of four studies. Within the use of live NHPs, Sweden accounted for about 3% of the total number of NHPs used in these studies. The Swedish sample was too small to draw any statistically valid conclusions, but results indicated that neuroscience, HIV, biochemistry, genetics and microbiology were among the most frequent areas of research.

The few longitudinal surveys elucidating the use of NHPs in research that have been published consist of database searches of either the general development (Brinkman, 2000), of an individual country (Guillén Salazar and Pons-Salvador, 1996) or an individual primate centre (Schwibbe, 2002). A longitudinal approach describing the use of live NHPs or some lower level of biological materi-

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al derived from NHPs in research in Sweden has, as far as the author knows, not been conducted. The aim of the present study was to elucidate the use of NHPs in research in Sweden during the past 25 years, between 1979 and 2003. Taxonomy, area of research, geographic location and type of procedure served as dependant variables.

2 Materials and methods

2.1 Data collection

All original articles that could be identified which 1) used live NHPs or some lower level of biological material derived from NHPs, 2) were published in the years 1979 to 2003, and 3) in which the work had been conducted in Sweden, were scrutinised. PrimateLit was searched to identify articles. A Medline search was also performed using the keyword *Vero* (Rhim et al., 1969). This search was performed because PrimateLit does not cover all articles that include the use of *Vero* cells (African green monkey cell line). Only those articles deriving from journals that had an impact factor according to ISI Journal Citation Reports were included in the final sample for analysis. Overall, 866 NHP articles containing 1038 studies in 321 different journals were identified and scrutinised.

2.2 Articles and studies

An article was considered to contain more than a single study if more than one species was used and/or more than one of the four experimental classes defined below were employed. This may be illustrated by the following example: if one animal was first subjected to some chronic procedure and then euthanised and its cells and tissues were utilised, the study was classified only as chronic. If cells or tissues were obtained from another animal than the one used in the chronic study, the research was classified as two studies. Articles related to genetics sometimes contained more than one species (Tab. 3 and related results).

2.3 Area of research

Sampling was done according to the focus of the journal. For multidisciplinary journals, articles were scored according to which heading they fit best.

2.4 Geographic location

The geographic location in which the project was undertaken was sampled rather than the address of the primary author. This was done because a minority of authors were based at geographic locations where there has never been any animal department containing NHPs. Swedish based scientists have also conducted projects abroad. These articles, when identified, were excluded from the final sample.

2.5 Experimental classes

All studies were sorted into four different classes as in Hagelin (2004a), apart from the exclusion of acute studies, i.e. experiments carried out under non-recovery anaesthesia, conducted during the sample period: 1) The chronic invasive study was characterised by the NHP being conscious during at least some part of the experiment. Procedures may have varied from routine blood sampling to more invasive procedures in which the subjects may have suffered pain, distress and/or lasting harm. All studies, except one veterinary case study (Ödkvist and Schauman, 1980), sorted into this class in the present sample involved procedures that were treated as animal experiments according to Swedish legislation (Djurskyddslagen, 1988). 2) The chronic non-invasive study implied that no physiological manipulation was performed on the subject. Some of the studies may have included psychological manipula-

