„Sozialverhalten, Reproduktionsbiologie und Wohlergehen der Mongolischen Wüstenrennmaus, *Meriones unguiculatus* (Milne Edwards 1867)“

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Inhaltsverzeichnis

1 Einleitung

1.1 Biologie und Lebensweise der Mongolischen Wüstenrennmaus

Die in dieser Habilitationsschrift berücksichtigten Veröffentlichungen befassen sich mit der Mongolischen Wüstenrennmaus (*Meriones unguiculatus* Milne-Edwards 1867). Ihr wissenschaftlicher Name entstammt sowohl dem Griechischen (Meriones=Krieger) als auch dem Lateinischen (unguiculatus=mit Krallen). Systematisch zählen sie zur größten Säugetierordnung, den Rodentia und werden aufgrund des einheitlichen Baus der Masseter und der Backenzähne der artenreichsten Unterordnung, den Myomorpha zugeordnet. Hinsichtlich der Familienzugehörigkeit ergeben sich je nach gewähltem Schrifttum unterschiedliche Auffassungen, wobei hier der derzeit im europäischen Raum verbreiteten Literatur der Vorzug gegeben wird, wonach diese Art zur Familie Cricetidae und Unterfamilie Gerbillinae gezählt wird (Corbet 1978).

Die Mongolische Wüstenrennmaus ist ein Steppentier und bewohnt in ihrer chinesisch-mongolischen Heimat die Randgebiete der Wüste und nicht etwa deren Inneres, wie man aus ihrem Namen schließen könnte. Ihr Lebensraum ist vor allem die Halbwüste und Steppe nördlich und östlich der Wüste Gobi. Ihr Verbreitungsgebiet erstreckt sich von den Randgebieten des Altaigebirges (südliche Mongolei) nach Osten bis zur Stadt Harbin (Mandschurei) und nach Süden in den Nordosten Chinas etwa 200 km nördlich von Peking. Die nordwestliche Verbreitung ist noch nicht eindeutig geklärt. Dieser Lebensraum ist durch ein kontinentales Klima geprägt. Es ist recht trocken, im Sommer sehr heiß und im Winter sehr kalt. Die jährliche Niederschlagsmenge beträgt lediglich 250 bis 500 mm/qm Regen und Schnee. Regen fällt überwiegend in den Monaten des Frühjahrmonsuns.

Die Mongolische Wüstenrennmaus hat sich durch ihren Körperbau und ihre Lebensweise gut an diese extremen Witterungsbedingungen angepasst. So gibt sie beispielsweise hochkonzentrierten Urin und nahezu trockenen Kot ab und kann ihren Wasserbedarf fast ausschließlich durch die Aufnahme von Tau oder pflanzlichem Material decken. (Bannikov 1954). Ihr Nahrungsspektrum erstreckt sich auf Wermut (*Artemisia*), verschiedene Grassamen, Buchweizen (*Fagopyrum*) und eine Vielzahl ein- und mehrjähriger Pflanzen. (Naumov & Lobachev

1975, Ågren et al. 1989a). Je nach Bodenbeschaffenheit legt die Mongolische Wüstenrennmaus unterirdische, netzartig verzweigte Baue variabler Tiefe an (Bannikov 1954, Naumov & Lobachev 1975, Roper & Polioudakis 1977, Ågren et al. 1989b). Obwohl sie nicht wie die Hamster über Backentaschen verfügt, zeigt sie ausgeprägtes Eintrageverhalten und legt in ihren Bauen umfangreiche Futterkammern an (Bannikov 1954, Naumov & Lobachev 1975).
Durch Untersuchungen unter Laborbedingungen ist bekannt, dass Mongolische Wüstenrennmäuse, die bei der Geburt zwei bis drei Gramm wiegen, als Nesthocker in ihren ersten Lebenstagen über eine nur ungenügend ausgeprägte Fähigkeit zur Thermoregulation verfügen. Daher sind sie auf die Wärme und den Schutz der Eltern im Nest überlebensnotwendig angewiesen. Nach etwa vier Wochen werden sie entwöhnt, und mit zehn (Weibchen) bzw. zwölf Wochen (Männchen) sind sie geschlechtsreif und bei permanenter Paarhaltung werfen sie erstmals in der 20. bis 28. Lebenswoche (Gattermann et al. 1986). Unter Laborbedingungen erreichen sie ein Lebensalter von drei bis vier Jahren. Weibchen haben einen Postpartum-Östrus mit einer mittleren Zykluslänge von vier bis sechs Tagen, nach einer Tragzeit von 24 – 26 Tagen werden etwa sechs Junge geboren. Adulte Gerbils erreichen Körpermassen von 60 bis 80 Gramm, wobei die Männchen im Mittel zehn Gramm schwerer sind als die Weibchen. Seit einigen Jahren wird die Mongolische Wüstenrennmaus in verstärktem Maße in tierexperimentellen Laboratorien gehalten. Sie dient unter anderem als Versuchstier für die medizinische Grundlagenforschung (z.B. in der Epilepsie- und Schlaganfallsforschung) und für verhaltensbiologische und soziobiologische Untersuchungen. Der erste Laborstamm wurde bereits 1935 in Japan etabliert und nach ihrer Einfuhr in die USA 1954 durch Victor Schwentker erfolgte die weltweite Verbreitung dieser in der angloamerikanischen Literatur auch als Gerbils bezeichneten Labortiere (Schwentker 1963, Marston & Chang 1965, Marston 1972, Wesley 1974).

1.2 Sozialverhalten und Reproduktionsbiologie

Betrachtet man Säugetiere aus verhaltensbiologischer Sicht, so ergibt sich nahezu zwangsläufig die wissenschaftliche Analyse ihres Sozialverhaltens. Die mehr als 4250 rezenten Säugetierarten (Ziswiler 1976) unterscheiden sich nicht nur in ihrem Aussehen, sondern auch in ihrem Verhalten und in ihrer sozialen Organisation. Da aber alle Säuger während der Fortpflanzungszeit mit ihrem Geschlechtspartner zusammenkommen, sind sie zumindest zu diesem Zeitpunkt auf Kontakte zu Artgenossen angewiesen, so dass kein Säugetier als nicht-sozial zu bezeichnen ist. Bei solitären Arten, wie zum Beispiel dem Goldhamster (*Mesocricetus auratus*) bleiben solche unmittelbaren Kontakte kurzfristig auf die Fortpflanzungsphase beschränkt (Gattermann et al. 2001). Dennoch gilt auch für diese Arten wie für Säugetiere insgesamt, dass ein wesentlicher Teil ihres Lebens auf Interaktionen mit Artgenossen beruht (Hendrichs 1978, von Holst 1987, Sachser 1994). Oft leben Säugetiere jedoch zeitlebens in Gruppen zusammen, wobei sie bei Feindabwehr, Brutpflege oder Nahrungserwerb über vielfältige Formen kooperativen Verhaltens verfügen können. Diese Kooperation setzt unter anderem auch eine zeitliche Synchronisation des Verhaltens voraus. Dabei kommt den circadianen Rhythmen eine zentrale Bedeutung zu, die bei Säugern durch ein hypothalamisches Kerngebiet, den suprachiasmatischen Nuklei (SCN) bzw. der „inneren Uhr“, generiert werden. Die Länge der resultierenden Periode ist genetisch fixiert und art- und individualspezifisch abweichend von 24 Stunden. Der Hauptzeitgeber, der die innere Uhr immer wieder auf die Tageslänge justiert, ist der Tag-Nacht-Wechsel. Daneben werden aber auch, insbesondere unter konstanten Lichtbedingungen (Dauerdunkel bzw. Dauerlicht) andere periodische Veränderungen als mögliche Zeitgeber postuliert. Dies schließt soziale Faktoren mit ein (Erkert & Schardt 1991, Goel & Lee 1995). Es erscheint plausibel, dass sich derartige soziale Zeitgeber insbesondere bei solchen Arten entwickelt haben, die in sozialen Verbänden leben und eine ausgeprägt hohe soziale Appetenz aufweisen. Um dies zu überprüfen, wurde eine mögliche soziale Synchronisation bei zwei Kleinsäugerarten mit unterschiedlicher sozialer Organisation, dem solitär lebenden Goldhamster und der in Familienverbänden lebenden Mongolischen Wüstenrennmaus (*Meriones unguiculatus*) im Rahmen einer vergleichenden chronobiologischen Studie untersucht (**Kap. 1.2: Lack of social entrainment of circadian activity rhythms in the solitary golden hamster and in the highly social Mongolian gerbil**).

Selektion findet auf der Individualebene statt (Dawkins 1976). Es ist das „Bestreben“ eines jeden Individuums, seine genetische Information durch erfolgreiche Erzeugung und Aufzucht von Nachkommen in die nächste Generation weiterzugeben. Die Reproduktionsbiologie nimmt aus diesem Grund eine zentrale Stellung im Sozialverhalten der Säugetiere ein. Im Zuge verhaltensökologischer und soziobiologischer Betrachtungsweisen stehen hierbei Nutzen/Kosten-Analysen der Individuen im Vordergrund, da beim Fortpflanzungsverhalten ein Interessenkonflikt zwischen den beteiligten Partnern besteht und sowohl Männchen als auch Weibchen auf die Maximierung des eigenen Fortpflanzungserfolges selektioniert sind, was in einen sexuellen Konflikt der Paarungspartner mündet (Übersicht in Birkhead & Parker 1997). Bei weiblichen Nagetieren sind Gravidität und Laktation häufige Ereignisse, die zumeist mit hohen physiologischen Kosten verbunden sind (Galef 1983). Als geeignete Parameter, um solche belastenden Lebensumstände messen zu können, haben sich unter anderem die Herzschlagfrequenz, die Körpertemperatur und die Aktivität erwiesen (Aschoff et al. 1971, Gattermann 1983, von Holst 1985, Tornatzky & Miczek 1993). Durch den Einsatz implantierter Transmitter in Verbindung mit einem telemetrischer Erfassungssystem konnten wir auf der Basis dieser drei Parameter erstmals den physiologischen Zustand und die Veränderungen während der Gravidität und der Laktation bei einer Mongolischen Wüstenrennmaus ohne erkennbare Nebeneffekte dokumentieren und darüber hinaus den potenziellen Nutzen dieser Methode für weitere ähnliche Studien darlegen (**Kap. 1.1: Measurement of physiological parameters and activity in a Mongolian gerbil during gravidity and lactation with an implanted transmitter**).

Soziale Einflüsse können die Fortpflanzungsfähigkeit hemmen - zum Beispiel durch einen negativen Einfluss von Glukokortikoiden auf das Luteinisierende Hormon (LH) - oder fördern – durch Zyklussteuerung, Synchronisation der Fortpflanzung und durch die Induktion der Ovulation (Bronson 1989, Sapolsky 1993) Der weibliche Sexualzyklus generell ist durch rhythmische Änderungen sowohl des physiologischen Status als auch des Verhaltens gekennzeichnet. Weibliche Säuger entwickeln Zyklen mit einer Periodenlänge von 24 Stunden < τ < 28 Tagen. Wie Studien an verschiedenen Kleinsäugern gezeigt haben (De

Kock & Rohn 1971, Carmichael et al. 1981, Wollnik & Turek 1988, Kerbeshian et al. 1994,) verändern sich sowohl physiologische als auch ethologische Parameter zyklusabhängig. Bei der Mongolischen Wüstenrennmaus liegt ein infradianer Sexualzyklus vor. Die verfügbare Literatur zur Zykluslänge und der Beschreibung der einzelnen Stadien war jedoch uneinheitlich und zum Teil widersprüchlich (Marston & Chang 1965, Nishino & Totsukawa 1996). Verlässliche Angaben zu dem aktuellen Zykluszustand eines Weibchens sind aber insbesondere dann von entscheidender Bedeutung, wenn Paare oder Gruppen neu formiert werden sollen, da vor allem das aggressive Verhalten des Weibchens im Verlauf des Zyklus starken Schwankungen unterliegt. Um hierzu zuverlässige Angaben zu erhalten, waren sowohl das Paarungsverhalten als auch die vaginalzytologische Determination der vier verschiedenen Stadien Gegenstand einer detaillierten Untersuchung (**Kap. 1.4: Mating behavior during the estrus cycle in female Mongolian gerbils (*Meriones unguiculatus*)**). Durch den erfolgreichen Abschluss dieser Studie und darauf aufbauend, konnte dann überprüft werden, ob auch die lokomotorische Aktivität als ein nicht-invasiver Marker geeignet ist, Aussagen zum Sexualstatus der Mongolischen Wüstenrennmaus zu treffen (**Kap. 1.5: The oestrus of female gerbils, *Meriones unguiculatus*, is indicated by locomotor activity and influenced by male presence**). Da Vertreter dieser Art unter Freilandbedingungen Stammpaare bilden und zwischen dem Stammweibchen und -männchen eine ausgeprägte soziale Beziehung besteht (Ågren 1984, Ågren et al. 1989a, 1989b), wurde darüber hinaus untersucht, welchen Einfluss die Anwesenheit von Männchen auf die Stabilität des Sexualzyklus der Weibchen hat.

Da gerade bei Säugetieren die endogenen und exogenen Faktoren in zum Teil sehr komplexer Weise miteinander in Beziehung und Wechselwirkung stehen, ist neben dem aktuellen Reproduktionserfolg eines Individuums auch dessen Fortpflanzungsleistung im Verlauf des gesamten Lebens in Betracht zu ziehen (Bronson 1989). Nur so ist es möglich, die jeweiligen reproduktiven Strategien zu erkennen und damit das aktuell beobachtete Verhalten zu verstehen. Dieser Ansatz, der im Rahmen von Life-history Studien verfolgt wird, analysiert die individuelle Lebensdauer, das Alter und die Größe zum Zeitpunkt der Geschlechtsreife, den Kompromiss zwischen eigenem Wachstum und der energieintensiven Reproduktion, die Entscheidung hinsichtlich Zahl und Größe der Nachkommen sowie dem Investment in den aktuellen Nachwuchs gegenüber zukünftigen Fortpflanzungsversuchen. Damit erlauben derartige Untersuchungen essentielle Einblicke in die zeitliche Organisation und Strategie sowohl innerhalb als auch zwischen den Arten (Übersicht in Daan & Tinbergen 1997). Insbesondere die auf Williams (1966) zurückgehende Auffassung, wonach die Kosten der Reproduktion einen trade-off zwischen aktueller und zukünftiger Fortpflanzung widerspiegeln, hat sich als ein herausragendes Element der Life-history Theorie erwiesen. Dabei wirkt sich nach Trivers (1972) das elterliche Investment in die bereits vorhandenen Nachkommen nachteilig auf die Reproduktion in der Zukunft aus, woraus ein Eltern-Jungtier Konflikt (parent-offspring conflict) um die vorhandenen limitierten Ressourcen resultiert (Trivers 1974). Als eine Möglichkeit, diese optimal auszunutzen, hat sich bei einigen Säugetierarten eine Tendenz zur Kooperation entwickelt. Obwohl sich hauptsächlich die Weibchen um den Nachwuchs kümmern, können auch die Väter die Überlebenschancen ihres Nachwuchses steigern. Dies kann sowohl direkt durch das Wärmen der Jungen als auch indirekt durch Nestbau oder die Verteidigung der Jungen realisiert werden. Die Mongolische Wüstenrennmaus bildet unter natürlichen Bedingungen Familienverbände von 2-15 Individuen unterschiedlichen Alters und Geschlechts (Ågren et al. 1989a). In diesen existiert nur ein reproduktiv aktives Weibchen, die anderen adulten Weibchen sind sexuell unterdrückt (Norris & Adams1974; Payman & Swanson 1980; Salo & French 1989). Dominante Männchen verteidigen das Territorium und es lagen nach Laborexperimenten erste Hinweise darauf vor, dass sie sich auch an der Aufzucht der Jungen beteiligen (Elwood 1975, 1979, Waring & Perper 1980). Darüber hinaus helfen weitere Familienmitglieder bei der Jungenaufzucht, z.B. durch länger andauernden Nestaufenthalt und damit verbundenem Wärmen der Jungen sowie dem Eintragen von Futter (Ostermeyer & Elwood 1984). Der Verbleib als Helfer in der Geburtsfamilie und die damit verbundene Brutpflegeerfahrung an den jüngeren Geschwistern führt zu einer vorzeitig einsetzenden und gesteigerten Reproduktion, einer beschleunigten Entwicklung der Helfer und damit letztlich zur Steigerung ihrer individuellen Fitness (Salo & French 1989). In Laborexperimenten wurden derartige Langzeiteffekte der Anwesenheit alloparentaler

Individuen auf die Reproduktion bestätigt (French 1994). Die bisher verfügbaren Daten, vor allem zu dem paternalen Anteil bei der Brutpflege, stammten allerdings nur aus Kurzzeitbeobachtungen (90 Minuten pro Familie), die darüber hinaus auch nur zur Lichtzeit, also der Ruhezeit der Tiere durchgeführt wurden. Im Rahmen einer Langzeitstudie wurde deshalb dieses für die Sozialstruktur der Mongolischen Wüstenrennmäuse so wesentliche Element, der mütterliche als auch der väterliche Anteil an der Jungenaufzucht von der Geburt bis zur Entwöhnung, detailliert über jeweils sechs Tage quantifiziert (**Kap. 1.3: Parental care and time sharing in the Mongolian gerbil**). Dabei lag das Hauptaugenmerk auf dem Nachweis einer zeitlichen Synchronisation bzw. eines „time sharings“ in der Brutpflege.

1.3 Wohlergehen

Insbesondere bei Tieren, die sich in menschlicher Obhut befinden, steht deren Wohlergehen im Zentrum verhaltensbiologischer Forschung. Reize aus der Umwelt können auch neuroendokrine Veränderungen hervorrufen, die langfristig zu chronischen Krankheiten bis hin zum Tod führen können (Henry & Stephens 1977, Henry 1982, Übersicht in Sachser 1994). Trotz der Vielzahl der einwirkenden Stressoren sind diese Reaktionen jedoch begrenzt. Die Adaptation an Bedrohungen erfolgt vor allem durch die Aktivierung des Sympathikus-Nebennierenmark- sowie des Hypophysen-Nebennierenrinden-Systems. Innerhalb von Sekunden nach Stressorwirkung erfolgt die Ausschüttung von Adrenalin und Noradrenalin aus dem Nebennierenmark und dem Sympathikus, was nach Cannon (1929) als „fight-flight-reaction“ bezeichnet wird. Mit einigen Minuten Verzögerung steigen dann auch die Glukokortikoidkonzentrationen an (Gärtner et al. 1980), die an einer Vielzahl biologischer Prozesse beteiligt sind (Glukoneogenese und -speicherung in der Leber, Eiweiß- und Fettabbau sowie die Hemmung des Körperwachstums) und in Belastungssituationen der schnellen Bereitstellung von Energieträgern dienen. Beide Stressachsen fungieren relativ unabhängig voneinander und bei chronischen Stimuli erfolgt eine langfristige Aktivierung eines der beiden Systeme, die zu vielfältigen Erkrankungen des Organismus führen kann (Übersicht in Sachser 1994). Das Hypophysen-Gonadensystem ist ebenfalls von sozialen Faktoren beeinflussbar (Benton et al.

1978, Coe et al. 1979, Sachser & Pröve 1986). Hohe Ränge und aktives Bemühen um Ressourcen und Fortpflanzungspartner führen zu erhöhter Gonadenaktivität, Niederlagen und niedrige Ränge dagegen vermindern diese Aktivität bis hin zu Fertilitätsstörungen und zur Sterilität (Henry 1982). Wie Henry in dieser Untersuchung zeigte, sind alle drei Systeme unabhängig voneinander aktivierbar. Von Holst wies (1987) jedoch darauf hin, dass nicht nur die soziale Stellung eines Individuums den endokrinen Status eines Tieres bestimmt, sondern auch die Art, wie diese Position errungen und erhalten wird. Darüber hinaus existieren bei höheren Vertebraten „Erwartungen“ („expectation copy“, Archer 1988) bezüglich ihrer sozialen Umwelt, die auf genetischen Faktoren und den während der Ontogenese gemachten frühen sozialen Erfahrungen basieren. Ist die Vorhersagbarkeit und die Kontrollierbarkeit von Umweltereignissen nicht mehr gewährleistet, so treten Differenzen zwischen erwarteten und realen Vorgängen auf, die ihrerseits zu Unsicherheit und akutem oder chronischem sozialen Stress führen. Nach Sachser (1994) sind für die Überprüfung von erwarteten und eingetretenen Ereignissen der Sicherheitsgrad, der Erregungszustand sowie der Autonomieanspruch eines Individuums entscheidend. Bei Veränderungen dieser drei Faktoren können Krankheiten entstehen (Henry & Stephens 1977, Henry 1982, Lick 1991). Für stressphysiologische Reaktionen eines Organismus sind also nicht die sozialen Ereignisse an sich, sondern deren „Bewertung“ durch das Individuum und die hieraus resultierenden Veränderungen seines Zustandes ausschlaggebend. Darüber hinaus kann auch die soziale Stellung eines Individuums deutliche Auswirkungen auf sein Verhalten, seinen hormonellen Status und seine endokrinen Reaktionen haben (Henry & Stephens 1977, Sachser 1994).

Versuchstiere leben in einer hoch standardisierten Umwelt, in der die Zahl der Stressoren limitiert ist. Dennoch mehren sich die Hinweise, dass auch unter solchen Bedingungen das Wohlergehen der Tiere beeinträchtigt sein kann, was sogar Veränderungen in bestimmten Gehirnregionen mit einschließt (Knight 2001, Würbel 2001). Darüber hinaus ist auch bekannt, dass sowohl Physiologie als auch Verhalten im Verlauf eines Tages rhythmischen Änderungen unterliegen, erkennbar an deutlich abgegrenzten Ruhe- und Aktivitätsphasen. Außerdem schwankt die Sensitivität gegenüber Pharmaka oder Pestiziden tageszeit-abhängig (Tkadlec & Gattermann 1993, Lemmer 1996). Es erscheint daher folgerichtig, dass auch die Empfindlichkeit der Tiere gegenüber Routineprozeduren in ähnlicher Weise variiert. Im Rahmen einer zweiteiligen Vergleichsstudie (Gattermann & Weinandy 19996/97, **Kap. 2.1: Time of day and stress response to different stressors in experimental animals. Part II: Mongolian gerbil (*Meriones unguiculatus*)**) wurden zunächst physiologische und ethologische Normwerte ermittelt und darauf aufbauend der Effekt verschiedener tierhaltungsüblicher nicht-sozialer und sozialer Stressoren wie das Handling, Käfigwechsel oder Gruppenbildung in Abhängigkeit vom Aktivitätszustand untersucht. Als anerkannte Indikatoren für das Wohlergehen der Tiere wurden die Herzschlagfrequenz, die Körpertemperatur sowie die lokomotorische Aktivität vor und nach Applikation der jeweiligen Stressoren erfasst.

Neben diesen Messgrößen können auch Störungen des endogenen Rhythmus, also der inneren Uhr, auf ein beeinträchtigtes Wohlergehen hindeuten (Folkard et al. 1993, Okawa et al. 1994). Obwohl die Mongolische Wüsterennmaus ein etabliertes Versuchstier ist, lagen zu wichtigen chronobiologischen Charakteristika wie der endogenen Spontanperiode überraschenderweise keine eindeutigen Daten in der Literatur vor (Roper & Polioudakis 1977, Lerwill 1974, Heinzeller & Aschauer 1989, Umezu et al. 1989). Weiterhin existierten keine Angaben über eine Beeinflussung der Circadianrhythmik durch nicht-photische und periodisch auftretende Faktoren wie z.B. Futtergabe oder Zugang zum Laufrad. Ziel einer daraufhin durchgeführten Reihe von Experimenten war zunächst, geeignete Bedingungen zu finden, um stabile und reproduzierbare freilaufende Rhythmen zu erhalten (**Kap. 2.2: Circadian activity rhythms and sensitivity to noise in the Mongolian gerbil (*Meriones unguiculatus*)**). Zusätzlich sollte die Empfindlichkeit der Mongolischen Wüstenrennmäuse gegenüber dem Umweltfaktor „Lärm“ näher untersucht werden, um weitere Hinweise auf eine für sie möglichst stressarme Lebenswelt zu erhalten.

Untersuchungen zum Wohlergehen zielen meist auch darauf ab, neben den bereits bekannten weitere Parameter zu finden, um Rückschlüsse auf den individuellen tierlichen Zustand ziehen zu können. Ihre Messung sollte möglichst rückwirkungsarm sein und das Tier nicht noch zusätzlich belasten. Die Bestimmung der Körpermasse ist dabei eine gängige und sehr einfache Methode, um derartige Aussagen treffen oder stützen zu können. Körpermassenunterschiede zwischen oder innerhalb von Individuen sind aber dennoch unter bestimmten Umständen oder für spezielle Fragestellungen ein zu grobes Maß, da es die Dynamik der Veränderungen der Körperzusammensetzung verdeckt. Um genauere Informationen für die Abschätzung der individuellen phänotypischen Fitness und damit dem Wohlergehen zu erhalten, empfiehlt sich daher die Bestimmung der Körperzusammensetzung, also des prozentualen Rohfettgehalts an der Gesamtkörpermasse bzw. die daraus resultierende fettfreie Körpermasse. Seit einigen Jahren gibt es nun erste Hinweise auf eine neue Untersuchungstechnik (total body electrical conductivity, TOBEC), die im Bezug zur genannten Problematik neue, ergänzende Daten erbringt und wiederholte Messungen an einem Tier zulässt, so dass kontinuierliche Untersuchungen mit weniger Versuchstieren möglich sind (van Loan & Koehler 1990, Fischer et al. 1996, Raffel et al. 1996). Sowohl für den Goldhamster als auch für die Mongolische Wüstenrennmaus lagen bisher derartige Daten nicht vor. Da diese Messtechnik eine jeweils artspezifische Kalibration erforderlich macht, wurde diese zunächst erstellt. Darüber hinaus konnten Normwerte der Körperzusammensetzung für beide Arten gefunden werden und die Methode wurde erfolgreich zur Indikation des Wohlergehens der Versuchstiere etabliert (**Kap. 2.3: Total body electrical conductivity (TOBEC) measurements in Mongolian gerbils (*Meriones unguiculatus*) and golden hamsters (*Mesocricetus auratus*)**).

Für Langzeitversuche, die für die unterschiedlichsten wissenschaftlichen Fragestellungen erforderlich sind, hat sich als eine nicht-invasive Technik die biotelemetrische Erfassung entwickelt (**Kap. 1.1: Measurement of physiological parameters and activity in a Mongolian gerbil during gravidity and lactation with an implanted transmitter**), die inzwischen weit verbreitet ist und bei verschiednen Tierarten zum Einsatz kam (Clement et al. 1989, Weinert & Waterhouse 1998, Waterhouse et al. 2000).

Erstaunlicherweise wurde dabei aber die erste Phase nach der mit der Transmitterimplantation verbundenen Operation noch nicht näher analysiert. Dies ist jedoch zwingend erforderlich, um Kriterien für eine Erholung des Versuchstieres nach einem solchen massiven Eingriff zu erhalten. In einer diesbezüglichen Studie wurden daher auf der Basis von Änderungen der Körpertemperatur und der Aktivität sowohl kurzzeitige als auch längerfristige Effekte der Narkose und der chirurgischen Prozedur ermittelt (**Kap. 2.4: Indicators for post surgery recovery in Mongolian gerbils**). Im Besonderen diente die Stabilität des Körpertemperaturrhythmus als ein Indikator für die Genesung des Individuums.

2 Sozialverhalten und Reproduktionsbiologie

2.1 Measurement of physiological parameters and activity in a Mongolian gerbil during gravidity and lactation with an implanted transmitter

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WEINANDY, R. AND R. GATTERMANN. Measurement of physiological parameters and activity in a Mongolian gerbil during gravidity and lactation with an implanted transmitter PHYSIOL BEHAV 58(4) 811-814, 1995.—This communication reports the first use of a transmitter implanted into the abdominal cavity of a small pregnant rodent to simultaneously measure the heart rate, core body temperature and general locomotory activity with no apparent side effects to the animals. Using this method the heart rate of one female Mongolian gerbil (*Meriones unguiculatus*) decreased from a mean of 303 ± 6 (SE) (beats per minute) during gravidity to a level of 248 ± 3 (SE) (beats per minute) during lactation. At the same time, the core body temperature increased from 37.7 ± 0.05 (SE) °C to 38.0 ± 0.03 (SE) °C. During pregnancy the general locomotory activity of this female followed the normal nocturnal pattern for this species (i.e., greater activity during the dark period) but there was an overall reduction in activity to 85% of the non-pregnant state. The level of activity was further reduced during lactation to about 43% of the nonpregnant state. This was largely the result of less activity during the dark period so that activity in the dark period and light period became more or less equivalent.

Heart rate   Core body temperature   Gravidity   Lactation   Case report

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[1] Weinandy, R. & R. Gattermann. 1995. Measurement of physiological parameters and activity in a Mongolian gerbil during gravidity and lactation with an implanted transmitter. Physiol. Behav. **58**: 811-814. Eigenanteil: Experimente, Auswertung und Manuskript

Meriones unguiculatus Circadian activity Implanted transmitter

INTRODUCTION

Gravidity and lactation are natural and frequent events in female rodents. These events are often associated with high physiological costs (6). In general, parameters such as heart rate, core body temperature, body and organ weights, hormone titres and locomotory activity are used to estimate the costs associated with such straining events (1,2,7,9,10,12,15,16,18). Due to technical difficulties in taking measurements there are very few reports describing the physiological state and the ongoing changes in small female rodents during gravidity and lactation (1,3,6,14). We report the first use of an implanted transmitter, in association with biotelemetry, to simultaneously measure the heart rate, core body temperature and general locomotory activity of a small pregnant rodent with no apparent side effects (for general description see 4). This communication indicates the potential use of this method for more extensive studies with small rodents during gravidity and lactation.

METHOD

ANIMALS AND HOUSING

Three adult females (age: 13 - 26 wk; body weight: 65 - 67 g) were selected from a laboratory colony of Mongolian gerbils [*Meriones unguiculatus* (CRW:(Mon) BR. Charles River, Wiga, Germany)]. The animals were caged separately in one room with a photoperiod of 12:12 hours Light/Dark (light period: 07.00 - 19.00 h). The room temperature was 24 ± 2° C and the light intensity varied from 200-300 lx (light period) to approximately 5 lx (dark period). The cages (size: 55 x 33 x 20 cm) were plastic with a wire mesh top and included a circular treadmill (30 cm in diameter). Water and food pellets were provided ad lib.

SURGERY AND PROCEDURE

At the same time on consecutive days, the three females were anesthetized with pentobarbital (5 mg per 100 gram body weight). A transmitter was placed in the abdominal cavity of each animal and sutured to the abdominal musculature. The transmitter (model TA10ETA-F20) had a weight of 3.9 g, a volume of 1.9 cc and two coiled wires working as sensing leads. The leads were pulled along two SC tunnels at opposite ends of the sternum. Receivers (model RA 1010), which were placed under each of the three cages, received signals from the transmitters and converted them into a computer readable form. The heart rate, core body temperature and general locomotory activity of these three females were measured using the computerized Telemetry and Acquisition System DATAQUEST IV (Data Sciences, Inc., St. Paul, MN). About 27 wk after the implantation (i.e., when the battery´s of the transmitters were exhausted) the three females were anesthetized, decapitated and dissected at approximately the same time on consecutive days (8.00 - 9.00 am). Within 3 min of death some blood was collected for the determination of hormone titres (glucocorticoids) by radioimmunoassays (for details see 5). Subsequently, the kidneys and adrenals were weighed. One female was paired with an adult male 3 wk after the implantation. Four weeks later the mated female gave birth to four healthy young. The male gerbil and the young were removed when the young were weaned (i.e., 4 wk later). This female was kept isolated for the remaining 16 wk. The other two non-mated females were housed singly or in unisexual groups (i.e., with two other females).

DATA ANALYSIS AND STATISTICS

The transmitters took measurements of the heart rate and the core body temperature every minute and a mean value for 5-minute-intervals was recorded by the computer. We calculated the daily mean values of the heart rate and the core body temperature from the 5-minute-intervals of the three females. The general locomotory activity was measured every minutes and a sum of 5-minute-intervals was recorded. We calculated the daily sum from these 5-minute-intervals. The statistical measure of the day-to-day variation is the standard error (SE). Recordings were initiated as soon as the transmitter was implanted until the animals were decapitated. The data of the general locomotory activity of the mated female was used to create a double-plot-actogram to show the distribution of activity during consecutive 24-hour-periods from day 11 until the end of the experiments. This plot uses lines of varying heights to show the activity amplitude of each 5-minute-block.

RESULTS AND DISCUSSION

All three females survived the implantation of the transmitters for the duration of the experiments. Most significantly, one female mated, conceived, became pregnant, gave birth and weaned four healthy offspring with no apparent serious side effects. This is significant because reproduction is easily disrupted by a number of stressors and its occurrence can be taken as an indication of an animal`s welfare (9,10). Furthermore, the reproductive behaviour (including the sexual activity, the length of the gravidity, the number of pups and the breeding behaviour) in this one mated female with the implanted transmitter is comparable to that reported in the literature for healthy female gerbils without implanted transmitters (6,8,13).

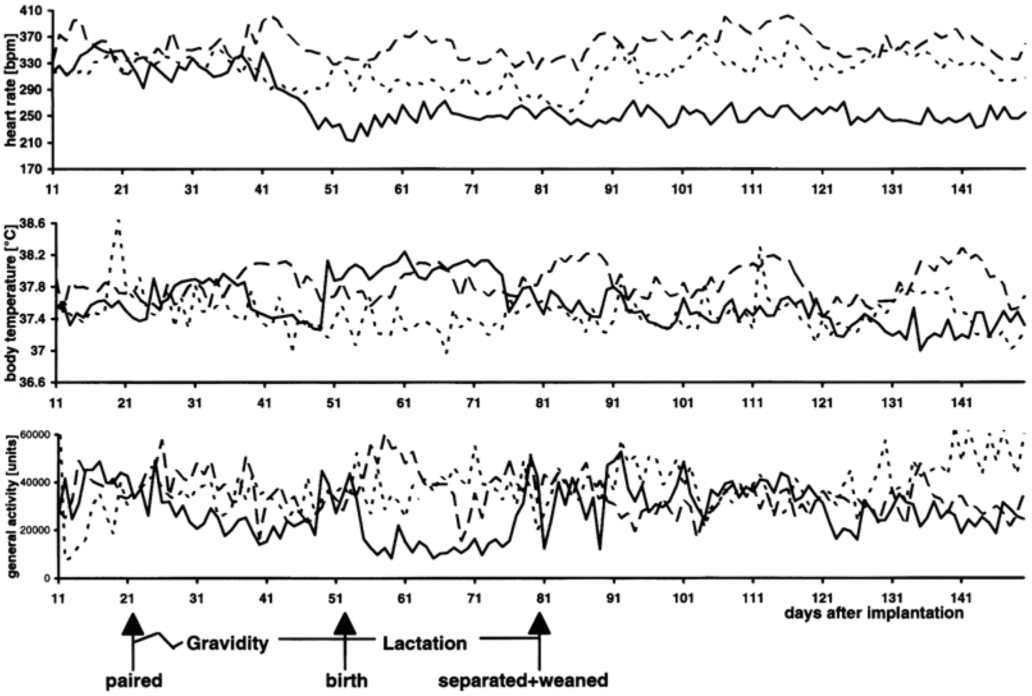

FIG. 1. Daily mean values of the heart rate, core body temperature and general locomotory activity of the mated female and two nonmated females. Mated female ——— Nonmated female 1— — — Nonmated female 2 – – –.

As well as these more general conclusions, this case report gives a description and evaluation of the ongoing changes in one female gerbil during pregnancy and lactation. Figure 1 shows the heart rate, core body temperature and general locomotory activity from day 11 after the implantation of the transmitters and until 4 wk before the animals were decapitated. Eleven days is considered to be a suitable time for the animals to become used to the transmitters (18).

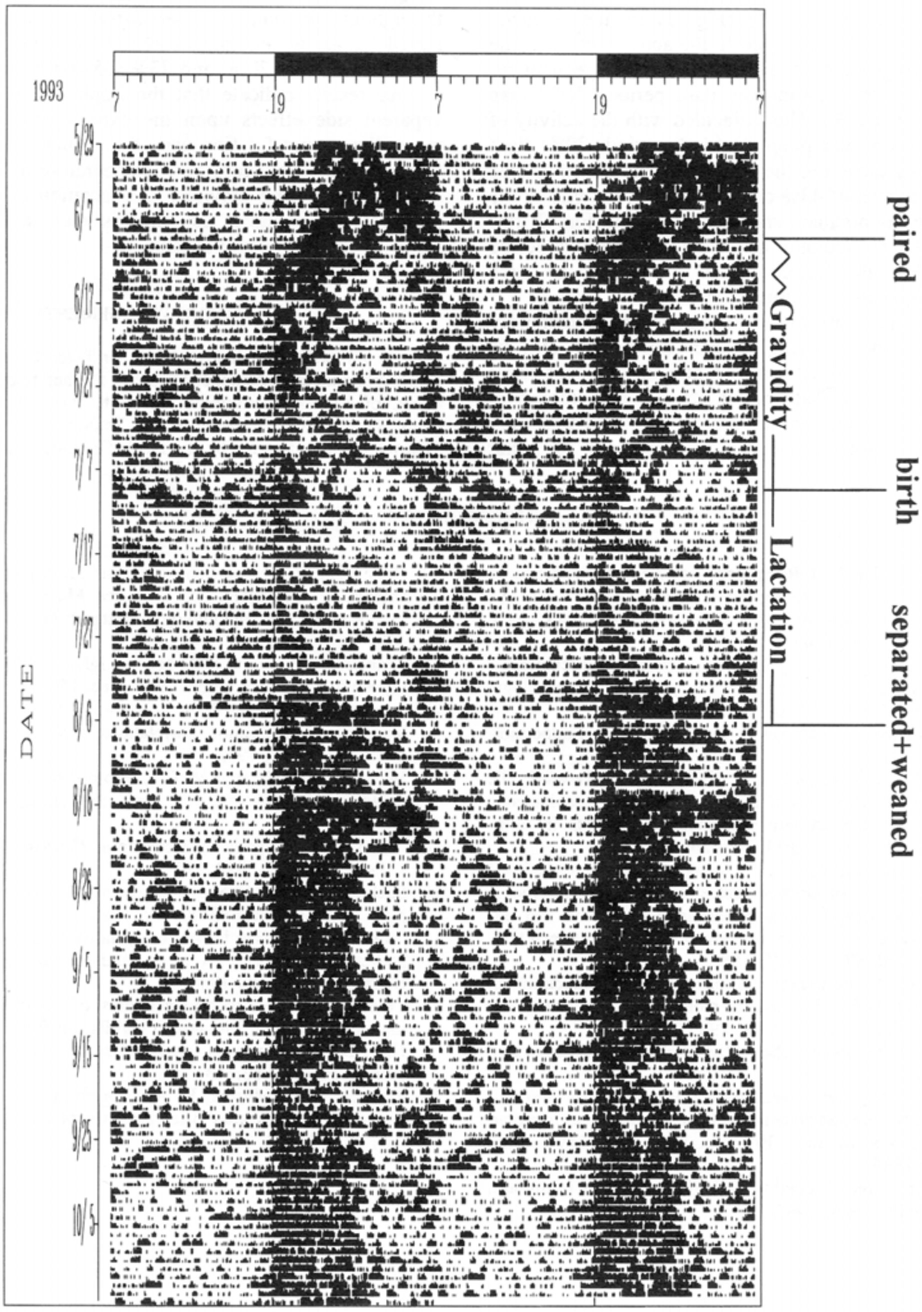

FIG. 2. Actogram of the general locomotory activity of the mated female.

Figure 1 shows that during gravidity, the daily mean heart rate of the mated female was 303 ± 6 (beats per minute) which was similar to the two nonmated females (Fig. 1). For the mated female, during the last ten days of gravidity, the heart rate declined steadily and reached its minimum of 213 (beats per minute) two days after the female gave birth to 4 healthy young. This low level remained unaltered for the following 100 days. It was lower than the daily mean heart rate of the two nonmated females over the period of registration.

During gravidity and lactation the mean values of the core body temperature profile for the mated female were marked by three different stages (FIG. 1). The first stage (approximately the first half of gravidity) was characterized by daily mean values of 37.8 ± 0.03 °C. The second stage (approximately the last 10 days of gravidity), the core body temperature declined and reached a minimum value of 37.3 °C three days before the female gave birth. Afterwards the core body temperature increased again and reached a plateau (mean 38.0 ± 0.02 °C) which was higher than the first stage. This third stage lasted for 23 days (approximately the duration of the lactation period). Finally the core body temperature declined again to a level of 37.4 ± 0.02 °C where it remained. These changes were similar to those of the two nonmated females, but there were no such distinct stages detectable for the core body temperature of the nonmated females.

The general locomotory activity (Fig. 1) of the pregnant female was reduced to 85% compared to the activity of the same female in the nonpregnant state. Nevertheless, the pregnant female was still more active during the dark period (71%) than during the light period (29%). This coincided with the activity of this animal when it was not pregnant (dark period: 79%; light period: 21%). During lactation, the activity of the mated female decreased again to a level of 43% compared to the activity of the same female in the nonpregnant state. As shown in Fig. 2, this was largely due to the reduction of activity during the dark period (dark period: 57%; light period: 43%). There was no such reduction of activity in the two nonmated females.

The decline in the heart rate and the general locomotory activity (Fig. 1), especially during the lactation period, may be due to the fact that the animal requires and hence saves more energy for its young during that time of life (2). This interpretation is supported by the increase of the core body temperature after the birth of the young, which is also reported for other rodents (1). In gerbils, this temperature increase may be relevant to the fact that pups are born completely naked and are warmed by their mother almost constantly for the first 10-15 days (see also 17).

The hormone titres (glucocorticoids), body, kidney and adrenal weights of the three animals with implanted transmitters did not differ from adult healthy females without transmitters (R. W. unpublished data). The body and organ weights of the three animals also coincides with measurements of these parameters in adult female gerbils reported elsewhere (8,11). Furthermore, the fact that the three females with implanted transmitters all put on weight during the experiment suggests that they remained healthy throughout (for comparison see also 8,11). The weight of the one mated female increased 18% (67 - 79 g), and the two nonmated females 22% (65 - 79 g) and 23% (65 - 80 g) respectively.

Our results indicate that the implanted transmitters had no apparent side effects upon the experimental animals. Furthermore, the successful first use of this method to simultaneously measure heart rate, core body temperature and general locomotory activity during gravidity and lactation in one female gerbil indicates the potential use of this method for similar larger studies with other small rodents.

ACKNOWLEDGEMENT

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2.2 Lack of social entrainment of circadian activity rhythms in the solitary golden hamster and in the highly social Mongolian gerbil

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Abstract

This study compared the capability for social entrainment of the circadian locomotory rhythms of solitary versus socially living nocturnal small mammals. Therefore single adult golden hamsters and Mongolian gerbils of both sexes were kept under LD 12:12 and then transferred in their cages to a dark chamber (DD), which was covered with an opaque material, but located permanently in a room with other conspecifics exposed to a light regime (LD 12:12). The animal in the chamber was able to detect acoustical and olfactorial stimuli of the conspecifics outside the chamber. Under these experimental conditions all the golden hamsters and gerbils investigated developed free-running rhythms of activity with an individual-specific spontaneous period. In golden hamsters these free-running rhythms were never entrained or masked. In 5 out of 6 gerbils the free-running of activity rhythms stopped after a few days and the rhythms were more or less pronounced entrained or masked. Unexpectedly the response was not induced via signals from the conspecifics but quite probably by the caretaking procedures in the animal room. Therefore, our results suggest that in neither investigated species did social entrainment of the circadian activity rhythms occur.

Abstracting keywords: circadian rhythm, entrainment, masking, solitary, social, conspecific, golden hamster, Mongolian gerbil

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[2] Gattermann, R.& R. Weinandy. 1997. Lack of social entrainment of circadian activity rhythms in the solitary golden hamster and in the highly social Mongolian gerbil. Biol. Rhythm Res. **28** (Suppl.): 85-93. Eigenanteil: Experimente, Auswertung und Manuskript

Introduction

The circadian rhythms in mammals are generated by the hypothalamic suprachiasmatic nuclei (SCN) via a photic oscillator. The length of the resulting period is genetically fixed and is species- and individual-specific different from 24 hours. The entrainment of these endogenous circadian rhythms to the geophysical determined 24 h day happens via so-called zeitgebers. The main zeitgeber is the natural day-night-cycle or the light-dark-cycle (LD), respectively. In absence of this zeitgeber, for example in constant light (LL) or in constant darkness (DD), other periodical changes (e.g. temperature, humidity, noise, feeding, cage cleaning and others) may take over the entrainment. Potential zeitgebers are also periodically occurring social cues with an entraining effect (for an overview see Erkert and Schardt, 1991; Goel and Lee, 1995). However, the effectiveness of these social zeitgebers is controversial. In contrast to the photic zeitgeber, general and definite evidence of direct and long lasting (i.e. weeks or months) effects of social stimuli for the entrainment of free-running circadian rhythms is still missing. In some cases it has been possible to influence the free-running circadian rhythm of single animals for days and weeks, but never of all animals in a study. For instance, Mrosovsky (1988) set an intruder for a maximum of 30 min at the same time of day into the home cage of a male golden hamster with a free-running activity rhythm. This led to entrainment in only 4 out of 9 animals. The other 5 and furthermore all other golden hamsters in the room were not influenced by the conspecific and the non-conspecific stimuli, which came along with the experimental manipulations. In diurnal degus (*Octodon degus*), living in family units, Goel and Lee (1995) investigated the time of resynchronisation of free-running rhythms of activity and temperature in an animal that was put with an LD synchronized animal. The resynchronisation of females occurred faster in the presence of another female. In males no such effects were registered. Erkert and Schardt (1991) found that in marmosets (*Callithrix jacchus*) social entrainment occurred only in particular cases and in related animals. They concluded therefore, that social entrainment in primates and other mammals is more likely to be a coincidental rather than an essential characteristic of the circadian system. Nevertheless, a critical review of the literature shows that the majority of the different experiments into social entrainment did not lead to positive results, e.g. in the social Australian sugar glider (Petaurus breviceps) and in golden hamsters (Kleinknecht, 1985; Davies et al., 1987).

All existing studies into social entrainment in blinded animals with damaged hypothalamic tracts and which therefore could not detect any photic stimuli also failed. Refinetti et al. (1992) kept blinded golden hamsters in pairs, in each case intact and by bilateral enucleation in a running wheel cage under a LD-cycle of 23.3 h. The individual activity and the body temperature of the blinded animals were measured via an implanted transmitter. While the intact animals adapted their circadian rhythms to the artificial 23.3 h day, the enucleated animals were unable to do so. Their running wheel and general activity as well as their body temperature were free-running. Besides the given close physical contact between the animals in this experiment, an entrainment was not demonstrable; there was merely a more or less pronounced masking. Furthermore, Aschoff and v. Goetz (1988) could influence neither the phase nor the period of free-running rhythms of blinded male golden hamsters through the periodic presence of females.

It is conceivable that in solitary living species social zeitgebers are only weak or not evolved at all. On the other hand, social zeitgebers should be more effective in social species with an innate social appetence. The design and the objective of our chrono- and sociobiological study was to test the hypothesis of social entrainment in small mammals with different social organization. Therefore we compared the solitary living golden hamster and the Mongolian gerbil, which lives socially in family units (Ågren, 1976; Weinandy, 1996).

Material and Methods

Adult golden hamsters (*Mesocricetus auratus* Waterhouse, 1839) and Mongolian gerbils (*Meriones unguiculatus* Milne Edwards, 1867) of both sexes were chosen for this study. All animals derived from our own laboratory stocks (Zoh:GOHA and Zoh:CRW respectively). The animals of both species were caged in different rooms with a LD 12:12 (250:5 lx) and lights on at 07.00 h. The room temperature was 24 ± 1 C and the relative humidity varied from 65-70%.

Water and food pellets ("Altromin 7024", Altromin GmbH, Lage) were provided ad lib.

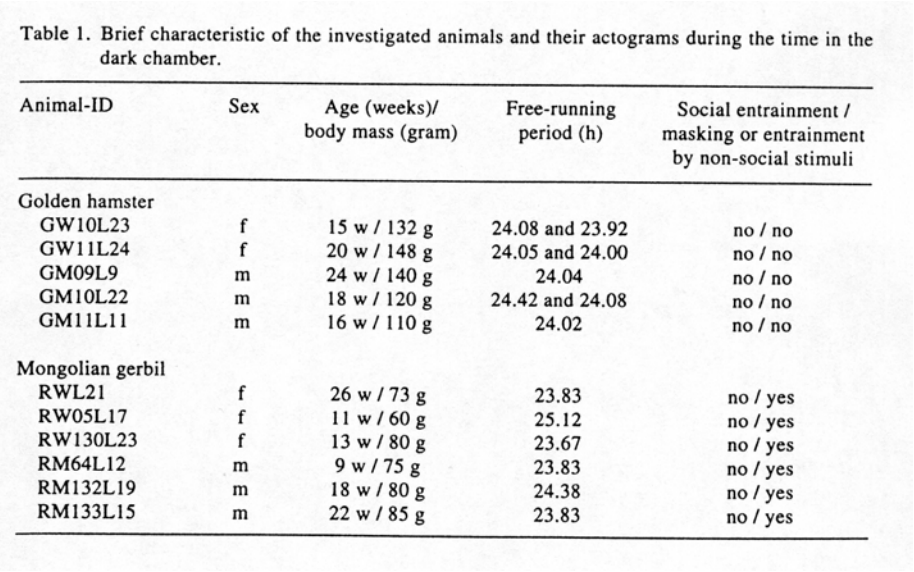

Table 1. Brief characteristic of the investigated animals and their actograms during the time in the dark chamber.

Animal-ID	Sex	Age (weeks)/ body mass (gram)	Free-running period (h)	Social entrainment / masking or entrainment by non-social stimuli
Golden hamster				
GW10L23	f	15 w / 132 g	24.08 and 23.92	no / no
GW11L24	f	20 w / 148 g	24.05 and 24.00	no / no
GM09L9	m	24 w / 140 g	24.04	no / no
GM10L22	m	18 w / 120 g	24.42 and 24.08	no / no
GM11L11	m	16 w / 110 g	24.02	no / no
Mongolian gerbil				
RWL21	f	26 w / 73 g	23.83	no / yes
RW05L17	f	11 w / 60 g	25.12	no / yes
RW130L23	f	13 w / 80 g	23.67	no / yes
RM64L12	m	9 w / 75 g	23.83	no / yes
RM132L19	m	18 w / 80 g	24.38	no / yes
RM133L15	m	22 w / 85 g	23.83	no / yes

The test animals for the social entrainment experiments (Table 1) were kept singly for at least 4 weeks under these housing conditions in a room together with 60-80 conspecifics (males, females and pups). The cages (size: 55 x 33 x 20 cm) were plastic with a wire mesh top and included a running wheel (30 cm in diameter and a running area width of 10 cm). Each wheel revolution triggered an impulse via a magnetic device which was stored and calculated by "The Chronobiological Kit" (Stanford, USA). With the same technical equipment each room-entering and the majority of the caretaking procedures in the room could be registered by passive infrared sensors which were installed above the doors of the animal rooms (fig. 4).

To evaluate the social entrainment, one animal was placed for at least six weeks with its home cage in a dark chamber (80 x 105 x 67 cm), which was covered with an opaque material (black cloth) and the dark chamber was located permanently in the room with the described conditions and together with the conspecifics. The investigated animal in the chamber was therefore maintained in constant darkness (DD) but could detect all the olfactorial, acoustical and other stimuli of the conspecifics. For the test animals water, food and bedding material ("Altromin-Versuchstiereinstreu") were renewed every 3-4 weeks and only during the dark period in the room. The calculation of the free-running periods occurred with the chi²- periodogram ("The Chronobiological Kit").

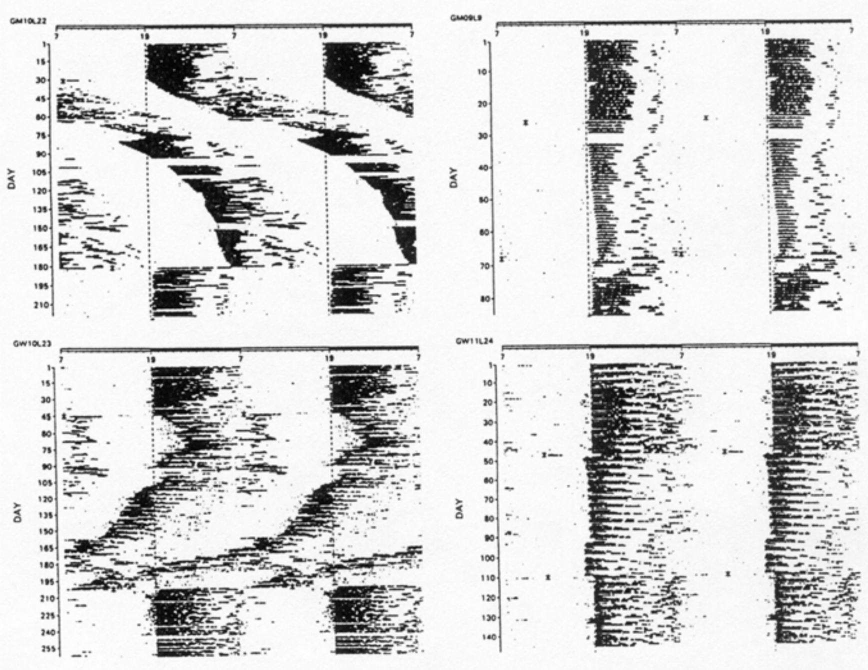

Fig. 1. Representative double plotted activity records of 4 golden hamsters. The animals were kept together with conspecifics in a room under the LD cycle, which is indicated by the bar. The two asterisks in the center of the figures indicate the beginning and the end of the stay in the dark chamber. Left hand side above: animal-ID, ordinate: days of experiment.

Results

The figures 1, 2 and 3 show typical actograms for both species. The free-running periods and the evaluation of the actograms are depicted in Table 1. Under LD 12:12 the golden hamsters entered the running wheel only in the dark. The beginning of activity was strictly connected to lights off. After the transfer into the dark chamber they showed robust free-running activity rhythms with individual-specific periods. In one female (GW10L23) an unclarified spontaneous phase occurred around day 80 of the investigation. In the other animals all pronounced changes in phase were due to direct caretaking procedures (e.g. cage changes, supply with fresh water and food) which were always carried out during the dark period in the room. These data of golden hamster activity rhythms show that there was neither entrainment nor a clear masking caused by conspecific or other stimuli detectable during the time in the dark chamber.

After reimposition of LD 12:12 the activity adjusted immediately. These masking effects were caused by the LD-cycle and it underlined the strength of the photic zeitgeber.

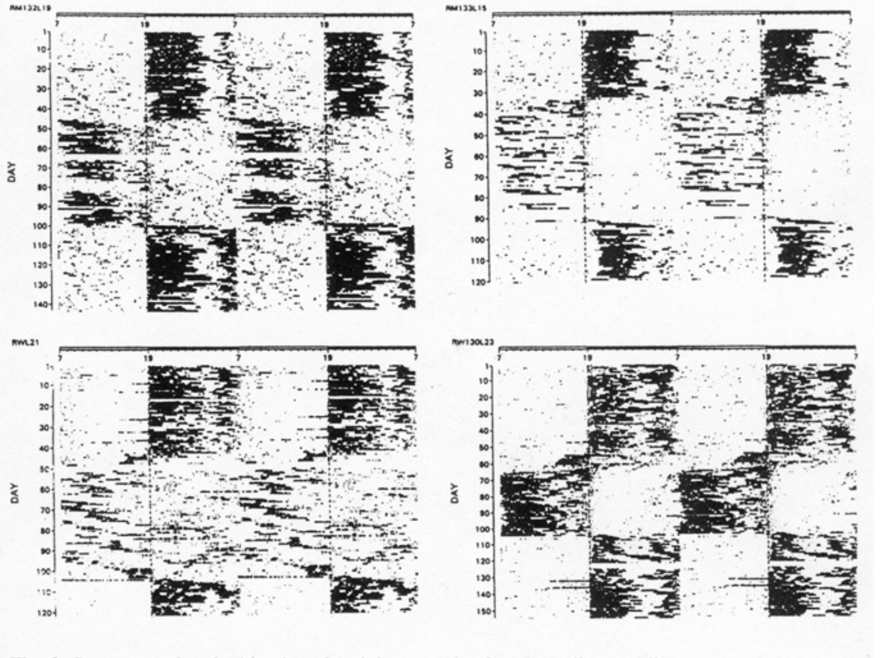

Fig. 2. Representative double plotted activity records of 4 Mongolian gerbils. See key to Figure 1 for further details.

Under LD 12:12 the Mongolian gerbils were nocturnal with a maximum of activity linked to lights off. However, in contrast to the golden hamsters they also entered the running wheel during the light period. After transferring them into the dark chamber all investigated gerbils showed free-running activity rhythms with individual-specific periods. In three gerbils (RWL21, RM64L12 and RM132L19) it was not possible to clearly identify these periods visually but they could be calculated by the chi²-periodogram. In 5 out of 6 gerbils the free-running of the rhythms were relatively quickly stopped by individually distinctive entrainment or masking (see discussion) and the rhythms were phase-shifted about 180° compared to the beginning of DD. As in the golden hamsters, the activity rhythms of all gerbils adjusted very quickly after the reinitiation of the LD-cycle, i. e. there were masking effects detectable which were triggered by the photic zeitgeber. The gerbil RW05L17 (fig. 3) exhibited a free-running activity rhythm for the whole test phase in the dark chamber and additionally a clear masking effect occurred, i.e. increased activity during the first hours of lights on outside the dark chamber.

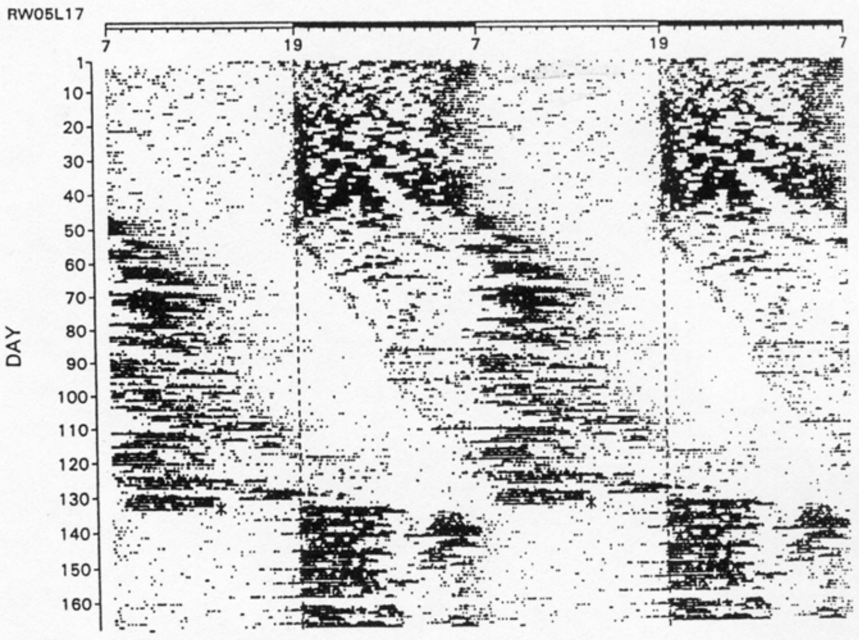

Fig. 3. Double plotted activity records of a female gerbil. It shows a dark chamber-phenomena, i.e. a free-running activity rhythm and a masking caused by the caretaking procedures during the light period outside the dark chamber.
See key to Figure 1 for further details.

A comparison with the caretaking procedures in the room (fig. 4) showed the probable cause of both entrainment and masking of activity rhythms during the experimental phase in the dark chamber. In this special DD condition the time of animal activity was just in phase with the human activity. The gerbils in the dark chamber seemed to have used the room entering and the activities of the animal care takers as a non-social zeitgeber and there were no hints for any response to the periodically stimuli of the conspecifics.

To sum up in brief, social zeitgebers or social entrainment cannot be proven for the solitary golden hamsters or for the highly social gerbils.

Discussion

An initial step in the analysis of the actograms was to check if there were really free-running conditions in the dark chamber. The detected free-running periods were evidence that the animals in the dark chamber could not receive any information about the LD periodicity in the animal room outside the chamber. As expected, and in agreement with the literature (Aschoff et al., 1988; Refinetti et al., 1992), the solitary living golden hamsters did not react to any conspecific stimuli under the free-running conditions in the dark chamber. Furthermore, they did not respond to non-conspecific stimuli like human activities in the room.

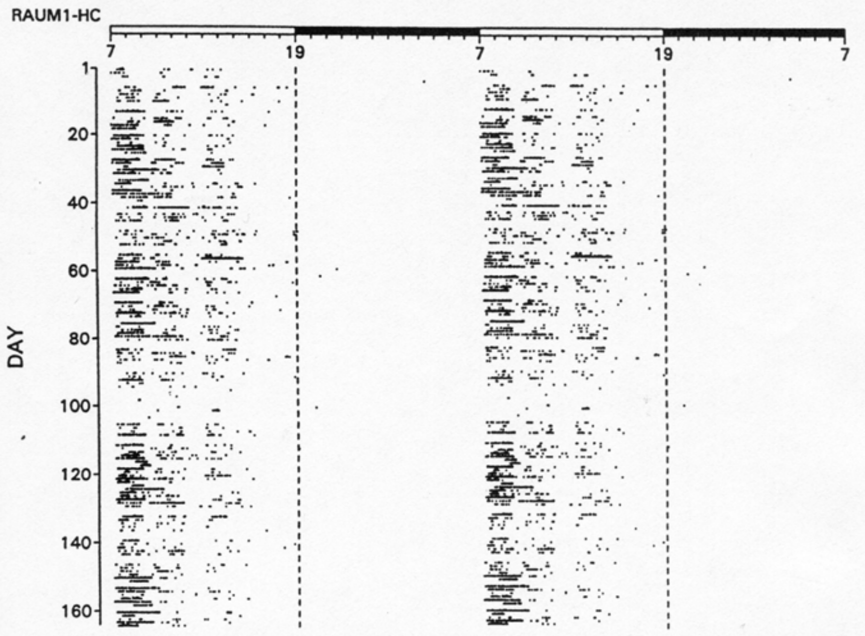

Fig. 4. Double plotted records of the presence and the activity of caretakers in the room for the daily routine visual control and care of the animals outside the dark chamber as well as the weekly cleaning of the floor.

For the Mongolian gerbils, which live socially in family units, we expected to find evidence for conspecific cues resulting in social entrainment or at least masking effects of the free-running activity rhythms induced by conspecifics. In one female (Fig. 3) there was a free-running activity rhythm which was clearly masked by the animal care taking procedures during its stay in the dark chamber. In the other investigated gerbils (Fig. 2) we also found spontaneous periods (Tab. 1) and transient phases at the beginning of the dark chamber experiment followed by an entrainment or a masking. With the used protocols we cannot discriminate distinctly between these phenomena. Contrary to expectations the activity rhythms of all tested gerbils were not in phase with these of the photically entrained conspecifics outside the dark chamber. Our data strongly suggest that the stimulus or zeitgeber was the regular and periodic presence of animal care takers and other members of the staff in the room, who on working days were in charge of the daily routine, control of the animals and their cages as well as the weekly cleaning of the floor and the cage changing (Fig. 4). It is important to mention that these activities were performed as quietly as possible and were directed only to the animals outside but obviously also affected the animals inside the dark chamber.

Above all, there were two quantitative levels of different potentially entraining agents in the room, prevailing social stimuli in the dark time vs non-social (human) stimuli in the light time. The question still remains open why the tested gerbils were responsive to the non-conspecific stimuli and not to the more regular appearing social cues from the photically entrained conspecifics. Our additional investigations may explain the differences between the golden hamsters and the gerbils (Gattermann and Weinandy in prep.). In these studies the solitary kept Mongolian gerbils had higher heart rates, increased masses of the adrenal glands and elevated levels of the glucocorticoids compared to group kept gerbils. All these are indicators of isolation caused stress and a higher tonic level of arousal (Weinandy, 1996), which did not occur in the solitary kept golden hamster. It may well be that the human presence additionally and periodically increased the level of arousal of the gerbils and this may led to a non-photic zeitgeber effect (discussions about arousal effects in Hastings et al., 1992; Mrosovsky, 1988).

To conclude, in the solitary living golden hamster and in the socially living Mongolian gerbil, periodic stimuli from the conspecifics have presumably not evolved as entraining agents or social zeitgebers. Under natural field conditions the internal circadian clock is primarily entrained via the steady and reliable occurring day/night periodicity and the conspecifics can not sufficiently replace this function.

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2.3 Parental care and time sharing in the Mongolian gerbil

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Abstract
The biparental care behaviour of the social Mongolian gerbil (*Meriones unguiculatus*) was quantified from birth to weaning of the young under laboratory conditions. Nestbuilding, nest-residence, and retrieving of the offspring were measured. The behaviour of the parents was registered per video-observation on days 2, 5, 8, 13, and 20 after the birth of the young, each for 24 h. To obtain control data, we additionally observed all pairs for 24 h without progeny. The objective of our study was to evaluate the paternal and maternal efforts in rearing the young and to focus on parental time sharing in the nest.
The female made the greatest contribution to care since there was no paternal support in building of the litter-nest and retrieving of the young. However, both adults cooperated via their synchronized presence with the young (temporal coordination or time sharing in the nest). We suggest that via this mutual behavioural synchronization the physiological strains of the female caused, e.g., by lactational hyperthermia are reduced.

Key words: *Meriones unguiculatus*, parental care, time sharing

Introduction
Although in mammals the females mainly care for the young, paternal investment can also increase their chances of survival via direct support, such as warming, and more indirect assistance like nest-building or defending the young. Investigations on cooperative breeding and paternal care mainly refers to primates and carnivores (for a review see: Solomon and French 1997; for rodents: Ostermeyer and Elwood 1984; Solomon and Getz 1997; Gerlach 1998).

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[3] Weinandy, R. & R. Gattermann. 1999. Parental care and time sharing in the Mongolian gerbil. Z. Säugetierkd. **64**: 169-175. Eigenanteil: Experimente, Auswertung und Manuskript

Based on short-term observations during the light period (90 minutes per family), Elwood (1979) showed that certain parental activities of the social Mongolian gerbils, e.g., nest-building, are influenced by the other parent. The aim of our long-term study was to quantify in greater detail paternal and maternal efforts from birth to weaning of the altricial young and to focus on parental time sharing in the nest.