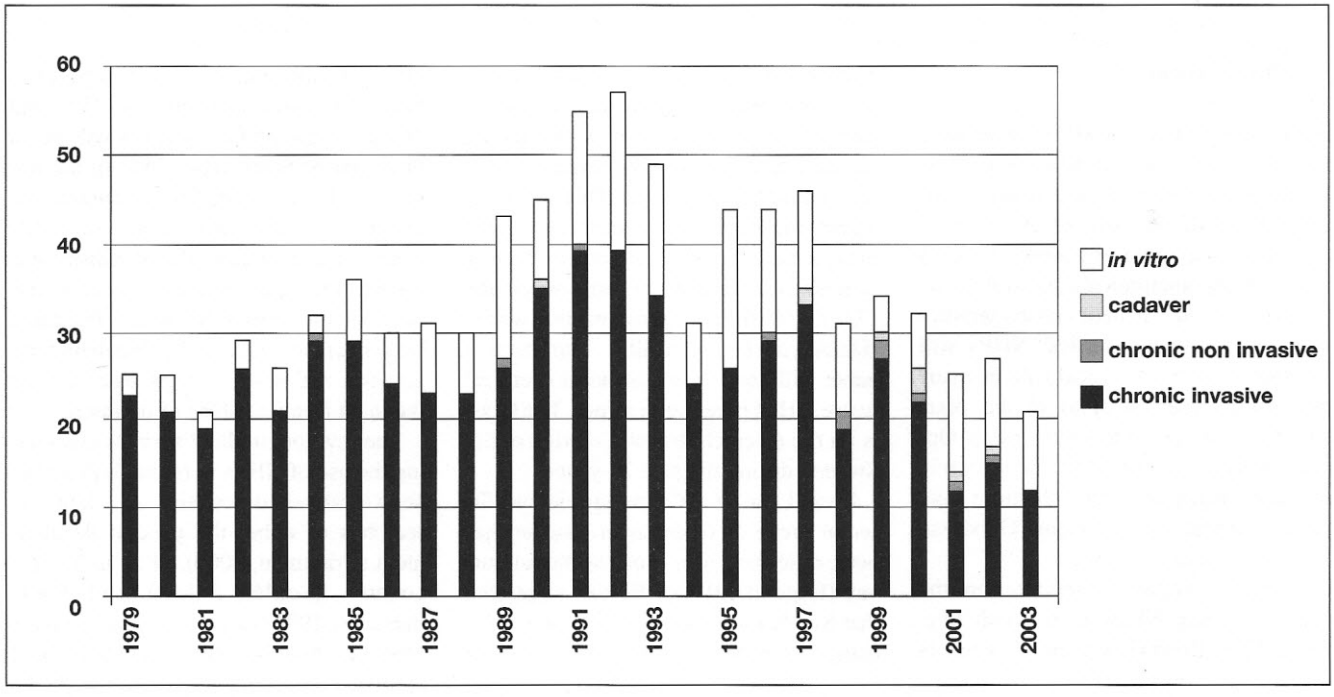

Fig. 1: Number of articles according to experimental class between the years of 1979 and 2003 (N=866).

tions induced by the scientists, but no needle penetrated the body surface of the NHP(s). All studies sorted into this class in the present sample involved procedures that were treated as animal experiments according to Swedish legislation (Djurskyddslagen, 1988). 3) The cadaver study, in which the NHP(s) were euthanised by the authors or a professional in the same setting prior to the actual experiment, comprised the third category. Unlike legislation in other countries, these studies, excluding veterinary case studies, are treated as animal experiments according to Swedish legislation (Djurskyddslagen, 1988). However, in the present sample, all cadaver studies utilised material from subjects that had been euthanised for unrelated medical reasons, and these are not considered animal experiments according to Swedish legislation. 4) The *in vitro* study, in which only cells and/or tissues were used, comprised the fourth category. The biological materials used were often obtained from commercial companies or museums, but could also be body hair or faeces collected from cages. Data derived from GenBank, a database that collects all publicly available DNA sequences, was also included in this category.

2.6 Statistics

Chi square analysis, using SAS/STAT (SAS Inc, Cary, NC, USA), was employed to measure differences between proportions. $P<0.05$ was considered significant.

3 Results

The results of Fig. 1 suggest that the number of published articles peaked between 1989 and 1997. Overall, 72.3% of the articles were classified as chronic invasive, 25.6% as *in vitro*, 1.2% as non-invasive, and 0.9% as cadaver. The number of articles classified as chronic invasive in relation to the total number of articles decreased in the last three years of the sampling period compared to the years 1998-2000 ($P<0.04$). The number of articles classified as *in vitro* in relation to the total number of studies increased from 1985 onwards ($P<0.001$).

The three most frequent areas of research were neuroscience (20.2%), dentistry (12.7%) and pharmacology (10.9%), cumulatively accounting for 43.8% of all articles (Tab. 1). Comparing the period 1979-1983 with 1999-2003, articles related to dentistry ($P<0.001$), ophthalmology ($P<0.05$) and physiology ($P<0.001$) decreased drastically. Articles related to genetics ($P<0.001$), HIV ($P<0.001$) and neuroscience ($P<0.001$) increased during the same period.