Material and methods

Animals and Housing

We selected six adult males and six females from a laboratory colony of Mongolian gerbils (*Meriones unguiculatus)*. The animals came from different litters and were caged in pairs after weaning at 6-8 weeks. They were kept in climatised rooms with a photoperiod of 12:12 h light (200-300 lx per cage): dark (5-10 lx per cage) (light period: 0700-1900 h Central European Time). The room temperature was 24 ± 2 °C and the relative humidity varied from 65-70 %. The cages (size: 55 x 33 x 20 cm) were plastic with a wire mesh top and included a circular treadmill (30 cm in diameter and a running wheel area width of 10 cm). Water and food pellets (Altromin® 7024, Altromin GmbH, Lage) were provided ad lib. The animal bedding was provided from Altromin GmbH, Lage. To facilitate nest-building, the animals were also provided with cellulose.

We confirm that the experiments have been performed in accordance with local animal welfare legislation and the legal requirements of Germany.

Data analysis and statistics

The parental care behaviour of five pairs towards their first litter and of one pair towards their third litter was observed over 1 600 h. The mean litter size was 5.1 pups (3-7). The behaviour of the parents was registered on days 2, 5, 8, 13, and 20 after the birth of the young (day of birth=day 0), each for 24 h. To obtain control data, we observed all pairs for 24 h without offspring, i.e., 2-3 weeks before birth or after weaning. We used the time-lapse videotechnic (Panasonic WV-CL352E u. AG-7350) and chose the 12 h mode. The analysis was performed using the software The Observer V 3.0 (Noldus, NL). For both pair partners we collected the following behaviours: nest-building (duration): time spent with the carrying-in and arranging of nesting material; nest-residence (duration): time spent in the nest; retrieving of the young (frequency): carrying the pups back to the nest. The parameters are given as mean values, the statistical measure of variance is the standard error. The Friedman analysis of variance and subsequently the two-tailed Wilcoxon test were used to assess the differences of the means. Differences were significant at $p < 0.05$ (* in the graphs). The computer package used for the statistical analyses was Winstat (V 3.1).

Results

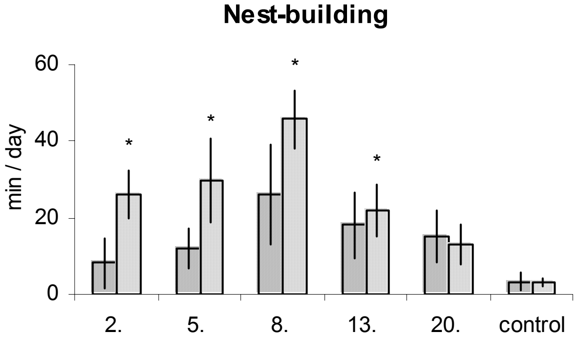
Nest-building

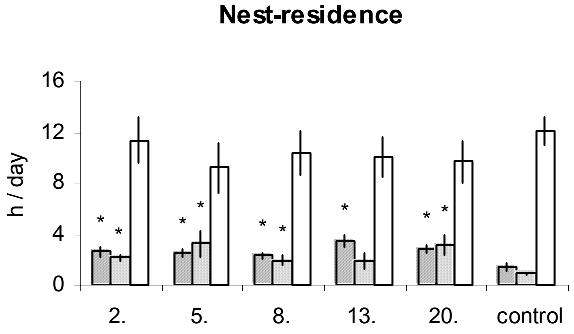
The time the gerbils spent on nest-building depended on whether it was used as a nest for resting (rest-nest) or as a nest for the approaching litter (litter-nest), i.e. the nest had two functions. When the adults lived without young under laboratory conditions, both males and females built a rest-nest as a depression in the animal bedding which was located in a corner of the cage (Fig. 1, males vs. females: Wilcoxon test, $z = -0.94$, $N = 6$, $p > 0.05$). It was only slightly enlarged in the last activity period before and completed just after birth of the pups. This litter-nest was more compact and was also built by both sexes.

Nevertheless, regarding the whole observation period, the respective effort of male and female was different (Fig. 1). In the mean a male invested 15.9 ± 7.4 and a female 27.3 ± 4.7 minutes per day (males vs. females: Wilcoxon test, $z = -1.99$, $N = 6$, $p < 0.05$). Whereas males did not intensify the nest-building behaviour (day 2 - day 20 vs. control: Friedman ANOVA, chi-square approximation, $\chi^2 = 8.3$, $N = 6$, $df = 5$, $p > 0.05$), it was significantly elevated in the females until day 13 (day 2 - day 20 vs. control: Friedman ANOVA, chi-square approximation, $\chi^2 = 15.64$, $N = 6$, $df = 5$, $p < 0.05$; Wilcoxon test, day 2 vs. control: $z = -1.99$, $N = 6$, $p < 0.05$; day 5 vs. control: $z = -1.99$, $N = 6$, $p < 0.05$; day 8 vs. control: $z = -2.2$, $N = 6$, $p < 0.05$; day 13 vs. control: $z = -1.99$, $N = 6$, $p < 0.05$; day 20 vs. control: $z = -1.36$, $N = 6$, $p > 0.05$).

Nest-residence

Both males and females stayed approximately the same time alone in the commonly established nest (Fig. 1). There were no intersexual differences in that respect either in the rest-nest (Wilcoxon test, z = -1.36, N = 6, p > 0.05) or in the litter-nest (Wilcoxon test, z = -0.52, N = 6, p > 0.05). In the presence of the young this separate nest-residence of the adults increased, i.e., the two parents showed time sharing in the litter-nest.





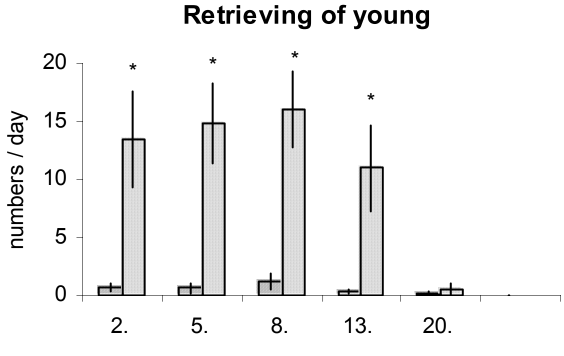


Fig. 1. Biparental care in Mongolian gerbils. Data of six pairs. Day 2 to day 20: with young (day 0 = day of birth); control: without young; scattered columns = males; dotted columns = females; blank columns = both parents together; bars = standard error of mean.* $p < 0.05$; The Friedman analysis of variance and subsequently the two-tailed Wilcoxon test were used to assess the differences of the means (for details, see text).

This increase of the sole care for the progeny was always significant for the males (day 2-day 20 vs. control: Friedman ANOVA, chi-square approximation, $\chi^2 = 13.38$, $N = 6$, $df = 5$, $p < 0.05$; Wilcoxon test, day 2 vs. control: $z = -1.99$, $N = 6$, $p < 0.05$; day 5 vs. control: $z = -1.99$, $N = 6$, $p < 0.05$; day 8 vs. control: $z = -2.2$, $N = 6$, $p < 0.05$; day 13 vs. control: $z = -2.2$, $N = 6$, $p < 0.05$; day 20 vs. control: $z = -1.99$, $N = 6$, $p < 0.05$). The same applies to the females except for day 13 (day 2-day 20 vs. control: Friedman ANOVA, chi-square approximation, $\chi^2 = 17.4$, $N = 6$, $df = 5$, $p < 0.05$; Wilcoxon test, day 2 vs. control: $z = -2.2$, $N = 6$, $p < 0.05$; day 5 vs. control: $z = -2.2$, $N = 6$, $p < 0.05$; day 8 vs. control: $z = -2.2$, $N = 6$, $p < 0.05$; day 13 vs. control: $z = -1.4$, $N = 6$, $p > 0.05$; day 20 vs. control: $z = -2.2$, $N = 6$, $p < 0.05$).

The common nest-residence, i.e. the time the adults spent together in the nest as a pair, was about four times longer than the separate stay (Fig. 1). However, in contrast to this, it was not affected by the offspring (day 2-day 20 vs. control: Friedman ANOVA, chi-square approximation, $\chi^2 = 11.5$, $N = 6$, $df = 5$, $p < 0.05$; Wilcoxon test, day 2 vs. control: $z = -0.94$, $N = 6$, $p > 0.05$; day 5 vs. control: $z = -1.57$, $N = 6$, $p > 0.05$; day 8 vs. control: $z = -1.36$, $N = 6$, $p > 0.05$; day 13 vs. control: $z = -1.57$, $N = 6$, $p > 0.05$; day 20 vs. control: $z = -1.78$, $N = 6$, $p > 0.05$).

Summing up the data of the separate and the paired nest-residence results in the total time the adult gerbils spent in the nest. It ranged from 14.4 ± 1.2 h per day (control) and 15.4 ± 1.8 h per day (mean of day 2 - day 20). As for the common nest-residence, the statistical analysis showed that there was no difference between these two periods (day 2-day 20 vs. control: Friedman ANOVA, chi-square approximation, $\chi^2 = 11.98$, $N = 6$, $df = 5$, $p < 0.05$; Wilcoxon test, day 2 vs. control: $z = -1.21$, $N = 6$, $p > 0.05$; day 5 vs. control: $z = -0.52$, $N = 6$, $p > 0.05$; day 8 vs. control: $z = -0.37$, $N = 6$, $p > 0.05$; day 13 vs. control: $z = -1.36$, $N = 6$, $p > 0.05$; day 20 vs. control: $z = -1.36$, $N = 6$, $p > 0.05$).

Retrieving of young

Until day 5, the progeny were passively dragged out of the nest while attached to the mothers nipples. Nevertheless, to an even greater extent they were thrown out of the nest by digging movements of the adults. With advancing age and increased locomotor activity the pups actively left the nest and were retrieved essentially by females (Fig. 1, males vs. females: Wilcoxon test, day 2: $z = -2.2$, $N = 6$, $p < 0.05$; day 5: $z = -2.2$, $N = 6$, $p < 0.05$; day 8: $z = -2.2$, $N = 6$, $p < 0.05$; day 13: $z = -2.2$, $N = 6$, $p < 0.05$; day 20: $z = -0.48$, $N = 6$, $p > 0.05$). The mothers grabbed the young in the neck or other parts of the body with their teeth. At day 20 the retrieving behaviour of females ended.

The males also tried to retrieve their offspring but they pushed the young back to the nest with their snout. However, they failed in all observed cases.

Discussion

In nature Mongolian gerbils live under territorial conditions in groups which are established by a founder pair (Bannikov 1954; Ågren 1984; Hendrie and Starkey 1998). In addition to the female, the male and the other family members also participate in promoting the development of the offspring (Elwood 1975; Ostermeyer and Elwood 1984). During their first days they have an incompleted ability to thermoregulate and are warmed and sheltered in a nest by the parents in order to stay alive. Paternal behaviour is described also in other rodent species. Wolff and Cicirello (1991) showed that *Peromyscus maniculatus* males retrieved pups and nested with females and newborn pups. In the laboratory adult gerbils built plain nests for common resting (rest-nest). For rearing the altricial young, the nests were enlarged and constructed more compactly mainly by the females (litter-nest). As shown in the golden hamster, the size of the nest and the amount of the female nest-building activity do not depend on the sexual cycle or the state of gravidity but on the environmental temperature (Richards 1966; Bhatia et al. 1995). Since the room temperature in our experiments was high (23-25 °C), the nest-building activity was relatively low. The increase in the female nest-building behaviour immediately after the birth of the pups is a response to parturition and the presence of the young. Besides the temperature, the nest-building behaviour in house mice and other myomorph rodents is intensified according to olfactorical and acoustical (ultrasonic-) stimuli of the young (Noirot 1972, 1974; Sales and Smith 1978). Two weeks after birth the coat of the pups is well developed and the reduced relative surface of the body diminishes the loss of body heat. This shift of the young gerbils from being "heat sinks" to "heat sources" is reflected in a reduction of the nest-building effort following day 13.

In contrast to the female-biased nest-building both males and females cared for the progeny in the nest. While one animal stayed in it and warmed the young, the other left it. This ensues from the increased separate nest-residence of the father and the mother after the litter, i.e., there was an intersexual time sharing in the nest. Even in the prairie vole, *Microtus ochrogaster*, the female does leave the nest more often when the male takes part in the care of the litter (Wang and Novak, 1992). Nevertheless, in this case the data were not calculated for a timed synchrony. Wynne-Edwards (1995) observed the care behaviour of *Phodopus campbelli* for 30 minutes per day during the activity phase and during rest, respectively. She was also able to prove a temporal synchronization in the care behaviour between the parents and additionally between mother and sister, i.e., the aunt of the offspring. The cooling down of the pups is prevented due to this temporal coordination of parental behaviour. Furthermore, the mother is able to satisfy her increased need for nutrients following the litter and during lactation (Galef 1983) and to reduce the physical strain caused by the lactational hyperthermia. This phenomenon of an increased core body temperature while in physical contact with the young is described in various small mammals (Adels and Leon 1986; Scribner and Wynne-Edwards 1994a, 1994b). In gerbils the daily mean values of core body temperature during the whole period of lactation are elevated by 0.6 °C (Weinandy and Gattermann 1995).

Although the common nest-residence of the pair partners was longer than the separate ones, it was not influenced by the pups. In accordance to their nocturnal activity pattern (Weinandy and Gattermann 1996/97) and their social behaviour gerbils rest together during most of the light phase, irrespective of the presence of young. They were left alone in the nest for about 8.6 h per day, i.e., the pups were not constantly warmed by the parents. Our assumption is that this is a consequence of the relatively high temperature conditions in the laboratory. Furthermore, the mutual warming of the young also reduced the loss of heat.

Retrieving the offspring is another direct nursing effort, which was in our study only successfully carried out by the female. The observed increase of retrieval behaviour was most likely triggered by ultrasonic vocalization and the growing locomotor activity of the young, which left the nest more frequently. Furthermore, they were thrown out of the nest due to the species specific stereotypic digging behaviour of both adults (Wiedenmayer 1997). Nevertheless during the rearing period, the females tended to dig more often (67 to 88 minutes per day; unpubl. obs.). Similar results for this species were obtained by Kaplan and Hyland (1972) and they considered this phenomenon an indication of female hyperactivity connected with litter and lactation.

In conclusion, in gerbils there is no paternal support in the building of the litter-nest and the retrieving of the young. Both adults cooperate via their synchronized presence with the young (temporal coordination or time sharing in the nest). We suggest that via this mutual behavioural synchronization the physiological strains of the female caused by lactational hyperthermia are reduced.

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Zusammenfassung

Elterliche Jungenpflege und zeitliche Kooperation bei der Mongolischen Wüstenrennmaus

Das elterliche Pflegeverhalten der sozial lebenden Mongolischen Wüstenrennmaus (*Meriones unguiculatus*) wurde von der Geburt bis zur Entwöhnung der Jungen unter Laborbedingungen quantitativ erfaßt. Nestbau, Nestaufenthalt und das Eintragen der Jungtiere wurde analysiert. Die Registrierung dieser Verhaltensweisen der Elterntiere erfolgte per Videobeobachtung an den Tagen 2, 5, 8, 13 und 20 nach der Geburt der Jungen (Tag der Geburt=Tag 0) für jeweils 24 Stunden. Als Kontrolle wurden alle Paare darüber hinaus einmalig für 24 Stunden ohne Nachwuchs beobachtet. Neugeborene Mongolische Wüstenrennmäuse sind typische Nesthocker. Ziel dieser Studie war es, den väterlichen und den mütterlichen Aufwand bei der Jungenaufzucht zu ermitteln. Das Weibchen leistete insgesamt den größten Anteil an der Brutpflege, da es keine männliche

Unterstützung hinsichtlich des Baues des Wurfnestes oder des Eintragens der Jungtiere gab. Dagegen kooperierten beide Elterntiere aufgrund ihrer synchronisierten Anwesenheit bei den Nestlingen miteinander (temporale Koordination). Wir vermuten, daß durch diese wechselseitige Verhaltenssynchronisation die physiologischen Belastungen des Muttertieres, beispielsweise verursacht durch die Laktationhyperthermie, reduziert werden können.

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2.4 Mating behavior during the estrus cycle in female Mongolian gerbils (*Meriones unguiculatus*)

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Key words: Mongolian gerbils, estrus cycle, mating behavior, vaginal smears

Mongolian gerbils (*Meriones unguiculatus*) are common socially living rodents in the steppe and semi-desert regions of Mongolia and Manchuria (Gromov 1990). In their natural habitat, families, grouped around a founder pair, are strictly territorial (Ågren et al. 1989). Male behavior has been shown to be influenced by females (Probst and Lorenz 1987). Since the current literature on the female estrus cycle is limited and ambiguous (Marston and Chang 1965; Nishino and Totsukawa 1996), a redescription appears to be necessary. The aim of the present study was to obtain detailed data on the four stages of the estrus cycle in the Mongolian gerbil.

Adult Mongolian gerbils *(Meriones* unguiculatus) of both sexes from different litters aged 12-28 weeks were selected for this study. They derived from our own laboratory stock (Zoh:CRW) and were kept in climatised windowless rooms under a photoperiod of LD=14:10 (lights on at 0500 h CET; 200-300 lx during the light phase, approximately 5 lx during the dark phase). The room temperature was 23±2 °C and the relative humidity varied between 65 and 70 %. The animals were housed in plastic cages (55x33x20 cm) with a wire mesh top. Tap-water and food pellets (Altromin® 7024, Altromin GmbH, Lage) were provided ad libitum. The animal bedding (Allspan®, NL) was renewed every two weeks.

Initially, the four different stages of the estrus cycle were defined in adult females (n = 18) by taking vaginal smears daily between two to four hours after

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[4] Weinandy, R., S. Hofmann & R. Gattermann. 2001. Mating behavior during the estrus cycle in female Mongolian gerbils (*Meriones unguiculatus*). Mamm. biol. **66**: 116-120. Eigenanteil: Planung Experimente, Auswertung und Manuskript

lights on, over a period of two months. The stained smears were microscopically analysed (Leica®, Type DMRBE, x200).

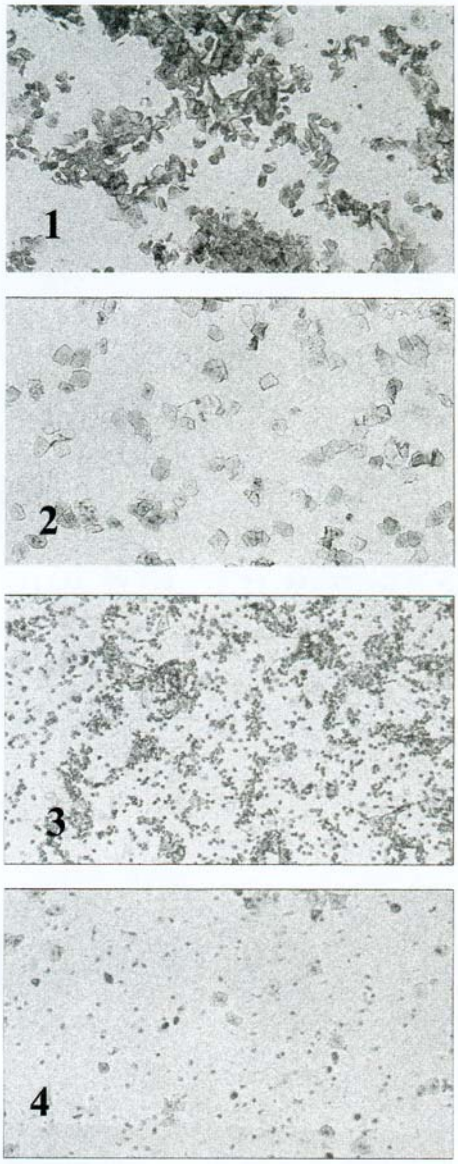

Fig. 1. Vaginal smears during the estrus cycle. Photographed under microscope (× 200).
1. Preestrus: high number of squamous epithelial cells, absence of leukocytes and almost no cornified epithelial cells; 2. Estrus: low number of squamous epithelial cells, high number of dispersed cornified epithelial cells and no leukocytes; 3. Metestrus: mainly leukocytes, isolated squamous epithelial cells and/or cornified epithelial cells; 4. Diestrus: low number of leukocytes, no or only a few squamous epithelial cells and/or cornified epithelial cells.

In figure 1 the respective pattern of the four stages of the estrus cycle is depicted. Some females remained in diestrus for up to 14 days, i.e., the cycle became irregular or was arrested for that period of time. However, it was always followed by the preestrus and the estrus cycle proceeded regularly. Mating tests were performed during the four different stages of the estrus cycle of the gerbils. To prevent gravidity, adult but sexually inexperienced males were sterilized by vasectomy. Two weeks after surgery they were taken to perform mating tests. Vaginal smears were taken from all 24 females to evaluate their stage of estrus cycle two hours before the start of the mating tests (20-30 minutes after lights off). The lowest number of females, to which a stage could be unambiguously assigned, counted 11. In the following, always 11 out of 24 females were randomly chosen before every mating test. For each stage the animals were tested in a clean cage with new animal bedding. Ten minutes before the female was introduced, a vasectomized male was put into the cage. Each test lasted for ten minutes and the frequency of the following activities of the females was registered: copulation [c]: female is mounted by the male combined with friction movements; copulation trials [ct]: female presses tail to bottom and prevents the male, which tries to mount the female; lordosis [l]: female remains in front of the male with bent hind paws and lifted tail; copulation avoidance behavior [cab]: female poses head towards the male, vocalizes and/or avoids the male, genitals and tail are directed away. Kruskal-Wallis analysis of variance and subsequent two-tailed Mann-Whitney U-test (Winstat V 3.1) were used to assess differences in the mating tests. Since multiple tests were run on the same basic dataset, the resulting p-values were corrected by the standard Bonferroni procedure. Differences were accepted as significant at p<0.05 (* in Fig. 2).

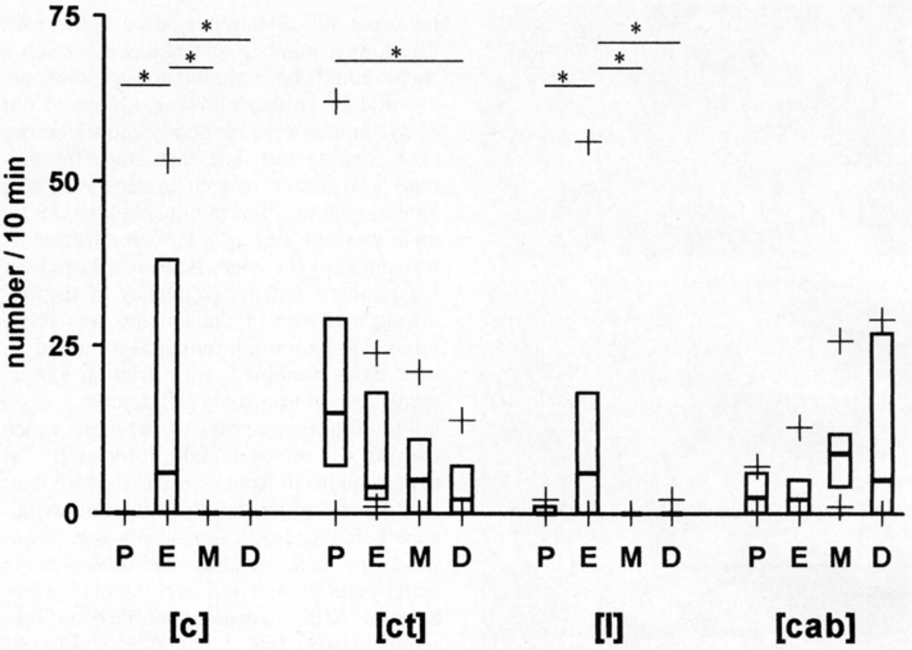

Fig. 2. Frequency of behavioral parameters during the mating tests. Females (n = 11) were tested during preestrus (P), estrus (E), metestrus (M) and diestrus (D).
[c] = copulation; [ct] = copulation trials; [l] = lordosis; [cab] = copulation avoidance behavior. Median values and the interquartils are shown, differences are significant at p < 0.05 and given as asterisks in the graph.

Figure 2 shows the results of the mating tests. The copulation behavior occurred exclusively in estrus (Kruskal-Wallis H-test: H-value = 20.23, n = 11, p < 0.05; Mann-Whitney U-Test estrus vs. preestrus, metestrus and diestrus: in all cases U = 27.5, p = 0.0346). The number of copulation trials was highest during the preestrus and lowest at diestrus. This difference was significant (Kruskal-Wallis H-test: H-value = 10.86, n = 11, p < 0.05; Mann-Whitney U-Test preestrus vs. estrus: U = 31, n.s.; preestrus vs. metestrus: U = 26.5, n.s.;

preestrus vs. diestrus: U = 16, p = 0.0188; estrus vs. metestrus: U = 48, n.s.; estrus vs. diestrus: U = 35.5, n.s.; metestrus vs. diestrus: U = 50.5, n.s.). The lordotic behavior was mainly shown in the estrus (Kruskal-Wallis H-test: H-value = 18.37, n = 11, $p < 0.05$; Mann-Whitney U-Test preestrus vs. estrus: U = 21.5, p = 0.0369; preestrus vs. metestrus: U = 44, n.s.; preestrus vs. diestrus: U = 57, n.s.; estrus vs. metestrus: U = 16.5, p = 0.0048; estrus vs. diestrus: U = 20.5, p = 0.0244; metestrus vs. diestrus: U = 12.5, n.s.). There were no significant differences concerning copulation avoidance behavior towards the males during the estrus cycle (Kruskal-Wallis H-test: H-value = 7.21, n = 11, n.s.). The morning after the females were tested in estrus, 7 of the 24 tested females developed a vaginal plug.

In various rodents the uterus and the vagina as targets of ovarian hormones show cycle-dependent proliferation and apoptosis of luminal and glandular epithelium (Sato et al. 1997). The periodical increase and decrease of squamous epithelial cells, leukocytes and cornified epithelial cells in vaginal smears is a consequence of these changes and has already been described for rats (Otha 1995) or golden hamsters (Sandow et al. 1979; Gattermann et al. 1985) and reliably indicates the estrus. In gerbils, the preestrus used to be characterized by an increased number of squamous epithelial cells and the absence of leukocytes (Nishino and Totsukawa 1996). The aggressiveness of the females was low and they displayed only minor copulation avoidance behavior towards the males. This belongs to precopulatory behavior which may have a proceptive function (Holman et al. 1985). The estrus stage is a period of characteristic behavior including sexual receptivity (lordotic posture) in confrontation with males and the related vaginal smear pattern have already been described (Barfield and Beemann 1968; Adams and Norris 1973; Vick and Banks 1969). A further indicator for the receptivity in *Meriones unguiculatus* is a vaginal plug (Marston and Chang 1965; Norris and Adams 1981). Due to the receptive stage, the interactions initiated by the females were not aggressive during the mating tests. The typical cellular pattern of metestrus was in some cases preceded by clustered cornified cells and isolated leukocytes. This has already been described in a previous study and classified as "estrus II" (Nishino and Totsukawa 1996). Our data do not confirm this suggestion, because our vaginal smear alike was always connected to metestrus behavior. A possible explanation for these contradictory results may be found in the diverging procedure, i.e., in the cited investigation the animals were injected with pregnant mare serum gonadotropin and human chorionic gonadotropin. The elevated level of gonadotrophin might have extended the estrus without affecting the vaginal epithelium. In the present study the females displayed no sexual behavior in that stage. The diestrus is generally defined as a "state of rest" between met- and preestrus, when the female was not fertilized. As described in an earlier study (Ågren and Meyerson 1977) the behavior of the females is agonistic and biased towards avoidance. Our analysis of the estrus cycle revealed characteristic changes in mating behavior of female gerbils.

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2.5 The oestrus of female gerbils, *Meriones unguiculatus*, is indicated by locomotor activity and influenced by male presence

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Abstract. Mongolian gerbils are territorial and live in family groups with established, stable male female pairs. The objectives of this study were to investigate whether locomotor activity indicates female receptivity, and to evaluate the impact of a male on the stability of the female sexual cycle. To prevent gravidity, males were sterilised by vasectomy. Nevertheless, they behaved like intact males, displayed copulatory behaviour, and had normal mean serum testosterone levels of 1.1 ± 0.2 ng/ml. Oestrus caused modulations in the activity pattern and hence influenced locomotor activity; female receptivity was reflected by an advance in the onset of activity. Male presence led to prolonged and irregular oestrus cycles in females. Three pairs were stable and nearly no attacks occurred for nine months. However, in four pairs, females suddenly attacked males. Males displayed flight and appeasement behaviours, but two died within 24 hours, and two were severely injured and had to be separated. Two of these females were paired again to other vasectomised males, but killed their cagemates after 3.5 and 12.5 weeks, respectively. In total, six of nine pairs were disrupted by female aggression, four males were killed and two males were separated but presumably would have otherwise died. Females presumably detected male infertility by repeated unsuccessful matings without sperm transmission and/or by physical impairments due to repeated pseudopregnancy. As a result they no longer invested in pair bonds that would eventually diminish their reproductive fitness.

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Key words: locomotor activity, vasectomised males, oestrus cycle, female aggression

Introduction

The female sexual cycle is defined by rhythmical changes in both physiological status and behaviour. Female mammals develop infradian sexual cycles with a length of 24 hours $< \tau <$ 28 days during their reproductive period. Studies of laboratory rats (De Kock & Rohn 1971, Wollnik & Turek 1988), *Microtus pennsylvanicus* (Kerbeshian et al. 1994*), Clethrionomys glareolus* and *Lemmus lemmus* (Carmichael et al. 1981) have shown that, depending on the receptive stage, various physiological and ethological parameters (e.g. wheel-running activity) change periodically. The most stable and predictable infradian rhythm occurs in golden hamsters (*Mesocricetus auratus*). This lasts for 96 hours and is reflected in the vaginal cells and sexual behaviour, as well as in fluctuations in body temperature, body mass and locomotor activity (Richards 1966, Gattermann et al. 1985, Fritzsche 1987). Mongolian gerbils (*Meriones unguiculatus* Milne-Edwards, 1867) are common in Mongolia and Manchuria (Gromov 1990, Thiessen & Yahr 1977). In their natural habitat they live in families established by stable male female pairs (Ågren 1976, 1984, Ågren et al. 1989), the disruption of which has consequences for subsequent social behaviour (Hendrie & Starkey 1998, Starkey & Hendrie 1998). Both sexes contribute to pup rearing - at least in the laboratory (Weinandy & Gattermann 1999). The female sexual cycle lasts for four to six days, its four stages are characterised by behavioural parameters, and by a fixed succession and number of epithelial cells, leukocytes and cornified cells in vaginal smears (Weinandy et al. 2001). One objective of this study was to show that locomotor activity can also serve as an indicator of female receptivity. Furthermore, we expected males to influence the female sexual cycle due to their sociobiology and pair bonds. To prevent females becoming gravid, males were sterilised by vasectomy. The second aim of our study therefore was to evaluate the impact of males on the stability of the sexual cycle in female gerbils.

Material and Methods

Animals and environmental conditions

Adult Mongolian gerbils from different litters aged 16 - 28 weeks were chosen for this study (female: N=14; male: N=9). Animals were derived from our own laboratory stock (Zoh:CRW) and were kept in acclimatised, windowless rooms under 14:10 L:D photoperiod (lights on at 05.00 h CET; 200-300 lx during the light phase, approximately 5 lx during the dark phase). The room temperature was 23°C ± 2°C and relative humidity varied between 65 - 70%. Animals were housed in plastic cages (55 x 33 x 20 cm) with a wire mesh top, and including an exercise wheel (diameter = 30cm, running wheel area width = 10cm). Tap water and food pellets (Altromin® 7024, Altromin GmbH, Lage) were provided ad libitum. Animal bedding (Allspan®, NL) was renewed every two weeks.

Activity recording

The activities of animals in their cages were recorded via the exercise wheels; each revolution triggered an impulse which was stored and analysed by ”The Chronobiological Kit” (Stanford Software Systems, Stanford, CA, USA). The double plotted actograms represent the distribution of locomotor activity during consecutive 24-hour-periods. This plot uses lines of varying heights to show the activity amplitude of each five minute block. To calculate the period τ of the infradian rhythms of the wheel-running activity, we determined the amount of female activity from 05.00 to 18.00 h and analysed this by cosinor analysis (Halle & Weinert 2000) for a range of three to 14 days. These calculations were based upon periods of 64 days each. The horizontal line in the graphs represents the level of significance ($p<0.05$). To compare infradian rhythms of paired and isolated females (N=5 in each case) any advance in the onset of activity was calculated. To further differentiate between oestrus and metoestrus, the difference in the mean amount of activity per day of each animal for the two stages was calculated.

Morphometrical parameters

Body mass was measured using digital balances (Kern® 440-45, precision 0.1 g). Ventral gland area measurements (length x width) were taken in cm² with standard sliding vernier callipers (precision 0.5 mm). The four stages of the sexual cycle were examined in each female by daily vaginal smears taken two to four hours after lights-on over a period of eight weeks. The stained smears were analysed and documented microscopically (LEICA®, Type DMRBE, x200) (for further details, see Weinandy et al. 2001). To characterise the testes and take blood samples (see below) animals were anaesthetised, decapitated and dissected at approximately the same time of day, i.e. between 8.00 and 10.00 h.

Steroid hormone determination

To determine serum titres of testosterone, progesterone and cortisol, a blood sample was collected within three minutes of death. This was centrifuged and the serum stored at -28 °C until determined by commercially available enzyme immunoassay kits (testosterone: SR 1 Testosterone; progesterone: SR 1 Progesterone, both kits supplied by BioChemImmunoSystems GmbH, Freiburg, Germany; cortisol: Cortisol-Immulite; kit supplied by DPC Biermann GmbH, Bad Nauheim, Germany).

Statistics

Data are given as means of the scores in the sample, and the statistical measure of variance is the standard error of estimate (SE). A two-tailed U test was used to assess the differences of the means, which were significant at $p<0.05$. Analyses were undertaken in Winstat (V 1999.2).

Results

Vasectomized males

Nine adult but sexually inexperienced males were vasectomised. At least two weeks after surgery they were paired with females aged 26 ± 3 weeks. They were subsequently monitored at regular intervals via direct observation, and appeared to behave like morphologically intact males - displaying copulatory behaviour when the females were in oestrus. Three pairs lasted until the end of the experiments, when the males were sacrificed. At this time, the mean male age was 66 ± 2 weeks, their body mass was 89.2 ± 2.8 g and the ventral gland area was 1.78 ± 0.07 cm². Due to female aggression in six pairs, four males were killed by their female cagemates at a mean age of 30 ± 6 weeks. Furthermore, two pairs had to be separated due to severe injuries to the males (mean age = 57 ± 1 weeks). The carcasses of some of the dead males were in such a poor condition that only the body mass of one and the ventral gland area of two could be measured. As a result, data on dead and injured males were pooled: body mass was 89.5 ± 7.3 g (N=3) and ventral gland area was 1.31 ± 0.24 cm² (N=4). Comparisons of the stably-paired (spm) and the dead males/injured males (dm/im) revealed that the body mass between both groups did not differ (U test: U=3, NS; N1=3, N2=3) but the ventral gland area was enlarged in the spm (U test: U=0.5, $p<0.05$; N1=3, N2=4). The mean serum concentrations of testosterone and cortisol were determined for six males (three spm, one dm/two im). The mean testosterone titre was 1.1 ± 0.2 ng/ml, whereas the values for spm (1.3 ± 0.4 ng/ml) and the dm/im (0.8 ± 0.2 ng/ml) did not differ (U test: U=1.5, NS; N1=3, N2=3). The mean serum cortisol concentration for the six males was 227.7 ± 70.6 ng/ml. Although there was a tendency for reduced cortisol concentrations in the spm (125.7 ± 51.4 ng/ml) vs. the dm/im (329.8 ± 109.1 ng/ml) the difference was not significant (U test: U=2, NS; N1=3, N2=3). The testes of five males (three spm, one dm/one im) were analysed histologically. In all cases the testes were fully developed but did not contain sperm cells, the lumina of the vesicles being partially filled with layers of connective tissue.

Females

The double-plotted actograms of the females' wheel-running activities revealed a nocturnal pattern of activity pattern typical of the species. Regularly, the activity phase started 4.7 hours (Fig. 1 a, isolated females) or 5.0 hours (Fig. 1 b, paired females) earlier than usual, i.e. females started to use the treadmills between 02.00 and 03.00 hours. As validated by vaginal smears (data not shown), this early onset coincided with the beginning of oestrus, i.e. it indicated the receptive stage. However, oestrus was not necessarily combined with an early onset.

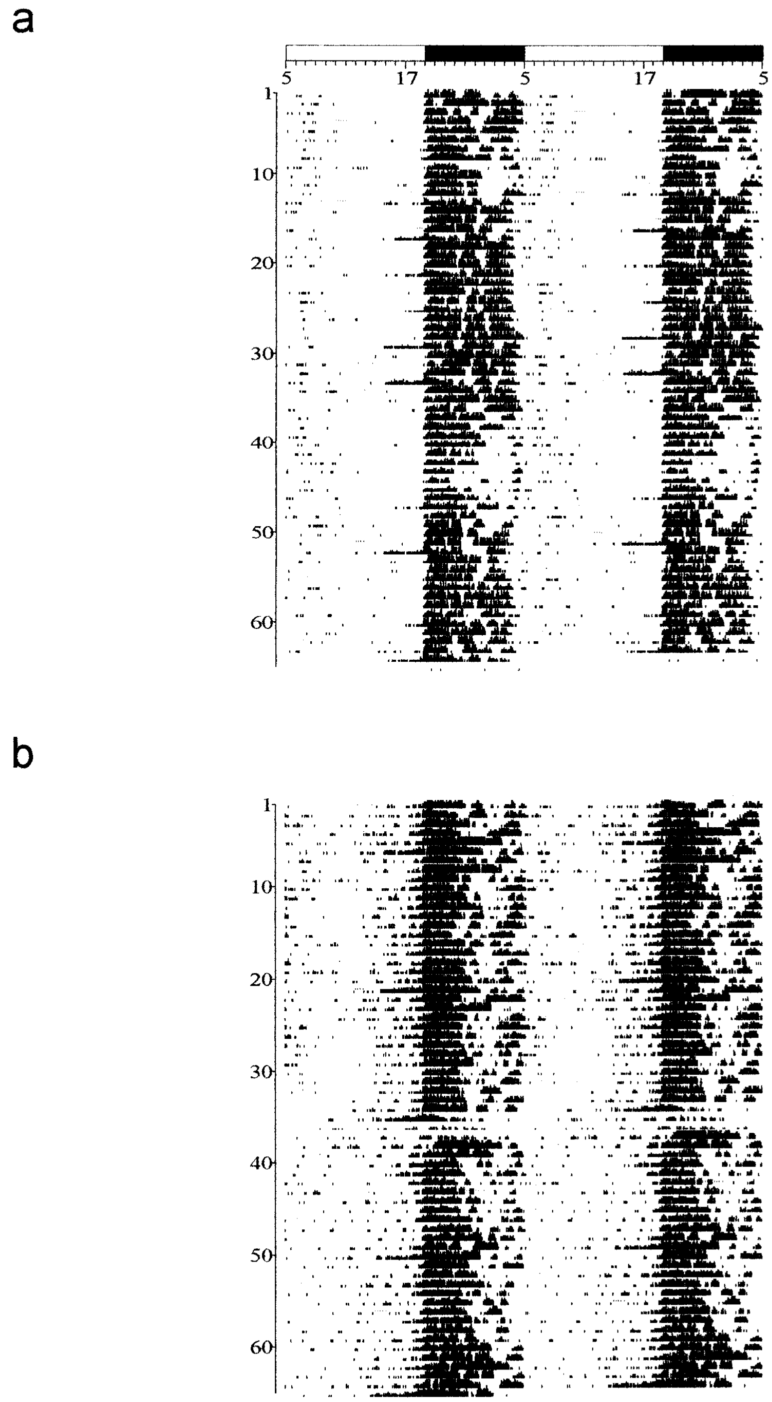
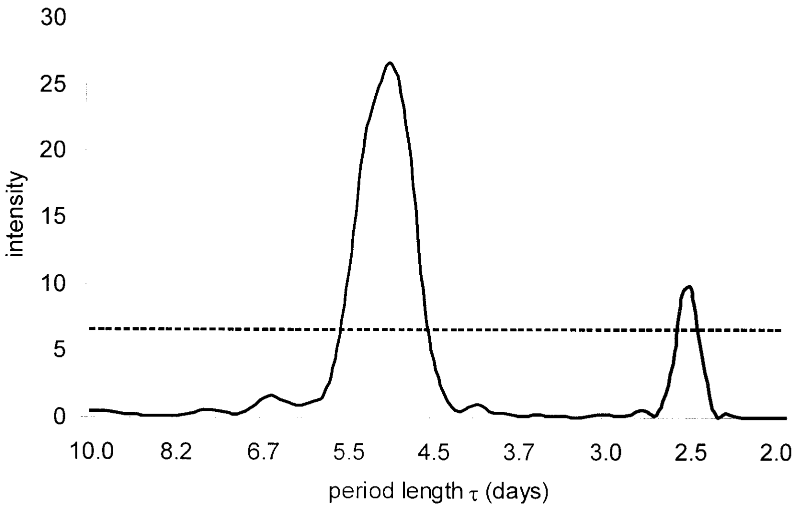


Fig. 1 a,b. Double-plotted actograms of wheel-running activity for (a) one female kept isolated and (b) for one female kept pairwise. The wheel-running activity was recorded for consecutive 24-hour-periods over 65 days. The white and black bars indicate the LD regime 14:10.

a



b

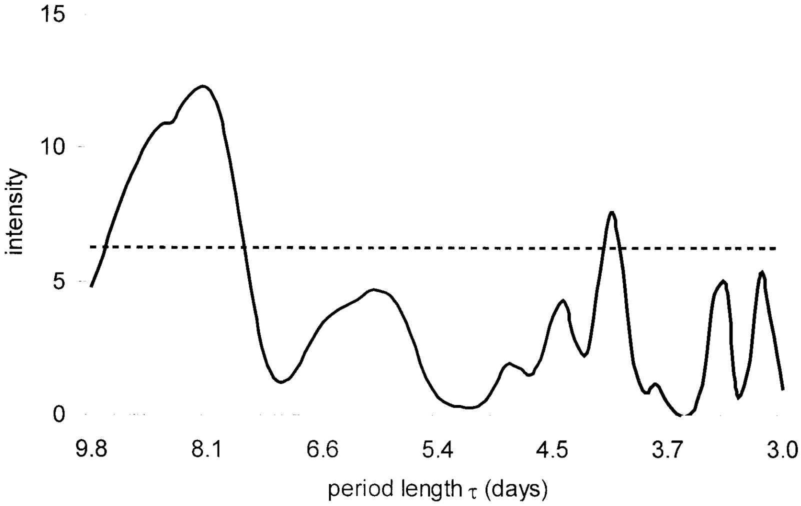


Fig. 2 a,b. Infradian rhythms of wheel-running activity for (a) one female kept isolated and (b) one female kept pairwise. Period length τ in days were analysed via cosinor analysis for a range of 3 to14 days. These calculations are based upon the amount of activity from 05.00 to 18.00 hours for a total period of 64 days each. The horizontal line in the graphs represents the level of significance ($p<0.05$).

The resulting mean period length τ in days of the infradian rhythms (i.e. cycles with a length of 24 hours < τ < 28 days) of wheel-running activity from 05.00 to 06.00 h were calculated via cosinor analysis for a range of three to 14 days. Fig. 2a shows a periodogram for a typical female that had been kept isolated. The infradian rhythm of the locomotor activity was clearly 5.1 ± 0.6 days. In contrast, the mean period of the infradian rhythms of paired females was prolonged and less stable (Fig. 2 b). Consecutive cycles varied from five to 14 days with a mean duration of 7.2 ± 1.3 days (N=7), whereas the females remained in dioestrus.

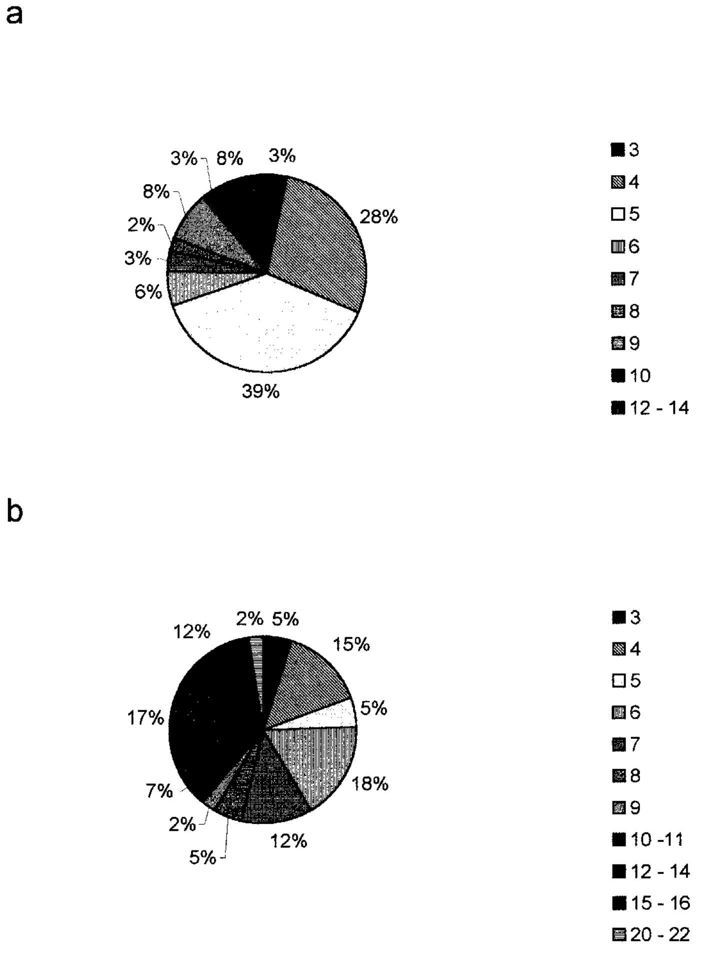


Fig. 3 a,b. Percentage of different inter-oestrus differences in (a) isolated females (N=6) and (b) females kept in pairs (N=4). Also given are the distances between consecutive oestrus stages in days, which were determined via daily vaginal smears for a total period of 56 days.

Fig. 3 shows the percentage of inter-oestrus variations in isolated (Fig. 3a; N=6) and paired females (Fig. 3b; N=4). The graphs reflect the relation of sexual cycle irregularities to social housing conditions. The inter-oestrus distances for isolated females were mainly four (28%) and five days (39%), whereas in the paired females they ranged from three to 22 days. These morphological findings coincide with the locomotor activity data, which show that only isolated females had pronouncedly stable infradian rhythms.

The stability of established gerbil pairs is shown in Fig. 4. Three females (F1, F2 and F4) were regularly-paired to their males for 260 days (F1) and 268 days (F2 and F4). Female F3 suddenly attacked her male after 74 days; severe biting occurred and the male died within 24 hours. Following that, the female remained isolated. Female F5 attacked her cagemate after only 20 days, which led to the death of the male 21 days post-pairing. Ten days later, the female was paired again to another vasectomised male, however, this was also killed after 90 days. The male that was paired to F6 had to be separated on the 74th day post-pairing, since it had been severely injured by biting by the female. Following a rest of 44 days, the female was re-paired, but the new male was killed after 26 days. Female F7 stayed with her male for 79 days, but eventually the pair had to be separated due to agonistic interactions initiated by the female. In total six of nine pairs were disrupted, four males were killed and two further males were separated, but presumably would have died otherwise. The infradian rhythms of all females that killed their cagemates stabilised immediately after being isolated. Three females became oestrous within three days of the death of their males and, moreover, had fixed infradian period lengths of four days (as verified by vaginal smears). When kept in pairs, the same females remained in dioestrus for up to 14 days, resulting in irregular sexual cycles of nine to more than 20 days. The mean serum concentrations of progesterone and cortisol were determined for all seven females. The mean progesterone titre was 10.4 ± 10.7 ng/ml, whereas the values for the three constantly-paired females (1.0 ± 0.5 ng/ml) and the four females that attacked their males (17.5 ± 15.8 ng/ml) did not differ (U test: U=4, NS; N1=3, N2=4). The mean serum cortisol concentrations of the seven females was 134.6 ± 47.5 ng/ml, whereas the cortisol titre of F1, F2 and F4 vs. F3, F5, F6 and F7 was nearly the same (137.8

± 65.3 vs. 132.2 ± 59.3 ng/ml). A male/female comparison showed that they were not significantly different (U test: U=15, NS; N1=6, N2=7).

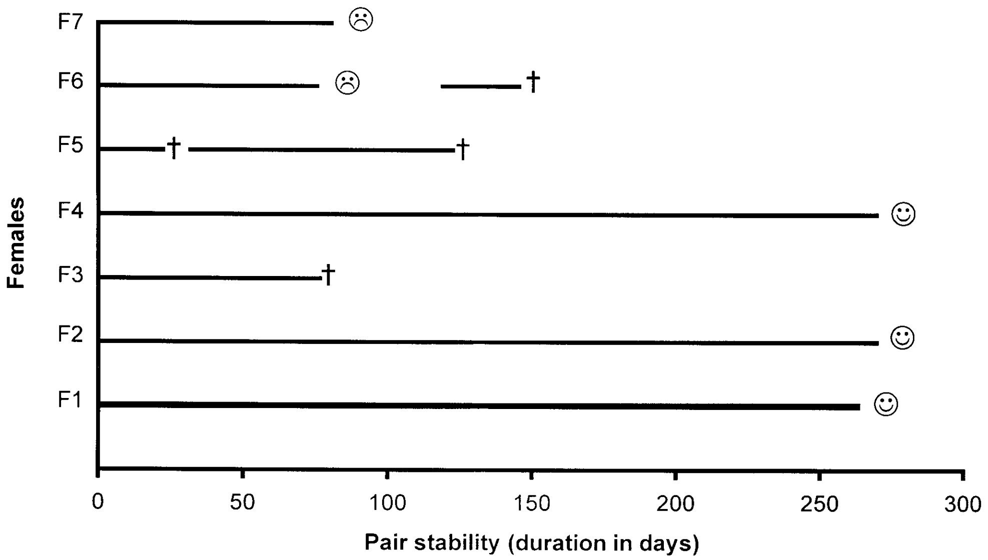


Fig. 4. Stability of established gerbil pairs. The female-IDs F1 to F7 (N=7) are given on the y-axis, the duration in days is shown on the x-axis. The horizontal lines represent the respective duration of stability in established pairs. Two females, F5 and F6 were paired to a second male. ☹ = Male was severely injured by the female and had to be separated; † = Male was killed by the female; ☺ = Male remained paired until the end of the experiment.

Discussion

The determination of oestrus via locomotor activity has been described as unreliable in Mongolian gerbils (Barfield & Beeman 1968, Umezu et al. 1989). In contrast, the actograms presented here based on wheel-running activity suggest that oestrus caused modulations in activity patterns, and hence influenced the locomotor activity of nocturnal gerbils. Female receptivity was reflected by an advance in the onset of activity which otherwise was correlated to lights-off times. Although not necessarily combined, such an advance coincided in each case with the beginning of oestrus, which has been shown to start earlier than in various other rodents (Barfield & Beeman 1968). Labyak & Lee (1995) found a two-hour advance in the onset of activity in degus (*Oc-*

todon degus) which recommenced the subsequent day five hours later. At the same stage of the sexual cycle, female golden hamsters display a similar advance, combined with an elevated oestradiol titre under both a 14:10 L:D lighting regime and under constant darkness (Morin 1977, Finkelstein et al. 1978). Experiments with gerbils maintained in colonies in semi-natural enclosures showed that females were more active and visited adjacent territories in oestrus, presumably triggered by endocrine changes (Vick & Banks 1969, Ågren 1976).

Male impact on the sexual cycle has been investigated repeatedly. In wood mice (*Apodemus sylvaticus*), Jonsson & Silverin (1997) found male presence to have no influence on females. However, Stopka & MacDonald (1998) investigated the signals responsible for the modulation of oestrus and the oestrus cycle in *A. sylvaticus*, showing that the prolongation of oestrus and curtailment of the oestrus cycle were typical in females caged with males from which they were separated by wire mesh. The main influence on the length of wood mouse oestrus was the presence of alternating-unfamiliar males.

House mice (*Mus musculus*) may arrest their cycle when olfactorial cues from the opposite sex are absent (Bruce 1970) whilst males stimulate female fertility (Bronson 1985). Moreover, conspecific chemical signals in M. musculus influence the length of the cycle and lead to irregular periods of three to seven days, anoestrus or pseudopregnancy (Whitten 1956, Marsden & Bronson 1965, De Leon & Barkley 1987). In our study male presence did not stabilise the infradian rhythms of females. The cycles of paired females were less regular and were prolonged to six to nine days, i.e. they were longer than those of isolated females, and longer compared to data in the literature. This was caused by the vasectomised males behaving like morphologically intact males and displaying copulatory behaviour. Moreover, in the females investigated, vaginal plugs were frequently found after copulation. According to Marston & Chang (1965) and Norris & Adams (1981) these plugs indicate receptivity in *M. unguiculatus* and show that copulation has occurred. The destabilising effect of males is further supported by the fact that those females that were isolated after they had killed their cagemates stabilised and shortened their period length to four or five days. Marston & Chang (1965) and

Barfield & Beeman (1968) found that copulation without fertilisation might be followed by a pseudopregnancy of 14 to 18 days [Toy (1985) reports a length of 14 to 16 days]. This period of time represents the range of variation in the paired females in our study, where one or two cycles with τ=5 ± 1 days were followed by up to 20 days in which females did not become receptive and did not show any oestrus behaviour, i.e. lordosis or copulation. However, the term pseudopregnancy must be used with caution since, although some females had the typical slightly bloody vaginal smear (Marston & Chang 1965), they did not display any signs of lactation. Moreover, Nishino & Totsukawa (1999) stated that although pseudopregnancy has been induced by mating with vasectomised males (30.0%), this method was uncertain because the incidence of pseudopregnancy was lower than that obtained in rats by other means, or by mechanical stimulation in gerbils (83.3%). Both authors differentiated between irregular cycles of a length of seven to 14 days and pseudopregnancy, in which the females remained in dioestrus for at least 12 days (Nishino & Totsukawa 1996). They pointed out that females with irregular cycles may overleap metoestrus, but that they always remain in dioestrus for up to seven days. Assuming that, the influence of the males would therefore lead to prolonged and irregular sexual cycles or pseudopregnancy as a consequence of copulation without fertilisation.

The spontaneous collapses of six of the nine pairs were caused by aggressive interactions initiated by females, and which ended in the death of the males within 24 hours, or in severe injury to the male. This phenomenon has never been described before. The males displayed flight behaviour and the ”paw thrust” (Yahr et al. 1977), an appeasement behaviour which is typical for submissive animals. Gerbil pairs are described as being distinctly stable and independent of endocrine fluctuations (Ågren & Meyerson 1977). In our own laboratory stock, only 13 % of all established breeding pairs (N=45) have had to be separated following aggression. Since these agonistic interactions were not attributed to either males or females, female-forced pair disruptions are supposed to be below 10%. Glucocorticoids are indicators for stress, but literature on the levels of these hormones is scarce and ambiguous. While Fenske (1986) found a titre of 176.7 ± 52.4 ng/ml plasma, Weinandy (1996) characterised gerbils (N=127) by a value of 77.8 ± 7.2 ng/ml serum with no sex differences. Compared to the latter study, at least the paired males in the present investigation must be regarded as stressed, although the six males and the seven females were not significantly different. The progesterone values found for females are difficult to interpret since the variation was high, even though still within the range described elsewhere (Weinandy 1996).

It is well known that sexually experienced female rats are able to differentiate between intact and castrated males via olfactorial cues, irrespective of the sexual cycle. Even inexperienced females prefer the odour of intact males when they are in oestrus (Carr et al. 1965). Castration of male rats leads to reduced aggression and a loss of social dominance due to a lack of testosterone (Albert et al. 1986). The males in our study were vasectomised, but the testes were fully developed and without sperm cells. They copulated with females and their ventral glands were well expressed. As has been shown for the desert gerbil, *Meriones hurrinae*, the endocrine state of the males should not have changed because of surgical interference (Lohiya & Dixit 1974). The testosterone values of the males tested in the present study were within the range reported by Clark & Galef (2000), who examined a possible correlation between testosterone levels and helping at the nest in male Mongolian gerbils, and found values of 0.77 ± 0.12 to 1.52 ± 0.13 ng/ml serum. Moreover, in an earlier study of 70 males, Weinandy (1996) found testosterone titres of 1.6 ± 1.3 ng/ml serum. The secretion of the ventral glands is used by gerbils in the context of reproductive behaviour, e.g. the attraction of potential mating mates, in territorial defence, and for individual recognition (Thiessen & Yahr 1977). Although the ventral glands were significantly enlarged in the stably-paired males (1.78 ± 0.07 cm² vs. 1.31 ± 0.24 cm²) both values were within the range described by Clark et al. (1990), who investigated the influence of foetal uterine position on copulation and scent marking in adult males, and found ventral gland areas of 1.78 ± 0.06 to 1.52 ± 0.08 cm².

It still has not been clarified how females detect male infertility. Most likely it is a mechanism triggered by repeated unsuccessful matings without sperm transmission and/or physical impairment due to repeated pseudopregnancy. As a consequence, females no longer invest in pair bonds with infertile males, which would reduce their reproductive success and eventually diminish their reproductive fitnesses.

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3 Wohlergehen

3.1 Time of day and stress response to different stressors in experimental animals. Part II: Mongolian gerbil (*Meriones unguiculatus* Milne Edwards, 1867)

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Summary

In this paper we report the results of studies on the circadian rhythmicity, physiological norm values and stress responses towards animal house routine stressors of the Mongolian gerbil, *Meriones unguiculatus* (Milne Edwards, 1867). The data were registered telemetrically via implanted transmitters. Under L:D = 12:12 conditions all parameters indicated that the animals were nocturnal. It resulted in bimodal circadian patterns with main peak values at the beginning of the dark period and side peaks at lights on. The mean heart rate over 24 hours ± SD was 312 +- 20 bpm, with significantly lower heart rates in males than females (293 +- 14 vs 326 +- 10 bpm). The mean core body temperature over 24 hours +- SD was 37.5 +- 0.3 degree C, and males had significantly lower values than females (37.3 +- 0.3 vs 37.8 +- 0.1 degree C). The mean activity over 24 hours +- SD was 83 +- 31 units per 5 min and no sex-dependent differences were detectable.

The stress responses to non-social stressors (i.e. handling and cage changing) and social stressors (i.e. effect of an intruder or resident animal and group formation) were investigated. After the application of these stressors animals exhibited significantly increased heart rate, core body temperature and general activity. The stressed animals showed significantly stronger responses and for a longer time when they were stressed during their rest time (light period) compared with their activity time (dark period). There were no sex-related stress response differences. The results of this study were compared with iden-

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[6] Weinandy, R. & R. Gattermann. 1996/97. Time of day and stress response to different stressors in experimental animals. Part II: Mongolian gerbil (*Meriones unguiculatus*). J. Exp. Anim. Sci. **38**: 109-122. Eigenanteil: Experimente, Auswertung und Manuskript

tical investigations on the solitary golden hamster (J. Exp. Anim. Sci. 1996/97: 38: 66-76).

Key words: chronobiology, handling, cage change, grouping, heart rate, body temperature, activity

Introduction

The Mongolian gerbil (*Meriones unguiculatus*) is kept increasingly as an experimental animal. The reasons are its tolerance of various housing conditions, stable reproduction, its low susceptibility to diseases and easy handling because of its docility (Thiessen and Yahr 1977).

The objective of the present study was to ascertain physiological norm values and to evaluate the effects of non-social and social stressors according to the activity state (activity vs rest) in the Mongolian gerbils. To register the stress indicators heart rate, core body temperature and general activity before and after the application of the different stressors, we implanted transmitters in adult males and females. A further aim was to compare these results of the highly social gerbils with that of the solitary golden hamster (part I of this paper). In both rodent species the influence of the activity-state on animal house routines such as cage change or handling has not yet been investigated. This is also true for social stressors like resident and intruder confrontations or grouping. However, findings on these questions are important and necessary for the proper care and management of these two common laboratory species.

Materials and methods

Four adult males and females of Mongolian gerbils (*Meriones unguiculatus* Milne Edwards, 1867) each were chosen at random from our laboratory colony (Zoh:CRW), which were founded from animals obtained from Charles River Germany (CRW/(Mon) BR). They were caged separately or in groups in one room with a L:D = 12:12 (light 250 lx, dark 5 lx, light period: 0700 - 1900 h CET). The room temperature was 24 ± 2 C and the relative humidity varied from 65 - 70 %. The cages (size: 55 x 33 x 20 cm) were plastic with a wire mesh lid and included a circular treadmill (30 cm in diameter and a running area width of

10 cm). Bedding material (Altromin® Animal Bedding, dust free softwood fibers) was exchanged regularly. Tap-water and food pellets (Altromin® 7014/7024, Altromin GmbH, Lage, Germany; crude proteine 19%, crude fat 4.2%, crude fiber 5.8%, ash 7.2%, moisture 13.5 and nitrogen-free extract 50.3%) were provided ad libitum.

All the other experimental procedures such as surgery, data acquisition, stressors and their application, and the statistics are described in detail in section Materials and methods of part I of this paper.

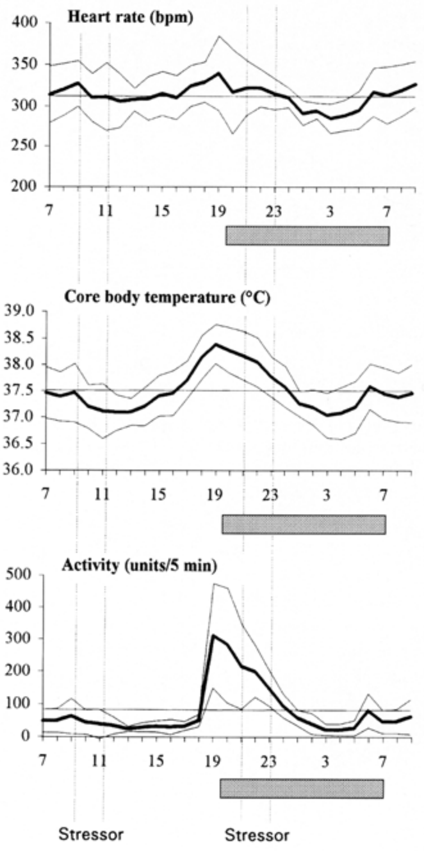


Fig. 1. Circadian patterns of the heart rate, the core body temperature and the general activity of male and female Mongolian gerbils and the times of stressor application. The graphs give mean values ± SEM. Horizontal lines represent the mean of 24 hours (24-hr-level or niveau). All data were calculated on the basis of 10 days per animal. Black bars indicate the dark period. n = 7–8 animals (for details see Table 1).

Results

Physiological norm values

The 24-hour patterns of the heart rate, the core body temperature and the general activity of group-housed and unstressed gerbils are given in Figure 1. All animals were nocturnal with a higher level of activity in the dark period. The circadian patterns of the three investigated parameters were quite similar.

They had a bimodal shape with a main maximum after lights off and a side maximum at the end of the dark period or at the beginning of the light period. The mean heart rate crossed the circadian level (horizontal line in Figure 1) three hours before lights off, the core body temperature two and a half hours and the general activity just one and a half hour before lights off.

On the basis of these data, Table 1 summarizes the physiological norm values of unstressed gerbils. The differences between the circadian maximum and minimum values were always significant ($p < 0.05$). The comparison of the sexes revealed that males had 10% lower mean heart rates and 0.5 °C lower mean and minimum core body temperature ($p < 0.05$). Such sexual-specific differences were not to be found in general activity.

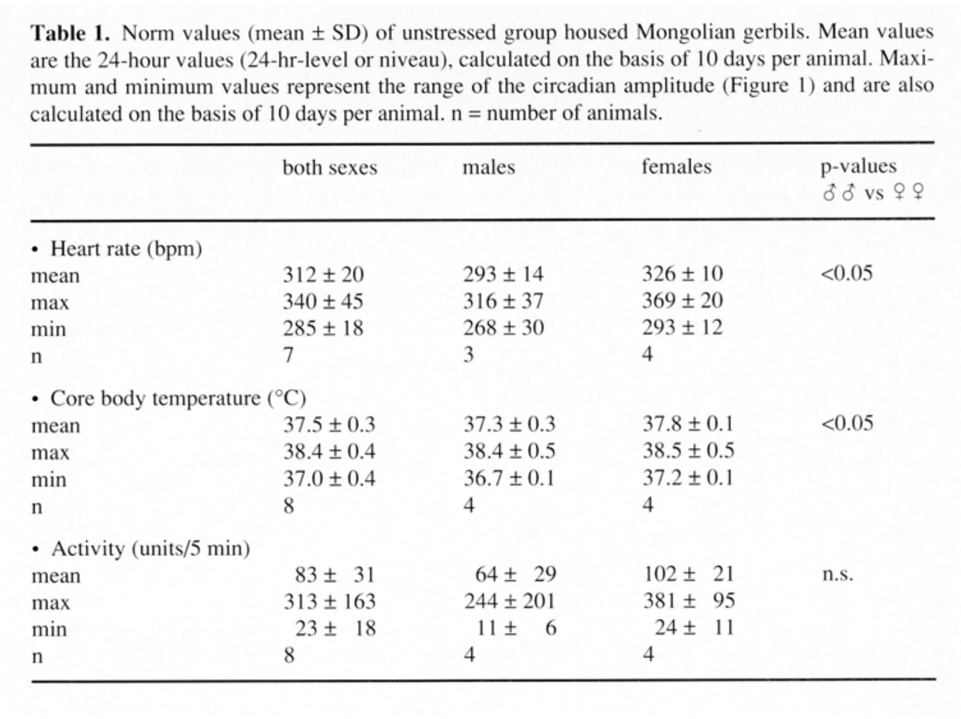


Table 1. Norm values (mean ± SD) of unstressed group housed Mongolian gerbils. Mean values are the 24-hour values (24-hr-level or niveau), calculated on the basis of 10 days per animal. Maximum and minimum values represent the range of the circadian amplitude (Figure 1) and are also calculated on the basis of 10 days per animal. n = number of animals.

	both sexes	males	females	p-values ♂♂ vs ♀♀
• Heart rate (bpm)				
mean	312 ± 20	293 ± 14	326 ± 10	<0.05
max	340 ± 45	316 ± 37	369 ± 20	
min	285 ± 18	268 ± 30	293 ± 12	
n	7	3	4	
• Core body temperature (°C)				
mean	37.5 ± 0.3	37.3 ± 0.3	37.8 ± 0.1	<0.05
max	38.4 ± 0.4	38.4 ± 0.5	38.5 ± 0.5	
min	37.0 ± 0.4	36.7 ± 0.1	37.2 ± 0.1	
n	8	4	4	
• Activity (units/5 min)				
mean	83 ± 31	64 ± 29	102 ± 21	n.s.
max	313 ± 163	244 ± 201	381 ± 95	
min	23 ± 18	11 ± 6	24 ± 11	
n	8	4	4	

Stressor experiments

The stress responses of the eight investigated gerbils with the implanted transmitters are depicted in Figure 2 and Table 2. The maximum stress response after its application and the mean duration of this stress response were the measures for the evaluation of the potentially straining effects of the non-social and social stressors (for details see Material and methods and Figure 1 of part I of this paper).