Most research published was carried out in Stockholm or Uppsala (Tab. 2). There were notable differences in areas of research between geographic locations. Use of live NHPs for dentistry research was more frequently conducted in Gothenburg ($P<0.001$) and Stockholm ($P<0.001$). Ophthalmologic research was primarily located in Uppsala, i.e. both use of live NHPs ($P<0.001$) and *in vitro* studies ($P<0.001$). Genetics primarily took place in Lund ($P<0.01$) or Uppsala ($P<0.01$). HIV research was conducted in Stockholm, both using live NHPs ($P<0.001$) and *in vitro* studies ($P<0.05$). Use of live NHPs in neuroscience was primarily conducted in Stockholm ($P<0.03$) and Uppsala ($P<0.02$). Use of live NHPs in pharmacology was primarily conducted in Uppsala ($P<0.03$). Microbiological research was primarily conducted in Stockholm, i.e. both use of live NHPs ($P<0.03$) and *in vitro* studies ($P<0.01$).

In total, 35 species were named in the study sample. Of these 35 species, 18 were involved in projects that included the use of live NHPs in research (Tab. 3).

Tab. 1: Developments of areas of research over time (N=866, frequencies and percentages)

	1979-1983	1984-1988	1989-1993	1994-1998	1999-2003	Total	%
Biochemistry, Chemistry	10	18	19	9	6	62	7.2
Dentistry	28	36	25	21		110	12.7
Endocrinology, Reproduction	10	13	12	4	4	43	5.0
Ophthalmology	18	19	24	13	9	83	9.6
Genetics	2	2	6	16	18	44	5.1
HIV		3	39	26	16	84	9.7
Neuroscience	8	28	49	51	39	175	20.2
Pharmacology, Toxicology	23	21	21	16	13	94	10.9
Physiology	12	9	6	4		31	3.6
Surgery, Transplantation	1	2	12	2	5	22	2.5
Microbiology	8	8	27	19	13	75	8.7
Other	9	4	9	9	12	43	5.0
Total	**129**	**163**	**249**	**190**	**135**	**866**	**100**
%	**14.9**	**18.8**	**28.8**	**21.9**	**15.6**	**100**	

M. fascicularis (46.4%) and *M. mulatta* (14.1%) cumulatively accounted for more than 60% of all studies, and live NHPs of these two species were involved in projects during the whole sampling period. Live *Aotus trivirgatus* were used primarily between 1986 and 1996. *Cercopithecus aethiops*, *Cebus apella* and *Papio* spp. were primarily used before 1990. *Callithrix jacchus* was used during the whole period except for the years 1985 to 1993. *Saguinus oedipus* was only used before 1982, and finally *Saimiri sciureus* was used from 1988 onwards.

4 Discussion

NHPs continue to be used for scientific purposes in Sweden. The number of articles published by Swedish based scientists using live NHPs in their research ranged from 12 to 39 per year during the sampled period. The number of articles using live NHPs has halved since 1993 (Fig. 1). By far the two most common species in studies published during the past 25 years were *M. fascicularis* and *M. mulatta* (Tab. 3). The number of studies classified as *in vitro* increased from the mid 1980s onwards. This seems to follow general trends in biomedical research. *C. aethiops* is the original source of kidney cells used for *in vitro* studies (Rhim et al., 1969). These cells are most often obtained from commercial companies and were frequently used in biochemistry and microbiology. Use of *Vero* cells seemed to have increased in recent years.

Comparing the present results with those reported in Hagelin (2004a), the research conducted in Sweden appears to be focused on a few areas of research; neuroscience, HIV, and genetics (Tab. 1 and 2). Further research areas such as behavioural studies, biochemistry, and microbiology (excluding HIV) may be less common compared to other countries in Europe.