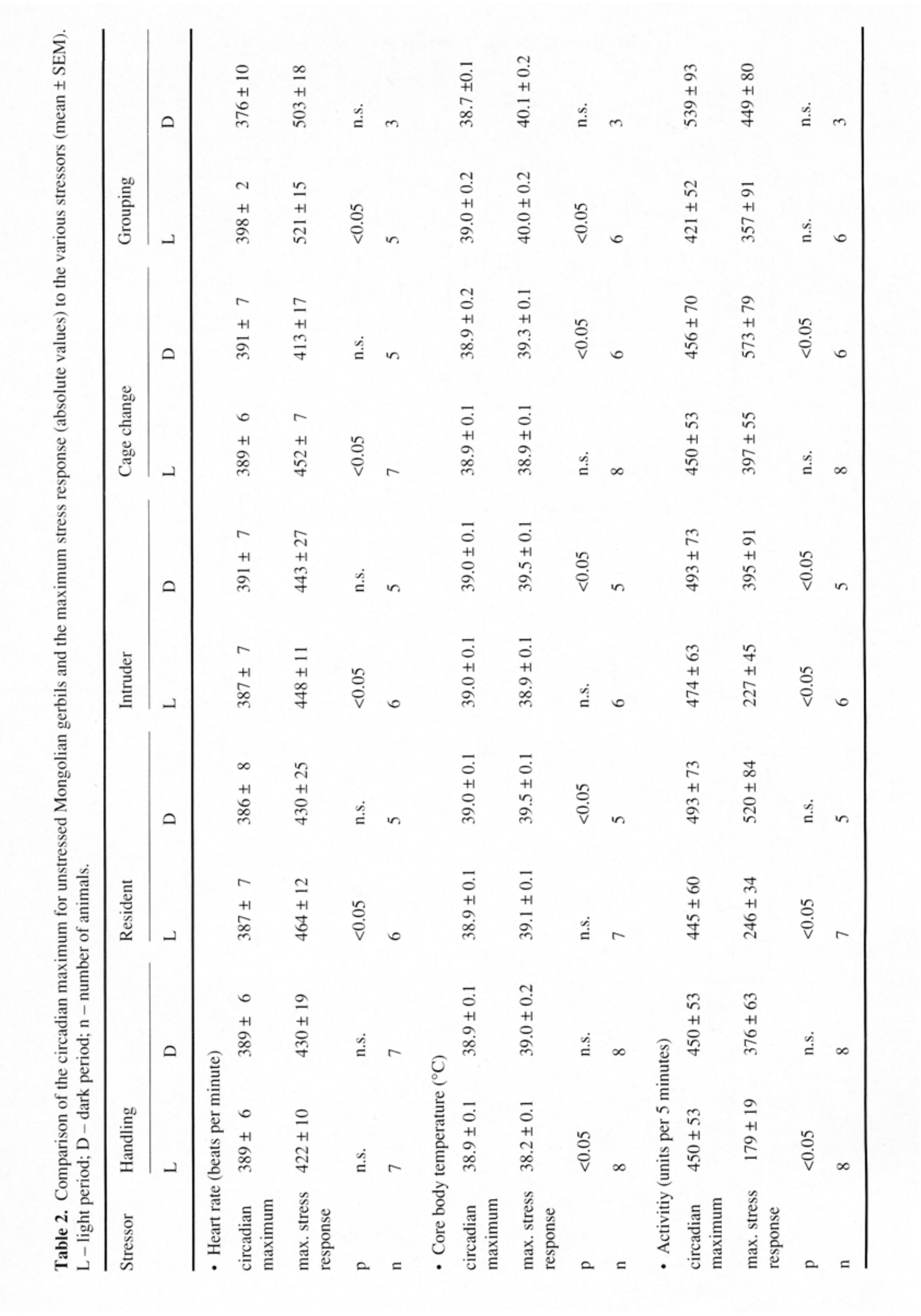


Table 2. Comparison of the circadian maximum for unstressed Mongolian gerbils and the maximum stress response (absolute values) to the various stressors (mean ± SEM). L – light period; D – dark period; n – number of animals.

Stressor	Handling		Resident		Intruder		Cage change		Grouping	
	L	D	L	D	L	D	L	D	L	D
• Heart rate (beats per minute)										
circadian maximum	389 ± 6	389 ± 6	387 ± 7	386 ± 8	387 ± 7	391 ± 7	389 ± 6	391 ± 7	398 ± 2	376 ± 10
max. stress response	422 ± 10	430 ± 19	464 ± 12	430 ± 25	448 ± 11	443 ± 27	452 ± 7	413 ± 17	521 ± 15	503 ± 18
p	n.s.	n.s.	<0.05	n.s.	<0.05	n.s.	<0.05	n.s.	<0.05	n.s.
n	7	7	6	5	6	5	7	5	5	3
• Core body temperature (°C)										
circadian maximum	38.9 ± 0.1	38.9 ± 0.1	38.9 ± 0.1	39.0 ± 0.1	39.0 ± 0.1	39.0 ± 0.1	38.9 ± 0.1	38.9 ± 0.2	39.0 ± 0.2	38.7 ±0.1
max. stress response	38.2 ± 0.1	39.0 ± 0.2	39.1 ± 0.1	39.5 ± 0.1	38.9 ± 0.1	39.5 ± 0.1	38.9 ± 0.1	39.3 ± 0.1	40.0 ± 0.2	40.1 ± 0.2
p	<0.05	n.s.	n.s.	<0.05	n.s.	<0.05	n.s.	<0.05	<0.05	n.s.
n	8	8	7	5	6	5	8	6	6	3
• Activity (units per 5 minutes)										
circadian maximum	450 ± 53	450 ± 53	445 ± 60	493 ± 73	474 ± 63	493 ± 73	450 ± 53	456 ± 70	421 ± 52	539 ± 93
max. stress response	179 ± 19	376 ± 63	246 ± 34	520 ± 84	227 ± 45	395 ± 91	397 ± 55	573 ± 79	357 ± 91	449 ± 80
p	<0.05	n.s.	<0.05	n.s.	<0.05	<0.05	n.s.	<0.05	n.s.	n.s.
n	8	8	7	5	6	5	8	6	6	3

As shown in Figure 2, the scale of the stress responses were strictly dependent on the light-dark-cycle. After handling, resident confrontation and cage change the maximum heart rate was significantly higher during the light period (i.e. the time of rest) than during the dark period (i.e. the time of activity). The stress response for this parameter was prolonged during the light period after resident and intruder confrontation and after changing the cage compared to the dark period. Except for the stressor grouping, the maximum and the duration of the raised core body temperature was always higher and prolonged during the time of rest (light period). Activity was significantly elevated after all stressors except for grouping when treated during the light period. The gerbils were less active than the circadian niveau (columns below the zero-line) when they were handled or confronted with an intruder during the dark period. Since the physiological parameters were elevated after the same stressor applications this indicated that heart rate or core body temperature alterations were not caused by a changed level of general activity. In case of the stress response duration, the gerbils reacted for a longer time after resident confrontation and cage change.

The more intense stress responses during the light period compared to the dark period were mainly caused by different mean basic values of the investigated parameters. The mean heart rate, core body temperature and general activity before the stressor application were significantly lower (in each case $p < 0.05$) in the light period (rest time) than in the dark period (activity time). However, the maximum heart rate after the cage change during the dark period was higher compared with the light period. The maximum core body temperature values after the dark period application of the stressors handling, intruder confrontation and cage change were higher then after the same stressors during the light period. This applied also to general activity: the animals were more active when the stressor experiments (except for grouping) were performed during the dark period than during the light period.

The applicated stressors had different effects on the animals but these differences were much more pronounced during the gerbils´ rest time than during their activity time. Taking all the data for the light period and the dark period stressor experiments together, it is possible to set up a general ranking of the stressors on the basis of their straining effects: handling < intruder confrontation < resident confrontation < cage change < grouping. A further question arising from the stressor experiments was whether the maximum increase in heart rate, core body temperature and general activity would rise under the influence of the stressors above the unstressed circadian maximum, i.e.: the normal physiological range. The respective results are given in Table 2. It shows the basic values for the measured parameters and the maximum values after the application of the different stressors during both the light and dark period. In the light period the maximum response rose above the unstrained circadian maximum after resident and intruder confrontation (heart rate), after changing the cage (heart rate) and after grouping (heart rate and core body temperature). During the animals´ activity time (dark period) the heart rate was never elevated after stressor application. After resident and intruder confrontation and after cage change, the core body temperature maximum was higher than the circadian maximum ($p < 0.05$). Activity was elevated only after the cage change.

Summarizing the results of the stressor experiments it can be said that the stressors had measurable effects on the animals which were more pronounced during the light period, i.e. the gerbils´ rest time.

Discussion

In the present study the stress responses of gerbils were investigated after the application of non-social and social stressors in the animal house routine. Furthermore, the circadian rhythms of the stress responses was evaluated. As in the golden hamsters (part I of this paper) the 24-hour patterns revealed that gerbils were nocturnal for the three parameters investigated (Figure 1). This finding is also supported by the circadian patterns of other parameters, such as running wheel activity or consumption (Weinandy 1996). The appearance of bimodal shaped curves (Figure 1) has already been described for various activity parameters of the gerbils (Stutz 1972, Gattermann 1980, Heinzeller and Aschauer 1989). The 24-hour rhythm of the heart rate has been investigated (Probst et al.1987) but the number of tested animals (only 3 male gerbils) and the duration of that earlier experiment (up to 34 days) is not comparable with that of the present study (7 animals for about 6 months). There have been no long-term data available until now on the 24-hour rhythm of the core body temperature of the Mongolian gerbils.

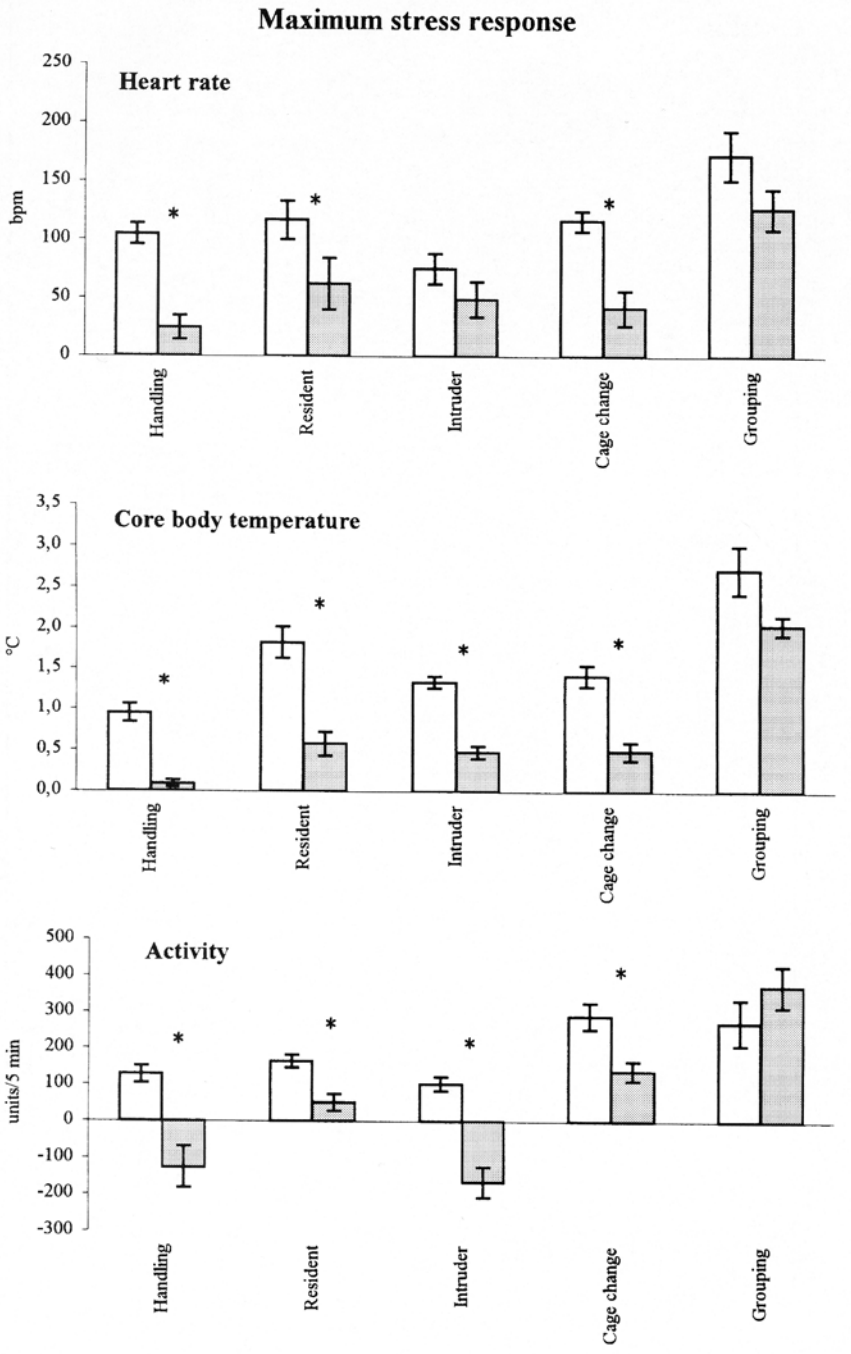
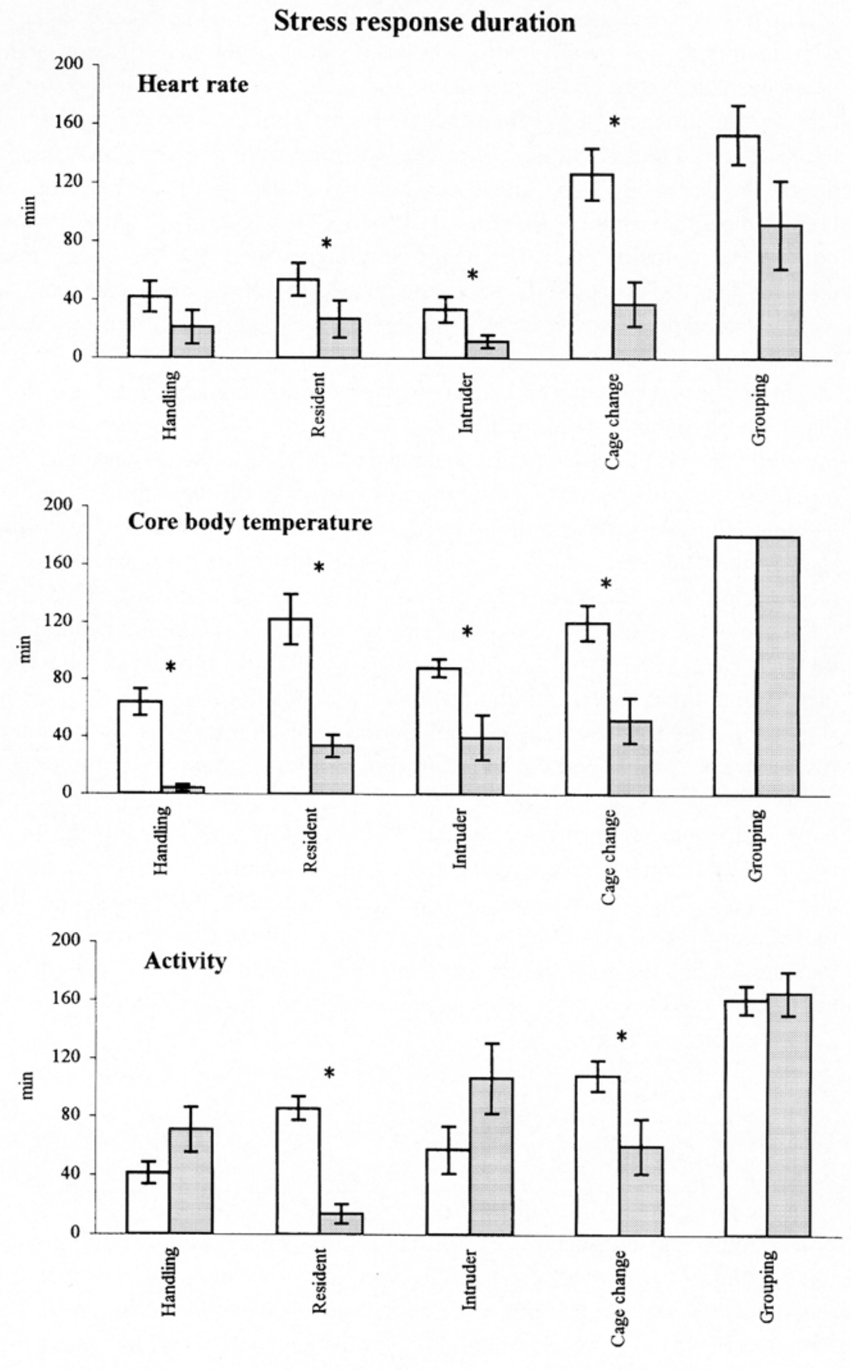


Fig. 2. Maximum and duration of stress responses in male and female Mongolian gerbils after application of the various stressors. Values are mean ± SEM. White and black columns represent



the stress responses during the light and dark period. n = 3–8 animals (for details see Table 2). * $p < 0.05$.

General discussion of part I and II

The golden hamster and the Mongolian gerbil are kept very frequently as experimental animals. In the wild both species live in semi-arid areas and are characterized as nocturnal and strictly territorial but with a different social organization. The golden hamster is solitary and attacks every intruder with the exception of oestrous females. The Mongolian gerbil lives socially in family units and the group defends its territory against any intruder, irrespective of sex (Ågren 1976, Weinandy 1996). In contrast to their natural lifestyle both species are kept under laboratory conditions singly (Mongolian gerbil) as well as in mixed or unisexual groups (golden hamster). Furthermore, the familiar environment of the animals is regularly destroyed or at least disturbed by animal house routines such as the new combination of existing groups or cage change. These and other manipulations may impair the well-being and reactivity of the animals and therefore distort the results of laboratory studies or make them more difficult to reproduce.

On the other hand there is a regular change in the general reactivity and the state of the animals even under optimal conditions, caused by the endogenous circadian clock. Therefore we investigated the effect of the usual stressors in an animal house during the animals´ rest and activity times in two comparative studies.

The various circadian rhythms of both investigated species (Figure 2 of part I and Figure 1 of part II) are linked to the external zeitgeber, the LD-change (external synchronization). Additionally, they are internally synchronized and are in stable phase relationships (internal synchronization). The external and internal synchronization of the three rhythms investigated and also of other physiological and ethological rhythms is necessary for the well-being and health of experimental animals and for organisms in general.

However, ongoing investigations in both species in our laboratory (in prep.) show that the basic structure of these rhythms and their synchronization is not affected even under the permanent stress situation of separation (Mongolian gerbil) or housing in unisexual groups (golden hamster), although endocrinological (glucocorticoids, testosterone and progesterone), histological (adrenals), and ethological parameters unambiguously indicate stress states in these animals (Weinandy 1996, Zimmer and Gattermann 1996).

The central generation and synchronization of the 24-hour rhythms is controlled by the hypothalamic suprachiasmatic nuclei (SCN), which receives the necessary photic information about the light-dark period (LD) primary via the retinohypothalamic tract (overview Wollnik 1989; Refinetti et al. 1994). This central 24-hour clock also synchronizes the rhythms of the three autonomic systems investigated. Therefore there are presumably no direct interdependencies between the circadian patterns, i.e. that an alteration of the heart rate or core body temperature is not simply a result of the circadian change of activity. Detailed analysis of the patterns of Figure 2 of part I and Figure 1 of part II shows that the nocturnal increase in heart rate and core body temperature anticipated the change in the general activity, clearly recognizable via the different waveforms of the three patterns in both species and the intersection of the curve and the circadian level (i.e. the mean 24-hour level). The mean heart rate crossed the circadian level line in the golden hamster two hours and in the gerbil three hours before lights off, the core body temperature one and a half hours (golden hamster) and two and a half hours (Mongolian gerbil) before lights off and the general activity just one hour (golden hamster) and 30 min (Mongolian gerbil) before lights off. In that respect even more meaningful are investigations of Golombek et al. (1993), Refinetti (1995) and Watts and Refinetti (1996). These studies show that the locomotor activity and the core body temperature are two different autonomic systems whose circadian time structure is controlled by the SCN. The same is likely for the heart rate and the function of other organic systems.

The chronobiological investigations under non-stressed conditions in the present study were necessary for the establishment of physiological norm values which form the basis of the subsequent stressor experiments. The parameters heart rate, core body temperature, and general activity are suitable indicators of the activation of the different stress axis and stress states (Lawler et al.1982, Alberts et al. 1983, von Holst 1985, Stöhr 1988, Kant et al. 1991, Tornatzky and Miczek 1993). It is important to mention that the implanted transmitters themselves had no apparent serious side effects upon the animals (Weinandy and Gattermann 1995). Therefore it was possible to take measurements of the physiological norm values for a period of approximately 6 months.

The results of the present two stress studies indicate that all the animal house routines tested did induce stress states but to a different extent. We always found an increase in the three measured parameters heart rate, core body temperature and general activity (except for the general activity after handling and intruder confrontation during the dark period). The increase in the core body temperature as a response to various stressors is a well known and widespread phenomenon, e.g. in rats (Briese and Cabanac 1991, Long et al. 1990), mice (Borsini et al. 1989, Zethof et al. 1994), hamsters (Conn et al. 1990), rabbits (Snow and Horita 1982) and humans (Marazitti et al. 1992). In mice the stressor-induced hyperthermia during the rest time took approximately 10 min to reach an elevated level, which was 1.0 to 1.5 °C higher then the baseline. This hyperthermia was long lasting, returning to the baseline after 40 to 60 min (Zethof et al. 1994). The same effect was found after transporting rats in their cages from warm to cold rooms. This stressor-induced elevation of the body temperature was 1.08 to 1.21 °C without any correlation with the ambient room temperature (Long et al. 1990). After a cage change during the rest time, performed 5 hours before the onset of darkness, the mean body temperature of female golden hamsters rose significantly between 1.4 and 1.7 °C in animals with and without access to running wheels. The difference between both groups was not significant, and the elevation in core body temperature lasted 40 to 60 min (Conn et al. 1990). This temperature-raising effect following cage change was similar to our results (Figure 3 of part I and Figure 2 of part II). Furthermore, we also confirmed with the present data that the running wheel activity and the general activity was not the reason for the hyperthermia. After the application of the stressors during the light period there was only an increase in the general activity detectable, whereas the running wheel was not entered (data not shown). The same experiments performed during the dark period led to an increase in both kinds of activity, i.e. the stressor-induced amount of activities was enlarged during the dark period, but the changes in the heart rate and the core body temperature were minor compared to the light period experiments. It is also remarkable that the mean heart rate after the stressor cage change during the light period rose significantly above the circadian maximum although the animals were inhibited and less active on the fresh animal bedding, which resulted in a significant lower activity level compared to the circadian maximum (Table 2 of part I). In gerbils, social stressors (resident and intruder confrontations) led to comparable effects: higher heart rates at reduced activity (Table 2 of part II). All these are strong indications of the psychological share of the measured stressor-induced changes. To sum up, one has to assume that the stressor-induced hyperthermia and tachycardia is a well-regulated increase (i.e. a change in the set point) and not simply a result of non-specific metabolic changes associated with the stress response itself.

Further evidence of such a set point change in the golden hamster is found in the comparison of the maximum stress responses and the circadian maximum (Table 2 of part I). The prominent elevations of the heart rate, the core body temperature and the general activity after the application of the various stressors during the light period were not above the physiological maximum, with the exception of the cage change and grouping already mentioned. As a coping strategy to deal with stressful situations or alterations of environmental factors in general the animals shifted acutely from the more vagotonic into the sympathicotonic axis. In gerbils the maximum stress response was in general above the circadian maximum (Table 2 of part II) which suggests stronger sensitivity.

The stressors can be classified according to their effects, mainly during the time of rest, because of their more pronounced impact in that period. The problem with this is that grouping and the changing of the cage took longer, while handling, vaginal smear, intruder and resident confrontation stopped after 3 min. Therefore, the ranking handling < resident/intruder confrontation < cage change < grouping is based more on practical aspects and directed towards the daily work with the hamsters and gerbils and applicable to other laboratory and experimental rodents.

One frequent routine is the cage change. Although it happens every week in our animal house, the stressor experiments over six months revealed that the animals did not adapt to this procedure. Studies on the direct physiological effects of such a periodical exchange of the olfactorial-familiar cage environment are not available. Our results lead to the assumption that species like the golden hamster and the Mongolian gerbil, which live territorially in their natural habitat, are affected quite severely by such manipulations. The fact that the natural social environment of a species may influence its behaviour in the laboratory has already been shown e.g. in monkeys (Mendoza and Mason 1986) and in mice (Brain 1975). The influence of the cage structure on the aggressive behaviour of laboratory mice was investigated by Haemisch et al.(1994) and Haemisch and Gärtner (1994).

The presence of an unknown conspecific of the same sex triggers aggressive behaviour in most mammals, independently of the actual behaviour of the unfamiliar individual. Even in the present studies these social interactions led to fights which were accompanied by elevated stress indicators. In the Mongolian gerbil this agonistic behaviour occurred irrespective of the sex of the resident or intruder. If the animals were not separated, as in the case of the grouUing, they remained on a higher physiological stress level for at least three hours. In our studies the registration was limited to three hours to clearly distinguish stressor-induced effects from physiological circadian alterations. The agonistic behaviour in newly established groups ended in nearly all cases after 24 hours at the most, which concurs with other references on the social behaviour of these rodents (Eisenberg 1967, Thiessen and Yahr 1977). The two acute conspecific-contacts after the resident and the intruder confrontations led to similar results. For the two investigated species the security-giving and stress-reducing effect of the home cage described (Hennessy and Levine 1978, Haemisch 1990), could therefore not be verified on the basis of the intruder-confrontations. On the other hand, the assumption that the defense of territory leads to stronger efforts and elevated physiological levels could also not be confirmed. Investigations of male gerbils by Wechkin and Cramer (1971) showed increased agonistic behaviour in confrontations in familiar surroundings compared to those in an unfamiliar area which may be an indication of such territorial defense. The data of the grouping and confrontation experiments suggest that group formation and changing of the group structure in the solitary golden hamster and also in the highly social Mongolian gerbil act as a strong stressor and should only be performed when absolutely necessary.

The principle conclusions are: i) all animal house routines investigated triggered stress responses, ii) the animals reacted differently depending on the time of day, i.e. their state of activity and rest, the same stressors having much stronger effects if performed in the rest time, and iii) a species comparison of the Mongolian gerbil and the golden hamster revealed that the gerbil was more susceptible to all stressors. The different reactivity of the experimental animals depending on the time of day may influence and distort the results of nearly all experiments. Therefore this must be generally taken into account when designing experiments.

Acknowledgement
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3.2 Circadian activity rhythms and sensitivity to noise in the Mongolian Gerbil (*Meriones unguiculatus*)

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Abstract

Since consistent data on endogenous circadian rhythms of Mongolian gerbils are not available, the main aim of our study was to identify suitable conditions to receive stable and reproducible free-running rhythms of activity under different light intensities. Another objective was to determine the role of social cues as an exogenous zeitgeber in the absence of a light-dark (LD) cycle. We performed two long-term sets of experiments with adult male gerbils kept in climatic chambers under various photoperiods of at least 30 days each. In all cases, the time of lights on in the chambers differed from the daily starting hour of work in the animal house. Always, two animals per chamber were kept separately in cages with a running wheel while their activity was monitored continuously. During the first set, only three of eight animals developed intra- and interindividual variable free-running rhythms. The activity patterns seemed to be influenced by human activities outside, indicating high sensitivity to external factors. Subsequently, we damped the chambers and the room and restricted access to the room. In the following noise-reduced set, all gerbils developed comparable free-running rhythms of activity. We determined the mean of the free-running period τ, the activity-rest relationship α/θ and the amount of running wheel activity per day: τ =23.7h ± 0.08h under low light (5 lux) and 25.5h ± 0.19h under high light intensities (450 lux); α/θ =0.53 ± 0.08 under 5 lux and 0.34 ± 0.04 under 450 lux. The amount of daily activity was 12 times as high under 5 lux as under 450 lux. There was no indication that the two animals in one chamber socially synchronized each other. In conclusion, the pronounced rhythm changes in accordance

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[7] Klaus, U., R. Weinandy & R. Gattermann. 2000. Circadian activity rhythms and sensitivity to noise in the Mongolian gerbil (Meriones unguiculatus). Chronobiol. Int. 17: 137-145. Eigenanteil: Planung Experimente, Auswertung und Manuskript

with Aschoff's theory support the view that gerbils are mainly nocturnal animals. (Chronobiology International, 17(2), 137–145, 2000)

Key Words: Circadian rhythms—Locomotor activity—Mongolian gerbils.

Introduction

In mammals, endogenous circadian rhythms are generated and controlled by a circadian oscillator, located in the hypothalamic suprachiasmatic nuclei (SCN). The entrainment to environmental periodicities is realized via exogenous zeitgebers, mainly the natural light-dark (LD) cycle. Although Mongolian gerbils are common laboratory animals, important chronobiological features of this species, like free-running circadian rhythms, are still unclarified. Even general statements range from diurnal to crepuscular to nocturnal (diurnal, see Roper and Polioudakis 1977; crepuscular, see Heinzeller and Aschauer 1989; nocturnal, see Lerwill 1974; Weinandy and Gattermann 1996/1997; all types, see Umezu et al. 1989). Apart from a recent study (Gattermann and Weinandy 1997), almost nothing is known so far about the influences of nonphotic factors on their circadian rhythmicity. Hastings et al. (1992) demonstrated effects of human-induced arousal on circadian activity rhythms in golden hamsters. They could trigger phase shifts by handling the animals. Amir and Stewart (1998) showed, in rats, that the resetting of the oscillator can be modified further by learning and environmental events that reliably precede the onset of light. Recently, nonphotic entrainment of activity and temperature rhythms were demonstrated in anophthalmic mice by using restricted access to an unlocked running wheel as the zeitgeber (Laemle and Ottenweller 1999).The role of periodically occurring social cues as a potential zeitgeber with entraining/masking effects has been investigated repeatedly. Crowley and Bovet (1980) showed that the circadian rhythms of wild-caught deer mice (*Peromyscus maniculatus*) can be synchronized socially when placing two previously isolated mice with different rhythms in a common enclosure. However, the majority of experiments on various species with different social organizations did not provide proof for social entrainment (golden hamster see Mrosovsky 1988; Refinetti et al. 1992; Gattermann and Weinandy 1997; degus,see Goel and Lee 1995; marmosets, see

Erkert and Schardt 1991; Australian sugar glider, see Kleinknecht 1985). Although Mongolian gerbils live in family units with an innate social appetence in which a social zeitgeber should be effective, there were no indications for social entrainment in this species. The observed entraining/masking effects were induced by human activities in the animal room and not by conspecifics (Gattermann and Weinandy 1997). In the present study, we investigated social entrainment under noise-reduced conditions in climatic chambers. The main aim of our study was to identify suitable conditions to receive stable and reproducible free-running rhythms of activity under different light intensities, giving further evidence on the activity type of the Mongolian gerbils.

Material and Methods

Animals and Their Maintenance

Adult male Mongolian gerbils (*Meriones unguiculatus* Milne Edwards, 1867) from different litters were chosen for this study. They were derived from our own laboratory stock (Zoh:CRW) and were kept singly in plastic cages (50 x 30 x 18 cm) with a wire mesh top; a running wheel (diameter 30 cm, area width 10 cm) was included. Water and food pellets (Altromin GmbH, Lage, FRG) were provided ad libitum. The animal bedding (Allspan® , NL, Karlsruhe, FRG) was renewed every 3–4 weeks.

Experimental Design

Always, two individually housed animals were transferred into one opaque air-conditioned climatic chamber (Ehret GmbH, Emmendingen, FRG). The various photoperiods lasted for at least 30 days each; in all cases, the time of lights on in the chambers differed from the daily starting hour of work in the animal house, around 07:00 (middle European time). The standard temperature regime was 23°C ± 1°C, and the relative humidity varied from 30% to 35%. The locomotor activity of the separated animals in their cages was recorded via running wheels; each revolution triggered an impulse that was stored and analyzed by the Chronobiological Kit (Stanford Software Systems, Santa Cruz, CA).

In the part I of the experiments, 8 males were kept for 5–11 months in four climatic chambers under LD 12:12 (450 lux/0 lux); LD 14:10 (450 lux/0 lux); LD

0:24 (0 lux). On the basis of the data obtained, we measured the human-caused external noises and low-frequency vibrations inside the climatic chambers with an acceleration detector (KD 31, Metra, Radebeul, FRG) and a spectrum analyzer (SI 1220, Schlumberger, Munich, FRG). In addition to the chamber-induced noises, especially higher frequencies due to voices or doors being locked with keys and low frequencies when closing doors entered the chambers. To reduce noise, we further damped and shielded the climatic chambers and the room with sound-absorbing materials and restricted the access to the room. In the part II, 6 males were kept for 5 months in three climatic chambers under LD 12:12 (450 lux/0 lux), LD 24:0 (450 lux/0 lux), and LD 24:0 (5 lux/0 lux).

Statistical Analyses

We determined the following chronobiological parameters: free-running period τ, activity-rest relationship α/θ, and amount of activity as revolutions per day. The data of days 1–10 of the respective photoperiod were excluded to allow the adaptation of the animals. The calculation of τ was carried out with a chi^2 periodogram analysis (Sokolve and Bushell 1978): data for LD 24:0 (5 lux/0 lux) for days 11–20; data for LD 24:0 (450 lux/0 lux) for days 11–35. In the latter case, the prolonged period was necessary due to the decreased amount of activity. We visually determined the α/θ ratio on actograms of the free-running rhythms, modified by the respective τ. The mean number of revolutions per day was averaged from these 10-day periods. All parameters are given as mean values; the statistical measure of variance is the standard error. The two-tailed Wilcoxon test was used to assess the differences of the means, which were significant at $p < .05$. The computer package for the statistical analyses was Winstat (version 3.1).

Results

In part I, all animals were entrained initially to the LD cycle with a main portion of activity during the dark period. Under LD 0:24, 5 gerbils did not show persistent endogenous rhythms, while only 3 exhibited distinct spontaneous periods with lengths both shorter and longer than 24h, that is, we found intra- and interindividual variable free runs under constant darkness. These opposed circadian rhythms are exemplified by 2 animals kept in the same chamber (Fig. 1, A and B). In the absence of light, animal A expressed three different and unstable free-running rhythms, all shorter than 24h. Under the four LD phases, its running wheel activity mainly occurred during the dark period, and it was synchronized to the LD cycle. In constant darkness, animal B developed τ >24h (2), τ =24h (4), and τ <24h (6).

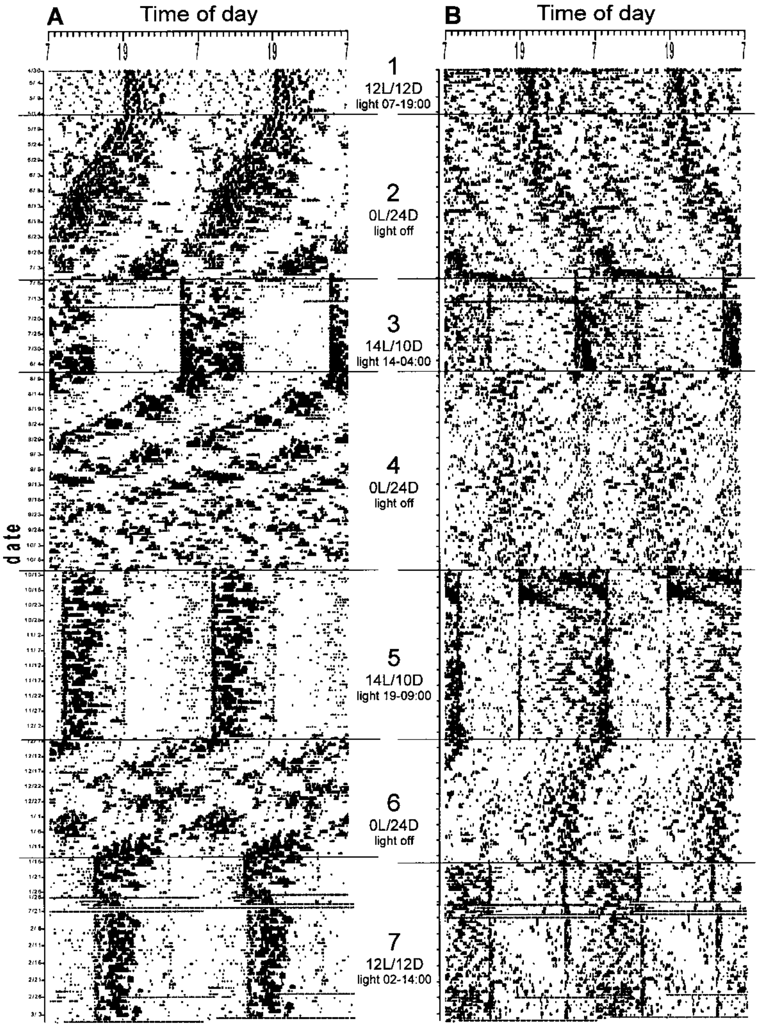

FIGURE 1. Double-plotted actograms of two gerbils (A and B) in one climatic chamber during part I. The running wheel activity was recorded for 11 months during seven different photoperiods (1–7), separated here by the horizontal lines. Every transition of the LD cycles to constant darkness was performed by prolonging the respective dark period. Dotted lines indicate missing data.

During the period 4, the activity pattern was biphasic, with a second maximum coinciding with the starting hour of work in the animal house. In LD periods 1 and 3, it was synchronized like animal A. In contrast, during periods 5 and 7, an inversion of the nocturnal activity profile occurred, and it was active during the light phase.

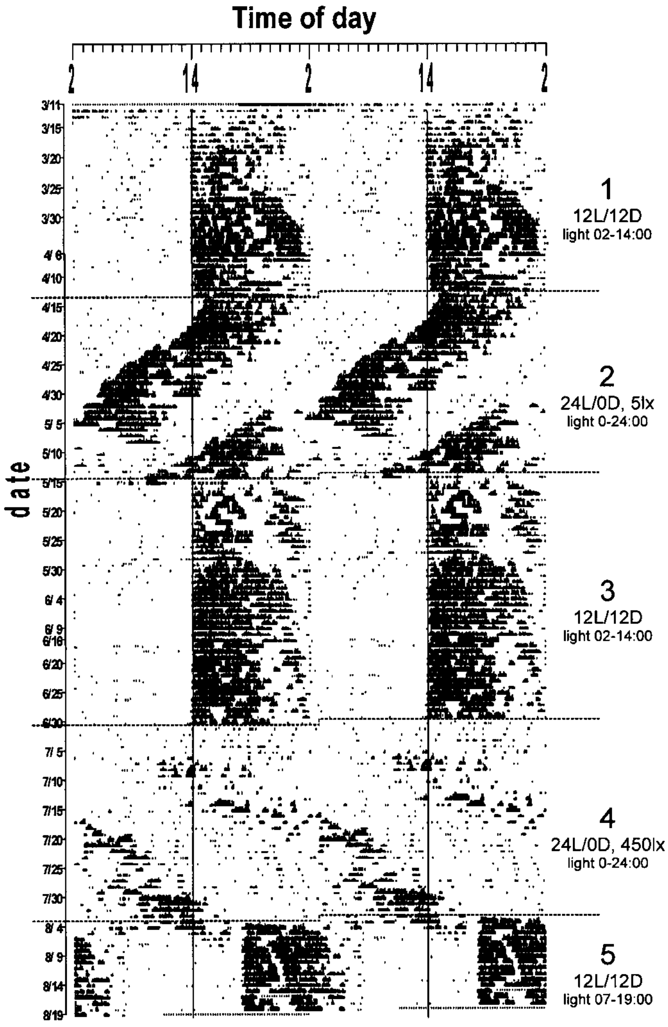

FIGURE 2. Double-plotted actogram of one gerbil in a climatic chamber under the noise-reduced conditions of part II. The running wheel activity was recorded for 5 months during five different photoperiods (1–5), separated here by the horizontal lines. The transition of period 1 to 2 was performed by reducing the light intensity to 5 lux at the end of a 450-lux period. Transition 2 to 3 was performed by increasing the light intensity from 5 lux to 450 lux for 12h and consecutive 0 lux–450 lux cycles. Transition 3 to 4 was performed by prolonging the 450-lux period. Transition 4 to 5 was perfomed by reducing the light intensity from 450 lux to 0 lux. Dotted lines indicate missing data.

In the part II, all gerbils developed stable free-running rhythms, but there was no indication that they socially synchronized each other. Figure 2 shows a representative actogram of one animal during this noise-reduced set. After being synchronized under LD 12:12 with an onset of activity at lights off and a main portion of activity during the dark period (1), it developed a free-running rhythm with an initial phase delay of about 1h under constant 5 lux (2). We calculated τ =23.5h and determined α/θ =0.77. In the course of this whole period 2, we observed two spontaneous phase shifts.

After a resynchronization of the onset of activity within a day under LD 12:12 (3), the pattern resembled that of period 1. During the following period, with the constant light intensity of 450 lux (4), the free running was prolonged, with τ =25.4h, a reduced α/θ =0.46, and a reduced mean amount of daily running wheel activity. The activity pattern in the final period 5 was similar to that of the other two LD cycles 1 and 3.

To sum, all 6 gerbils showed comparable free-running activity rhythms depending on the light intensities: under constant 5 lux (Fig. 2, period 2), τ =23.7 ± 0.08h, and α/θ =0.53 ± 0.08; under constant 450 lux (Fig. 2, period 4), τ =25.5 ± 0.19h, and α/θ =0.34 ± 0.04 (Wilcoxon test for τ: $z = -2.2$, $p < .05$; Wilcoxon test for α/θ: $z = -1.99$, $p < .05$). The mean amount of activity per day was 12 times higher under 5 lux compared to 450 lux (Wilcoxon: $z = -2.2$, $p < .05$).

Discussion

All gerbils investigated were entrained to the initial LD cycles and showed, under these conditions, the pattern of mainly nocturnal animals. This confirmed the consistent activity data obtained during long-term studies under standard lighting regimes in our laboratory (Weinandy 1996). Under constant darkness in climatic chambers, we found rare and unstable free-running rhythms both shorter and longer than 24h. There was a pronounced intra- and interindividual variability, including activity parameters like shape, amount, and distribution, as formerly reported for free-running experiments under less shielded constant conditions (Gattermann and Weinandy 1997). However, this current result was unexpected since the same climatic chambers were used frequently for chronobiological experiments in our animal house and were found to be suitable for supplying free-running conditions in other rodents (e.g., in mice: Weinert and Weiss 1997; Weinert and Kompauerova 1998). High variability was found even under LD conditions in the laboratory by Umezu et al. (1989), but without providing a convincing explanation.

In our opinion, these findings of more or less variable activity patterns generally indicate the high attentiveness and sensitivity of gerbils toward external nonphotic factors, allowing them to adapt quickly to environmental changes. Its extent may depend both on the distinct level of individual sensitivity and the intensity of the factors. In the absence of the main zeitgeber light in the climatic chambers during part I and in the aforementioned former study (Gattermann and Weinandy 1997), the gerbils were found to be affected by human-caused noise. Periodically commencing at start of work, the human activity repeatedly generated masking/entrainment, that is, it was used as an exogenous nonphotic zeitgeber by the animals. For hamsters (Janik and Mrosovsky 1993; Mrosovsky 1993) and rats (Barrington et al. 1993), it was reported that nonphotic events served as time cues and led to masking and entraining effects as well. Fundamental characteristics of such rhythm-influencing factors are reviewed by Rietveld et al. (1993). Further evidence for the causality of varying activity patterns and exogenous noise emerged from part II. As a consequence of the drastic reduction of outer acoustical disturbances, all gerbils kept under these more shielded conditions developed comparable free-running activity rhythms. Applying this to the ambiguous data of Umezu et al. (1989) and considering that locomotor activity is a bioindicator (Gattermann 1983), we suggest an increased sensitivity that caused their animals to have been influenced or even disturbed under the LD conditions.

A more detailed explanation for the multiple and concurrent phenomena observed in the gerbils may be deduced from Vilaplana et al. (1997). They investigated male rats under LD cycles and found masking, entrainment, and free-running rhythm at the same time. The authors attributed this to a multioscillator system, in which some oscillators were entrained to the L/D cycles, while others remained nonentrained. Though the highly social gerbils possess a well-developed system of acoustical and olfactorial communication (Thiessen and Yahr 1977; Yapa 1995), in accordance with our former study (Gattermann and Weinandy 1997), we did not find any indication for social entrainment in the gerbils, even under free-running conditions in part II. One possible reason, we assume, is that there was no evolutionary necessity to use spatially distant conspecifics as a zeitgeber. In addition, we cannot exclude completely the existence of timing signals according to close body contacts, which seem to be the important feature for the maintenance of the family unit.

In conclusion, the main aim we achieved with the present study was to establish suitable conditions to obtain stable and reproducible free-running rhythms under different light intensities. With respect to the circadian rule (Aschoff 1960), the shortened free-running periods and the increased amount of activity with an increased a/q under low light compared to high light indicate that gerbils are mainly nocturnal animals.

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3.3 Total body electrical conductivity (TOBEC) measurements in Mongolian gerbils (*Meriones unguiculatus*) and golden hamsters (*Mesocricetus auratus*)

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We confirm that the coauthor knows that the manuscript has been submitted for publication and consents to it. All experiments have been performed in accordance with local animal welfare legislation.

Dr. R. Weinandy   Prof. Dr. R. Gattermann

Summary

Body composition of adult Mongolian gerbils (*Meriones unguiculatus*) and golden hamsters (*Mesocricetus auratus*) was determined using the non-invasive measurement of the total body electrical conductivity (TOBEC). A regression model was developed based on direct chemical analysis of the carcass and calibration equations were defined to predict the fat-free mass (FFM) and the total body water (TBW) in these two species. The closest correlation was obtained by calculating a linear regression of FFM and TBW as a function of TO-

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[8] Weinandy, R. & R. Gattermann. 2001. Total body electrical conductivity (TOBEC) measurements in Mongolian gerbils (*Meriones unguiculatus*) and golden hamsters (*Mesocricetus auratus*). J. Exp. Anim. Sci. 42: 21-30. Eigenanteil: Experimente, Auswertung und Manuskript

BEC: gerbils: FFM (g) = 0.20548 x TOBEC + 10.0224; TBW (g) = 0.14485 x TOBEC + 6.51824; golden hamsters: FFM (g) = 0.18043 x TOBEC + 18.5806; TBW (g) = 0.14817 x TOBEC + 4.50379. Based on these equations and the respective TOBEC values, the body composition was analysed. Male golden hamsters had a higher crude fat mass (CFM, obtained by substracting the FFM from the body mass) and a higher dried body mass (DBM, obtained by substracting the TBW from the body mass) than females, which had a higher percentage of FFM and TBW. In Mongolian gerbils, males had higher CFM, higher DBM and the absolute FFM and TBW was elevated. As in female golden hamsters, female gerbils had a higher percentage of FFM and TBW. Results are discussed in relation to the usefulness of this non-invasive technique, which allows repeated individual measurements of body composition. It helps to reduce the number of animals necessary when estimating body condition and welfare issues, male/female differences or for the evaluation of seasonal effects and housing conditions.

Key words: Mongolian gerbils; golden hamsters; fat-free mass; total body water; TOBEC

Introduction

The technique of measuring total body electrical conductivity (TOBEC) makes it possible to determine the body chemical composition of animals and humans. It is a rapid and safe non-invasive in vivo method and has been recommended for serial measurements of an individual. TOBEC measures changes of the electromagnetic field that are proportional e.g. to the fat-free mass or total body water (for further details see: FIOROTTO et al. 1987), and has been applied to measure the body composition in a variety of species including humans. HERGENROEDER et al. (1991, 1993) performed anthropometric measurements to estimate differences in the total body water and the fat-free mass in ballet dancers and non-dancers. VAN LOAN (1990) showed that the TOBEC methodology is suitable to assess the body composition in teenagers. In human infants, DEBRUIN et al. (1995) found an excellent correlation between TOBEC-derived fat-free mass and fat mass with reference data. In an ecological study concerning the basal metabolic rate of redshanks (*Tringa totanus*), SCOTT et al. (1996) examined the effect of seasonal variation in the amount of fat carried by an individual on its basal metabolic rate. They concluded that total body mass and not total lean mass is the best value to be used in predictive interspecific allometric equations. Based on repeated TOBEC measurements, MEIJER et al. (1994) showed that lean mass of both sexes in starlings (*Sturnus vulgaris*), decreased over the breeding season, increased again during the first half of the moult, and remained constant for the rest of the year. In laboratory rats, numerous investigations have been performed to evaluate the reliability of TOBEC readings (CUNNINGHAM et al. 1986; GOSSELIN and CABANAC 1996; STENGER and BIELAJEW 1995; TROCKI et al. 1995). MICHEL and CABANAC (1999) found that in lipectomized and sham-lipectomized adult male Wistar rats the body weight set point was not mainly modulated by fat depots but to be due to surgical stress. RAFFEL et al. (1996) applied this method to study the allocation of the fat-free mass in young and adult female guinea pigs (*Cavia porcellus*). They found that the young in large litters had less fat reserves than that of small litters and revealed that reproducing females allocated as much of available resources to growth in fat-free mass as non-reproducing females, but reduced fat storage. As shown for a number of species (rats: YI et al.1996; SMAGIN et al. 1999; ZHOU et al. 1999; humans: SHEPHERD et al. 1991; BLANC et al.1998; golden hamsters: WADE et al. 1986; MEISEL et al. 1990; mice: LAUGERO and MOBERG 2000), changes in body composition reflect metabolic stress states caused by behavioural, social or pharmacological stressors.

Although golden hamsters and Mongolian gerbils are well-established laboratory animals, there are no data available using this technique until now; information on their body composition is necessary to improve studies on stress and welfare issues in these two species (e.g. GATTERMANN and WEINANDY 1996/1997; WEINANDY and GATTERMANN 1996/1997; KORZ and GATTERMANN 1999). Since the application of the TOBEC method requires a preceding calibration of body composition for each species, the aim of the present study was to develop a regression model for each species. Correlation equations based on direct carcass chemical analysis were defined for predicting the fat-free mass, the crude fat mass, the total body water and the dried body mass in Mongolian gerbils and golden hamsters.

Materials and methods

Animals and housing conditions

Mongolian gerbils (*Meriones unguiculatus*) and golden hamsters (*Mesocricetus auratus*) of both sexes were selected for this study (for the respective age see below). They derived from our own laboratory outbred stocks (gerbils: Zoh:CRW; golden hamsters: Zoh:GOHA) and were kept separately or in unisexual groups of 3 animals in climatised windowless indoor rooms in standard plastic cages (Makrolon type IV, 55 x 33 x 20 cm) lined with wood shavings (Allspan® Animal bedding, The Netherlands) with a wire mesh top. The animal bedding was renewed every two weeks. Food pellets (one part breeding diet Altromin 7014, two parts maintenance diet Altromin 7024; crude protein 19 %, crude fat 4.2 %, crude fibre 5.8 %, ash 7.2 %, moisture 13.5 % and nitrogen-free extract 50.3 %; Altromin GmbH, Lage, Germany) and tap water were provided ad libitum. The light/dark conditions were 14:10 h, the light intensity varied from 200-300 lux (light period) to 5 lux (dark period). The light period lasted from 05:00 -19:00 h Central European Time. The room temperature was 24°C ± 2°C, the relative humidity varied between 60 and 65 %.

TOBEC measurements

The non-invasive determination of fat-free mass (FFM) and total body water (TBW) was performed using an EM-SCAN SA-3000 small animal body composition analyzer (EM-SCAN Inc. Springfield, Illinois, U.S.A.), including a base unit (No. SA3B06996) and a detection chamber (No. SA3057, chamber diameter 57 mm). The internal electronic circuitry and detection chambers have been designed to operate efficiently at 12 Volts (350 mA). The device measures the total body electrical conductivity (TOBEC) following the Harker Principle. The electronic circuitry drives a 10 megahertz oscillating magnetic field within its chamber, and senses the nature of the conductive material within the measurement chamber by detecting changes in the radiating coil's impedance. The conductivity index, i.e. the TOBEC value, is defined as the energy loss at the detector between background and subject measurements. Initially the body mass of the animals was measured using digital balances (Kern® 440-45, precision 0.1 g) and they were then anaesthetized by transferring them into a glass container (volume: 4 litre) which was supplied with 10-15 ml Forene (active component: Isofluran; supplied by Abbott GmbH, Wiesbaden, Germany). Animals were fully sedated after 1 minute and were then placed in the measuring chamber, positioned with the widest part of the body in its middle and lying prone on the plastic holder. Three to six 20-seconds measurements per animal were taken over a 4- to 7-minute period, depending on the sedation, and the resulting mean value was used for further analyses. In order to obtain intra-individual reliable TOBEC data, i.e. an intra-individual variability of less than 3 %, it was important that the animal´s position did not change during the whole scanning procedure. After the animals had recovered from anaesthesia 10 minutes later, they were re-introduced into their cages.

Direct carcass chemical analysis

To evaluate the predictive value of TOBEC, a regression analysis was established for FFM and for TBW. Therefore, the TOBEC results of 40 gerbils (mean ± SEM age: 25 ± 4 weeks) and 13 golden hamsters (mean ± SEM age: 16 ± 3 weeks) of both sexes were correlated via direct carcass chemical analysis. After TOBEC measurement, the animals were killed by an overdose of isofluran, minced with scissors and homogenized by a laboratory mixer (Ultra-Turrax T 25, Janke & Kunkel GmbH, IKA®-Labortechnik, Staufen, Germany). The carcasses were desiccated to constant mass in a convection oven at 60 °C and ground with a pestle. Next, 2.5 g aliquots of the dried powdered carcass were heated with 3 M hydrochloric acid (HCl) for 1 hour. The resulting solution was filtrated through paper filters (No. 595 ½, 185 mm, Ref-No. 311647, supplied by Schleicher & Schuell GmbH, Einbeck, Germany), washed with double distilled water and dried. The non-polar lipid components of the filtered effluent were extracted using a Soxhlet-apparatus by multiple bathings in petroleum ether for at least five hours and dried again to constant mass. The content of the FFM was calculated as the difference between the total mass before and after the fat extraction and the TBW was determined as fresh mass minus dry mass. All analyses were performed in triplicate of three times 2.5 g aliquots per animal. The mean ± SEM crude fat mass extracted in these aliquots was 0.70 ± 0.06 g for the gerbils and 1.01 ± 0.04 g for the golden hamsters. The mean intra-individual variability (expressed as SD of the triplicates) was in both cases low: gerbils: 0.01 g (1.89 %); golden hamsters: 0.02 g (1.79 %), i.e. the accuracy of the threefold determinations was high. Subsequently, the quality of the prediction was determined by plotting the relative residuals from the regression equation versus the determination from chemical analysis and the body composition of golden hamsters (n = 103) and Mongolian gerbils (n = 70) as a function of TOBEC values.

Statistics

Data are given as mean values, the statistical measure of variance is the standard error of mean (SEM). For the calculation of the mean intra-individual variability of the triplicate determinations and the three to six 20-seconds TOBEC measurements per animal the standard deviation (SD) was used. To establish the calibration curves, a linear regression analysis was performed and the Pearson r correlation coefficient was determined. The two-tailed Mann Whitney U test (Winstat V 3.1) was used to assess the differences of the means, which were accepted as significant at $p < 0.05$.

Results

Calibration curves for the TOBEC values

Based on the direct carcass chemical analysis of Mongolian gerbils and golden hamsters, different regression models were performed to predict the FFM and the TBW from the TOBEC value. The closest correlation of the data was obtained by calculating a linear regression of FFM and TBW as a function of TOBEC. The resulting correlation equations were as follows:

Gerbils

[1] FFM (g) = 0.20548 x TOBEC + 10.0224
(Pearson r correlation coefficient = 0.96467, $p < 0.0001$)

[2] TBW (g) = 0.14485 x TOBEC + 6.51824
(Pearson r correlation coefficient = 0.92543, $p < 0.0001$)

Golden hamsters

[3] FFM (g) = 0.18043 x TOBEC + 18.5806

(Pearson r correlation coefficient = 0.97674, $p < 0.0001$)

[4] TBW (g) = 0.14817 x TOBEC + 4.50379

(Pearson r correlation coefficient = 0.98043, $p < 0.0001$)

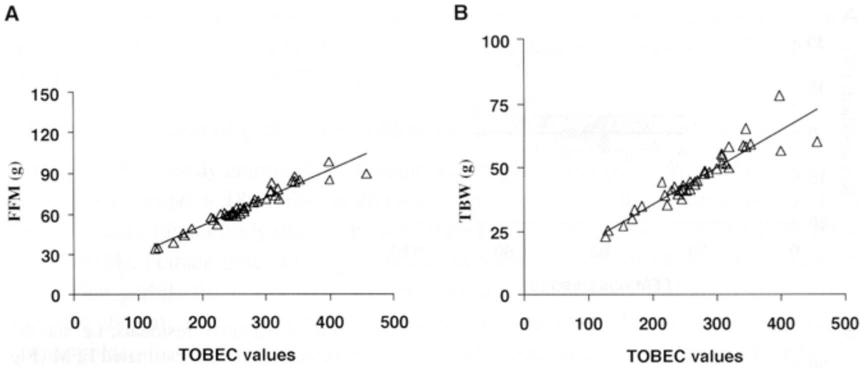

Fig. 1. Calibration curves for Mongolian gerbils (n = 40) based on a linear regression of the fat-free mass (FFM) (**Fig. 1 A**) and the total body water (TBW) (**Fig. 1 B**) as a function of TOBEC. Open triangles: individual gerbil data. For the respective calibration equations see text ([1] and [2]).

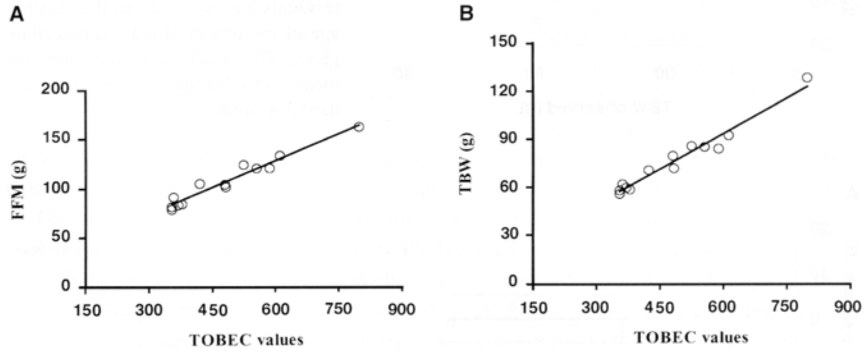

Fig. 2. Calibration curves for golden hamsters (n = 13) based on a linear regression of the fat-free mass (FFM) (**Fig. 2 A**) and the total body water (TBW) (**Fig. 2 B**) as a function of TOBEC. Open circles: individual golden hamster data. For the respective calibration equations see text ([3] and [4]).

Fig. 1 gives the resulting calibration curves for all individual gerbils (n = 40) for FFM (Fig. 1 A) and for TBW (Fig. 1 B). The mean body mass ± SEM of the animals was 75.6 ± 3.8 g with a minmum of 36.0 g and a maximum of 136.9 g. The mean ± SEM of the TOBEC values was 270 ± 11 and the mean intra-individual variability of the repeated TOBEC measurements per animal, expressed as SD, was 4.2.

For the golden hamsters (n = 13), the resulting calibration curves for FFM and TBW are given in Fig. 2 A and B. The mean body mass ± SEM of the animals was 125.8 ± 7.0 g with a minmum of 96.7 g and a maximum of 180.0 g. The mean ± SEM of the TOBEC values was 484 ± 37 and the mean intra-individual variability of the repeated TOBEC measurements per animal, expressed as SD, was 11.2.

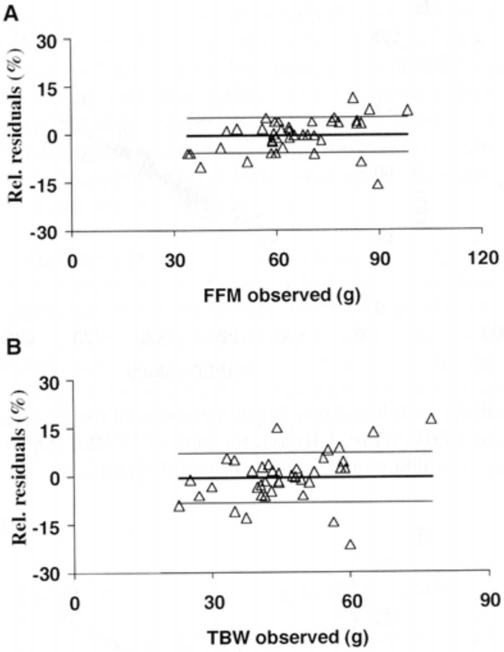

Fig. 3. Relative residuals, i.e. the differences between estimated FFM (**Fig. 3 A**) or TBW (**Fig. 3 B**) based on the calibration curve and the observed TBW or FFM determined by the direct carcass chemical analysis of the Mongolian gerbils (n = 40). The relative residuals are expressed as the percentage of the observed mass (open triangles). The thick line indicates the mean, the solid line indicates the standard deviation.

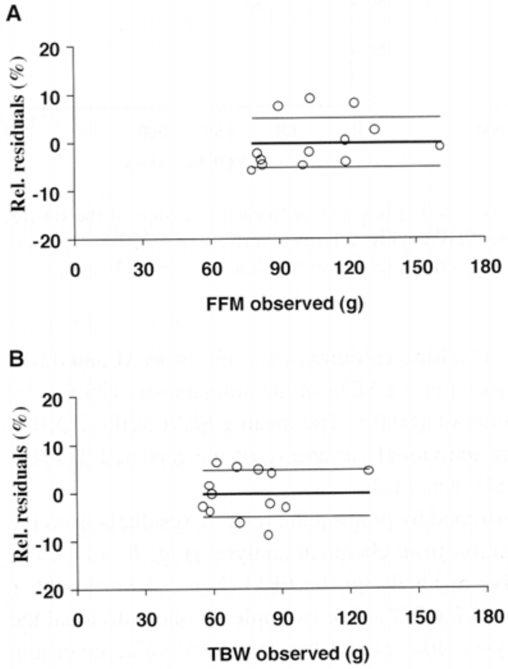

Fig. 4. Relative residuals, i.e. the differences between estimated FFM (**Fig. 4 A**) or TBW (**Fig. 4 B**) based on the calibration curve and the observed TBW or FFM determined by the direct carcass chemical analysis of the golden hamsters (n = 13). The relative residuals are expressed as the percentage of the observed mass (open circles). The thick line indicates the mean, the solid line indicates the standard deviation.

The quality of the prediction was determined by plotting the relative residuals from the regression equation versus the determination from chemical analysis (Fig. 3 and 4).

For the gerbils, the mean ± SD of the relative residuals for the FFM (Fig. 3 A) was -0.6 ± 5.5 % and for the TBW (Fig. 3 B) it was -0.7 ± 7.7 %. For example, in one individual the difference of TBW from chemical analysis (40.8 g) and the calculated value emerging from the regression equation (43.3 g) was -2.5 g, that corresponded to a relative residual of -6.1 % (-2.5 g / 40.8 g x 100). In case of the golden hamsters it was -0.2 ± 5.2 % (FFM, Fig. 4 A) and -0.2 ± 4.9 % (TBW, Fig. 4 B).

Body composition of gerbils and golden hamsters based on TOBEC values

In Table 1 the body composition of adult golden hamsters (age: > 21 weeks) and Mongolian gerbils (age: > 18 weeks) is given as a function of TOBEC values. Male golden hamsters had a higher body mass, a higher crude fat mass (CFM) and a higher dried body mass (DBM). Female golden hamsters had a higher percentage of FFM and of TBW. In Mongolian gerbils the males were also heavier and had higher CFM and DBM. Moreover, the absolute FFM and TBW was elevated when compared with females. As in the female golden hamsters, female gerbils had a higher percentage of FFM and of TBW.

Discussion

In the present study, body composition of Mongolian gerbils and golden hamsters was analysed via the total body electrical conductivity (TOBEC). This methodology is based upon the different electrical properties of the various body compartments. In accordance with data from the literature (GUGGENBUHL 1995; RAFFEL et al. 1996; YASUI et al. 1998) and based on a number of different regression models performed, a linear regression of FFM and TBW as a function of TOBEC was chosen as the most adequate model. Moreover, it coincides with the instrument´s measuring principle, described e.g. by FIOROTTO et al. (1987).