NHPs are diverse and each species occupies a distinct ecological niche. Housing and husbandry should take into account both species and individual differences (Segal, 1989). Sufficient veterinary skills and responsible management may enhance animal welfare and minimise the number of subjects used in research projects and avoid problems with behavioural and nutritional deficiencies. Currently there are two major facilities which house NHPs used for scientific purposes in Sweden. These two facilities are located in Stockholm (opened in 2002) and Uppsala (opened in 1994). A number of smaller facilities located at universities have been closed since the mid 1990s (Erlandsson, 1994).

All use of live apes in research was recently banned in Sweden (Statens Jordbruksverk, 2003). In the present sample, two articles classified as chronic invasive involved the use of live apes (Tab. 3). One was an endocrinological study (Axelsson et al., 1984), and the other was a veterinary case study (Ödkvist and Schauman, 1980). There were also two articles, classified as chronic non-invasive, in which live apes were involved (Gustavsson et al., 1990; Schedin et al., 1997). All four articles seemed to be of relatively mild severity and subjects involved were zoo animals. Considering that live apes were hardly used at all during the past 25 years (Tab. 3), this regulatory change will not have any major impact on the use of NHPs in research in Sweden. However, it should be noted that a few Swedish based scientists have conducted projects in the US using *Pan* spp. during the sample period (data not shown).

Comparisons over time revealed that studies on HIV and studies utilising Positron Emission Tomography (PET) techniques became more frequent over the sample period (Tab. 1). The vast majority of the former projects involved *M. fascicularis* and were carried out at the Swedish Institute for Infectious Control (SMI) or its predecessor before 1994, the National Bacteriological Laboratory (NBL). HIV is currently one of the main areas of invasive research in which live NHPs are used (Smith, 2002; Hagelin,

Tab. 2: Geographic location and area of research (N=866, research classes 1+2 (i.e. use of live NHPs) and 3+4 (i.e. use of lower level of biological material derived from NHPs))

	Gothenburg		Lund/Malmö		Stockholm/Linköping[1]		Uppsala/ Umeå[1]	
	1+2	3+4	1+2	3+4	1+2	3+4	1+2	3+4
Biochemistry, Chemistry		4	5	11	2	13	8	19
Dentistry	25	2	9		72	2		
Endocrinol., Reproduction	1		7	2	16	5	11	1
Ophthalmology		1	9	3	17		43	10
Genetics		2		16		4		21
HIV	2	2	2	1	68	11	1	2
Neuroscience	2		11	1	90	13	50	3
Pharmacology, Toxicology	6	1	6		39	4	35	3
Physiology	6		4	3	6	2	9	1
Surgery, Transplantation			6		11		5	
Microbiology	1	5	1	10	18	32	2	6
Other	3	1	4	2	10	8	12	3
Total	**46**	**18**	**64**	**49**	**349**	**94**	**176**	**69**

[1]Linköping had 13 articles (eleven in column 1+2, and two in column 3+4). Umeå had six articles (all in column 3+4).

2005). Worldwide the main body of this research is carried out in the US. The PET technique has been available since the late 1970s. In the present sample, PET scanning was most commonly utilised in studies related to neuroscience and pharmacology and is the explanation for the relative increase in studies observed during the study period. All PET studies were carried out in Stockholm or Uppsala.

Over the last 25 years research related to dentistry, ophthalmology and physiology using NHPs has almost vanished (Tab. 1). There may be several explanations for this decline: 1) NHPs may have been replaced by other types of models. 2) Mergers and acquisitions in the pharmaceutical industry may have cancelled programmes or research may have been transferred to other countries. For example, the ophthalmology research programme conducted by Kabi Pharmacia (now part of Pfizer) was cancelled in the mid 1990s. 3) The scientists may have gone into retirement or relocated abroad and no one has continued this type of research in Sweden.

Scientists at universities or national governmental centres led the vast majority of the published projects, but some were also led by the pharmaceutical industry. The other major Swedish based pharmaceutical company during the study period, Astra (now Astra-Zeneca), primarily conducted their research using NHPs in Germany or the UK, although some research programmes were also conducted in Stockholm (Erlandsson, 1993). Other pharmaceutical companies, e.g. Leo and Medivir, also conducted some NHP research during the period.