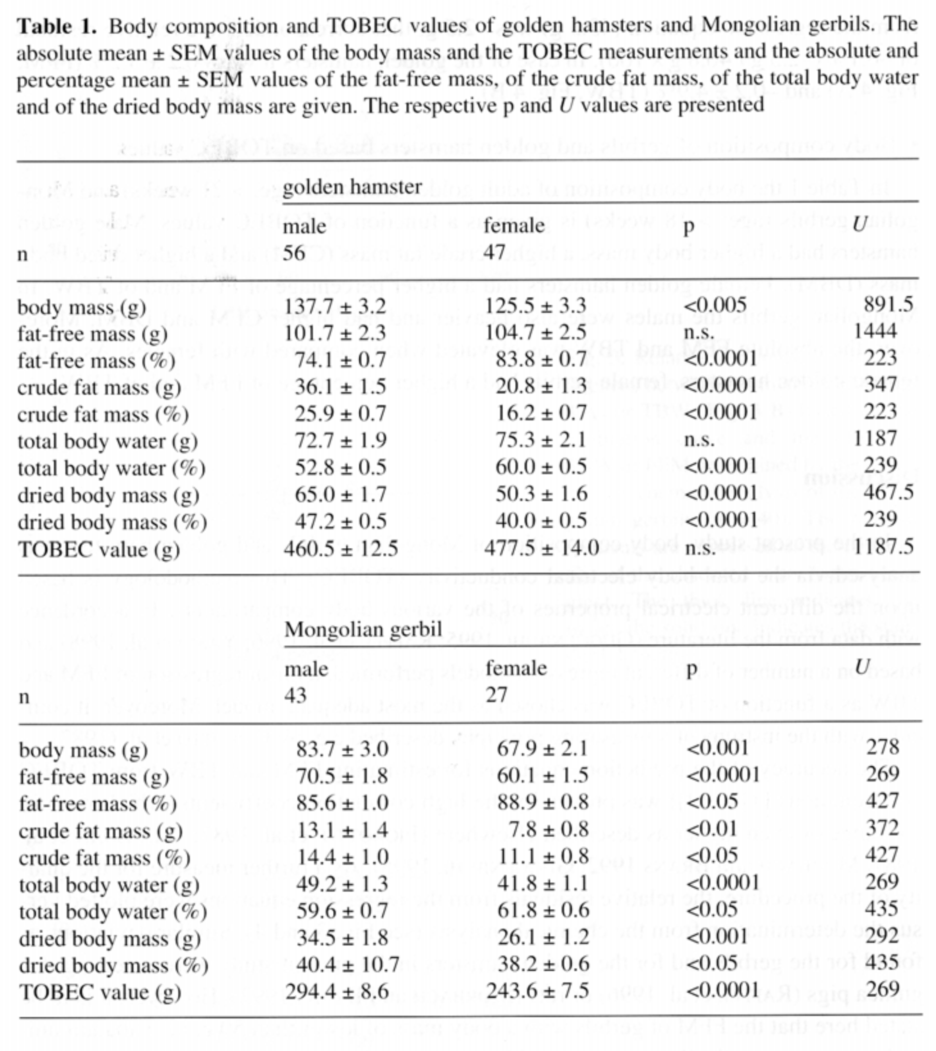

Table 1. Body composition and TOBEC values of golden hamsters and Mongolian gerbils. The absolute mean ± SEM values of the body mass and the TOBEC measurements and the absolute and percentage mean ± SEM values of the fat-free mass, of the crude fat mass, of the total body water and of the dried body mass are given. The respective p and *U* values are presented

	golden hamster			
	male	female	p	*U*
n	56	47		
body mass (g)	137.7 ± 3.2	125.5 ± 3.3	<0.005	891.5
fat-free mass (g)	101.7 ± 2.3	104.7 ± 2.5	n.s.	1444
fat-free mass (%)	74.1 ± 0.7	83.8 ± 0.7	<0.0001	223
crude fat mass (g)	36.1 ± 1.5	20.8 ± 1.3	<0.0001	347
crude fat mass (%)	25.9 ± 0.7	16.2 ± 0.7	<0.0001	223
total body water (g)	72.7 ± 1.9	75.3 ± 2.1	n.s.	1187
total body water (%)	52.8 ± 0.5	60.0 ± 0.5	<0.0001	239
dried body mass (g)	65.0 ± 1.7	50.3 ± 1.6	<0.0001	467.5
dried body mass (%)	47.2 ± 0.5	40.0 ± 0.5	<0.0001	239
TOBEC value (g)	460.5 ± 12.5	477.5 ± 14.0	n.s.	1187.5

	Mongolian gerbil			
	male	female	p	*U*
n	43	27		
body mass (g)	83.7 ± 3.0	67.9 ± 2.1	<0.001	278
fat-free mass (g)	70.5 ± 1.8	60.1 ± 1.5	<0.0001	269
fat-free mass (%)	85.6 ± 1.0	88.9 ± 0.8	<0.05	427
crude fat mass (g)	13.1 ± 1.4	7.8 ± 0.8	<0.01	372
crude fat mass (%)	14.4 ± 1.0	11.1 ± 0.8	<0.05	427
total body water (g)	49.2 ± 1.3	41.8 ± 1.1	<0.0001	269
total body water (%)	59.6 ± 0.7	61.8 ± 0.6	<0.05	435
dried body mass (g)	34.5 ± 1.8	26.1 ± 1.2	<0.001	292
dried body mass (%)	40.4 ± 10.7	38.2 ± 0.6	<0.05	435
TOBEC value (g)	294.4 ± 8.6	243.6 ± 7.5	<0.0001	269

The accuracy of the prediction equations for estimating FFM and TBW from TOBEC (see equations [1] to [4]) was proven by the high correlation coefficients, which were in the range or even higher as described elsewhere (FIOROTTO et al. 1987; VAN LOAN et al. 1987, MORBACH and BRANS 1992; GUGGENBUHL 1995). As a further measure for the quality of the procedure, the relative residuals from the regression equations were plotted versus the determination from the chemical analysis (see Fig. 3 and 4). Similar deviations as found for the gerbils and for the golden hamsters in the present study were noted e.g. for guinea pigs (RAFFEL et al. 1996) or rats (MORBACH and BRANS 1992). However, it is to be stated here that the FFM of gerbils with a body mass of lower than 50 g, i.e. subadult animals were systematically overestimated by the regression equation. The basic direct carcass chemical analysis of both species revealed a low intra-individual variability of less than 2 %, i.e. also a high accuracy of the threefold determinations was recorded. For the appropriate use of the TOBEC technique repeated measurements per animal are required to increase the reliability of the obtained values. The calculated intra-individual variability of all TOBEC values in the current study was low for gerbils (1.5 %) and golden hamsters (2.3 %), proving the validity of the taken data.

As shown in Table 1, males of both species were heavier than females, which is due to the higher CFM in males. In contrast, female gerbils and golden hamsters revealed a higher percentage of FFM and TBW than males. For neither species are comparable data available in the literature.

In conclusion, the electromagnetic scanning technique appears to be a useful tool for the evaluation of the body composition of golden hamsters and gerbils.

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3.4 Indicators for post surgery recovery in Mongolian gerbils.

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Short title: Surgery recovery in Mongolian gerbils.

Summary
Telemetric investigations of various parameters are widely used to estimate an animals state. However, the implantation of the transmitters including anaesthetization and surgery procedures has short and longer lasting impacts on the studied object. The aim of the present paper was to evaluate these effects in Mongolian gerbils, namely the hypothermia caused by the anaesthetic and the enduring disturbance of daily rhythmicity until complete recovery. The surgery associated with the implantation of the transmitters differed both in severity and narcosis. Whereas normal values of body temperature were restored within hours, the daily rhythm did need several days, depending on the severity of the surgical procedure about 7 and 14 days, respectively. Also, the sensitivity of the body temperature to activity changes was different until the rhythms have been re-established. A method, based on the rhythm magnitude and shape was proposed to estimate the time until complete reconstitution of the animals.

Keywords
telemetry, transmitter implantation, body temperature, activity, circadian rhythms, anaesthetization, surgery effects, Mongolian gerbils, reconstitution

For different scientific purposes animals have to be investigated over a longer time. The measured values must not only be informative but also obtainable without impairment of the studied object, i.e. non-invasive methods are pre-

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[9] Weinandy, R., P. Fritzsche, D. Weinert & R. Gattermann. 2002. Indicators for post surgery recovery in Mongolian gerbils. Lab. Anim. (submitted). Eigenanteil: Experimente, Auswertung und Manuskript

ferred. Good examples for this are the motor activity, body temperature and cardio-vascular parameters. They are used to characterize an organism in pharmacological and toxicological studies (Witte et al. 1999, Gordon & Mack 2001) or to estimate the costs associated with impairing events and stress (Gattermann 1983, Tornatzky & Miczek 1993, Moberg 2000, for a review see Broom & Johnson 1993). Monitoring of these parameters also allows to evaluate the housing conditions of laboratory animals, thus preventing stress effects and provide conditions for a maximum animal welfare. This in its turn will reduce the variance in normal physiological values and hence improve the results of experimental procedures (Gattermann & Weinandy 1996/97, Würbel 2000, 2001). In chronobiological studies these parameters are used to characterize the endogenous body clock and its interaction with the periodic environment (Refinetti 1999, Weinert et al. 2002, Waterhouse et al. 2000, Witte et al. 2001). Telemetry systems are suitable and currently widely used to investigate these parameters in different animal species (Clement et al. 1989, Weinandy & Gattermann 1995, Weinert & Waterhouse 1998, Waterhouse et al. 2000). Once implanted, they allow long-term measurements without apparent serious side effects to the animal. Moreover, an individual can be monitored while it lives in its group or family. Whereas a wealth of literature data does exist for laboratory mice, rats and hamsters, Mongolian gerbils scarcely have been used in telemetric studies (Weinandy & Gattermann 1996/97). Nevertheless, to our knowledge the initial phase after the surgery was hitherto not analyzed in further detail in order to assess criteria for an animals recovery. The aim of the present study therefore was to evaluate the influence of anaesthetization and surgery which both are associated with the implantation of the transmitters. Based on the changes of core body temperature and activity we estimated short- and long-term effects with respect to two different anaesthetics and surgical procedures. In particular, temperature rhythm characteristics were taken to measure the necessary recovery time for this species.

Materials and Methods

Animals and housing conditions

Eight adult males and three adult females of Mongolian gerbils (*Meriones unguiculatus*) each were chosen at random from our laboratory colony (Zoh:CRW), which were founded from animals obtained from Charles River Germany (CRW/(Mon) BR). They were kept separately in climatised windowless indoor rooms in standard plastic cages (Makrolon type IV, 55 x 33 x 20 cm) lined with wood shavings (Allspan® Animal bedding, The Netherlands) with a wire mesh top. The animal bedding was renewed every two weeks. Food pellets (one part breeding diet Altromin 7014, two parts maintenance diet Altromin 7024; crude protein 19 %, crude fat 4.2 %, crude fibre 5.8 %, ash 7.2 %, moisture 13.5 % and nitrogen-free extract 50.3 %; Altromin GmbH, Lage, Germany) and tap water were provided ad libitum. The light/dark conditions were 12:12 h, the light intensity varied from 200-300 lux (light period) to 5 lux (dark period). The light period lasted from 07:00 -19:00 h Central European Time. The room temperature was 24 °C ± 2 °C, the relative humidity varied between 60 - 65 %.

Surgery and data acquisition

Two different telemetry systems have been used - the PhysioTel® ETA Telemetry and Acquisition System DATAQUEST IV (Data Sciences, Inc., St. Paul, MN, U.S.A.) and the VitalView system (Mini Mitter Co., Inc., Bend, OR, U.S.A.). In the first case battery-powered transmitters (TA10ETA-F20) were implanted. They allowed telemetric measurement of heart rate, core body temperature and general activity. A receiver (RA1010), placed under the home cage, picked up the signal and converted it to a computer-readable form. In the second case E-Mitters (PDT-4000), which allowed the measurement of core body temperature and general activity and working without batteries were implanted. The receiver (ER-4000 Energizer Receiver) was placed under the home cage. It not only energized continuously the transmitters but also picked up the signal and converted it to a computer-readable form. Recordings were performed at 5-min intervals. Activity impulses were summed over 5 minutes, body temperature and heart rate were taken at the end of this interval. Recordings were initiated as soon as the surgical operation was completed. Due to the differences in the transmitters the severity of the surgical operation was different. Further differences concerned the anaesthesia. Accordingly, these two procedures were described separately.

Procedure 1

The battery-powered transmitters (TA10ETA-F20) had a weight of 3.9 g, a volume of 1.9 cc and two coiled wires working as sensing leads for heart-rate measurement. Animals were anaesthetized with an intraperitoneal injection of pentobarbital (80 mg/kg body mass). The body of the transmitter was placed into the abdominal cavity and sutured to the abdominal musculature. The leads were pulled along two subcutaneous tunnels at 45-60 degree angle to transverse plane of the heart.

Procedure 2

The implanted E-Mitters (PDT-4000) had a weight of 1.5 g and a volume of 1.0 cc. The animals were anaesthetized using the SpartanTM VMC small animal anesthesia machine (Matrx Medical Inc., Orchard Park, NY, U.S.A.). A tight-fitting face mask was selected and placed on the face of the animal. Subsequently, 2 vol. % isofluran and an oxygen flow of 0.5 l / min were administered. It is to be mentioned here that the animals were not intubated.

The manufacturer of the telemetry system guarantee a linear dependence of the transmitter frequency from temperature between 35° and 39°C. In the present study we have to deal with lower temperatures, namely in the course of the first hours following surgery. In a former study (Weinert et al. 1994) we did investigate the frequency characteristic of the transmitters for temperatures below 35°C. According to these results the low temperatures observed immediately after implantation (see Results) are rather too high than too low. As it was not the aim of the present investigation to measure how deep the temperature falls but to get an idea how long it needs to normalize, the obtained values were not corrected.

To characterize the reconstitution of the daily rhythms the 24-h mean, the minimum and the maximum values were calculated using hourly data. The difference between minimum and maximum was called the magnitude of oscillation.

Coefficients of correlation were used to characterize the similarity of the shape of the rhythms. For that purpose, the days 15 till 21, when the rhythm was assumed to be restored, were averaged in order to get a reference pattern. In a next step, this curve was correlated subsequently with the curves of each single day. Again, hourly data were used.

The sensitivity of the body temperature to motor activity changes was investigated using a slightly modified version of a method described by us before (Weinert & Waterhouse 1998). Briefly, this method calculated the linear regression of raw temperature on activity in a 1.5-h “window” (18 data pairs, each consisting of activity counts summed over 30 min and the temperature measured at the end of this 30-min interval). The “window” was then moved in 5-min steps (288 points/day). This analysis was performed for all days. The gradient (slope) was taken as a measure of the sensitivity of temperature to activity. For the purpose of the current study only the values of the middle of dark time (0:05-2:00) and of the light time (12:05-14:00) were considered.

Results

In the present study both male and female animals were investigated. As we could not obtain sex differences with respect to the values of interest for the present study, all data were averaged.

The surgical operation (Procedure 1) lasted on average 55 ± 11 min. At the end of the transmitter implantation the body temperature was far below normal values (Fig. 1). The lowest measured temperature was 30.5 °C, and normal values were restored only about 8 hours after first application of the anaesthetic. Motor activity was detected on average 1-2 hours after the implantation was completed.

To better illustrate some details of the temperature change following transmitter implantation, Fig. 2 shows as an example the data of one of the seven animals from Fig. 1. The steep increase at the beginning was caused by an infrared bulb, installed to warm up the animals. As only the gerbil started to move around it left the warm area below the lamp. As a consequence and despite of being locomotory active, the body temperature decreased from 37 °C to 33 °C.

The transmitter implantation according to Procedure 2 (Fig. 3) lasted on average 15 ± 5 min. The body temperature decreased much less compared with Procedure 1 (cf. Fig. 1). The lowest measured temperature was 35.2 °C. However, it took nearly the same time (7 to 8 hours) to restore normal values. The animals woke up immediately after finishing implantation and started to be active within the subsequent 10 minutes, and their general behaviour was rather normal.

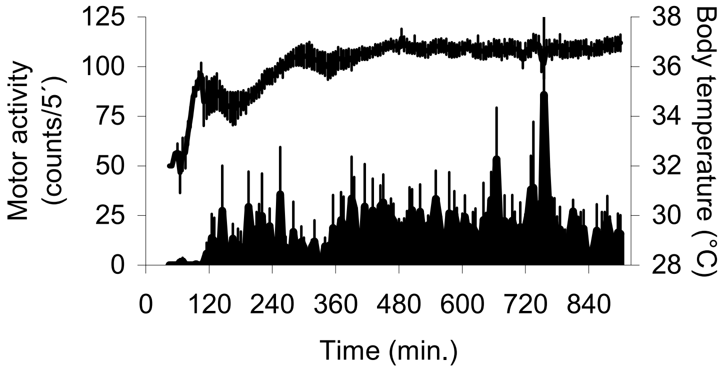

Fig. 1: Changes of body temperature (line) and motor activity (bars) following transmitter implantation (Procedure 1). Mean values and standard errors (n=7) are shown. Values on the abscissa indicate the time in minutes beginning at the start of the surgery (application of the anaesthetic).

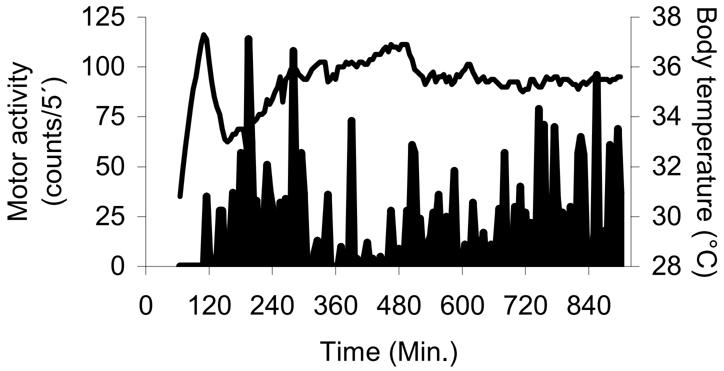

Fig. 2: Changes of body temperature (line) and motor activity (bars) following transmitter implantation. The data of one of the seven animals from Fig. 1 are shown. Values on the abscissa indicate the time in minutes beginning at the start of the surgery (application of the anaesthetic).

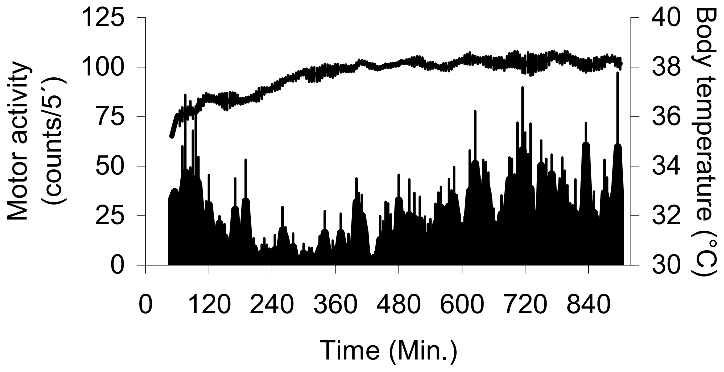

Fig. 3: Changes of body temperature (line) and motor activity (bars) following transmitter implantation (Procedure 2). Mean values and standard errors (n=4) are shown. Values on the abscissa indicate the time in minutes beginning at the start of the surgery (application of the anaesthetic).

Whereas mean values of motor activity and core body temperature were restored within hours, the daily rhythms did need several days. This is shown in Fig. 4.

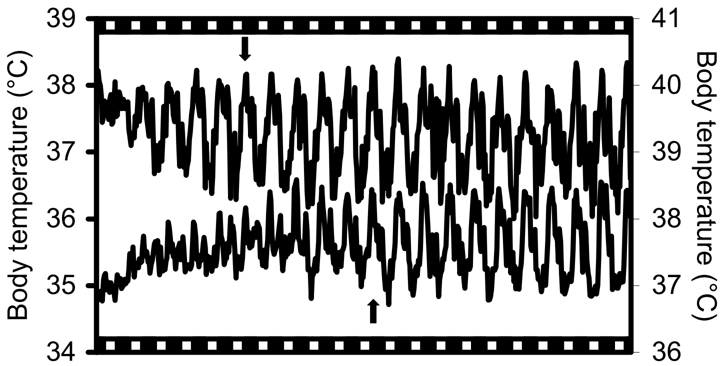

Fig. 4: Reconstitution of daily temperature rhythms. The curves start at 0:00 of the day after the transmitters were implanted and show hourly values. Subsequent days are indicated by the black (dark time) and white (light time) bars. The upper curve (left ordinate) shows the mean value of the four gerbils with implants measuring motor activity and core temperature (Procedure 2). The lower curve (right ordinate) represents the seven animals with transmitters measuring also the heart rate (Procedure 1). For clarity, the SE have not been shown. The arrows indicate the first day of a stable 24-h rhythm (estimated independently by the authors of the paper).

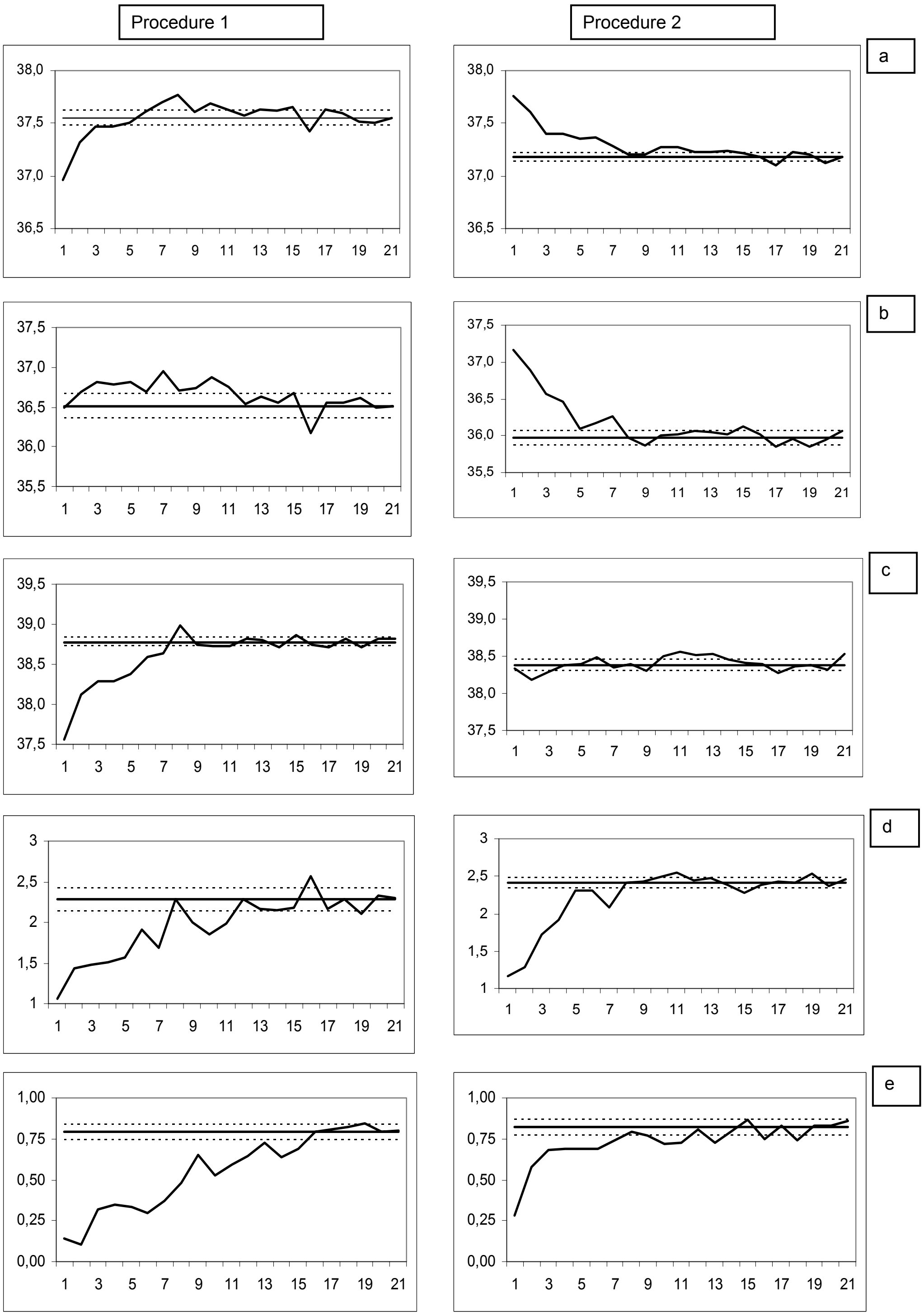

Fig. 5: Changes of rhythm characteristics following transmitter implantation. Mean curves of the seven animals implanted with transmitters according to Procedure 1 (5 panels on the left) and of the four gerbils with implantation according to Procedure 2 (5 panels on the right) are depicted. Also, the mean values (solid lines) and 95-% confidence intervals (dotted lines) of the last seven days, when the rhythm has normalized, are shown. From the top: 24-h mean (a), minimum value per day (b), maximum value per day (c), magnitude of the daily rhythm (d), coefficient of correlation (e). For further details, see text.

The core temperature rhythm was chosen to demonstrate this, as it is a good estimate of the body clock. Also, compared to motor activity, it is less affected (masked) by exogenous factors. As can be seen very clearly, the time of reconstitution was different between the two surgical procedures (6 vs. 11 days). To quantify this more formally, several rhythm characteristics were estimated (Fig. 5). Results confirm the differences obtained by visual inspection of the time series not only with respect to the time necessary for reconstitution but also concerning its mechanism. Whereas in the first case (Procedure 1) the minimum values remained nearly constant, and the maximum values increased, in the other case (Procedure 2) the maximum values did not change but the minimum values decreased. As a consequence the daily mean increased in one case and decreased in the other one. Considering that the daily mean increased first above the value of the reference days and only thereafter decreased to normal values, then the reconstitution time was somewhat longer. Altogether, the daily mean, the minimum and the maximum values were not as suitable to quantify the time of reconstitution, particularly the difference between the two surgical procedures. On contrary, the changes of the magnitude and of the coefficients of correlation turned out as very useful. They were less influenced by the mechanism of rhythm reconstitution, that means whether there was an increase of maximum values or a decrease of minimum values. Both, the magnitude and the coefficient of correlation did show a steeper increase and did reach the 95%-confidence limit earlier following Procedure 2. This was confirmed by fitting an exponential function (Tab. 1).

The estimated magnitude on day 1 following surgery was reduced though similar in both cases. However, the initial velocity was less following Procedure 1 and therefore rhythm reconstitution lasted longer. The coefficient of correlation on day 1 was also lower in that case, what means, the circadian rhythm was more disturbed. In addition, the initial velocity was much less. As a consequence, also with respect to the general shape (coefficient of correlation) the reconstitution of the rhythm was completed much later as compared with Procedure 2.

Table 1: Changes of rhythm magnitude and shape. Results of fitting an exponential function Y=A+B*exp(C*X)

	Magnitude		Coefficient of correlation	
Procedure	1	2	1	2
A	2.28	2.41	0.79	0.82
95%-limit (+/-)	0.14	0.07	0.05	0.05
calculated value on day 1	1.09	1.03	6	36
C	-0.19	-0.39	-0.15	-36
Reconstitution (days)	13	9	20	8

Y - corresponding value on day X after implantation
A - asymptote of the function (the mean value of the last seven days, see Fig. 5, was taken)
B - difference between asymptote (A) and the value Y on day zero; instead of B, what is a rather theoretical value, the Y value on day 1 following the implantation was included in the table
C - characterizes the steepness of the curve, and the absolute value can be used as a measure of the initial velocity of reconstitution
Reconstitution (days) - the day after the fitted curve crossed the 95% confidence limit

In a next step the sensitivity of the body temperature to activity changes was investigated. Results are shown in Fig. 6. Whereas the gradient obtained in the middle of the light time did not change in the course of the three weeks following transmitter implantation, it was elevated in the dark time during the first two weeks. The difference between dark and light was first significant in the second week. It was small, however, compared to the difference obtained when rhythms were restored completely (third week).

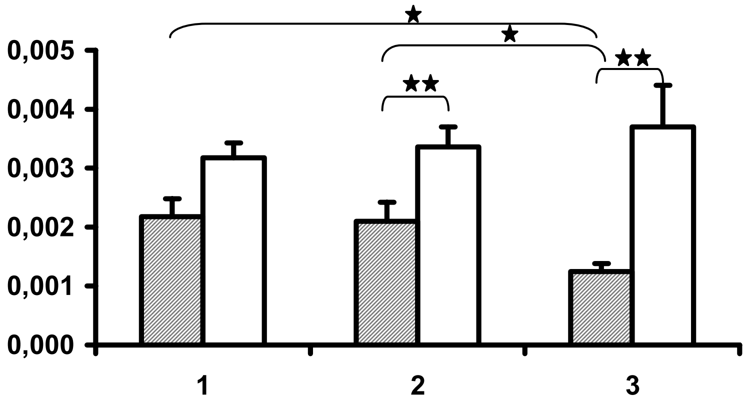

Fig. 6: Sensitivity of body temperature to activity changes depending on circadian phase and on the time after transmitter implantation. The gradients (slopes) of linear regression lines are shown (mean values ± SE; n = 7). Hatched bars - middle of the dark time; open bars - middle of the light time (for details, see text.). The abscissa indicates the 1st, 2nd and 3rd weeks after transmitter implantation. * $p < 0.05$; ** $p < 0.01$ (two-tailed, paired t-test).

Discussion

The surgical procedure when implanting telemetry transmitters has a great impact on the animals. Besides the short lasting anaesthetic effect together with a hypothermia, one has to consider a longer lasting disturbance of daily rhythmicity. The hypothermia following narcosis and transmitter implantation might have several causes. So, a heat loss as a result of depilating and opening the abdomen and resulting from inactivity of animals during the surgery must be taken into account (Weinert et al. 1994). However, the main effect to be considered is that of the anesthetic on the mechanisms of thermoregulation (Sessler 1993). Therefore, the differences in the temperature drop and the subsequent rise were caused mainly by the anesthetic. Isofluran, used in Procedure 2, turned out as superior compared to pentobarbital. Hypothermia was less strong. The animals woke up immediately after the implantation was completed and did behave in a rather normal manner. On the contrast with pentobarbital the body temperature decreased much more and the animals began to be active later. Pentobarbital, an oxybarbiturate, starts to work within 30 - 60 seconds, but its effect lasted as long as 1-2 hours. The body temperature decreases due to a reduced metabolic rate, peripheral vasodilatation and general dampening of thermoregulation. Pentobarbital is metabolized in the liver and excreted with the urine (about 15 % / hour). This is consistent with the time of recovery of normal body temperature (see below). The effect of isofluran is also very fast but in this case it does stop within 5 minutes. It has only a low impact on the cardiovascular system. Nearly all of the anaesthetic will be eliminated with expiration. Only about 0.2 % will be metabolized in the liver, what is remarkable considering that several hours are necessary to restore the normal body temperature.

Despite of the different degree of hypothermia, with both anaesthetics the normal temperature was reached only after 7-8 hours. This is consistent with results on laboratory mice. These were anaesthesized injecting ketamin-hydrochlorid intraperitoneally. The body temperature decreased to 31.9 ° C ± 0.3 °C., and about 8 hours after the surgery the normal body temperature was restored (Weinert et al. 1994). Thus, the recovery of the normal body temperature seems to depend mainly on the elimination rate of the drug. It was nearly unaffected by the amount of motor activity the animals showed.

Whereas normal body temperatures were restored within hours circadian rhythms were disturbed for several days. This is consistent with our own earlier investigations on mice (Weinert et al. 1994) and some literature data (Clement et al. 1989, Drijfhout et al. 1995). Disturbances of circadian temperature rhythms were also found caused as a consequence of stress (Kant et al. 1991). In the present paper we found that the time of rhythm reconstitution depends on the severity of the surgical operation. Due to the complexity of the matter it is not possible to give an exact value. Also, there is certain inter-individual variability. However, we can state that at least 7 days were necessary after implantation of transmitters measuring locomotor activity and body temperature. In the case of transmitters measuring also heart rate, even two weeks were necessary. In our investigations on laboratory mice (Weinert et al. 1994) we also found a recovery time of about 7 days. The transmitters were similar to those implanted in the present study in procedure 2.

In the present study a mean chronogram calculated from days 15-21 was taken as a reference, since it was consistent with formerly described temperature rhythms (Weinandy & Gattermann 1996/97, Refinetti 1996). Several rhythm characteristics have been calculated, the magnitude and the rhythm shape as evaluated by the mean of the coefficient of correlation turned out to be most appropriate to estimate the time of recovery. As long as the daily temperature rhythm is not recovered, also other physiological function are disturbed. This we could show investigating the sensitivity of body temperature to activity changes. In intact organisms there is an distinct daily rhythm with higher sensitivity in the light (resting period) compared with the dark time (activity period). This daytime difference was absent as long as the temperature rhythm was not yet restored. Since the different sensitivity of body temperature to activity changes is a consequence of different efficacy of thermoregulation (Weinert et al. 2002), one has to consider, also differences in other physiological reactions. That is a further reason not to start with the experiment until stable rhythms can be obtained.

In summary, it was shown that daily rhythms need several days to recover following transmitter implantation and that this time depends on the severity of surgical operation. It follows, that one has to wait until stable rhythms can be obtained as they are essential for an animal organism. At the same time, these rhythms can be taken as an appropriate indicator to estimate the start time for an experiment.

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4 Diskussion, Schlussfolgerungen und Ausblick

Natürlicherweise leben Mongolische Wüstenrennmäuse territorial in Familienverbänden, die auf ein Stammpaar zurückgehen (Bannikov 1954, Ågren 1984, Ågren 1989a, Hendrie & Starkey 1998). Da sich Familien erst dadurch bilden, dass die Jungen bei den Eltern verbleiben, resultiert in diesen sozialen Einheiten ein sehr hoher Verwandtschaftsgrad und sie unterscheiden sich dadurch erheblich von allen anderen Formen sozialer Verbände. Die Untersuchung der spezifischen Fortpflanzungsstrategien, des Sozialverhaltens und der zeitlichen Organisation in derartigen Verbänden mit hoher genetischer Verwandtschaft war Gegenstand der vorliegenden Arbeit.

Wesentlich für die Stabilität solcher Sozialstrukturen ist die zeitliche Verhaltensabstimmung, da diese für ein Leben im Verband, z.B. durch gemeinsame und koordinierte Verteidigung des Territoriums oder der Aufzucht der Jungen (**Kap. 1.3: Parental care and time sharing in the Mongolian gerbil**), essentiell ist. Daher und auf Grund entsprechender Literaturdaten zur Thematik insgesamt (Mrosovsky 1988, Erkert & Schardt 1991, Goel & Lee 1995) erwarteten wir auch bei den sozial lebenden Mongolischen Wüstenrennmäusen Belege für eine Beeinflussung der Circadianrhythmik durch soziale Stimuli (**Kap. 1.2: Lack of social entrainment of circadian activity rhythms in the solitary golden hamster and in the highly social Mongolian gerbil**). Jedoch konnte in keinem Fall ein solcher Effekt nachgewiesen werden. Während dieses Ergebnis für den untersuchten solitär lebenden Goldhamster auf Grund seiner Lebensweise den Erwartungen entsprach, war das Fehlen einer sozialen Synchronisation für die Mongolische Wüstenrennmaus überraschend. Weder akustische noch olfaktorische Signale der Artgenossen führten dazu, dass Tiere, die sich unter konstanten Lichtbedingungen (Dauerdunkel) befanden, ihre freilaufenden endogenen Rhythmen anpassten und sich synchronisierten. Dagegen fanden sich klare Hinweise darauf, dass die menschliche Anwesenheit im Rahmen der regelmäßig und periodisch erfolgenden Routinekontrollen in der Tierhaltung und die damit verbundenen Geräusche einen Zeitgeber für die Tiere darstellte. Dass derartige nicht-photische Zeitgeber in dieser Weise wirksam werden können, wurde bereits beim Goldhamster gezeigt (Hastings et al. 1992). Nach den vorliegenden Daten zu Goldhamstern und Mongolischen Wüstenrennmäusen muss davon ausgegangen werde, dass sich Stimuli der Artgenossen nicht als verlässliche Zeitgeber im Verlauf der Evolution erwiesen haben und somit auch unter Laborbedingungen nicht den Licht-Dunkel-Wechsel als Hauptzeitgeber ersetzen können.

Die Fortpflanzung eines potenziell reproduktiven Individuums kann durch eine Reihe von Stressoren gestört oder sogar unterdrückt werden. Daher stellt eine erfolgreiche Reproduktion einen Indikator für das Wohlergehen eines Tieres dar (Henry 1982, von Holst 1985). Aus diesem Grund wurden die physiologischen und ethologischen Veränderungen, die bei Weibchen mit der Gravidität und Laktation einhergehen, mit Hilfe eines biotelemetrischen Systems untersucht (**Kap. 1.1: Measurement of physiological parameters and activity in a Mongolian gerbil during gravidity and lactation with an implanted transmitter**). Trotz des implantierten Transmitters unterschied sich das reproduktive Verhalten des untersuchten Weibchens in keiner Weise von dem gesunder Artgenossen ohne implantierten Sendern, wie ein Vergleich mit Literaturdaten ergab (Galef 1983, Gattermann et al. 1986). Dies ist bedeutsam, da es darauf hinweist, dass mit dieser Technik Langzeituntersuchungen ohne erkennbare Nebeneffekte möglich sind (**Kap. 2.1: Time of day and stress response to different stressors in experimental animals. Part II: Mongolian gerbil (*Meriones unguiculatus*)**). Gestützt wird diese Auffassung auch durch die am Ende des Versuches erhobenen Daten zu Stresshormonen (Glukokortikoiden), sowie Körper- und Organmassen, die nicht von denen anderer adulter Weibchen abwichen (unpublizierte Daten). Ein Vergleich mit Literaturdaten zu Körper- und Organmassen (Kramer 1964, Gattermann et al. 1986) ergab ebenfalls eine gute Übereinstimmung. Neben dieser generellen Erkenntnis zur Validität der Methode zeigte sich, dass die Herzschlagfrequenz des Weibchens in den letzten Tagen der Gravidität deutlich abfiel und auch nach der Geburt der vier Jungtiere für mehr als drei Monate auf diesem erniedrigten Niveau verblieb. Dies weist daraufhin, dass das Weibchen in dieser physiologisch belastenden Phase Energie einsparte und diese den neugeborenen Jungen in Form von Wärme zur Verfügung stellte. Diese Interpretation wird durch den Befund, wonach das Weibchen während der gesamten Laktationsperiode eine um 0,6 °C erhöhte Körpertemperatur aufwies, überzeugend gestützt. Für die Jungtiere, die nackt als Nesthocker geboren werden und über eine nur ungenügend ausgeprägte Fähigkeit zur Thermoregulation verfügen, sind die Wärme und der Schutz durch die Mutter in den ersten beiden Lebenswochen überlebensnotwendig. Dass dies für das Weibchen massive Konsequenzen hat, zeigen erste, noch unpublizierte Daten zur Körpermasseentwicklung laktierender Weibchen, die ihre Jungen entweder mit oder ohne Männchen aufzogen. Während die Weibchen in Paarhaltung ihre Körpermasse während der Laktation konstant hielten, verloren die ihre Jungen alleine aufziehenden Weibchen fast zehn Prozent ihrer Ausgangskörpermasse, was insbesondere für die Betrachtung des Reproduktionserfolges im Verlauf des gesamten Lebens von Bedeutung sein könnte. Dies erklärt auch, warum sich bei dieser Art neben dem Weibchen auch das Männchen und weitere Familienmitglieder an der Aufzucht der Jungen beteiligen (Elwood 1975, Ostermeyer & Elwood 1984). Väterliches Brutpflegeverhalten wurde aber auch bei anderen Nagetieren beschrieben. So zeigten Wolff und Cicirello (1991), dass Männchen der Hirschmaus (*Peromyscus maniculatus*) ihre Jungen eintrugen und sich gemeinsam mit dem Weibchen und den Jungtieren im Nest aufhielten. Da die für die Mongolische Wüstenrennmaus publizierten Befunde auf Kurzzeitbeobachtungen basierten, war die elterliche Jungenpflege Gegenstand einer detaillierten quantitativen Analyse (**Kap. 1.3: Parental care and time sharing in the Mongolian gerbil**). Dabei standen von der Geburt der Jungen bis zu ihrer Entwöhnung die Verhaltensweisen Nestbau, Nestaufenthalt und das Eintragen der Jungtiere sowie die zeitliche Kooperation der Elterntiere im Mittelpunkt der Betrachtungen. Der Ausbau des Wurfnestes nach Geburt der Jungen erfolgte im Wesentlichen durch die Weibchen. Dies ist als eine Reaktion auf die Anwesenheit der Nachkommen zu verstehen, da das Nestbauverhalten von Nagetieren neben der hormonellen Steuerung auch durch olfaktorische und akustische Stimuli der Jungen intensiviert wird (Noirot 1972, 1974, Sales & Smith 1978). Ebenfalls fast ausschließlich durch die Weibchen erfolgte das Eintragen der Jungen ins Nest. Auch hier wurde das Verhalten höchstwahrscheinlich durch die Ultraschall-Vokalisation der Jungen ausgelöst. Obwohl auch Männchen versuchten, ihre Jungen wieder ins Nest zu transportieren, gelang ihnen der Nackengriff nicht, so dass sie sie nur mit ihrer

Schnauze in Richtung Nest schieben konnten. Im Gegensatz zu diesen beiden hauptsächlich von den Weibchen ausgeführten Verhaltensweisen kümmerten sich sowohl die Männchen als auch die Weibchen gemeinsam um ihre Jungen im Nest. Während ein Elterntier bei den Jungen blieb und diese wärmte, konnte das andere es verlassen und beispielsweise Nahrung oder Wasser aufnehmen. Durch die zeitliche Kooperation und die synchronisierte Anwesenheit der adulten Tiere bei den Nestlingen wird zum einen deren Auskühlen verhindert. Zum anderen kann die Mutter ihren gesteigerten Wasser- und Nährstoffbedarf nach der Geburt und während des Säugens decken (Galef 1983). So werden vermutlich die physiologischen Belastungen des Muttertieres post-partum, beispielsweise auch verursacht durch die Laktationhyperthermie (**Kap. 1.1: Measurement of physiological parameters and activity in a Mongolian gerbil during gravidity and lactation with an implanted transmitter**), reduziert. Eine derartige zeitliche Synchronisation beschrieb Wynne-Edwards (1995) auch für den Zwerghamster *Phodopus campbelli*.

Eine wechselseitige Verhaltensbeeinflussung findet bei den sozial lebenden Mongolischen Wüstenrennmäusen aber natürlich nicht nur bei Anwesenheit des Nachwuchses statt, sondern ist ein generelles Merkmal, das insbesondere für die Männchen-Weibchen Beziehung kennzeichnend ist. Es ist nahe liegend, dass in diesem Zusammenhang dem weiblichen Zyklus und den damit einhergehenden physiologischen und ethologischen Veränderungen eine zentrale Bedeutung zukommt. Aus der Literatur war bekannt, dass das männliche Markierverhalten, das bei ranghohen Tieren verstärkt zu beobachten ist und unter anderem zur Sicherung des Territoriums genutzt wird, durch die Anwesenheit von Weibchen im selben Haltungsraum signifikant zunimmt (Probst & Lorenz 1987). Als auslösendes Signal konnten Polypeptide im weiblichen Urin identifiziert werden. Allerdings wurde in diesem Fall nicht nach den verschiedenen Zyklusstadien unterschieden. Der Grund ist vermutlich der, das der weibliche Sexualzyklus der Mongolischen Wüstenrennmäuse, im Gegensatz zum Beispiel zum Goldhamster (Gattermann et al. 1985), eher variabel und daher weniger präzise vorhersagbar ist. Dem entsprechend sind auch die in der Literatur verfügbaren Daten eher spärlich und zum Teil hinsichtlich Länge und Klassifizierung der einzelnen Stadien widersprüchlich (Marston & Chang 1965, Nishino &

Totsukawa 1996). Um dem abzuhelfen, wurden die vier Zyklusstadien Pröostrus, Östrus, Metöstrus und Diöstrus anhand von vaginalzytologischen Kriterien und ausgewählten Verhaltensweisen genau beschrieben (**Kap. 1.4: Mating behavior during the estrus cycle in female Mongolian gerbils (*Meriones unguiculatus*)**). Bei Nagetieren zeigen Uterus und Vagina durch die Einwirkung ovarieller Hormone zyklusabhängig sowohl Proliferation als auch Apoptose des Epithels (Sato et al. 1997). Die periodische Zu- und Abnahme der unverhornten Epithelzellen, der Leukozyten und der verhornten Epithelzellen im Vaginalabstrich ist die Konsequenz dieser Veränderungen und wurde bereits für Ratten (Otha 1995) und Goldhamster (Sandow et al. 1979, Gattermann et al. 1985) als zuverlässige Indikatoren des Östruszustands des Weibchens beschrieben.
Für die Mongolische Wüstenrennmaus konnte der Proöstrus in Übereinstimmung mit der Literatur (Nishino & Totsukawa 1996) durch eine Zunahme der unverhornten Epithelzellen, dem Fehlen der Leukozyten und das nur vereinzelte Auftreten verhornter Epithelzellen charakterisiert werden. Die Aggressivität der Weibchen war in dieser Phase niedrig und sie zeigten den Männchen gegenüber nur noch seltenes Kopulationsvermeidungsverhalten. Dies wird als präkopulatorisches Verhalten bezeichnet und signalisiert den Männchen den bevorstehenden Östrus (Holman et al. 1985). Im Östrus ging die Anzahl unverhornten Epithelzellen zurück und es dominierten verstreut liegende verhornte Epithelzellen. Leukozyten traten in diesem Stadium nicht mehr auf. Das Verhalten wurde durch die sexuelle Rezeptivität bestimmt, das Weibchen zeigte Lordose und keine Aggressivität dem Männchen gegenüber. Als ein weiterer Indikator für das Vorliegen eines Östrus und erfolgte Kopulationen war das Auftreten eines Vaginalpfropfs zu sehen. Typisch für den Metöstrus war, dass Leukozyten überwogen und von verstreut liegenden unverhornten und verhornten Epithelzellen umgeben waren. Dies steht im Gegensatz zur Literatur (Nishino & Totsukawa), wonach der durch dieses Zellmuster indizierte Zustand als „Östrus II“ bezeichnet wird. Auf Grund des Verhaltens, insbesondere durch das Fehlen jedweder sexueller Aktivität und der Zunahme der Kopulationsvermeidung, konnte dies allerdings nicht bestätigt werden. Der Diöstrus, also der Ruhezustand, der zwischen Met- und Proöstrus eintritt, wenn ein Weibchen nicht befruchtet wurde, war durch agonistisches Verhalten der Weibchen charakteri-

siert. Dies stimmt weitgehend mit Literaturdaten überein (Ågren & Meyerson 1977). Im Abstrichbild war die Zahl der Leukozyten verringert und es traten keine oder nur wenige Epithelzellen auf. Durch die kombinierte Erfassung der Vaginalzytologie und des Paarungsverhaltens konnten somit die vier Zyklusstadien weiblicher Mongolischer Wüstenrennmäuse eindeutig beschrieben werden. Neben der generellen Bedeutung dieser Befunde für Experimente, die mit Paar- bzw. verschiedengeschlechtlicher Gruppenbildung einhergehen, war es nun möglich, nach weiteren Indikatoren für den Sexualzustand der Weibchen zu suchen (**Kap. 1.5: The oestrus of female gerbils, *Meriones unguiculatus*, is indicated by locomotor activity and influenced by male presence**). Dabei stand die chronobiologische Analyse der lokomotorischen Aktivität, ein im Gegensatz zum Vaginalabstrich nicht-invasiver Marker, im Vordergrund. Obwohl laut Literatur dieses Verfahren als wenig verlässlich bezeichnet wurde (Barfield & Beeman 1968, Umezu et al. 1989), konnten östrusbedingte Veränderungen der Laufradaktivität festgestellt werden. Weibliche Rezeptivität spiegelte sich in einem um vier bis fünf Stunden vorverlagerten Aktivitätsbeginn wider, während dieser ansonsten mit dem Zeitpunkt Licht-aus korrelierte (für das typische Aktivitätsmuster dieser nachtaktiven Tiere vgl. auch **Kap. 1.1: Measurement of physiological parameters and activity in a Mongolian gerbil during gravidity and lactation with an implanted transmitter** und **Kap. 2.2: Circadian activity rhythms and sensitivity to noise in the Mongolian gerbil (*Meriones unguiculatus*)**). Somit konnte die lokomotorische Aktivität als ein weiterer Indikator für den weiblichen Sexualzustand identifiziert und verifiziert werden. Auf Grund der bereits beschriebenen ausgeprägten sozialen Beziehung zwischen Männchen und Weibchen (siehe Einleitung) lag es nahe, einen männlichen Einfluss auf diesen für die Reproduktion so wesentlichen Zyklus zu postulieren. Literaturdaten zu diesem Phänomen sind widersprüchlich, zum Teil sogar innerhalb derselben Art. Für Waldmäuse (*Apodemus sylvaticus*) ließ sich zum einen kein Effekt der männlichen Anwesenheit auf die Weibchen nachweisen (Jonsson & Silverin 1997), während Stopka & Mac Donald (1998) eine männchen-induzierte Zyklusverlängerung beschrieben.

Bei Hausmäusen kommt es im Extremfall sogar zur Aussetzung des Zyklus, wenn olfaktorische Signale des anderen Geschlechts fehlen (Bruce 1970), andererseits stimuliert die Existenz von Männchen die weibliche Fertilität (Bronson 1985).

Die in der vorliegenden Untersuchungen (**Kap. 1.5: The oestrus of female gerbils, *Meriones unguiculatus*, is indicated by locomotor activity and influenced by male presence**) eingesetzten Männchen wurden sterilisiert, um eine Gravidität der Weibchen auszuschließen und so mehrere individuelle Zyklen auswerten zu können. Die vasektomierten aber sich geschlechtstypisch verhaltenden Männchen stabilisierten die Zyklen der Weibchen nicht. Im Gegenteil, Weibchen die mit diesen Männchen verpaart wurden, wiesen irreguläre und verlängerte Zyklen auf. Darüber hinaus kam es als Konsequenz der Kopulationen ohne erfolgte Befruchtung und Gravidität zu längeren Zykluspausen, die in Übereinstimmung mit der Literatur als Pseudogravidität bezeichnet werden können (Marston & Chang 1965, Barfield & Beeman 1968, Nishino & Totsukawa 1999). Weitaus gravierender und unerwartet war jedoch, dass es in sechs der neun formierten Paare zu spontanen und nicht absehbaren Zusammenbrüchen kam, die durch die weibliche Aggressivität ausgelöst wurden. Die betroffenen Männchen starben entweder innerhalb von 24 Stunden oder wurden so schwer verletzt, dass sie entfernt werden mussten. Etwas derartiges wurde zuvor noch nie beschrieben. Es ist derzeit noch unklar, auf welche Weise die Weibchen die männliche Unfruchtbarkeit detektierten. Möglicherweise spielen veränderte olfaktorische Signale eine Rolle, obwohl die Männchen intakte Hoden besaßen und arttypische Testosteronkonzentrationen von 1.1 ± 0.2 ng/ml Serum aufwiesen (vgl. auch Clark & Galef 2000). Vermutlich wurde die weibliche Aggression durch die wiederholten erfolglosen Paarungen ohne Spermienübertragung, verbunden mit möglichen physiologischen Beeinträchtigungen der Weibchen durch die wiederholten Pseudograviditäten ausgelöst. Somit investierten die Weibchen nicht länger in diese Paarbindungen, die ihren Fortpflanzungserfolg minderten und damit letztlich ihre reproduktive Fitness reduzierten.

Neben einer Reihe weiterer Faktoren hat auch die soziale Lebensweise eine Auswirkung darauf, ob und wie stark ein Individuum auf eine belastende Situation reagiert (Übersicht in Sachser 1994). Daher wurden im Rahmen einer vergleichenden zweiteiligen Studie die Stressreaktionen des solitär lebenden

Goldhamsters (Gattermann & Weinandy 1996/97) und der sozialen Mongolischen Wüstenrennmaus (**Kap. 2.1: Time of day and stress response to different stressors in experimental animals. Part II: Mongolian gerbil (*Meriones unguiculatus*)**) untersucht. Versuchstiere leben unter Laborbedingungen in standardisierten Umwelten. Ging man früher davon aus, durch diese Haltungsform die Vergleichbarkeit von Studien besser zu gewährleisten, so mehren sich die Hinweise, dass diese Standardisierung selbst massive Veränderungen in den Tieren auslöst, die die Interpretation der Resultate erschweren und die Variabilität in den untersuchten Versuchsgruppen erhöhen können (Würbel 2000, 2001, Knight 2001). Darüber hinaus kann es unter solchen Bedingungen auch zur Ausprägung von Stereotypien kommen, wie es unter anderem für die Mongolischen Wüstenrennmaus am Beispiel des Scharrens an der Käfigwand und dem Nagen an den Gitterstäben des Käfigdeckels beschrieben wurde (Wiedenmayer 1997). Gegenstand der Untersuchungen am Goldhamster und der Mongolischen Wüstenrennmaus war die Evaluierung ihres Wohlergehens nach Einwirkung tierhaltungsüblicher Maßnahmen wie dem Handling, der Neuformation von Gruppen oder dem Käfigwechsel, die nachfolgend als soziale bzw. nicht-soziale Stressoren bezeichnet werden. Da aus verschiedensten Studien bekannt ist, dass die Tageszeit sowohl die Physiologie als auch das Verhalten beeinflusst (Tkadlec & Gattermann 1993, Lemmer 1996, Waterhouse et al. 2000, 2001), war zu erwarten, dass auch die Stressreaktionen in Abhängigkeit vom Aktivitätszustand unterschiedlich stark ausfallen. Dem entsprechend fanden die Experimente zum einen in der Aktivitätszeit (also der Dunkelzeit) und zum anderen in der Ruhezeit statt. Die Erfassung der anerkannten Belastungsindikatoren Herzschlagfrequenz, Körpertemperatur und Aktivität (Aschoff et al. 1971, Gattermann 1983, Stöhr 1988, Tornatzky & Miczek 1993), erfolgte telemetrisch durch intraperitoneal implantierte Transmitter ohne erkennbare Nebenwirkungen (**Kap. 1.1: Measurement of physiological parameters and activity in a Mongolian gerbil during gravidity and lactation with an implanted transmitter**). Im Rahmen dieser Studie konnten für die genannten Parameter Normwerte erhoben werden, die nicht nur für die konkrete Fragestellung essentiell sind, sondern darüber hinaus eine Basis für weitere Untersuchungen zum Wohlergehen beider Arten darstellen. Es zeigte sich, dass das grundsätzliche Muster der circadianen Rhythmen durch die induzierten Belastungen nicht beeinflusst war. Die Synchronisation der „inneren Uhr“ durch den Licht-Dunkel-Wechsel blieb unverändert erhalten. Diese Robustheit deckt sich mit früheren Befunden (Weinandy 1996) und ist nicht überraschend, da die interne und externe Synchronisation eines Individuums mit seiner Umwelt einen entscheidenden Faktor für seine Gesundheit und sein Wohlergehen darstellt und daher ein Zusammenbruch dieses Systems, wenn überhaupt, vermutlich nur in lebensbedrohlichen Situationen erfolgt. Davon abgesehen, resultierte die Einwirkung aller gewählten tierhaltungsüblichen akuten Stressoren bei beiden Arten in einer Erhöhung der Herzschlagfrequenz, der Körpertemperatur sowie der Aktivität. Studien an Tupajas und Wildkaninchen (von Holst 1986, Eisermann 1992) ergaben, dass erhöhte Herzschlagfrequenzen ein sehr deutliches Signal für kurz- und langfristige Belastungen darstellen. Ein Anstieg der Körpertemperatur als physiologische Antwort auf die Einwirkung unterschiedlichster Stressoren ist ein allgemein bekanntes und verbreitetes Phänomen (Borsini et al. 1989, Briese & Cabanac 1991, Zethof et al. 1994). Aktivitätsänderungen können ebenfalls als ein Bioindikator für das Wohlergehen von Versuchstieren angesehen werden (Gattermann 1983).

Der Vergleich von Goldhamster und Mongolischer Wüstenrennmaus ergab, wenn auch ohne statistische Sicherung, dass letztere empfindlicher auf die applizierten Stressoren reagierten. Ob dies mit der unterschiedlichen sozialen Lebensweise zusammenhängt, darüber kann gegenwärtig auf Grund fehlender Referenzen nur spekuliert werden. Für beide Arten aber zeigte sich, dass die Stressreaktionen sowohl von der Tageszeit als auch von den eingesetzten Belastungen abhängig waren. Wurden die Experimente in der Aktivitätszeit der Tiere durchgeführt, so reagierten sie nur kurz und die Reaktionswerte der Stunde vor und der Stunde nach der Belastung waren nicht zu unterscheiden. Dies gilt für alle Stressoren bis auf die Gruppenbildung, die unabhängig vom Aktivitätszustand zu heftigen Reaktionen führte. Erfolgten die gleichen Belastungen dagegen in der Ruhezeit, so dauerte es mindestens 50 Minuten, bis die Tiere wieder ihr Ausgangsniveau erreichten. Darüber hinaus ergab sich auf Grund des Ausmaßes der Stressantwort eine abgestufte Wirksamkeit der Stressoren, wobei das Handling die Tiere am wenigsten belastete, während die Gruppenbildung die stärksten Effekte hatte. Zieht man jedoch die unterschiedliche Einwirkungsdauer in Betracht, kann eine solche Klassifizierung lediglich eine Hilfestellung für die praktische Arbeit mit diesen (und womöglich auch anderen Nagetieren) Tieren im Labor sein.

Störungen des endogenen Rhythmus beeinträchtigten das Wohlergehen (Folkard et al. 1993, Okawa et al. 1994). Die Nutzung und Validität dieses Parameters setzt jedoch das Vorhandensein artspezifischer Basiswerte voraus. Diese lagen für die Mongolische Wüstenrennmaus bis dato nicht vor, was vermutlich auch auf ihre hohe Sensitivität und Störanfälligkeit gegenüber nicht-photischen Reizen zurückzuführen ist (**Kap. 1.2: Lack of social entrainment of circadian activity rhythms in the solitary golden hamster and in the highly social Mongolian gerbil**). Um die Tiere trotz dieser Schwierigkeiten chronobiologisch zu charakterisieren, wurden Langzeitversuche unter abgeschirmten Bedingungen in Klimaschränken durchgeführt. (**Kap. 2.2: Circadian activity rhythms and sensitivity to noise in the Mongolian gerbil (*Meriones unguiculatus*)**). Weiterhin sollte eine mögliche Ursache für die hohe Empfindlichkeit der Mongolischen Wüstenrennmäuse gefunden werden. Obwohl sich das Versuchsdesign für ähnliche Fragestellungen bei Mäusen bereits als geeignet erwiesen hatte (Weinert & Kompauerova 1998), lies im Falle der Mongolischen Wüstenrennmaus die hohe intra- und interindividuelle Variabilität der Aktivitätsmuster eine eindeutige Interpretation der Daten nicht zu. Wie schon in einer früheren Studie (**Kap. 1.2: Lack of social entrainment of circadian activity rhythms in the solitary golden hamster and in the highly social Mongolian gerbil**), ergab sich auch hier zunächst, dass der circadiane Rhythmus der Tiere durch unspezifische Geräusche beeinflusst wurde. Dies erklärt sich vermutlich daher, dass die Tiere unter Freilandbedingungen ein gut entwickeltes System der olfaktorischen und akustischen Kommunikation etabliert haben (Thiessen & Yahr 1977) und daher auf Reize entsprechender Qualität sehr sensibel reagieren. Dass nicht-photische Ereignisse unter bestimmten Bedingungen eine derartige Potenz besitzen, konnte bereits für andere Tierarten beschrieben werden (Goldhamster: Janik & Mrosovsky 1993, Mrosovsky 1993, Ratten: Barrington et al. 1993, Übersicht in Rietveld et al. 1993). Erst nach einer weitgehenden drastischen Reduzierung jedweder äußerer akustischer Störungen durch eine massive Dämmung der Klimaschränke entwickelten die Tiere schließlich vergleichbare und stabile freilaufende Aktivitätsrhythmen. Die so identifizierte Störquelle „Lärm“ ist vermutlich auch der Grund für die gesteigerte Sensitivität der Tiere unter den normalen Laborbedingungen und sollte daher im Hinblick auf eine belastungsarme Haltung als ein möglicher Stressor berücksichtigt werden.

Neben physiologischen, neuroendokrinen und morphologischen Parametern kann auch die Körperzusammensetzung Aufschlüsse über den Zustand eines Individuums geben. Durch die in vivo Messtechnik des elektromagnetischen Scannings (total body electrical conductivity, TOBEC), die auf der unterschiedlichen elektrischen Leitfähigkeit der einzelnen Körperbestandteile basiert, wird der prozentuale Rohfettgehalt an der Gesamtkörpermasse, eine wichtige stoffwechselphysiologische Kenngröße, bestimmt. Änderungen der Körperzusammensetzung können auf metabolische Stresszustände hindeuten, z.B. nach Kontakt mit sozialen oder pharmakologischen Stressoren (Zhou et al. 1999, Laugero & Moberg 2000). Da die Körperzusammensetzung artspezifisch variiert, ist vor dem erfolgreichen Einsatz eine Kalibration des Gerätes und des Verfahrens insgesamt erforderlich. Diese wurde sowohl für den Goldhamster als auch die Mongolische Wüstenrennmaus in einer methodisch orientierten Untersuchung erstellt (**Kap. 2.3: Total body electrical conductivity (TOBEC) measurements in Mongolian gerbils (*Meriones unguiculatus*) and golden hamsters (*Mesocricetus auratus*)**). In Übereinstimmung mit Literaturdaten (Guggenbuhl 1995, Yasui et al. 1998) wurde eine lineare Beziehung des Rohfettgehaltes und des Gesamtkörperwassers als Funktion des dimensionslosen TOBEC-Wertes ermittelt, die sich bei beiden Arten und für beide Parameter durch eine hohe Präzision auszeichnete. Nachfolgend konnten erstmals Basiswerte angegeben werden, die für andere Studien zum Wohlergehen oder für soziobiologische Analysen phänotypischer Fitnessparameter, z.B. beim Partnerwahlverhalten, von Bedeutung sein können und so die Nützlichkeit dieser Technik verdeutlichen.

Bereits mehrfach wurde auf die vielfältigen Einsatzmöglichkeiten telemetrischer Systeme hingewiesen (**Kap. 1.1: Measurement of physiological parameters and activity in a Mongolian gerbil during gravidity and lactation with an implanted transmitter** und **Kap. 2.1: Time of day and stress response to**

different stressors in experimental animals. Part II: Mongolian gerbil (*Meriones unguiculatus*)). Sobald die dafür notwendigen Transmitter implantiert sind, funktioniert diese Technik meist nahezu rückwirkungsfrei und ohne erkennbare Nebenwirkungen. Für das Wohlergehen des Tieres ist aber die Operation zu Beginn sehr belastend, die mit einer Narkose und zum Teil erheblichen Verletzungen, wie dem Eröffnen des Bauchraumes oder der subkutanen Tunnelung einhergeht. Um zu ermitteln, wie schnell sich ein Tier von diesen Strapazen erholt und wann es für die geplanten Experimente einsatzbereit ist, wurden sowohl die kurzfristigen als auch die länger andauernden Effekte eines solchen chirurgischen Eingriffs ermittelt (**Kap. 2.4: Indicators for post surgery recovery in Mongolian gerbils**). Grundsätzlich wurde dabei zwischen zwei Faktoren, die sich natürlich auch wechselseitig verstärken können, unterschieden: 1. Art und Applikation des Anästhetikums und 2. Art und Dauer der Operation. Unmittelbar nach dem Eingriff kam es zu einem Abfall der Körpertemperatur, der durch die Betäubung des Tieres ausgelöst wurde (Sessler 1993) und je nach eingesetztem Narkotikum unterschiedlich deutlich ausfiel. Während Isofluran, das gasförmig als Rauschnarkose verabreicht wurde, nur einen leichten Temperaturrückgang auf etwa 35 °C bewirkte, war die Hypothermie nach intraperitonealer Applikation von Pentobarbital, einem Oxybarbiturat, mit ca. 30 °C weitaus massiver. Daher und weil die Tiere im erstem Fall bereits unmittelbar nach dem Ende der Operation wieder lokomotorisch aktiv waren, ist Isofluran als geeigneter anzusehen. Der Grund für diese unterschiedliche Wirksamkeit ist wahrscheinlich in der jeweiligen Metabolisierung zu sehen. Isofluran wird nahezu vollständig wieder abgeatmet, so dass nur 0,2 % der Substanz in der Leber abgebaut werden müssen. Pentobarbital dagegen wird ausschliesslich in der Leber metabolisiert, wobei die Stoffwechselrate laut pharmakologischen Lehrbüchern mit 15 % / Stunde angegeben wird. Unabhängig von dem Grad der Hypothermie erreichten die Tiere sowohl nach Isofluran- als auch nach Pentobarbital ihre Ausgangskörpertemperatur erst nach etwa sieben bis acht Stunden, was mit Daten von Labormäusen übereinstimmt (Weinert et al. 1994). Während sich die Änderung der Körpertemperatur als ein zuverlässiger Indikator für die kurzfristigen Reaktionen post operativ erwies, war erst durch die chronobiologische Analyse des circadianen Rhythmus dieses Parameters sowie der lokomotorischen Aktivität der Zeitpunkt der vollständigen Genesung feststellbar. Erst nach Tagen war das Grundmuster wieder hergestellt, was sich mit Angaben aus der Literatur deckt (Clement et al. 1989, Drijfhout et al. 1995). Es zeigte sich, dass für die jeweilige Dauer insbesondere die Schwere des chirurgischen Eingriffs verantwortlich war. Nach der Implantation des Transmitters in die Bauchhöhle waren etwa sieben Tage notwendig; mussten zusätzlich subkutane Tunnel für die Erfassung der Herzschlagfrequenz angelegt werden, was unvermeidbar mit erheblicher Gewebsverletzung verbunden war, dauerte es sogar bis zu zwei Wochen. Solange der Körpertemperaturrhythmus als solcher nicht wieder hergestellt ist, sind auch andere Körperfunktionen beeinträchtigt. Dies konnte anhand der Sensitivität der Körpertemperatur gegenüber Aktivitätsänderungen gezeigt werden, die in intakten Organismen in der Ruhezeit ausgeprägter ist. Bei den operierten Tieren war dies erst nach der Wiederherstellung des Körpertemperaturrhythmus der Fall, was auf eine bis zu diesem Zeitpunkt gestörte Thermoregulation hindeutet (Weinert et al. 2002), und wiederum als ein Indikator für eine generelle Beeinträchtigung des Individuums anzusehen ist. Für eine möglichst umfassende Bestimmung des Wohlergehens eines Tieres sind somit neben den genannten Indikatoren auch die tagesrhythmischen Lebensäußerungen zu beachten.

Durch die in dieser Arbeit vorgestellten Publikationen konnten wesentliche Erkenntnisse zum Sozialverhalten, den Fortpflanzungsstrategien und dem Wohlergehen der Mongolischen Wüstenrennmäuse unter Laborbedingungen gewonnen werden. Die dabei etablierten Methoden lassen fundierte Aussagen für die Bewertung von Belastungen zu, die mit zentralen Lebensereignissen wie beispielsweise der Gravidität, Geburt und Aufzucht des Nachwuchses verknüpft sind. Um ein noch tieferes Verständnis über die komplexen Verhaltensmechanismen dieser Tierart zu erlangen, soll darauf aufbauend in weiteren Experimenten die Dynamik in den Familienverbänden unter naturnahen und natürlichen Freilandbedingungen analysiert werden. Neben dem vorhandenen Methodenrepertoire sind die dazu notwendigen molekulargenetischen Verfahren ebenfalls verfügbar und wurden bereits erfolgreich zur Charakterisierung von Wild- und Laborpopulationen eingesetzt (Neumann et al. 2001). Für die Beantwortung populations- und ethökologischer Fragestellungen kann darüber hinaus auf Freilandtelemetrie zurückgegriffen werden, die sich derzeit für Untersuchungen am Feldhamster bereits im Einsatz befindet. Schließlich soll in einem positiv begutachteten Projekt das Investment der Eltern- und Geschwister gegenüber den Nachkommen erfasst werden und so direkte und indirekte Fitnessvorteile kooperativer Brutpflege anhand des Reproduktionserfolges abgeschätzt werden. Dabei ist die Arbeitshypothese zu prüfen, wonach sich die Paare erfolgreicher reproduzieren und intensiver um ihren Nachwuchs kümmern, in denen Weibchen die freie Wahl zwischen Männchen als Paarungspartner haben.

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6 Anhang

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Eidestattliche Erklärung

Hiermit erkläre ich an Eides statt, dass die Habilitationsschrift selbständig und ohne fremde Hilfe verfasst, andere als die angegebenen Quellen und Hilfsmittel nicht benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht wurden.

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