Overall, it is likely that the species used and the magnitude of research conducted during the past 25 years reflect the availability of animals, scientists and funding of research, and to some extent public opinion towards the use of animals in research (Hagelin et al., 2003a; Hagelin, 2004b) as well as regulatory hurdles that must be overcome before a project is approved (Hagelin et al., 2003b). The results of Hagelin et al. (2003b) indicated that there were 52 applications involving the use of NHPs for which ethical committees requested changes prior to project approval between the years of 1989 and 2000 (an approved application is valid for three years and may result in more than one publication or none at all). Considering that only projects belonging to experimental classes 1 and 2 in the present sample were subjected to review by ethics committees, the present results indicate that the use of NHPs in research

Tab. 3. Species use according to areas of research (N=1038, experimental classes in columns: 1+2 (i.e. use of live NHPs), and 3+4 (i.e. use of lower level of biological material derived from NHPs), frequencies and percentages)

	B/C		D		E/R		O		G		H		N		P/T		P		S/T		M		Other	
	1+2	3+4	1+2	3+4	1+2	3+4	1+2	3+4	1+2	3+4	1+2	3+4	1+2	3+4	1+2	3+4	1+2	3+4	1+2	3+4	1+2	3+4	1+2	3+4
Apes																								
Gorilla spp.					1					17											1	3	1	
Pan spp.		1				3			5	33		3										8	2	1
Pongo spp.						2			1	10												4		
Lower apes																								
Hylobates spp.						1			1	8		4												
Old World																								
C. aethiops	1	8	10		5	5	1			4	1	3	3	2	1		2	1			2	38		1
M. fascicularis	3	20	92	4	11	3	61	12		2	67	9	78	9	43	3	10	1	13		17	9	10	6
M. mulatta	3	7	4		15	3	2	2	1	5		3	51	4	21	4	7	1	2		1	3	7	
Other *Macaca* spp.							1	1		3		1		1		1					1	2		
Papio spp.	1	5			2	1	1	3	1	14			3	1	1		3	1	2		1	5	3	6
Other Old World	1				2					1		2		1									1	
New World																								
C. jacchus	1	1							1	3			8	1	7							1	1	
C. apella	3				2	1	1			2			2		9		1						1	
A. trivirgatus								1		1			4								1		1	1
S. oedipus		1				1		1		3												1	1	
S. sciureus	2	4			1	1	3			2		1	10	1	6		1	1	5			2	2	
Other New World						1				5												1		
Prosimians																								
All prosimians									6											2	1			
Tarsiers																								
T. bancanus									1	1														
Unspecified NHP		6				1		1				1	1	1			1	2					1	1
Total	**15**	**53**	**106**	**4**	**39**	**23**	**70**	**21**	**11**	**120**	**68**	**27**	**160**	**21**	**88**	**8**	**25**	**7**	**22**	**0**	**26**	**78**	**30**	**16**
%	**1.4**	**5.1**	**10.2**	**0.4**	**3.8**	**2.2**	**6.7**	**2.0**	**1.1**	**11.6**	**6.6**	**2.6**	**15.4**	**2.0**	**8.5**	**0.8**	**2.4**	**0.7**	**2.1**	**0**	**2.5**	**7.5**	**2.9**	**1.5**

Abbreviations used: B/C = biochemistry, chemistry; D = dentistry; E/R = endocrinology, reproduction; O = ophthalmology; G = genetics; H = HIV; N = neuroscience; P/T = pharmacology, toxicology; P = physiology; S/T = surgery, transplantation; M = microbiology.

has received considerable attention from the committees during this period. Further research is needed to verify this.

The present study has some shortcomings. Although most NHP research in Sweden is likely to be conducted in academic or governmental settings nowadays and is likely to be designed for publication of results in peer reviewed journals, some other types of users have conducted a proportion of this research in the past. These include various industries and governmental institutes (e.g. NBL/SMI used NHPs for vaccine safety testing up until the mid 1990s).

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